DEEP DIVE

Gut feeling: How microbes shape autism

BY **NICHOLETTE ZELIADT**

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Animations & Illustrations by Abigail Goh

Around the time that Sam was diagnosed with autism at age 3, he began to throw hour-long crying fits. When he wasn't plagued by lengthy spells of constipation, the Michigan toddler endured terrible bouts of diarrhea.

By the time Sam was 4, his parents suspected that it might be something besides autism that made him so miserable. About half an hour before every bowel movement, Sam doubled over, clutching his belly and crying out in pain. He sometimes draped his abdomen over toys or furniture, as if trying to ease his aching gut.

Sam's parents took him to numerous pediatricians and gastroenterologists, but physical exams and blood tests revealed no obvious problems that would affect his digestive system. His parents reluctantly began to accept that his gut problems might simply be yet another manifestation of his autism. "We kind of gave up," says Sharon, Sam's mother. (Their names have been changed to protect their privacy.)

Unfortunately, Sam's experience is common among children with autism. About 40 to 60 percent of these children **cope with gastrointestinal (GI) problems**, ranging from frequent abdominal pain and bloating to diarrhea and constipation. But why and how their distress develops — and what to do about it — remain a mystery.

Despite their pain, these children's abdominal tracts generally appear normal, says **Emeran Mayer**, director of the Center for Neurobiology of Stress at the University of California, Los Angeles. In some children, GI problems may result from stress and anxiety, or emerge as a consequence of behavior. If a child's insistence on sameness spills over into eating habits, for example, she might not consume enough fiber or liquid, and may become constipated as a result.

It's also possible that the real culprit of these digestive symptoms is not human at all. Evidence from the past decade suggests that GI problems in some people with autism stem from disruptions in the gut microbiome — the complex stew of bacteria and other microbes that help to digest food,

make vitamins and protect against pathogens. Scientists have found tantalizing clues that the types of microbes that live in the guts of people with autism differ from those in people without the condition.

So far, this research poses more questions than it answers, but the need for clarity is urgent: Against the advice of experts, and in a desperate attempt to help their pain-wracked children, some parents are performing do-it-yourself fecal transplants, overhauling the gut microbiome by transferring stool (and the intestinal bacteria it contains) from a healthy donor into a child with autism.

Sharon began giving her son fecal transplants in July 2014, after a doctor in Florida showed her how to do it safely. She pumps her husband's liquefied stool into Sam's colon through his rectum using a syringe and catheter. She also squirts the concoction into gelatin capsules — a preparation sometimes called 'crapsules' — that her son takes by mouth. Initially, she gave the transplant and pills every day, but has since reduced the frequency to twice per week.

Clinicians caution that such do-it-yourself fecal transplants are potentially dangerous, as researchers are only beginning to explore the technique. One group of researchers at Arizona State University is now wrapping up a small clinical trial of fecal transplantation, in the hopes that changing the gut microbiome can help to alleviate GI problems in children with autism.

Meanwhile, some researchers are exploring an even more controversial idea: that the gut microbiome may also shape the brain and be a root cause of autism's core features. According to this hypothesis, having a **leaky gut** allows compounds produced by gut bacteria to escape from the intestines; these compounds then alter brain function and contribute to the social problems and **repetitive behaviors** characteristic of autism.

So far, this idea is supported primarily by **studies in mice**, but some are thrilled by its potential. "I think it's exciting; it's a new hypothesis for the cause of autism and could potentially have a lot of power to help kids," says **Jason Shepherd**, assistant professor of neurobiology and anatomy at the University of Utah.

Ultimately, understanding how the microbiome differs in people with autism and how it affects the function of the gut or the brain could help clinicians diagnose and treat children like Sam. The composition of a particular child's gut microbiome could serve as the basis for personalized treatment. "It's very early days," says Shepherd. "There's just so much information that we don't know yet."

"It's like a fingerprint — we're all very different." Sarkis Mazmanian

Thinking small:

In the average human, gut microbes — mostly bacteria, but also fungi, viruses and other microorganisms — outnumber cells that make up the human body by nearly 10 to 1. These microscopic residents are staggeringly diverse, including more than 1,000 different types of bacteria, which collectively have an estimated 3 million or more genes, vastly more than the 23,000 or so in the human genome.

