Report published in the Super Saudável magazine (issue 83 - July to September/2019), publication from Yakult do Brasil directed to doctors and health professionals. www.yakult.com.br

THE ROLE OF MICROBIOTA IN ASD SYMPTOMS

SCIENTISTS TRY TO DECIPHER THE EXTENT TO WHICH THE COMPOSITION OF INTESTINAL BACTERIA INTERFERES WITH THE DEVELOPMENT OR WORSENING OF THE DISORDER

Adenilde Bringel

The brain-gut-microbiota axis has been extensively investigated in several parts of the world and studies suggest that the close relationship between these organs and systems may be the answer for the development of several brain disorders, including autism spectrum disorder (ASD). Recent scientific findings have indicated that abnormalities in the gut microbiota composition may be potentially involved with the development or worsening of ASD symptoms because of their role in modulating the immune system and gut microbiota. It has also been shown by different studies that these individuals have a population of pathogenic bacteria in the gut microbiota in a quantity much higher than expected, which generates a dysbiosis and consequently an immunoregulatory imbalance. Children with ASD have 3.5 times more bowel problems than neurotypical ones – second major disorder comorbidity (the first is epilepsy) – and often report abdominal pain, constipation, chronic diarrhea, reflux, colitis and other gastrointestinal tract dysfunctions which significantly worsen behavior and quality of life.

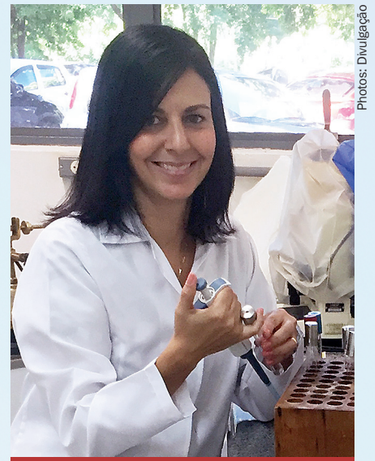
It is not yet clearly established by science how gastrointestinal factors are related to ASD. However, many children with the disorder have a history of prior exposure to antibiotics or hospitalization, frequent gastrointestinal symptoms, abnormal eating cravings and unique intestinal bacterial populations that may be related to the varying severity of symptoms. Another factor considered important is the delivery form, the natural one being more recommended because the child receives, at the time of birth, a high beneficial bacterial load from the mother in the passage through the vaginal canal. "Brazil certainly has a high risk of autism and related disorders due to high numbers of elective C-sections, high antibiotics use in medicine and agriculture, increased consumption of processed and industrialized foods and genetic populations at risk, with high prevalence of organic acidaemia polymorphisms (elevation of acidity in the blood), besides migration, conditions that lead to alteration of the immunity, metabolism and brain development due to microbiome modification", warns Derrick MacFabe PhD, director of the American College of Nutrition and the Kilee Patchell-Evans Autism Research Group (KPEARG), one of the largest ASD research centers in the world, founded at the University of Western Ontario, in Canada, whose work is listed among Canada's top 50 scientific discoveries by the Council Research in Natural Sciences and Engineering of the country.

Researches on microbiota and ASD gained prominence in the year 2000, when Dr. Sidney Feingold's group of Infections Disease Sections, Veterans Affairs Medical Center, California, United States, found that abnormal populations of intestinal bacteria, especially certain species of *Clostridium difficile* – mainly *C. perfringens*, which are pathogenic and toxin producers – were found in large numbers in fecal samples of children with ASD compared to controls. At the time, scientists identified 25 different species of *Clostridium* in samples from children with autism. In gastric and duodenal specimens, the most striking finding in the 'Gastrointestinal microflora studies in late-onset autism' study was the total absence of non-spore-forming anaerobes and microaerophilic bacteria in the controls, and a significant number of these bacterias in children with ASD. By demonstrating these changes in the gut microbiota of children with late-onset ASD, the experiment triggered a number of researches to understand the real role of the microbiota in the nature of the disorder.

In the studies developed by the group of Katia Sivieri PhD, from Department of Food and Nutrition of the Faculty of Pharmaceutical Sciences of the Júlio de Mesquita Filho State University (UNESP) – Araraquara campus, some of these findings were confirmed. The scientists evaluated the microbiota of children with ASD and neurotypics and realized that the group with ASD had a higher concentration of *Desulfovibrio* – considered an important microbiota marker – and *Clostridium*, including *C. perfringens*, besides a lower concentration of *Bifidobacterium* and an intermediate concentration of *Lactobacillus*, which was used as a marker in the assay. "We performed an *in vitro* experiment using the Shime® simulator with different mixtures, and the most significant was that involving *Bifidobacterium longum*, *Lactobacillus reuteri* and Vivinal® GOS, which is a galactooligosaccharide. After 24 hours in fermentation there was a change in this microbiota with expressive presence of *Bifidobacterium* and increased amount of *Lactobacillus*", she details.

