Gut-Brain Axis in Autism Spectrum Disorder

Subjects: Nutrition & Dietetics | Microbiology
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Autism spectrum disorder (ASD) is a complex behavioral syndrome that is characterized by speech and language disorders, intellectual impairment, learning and motor dysfunctions. Several genetic and environmental factors are suspected to affect the ASD phenotype including air pollution, exposure to pesticides, maternal infections, inflammatory conditions, dietary factors or consumption of antibiotics during pregnancy. Many children with ASD shows abnormalities in gastrointestinal (GI) physiology, including increased intestinal permeability, overall microbiota alterations, and gut infection. Moreover, they are “picky eaters” and the existence of specific sensory patterns in ASD patients could represent one of the main aspects in hampering feeding. GI disorders are associated with an altered composition of the gut microbiota. Gut microbiome is able to communicate with brain activities through microbiota-derived signaling molecules, immune mediators, gut hormones as well as vagal and spinal afferent neurons. Since the diet induces changes in the intestinal microbiota and in the production of molecules, such as the SCFA, we wanted to investigate the role that nutritional intervention can have on GI microbiota composition and thus on its influence on behavior, GI symptoms and microbiota composition and report which are the beneficial effect on ASD condition.

Keywords: autism spectrum disorders (ASD) ; diet ; nutritional status ; anthropometry ; metabolites ; gastrointestinal symptoms ; gut microbiome

1. Introduction

Autism spectrum disorder (ASD) is a complex behavioral syndrome that occurs before the third year of life and which affects several spheres of the normal mental development. Children with ASD are characterized by speech and language disorders, intellectual impairment, learning and motor dysfunctions [1]. The effects and the severity of symptoms of ASD are different in each person, with a wide range of types and severity of behavior. Verbal and nonverbal intelligence quotients (IQs) are highly variable in ASD [2] and Repetitive and Restricted Behaviors (RRBs) can range from low-level stereotyped motor behaviors to higher order behaviors, such as insistence on sameness [1]. Recently, an increase in the diagnosis of ASD has been reported with an average of 1 case for every 88 children [1][3]. It is currently believed that these disorders result from alterations in pre- and/or post-natal neurological development [1]. Indeed, it has been proposed that these complex behavioral features are associated with atypical patterns of functional connectivity (FC), compared with typically developing (TD) individuals [4][5]. These neurodevelopmental abnormalities lead to the child’s impairment in the ability to relate with others in the first years of life, causing dramatic cognitive, affective and behavioral effects, which need to be approached in the family and at school.

Among the pathogenic factors of ASD there are very strong genetic components, where heritability has been estimated to be from 60% [6][7] to more than 80% [8]. The genetic factors affecting ASD are very heterogeneous [9][10] and there are few genes whose association to ASD have been well characterized [11][12], for example SH3 and multiple ankyrin repeat domain 3 (SHANK3) [11][13][14][15], contactin associated protein-like 2 (CNTNAP2) [16][17], and more recently, chromodomain helicase DNA binding protein 8 (CHD8) [18]. In particular, both de novo mutations and deletions in the SHANK3 gene have been related to autism. Furthermore, Mark E. Obrenovich et al. have shown that metal ion homeostasis is altered in ASD children and involve the deposition of several divalent cations, as demonstrated in a complex autosomal dominant disorder characterized by ASD, which is known as Timothy syndrome [19][20].

Next to genetic factors, the environmental elements that are implicated in the increase of ASD risk seem to include: air pollution, exposure to pesticides, maternal infections, dietary factors, maternal diabetes, stress, medications, infections, inflammatory conditions or consumption of antibiotics during pregnancy [21][22]. Proposed dietary risk factors include also maternal prenatal and perinatal folate and iron status or polyunsaturated fatty acid (PUFA) intake [23][24][25].

