The role of microbiota in autism spectrum disorders

Title: ROLE OF MICROBIOTA IN THE AUTISM SPECTRUM DISORDERS

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ABSTRACT

Autism Spectrum Disorder (ASD) defines a set of neurodevelopmental disorders characterized by persistent deficits in social communication and interaction, along with repetitive patterns of behavior. Symptoms generally appear in the early developmental period and cause significant impairment in individual and social functioning. In recent years the increased prevalence of ASD, along with the evidence of a significant link between autism and gastrointestinal (GI) disturbances, raised a special interest in exploring the reciprocal influences between gut and brain. Investigators highlighted the existence of a so-called “gut-brain axis”, empowering the hypothesis that GI abnormalities could trigger neuropsychiatric symptoms in ASD. Intestinal microbiota is thought to play a pivotal role in gut and systemic homeostasis, in CNS development, as well as in behavioral modulation and recurrent microbial imbalances have been shown in gut microbiota of autistic people. In this review we analyze current knowledge about intestinal microbiota and the

relevance and role of dysbiosis in ASD. The most accredited theories about gut-brain interaction will be reviewed, along with current scientific evidence supporting the relationship between microbial imbalances and impairment of neurodevelopment. Finally, we will focus on the results of different therapeutic approaches in this context: administration of pre- and probiotics, antibiotics, fecal microbiota transplantation and special diets and dietary supplements.

Key-words: autism spectrum disorder, microbiota, dysbiosis, probiotics, fecal microbiota transplantation

INTRODUCTION

Autism Spectrum Disorder (ASD) encompasses a wide range of conditions characterized by an impairment in neurodevelopment leading to persistent deficits in social communication and social interaction, along with repetitive patterns of behavior, interests and activities. Symptoms generally appear in the early developmental

period and persist life-long causing significant impairment in individual and social functioning. The term “spectrum” refers to the wide variability of presentations. Indeed, in 2013 the Diagnostic and statistical manual of mental disorders (5th ed., DSM V) introduced this new definition to merge some previously separate entities: autism, Asperger's syndrome, childhood disintegrative disorder and a pervasive developmental disorder not otherwise specified.

ASD is diagnosed in all racial, ethnic and socio-economic groups and it is three to four times more common in boys than in girls. A progressive increase of ASD prevalence has been reported worldwide over the last decades. Among US children a rise in prevalence from 0.67% in 2000 to 1.47% in 2010 has been reported, with a plateau in 2012 (1.46%). A recent large, nationwide population-based study reported an estimated ASD prevalence of 2.47% among US children and adolescents in 2014-2016, with no statistically significant increase over the 3 years.[1] It is postulated that at least part of the previously reported increase in ASD prevalence could reflect the effects of

expanded definitions of the disorder, increased public awareness and clinician referral, along with a probable true increase in risk factors.

To date, the etiology still remains unknown. ASD is probably a multifactorial disorder, resulting from the interaction of environmental, immune, genetic and epigenetic factors. Various environmental risk factors have been suggested and are currently under investigation, including advanced parental age, pregnancy-related complications, use of medications during pregnancy (e.g. valproate), pre- or postnatal exposure to chemicals (e.g. organophosphates and heavy metals), drugs or pollutants, viral infections and metabolic imbalances, along with the evidence of an important genetic influence, as demonstrated by a strong aggregation in identical twins.[2,3]

Children with ASD are at increased risk for a broad spectrum of concomitant medical issues, the most prevalent being sleeping problems, epilepsy, immune dysregulation and gastro-intestinal (GI) disturbances. The co-occurrence of these disorders appears to be

associated with more severe behavioral symptoms in the affected child.[4]

The most commonly reported GI disturbances among ASD children are chronic constipation, abdominal pain, diarrhea, bloating, gastro-esophageal reflux, disaccharidase deficiencies, as well as pathologic findings such as inflammation of the GI tract and abnormalities of the enteric nervous system.[5]

According to a consensus report of 2010 the reported prevalence of GI disorders in ASD patients aged 1–18 years ranged from 9 to 91%.[5] This relevant heterogeneity may be explained by different methodological approaches and study designs, different criteria to define GI problems and also difficulties for ASD subjects to communicate their symptoms. A recent meta-analysis confirmed a higher prevalence of GI symptoms among ASD children compared to control peers.[6]

GI comorbidities have raised increasing interest not only due to their frequency and secondary impact in worsening ASD behavioral

problems, but also because of their possible primary role in contributing to the genesis of the neurodevelopmental disorder.

Indeed anxiety problems, irritability, sleep disorders, self-injury and oppositional behaviors appear to be significantly more common among ASD children with GI symptoms, compared to peers without GI disturbances.[7, 8] It seems plausible that behavioral problems could be triggered or exacerbated by GI distress.

On the other hand recent theories propose gut dysregulation as a possible primary cause of disruption of the neurodevelopment process. This theories rely on the existence of a so-called “gut-brain axis”, a bidirectional complex network of communication between the central nervous system (CNS), the enteric nervous system (ENS) and the gut, that seems to play a key role in the physiological neurodevelopment. The exact mechanisms by which gut and brain reciprocally influence each other are still unclear.

Several factors co-operate to the normal functioning of the gut-brain axis, among them: the integrity of the gut mucosal lining, the luminal enzymatic activity and the regular absorption of nutrients, the

secretion of neurotransmitters and their precursors, as well as many other signaling molecules, cytokines and bacterial products. In this context the intestinal microbiota seems to play a key role in maintaining gut homeostasis and probably in preserving a normal gut-brain inter-relation. These evidences led to focus research efforts on the putative pathophysiological relevance of dysbiosis in ASD.[9, 10] Dysbiosis is defined as any qualitative or quantitative change in the composition of resident microbial communities compared to the communities found in healthy individuals.

The advances in DNA sequencing technologies have revolutionized the field of microbiomics, supplanting culture-based approaches and expanding investigational fields. Thanks to the amplification and sequencing of target microbial DNA regions and the more recent whole-genome shotgun sequencing techniques, investigators can identify the constituents of the microbiome, analyze their microbial genome, identify genetic variability and obtain taxonomic analysis.

In this review we summarize current knowledge of intestinal microbiota and the role of dysbiosis in autism spectrum disorders. The

most accredited theories about gut-brain interaction will be reviewed, along with current scientific evidence supporting the relationship between microbial imbalances and impairment of neurodevelopment. Finally, we will focus on the results of different therapeutic approaches that have been proposed to target gut dysregulation in ASD patients.