From this result, the group performed a long-term experiment in which the reactor was fed with this mixture for two weeks. The same behavior was observed, with an increase of *Bifidobacterium* and *Lactobacillus*, decrease *Clostridium perfringens* and *Desulfovibrio*, besides increase of butyric acid that, in children with autism, is almost not found. "Depending on the microbiota, we cannot measure

DERRICK MACFABE



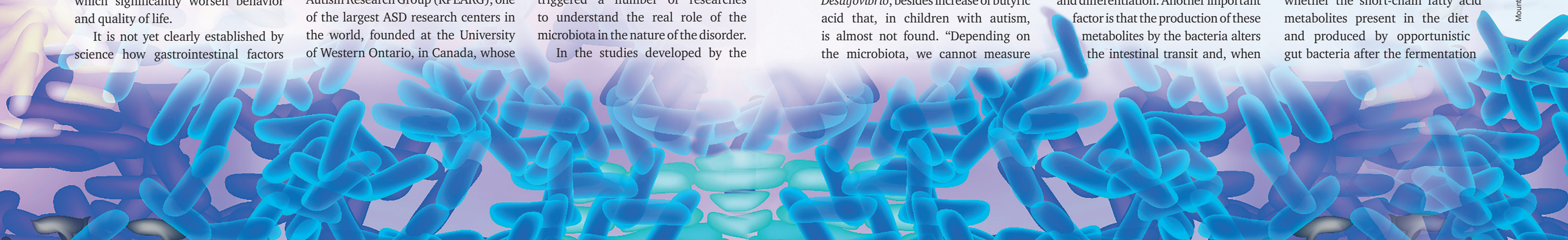
KATIA SIVIERI

Photos: Divulgação

butyric acid in these children because the amount is too small. And this is a very important metabolite. We also identified increased acetic acid and decreased propionic acid, which is generally increased in children with autism", she describes.

The researcher points out those short-chain fatty acids (SCFA) – mainly acetate, butyrate and propionate, produced from the fermentation of fibers in the bowel – have different roles for the health of the body. When SCFA are absorbed, they enhance the uptake of water and salts, and are used as the energy source by the host. Butyric acid is the main source of energy for epithelial cells lining the colon and can influence cell growth and differentiation. Another important factor is that the production of these metabolites by the bacteria alters the intestinal transit and, when the transit time is prolonged, usually constipation often occurs or some other gastrointestinal disorder, increased intestinal permeability and consequently modification in the production of these microbial metabolites.

Studies with SCFA and autism spectrum disorder have been a focus of the KPEARG international multidisciplinary research group, which aims to understand how the gut microbiome metabolites products control brain function and behavior in ASD, and how neuropsychiatric conditions are related, such as obsessive-compulsive disorder, anxiety, eating and learning disorders. "We are particularly interested in understanding whether the short-chain fatty acid metabolites present in the diet and produced by opportunistic gut bacteria after the fermentation



Mounting from the image: Depositphotos/mikrostoker

of dietary carbohydrates can be environmental triggers, particularly propionic acid. We want to understand its role in autism and the development of new clinical biomarkers and therapies to prevent, identify, track and treat the disorder", says the professor. SCFA represent a group of compounds derived from the host microbiome that are plausibly linked to ASD and can induce generalized effects in the gut, brain and behavior.

In the review article 'Enteric short-chain fatty acids: microbial messengers of metabolism, mitochondria and mind: implications in autism spectrum disorders', the researcher reports that propionic acid, an important SCFA produced by gastrointestinal bacteria associated with ASD and a common food preservative, may produce behavioral, electrogenic, neuroinflammatory, metabolic and reversible epigenetic changes that resemble those found in autism when these strains are administrated in rodent studies. In the experiment, intraventricular administration of propionic acid in rats induced abnormal motor movements, repetitive interests, electrographic changes, cognitive deficits, perseveration and impaired social interactions. "Brain tissue from mice receiving propionic acid shows a number of neurochemical changes related to ASD, including innate neuroinflammation, increased oxidative stress, glutathione depletion and altered phospholipid/acylcarnitine profiles. These changes contribute directly or indirectly to mitochondrial dysfunction acquired through the involvement of carnitine and glutathione-dependent pathways consistent with findings in patients", he describes. The scientist recalls that glutathione is low in autism, and common medications, such as acetaminophen – commonly given to children with ASD – can further harm this system, leading to increased oxidative stress and metabolic problems.