Amongst the others, food restriction, difficult eating behaviors and GI disorders were easily recorded among medical conditions associated to ASDs. Indeed, children with ASD are very selective eaters (“picky eaters”) and most of them show aversions to specific food colors, texture, smells or other foods’ characteristics [26][27]. This exert a direct adversely

effect on diet quality, nutritional deficiency and, on gut microbiota composition. Most of ASD patients that have a co-occurrence of GI disorders could be influenced by particular dietary habits that may exacerbate ASD symptomatology [28][29][30][31]. Immune dysfunction and gastrointestinal (GI) inflammation are also common in individuals with ASD and contribute to severity of behaviors [28][32][33]. Many ASD children have also been shown to carry abnormalities in GI physiology, including: increased intestinal permeability [34][35], overall microbiota alterations [36][37][38][39][40], and gut infection with cresol-producing Clostridium difficile [39][41][42][43][44].Recent evidences in human gut microbiota studies highlighted the existence of a close connection between gut and brain functions, the so called “gut-brain axis”, including neural, hormonal, immune, and metabolic pathways [45]. Neuroimmune pathways can contribute to ASD symptomatology via the gut–brain axis [46]. It has been proposed that cytokines associated with ASD, due to an inflamed gastrointestinal tract, may cross the blood-brain barrier and help an immune response in the brain, thus influencing behavior [46].

2. The role of Nutrition and Interventions in ASD

2.1. Food Selectivity and ASD

Neurotypical children, especially preschoolers, are often referred to as “picky eater” and often show an attitude of preference towards certain foods and rejection of others. This alimentary conduct usually falls around the age of six and can be part of an adequate developmental framework typical of the developmental age [47][48]. In children with ASD, this picture is intensified, begins in a very early age and results in a real food selectivity framework. In addition, food problems tend to remain stable over time, with negative consequences on health and nutritional status. From a nutritional point of view this leads to an inadequate caloric intake and hence to nutritional deficits [49][50]. The importance of food regulation in children with ASD is emphasized in DSM-5, although it is not a diagnostic criterion [1]. However, one of the major issues concerns the definition of selectivity, which complicates the evaluation and the comparison of the results of different studies. Atypical eating behaviors and the peculiar lifestyle of ASD (i.e., different levels of physical activity; idiosyncratic social skills; poor social interaction) are factors that imply risks of malnutrition, both in excess and in default [51]. Furthermore, studies have indicated that food selectivity is being determined by the following factors: texture (69%), appearance (58%), taste (45%), smell (36%), and temperature (22%), as well as reluctance to try new foods (69%) and a small repertoire of accepted foods (60%) (**Figure 1**) [52][53][54][55]. A strong preference for starches, snacks and processed foods, along with a rejection of fruits, vegetables or protein, is particularly common [56][57]. Increased consumption of snack foods and calorie-dense foods can lead to excessive weight gain, with related higher rates of obesity in ASD children than in unaffected children [58]. Indeed, obesity-related complications (e.g., hypertension, diabetes) are generally more prevalent among adults with ASD [59]. Nadon et al. found that nearly 90% of preschool and school age ASD children do not process sensory information, in particular related to touch, smell, sight, and hearing, in the same way as their typically developing peers [60]. Some studies reported that ASD children had strong food preferences [61].

Figure 1. Factors that could determine food selectivity (data taken from the studies of Williams, Schreck and Klein [52][53][54]).

Other factors related to food selectivity are linked to the way the food is presented (48.6%), to the use of certain utensils and to the characteristics of the tableware (13.8%) [53][62]. The study of Spek et al. [63] examined eating problems in the context of the Swedish Eating Assessment for Autism spectrum disorders [SWEAA] [64]. It has been shown that males with ASD can't adapt their eating behavior to other people present and have problems doing two things simultaneously during a meal. Besides these, women with ASD showed eating rituals, a pronounced sensory sensitivity to the smell, taste, texture and visual appearance of food and were uncomfortable in sharing meals with other people [63]. Indeed, there are studies on the identification of specific sensorial patterns in ASD focused mainly on visual and auditory perception. A study on sensory profiles highlighted the existence of different clusters of sensory expression in ASD people [65]. In particular, the study identified a subpopulation of subjects among ASDs with particular taste/smell sensitive clusters, which could represent one of the major aspect in hampering feeding and introducing new foods. In another study, Miller identified three different cluster of modulating sensory sensitivity in ASDs and found a positive correlation between sensory overresponsivity (SOR) in ASDs and the severity level of food selectivity, expressed by number of foods accepted by the child [66]. Overall, the available evidence suggests that this food selectivity and an altered elaboration of sensory *stimuli* could imply a higher risk of nutritional deficiencies that could, in turn, affect gastrointestinal symptom and microbiota.

