Bacteria On The Autism Brain

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Scientists leverage the gut microbiome-brain connection to develop new treatments for autism spectrum disorders.

NEW TREATMENTS FOR AUTISM may soon come from a surprising place: gut microbes.

While gut bacteria help us digest food and prime our immune system, research over the past ten years has shown that they also communicate with our brain to influence behavior. Scientists have now linked changes in the gut microbiome to a plethora of neurological disorders, from epilepsy and depression to autism.

Characterized by difficulties in social communication and by restricted or repetitive behaviors, autism spectrum disorders have a wide range of severity. Autism symptoms usually manifest in a child's first two years of life, and while some children can manage their symptoms without too much help, others may need full time care.

While the causes of autism are not well-understood, scientists think that both genetics and the environment contribute. Some autism spectrum disorders such as Rett syndrome and Fragile X syndrome, two rare genetic diseases, have a clear genetic cause, but for most other autism disorders, the relative contributions of genetics and the environment are less clear.

"Almost all of the risk alleles associated with autism are found in larger populations than those who have autism," said Sarkis Mazmanian, a microbiologist at the California Institute of Technology. "In other words, there are mutations that are found in 4, 5, 6% of the human population, most of whom do not have an autism diagnosis, so the genetics alone aren't enough."

Lending support to the contribution of environmental factors in autism, the incidence of autism has increased dramatically in western countries in the past 30 years. Once occurring in 1 in 5000 kids, it now appears in 1 in 54 children in the United States.

Research over the past decade or so points to the gut microbiome as one of the important environmental factors contributing to autism. The revelation that microbes in the gut can signal to the brain to alter behavior has led to the development of novel therapeutics for autism that work not by targeting the brain, but the bacteria in the gut.

The gut is not Las Vegas

Microbes begin colonizing our guts pretty much right after birth. Through breastmilk, we gain even more microbes, and as we age, the microbes we encounter in our household and through our diet contribute to the thriving flora that makes itself at home in our digestive system.

Scientists have known for a long time that our brain communicates with our gut. For example, Alessio Fasano, a pediatric gastroenterologist at Massachusetts General Hospital, explained, "if you got nervous or upset, you [would] have a stomachache, and that means that there is this communication." But, he added, "what we didn't know was that this is a two-way communication — that the gut can communicate with the brain."

Scientists have since discovered that gut microbes send signals to the brain in numerous ways: via the immune system, through the vagus nerve, as hormones, and as neurotransmitters. They also alter the integrity of the gut barrier, making it easier for microbial signals to cross from the gut lumen into the lamina propria, a space rich in immune cells and enteric nervous system neurons.

“The gut is not like Las Vegas. What happens in the gut, does not stay in the gut,” Fasano added.

Early hints that the gut microbiome might play a role in autism came from small studies that reported that some children diagnosed with autism suffered from severe gastrointestinal (GI) problems (1-3).

Depending on the study, “it could be anywhere from 40 to 70% of kids with autism tend to have GI issues,” said Stewart Campbell, CEO of Axial Therapeutics, a biotechnology company developing guttargeted therapeutics for autism. “They’re often variable even within an individual, so it makes life pretty unpredictable for them, even separate from the core autism.”

Of mice and metabolites

To understand how the gut microbiome affects autism, researchers investigate the guts of mice. While there is no way for a mouse to recapitulate every feature of autism that presents in humans, mouse studies have led to important insights into the role of gut microbes and metabolites in autism.

Sitting together in 2009, California Institute of Technology neuroscientist, Paul Patterson and Mazmanian discussed Patterson’s new mouse model for autism. Epidemiological data had shown that mothers who experienced a severe infection resulting in a fever during pregnancy had an increased risk of autism in their child. In Patterson’s autism mouse model, called a maternal immune activation model (MIA), researchers induced an immune response in pregnant mice and studied the resulting offspring, which exhibited autism-like symptoms (4).

Mazmanian told Patterson about his research on how the microbiome influences immune responses in the gut, and Patterson mentioned that kids with autism often have gastrointestinal symptoms.

That small observation, Mazmanian said, changed the course of his career.

