

# BARRIERS TO SIMILI

Children with the subtlest form of autism suffer social isolation; those with more severe disease face a much tougher road. New genetic clues put the spotlight on the communication hubs of the brain's neurons—the synapses.

by **Richard Saltus** | photography by Fredrik Brodén



# UNLIKE HER TWIN SISTER, CARLY, 12-YEAR-OLD NICOLE BRANCONNIER DOESN'T HAVE CLOSE FRIENDS.

She doesn't share her sister's pre-teen obsession with *High School Musical* or instant messaging. She goes to a special school; she speaks in choppy, incomplete sentences, unable to explain anything complicated; and when she walks past people in her home in Danvers, Massachusetts, she shows little interest and doesn't make eye contact.

Like many children with autism spectrum disorders, Nicole's connection to the world is an extremely narrow one. Her single overwhelming interest is animals—her dog Lulu (she will read out loud only to Lulu), zoo animals, animals in photos, stuffed or plastic ones that she used to arrange in particular, ritualistic ways. “She has her own agenda,” says her mother, Maureen. “She can't take other

people's perspective and doesn't understand why they don't go along with what she wants to do.”

Sixty-five years after child psychiatrist Leo Kanner described the puzzling cluster of cognitive, emotional, and social disturbances of 11 children with what he dubbed “infantile autism,” much about autism remains an enigma. An ever-widening array of disturbed behaviors and developmental obstacles—from mild to devastating—now fits under the umbrella term “autism spectrum disorders” (see sidebar).

Research into this mysterious disease, however, has gotten a recent “kick in the pants,” says Gerald Fischbach, former director of the National Institute of Neurological Diseases and Stroke who has served on the HHMI scientific review board. A convergence of funding, a handful of key discoveries about the autistic brain, and technology advances that enable fine-grained DNA searches have attracted major scientific players who've narrowed

the search for causes, according to Fischbach, now scientific director of the autism-focused Simons Foundation, a private philanthropy launched by billionaire hedge fund manager James H. Simons, whose daughter has mild autism.

Discoveries of gene mutations in some autistic individuals support the growing suspicion that the key problem in autism may lie within the synapse—the tiny, chemical-filled gap between the tip of a transmitting neuron's long, spindly arm and the receiving end of the next.

An estimated 100 billion neurons make up the human brain, connecting at synapses to create powerful information-processing networks that enable humans to think and remember, interpret sensory information from the outside world, and navigate the challenges of social relationships.

Sir Charles Sherrington, the neurologist and Nobel laureate who coined the term “synapse,” famously likened the brain to “an enchanted loom where millions

## A SPECTRUM OF BEHAVIORS

In his classic 1943 paper, child psychiatrist Leo Kanner described the peculiar behaviors of 11 children he had observed in the late 1930s and early 1940s. He was most dismayed—and intrigued—by what he called their “profound aloneness.” These self-absorbed children didn't respond to their names or make eye contact, and their isolation was evident from their earliest days. In general, Kanner noted, they showed no more interest in people than in “the desk, the bookshelf, or the filing cabinet.” The disease, it was thought at the time, was rare, the cause unknown.

Today, the label “autism” is applied to a spectrum of what are called “pervasive developmental disorders” (PDD), and it is still based on symptoms and behaviors. Disorders on the spectrum—including autistic disorder, PDD-NOS (not otherwise specified), childhood disintegration disorder, and Asperger's syndrome—range in severity from devastatingly disabling

to so-called “high-functioning autism.” Rett syndrome is sometimes included on the more severe end of the spectrum.

Autistic disorders, once considered rare, are now diagnosed in about 1 in 150 children. The Simons Foundation's Gerald Fischbach believes that the broadening definition of autism accounts for most of the increase. In addition, he says, the expansion of services for children with autism could be promoting more-frequent diagnoses.

Delays in normal development, causing cognitive and social deficit, usually appear in the first two or three years. Autistic children may walk on their toes, flap their hands, or become fascinated with spinning things. They may be absorbed in obsessive rituals, such as lining up toys in peculiar ways, flipping light switches on and off, or unraveling their socks thread by thread. Boys are diagnosed with autism four times more often than girls. —R.S.

of flashing shuttles weave a dissolving pattern, always a meaningful pattern though never an abiding one.” Perhaps the autistic brain is a loom that creates flawed patterns or can’t easily be programmed to weave new designs.