MICROBIOTA: DEFINITION AND FUNCTIONS

Humans are complex ecosystems, in which a large number of microorganisms live in symbiosis with the host. As a whole "superorganism", our body is composed of 10% of human cells and 90% of microbial cells, the latter populating almost every cutaneous and mucosal surface of the body. These microorganisms (mainly bacteria, but also viruses, fungi and archeas) constitute the human microbiota.[11]

The most heavily colonized area of the human body is the digestive tract, particularly the colon. The whole genome of gut microorganisms, the so-called gut microbioma, comprises nearly 150 times more genes than the human genome.

The gut microbiota is composed by 10^{14} different bacteria, divided into 4 different phyla: Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria. Firmicutes and Bacteroidetes are the predominant phyla (60-75% and 30-40% of the gut microbiota, respectively).[12]

The Firmicutes phylum is composed of Gram-positive bacteria (Lactobacillus, Enterococcus, Clostridium, Ruminococcus, Streptococcus, Staphylococcus); Bacteroidetes are Gram-negative bacteria such as Bacteroides and Prevotella.[13]

Gut microbiota plays an important role in maintaining the homeostasis of the human body, contributing to several fundamental functions, such as fermentation of otherwise indigestible carbohydrates, hydrolysis of proteins into peptides and free amino acids, deconjugation of bile salts and synthesis of some vitamins (K and biotin). Moreover, it is essential for the development of the immune system and to keep an efficient immune control: bacterial metabolites stimulate the epithelial production of IgA, regulate cytokines synthesis, assure a balance between regulatory T cells and effector T lymphocytes and play a central role in maintaining the integrity of the

intestinal barrier. For instance, germ-free (GF) mice display Peyer's patches hypoplasia, depletion of intra-epithelial lymphocytes, reduced secretion of IgA and cytokines and decreased serum levels of immunoglobulins. These abnormalities can be reverted after colonization.[12]

Moreover, growing evidences suggest that gut microbiota modulates CNS development and function, thus influencing human behavior and concurring to the pathogenesis of neuropsychiatric disorders, as discussed in greater detail hereunder.

MICROBIOTA: DEVELOPMENT

Human microbiota develops during the early phases of life, changes with breast/formula feeding and weaning and it achieves its definitive composition in the third year of life.

In the past, the intrauterine environment was considered completely sterile. Recent evidence demonstrated the presence of bacteria in amniotic fluid, placenta, umbilical cord blood and meconium.[14] Bacterial composition is similar in placenta and meconium, thus

suggesting a microbial transfer from mother to fetus via bloodstream and placenta.[13]

Maternal stress and diet, prenatal infections and exposure to antibiotics can modify both mother's and offspring's gut microbiota. Gestational age and delivery mode highly influence infants' gut microbial composition as well. Caesarean delivery exposes infants to mother's skin microbiota (dominated by Staphylococcus, Corynebacterium and Propionibacterium), whilst vaginally born infants harbor bacteria similar to their mothers' fecal and vaginal microbiota (mainly Lactobacillus and Prevotella).[14]

Additional modifications are induced by diet. Breast milk contains a great amount of Streptococci and Staphylococci and complex oligosaccharides that stimulate the growth of Staphylococci and Bifidobacteria.[13] Breast-fed infants have different, more stable and uniform microbial populations when compared to the formula-fed ones.[15]

Further changes occur after the introduction of solid food. The definitive composition of gut microbiota is achieved at the end of the

third year of life, and tends to remain stable in adulthood: 60%-70% of microbiota remains unchanged throughout life.

Intercurrent events like illnesses, diet modifications, infections and antibiotic treatments may cause imbalances in the intestinal microflora, potentially resulting in dysbiosis.[14]

MICROBIOTA AND CNS DEVELOPMENT

Gut microbiota and CNS evolve in parallel during fetal life and after birth, and several evidences suggest a fundamental role of the microflora in warranting a correct neurodevelopment. Deviances in gut microbiota composition have been linked to abnormalities in CNS development in different studies on animal models.

For instance, multiple alterations in neurogenesis have been described in GF mice, resulting in abnormally increased synaptic maturation and myelination, as well as in volumetric changes in hippocampus and amigdala.[13, 16]

Recent studies demonstrated an influence of microbiota on microglial maturation and blood-brain-barrier (BBB) permeability. Indeed, microglia displays an immature profile in GF mice, with a reduced ability to react to inflammatory stimuli, whilst a decreased expression of tight-junctions in the brain endothelium has been described, leading to increased BBB permeability. These abnormalities are reversed after administration of short-chain fatty acids (SCFA) or after gut colonization.[16]

MICROBIOTA AND BEHAVIORAL CHANGES

Dysregulation of gut microbiota can result not only in structural CNS abnormalities, but also in behavioral alterations, described both in animal models and humans.

Various pre-natal and early life events have been related to imbalances in microbiota composition and concomitant behavioral changes. For example, exposure to non-absorbable antibiotics in early gestational age in rats caused reduction in social interactions and increased anxiety in offspring.[17] In another study, the administration of non-absorbable antibiotics to pregnant mice induced variations in

microbiota composition in offspring, which, from a behavioral point of view, showed low locomotor activity.[18]

In mice, a high-fat diet during pregnancy resulted in abnormalities of gut microbiota (particularly with a reduction of Lactobacillus reuteri) and reduced social interactions in offspring. Treatment with L. reuteri improved offspring's sociability. Recently, obesity and diabetes mellitus in pregnant women have been linked to a higher risk of ASD.[16, 19]

Concerning prenatal infections, immune activation during pregnancy has potentially severe outcomes on offspring behavior and neurodevelopment. Some studies found a correlation between prenatal infections and a higher risk of schizophrenia and ASD in humans. Based on these findings, rodent models of Maternal Immune Activation (MIA) were developed. MIA offspring show abnormal communication and sociability, repetitive behaviors and increased anxiety.[20]

Hsiao et al. demonstrated microbiota alterations and higher gut permeability in MIA offspring. Treatment with Bacteroides fragilis

corrected gut permeability, restored microbial composition and improved communicative behaviors.[21]

Maternal separation is a typical model of early life stress employed in animal studies. In rats, early maternal separation induced higher plasma corticosterone levels (a common marker of stress), anxiety-like behaviors, alterations in gut microbiota and functional GI symptoms.[22] Treatment with Lactobacillus during maternal separation reduced corticosterone levels.[23] In macaques, early maternal separation reduced levels of Lactobacillus in offspring's gut microbiota and increased distress-related behaviors.[24]

GF mice show various behavioral abnormalities, among them: exacerbated response to stress, reversed by reconstitution with Bifidobacterium infantis; and reduced social interactions, normalized after recolonization.[25, 26] Conflicting results are reported by other authors, describing reduced anxiety in GF mice.[27, 28]

Several data in animal models and humans suggest that probiotic and antibiotic administration can modify microbiota composition transiently or permanently, possibly resulting in behavioral changes.[16]

GUT DYSBIOSIS AND ASD

The evidence of the impact of dysbiosis on CNS raised interest in the analysis of gut microbiota in neurodevelopmental, psychiatric and neurodegenerative disorders: depression, anxiety, schizophrenia, Attention Deficit Hyperactivity Disorder (ADHD) and ASD; Parkinson's and Alzheimer's disease were also investigated in this context.