“My response to him was, ‘Paul, I’m sure you’re throwing the intestines of your mice away when you do your experiments.’ He said, ‘of course, we are.’ I said, ‘why don’t you, next time you have a group of mice — you don’t need to go out of your way, but when you’re studying the brains, let me know. I’ll send a student over to your lab, and we’ll collect the intestines and see if there’s anything going on.’”

It turned out that there was.

Not only did the gut permeability of the autism mouse model differ from healthy control mice, but Mazmanian’s team found that the microbiomes of the mice differed too (5). They discovered that if they gave the MIA offspring a probiotic of the bacterial species Bacteroides fragilis, many of their autism-like behaviors — anxiety, impaired communication, and repetitive behavior — improved.

The team also found that the MIA offspring had altered levels of specific serum metabolites, with the clearest example being 4-ethylphenolsulfate (4EPS), which was present at incredibly high levels compared to the healthy mice.

“We, mice, worms, [and] flies don’t make this molecule. Only bacteria do, and only certain bacteria do,” said Mazmanian.

Treatment with B. fragilis restored blood serum levels of 4EPS to control levels in the MIA offspring, likely by inducing an increase in specific gut microbes and restoring gut permeability. Additionally, healthy mice treated with 4EPS induced anxiety-like behaviors, indicating that the bacterial metabolite likely contributes to the anxiety-like behavior seen in the autism mouse model.

In a new study that is in the late stages of peer-review, Mazmanian’s team showed that 4EPS gets into the brain.

“This metabolite, we believe, arrests the maturation of brain cells called oligodendrocytes. Oligodendrocytes are cells in the brain that myelinate neurons,” he said. By reducing myelination in a mouse brain, “it essentially changes the connectivity of the brain. Different regions of the brain don’t talk to each other in the mouse as they would otherwise, and that leads to anxiety and autism-like behaviors.”

Further supporting the role of the microbiome in autism, Mazmanian’s team reported in 2019 that treating female germ-free mice with fecal samples from children with autism led to offspring with autism-like symptoms: increased repetitive behavior, decreased movement, and decreased communication, with some mice also displaying decreased sociability (6).

The team found that levels of the metabolites taurine and 5-aminovaleric acid (5AV) were low in the metabolome of mice displaying autism symptoms compared to controls. Treating a mouse model of autism with taurine or 5AV reduced their repetitive behaviors and improved their social behavior, while also reducing the neuronal excitability in the prefrontal cortex, directly linking metabolites induced by the microbiome to brain function.

The microbiome meets genetics

Around the same time at Baylor College of Medicine, Mauro Costa-Mattioli’s team also revealed a role for the microbiome in a mouse model of autism.

Interested in the genetic and environmental factors that contribute to neurodevelopmental disorders like autism, Costa- Mattioli’s team found that female mice fed a high-fat diet have offspring with autism-like symptoms and an altered gut microbiome (7). The mice have dramatically reduced numbers of the bacterial species Lactobacillus reuteri in their gut, and they have deficient synaptic plasticity and reduced oxytocin production in the ventral tegmental area (VTA) of the brain, an area involved in regulating social behavior.

Treatment with an L. reuteri probiotic improved social behaviors and increased oxytocin production in the brains of these mice. In a follow up study, the team discovered that L. reuteri signals from the gut to the brain through the vagus nerve to promote oxytocin production, improving social behavior deficits (8).

The team’s latest finding — a “serendipitous discovery,” according to Costa- Mattioli — further implicates the microbiome in autism (9).

Mice deficient in the gene Cntnap2, which carries a genetic mutation implicated in autism, are hyperactive and have impaired social behavior. These mice also have fewer oxytocin producing neurons in the VTA.

Costa-Mattioli's team saw that when they bred and housed wildtype mice separately from the Cntnap2 knockout mice, the Cntnap2 knockout mice exhibited hyperactive and social behavior defects as expected and had distinct microbiomes.

"But then when we did the experiment the right way...with littermates, where essentially the mutant and the wildtype animals are in the same cage, we discovered that the social behavior [defect] completely disappeared," said Costa-Mattioli, while the hyperactivity behavior remained. "We said, 'What? How can that possibly be?'"