2.2. Nutrient Intake and ASDs.

The ASD has been included among the psychiatric conditions associated with nutritional deficiencies due to food selectivity [67][68][69]. However, literature still shows conflicting results regarding the risk of nutritional deficits in children with ASD [70], especially because ASDs are compared with NTs. However, in many studies children with ASDs show a considerably smaller variety of foods, but authors report no overall differences in their total calories, carbohydrates, or fat intakes [62][71][72][73][74], suggesting that their satiety mechanisms are not impaired. Protein intake was adequate or quite similar to that of typically developing children [69][72][73][74][75][76][77]. Children with ASDs eat fewer vegetables and eat more energy-dense foods [76][78], so fiber intake was inadequate in a considerable number of children with ASDs [71][73][79][80]. Substantial number of subjects with ASDs had inadequate intakes of micronutrients. In particularly they showed deficiencies of few minerals such as calcium [67][69][71][73][75][77][79], iron [73][77], zinc [75][77][80], potassium [81], copper [81] and vitamins as vitamin A [71][75][77], vitamin D [67][69][73][78], vitamin E [71][73], riboflavin [77], vitamin C [75][78], vitamin B-12 [69][77] [82], folic acid [75][82], and choline [80][83]. Excessive consumption of sodium was reported [79][84], probably due to the consumption of packaged foods. Some studies reported decreased bone development lower mineral density and a greater risk of fractures in children with ASD compared to controls (TDs), linked to a lack of calcium and vitamin D in the diet, despite good anthropometric growth [85][86][87][88]. Very interesting is the case of beta carotene excess reported in a case report of a 4-year-old ASD child with selective feeding and excessive consumption of carrot juice (>2.5 L/day) [89]. Cases of vitamin C deficiency with scurvy have been described in the literature [90][91][92][93][94][95]. However, dietary data obtained in the studies may be inaccurate due to the influence of parents, who, being concerned about the nutritional behavior of their children, do not actually reflect the correct nutritional approaches of their children. A schematic overview of food selectivity in ASDs' children is provided below (**Figure 2**).

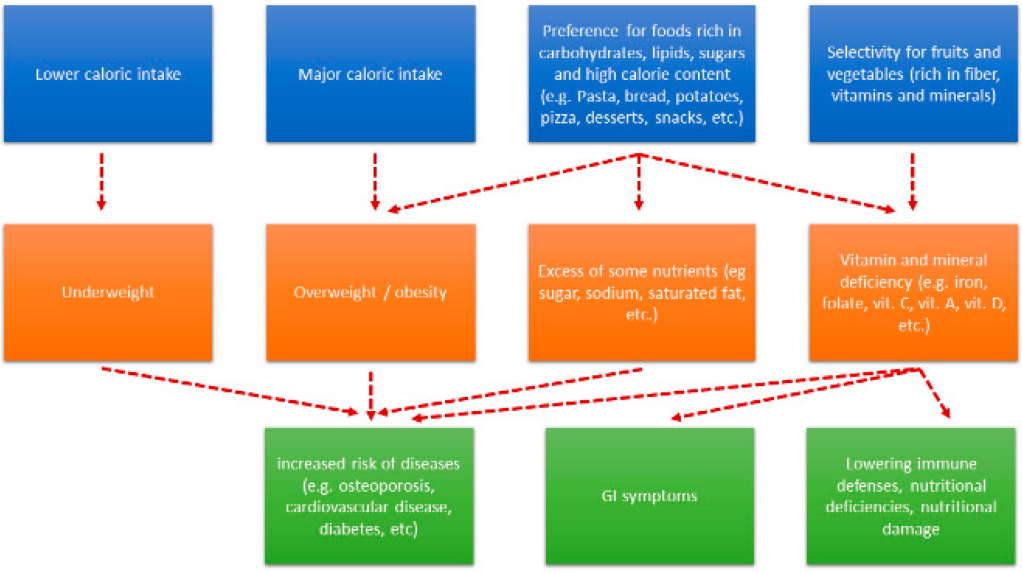

Figure 2. Synopsis of ASD food selectivity on nutritional status, anthropometric features and clinical conditions.

