Microbiota disorders and food hypersensitivity in autism spectrum disorders; what do we know?

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ABSTRACT
Pathogenesis of autism spectrum disorders (ASD) is probably multifactorial. Many studies have shown intestinal dysbiosis in children with autism. Moreover, gastrointestinal disturbances, probably resulting from abnormal microbiota composition, are commonly reported in children with autism.

Another aspect, that may have negative influence on ASD children’s behavior are enzymopathies (enzymatic deficiency). Lack of selected gastrointestinal enzymes leads to inappropriate nutrients decomposition (mainly gluten and casein) and formation of so called exorphins, i.e. substances with opioid activity (opioid therapy). Because of increased permeability of the intestinal barrier and cerebrospinal axis, exorphins and others improperly digested food particles may be transported to the central nervous system. Consequently, disorders in brain development and children’s behavior deterioration may be observed.

Studies have shown that both microflora disorders and increased intestinal permeability may contribute to behavioral impairment.

KEY WORDS: Autism spectrum disorders, IgG-dependent food hypersensitivity, microbiota, opioid therapy

MICROBIOTA DISORDERS IN AUTISM SPECTRUM DISORDERS
The intestinal microbiota plays a strategic role to keep the homeostasis of the body. Gut dysbiosis, i.e. state of qualitative and quantitative imbalance in the gut microbial ecosystem, is the risk factor for several diseases including inflammatory bowel diseases, atopic diseases and obesity [1, 2].

Many-sided influence of gut microbiota on human health has captured the attention of scientists from multiple research fields. In light of this publication it is advisable to discuss the importance of intestinal microbes in the pathogenesis of autism and other neurodevelopmental disorders. The significance of microbial factors in the pathogenesis of autism spectrum disorders (ASD) is analyzed in the aspect of bacterial dysbiosis, i.e. the impact of metabolites produced by intestinal bacteria and the effect of antibiotic therapy and probiotic administration. The latest studies evaluating the gastrointestinal microbiome allowed to gain knowledge about interdependence and interaction between the human body and gut’s microorganisms. Direct connections occur between intestinal microbiota and enteric neurons forming the axis of microbes-intestine-brain. Regulatory activity of microbiota on the central nervous system takes place via neuronal, endocrine, metabolic and immune mechanisms [3]. Shaw et al [4], as the first, has taken attempt on the correlation of intestinal microbiota with occurrence of clinical symptoms of autism. In that mentioned study, the presence of analogs of Krebs cycle metabolites and high concentrations of arabinose have been shown in the urine of two brothers with autistic features [4]; reported compounds are not present in the urine of healthy subjects. One of the possible explanation for this unusual condition is colonization of the gastrointestinal tract by selected bacteria and/or yeasts, where the metabolites are inhibitors of mitochondrial Krebs cycle. High concentrations of arabinose may interfere normal carbohydrate metabolism. Both conditions may lead to reduction of cellular energy production.

Significant improvement in children behavior after vancomycin administration also favors the potential impact of the microbiota in initiation/deterioration of the patient with ASD. Vancomycin therapy, as a type of ASD treatment, began essentially in 1998 when Ellen Bolte [5], mother of an autistic boy, has published a paper on the potential contribution of Clostridium tetani in the pathogenesis of ASD. Her hypothesis based on the fact, that a significant percentage of children with ASD is subjected to frequent broad-spectrum antibiotic therapy, mainly due to recurrent ear infections. Oversupply of antibiotics in ASD individuals strongly disturbs the intestinal ecosystem, which consequently promotes overgrowth of the pathogenic microorganisms including toxigenic strains of Clostridium genus. In the pathogenesis of many multifactorial diseases, including inflammatory bowel diseases and necrotising enterocolitis, involvement of Clostridium species is confirmed.

In the above mentioned publication Bolte has postulated, that the neurological symptoms in some children with ASD, developed following the direct effect of tetanus neurotoxin, namely tetanospasmin [5]. It is produced by the vegetative cell of C. tetani and is known as to be extremely potent for