They also noticed that the microbiomes of the Cntnap2 knockout mice were distinct from those of the wildtype mice, but when the knockout and wildtype mice were bred and housed together as littermates, their microbiomes no longer looked different.

"Then we said, 'Could it be the microbiome at play?' And that's how the entire story essentially emerged," said Costa-Mattioli.

The researchers found that co-housing independently bred wildtype and knockout mice also made the social behavior deficit disappear in knockout mice. Alternately, separating wildtype and knockout littermates and breeding them independently resulted in offspring of the knockout mice displaying impaired social behavior again.

"Then we did that amazing experiment that completely takes care of everything, which is called the fecal material transfer, or poop transplant, where we transfer the different microbiomes into the germ-free mice," said Costa-Mattioli.

The team saw that the behavior of the germ-free mice directly reflected that of the mice whose microbiome they received. The evidence indicated that something in the microbiome of wildtype mice could rescue the social behavior defect, but not the hyperactivity, in Cntnap2 knockout mice.

That something, they discovered, was the bacterial species L. reuteri, specifically L. reuteri's induction of metabolites in the tetrahydrobiopterin (BH4) metabolic pathway. The team found that treatment with either L. reuteri or BH4 reversed the social behavior defects and the impaired synaptic plasticity in the VTA of Cntnap2 knockout mice, indicating that L. reuteri's induction of BH4 in the gut relieves the signaling and behavioral defects in this mouse model of autism.

Costa-Mattioli noted that if they had taken a traditional neuroscience approach and only looked at the brain, "out of the two symptoms — hyperactivity and social behavior — you might likely reverse only one, which is the hyperactivity, not the social behavior."

Clinical trials for BH4 in the treatment of autism showed some promising results in improving the social behavior of kids with autism, but Costa-Mattioli said, "the problem when you administer biopterin is that the molecules get oxidized very quickly, super quickly, and as a consequence, lose efficacy."

If scientists could induce the gut microbes in a child with autism to increase the production of endogenous BH4 via L. reuteri, that may overcome BH4's quick inactivation and offer an effective

treatment.

“This opens up the possibility of now leveraging that knowledge, and perhaps developing new therapeutics, which if we were to have these discussions 10 years ago, would be inconceivable,” said Costa-Mattioli. “You will quote me in the article saying, ‘this guy is out of his mind! How the heck is he going to treat the brain through the gut?’ But now, the evidence is emerging as a way [for it] to modulate the brain.”

From bugs to drugs

While the microbiomes of mouse models of autism have allowed researchers to identify molecular signals induced by gut bacteria that influence behavior, many scientists are also investigating the microbiomes of humans to develop new therapeutics targeting autism’s core symptoms.

Rosa Krajmalnik-Brown, an environmental engineer at Arizona State University (ASU), started studying the connection between the microbiome and autism when she met fellow ASU scientist, Jim Adams.

“He had the hypothesis that the microbiome is involved in autism,” she said. In one of Adams’ early studies, “he showed that kids with autism who had gastrointestinal symptoms, the more severe the gastrointestinal symptoms were, the more severe the diagnosis was” (3).

A small clinical trial also revealed that when researchers treated children with autism with the antibiotic vancomycin, their autism-associated behaviors improved (10). However, after vancomycin treatment stopped, their symptoms returned, indicating a potential role for gut microbes.

Krajmalnik-Brown and Adams teamed up to investigate how the microbiome contributes to autism, and in July 2014, they initiated an open-label clinical trial to investigate if microbial transfer therapy (MTT) — a modified version of fecal microbiota transplant, where gut microbes from healthy pre-screened donors are administered to others — could relieve autism symptoms in children.

They first treated 18 children with autism and GI problems, aged 7 to 16, with vancomycin for two weeks to get rid of their gut microbes, along with a proton pump inhibitor to help promote the colonization of the donor microbes. Adams and Krajmalnik- Brown’s team then treated the children with donor microbes for 7-8 weeks and monitored them for 8 weeks following treatment. They compared the microbiomes of these children with those of untreated, age-matched neurotypical children.