Therefore food selectivity and an inadequate nutrient intake could increase the risk of malnutrition in ASDs that ultimately leads to either obesity or undernutrition. In fact, it has been shown that this two conditions are associated with an altered composition and diversity of the gut microbiota compared to healthy individuals [96][97][98]. Furthermore this changes has

been associated with altered SCFA composition, energy homeostasis, and inflammation [99]. Therefore it is important to take into account this influence that has the nutritional status on the intestinal microbiota, in order to choose the best nutritional approach for patients with ASD.

2.3. Effects of Dietary Interventions in ASD

Effects of dietary interventions in ASD have recently begun to emerge. It is important to understand what physiological effects dietary interventions may have, because individuals with ASD already exhibit difficult and picky eating behaviors [26][27]. So it is most important to investigate diets, because they could also aggravate the imbalances in gut microbiota composition and the GI problems. In the literature the most studied nutritional approaches are gluten-free/casein-free diet (GF/CFD), ketogenic diet (KD), the specific carbohydrate diet (SCD), and the Mediterranean diet (MD).

3. The role of GI Symptoms, Gut Microbiota and Gut-Brain Axis in ASD

3.1. GI symptoms in Children with Autism

Individuals with ASD often suffer from gastrointestinal (GI) symptoms [30][100]. Frequent reports of GI symptoms in children with ASD are beginning to be clarified by research efforts examining the issue. Although the connection between gastrointestinal problems and autism is still not resolved and the prevalence of gastrointestinal symptoms varies from 23 to 70% [31][101][102][103][104][105].This demonstrates a high variability in prevalence of GI problems that may be due to several differences across studies including: variations in the criteria used to define a GI symptom; the number of different GI symptoms considered; the definition of any particular GI symptom or lack of variations in methodology such as data source (medical chart versus self-report) or time period for reporting (last few months, lifetime, etc.); and study population characteristics such as age and other criteria for participation [106]. In the literature, we have found in-depth studies on 140–170 children with ASD, of which 24–63% had a history of at least one GI symptom, including: diarrhea or unformed stools, constipation, bloating, and/or gastroesophageal reflux (GERD) [31][101][107][108]. Another study on 150 children (50 ASD, 50 controls, and 50 children with other developmental disabilities (DD)) found that 70% of children with ASD presented GI symptoms, compared to 28% of typically developing children and 42% of DD children [102]. However, a study conducted in 2009 on people with ASD followed longitudinally up to 18 years, did not report an increased risk of GI diseases of an inflammatory and/or malabsorption nature compared to the typical development controls; the only significant difference found was the higher incidence of food selectivity and constipation in ASD people [103]. Therefore, it is not clear which kind of relationship correlates GI disorders and food selectivity, in fact, the malaise associated with GI disorders could increase feeding difficulties. Few evidences have been collected, at the moment, to deeply understand if GI symptoms may affect picky attitudes or if are principally the ASD dietary habits to influence GI disorders. Indeed, it could hypothesize that the picky attitude in ASD behavior could be due to a protective attitude, which the child implements to avoid discomfort resulting from the diet [109]. Interestingly other studies have shown that people with verbal and intestinal problems show poor appetite and react by rejecting a wide range of foods, and find it difficult to communicate their discomfort [51][110]. Moreover, food selectivity can exacerbate or determine GI symptoms (e.g., constipation) due to a diet rich in carbohydrates and poor in fiber that do not promote intestinal transit and can lead to constipation [103]. The presence of GI disorders together with food selectivity could constitute a specific clinical phenotype [31][103][111], characterized by frequent problematic behaviors, such as anxiety, self-aggression, sleep problems, resulting from both conditions [112]. Indeed correlation between certain behavioral problems, such as anxiety and aggression, and the increase in GI disorders is now known [113]. Indeed, abdominal pain, constipation, and/or diarrhea likely to produce frustration and may contribute to the severity of the disorder, with decreased ability to concentrate on tasks, behavior problems, and possibly aggression and self-abuse, especially in children unable to communicate their discomfort [29]. GI disorders also result in a decreased ability to learn toilet training, leading to increased frustration for the child and their parents/caregivers. However, at the moment, it is difficult to precisely decipher the physiological processes that link together food selectivity and GI problems. What is certain is that both conditions, food selectivity and gastrointestinal disorders require attention from the clinician. Further studies characterized by a more accurate methodology, both in the selection of the samples and in the development and use of more accurate diagnostic tools, could allow a more precise estimation of the prevalence of GI disorders in the ASD [114][115][116].