In the past decade, research into what these creatures are and what they do has moved from the sidelines to the mainstream, and it has become evident that a robust microbiome is essential to good health. Dysfunctions in the gut microbiome may not merely interfere with digestion, but may also play a crucial role in **conditions as diverse** as obesity, asthma, cancer and even neurological conditions such as multiple sclerosis.

In autism, the first hint of a connection between microbial communities and behavioral symptoms emerged from a study in 2000 that focused on 11 children whose parents first noticed autism symptoms after their child **took antibiotics**. These children had diagnoses of ‘regressive’ autism, meaning they appeared to develop typically from birth but then abruptly lost language, social skills or other abilities. (Researchers are divided on whether **regressive autism** differs from other forms of the condition.)

The researchers, led by **Sydney Finegold** at the University of California, Los Angeles, hypothesized that the antibiotic treatment kills off a few essential species of gut bacteria, allowing the intestines to be invaded by a group of bacteria called *Clostridia* that produce neurotoxins, resulting in behavioral problems.

Finegold’s team treated the children for eight weeks with a second antibiotic capable of wiping out harmful bacteria, reasoning that it might provide some relief. Almost none of the drug, vancomycin, gets across the intestinal lining into the bloodstream, and it is therefore thought to act only on the digestive system.

A clinical child psychologist then viewed 30-minute videos of each child playing before and during treatment. Unaware of when each video was taken, the psychologist determined that eight of the children improved in their behavior and communication while taking vancomycin. When the researchers reevaluated the children two to eight months after the end of the trial, however, all but one of them seemed to have returned to their pre-treatment state. The researchers suggested that in the absence of vancomycin, the harmful microbes had returned.

“This was a good piece of evidence showing that there is definitely a correlation between the gut microbes and behavioral changes,” says **Rosa Krajmalnik-Brown**, associate professor of civil and environmental engineering at Arizona State University. Krajmalnik-Brown studies human gut microbes but was not involved in this work.

For Ellen Bolte's son, who participated in the study, the vancomycin made a big difference. Although it did not take away his autism, he became much calmer. "If you have a child on the spectrum, you actually don't need them to be cured to have a huge difference in the quality of their life and yours," says Bolte. "When you can go from screaming and misery to calm, just taking that irritability away is huge."

The study was small and lacked a control group, however, so the notion that gut microbes shape behavior remained a fringe theory until about a decade ago, when researchers began manipulating the microbiomes of animals in more controlled ways.

In 2004, researchers in Japan found that 'germ-free' mice, which live in a sterile environment and lack a microbiome, produce larger amounts of **stress hormones** than control mice do when restrained in a narrow tube. If the mice were dosed with just one species of bacteria — *Bifidobacterium infantis*, a normal resident of the mouse gut — before the experiment, though, their stress responses were normal.

Last year, researchers in Ireland found that germ-free mice **don't show the normal preference** for interacting with an unfamiliar mouse rather than a familiar one, and they compulsively **groom themselves** — mouse behaviors often used as proxies for autism symptoms.

Together, the studies indicated that it was worth looking at whether gut microbes affect the brain and behavior in people.

Gut reactions:

So far, a link between microbes and mental function in people has been harder to pin down than it was in mice. To begin with, it's still unclear whether there are consistent differences between the gut microbiomes of people with autism and those without.

About a half-dozen reports have found that the **composition of the intestinal flora** — measured via fecal samples — in children with autism differs from that in children without the condition. But each finding seems to implicate a different set of microbes, and a few report **no differences at all**. It doesn't help that each individual seems to have a different characteristic microbial pattern.

"It's like a fingerprint — we're all very different," says **Sarkis Mazmanian**, professor of biology at the California Institute of Technology in Pasadena. The microbial community may also change with age. Given that natural variability, it's difficult to identify a single microbial strain or a grouping of

microbes that is consistently associated with autism, he says.

The studies in this field so far are small; one of the largest to date included only 58 children with autism and 22 controls. “Statistically, it’s very difficult to identify any trends if you’ve only got 20 people to look at,” says **Jack Gilbert**, a microbial ecologist at Argonne National Laboratory outside of Chicago.