At the end of the trial, the pair noted that the microbiomes of the children with autism showed the same level of diversity as those of neurotypical children, and they also saw improvement in the children’s behavioral symptoms (11). Although, because this was an open-label study — all participants knew they were taking the drug — the placebo effect may have influenced the behavioral results, Krajmalnik-Brown cautioned.

After the trial, Krajmalnik-Brown and Adams heard from the study coordinator that the kids were doing very well, even long after the end of the study, so they decided to assess the children again two years later (12).

"We saw at the follow up that there were still huge improvements and that the benefits were not lost, which is one of the amazing, surprising things of our study," said Krajmalnik-Brown. Right after the trial, many of the kids' microbiomes looked like those of their donors. Two years later, their microbiomes looked less like their donors but were still very diverse, an indication of a healthy microbiome.

Surprisingly, the children's behavioral symptoms had improved even more since the end of the trial two years before. At the beginning of the trial, 83% of the children had a diagnosis of severe autism according to the Childhood Autism Rating Scale (CARS), and after two years, only 17% had a severe diagnosis.

"Some of the older children who participated in our study are in college now," said Krajmalnik-Brown. "They're in the normal classes. They're doing great."

Krajmalnik-Brown thinks that the treatment changed the children's gut environment into a place where a healthy microbial community could thrive. "By changing that growth environment, now it was an environment more prone to acquire and grow beneficial microbes," she said.

Adams and Krajmalnik-Brown are currently running a similar trial with a placebo control, and due to the beneficial effects that the first trial showed in teenagers, they are in the process of running an MTT trial in adults with autism.

Additionally, they are collaborating with Sun Genomics, a biotech company located in San Diego, to explore how a customized probiotic may influence the behavioral symptoms of kids with autism. Parents of children send in an initial stool sample from their child, and Sun Genomics produces a customized probiotic that the child takes for two months. After two months, parents send the company another stool sample.

"Just with the initial baseline sample, we have now a lot of samples of kids on the spectrum that are being sequenced," said Krajmalnik-Brown. "Ideally, we could maybe see who is prone to responding to this probiotic and who's not because not everyone improves the same on these types of therapies."

Capturing gut metabolites goes to the clinic

Mazmanian and the biotech company he co-founded, Axial Therapeutics, also hope to translate changes in the gut microbiome to treatments for autism.

In 2006, scientists at the University of California (UC), Davis enrolled 130 children with autism and 101 typically developing children into the Childhood Autism Risk from Genetics and Environment (CHARGE) study (13). Compared to other studies of kids with autism, which usually consist of 30-40 participants, this study was huge.

The CHARGE study also proved particularly valuable to microbiome researchers because it was the only known autism study at the time to collect fecal material. "Who would think to collect feces in an autism study a decade or two ago?" asked Mazmanian.

Through a collaboration with immunologist Paul Ashwood at UC Davis, Mazmanian's team, along with scientists at Axial Therapeutics and Massachusetts General Hospital, analyzed the metabolites present in the plasma and feces from the children with autism compared to the neurotypical children (14).

They identified significant differences in both the plasma and feces. In particular, they found that phenolic metabolites, including 4EPS, were elevated in some children with autism.

“4EPS isn’t a beginning or end of the story,” Mazmanian said. Rather it is “just one of the dozen or fourteen molecules that seems to have a very similar profile, where they’re not just structurally similar, but they co-vary with [autism] behaviors.”

Based on Mazmanian’s early mouse work on 4EPS and bolstered by these new findings, Axial Therapeutics decided to develop a therapeutic to target 4EPS and similar phenolic compounds in the gut to improve autism symptoms in children.

“We could go in and try to knock out the bugs that are making these metabolites,” said Campbell, but there is “this functional redundancy problem. You pull out one microbe; another one slides in and performs that function.”

Instead, the scientists at Axial Therapeutics took a simple, but clever approach. Regardless of where the metabolites came from, they would capture them in the gut. The team developed a molecule called AB-2004 that bound these phenolic compounds and sequestered them. People would then simply pass AB-2004 with its bound metabolites in their stool, removing them from the gut.

“It’s a really simple concept, but sometimes the simplest approach is the one that works,” said Campbell.