3.2. GI Disorders, Microbiota and Microbiota-Gut-Brain Axis Alteration in ASD

GI disorders such as intestinal pain, constipation and diarrhea are often associated with an altered composition of the gut microbiota [28][108][116][117][118]. A possible mechanism could be that this condition would allow macromolecules coming from GI tract to pass into the blood stream and exert an important systemic action; in particular, this action would apply at the level of the Central Nervous System (CNS) [119]. Indeed, microbiota and their ligands are crucial in maintaining the cell–cell junctions critical to barrier integrity, with GI barrier defects seen with dysbiosis [120]. Moreover, a higher intestinal

permeability allow the increase in circulating bacteria-derived lipopolysaccharide (LPS) which leads to an immunological and inflammatory response, with an augmented systemic pro-inflammatory cytokines [121]. High levels of cytokines (e.g., IL-1B, IL-6, IL-8, and IL-12p40) have been reported in ASD children associated with poor communication and impaired social communication [32][122]. In a study that analyzed autopsy and cerebrospinal fluid (CSF) of individuals with ASD, a neuroinflammatory response involving excess microglial activation and increased proinflammatory cytokine profiles as compared to non-ASD controls was found [123]. The role of microglia deficits in neurological development disorders in a mouse model has emerged [124]. Therefore, this leads to the hypothesis that the leaky gut may play an important role in some behavioral manifestations of ASD children. Thus the existence of a close connection between the gut and brain was highlighted, and that cross-communication occurs regularly. In fact, the CNS control the gut microbiome composition through peptides, which are sent upon satiation and thus affect nutrient availability. Furthermore, the hypothalamic–pituitary–adrenal (HPA) axis releases cortisol, which regulates intestinal motility, integrity and hypersecretion of CRH is a crucial factor in depression and anxiety disorders [125]. In turn, the immune and neuronal pathways regulate the secretion of mucin from intestinal epithelial cells, which control microbial populations within the intestine. However, communication is bidirectional and the intestinal microbiota is able to control the activity of the CNS through neural, endocrine, immune, and metabolic mechanisms that could have a possible influence on behaviours typical of ASD patients. [126]. A further confirmation of the possible central regulation mechanism of the gut-brain axis comes from studies on animal models, where it has been observed that an alteration of autonomic nervous system activity, such as anxiety and stress, could play a key role in the pathogenesis of increased permeability of the intestinal epithelium, found in the ASD population [40][127][128]. For example, germ-free (GF) mice showed reduced anxiety-like behavior and no spatial memory, altered neurotransmitter levels in the brain, and altered hypothalamic–pituitary–adrenal (HPA) axis activity [129][130][131][132]. Particularly intriguing for ASD is the influence of gut microbiota on the development of social behavior [133][134]. Indeed, the gut microbiota is reported to modulate structural and functional changes in the amygdala, a critical brain area for social and fear-related behaviors, which are associated with a variety of neuropsychiatric disorders [135]. A study conducted on early adolescence in mice showed that the modification of the intestinal microbiota alters their behavior and significantly reduces the neurotrophic factor (BDNF), oxytocin and vasopressin expression in the adult brain [136]. A study demonstrated that treatment with microbial-produced short-chain fatty acids (SCFAs) could rescue microglial function impaired in GF animals [137]. Furthermore, the microbiota affects the circulating levels of other mediators and substances, such as melatonin, serotonin, histamine and acetylcholine [138][139], which are important for brain maturation [140]. We can assume that if the hypothesis of a connection between symptoms related to autism and gastrointestinal disorders was confirmed, the manipulation of the intestinal microbiota, with supplementation with probiotics and treatment with Fecal Microbiota Transplantation (FMT), could constitute a therapeutic approach for the symptoms of autism and the associated medical comorbidities [141].