Yet another limitation is that none of the studies have controlled for differences in diet, which may **shape the microbiome’s profile**. Many children with autism are **picky eaters**, and others **avoid gluten or casein** — proteins found in wheat and dairy, respectively. That means the microbial differences reported in studies could simply reflect these children’s dietary quirks, rather than something fundamental about autism.

Clearer answers may be forthcoming. Several teams are analyzing the intestinal flora of hundreds of children and documenting what each participant eats. Gilbert and his colleagues aim to recruit a thousand people with autism as part of a large study called **American Gut**. That project, launched in 2012, has already catalogued microbes in fecal samples from more than 3,000 people worldwide (despite the project’s name, the team accepts samples from across the globe). It is open to anyone willing to pay $99 to cover the costs of the fecal analysis, mail in a stool sample and complete a detailed online questionnaire that asks about diet and other lifestyle factors.

Last year, Gilbert and his colleagues began **waiving the fee** for participants with an autism diagnosis and have so far enrolled 220 people with autism. “A thousand will give us the statistical power to have a real impact,” Gilbert says.

Gilbert is also working on a **similar project** led by **Dennis Wall**, associate professor of pediatrics and psychiatry at Stanford University in California. Wall also aims to recruit a thousand or more children with autism, and plans to collect detailed medical histories and symptom information. In addition, Wall’s study aims to analyze the children’s DNA to link genetic variants to particular microbiome profiles.

Other researchers are addressing the problem of variability over time, investigating how the gut flora change in children with autism as their GI symptoms wax and wane, to try to identify strains that cause symptoms. In one such study, a team led by **Catherine Lozupone** at the University of Colorado, Denver, has assembled a group of 30 children, half of whom have autism. The researchers are aiming to collect four samples from each participant, one every three to six months.

Mind-altering microbes:

While researchers grapple with designing studies that can spot real differences among people with autism, other teams working with mice have begun to identify specific microbes or their metabolites that can trigger autism-like symptoms.

For example, mice exposed prenatally to the **epilepsy** drug valproic acid **have autism-like symptoms**, such as preferring an inanimate object over interacting with a cage-mate. In 2013, a group based in the Netherlands found that these animals also have an **altered profile** of gut bacteria compared with controls.

Another autism model follows a similar pattern. Here, pregnant mice are injected with a virus-like molecule to stimulate an immune response, and the pups **exhibit autism-like behaviors** such as excessive self-grooming and a disinterest in other mice. In a 2013 study, Mazmanian's team found that these pups **have different types** of gut bacteria than control mice do. They also develop leaky guts, with increased blood levels of the bacterial compound 4-ethylphenyl sulfate (4EPS). Treating the pups with *Bacteroides fragilis*, a bacteria common in healthy human microbiomes, seals their leaky guts. The mice lose their autism-like repetitive behaviors, although not their social deficits.

It's unclear why the bacterial treatment works, but "I don't see this per se as implausible," says **Jonathan Eisen**, professor of medical microbiology and immunology at the University of California, Davis, who was not involved in the work. "There are lots of examples of how adding something unnatural can modify behavior: Opium. Rabies. Caffeine. Why not a microbe?"

Still, Eisen and others caution against making too much of the results of the study, citing arguments often made in applying results from mouse studies to conditions in people. "The absolute key to me is the overselling of the relevance to autism," he says. "This is not autism; it is some behaviors that resemble behaviors seen in human autism."

Without knowing exactly how gut microbes might affect the brain, it's difficult to determine whether the microbial treatment improves behavior indirectly by resolving GI problems, or whether it has a more direct effect on the brain and behavior. To address this question, Mazmanian's team is introducing bacteria that produce 4EPS into normal mice to see whether this induces anxiety-like behavior, in the hopes of pinpointing a molecule that mediates the effect.

Mazmanian is also investigating the role of gut microbes in several other mouse models of autism, to see whether these animals also have gut problems and autism-like behaviors that improve after treatment with *B. fragilis*. If the findings turn out to be common to multiple mouse models of autism, that may help to convince skeptics that the findings are relevant to people with the condition.

Testing transplants:

Even with these scientific questions left unresolved, some researchers are testing out a blunt approach: introducing a new microbiome into children with autism via fecal transplant.