The team tested AB-2004’s effect on autism symptoms in boys with autism aged 12 to 17 in a Phase 1b/2a open-label clinical trial. In addition to measuring safety and tolerability data on the drug, they also looked at its effect on irritability, anxiety, and adaptive and repetitive behaviors.

“We were blown away by what we saw,” said Campbell. AB-2004 significantly reduced irritability and anxiety in kids with high irritability or anxiety at the outset of the trial. They were even more encouraged by some of the feedback they received from parents of boys in the study.

One of the teenagers had severe germophobia and refused to eat food that wasn’t prepared in front of him, but “when he was on our drug, his family was able to go out to a restaurant for the first time, and he ordered off the menu and ate the food. And they’d never seen that happen before,” Campbell said.

Another teenage boy needed his parents’ help to bathe, and one day while on the trial, his father heard the water running. When he went to check, he found that his son was showering himself for the first time.

“You talk about all the signals and the measurements and everything, but when you hear that, and you say, ‘that means something to that family,’ that just brings you to work. You’re excited every single day,” said Campbell.

Axial Therapeutics is starting a Phase 2b randomized, placebo-controlled clinical trial of AB-2004 for children aged 13 to 17 with autism soon, with a specific focus on reducing irritability. The current drugs used to treat irritability in autism are anti-psychotics, but these can have many side effects, making them difficult for kids to tolerate, especially in the long-term. So, if AB-2004 proves effective and has a more favorable safety profile, it could offer an exciting new treatment for the autism community, Campbell added.

The scientists are also developing new molecules that function similarly to AB-2004 but have different binding profiles so that they can sequester or modulate different gut metabolites.

To Mazmanian, the take home message of the AB-2004 clinical trial is simply that “Something in the gut is affecting behaviors associated with autism. That in and of itself is a huge win because it starts to add support for the fact that the gut may play a role in autism.”

A longitudinal view of autism

With exciting new treatments for autism targeting the gut microbiome in the pipeline, researchers are taking a step back to unravel the complex causes of autism from before birth to diagnosis.

Physicians use behavioral symptoms to diagnose children with autism because there is no biochemical marker for autism yet. Identifying an autism biomarker would allow for earlier diagnosis and earlier intervention. Behavioral therapy for autism, for example, is more effective when started earlier in life.

One way to find these biomarkers is by studying children before they are diagnosed with autism. For that, scientists need to perform longitudinal studies to track at-risk pregnancies through birth.

Alessio Fasano and his colleagues at research sites in Ireland, Italy, and the United States are doing just that with their longitudinal study Genome, Environment, Microbiome, and Metabolome in Autism (GEMMA) (15).

“We realize that the microbiome is definitely important, but if we study just the microbiome, we’re not going to understand the mechanism that leads to the neuroinflammatory process that characterizes autism,” he said.

The researchers are enrolling infants with an older sibling with autism due to their increased risk of also developing autism. Fasano and his team plan to assess multiple factors that contribute to autism. In addition to collecting stool, blood, urine, saliva, and serum samples from the children, Fasano’s team also documents how the child was born (vaginal delivery vs cesarean-section), any infections the child had along with any antibiotic treatment, and a detailed diary of what the child eats, among other information.

By partnering with machine learning and AI experts to integrate these data into models, Fasano hopes that one day, scientists may be able to predict “who on a specific genetic background with a specific microbiome will take the wrong turn and will develop autism, and who will stay on the course,” he said. “We want to know months before, who is taking the wrong turn.”

While the researchers are currently only about halfway through the GEMMA trial, Fasano hopes that they will have some meaningful data to share from their observational study in a couple of years. Fasano hopes that their findings may lead to the prevention of autism in at-risk children.

With the many observational and investigational trials underway, the overall feeling in the gut microbiome and autism research community is one of hope. “If the microbiome has this strong modifying effect on a person’s genetics, well, the microbiome is something we can change … maybe even correct,” said Mazmanian. “At this point in the autism community, we need some wins.”

With microbiome-targeted therapies, “I think we can deliver hope,” Mazmanian added, “and really start helping out families in huge ways.”