It Takes Guts

Scientists Delve Into the Role of Intestinal Microbes in Autism

by Tim Paul
Seven decades ago, child psychiatrist Leo Kanner described Richard M., an anti-social 3-year-old with obsessive preoccupations and an inability to talk, despite obvious intelligence and good hearing. Kanner's text would become the first case report of autism, a previously unknown condition. In addition to the behavioral and developmental symptoms familiar to any modern reader, Kanner's 1943 case study also details an array of digestive ills, including the boy's need for daily suppositories during infancy—administered, his mother said, to "train" him "so his bowels would move by the clock"—and subsequent diarrhea. Richard M. wasn't the last child with autism to suffer gastrointestinal distress, and yet doctors have tended to dismiss such symptoms as mere extensions of picky eating, a classic trait of autism typically lumped among the children's other characteristic behaviors.

In March, *JAMA Psychiatry* published a report with the most robust evidence to date that intestinal distress is in fact very real for many autistic children. Authored by scientists at the Mailman School's Center for Infection and Immunity (CII), the Department of Epidemiology, and the New York State Psychiatric Institute (NYSPI), the
report confirms what parents have long suspected. Michaeline Bresnahan, PhD ’99, with co-authors Mady Hornig, MD, Ezra Susser, MD, MPH ’82, DrPH ’82, and W. Ian Lipkin, MD, document that among children with autism, gastrointestinal complaints often begin in infancy—a year or more prior to presentation of behavioral and neurological symptoms.

A related CII study suggests why: unique features of the microbial ecosystem within the intestines of children who have both autism and gastrointestinal symptoms. Those findings, together with other studies by the team and their colleagues—funded by Autism Speaks, the Jane Botsford Johnson Foundation, the National Institutes of Health, and the Simons Foundation Autism Research Initiative—reflect the culmination of 15 years’ investigation motivated by the alarming rise in the number of children with autism. They also provide tantalizing clues about the origins of the disorder and how to treat it.

Is This Really Happening?

Today, 1 in 70 American children is diagnosed with autism. Over the last three decades, that rate has climbed precipitously—more than tenfold. Such a trend is highly unusual for any disease, let alone a developmental disorder, and it’s left many wondering whether the epidemic of children with a common pattern of atypical social and cognitive development is merely a mirage, attributable to heightened awareness about the disorder and revisions to the official diagnostic criteria in 2000.

A 2007 simulation by Bresnahan—now an assistant professor of Epidemiology and an NYSPH research scientist—and others concluded that diagnostic revisions account for only a portion of the precipitous rise. While the issue remains contentious, one recent estimate suggests that such changes account for less than half of the shift. What, then, explains the bulk of the trend?

Genetic studies may hold a clue: Autism is among the most heritable of developmental disorders. Yet a focus on genes alone won’t be sufficient. “The rate of genetic mutations doesn’t rise on its own,” says Hornig, an associate professor of Epidemiology and director of translational research at CII. Something in the environment, she says, must have changed in the last 30 years that—when combined with genetic susceptibility—gives rise to the increased number of cases. “You need a trigger.”

Search for a Smoking Gun

In 1998, The Lancet published a paper by British physician Andrew Wakefield reporting measles virus in the intestinal tracts of children with autism who also had gastrointestinal symptoms. The popular press fixated on the safety of the measles vaccine. Scientists focused on the study’s limited size—only 12 children—and its lack of rigor. Amid the furor, the gut symptoms went relatively unmentioned.

Even as parents worried about the hazard of vaccines, pediatricians and public health officials cautioned that further investigation of the questions Wakefield had raised could be devastating to vaccination programs. “Any research related to the idea that vaccines could have some negative effect was discouraged,” recalls Lipkin, director of CII and the John Snow Professor of
Epidemiology. “Even looking into the issue was seen as pointing the finger at vaccines.”

Hornig and Lipkin were undeterred. In 2003, they set out to painstakingly replicate Wakefield’s original study, correcting methodological flaws and draining any apparent sources of bias. They worked with three separate laboratories—including, for added rigor, the original facility that produced Wakefield’s results—to search for genetic evidence of measles in the intestines of 47 children. To minimize the risk of inadvertent introduction of a measles virus contaminant, they even used a separate, virus-free laboratory to prepare specimens for analysis. “To get clarity,” says Hornig, “required a watertight, blinded, pristinely controlled and conducted study.

The results—five years in the making—appeared in 2008 in the journal *PLOS ONE*: There was no difference in the levels of measles virus in children with autism compared with those without, no link between measles and autism. Three years later, following a British medical disciplinary investigation, *The Lancet* retracted Wakefield’s paper.

**Gut Feeling**

Hornig and Lipkin’s 2008 *PLOS ONE* paper closed one chapter of autism research and opened another. In a follow-up study published by the same journal in 2011, Brent Williams, an assistant professor of Pathology and Cell Biology at CII, with Hornig and Lipkin, re-examined tissue samples from the intestines of children in the earlier study. They found marked reductions in the expression of the genes that create enzymes to break down carbohydrates and transport simple sugars into the bloodstream; without them, a child with autism might be unable to absorb even glucose, the simplest of sugars. Using an assay custom-built by Williams, they recorded a shift in the bacterial population of the intestines. In other words, there was a disturbance in the gut microbiome, the delicate ecosystem within our intestines. “Because these children can’t break down carbohydrates,” says Lipkin, “all those sugars flow into the colon, where they wreak havoc with the microflora.” But whether the gut made a difference on the macro scale in the lives of children with autism was still unknown.