Common antibiotics can impair carnitine-dependent processes by altering the gut microbiota and consequently favoring the bacteria producing propionic acid and inhibiting the carnitine transport through the intestine. According to the professor Derrick MacFabe, propionic acid also produces bioactive effects on neurotransmitter systems, intracellular acidification and calcium release, fatty acid metabolism, immune function and altered gene expression. "These findings are consistent with the underlying symptoms and mechanisms proposed in ASD. Collectively, they provide additional support for which factors derived from the bowel, such as bacterial or enteric SCFA produced by bactericides, may be plausible environmental agents that may trigger ASD or behavior related to the disorder, and therefore deserve further exploration in basic science and clinical medicine", he emphasizes.

Another KPEARG study investigates butyrate, a short-chain fatty acid derived primarily from the enteric microbiome that positively modulates mitochondrial function – including increased oxidative phosphorylation and beta-oxidation – and has been proposed as a neuroprotective. As well as other SCFA, butyrate was associated with ASD for being a condition related to mitochondrial dysfunction. However, future clinical studies in humans are needed to help define the practical implications of these physiological findings. "In autism we see many strange situations, such as different bacteria in the microbiota, brain inflammation, changes in lipids and differences in the mitochondria. And what we want to know is how we can link all these changes to better understand the disorder", he emphasizes. The researcher stands out that not all SCFA are the same and therefore their effects may vary in different types and doses, diet, microbiome composition, inheritance and acquired changes in immune and mitochondrial function, including those found in people with ASD.

OTHER EVIDENCES

Many other studies have been able to demonstrate microbial changes in children with ASD. One of them was presented in 2013 by researchers from Arizona State University, USA, who measured the levels of various microbial by-products in the feces of children with autism and control and found significant differences between the two groups. In the same year, Italian scientists reported that, compared to healthy children, those who had ASD had altered levels of various bacterial intestinal species, with lesser amounts of the *Bifidobacterium* species, admittedly one of the most important for bowel health. An experiment published in 2017 suggested that the use of fecal transplantation in children with autism could relieve digestive problems and social disturbances.

Researchers from Shanghai Mental Health Center in China published a systematic review of the characteristics of the gut microbiota of children with autism in the Shanghai Archives of Psychiatry (2013-6). Of the 15 cross-sectional studies analyzed, 11 reported significant differences in the prevalence of gut bacteria among children with ASD and those in the control group, with emphasis on *Firmicutes*, *Bacteroides* and *Proteobacteria*. In 2018, scientists from Peking University First Hospital in Beijing published a meta-analysis of 150 studies, conducted in several countries since the 1960s, relating bowel health and autism. According to the studies cited in the meta-analysis, published in the scientific journal *Frontiers*, the balance of the gut microbiota improves the symptoms of ASD.

THE BENEFICIAL ACTION OF PROBIOTICS

Studies already carried out about the human microbiota showed that the trillions microorganisms that inhabit the digestive tract are responsible for a significant effect on health and disease. For this reason, researchers claim that the greater the microbial diversity, the healthier this microbiota will be and, consequently, the organism will have more resistance to diseases. However, it is still premature to claim that an intervention with probiotic strains may alter the history of autism spectrum disorder. Professor Katia Sivieri from UNESP says it is not to be forgotten that a microbiota is built throughout life, especially during the first 1,000 days. "We are born with a small amount of microorganisms, we have contamination at the time of delivery, through breast milk, with all diet in the first years of life. And an important point is the quality of the child's food and the way this microbiota makes its first three years of life", she teaches.

The scientists believe that the formation of the microbiota, from the child-birth and during the first 1,000 days, will be fundamental for the development of all points of view, including the possibility of autism. The researcher adds that one of the risks is the use of antibiotics in the first three years because they end up destroying all the microorganisms, beneficial and pathogenic, that are part of the microbiota that is still being formed. "I believe that the microbiota is a central point of several modernity diseases, not just autism. What is to find probiotic strains to help improve the typical symptoms of the disorder", she reinforces. The study 'Microbial-based treatment reverses autism spectrum social deficits in mouse models', by Professor Mauro Costa Mattioli, developed at the Baylor College of Medicine in Houston, USA, indicates that *L. reuteri*, for example, could have an action in control ASD symptoms.