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human. Its mechanism is based on blocking the release of y-aminobutyric acid and glycine, neurotransmitters which are inhibitors of acetylcholine. The consequence is an accumulation of acetylcholine which leads to muscle contraction, conduction disorder of the central nervous system and spastic paralysis. Accumulation of bacterial toxins and excessive intestinal permeability which are observed in children with ASD leads to their increased blood concentration, resulting in systemic symptoms. Based on Bolte’s observations, Sandler and colleagues [6] conducted a study for testing her hypothesis. They demonstrated significant clinical improvement in children with ASD after oral vancomycin administration [6]. Because of its pharmacokinetic properties, vancomycin is the treatment of choice for management of diarrhea caused by pathogenic clostridia such as Clostridium difficile. When administered orally it is poorly absorbed from the gastrointestinal tract. This fact suggests that its temporal improvement of clinical symptoms of autism is strictly associated with its influence on the gut microbiota. ASD progression when vancomycin was withdrawn confirmed this theory. In addition, each implementation of antimicrobials active strictly for anaerobic microorganisms (such as vancomycin or metronidazole) caused significant improvement in behavior of these patients. However, it should be noted that this improvement is short-lived generating a vicious circle. Each supplementation of antimicrobial drugs led to disruption of protective indigenous bacteria extremely beneficial for the proper function of the body. In the study mentioned above [6], it was ascertained that children’s behavior deteriorated significantly within approximately two weeks of ending vancomycin therapy. This is explained by the fact, that vancomycin shows activity against vegetative cells of bacteria of the Clostridium genus, but not for the spores; so, is the symptoms of autism return after antibiotic therapy.

Prolonged use of vancomycin is not recommended due to the risk of selection of vancomycin resistant pathogens. Also it has been suggested that the dose of vancomycin used for the study could be insufficient to make a full eradication of the said bacterial population. In subsequent studies assessing the intestinal ecosystem in children with ASD, a significant increase in the number of species under the Clostridium genus in autistic patients compared to control group has also been shown [7-10]. These findings are confirmed by the analysis of Parracho et al [11], in which a greater number of Clostridium histolyticum in the stool of children with ASD has been reported. Children diagnosed with ASD and two control groups, a non-autistic sibling group and an unrelated healthy group, were enrolled to the study; interestingly, the non-autistic sibling groups had an intermediate level of the C. histolyticum, but wasn’t significantly different from the other subject groups.

Based on the analysis conducted by Finegold et al [10], demonstrating the overgrowth of Clostridium bolteae in the stool of individuals with ASD, Pequegnat et al [12] decided to investigate the cell-wall polysaccharides of C.bolteae. The purpose of the study was to determine their structure and immunogenicity. The researchers, as the first in the world, showed that C.bolteae produces a conserved specific capsular polysaccharide, composed of rhamnose and mannose units, which is immunogenic in rabbits. These findings are promising for the possibility of a vaccine development to reduce or prevent C.bolteae colonization in autistic patients gut’s. It could be also useful to create a diagnostic marker for the rapid detection of C.bolteae in clinical settings.

New ways of modulating the intestinal microbiota with particular attention to the bacteria of the Clostridium genus seem to be extremely valuable. In addition to the well documented overgrowth of Clostridium spp in patients with ASD, there are also studies suggesting abnormal relations in commensal bacteria. Finegold et al [10] demonstrated that in children with severe symptoms of autism predominate Bacteroidetes, whereas in the healthy individuals Firmicutes. Disruption in the composition of other groups of bacteria, such as Bifidobacterium, Lactobacillus, Sutterella, Prevotella, and Ruminococcus, have also been reported in autistic patients [13-16]. Furthermore, as a potential risk factor in ASD pathogenesis, sulfate-reducing bacteria belonging to the Desulfovibrio genus have also been analyzed; through production of hydrogen sulfide they negatively affect the mitochondria function [17]. It has been shown that this microorganism occurred in 50% individuals with autism, while not present in healthy people [18].

For a thesis about the potential bacterial factor involvement in ASD speaks also the fact that a significant percentage of children with ASD have symptoms of the gastrointestinal tract, such as excessive production of intestinal gases, bloating, abdominal pain, diarrhea, belching, symptoms of gastroesophageal reflux or obstructions. A multicenter study of individuals with ASD revealed also a higher incidence of inflammatory bowel diseases and other gastrointestinal tract disorders, compared to healthy control group [19]. This relationship may indirectly suggest the involvement of intestinal microbiota in the initiation of ASD. Interestingly, in direct conversation with the parents of autistic children, many of them reported that behavioral symptoms are overlapped with gastrointestinal problems.

Not only the overgrowth of pathogenic microorganisms is involved in the pathogenesis of ASD, but also the lack of health-related groups of bacteria. Therefore, probiotics administration and other therapy alternatives (e.g. stool transplantation) for modulating the host microbiota are extremely important [20]. Promoting the beneficial intestinal microflora prevent colonization of the pathogenic microorganisms including toxin-producing bacteria such as Clostridium. Hsiao et al [21], in an animal model, have shown that the supply of Bacteroides fragilis, commensal intestinal bacteria, significantly reduced gastrointestinal symptoms in mice with ASD. Moreover, further studies have shown that the supply of B.fragilis improves intestinal barrier permeability and reduces behavioral disturbances in mice with ASD manifesting gastrointestinal and...
neurological symptoms. Beneficial effects of *B. fragilis* in mouse models with colitis and multiple sclerosis have also been documented [22, 23].