Anecdotally, parents of children with regressive autism reported a relationship between the onset of neurobehavioral changes and repeated courses of antibiotics, followed by chronic diarrhea.[29] Moreover, different studies showed a higher frequency of antibiotic use in early life in children who later developed ASD.[30] Therefore, the disruption of gut microbiota has been proposed as a potential contributory cause of ASD.

Various studies have analyzed the microbiota composition in ASD suggesting several major abnormalities, but so far results are often conflicting, failing to generate a coherent picture. Other studies showed no differences in gut microbiota composition between

children with ASD and their siblings, rejecting the hypothesis of a bacterial role in triggering or maintaining ASD. According to the authors, other explanations for the GI dysfunction in this population should be considered, including elevated levels of anxiety and self-restricted diets.[10]

A recent study showed a higher prevalence of small intestinal bacterial overgrowth (SIBO) in ASD patients compared to controls, associated to worse behavioral manifestations.[31] SIBO consists in an increase in number and/or alteration in type of bacteria colonizing the small bowel.

Large-scale studies, using standardized methodologies, are needed to overcome these conflicting results.

Hereafter we summarize the main abnormalities of gut microbiota described in literature among ASD patients.

Clostridium

Investigations initially focused on the exploration of possible alterations in gut-populating Clostridium species, because of their

known ability to produce potentially neurotoxic compounds. Finegold et al. (2002) demonstrated higher counts of Clostridium and Ruminococcus spp in children with autism; nevertheless these data were not confirmed in a subsequent study.[32,33]

Song et al. demonstrated high abundance of a novel species of Clostridium (C. bolteae) in ASD patients and higher levels of clostridial cluster I e XI (which include toxin-producing clostridia), and decreased XIVab cluster.[34,35]

Parracho et al. demonstrated higher levels of C. histolyticum in ASD patients, and higher levels of Clostridia were associated to the presence of GI symptoms.[36]

Higher levels of Clostridia cluster I were confirmed by Tomova et al.[37]

Bacteroidetes/Firmicutes ratio

Finegold et al. (2010) described higher levels of Bacteroidetes in ASD patients, while Firmicutes were predominant in the control group.[33]

Conversely, Tomova et al., Williams et al. and Strati et al. showed a lower Bacteroidetes/Firmicutes ratio in autistic children. A lower ratio was evidenced in patients with more severe GI symptoms.[37-39]

Lactobacillus

Adams et al. described higher levels of Lactobacillus spp in autistic children, data confirmed by Tomova et al.[9,37]

Bifidobacter

De Angelis et al. and Adams et al. showed lower levels of Bifidobacterium spp in ASD.[9,40]

Desulfovibrio

A role of Desulfovibrio in the pathogenesis of ASD has been suggested, mainly because of its ability to release lipopolysaccharides (LPS) and to produce potentially toxic metabolites like hydrogen sulphide.

Finegold et al. (2010) described higher levels of Desulfovibrio in autistic subjects.[33] These data were confirmed by Tomova et al., who

described a relationship between Desulfovibrio levels and ASD symptoms and a significant decrease in Desulfovibrio levels after probiotic administration.[37]

Sutterella

Williams et al. (2012) described a higher prevalence of Sutterella species in GI biopsies of ASD patients with GI symptoms compared to non-autistic children with functional GI disorders.[41]

Wang et al. and De Angelis et al. confirmed elevated levels of Sutterella in feces of ASD patients compared to neurotypical controls.[40,42]

Fungi

Yeast proliferation can lead to lower absorption of carbohydrates and higher release of ammonia and toxins, which are thought to contribute to autistic behaviors. Few studies have investigated the relationship between intestinal fungi and ASD. Kantarcioglu et al. described a higher prevalence of yeasts in feces of ASD patients compared to controls, the most commonly isolated species being Candida albicans.

These data were confirmed by Strati et al. and Iovene et al.[38, 42] Iovene et al. found no relationship between GI symptoms and Candida levels in ASD patients.[43]

DYSBIOSIS IN THE PATHOGENESIS OF ASD: PROPOSED MECHANISMS

Brain and gut, including gut microbioma, communicate reciprocally by means of complex and not fully elucidated ways of signal transmission, including metabolic, neural, endocrine and immune pathways. In this context, different mechanisms have been proposed to explain the influence of gut dysbiosis on ASD pathogenesis or exacerbation. The absorption of microbial components or metabolites seems to play a pivotal role, probably fostered by the increased intestinal permeability described both in humans and animal models of ASD, the so-called “leaky gut syndrome”.

Leaky gut syndrome

The intestinal barrier is a selective and dynamic filter which regulates the absorption of nutrients from the lumen into the circulation and simultaneously prevents the penetration of pathogens or their metabolites. Recently, several studies have been investigating the intestinal permeability (IPT) in patients suffering from neurodevelopmental and psychiatric disorders, including ASD.

IPT is generally evaluated by dosing the urinary excretion of two orally administered indigestible saccharides with different molecular size and absorption routes. One (usually lactulose) passes the intestinal barrier through paracellular pathways, mainly regulated by tight junctions, and in physiological conditions it is minimally absorbed (1%). The co-administered sugar (usually mannitol) uses the transcellular transport and its absorption is proportional to the small bowel surface and absorptive capacity. An increased urinary excretion of lactulose reflects a higher intestinal permeability, whereas an intestinal inflammation with mucosal damage results in a decreased mannitol absorption. Hence, a higher urinary lactulose/mannitol ratio reveals an increased IPT.

In 1996, D'Eufemia et al. firstly demonstrated a high frequency of altered IPT in autistic children, mainly due to the increased lactulose recovery, thus suggesting mostly a damage of the tight junctions.[44] An increase in IPT was subsequently confirmed by de Magistris et al., Iovene et al. and Horvath et al.[43, 45, 46]

Fiorentino et al. described a reduction of barrier-forming tight junction components (Claudin-1, Occludin, Tricellulin) and an increase in pore-forming elements (Claudin-2, -10, -15) in duodenal biopsies of ASD patients compared to healthy controls, strengthening the hypothesis of an impaired intestinal barrier in autism.