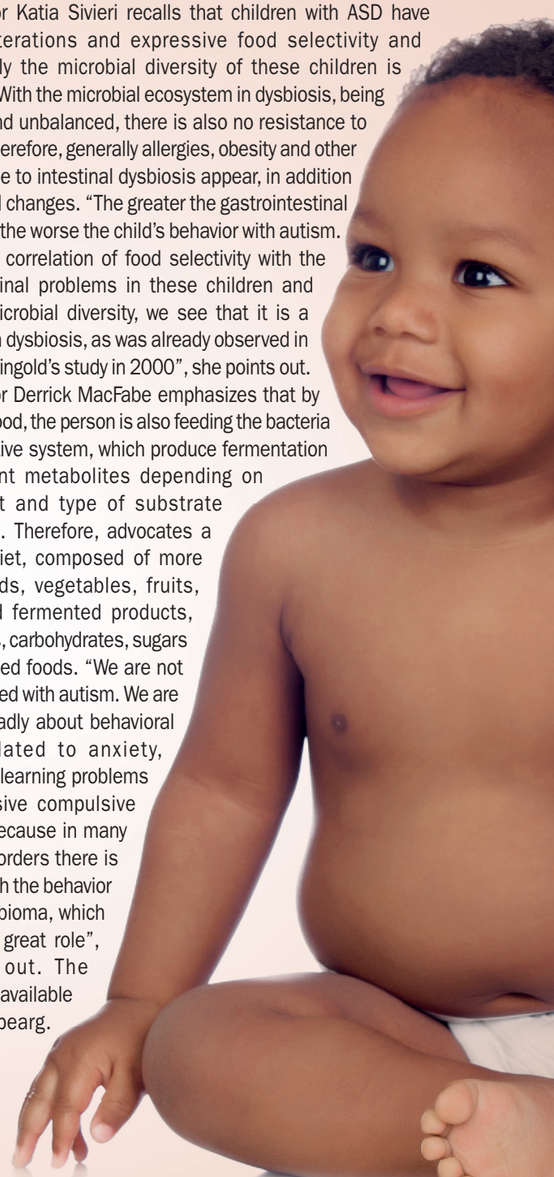
While studying females of mice that ate a high fat diet to evaluate the fat content of the offspring, the researcher observed that some individuals of the offspring had behavior similar to children with ASD. "In analyzing the microbiota of this offspring, the scientist saw that *Lactobacillus reuteri* was missing and, upon administration, the animals returned to normal behavior. But it is important to note that not all *L. reuteri* have this same behavior in the gut, which indicates that the positive action is strain-dependent", explains the UNESP researcher. Master student Ana Luiza Rocha Duque will go to Baylor College of Medicine to follow the *in vitro* work developed at UNESP, but now using an animal model in partnership with Professor Mauro Costa Mattioli.

Researcher Derrick MacFabe recalls that some studies show that changes in the microbiome may be a consequence of events that occur during childhood, such as prematurity, cesarean sections and nosocomial infections. In addition, certain childhood diseases have been associated with changes in the microbiome, such as necrotizing enterocolitis, childhood colic, asthma, atopic disease, gastrointestinal disease, diabetes, malnutrition, mood/anxiety disorders and autism spectrum disorder. "We reviewed some of the evidences of microbiome modification in childhood illness and discussed clinical trials that examined microbioma manipulation in an effort to prevent or treat these diseases with a primary focus on probiotics, prebiotics, and/or symbiotics. Treatment studies suggest that probiotics are potentially protective against the development of some of these diseases", he reports. However, the time and duration of treatment, probiotic strain and factors that may alter the microbiome composition and function still require further researches.

DYSBIOSIS RISKS

Professor Katia Sivieri recalls that children with ASD have sensory alterations and expressive food selectivity and consequently the microbial diversity of these children is diminished. With the microbial ecosystem in dysbiosis, being unhealthy and unbalanced, there is also no resistance to diseases. Therefore, generally allergies, obesity and other problems due to intestinal dysbiosis appear, in addition to behavioral changes. "The greater the gastrointestinal dysfunction, the worse the child's behavior with autism. In making a correlation of food selectivity with the gastrointestinal problems in these children and with little microbial diversity, we see that it is a microbiota in dysbiosis, as was already observed in Dr. Sidney Feingold's study in 2000", she points out.

Professor Derrick MacFabe emphasizes that by ingesting a food, the person is also feeding the bacteria in the digestive system, which produce fermentation and different metabolites depending on the amount and type of substrate they receive. Therefore, advocates a moderate diet, composed of more natural foods, vegetables, fruits, yogurts and fermented products, and less fats, carbohydrates, sugars and processed foods. "We are not only concerned with autism. We are thinking broadly about behavioral effects related to anxiety, depression, learning problems and obsessive compulsive disorders, because in many of these disorders there is a relation with the behavior of the microbioma, which is playing a great role", he points out. The studies are available at http://kpearg.com. ◆



Depositphotos/Gelpi