3.3. Focus on Bacterial Metabolites and Gut-Brain Axis

As we discussed, it is known that certain bacteria are able to produce different essential neurotransmitters and specific neuromodulators. Indeed, several neurotransmitters such as gamma-aminobutyric acid (GABA), serotonin, catecholamines and acetylcholine are produced by bacteria, some of which are inhabitants of the human gut. Indeed, researchers report that *Lactobacillus spp*. and *Bifidobacterium spp*. produce GABA [139]; *Escherichia spp*., *Bacillus spp.* and *Saccharomyces spp.* produce noradrenalin; *Candida spp*., *Streptococcus spp*., *Escherichia spp*. and *Enterococcus spp*. produce serotonin; *Bacillus spp*. produce dopamine; and *Lactobacillus spp*. produce acetylcholine [142]. Neurotransmitters secreted from gut bacteria may induce cells to release molecules that have the ability to modulate neural signaling within the enteric nervous system and subsequently control brain function and behavior, trough the microbiome-gut-brain axis. Significant deviations in the bacterial metabolites present in faeces and urine of children with ASD were seen [143]. Two possible pathways we hypothesize may be principally involved which are reviewed below.

3.4. Short-Chain Fatty Acids (SCFAs) and Gut-Microbial Metabolites

Short-chain fatty acids (SCFAs) as acetic acid (AA), propionic acid (PPA), and butyric acid (BA), are the fermatation end-products of non-digested carbohydrates in the colon and have been suggested to have various health benefits to the host related to weight control, lipid profiles, and colon health [144]. However, the accumulation of SCFAs, and specifically of propionate, has also been shown to have broad effects on the nervous system physiology, and it is associated to the pathogenesis of ASD [145][146]. In fact, higher levels of AA and PPA that is used as a preservative in the food industry and can also induce autistic-like behaviors in rodents have been reported in ASD children [147][148]. At the same time, lower levels of BA, that can positively modulates neurotransmitter gene expression and can rescue behavioural abnormalities in mouse model, have been reported in ASD [149]. Moreover, ASD patients seem to be characterized by both elevated levels of SCFA concentrations in stool and serum, and increased level of SCFA-producing bacteria (e.g., *Clostridia*, *Desulfovibrio*, and *Bacteroides*) [29][36][150]. Thereby, translocation through the blood–brain barrier by transporters or by

passive diffusion could cause potential effects on the brain and lead to development of some ASD symptoms [151]. The precise mechanisms of how SCFAs alter behavior in ASD are unknown, but effects on mitochondrial function (e.g., Krebs cycle) or epigenetic alterations may be involved [152]. In addition to direct effects on the brain, propionate has been shown to modulate 5-hydroxytryptamine (5'-HT) secretion in the gut and deplete 5'-HT and dopamine levels in the brain, which could potentially contribute to the hyperserotonemia observed in children with ASD [152][153][154]. Another metabolite that we could considered is *p*-cresol and its co-metabolite *p*-cresyl sulfate, which are phenolic compounds that are produced by bacteria such as *C. difficile* and *Bifidobacterium* [155][156][157]. It has been demonstrated that an early exposure to *p*-cresol may contribute to the severity of behavioral symptoms and cognitive impairment in ASD [155]. Furthermore, ASD patients have high level of free amino acids (FAAs) [156], which are derived from hydrolysis of proteins and peptides, like glutamate that may be involved in the etiopathogenesis of neurodevelopmental disorders [157]. This picture shows how there is a bidirectional influence between microbiota and diet, through the production of metabolites, which can be characterized through metabolomics and can help to delineate new therapeutical strategies in autistic patients.