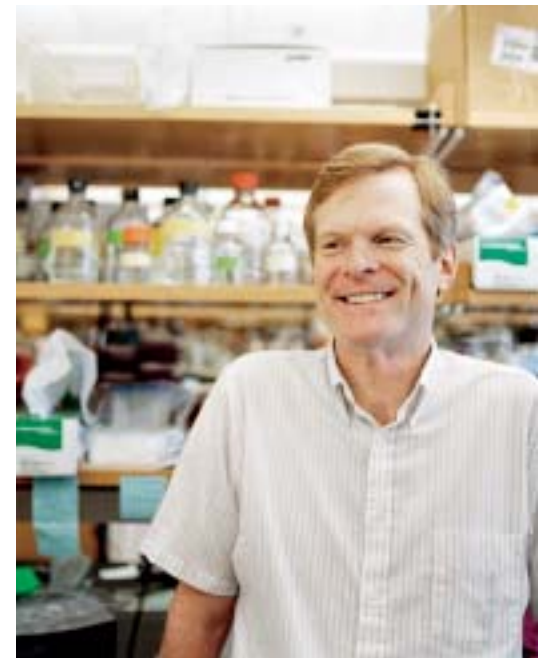
### BAD GENES, NOT BAD PARENTS

Though Kanner believed autism was inborn, many psychiatrists blamed it on cold, unloving mothers whose inadequate parenting marred their autistic children’s developing psyches. But studies in the 1970s showed that among twins with autistic disorders, identical twins are very likely to both be affected, whereas fraternal twins—like Nicole and Carly Branconnier—are rarely both affected. These studies provide strong evidence that faulty genes are largely responsible, most likely combined with unknown and unmeasurable inputs from environmental factors.

“Genetic” doesn’t always mean “inherited.” Although the less-disabling forms of autism can often be traced in families, scientists believe severe cases most often arise from spontaneous, or *de novo*, mutations. These are DNA mutations—present in the child but not in the parents—that occurred during the formation of the eggs or sperm before conception.

“This is not surprising, as autistic children rarely marry and have children,” says Christopher Walsh, an HHMI investigator at Children’s Hospital Boston and Beth Israel Deaconess Medical Center, who is searching for relevant genes.

An estimated 5 to 15 percent of cases stem from chromosomal abnormalities causing rare diseases with autistic features, such as Rett syndrome and fragile X syndrome. As for the remainder, no single gene has been linked to a large



Li-Huei Tsai’s studies of protein interactions and Christopher Walsh’s gene-seeking strategy are beginning to provide clues to autism’s origins.

portion of cases. A likely scenario is that mutations or slight variants in a handful of genes, or maybe in hundreds of different genes, acting in combination account for most autism.

In the absence of any known biological abnormality or “biomarker” in autism, scientists’ best bet is to hunt for altered genes in patients and families. Just such a discovery, in a rare syndrome caused by a single damaged gene, opened a new window on autism less than a decade ago.

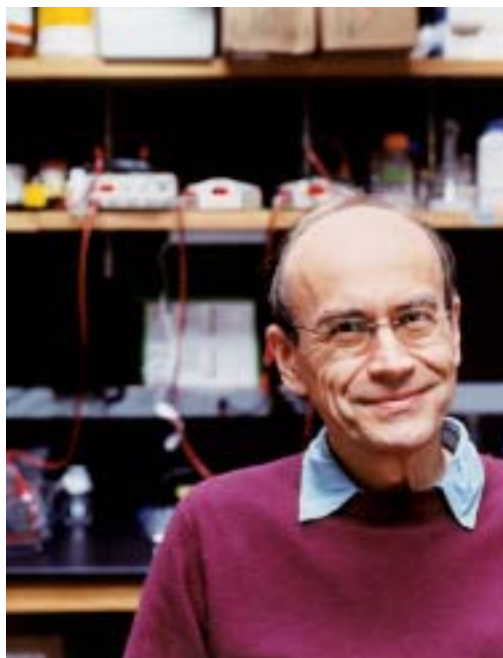
In 1999, HHMI investigator Huda Zoghbi identified gene mutations that cause Rett syndrome—a rare, devastating “autism spectrum” disease that affects girls. After normal development for the first 6 to 18 months, affected girls’ speech, motor control, and social development plateau and then deteriorate, accompanied by the onset of tremors, seizures, and stereotypic hand-wringing movements. Zoghbi, a pediatric neurologist and geneticist at Baylor College of Medicine, found that 95 percent of Rett cases involve mutations in a gene on the X chromosome called *MECP2*. “This was the first identification of a gene [mutation] in any developmental cognitive disorder,” observes Fischbach.