People **have experimented** with fecal transplants since the 4th century, when doctors treated diarrhea with a concoction called 'yellow soup,' an oral remedy made of fecal matter from a healthy person. In modern medicine, the technique began gaining credibility a few years ago, after Dutch researchers found that a single dose of stool from healthy donors **cured more than 80 percent** of people infected with the deadly intestinal pathogen *Clostridium difficile*.

Still, the procedure is used only as last resort, when antibiotics or other conventional treatments fail, and has only been shown to be effective for recurrent, dangerous GI infections.

"Fecal transplants are extraordinary, because they fix a disease we don't have another fix for," says **Neil Stollman**, chairman of the department of medicine at Alta Bates Summit Medical Center in Oakland, California, who has performed more than 100 fecal transplants to treat *C. difficile* infections. But, Stollman himself says, "They are really the wrong answer."

To be a viable option, says Stollman, transplants will need to be more precise, providing only those microbes a person actually needs rather than replacing their gut microbiome entirely. The procedure appears to have relatively few harmful side effects, but there isn't yet enough evidence to rule out risks. In one case, a woman with a *C. difficile* infection who was cured by a fecal transplant from an overweight donor later **became obese**. Others have **developed immune disorders** such as rheumatoid arthritis and nerve damage after receiving fecal transplant. However, it is unclear whether the conditions developed as a result of the treatment.

This year, a team led by **James Adams**, who directs the Autism/Asperger's Research Program at Arizona State University, tested the procedure **in 20 children** who have both autism and gastrointestinal problems. The children, who range in age from 7 to 17 years old, received a course of vancomycin to knock out the bacteria resident in their guts, followed by a fecal transplant from a donor.

> "It's a new hypothesis for the cause of autism, and could potentially have a lot of power to help kids." Jason Shepherd

The trial is designed to test whether the treatment improves the children's digestion. "If we can at

least understand what's happening with these gastrointestinal problems, and we can modulate them and give these kids a better quality of life, that's a big gain," says Krajmalnik-Brown, who is also working on the project.

The team is also assessing whether the treatment alleviates symptoms of autism. (They have declined to discuss their findings before publication, which they anticipate will happen next summer.)

Few researchers not involved in the trial expect to see any changes in this realm, however. "I think it's unlikely that interventions like fecal transplant would affect the core symptoms of autism," says California gastroenterologist Emeran Mayer.

In the meantime, though, parents like Sharon are forging forward, unwilling to wait for the slow progress of science.

Sharon says that thanks to the do-it-yourself fecal transplants, Sam's gut symptoms have almost completely disappeared — with the exception of a few flare-ups — and his stools are normal. The treatments have not changed his autism symptoms, but they have made a big difference in Sam's life. "He is a completely different kid," she says. "He still has autism, but he's happy and much more interactive. He's no longer in so much pain, so he can actually focus on what's going on around him."

Michelle Roberta, the mother of a 4-year-old boy with autism troubled by frequent bouts of diarrhea, began giving her son rectal fecal transplants a year ago with the help of instructional videos on the Internet. She says the treatment not only got rid of his diarrhea but even alleviated her son's repetitive behaviors and improved his social skills.

"He started playing more with my younger son, he started acknowledging other people at the park and observing them, his eye contact increased a lot, and he got some language, too," Roberta says.

She is encouraged by his progress. "Once you start doing social things and interacting, that's the beginning of language development, and I see that happening in him," she says.

Still, Stollman and others strongly advise against do-it-yourself fecal transplants, given the lack of evidence supporting its use for autism and the potential for harm. "I think there's this perception that transplants are benign, and they may not be," Stollman says.

Mayer agrees, explaining that the primary concern is that feces, if not properly screened for pathogens, could trigger diseases. Although a person's poop may be tolerated by his own immune system, it could trigger a harmful immune response in someone whose body has never before encountered that specific mix of organisms. The procedure could also injure the recipient's ano-

rectal area.

Sharon says she recognizes the risks but has no other treatment options, and has little to lose by tinkering with her son’s microbiome. “When your child is in that kind of pain and medicine isn’t helping, you’re willing to try things like this.”

So far, neither research studies nor home-based experiments explain why the microbiome seems to play such an important role in autism. But the research is only getting started, and already, it offers parents such as Sharon a vision for the future that is much easier to digest.