3.5. Neurotransmitters

In the last few years a role of the serotonin pathway in ASD, especially in the gut-brain axis, is emerging in the literature. Although most serotonin, or 5′-HT, is produced in the GI tract and can also be metabolized directly by the gut microbiota, it modulates neurodevelopment and might be important in social function and repetitive behavior [158]. High levels of 5′HT may be caused by a gastrointestinal 5′HT hypersecretion, produced by the enterochromaffin cells in the gut and it is involved in functions such as motility and secretion [159]. Furthermore, a study show the role of the 5′-HT as the link for the gut-brain-axis in ASD [160]. However, hyposerotonemia and lower synthesis of 5′HT in the brain in ASD children has been reported [161]. Some bacterial species that are known to influence 5′-HT metabolism (e.g., *Clostridium spp, Lactobacillus spp*) were observed to be increased in stool samples from ASD children. In patients with ASD, altered function and metabolism of neurotransmitters, such as 5′-HT and catecholamines, and dysfunction of the serotonergic system have been reported to contribute to symptomatology [158][162][163][164][165][166]. 5′-HT is elevated in whole blood and platelets in approximately 30% of children with ASD, making it a potential candidate as a biomarker for ASD [163]. Interestingly, administration of *Bacteroides fragilis* normalized plasma levels of 5′-HT in an animal model of ASD [167][168]. These data indicate that the gut microbiota could be involved in higher 5′-HT production, thus identifying 5′-HT as a potential pathway through which the gut microbiota and brain communicate in ASD. In ASD, abnormal intestinal permeability could allow 5′-HT to translocate into the systemic circulation, leading to elevated levels of blood 5′-HT [34][35][103][163]. Increased 5′-HT production by some species of the gut microbiota in ASD could deplete peripheral tryptophan availability. This corresponds to data showing decreased capacity for 5′-HT synthesis in children with ASD as well as to reports showing a worsening in repetitive behaviors in individuals with ASD after tryptophan depletion [161][169]. Lastly, higher levels of 5′-HT in children with ASD can be linked to intestinal inflammation and play an important role in intestinal inflammatory responses [170], so there is a connection between enteric serotonin production and dysbiosis. On the other hand, dysbiosis can decrease the number of amino acids that are absorbed from the diet and reduce the availability of tryptophan [171], that is a precursor for a number of metabolites as serotonin, thus creating a vicious cycle. Indeed, a lower level of tryptophan may influence the synthesis of serotonin in the brain, playing a role on the mood and cognitive impairment which characterize ASD children [172]. Thus, it can be proposed that the intestinal inflammatory response in children with ASD, which is exacerbated by gut microbiota, can lead to a further increase in 5′-HT levels and, ultimately, to upstream behavioral effects on the brain.

4. Conclusions

It has been observed that ASD children are characterized by a strong food selectivity that consequently deeply influences their gut microbiota composition. Indeed, an increase in SCFA and 5′-HT-producing bacteria was observed in several studies on ASD patients. Increased levels of 5′-HT result in a different modulation of 5′-HT metabolism in the host, leading to tryptophan depletion and hyperserotoninemia, which may affect GI symptoms. Moreover, some ASDs are even characterized by higher levels of intestinal permeability which allow passive diffusion of bacteria-derived lipopolysaccharides (LPS) and metabolites through the intestinal barrier. As a consequence, an increase in pro-inflammatory cytokines (e.g., IL-1B, IL-6, IL-8, and IL-12p40) was observed, which are associated with impaired social communication and neurodevelopmental disorders. At the same time, gut-brain cross-talk through the vagus nerve and the hypothalamus-pituitary-adrenal (HPA) glands, influences vagal chemo- and mechanoreceptors on the mucosal villi and systemic cortisol levels, leading to an exacerbation of GI symptoms and inflammatory status (**Figure 3**). Further studies are needed to assess the effect of different dietary interventions (such as the Mediterranean diet) on GI symptoms and, as a consequence, how they may affect behavioral patterns associated to ASD conditions.