Zoghbi created a mouse model in which she has been studying the effects of the mutant gene. “MeCP2 is present in every mature neuron—thus, it is not surprising that it is important for social behavior and communication,” says Zoghbi. The MeCP2 protein regulates the activity of other genes in its pathway and also controls variable splicing of the genes’ RNA blueprints to make different forms of the protein.

### TROUBLE AT THE JUNCTION

Zoghbi’s group later described *MECP2* mutations in a handful of girls and boys with autistic features who did not have Rett syndrome. Although *MECP2* mutations don’t appear to be very common in “pure” autism, findings by Zoghbi and others have shown that they can occur. These discoveries point a suspicious finger at synapses.

In 2007, Zoghbi and colleagues reported in *Neuron* that MeCP2 regulates the formation of synapses connecting neurons that secrete glutamate—an “excitatory” chemical messenger that causes neurons to fire. Glutamate is like a green light that encourages nerve signals to jump across the synapse; its opposite “red light” neurotransmitter is GABA, an inhibitor that quickly halts nerve firing when appropriate.



The relevance of the synapse is becoming clear from Huda Zoghbi's findings in Rett syndrome and Thomas Südhof's mouse model of autism.

A proper balance of excitatory and inhibitory events is key for learning, memory, and other information-processing tasks. If there's a generalized excitatory-inhibitory imbalance, it might well explain why the autistic brain falters in trying to build networks for learning, language, and social awareness. In 2003, Zoghbi proposed that changes in the function of synapses may be a fundamental cause of neurological disorders—including autism.

That same year, another set of mutations in autism came to light, causing excitement because they, too, pointed to components of the synapse-making machinery. In the 1990s, Thomas Südhof, an HHMI investigator at University of Texas Southwestern Medical Center at Dallas, identified genes for two key families of proteins involved in creating the brain's synaptic nerve connections.

The two gene-protein families, called neurexins and neuroligins, are located on opposite sides of the "cleft" or tiny space where the neurons meet at a synapse. These two protein complexes extend out of the nerve cells and physically bridge the synaptic divide, but they also affect the excitatory-inhibitory balance of nerve signal traffic.

Thomas Bourgeron at the Pasteur Institute in Paris first reported mutations affecting these proteins in autistic patients

in 2003. He found that two pairs of Swedish brothers with autism disorders had mutations in the neuroligin proteins that Südhof had identified seven years earlier.

More recently, a larger international study revealed a gene for one of the neurexins in a chromosomal region linked to autism. Bourgeron has also found mutations in another gene expressed in synapses, *Shank3*, which interacts with neuroligins. Other scientists have uncovered evidence that links the gene with autism.

"I suspect that *Shank3* is one of at least a dozen genes that have rare variants in them which likely are causative for the disease," comments Louis Kunkel, an HHMI investigator at Children's Hospital Boston, who is hunting for autism genes. "These will all likely be important in neuronal maturation and learning."

Another piece of the synaptic puzzle recently emerged from the lab of HHMI investigator Li-Huei Tsai at the Picower Institute for Learning and Memory at MIT. Publishing in *Neuron* in 2007, she reported that a protein, *Cdk5*, modifies another protein called *CASK* and promotes the interaction of *CASK* with neurexin proteins at newly forming synapses.

"This general process seems to be extremely relevant to autism," Tsai says, "because a lot of the proteins implicated

in autism spectrum disorders all seem to overlap in this particular area of synapse development."

A dramatic demonstration of how a single mutation can cause autistic symptoms came when Südhof created lab mice containing the *neuroligin-3* gene mutation previously found in humans. The mutation lowered the amount of neuroligin-3 protein in the animals' forebrains by 90 percent, with a surprising consequence for their behavior.

Compared with control mice, the gene-altered rodents were less social: they spent less time interacting with a new mouse placed in their cage. But they became smarter: they took fewer days to learn the location of a platform submerged in murky water, indicating enhanced spatial memory.

"This is incredibly exciting," Südhof says. "Usually when you impair mouse cognitive function, they're just stupid. These mice are not stupid—they have a huge positive change in learning along with a modest social deficit. This is the first genetic dissection of circuits that underlie these different effects."

The events leading from mutation to altered behavior aren't fully understood, Südhof says, but the results "validate the whole idea that autism is related to synapses."