Studies that undermine the theory of intestinal microflora involvement in the initiation of ASD are in minority. Researchers reported comparable results in the composition of the gut microbiota between ASD and control groups [24]. Further studies concentrated on the role of gut microbiome in the initiation of autism are of special importance warranting promising results for the prediction and treatment of a number of diseases, including neurodevelopmental one’s.

**INCREASED GUT PERMEABILITY, ENZYMOPATHIES AND FOOD HYPERSENSITIVITY IN AUTISM SPECTRUM DISORDERS**

As previously mentioned, the dysfunction of gastrointestinal tract is ascertained particularly often in children with ASD. These conditions relate to even more than 80% of all patients. In the described group of patients, occurrence of abdominal pain, heartburn, flatulence, constipation and/or diarrhea have been observed. It appears that the real problem is the inability to diagnose the disorder and its severity, due to absence or hindered communication with the patient [25]. Furthermore, observed problems are not only defined as functional disorders, but also as organic changes such as gastritis, presence of lymphoid follicles in the intestine, eosinophilic infiltration and others [26]. Due to the fact that the observed changes (eosinophilic infiltration) could be partially resolved by dietary intervention (gluten- and casein-free diet) researchers have taken more attention to food hypersensitivity in children with autism. This is especially purposeful in ASD patients who were confirmed for increasing permeability of small intestine mucous membrane, also observed in patients with non-specific inflammatory bowel disease, cystic fibrosis or celiac disease [26, 27].

Homeostasis of the intestinal barrier provides selective and preferential absorption of nutrients from the intestinal lumen, thereby preventing migration of pathogens and toxic agents to the bloodstream. Selective loss of barrier function may be the cause of immunoglobulin (Ig)E-independent food hypersensitivity, developed with the delayed response mechanism. Penetration of undigested food particles through the intestinal barrier activates the immune system, leading to specific IgG antibodies production, immune complexes formation and development of local inflammation [28]. This type of reaction results in the development of symptoms from the gastrointestinal tract, but also, based on the location of immune complexes, could result in skin problems, headaches, depression etc [29]. In children with ASD, selected food particles pass through the intestinal barrier with increased permeability into the bloodstream and then penetrate the blood-brain barrier, which has a negative impact on the development and functioning of the central nervous system. Therefore, an increasing number of reports, based on observations of both doctors/therapists and parents of children with ASD indicate the effectiveness of elimination diet, which improves the functioning of patients with the neurodevelopmental disorders [50-33]. At the moment, however, there is no scientific basis for the implementation of the described nutrition procedure in patients with ASD, due to the lack of adequately designed and conducted clinical trials. Therefore, there is urgent need to conduct randomized clinical studies, designed to estimate the actual effectiveness of elimination diet in patients with ASD. The most common dietary practice is restrictive elimination of gluten and casein, because of dipeptidyl peptidase (DPP)-4 deficiency is reported in ASD patients. The described enzyme is a serine peptidase located in brush border of the small intestine epithelium, involved in the gliadomorphin and casomorphin degradation, products of partial gliadin and casein degradation; molecules with opioid properties. Deficiency of the enzyme responsible for opioid degradation to further non-opioid particles, lead to casomorphin and gliadomorphin permeation into the central nervous system. The opioid-like particles lead to develop symptoms similar to the condition after taking drugs: fear, hunger, hallucinations, or changes in temperature. Such a negative influence on the brain development and functioning contributes to additional severity of behaviors specific to ASD [25]. Analysis carried out so far have shown significantly increased levels of specific IgG antibodies to both gluten and casein in children with ASD compared to healthy children [34]. The effectiveness of an elimination diet in the described group of patients probably results from two overlapping coexisting elements [35]:

1. increased permeability of the intestinal barrier, penetration of incompletely digested food particles into the bloodstream and the appearance of IgG-dependent food hypersensitivity as a consequence

2. enzymopathies (described above)

Currently, it is unknown whether the background of observed enzyme deficiency is genetic or acquired [36]. It seems however, that the exclusion of gluten and casein only is not always the optimal way of nutrition proceeding, as the distribution of other food particles can lead to the formation of exorphins as well. Other products intensively studied in this study area are soy and rice [25].

DPP-4 deficiency may also lead to the formation of other than opioid antigenic structures, generating an additional inflammation state in the organism. Therefore, it seems appropriate to evaluate all components permeating through the intestinal barrier, mainly allergens in IgG-dependent food hypersensitivity and implementing targeted elimination diet. There are also additional difficulties in determining the actual impact of an elimination diet on the functioning of patients with autism. It is known, that only a certain group of patients derive profit from this type of nutrition proceeding, while in the rest of the group elimination diet has no effect on both the ailments of the gastrointestinal tract, as well as the child’s functioning level [31].
REFERENCES