Conflicting results have been published in this field as well. Kushak et al., Robertson et al., Dalton et al., Kemperman et al. and Pusponegoro et al. failed to demonstrate an increased IPT in ASD children.[47-51]

Several studies focused on the analysis of fecal calprotectin (FC) levels in ASD patients. FC is a protein synthetized by intestinal granulocytes. Higher calprotectin levels in feces indicate neutrophil migration and infiltration in the intestinal tract, thus it is commonly used as a marker of local inflammation in inflammatory bowel

diseases. These investigations in ASD were aimed at evaluating if an increased IPT in autistic children could be related to intestinal inflammation. De Magistris et al. showed abnormal FC levels in 24.6% of the autistic patients, but there was no correlation between IPT and FC values.[45] Kushak et al. described no differences in FC values in ASD children compared to controls, data confirmed by Pusponegoro et al., Iovene et al., Fernell et al. and Babinska et al.[43, 47, 51-53] All together, these results coherently reject the hypothesis of an increased intestinal inflammation as a cause of higher IPT in autistic patients.

Recently, other studies tried to evaluate if IPT variations could be explained by higher circulating levels of Zonulin. Zonulin is a protein which physiologically modulates IPT, inducing tight junction disassembly, by modifying protein-protein interaction. Several stimuli, including intestinal dysbiosis, can induce Zonulin synthesis, therefore influencing IPT. Esnafoglu et al. demonstrated higher levels of Zonulin in ASD patients compared to neurotypical controls, whereas no statistically significant difference was described by

Józefczuk et al. Further studies are needed in this field, to assess the effective role of Zonulin in modulating IPT in ASD children and the potential usefulness of Zonulin inhibitors (such as larazotide acetate) in these patients.[54, 55]

To summarize, whether IPT is increased or not in autism is still debatable, and the influence of the so-called "leaky gut syndrome" on ASD pathogenesis and symptoms has yet to be demonstrated.

Cytokines and Lipopolysaccharides (LPS)

Immune system appears to be an important factor intervening in gut-brain intercommunication. Intestinal bacteria exert either pro- or anti-inflammatory effects depending on the type of bacteria (pathogenic or commensal) and the type of immune response elicited (T helper cells or regulatory T cells). Hence pro- or anti-inflammatory cytokines are secreted and, apart from their local actions, they can reach the brain via the bloodstream and, in certain conditions, it is thought that they can pass the BBB and exert central actions.

In detail, cytokine signaling seems to especially affect the hypothalamus, an area of the CNS where the BBB is deficient. Here pro-inflammatory cytokines such as IL-1 and IL-6 can activate the hypothalamic-pituitary-adrenal (HPA) axis, inducing cortisol release and activation of the stress system, that can in turn influence gut function.[56]

GF mice show a higher elevation of plasma ACTH and corticosterone levels in response to stress, normalized by reconstitution.[25] This evidence could be interpreted as an hyper-reactivity of the HPA axis, with amplified response to stress in GF mice.

In ASD patients, increased levels of inflammatory cytokines have been reported and were associated to impaired social skills. Moreover, a marked neuroinflammation has been described in brain specimens of ASD individuals.[29, 30]

Immune activation, with the production of inflammatory cytokines, can also be elicited by the translocation of LPS (a component of the cell wall of Gram-negative bacteria) through the intestinal wall into the systemic circulation. Some studies described elevated serum levels

of LPS in ASD patients compared to healthy controls, and higher levels were associated to impaired social behavior, probably suggesting the role of inflammation and immune activation in promoting ASD.[29, 30]

Hence, the correct balance in circulating levels of cytokines seems to be essential for CNS development and HPA axis normal response to stress: a higher systemic inflammation can alter this process, leading to neurodevelopmental disorders.

Short-chain fatty acids (SCFA)

Short-chain fatty acids (SCFA), including acetate, proprionate and butyrate, are produced by microbial fermentation of complex carbohydrates (e.g. resistant starch, inulin, pectin). They represent the primary energy source for colonocytes and play an essential role in maintaining the gut epithelial barrier by strengthening the tight junctions and stimulating the production of the mucus layer. They also regulate transepithelial transport, particularly of water and electrolytes, as well as intestinal motility and modulate the inflammatory state of the intestinal mucosa by stimulating the

production of anti-inflammatory compounds. Furthermore SCFAs show an inhibitory effect on neutrophil migration, leading to limitation of the inflammatory processes.

The demonstration of quantitative imbalances in SCFAs levels in ASD children would provide further indication for a role of dysbiosis in this neurodevelopmental disorder. Unfortunately there is conflicting evidence about this issue. Wang et al. found significantly increased levels of SCFAs in fecal samples from autistic children compared to controls, while another study by Adams et al. reported opposite results.[9, 57]

De Angelis et al. found significantly lower levels of butyric, but not acetic or propionic acid, in ASD children.[58]

Although SCFAs are known to have neuroactive properties, their exact role in ASD remains poorly understood. Butyrate can cross the BBB and is known to act as a histone deacetylase inhibitor, thus being able to modulate gene silencing and gene expression in the CNS. It is believed to play a role in the epigenetic pathways of transcriptional regulation of neurotransmission, particularly in the prefrontal cortex.

The prefrontal cortex is an area of the CNS with primary roles in cognitive, emotional and social behaviors, frequently affected by morphological, transcriptional, and epigenetic dysregulation in autistic individuals.[59]

Intraperitoneal administration of butyrate has been shown to improve social behavior and repetitive symptoms in a murine model of ASD[59], whereas intra-cerebroventricular infusions of propionic acid induce autistic-like behaviors in rats.[60]

Furthermore SCFAs seem to be involved in multiple pathways of neurodevelopment; for instance they regulate microglial maturation and activation, and oral treatment with SCFAs can rescue impaired microglial function in GF animals.[61]

However, since the expression of SCFA receptors is low or absent in the brain[61], some authors argue that these compounds are likely to have mainly indirect effects on the CNS, probably mediated by glucagon-like peptide-1 (GLP-1), a gut hormone with both neurotrophic and anti-inflammatory actions, or via the vagus nerve.[62]

Further research is warranted to fully understand the role, if any, of SCFAs in ASD and other neurologic disorders.