#### CASTING A WIDE NET FOR GENES

Further progress in autism research means continuing the gene hunt on a broad front, deploying a variety of strategies. Many different kinds of genetic flaws appear to be involved—mutations, deletions, copy number variations (too many or too few copies of critical genes), large and small chromosome effects. Fortunately, new genomic tools such as single nucleotide polymorphism "chips" can spot increasingly small genetic flaws.

A genome search using these methods led to a January report by the Boston-based

Autism Consortium (a research collaboration involving 14 Boston-area institutions) of an abnormal chunk of chromosome 16 found in several autistic patients. The segment harbors a mixture of gene deletions and extra copies of genes that is estimated to account for 1 percent of unexplained cases of autism spectrum disorder. Several of the genes are known to be expressed in the brain or in early nervous system development. Researchers believe that a large number of rare “copy number variations” such as these may be waiting to be found.

Working with clinical genetics collaborators in several Middle Eastern countries where intermarriage between cousins and large family size are common, HHMI investigator Walsh, a member of the Autism Consortium, has been trying another gene-seeking strategy. He is attempting to find rare recessive gene mutations, which are more likely to be expressed when intermarriage occurs over generations. “My collaborators bring in their tough cases,” says Walsh, who has visited countries including Dubai, Kuwait, Saudi Arabia, and Oman. “Our team of genetic counselors, pediatric neurologists, clinical psychologists, and others provide a second opinion, draw blood for DNA samples, and say that we’ll try to figure out the genetic picture.”

Walsh’s strategy has begun to pay off. He has found several families in which a member with autism is missing both the paternally and maternally inherited copies of genes, making those genes likely suspects.

“We don’t yet know what the deletions do,” he says. But three of the missing genes turned out to be among those being studied by Michael Greenberg, a Children’s Hospital Boston colleague. Greenberg is looking for autism clues by studying nervous system genes that are silent until activated, during learning, to make more synapses and are logical candidates for autism research.

Also collaborating with Greenberg at Children’s Hospital Boston is Kunkel, who previously discovered the gene for Duchenne muscular dystrophy. He and Children’s Hospital colleague Isaac Kohane have devised an unorthodox gene-hunting approach—looking for gene “signatures” of autism in blood cells, rather than in the brain.

“We’re using microarrays to see if there is a signature of gene expression in whole blood that distinguishes autism, and we are starting to see such signatures,” says Kunkel. If gene expression in the blood cells is similar enough to that in brain cells to be a useful surrogate measure, researchers could avoid the need to obtain and test brain tissue, which is impossible in living humans. Ultimately, a “proxy” signature for autism could be used diagnostically and in testing candidate drug therapies.

With a variety of intensive behavioral therapies, the capabilities and lives of many autistic children, like Nicole Branconnier, have improved. There is no definitive treatment for the multifaceted, complex disorders of autism, and a cure seems a distant prospect.

However, the biological underpinnings of autism are becoming clearer. And among the recent findings, one in particular may bode well for reversing symptoms with future therapies.

In 2007, Adrian Bird, a geneticist at the University of Edinburgh, showed that Rett syndrome mice with a silenced *MECP2* gene could recover many of their lost functions when the gene was reactivated in adulthood. This couldn’t be attempted in humans with Bird’s experimental methods, but the implications are cause for optimism: apparently the *MECP2* mutations don’t irreparably harm the neurons themselves, which develop very early; rather, the mutations cause malfunctions in the synapses that form later on.

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—Maureen Branconnier

Bird’s finding offers another reason why the synapse makes sense as the culprit in autism, says Walsh. “It may explain the later age of diagnosis of autism, and its preferential effects on later-appearing skills—like language and social behavior—and perhaps its greater likelihood of improvement in some fortunate children,” speculates Walsh. “If we can develop medications to modulate these synaptic changes, we may be able to provide better therapies for this devastating disorder.”

Fischbach agrees: “The possibilities of reversal are very real.” That’s encouraging, and so is the quickening pace of gene discovery, which ultimately should shed further light on causative mechanisms. How long this will take, though, is anyone’s guess.

“The hunt is on,” says Fischbach, “but it’s going to be a while.” ■

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FOR MORE INFORMATION: To learn more about the latest approaches to autism, visit the Websites for the Simons Foundation (<http://www.simonsfoundation.org/>) and the Autism Consortium (<http://www.autismconsortium.org/>).

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