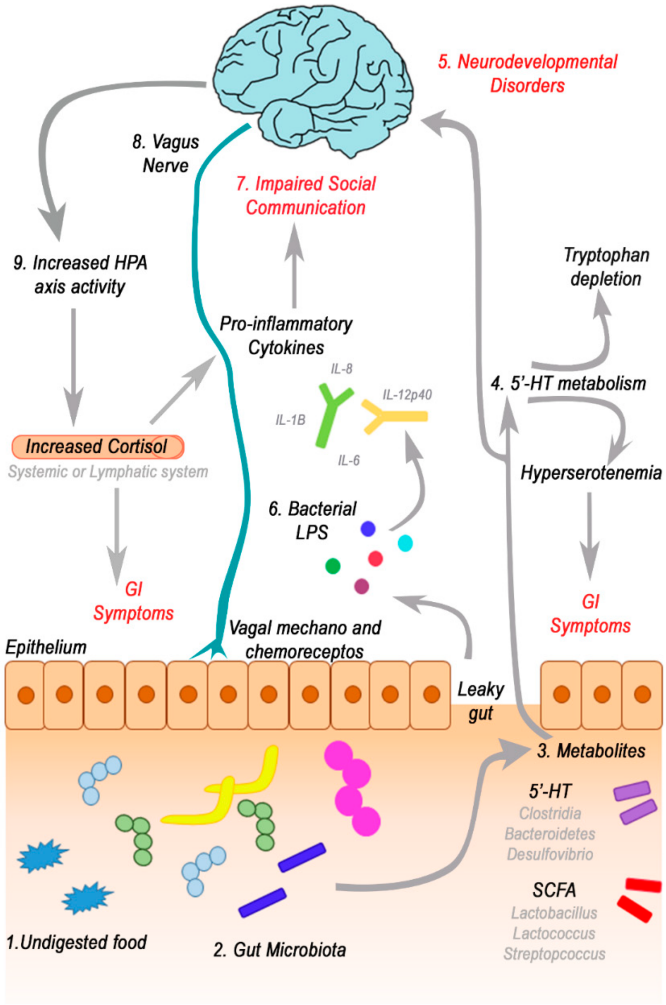

Figure 3. Role of the gut-brain axis in the etiology of ASD. (**1**,**2**) Food that escapes digestion can be used by the gut microbiota bacteria to produce metabolites (e.g., SCFAs and/or 5′-HT) that can be used by the host. Among metabolites (**3**) 5′-HT is produced particularly by *Lactobacillus*, *Streptococcus*, and *Lactococcus* species, while SCFAs (e.g., propionate) are produced by Clostridia, Bacteroidetes, and *Desulfovibrio* species. (**4**) Increased 5′-HT production by the microbiota acts on the metabolism of 5'-HT, leading to tryptophan depletion and contributing to hyperserotonemia, which is associated with GI Symptoms. (**5**) Intestinal permeability in children with ASD could allow passive diffusion of metabolites, and cause neurodevelopment disorders, such as behavioral and chemical changes (e.g., mood, cognitive state and emotion). (**6**,**7**) Moreover, higher intestinal permeability allow the increase in circulating bacteria-derived lipopolysaccharide (LPS), thus stimulating systemic pro-inflammatory cytokines production (e.g., IL-1B, IL-6, IL-8, and IL-12p40), which is associated with impaired social communication. (**8**) The vagal-mediated signaling from the gut microbiota to the brain can be transmitted through vagal chemoreceptors on mucosal villi that are activated by bacterial metabolites (e.g., 5′-HT, SCFAs) or by vagal mechanoreceptors that sense motility changes induced by bacterial species. (**9**) Gut microbiota influences the activity of Hypothalamus-Pituitary-Adrenal glands (HPA) axis that increased levels of cortisol in the systemic system. As a consequence, higher levels of cortisol may affect cytokines response and exacerbate GI symptoms.

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