Glutamate, serotonin and other neurotransmitters

The aminoacid glutamate acts as an excitatory neurotransmitter and an excess of glutamate is known to induce neuronal cell death in the CNS. In the gut glutamate can also be produced from the hydrolysis of proteins and peptides by proteolytic bacteria (e.g. Clostridium and Bacteroides). High levels of glutamate have been found in fecal and blood samples of children with ASD compared to healthy controls and this hyperglutamatergic state has been suggested to play a pivotal role in the pathophysiology of autism.[7, 58, 63]

Besides glutamate, gut microbiota can indirectly regulate many other central neurotransmitters, such as serotonin, by altering the levels of precursors. Serotonin is known to be involved in the modulation of anxiety, stress responses, reward, and social behavior. Bifidobacterium infantis has been shown to increase plasma tryptophan levels, thus empowering serotoninergic transmission in the CNS.[64] Reduced levels of Bifidobacterium spp have been

demonstrated in autistic patients, supporting their protective role in CNS development and functioning. In a metabolomics study the colonization of GF mice by gut microbiota determined an almost three-fold increase in plasma serotonin levels.[65] In animal models, a defective serotoninergic neurotransmission has been linked to the pathogenesis of several behavioral abnormalities and CNS structural changes. Mutant mice for genes involved in the serotonin signaling or in its degradation show abnormal serotoninergic transmission and display social deficits.[66] Furthermore, mice manipulation leading to excess serotonin clearance during early stages of neurodevelopment has been shown to influence neuronal migration, axonal projections, and synapse development.[67]

Interestingly, synthesis and direct release of various neurotransmitters from bacteria has been reported: specific strains of Lactobacillus and Bifidobacterium spp can produce γ-aminobutyric acid (GABA); Escherichia, Bacillus and Saccharomyces spp can produce noradrenaline; Candida, Streptococcus, Escherichia and Enterococcus spp can produce serotonin; Bacillus can produce dopamine and

Lactobacillus acetylcholine.[56] These neurotransmitters, once synthesized in the gut, cannot cross the BBB, but their central effects are likely to be mediated by an action on the ENS via the vagus nerve.

Oxytocin

Oxytocin is a neuropeptide secreted from the hypothalamus and implicated in the regulation of various social behaviors in mammals such as emotional bonding, maternal care, affiliation and social attachment. Reduced oxytocin blood levels, genetic variants of the oxytocin receptor and mutations of proteins involved in oxytocin release have been demonstrated in ASD individuals, leading to the hypothesis that oxytocin could play a role in modulating autistic behaviors and thus suggesting a potential therapeutic effect of oxytocin administration.[68]

Intriguingly, a recent study indicates that probiotic bacteria can increase posterior pituitary release of oxytocin and thus circulating levels.[69] This evidence suggests the possibility of influencing oxytocin signaling by targeting gut microbiota.

Other metabolites

Significant differences in the plasma levels of several metabolites have been reported in studies comparing the plasma metabolomes of germ-free and conventional mice, thus underlining the importance of intestinal microbiota in the regulation of several metabolic pathways.[65] Further studies are needed to investigate the influence of these metabolic imbalances on CNS.

When comparing ASD children to healthy controls, higher levels of p-cresol, a phenol compound produced by certain intestinal bacteria and probably related to neuropsychiatric impairment, as well as higher levels of indole, a precursor of serotonin and melatonin synthesized by several commensal bacteria, have been reported.[58] The clinical relevance of these findings is not known.

THERAPEUTIC MODULATION OF MICROBIOTA

Considered the high prevalence of GI disorders in ASD individuals and the evidences linking intestinal dysbiosis to GI symptoms and to behavioral disturbances in these patients, therapeutic manipulation of

the intestinal microbiome has been proposed to restore the microbial balance in the gut, in order to improve GI and neuropsychiatric symptoms. The restoration of a balanced microflora can be targeted through the administration of pre- and probiotics, antibiotics, nutritional approaches or more recently, through fecal microbiome transplantation (FMT).[70]

Prebiotics

The concept of "prebiotic" was firstly introduced in 1995 by Gibson and Roberfroid, who defined a prebiotic as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria already resident in the colon, and thus improves host health". At that time fructans, comprising fructooligosaccharides (FOS) and inulin, and galactans (galactooligosaccharides or GOS) were recognized as prebiotics, acting through the enrichment of Lactobacillus and/or Bifidobacterium spp. Since then the definition has progressively expanded to include additional non-food and non-carbohydrate

substances with recognized ability to influence gut colonization, as well as to consider extra-intestinal effects or applications of prebiotics.

Thus in 2017 an expert consensus document of the International Scientific Association for Probiotics and Prebiotics (ISAPP) released a revised definition of prebiotic as "a substrate that is selectively utilized by host microorganisms conferring a health benefit".[71]

According to this definition, prebiotics are non-viable substrates that serve as nutrients for beneficial microorganisms harbored by the host, including administered probiotic strains and resident microbiota. Differently from most dietary fibers, which promote growth of a wide variety of microorganisms, prebiotics should display a selective effect, being substrate for beneficial strains only, thus excluding metabolism by pathogenic bacteria such as Clostridium spp and Escherichia coli.

So far the most studied prebiotics are the non-digestible oligosaccharides fructans and galactans, which are mainly found in foods like fruit, vegetables, legumes and cereals, cannot be metabolized by human gut enzymes, pass across the small bowel without being absorbed and reach the colon where they are

preferentially metabolized by Bifidobacteria. Other substances with a recognized prebiotic function are the human milk oligosaccharides (HMOs), that are particularly important for the development of the intestinal microbiota of the newborn. Consumption of maternal milk containing HMOs increases the proportion of intestinal Bifidobacteriaceae and Bacteroidaceae.[72] Furthermore, fucosylated and sialylated HMOs can prevent adhesion of pathogens to the intestinal epithelium, thus protecting the baby from infections. Plant polyphenols too, although less investigated, are recognized as having prebiotic action, particularly after extensive biotransformation by the colonic microbiota.[71]

As stated in the definition itself, a substance can be considered prebiotic if it confers a health benefit to the host. Such benefits are not limited to the gut homeostasis but can extend elsewhere in the organism, leading to improvements of the immune, metabolic, endocrine or nervous functions. Besides enrichment of beneficial microbial strains, various other mechanisms have been identified through which prebiotics can act: generation of SCFA from their

bacterial fermentation, elongation of microvilli, increase in mucus layer thickness and consequent protection of gut epithelium.[71]

At present no clear data are available to demonstrate a role of prebiotics in the treatment of autistic patients, although their known beneficial effects strongly suggest a potential for their application in the management of gut dysbiosis and GI disturbances of ASD children.