To dig deeper into the gut-autism question, the scientists needed a more robust data set. They turned to the Autism Birth Cohort (ABC) Study, a long-standing project that originated in work by Susser in 1999. Then chair of Epidemiology, Susser had established a partnership with the Norwegian Institute of Public Health to develop studies of neurodevelopmental disorders in a large sample of pregnant women and their children. A major outcome of the collaboration was the ABC Study, led by Lipkin, that has now followed 115,000 children since before their birth, collecting biological samples and questionnaire data (see “Learning from ABC,” page 30).

When the ABC Study children were still infants, researchers asked their parents about gastrointestinal issues—long before symptoms of autism might manifest and likely before the child was old enough to be a picky eater. The results were clear: Children who would later be diagnosed with autism had 2.5 times the odds of persistent gastrointestinal complaints, compared with children who didn’t develop autism. “The symptoms started early and in many cases persisted,” says Bresnahan, who co-authored the report with Susser, Hornig, and Lipkin. “It wasn’t just a transient issue.”
Learning From ABC

Just as there likely are many kinds of autism, researchers also believe there are many autism triggers. Over the last 15 years, Mailman School researchers and their international collaborators have reported on a number of discoveries drawn from the Autism Birth Cohort (ABC) Study, as well as the International Collaboration for Autism Registry Epidemiology (ICARE) and its offshoot, the Multigenerational Familial and Environmental Risk for Autism (MINerva) Network, which collate birth registry data from six countries and the state of California. So far, the biggest findings have focused on differences in the ages and weights of parents of autistic children as well as the mother’s nutrition around the time of conception.

As they follow the ABC children into adulthood—the oldest participants are just 16—researchers hope to learn whether people with autism are more likely to be diagnosed with other neurological disorders—Alzheimer’s, for example—and how many of them spontaneously recover. “We’re only just beginning,” says senior investigator Ezra Susser, MD, MPH ’82, DrPH ’92, a professor of Epidemiology and Psychiatry who has authored multiple papers derived from the data.

Folic Acid Supplementation

Susser compared women who took prenatal supplements that contained folic acid with women who went without. Those who took the supplements around the time of conception reduced by more than one-third the risk that their child would develop autism. The results—published by JAMA in 2013—raised new questions. If folic acid is protective, how does it work? Says Susser: “Is it counteracting an environmental toxin that’s been on the rise?”

Parental Obesity

Could epidemic levels of obesity among adults be related to the explosion of autism diagnoses? At first, researchers hypothesized that a mother’s weight was a factor in her child’s risk for autism. The ABC Study supplied a crucial piece of information: the corresponding paternal data. Assistant Professor of Epidemiology Michaeline Bresnahan, PhD ’99, a co-author of the resulting 2014 Pediatrics paper, says her team’s findings were a surprise. “The father’s weight, not the mother’s, appeared to be the deciding factor,” she says. “And the higher the father’s BMI, the greater the risk.”

Parents’ Ages

Data published earlier this year by Molecular Psychiatry showed that mothers in their 40s were 15 percent more likely to give birth to an autistic child than mothers in their 20s. Children conceived after their fathers had marked their 50th birthday were 66 percent more likely to develop autism, compared to those with younger fathers. “We know that mutations in sperm go up with paternal age,” says Susser, a co-author of the study. “Maternal age is more of a mystery.”
Not all children who develop autism experience gastrointestinal issues, although one recent estimate puts the incidence as high as 70 percent. The CII investigators suspect there is a specific subtype of autism involving the gut, perhaps with its own causal pathway. Pulling back the curtain on the gut’s role in autism promises to do more than clear up confusion between doctors and parents. It could open the door to new treatments.

Tractable Information

The intestinal tract has the largest cluster of nerves in the body after the brain. It is also home to trillions of microbes that release vital chemical building blocks to form, among other things, the neurotransmitter serotonin. Serotonin in turn regulates the enteric nervous system—the nerve network found throughout the intestines—which signals pain and controls the reflexes that move food products throughout the bowel for digestion. Serotonin also has a vital role in communicating with parts of the brain involved in mood, memory, sleep, and learning. “Disturbances in the gut microbiome lead to a dysregulation of neuroactive molecules in the bloodstream that either signal or fail to signal in the appropriate way,” says Hornig. “That’s a potential source of behavioral disruption.”

Bringing relief to autistic children with gastrointestinal troubles might be as simple as modifying their diets—and the benefits extend beyond their guts to their behavioral and neurological symptoms. For example, given the findings by Williams, Hornig, and Lipkin about gene expression abnormalities within the intestines of some kids with autism, a nutritionist might recommend altering the types and quantities of carbohydrates a child is offered. If the CII team substantiates their hypothesis that disturbances in metabolism and altered gut bacteria in the intestines of children with autism may drive chemical imbalances in the blood and brains of these children, says Hornig, dietary changes that reestablish a normal biochemical state could also modify autistic behaviors.

For Hornig and Lipkin, the next step is homing in on the specific gut bacteria connected to autism symptoms. In one study, their team is conducting a census of the microbes in the mother’s placenta and in her baby’s meconium, the first bowel movement. In another, they are measuring levels of 181 metabolites in women during pregnancy and in the same mothers and their infants at birth. A third explores whether antibiotics prescribed in the first six months of life, which might precipitate changes in the gut, raise a child’s risk of developing autism.

Increasingly, scientists anticipate that it may be feasible to put the microbiome back on track. While a fetus has been thought to have a relatively sterile gut, delivery and breast-feeding bestow starter cultures from the mother’s microbiome on her newborn. Hornig envisions that there may be a way to adjust a woman’s commensal bacteria prior to pregnancy, softening the blow of environmental toxins and staving off disruptions that could alter fetal brain development. “I like the custom perfume approach,” she says. “A lab grows the bacteria to make a blend that works for the individual.”

The goal is to hit on something tractable, says Hornig. “I’m excited by things we can actually do something about.”

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