Furthermore, an anxiolytic effect of prebiotics has been suggested, both in humans and mice models. In human healthy volunteers GOS administration has been shown to reduce the cortisol awakening response and to modulate some emotional processes.[73] The cortisol awakening response is a reliable marker of HPA axis activity. Prebiotic administration may modulate HPA activity similarly to direct administration of probiotic strains in rodents and humans.[23, 25, 74]

In 2016 Savignac et al also demonstrated that GOS administration led to modulation of cortical IL-1β levels and 5-HT2A receptor expression and reduced anxiety behaviors in mice.[75] These evidences suggest a potential role for prebiotics in ASD and other

neuropsychiatric disorders where anxiety and neuroinflammation are prominent clinical features.

Recent studies demonstrated an influence of prebiotic administration on central Brain Derived Neurotrophic Factor (BDNF) expression. BDNF is important for survival and differentiation of dopaminergic neurons in the developing brain, regulates the formation and plasticity of synaptic connections and is also trophic for serotonergic neurons. Impairment in BDNF signaling in early developmental phases is thought to be related to CNS abnormalities, severer forms of autism and intellectual disability, as well as epileptic manifestations. Studies in autistic patients reported conflicting data about BDNF levels, showing often higher, but sometimes lower or not significantly different levels compared to controls.[76] In GF rodents the central expression of BDNF and N-methyl-d-aspartate receptor (NMDAR) subunits is reduced, whereas administration of oral probiotics is known to increase BDNF levels. In 2013 Savignac et al demonstrated that prebiotic feeding too elevates hippocampal BDNF and NMDAR subunits expression in rats, confirming a role of gut microbiota in

regulating BDNF synthesis.[62] These preliminary results warrant further investigation to better understand the role of BDNF in autism pathogenesis and the potential usefulness of its modulation through pre and probiotic administration in early life.

Further investigations are needed to ascertain the beneficial neurotropic effects of prebiotics, particularly in ASD individuals.

Probiotics

According to the Food and Agriculture Organization (FAO)/World Health Organization (WHO) definition of 2001, probiotics are products that contain live microorganisms, which have beneficial and desirable effects on humans and animals when provided in adequate amounts.

As previously observed, several data suggest that probiotic administration can not only reverse gut dysbiosis and relieve GI disturbances, but also exert central actions, promoting positive behavioral changes both in animal models and humans with or without ASD.[19, 21, 25, 77-81] Probiotic intake has been linked to reduced

subjective feelings of anxiety and improved well-being.[74, 82] For instance, the administration of Lactobacillus helveticus and Bifidobacterium longum reduced anxiety-like behaviors in rats and relieved psychological distress in healthy humans.[74] Another study showed that administration of a 4-week course of a multispecies probiotic containing B. bifidum, B. lactis, L. acidophilus, L. brevis, L. casei, L. salivarius and L. lactis decreased cognitive reactivity to sad mood and aggressive thoughts in humans.[83] Furthermore, a functional MRI investigation revealed that administration of a fermented milk product with probiotics resulted in measurable changes in emotional processing in the healthy human brain.[84]

In rats, the oral administration of Bifidobacteria increased levels of hippocampal BDNF, which plays an important role in neurodevelopment and may also exert some antidepressant actions.[85] Similarly, L. reuteri administration has been showed to elevate oxytocin levels, a key regulator of social behaviours.[86] Finally, as mentioned before, probiotic therapy could lead to a modulation of the HPA axis functioning and regulation of the cortisol stress response, as

well as to a down-regulation of inflammatory pathways in the gut and CNS.[25]

In 2015 a randomized trial showed that early postnatal probiotic treatment decreases ASD risk. The enrolled children were randomly assigned to the probiotic or placebo groups during the first six months of life and results showed that none in the probiotic and 17% in the placebo group was diagnosed with ASD or ADHD.[87] The authors noted that no significant modification of the microbiota composition was determined by probiotic therapy in their cohort, thus suggesting that the central effects of probiotics are likely mediated by other mechanisms such as reduced inflammatory milieu, reduced gut permeability, strengthened BBB, restoration of a normal balance between harmful and beneficial gut bacterial products.

Antibiotics

Further support for the hypothesis of a microbiota-gut-brain axis comes from studies on autistic children who were treated with antibiotics. A subgroup of autistic children appears to develop normally until a cognitive deterioration is observed. In some cases the

onset of these regressive forms of autism is preceded by antibiotic exposure and chronic diarrhea reported by parents. Consequently, a potential pathogenetic role of certain antibiotics has been postulated, as they could dysregulate intestinal microflora, promoting gut colonization by neurotoxin-producing bacteria, such as Clostridium spp, resulting in disruption of neurodevelopmental processes.[88] Specifically, a correlation between the use of broad-spectrum antibiotics, such as amoxicillin-clavulanate, and ASD onset has been noticed. The increased incidence of middle ear infections in ASD children, together with the extensive use of broad-spectrum antibiotics in contemporary society, have been advocated as possible causes of the recent increases in ASD epidemiology.

A trial with 8-week oral vancomycin administration in a small group of children with regressive-onset autism determined a transient but significant improvement in both GI and behavioral disturbances.[88] Indeed vancomycin is a minimally absorbed antibiotic, active against Clostridia, and its antibacterial activity can explain the observed benefits as a consequence of the modulation of the gut microbiota.

The symptomatic relapse after therapy discontinuation could be linked to the conversion of Clostridia to a spore-form, highly resistant to antibiotics, which can later newly germinate into vital forms.

Extensive and prolonged use of vancomycin or other antibiotics is therefore not advisable, because of the risk of selecting resistant strains.

Fecal Microbiota Transplantation (FMT)

The process of FMT consists in the delivery of feces from a donor to a recipient, with the aim of replacing an impaired microbiota with a healthy one.

A Chinese physician, Ge Hong, described the use of FMT in human medicine for the first time approximately 1700 years ago for treating severe diarrhea. In 1958 FMT has been introduced in modern medicine for the management of antibiotic-associated pseudomembranous enterocolitis. Recently, in several randomized clinical trials FMT demonstrated its efficacy in treating antibiotic-

refractory C. difficile infection, a severe life-threatening condition.[89, 90]

Currently, interest about the use of FMT as a therapeutic strategy for diseases related to gut dysbiosis has grown. Unlike probiotics, which contain only a restricted number of bacterial species, FMT allows the transplantation of thousands of different components of the healthy gut microbiota, resulting therefore a promising therapeutic approach for GI diseases such as inflammatory bowel diseases, irritable bowel syndrome and chronic constipation, and non-GI diseases like autoimmune disorders, obesity and insulin sensitivity. Moreover, it is a safe and well-tolerated treatment: the most commonly reported adverse events include mild diarrhea, abdominal tenderness, bloating, flatulence and nausea.[89]

Since, as previously explained, ASD subjects often display dysbiosis, FMT can be considered a potential therapeutic intervention for these patients, thanks to its ability to revert gut microbiota abnormalities.[91]

Recently, Kang et al. published an open-label study investigating safety, tolerability and efficacy of FMT in autistic patients. The study

involved 18 ASD patients with GI symptoms and a control group of 20 neurotypical subjects. ASD patients underwent a modified FMT protocol, named Microbiota Transfer Therapy: oral vancomycin treatment, followed by laxatives administration and a subsequent high initial dose of Standardized Human Gut Microbiota-SHGM (orally or rectally). Afterwards, a daily lower maintenance oral dose of SHGM associated to proton pump inhibitors (PPI) was given for 7-8 weeks. Patients in the control group did not receive any therapeutic intervention. GI and ASD-related symptoms improved in the treatment group, with response maintenance during follow-up. FMT confirmed its ability in modifying gut microbiota composition: levels of Bifidobacteria, Prevotella and Desulfovibrio significantly increased. In line with previous reports, few adverse events were described, thus confirming treatment safety also in autistic patients.[92]

According to these results, FMT can be considered an effective and well-tolerated approach in ASD. However, the study was neither blinded nor placebo controlled. Hence, the amelioration of GI and ASD–related symptoms should be carefully interpreted as it could

derive from placebo effect. Moreover, it described the effects of four combined treatments, and it has to be determined if these results derive solely from one of the elements (vancomycin, laxatives, SHGM, PPI) or from their combination.

In conclusion, results are promising, but randomized, placebo-controlled, double blinded studies are needed to confirm these outcomes. A larger sample size and a longer follow-up period will be essential to clarify long-term safety and tolerability, particularly to clear up concerns about the potential risk of transmission of pathogenic agents or the possible epigenetic influences on human genoma over time.

Diet

In recent years research focusing on nutritional approaches in autism has increased. The rising interest on the potential role of dietary interventions in ASD originates from three main elements.

First, ASD patients are more frequently selective eaters then neurotypical children of the same age. Indeed, they usually display

restricted diets, with a limited food repertoire, resulting in reduced fruit, vegetables and protein intake. Kanner firstly described feeding difficulties in autistic patients in 1943: “Our patients, anxious to keep the outside world away, indicated this by the refusal of food”.

Second, some reports showed improvement of ASD symptoms after specific dietary changes. Third, diet highly influences gut microbiota composition, thus suggesting that a rebalance in intestinal microbiota induced by diet could explain symptoms amelioration.

So far, several dietary interventions have been described in ASD: ketogenic diet, gluten-free casein-free (GF/CF) diet, ω-3 fatty acids, digestive enzymes or vitamins supplementation, consumption of curcumin or camel milk.[93]

Below, we discuss in detail the most relevant nutritional approaches investigated in autistic patients.

Ketogenic Diet (KD)

KD is a nutritional regimen very low in carbohydrates, high in fat and with an adequate proportion of proteins. KD causes a metabolism shift, mimicking the fasting state and resulting in higher synthesis of ketone bodies. It is commonly used in pediatric neurology owing to its effectiveness in treating drug-resistant epilepsy, even though its exact mechanism of action remains unclear. Modification of gut microbiota composition after the KD has been described in several studies in neurologic patients.[94]

In animal models of ASD, both improvement of behavioral symptoms and compositional remodeling of gut microbiota after KD have been described.[93, 95]

In humans, several case reports evidenced a potential role of KD in ameliorating autistic symptoms. Two non placebo-controlled studies were conducted (recruiting respectively 30 and 15 patients), both confirming the beneficial effects of KD in autistic patients.[96, 97] Further studies are required to confirm these preliminary data and to analyze the possible impact of KD on autistic behavior.

Gluten-Free Casein-Free Diet (GF/CF)

Hans Asperger was the first one to suggest a potential relationship between autism and celiac disease in 1961, paving the way for attempting gluten-free nutritional interventions in ASD patients.

Afterwards, theories enlightening the potential harmful effect of diets containing gluten and casein in autistic patients developed. According to these theories, ASD pathogenesis could be explained, at least in part, by an excess of opioid activity in the CNS. As stated by these models, a reduced activity of peptidase enzymes results in gluten and casein abnormal metabolism, with an excessive production of incompletely metabolized peptides. These peptides, thanks to the increased permeability of the intestinal and blood-brain barriers, can enter the bloodstream and reach the CNS. Here, they can act like opioid neurotransmitters or interfere with the hydrolysis of other neurotransmitters binding central peptidase enzymes, halting their metabolic activity. Furthermore, gluten and casein peptides have been suggested to unbalance the immune response, triggering local inflammation, thus enhancing the increased intestinal permeability, and also promoting systemic cytokine synthesis.[93]

Both excessive central opioid activity and unbalanced inflammatory response can adversely affect CNS development and function. Consequently, the GF/CF diet was proposed as a possible therapeutic approach for patients with ASD.

In 1995, a non-randomized, non-blinded study by Knivsberg et al., conducted on 15 ASD patients, showed a potential benefit of GF/CF diet on autistic-related behaviors. These results added scientific significance to the great number of previous anecdotal observations.

Subsequently, numerous research studies worldwide, including randomized clinical trials (RCT), have examined the role of GF/CF diet in autism, and several systematic reviews have been published.

When analyzing these studies, many methodological issues stand out: a lack of placebo in some trials, small sample sizes, wide age ranges, short duration and follow up, concomitant administration of other therapies, difficult assessment of dietary adherence and outcomes (mostly based on parental observations).

Moreover, results about the efficacy of GF/CF diet on behavioral symptoms are conflicting and often not statistically significant. At the moment, the limited available data suggest that there is no sufficient evidence to support the adoption of GF/CF diet in ASD children. These results have to be carefully interpreted, as the number of high quality studies is limited to draw a clear conclusion; therefore, the need for further research has to be stressed.[93, 98]

ω -3 fatty acid supplementation

ω-3 fatty acids are essential fatty acids, considered to be fundamental for a balanced and physiological neurodevelopment. Indeed, deficiency in ω-3 fatty acids has been described in different psychiatric disorders, including ASD.

Therefore, several studies were conducted to analyze behavioral improvement in autistic children after dietary supplementation of ω-3 fatty acids.

Case control and open label studies initially provided evidence of a potential efficacy of ω-3 fatty acids, whereas RCT yielded contrasting and heterogeneous outcomes. Administered doses were highly dissimilar among different studies, with often limited sample size. On the basis of current evidence, ω-3 fatty acids could be used as a complementary therapy in ASD, but their effect on ASD symptoms has still to be clarified.[99]

Digestive enzymes supplementation

Deficiency in digestive enzymes has been described in ASD patients, in particular a lack in those responsible for carbohydrate digestion. [89] Therefore, undigested elements can be used as a source of energy for intestinal bacteria, potentially contributing to gut dysbiosis.

Hence, digestive enzymes supplementation has been tested as a possible therapy for autistic children. A first pilot, non placebo-controlled study showed an improvement in behavioral disturbances in ASD patients after enzymes administration. This evidence was not

confirmed in a subsequent randomized placebo-controlled clinical trial. [89]

It should be noted, however, that this two studies cannot be properly compared, as they used different enzymes formula. In the first case a mixture of enzymes digesting both carbohydrates and proteins was used, whilst in the RCT only proteolytic enzymes were administered. We may argue that failure to demonstrate a beneficial effect of enzyme supplementation in the RCT might have been determined by the absence of glycolytic enzymes. According to these partial and preliminary results, the matter remains an interesting area of research, which deserves further investigations.[89]

Vitamins supplementation

Vitamins are essential for our organism homeostasis and, as previously said, several of them can be synthetized by gut microbiota, such as biotin and vitamin K. Various vitamin deficiencies have been described in ASD patients, generating a wider interest in the possible

efficacy of their supplementation as a therapeutic approach. For instance recent studies highlighted the possible role of vitamin D deficiency in ASD, as it is frequently described in autistic patients and a maternal deficit during pregnancy has been linked to a higher risk of autism in the newborn.

In a placebo-controlled study conducted by Adams et al., a formulation of vitamins and minerals, including vitamin K and biotin, was administered to autistic patients for 3 months. After supplementation, a behavioral improvement was demonstrated. Regression analysis showed that low pre-treatment levels of biotin and vitamin K were highly related to symptoms amelioration, thus being the best predictors of improvement.[100]

In conclusion, autistic patients may commonly have hypovitaminosis, partly due to a lower GI microbial synthesis, partly due to a reduced intake; so we can hypothesize that vitamins supplementation could be beneficial in these patients. Further research is needed.

Curcumin

Curcumin has been used for centuries in traditional medicine. Recently, interest concerning the use of curcumin has raised, as a consequence of growing evidences of its anti-inflammatory, antioxidant, neuroprotective and monoaminergic effects. In murine models of ASD, curcumin administration over a period of 4 weeks improved ASD-related symptoms, namely restoring social interactions and reducing anxiety and repetitive behaviors.

Studies on humans have yet to be conducted, but curcumin could be a promising therapeutic approach in these patients. [89]

Camel milk

Camel's milk has a unique composition: compared to cow's milk, it contains more minerals, more vitamins, less fat and less lactose; it lacks of β-lactoglobulin and β-casein, potentially allergenic, and contains various components with antibacterial and immunological

properties. Furthermore, it may, at least in part, influence gut microbiota composition owing to its content of bacteria and prebiotics.

A first study by Al-Ayadhi et al. displayed a potential benefit of camel's milk compared to cow's milk: in the treatment group, ASD symptoms improved and oxidative stress decreased, as demonstrated by an increase in plasma levels of superoxide dismutase, myeloperoxidase and gluthatione. Behavioral improvement induced by camel's milk was confirmed by two subsequent studies. It should be noted that the use of cow's milk as a control could be a potential confounder, as it is believed to stimulate the immune function and increase the oxidative stress. [89]

Further studies are requested to confirm the potential efficacy of this dietary intervention, better if not using cow's milk as a control, in order to clarify whether the observed effects are merely due to the lack of cow's milk in the diet or to a specific effect of camel's milk.

No evidence about the effectiveness of other nutritional approaches, such as low FODMAP (Fermentable Oligosaccharides, Disaccharides,

Monosaccharides and Polyiols) diets or food additives exclusion diets in ASD exists.

Generally speaking, we can argue that very little is known about the impact of dietary interventions on symptoms and microbiota composition in ASD patients. Unfortunately, available data are limited and frequently contradictory. Furthermore, the risk of provoking nutritional deficiencies in developing children as well as the risk of low adherence, require additional attention when introducing diet changes in our patients, carefully balancing risks and benefits. Well-designed clinical trials, focusing on the consequences of dietary interventions on symptoms, gut microbiota composition and nutritional state in autistic patients are required.

CONCLUSION

In recent years the increased prevalence of autism spectrum disorders, along with the evidence of a significant link between autism and GI disturbances, raised a special interest in exploring the reciprocal

influences between gut and brain in ASD. At the same time, investigators highlighted the existence of a so-called “gut-brain axis”, empowering the hypothesis that symptoms of neuropsychiatric disorders, like ASD, could be triggered by GI abnormalities. Intestinal microbiota is thought to play a pivotal role in gut and systemic homeostasis, in CNS development, as well as in behavioral modulation. In most studies comparing ASD children versus healthy peers, significant differences in microbiota composition have been identified, mainly consisting in reduced levels of Bifidobacter and increased levels of Clostridium spp and Desulfovibrio. It is still a matter of debate if these microbial imbalances can be primarily involved in ASD pathogenesis or if they are just a consequence of dietary dysregulation, a common issue in autistic children. However consistent data, mainly in animal models, suggest a strong potential for dysbiosis to induce alterations in CNS development and functioning. On the basis of the actual knowledge, it can be postulated that a dysregulated microbiota could generate a negative environment, able to disrupt the physiological neurodevelopment. Multiple factors

could be involved in unbalancing this process: the bacterial production of neurotoxic compounds, whose absorption can be fostered by the presence of a leaky gut; the induction of a pro-inflammatory milieu, stimulating itself the HPA axis and amplifying stress response; the impairment in protective SCFA generation; the imbalances in neurotransmitters synthesis and signaling; the alteration of oxytocin release.

In accordance with these findings, studies analyzing the modulation of intestinal microbiota through different approaches (administration of pre- and probiotics, antibiotics, FMT, special diets) show promising results in relieving some autistic disturbances mainly in animal models but also in humans. At present robust data are lacking and no clear recommendation can be made concerning the use of such therapeutic interventions in ASD patients. In the near future new well-designed trials are awaited, investigating the role of dysbiosis and its correction on larger populations of ASD patients.

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