Autism's origins lie hidden in a perplexing maze of behaviors and biology. Step by step, researchers are finding their way inside.

Through

Ricardo Dolmetsch was studying the basic biology of nerve cells when two events propelled him into autism research. In 2004, a mutation in one of the proteins he specialized in was pinpointed as the cause of Timothy syndrome, a rare ▶

by Kristin Saxton

Photograph by Timothy Archibald

Eli Archibald, photographed by his father, from the book *Echolilia*.

genetic disorder associated with autism. Then in 2006, Dolmetsch's oldest son, who was 4, was diagnosed with autism.

"At the time, we were very worried. We didn't know what was happening," says Dolmetsch, PhD '97, assistant professor of neurobiology. "So I went home and sat down with my wife, [Asha Nigh, '93,] who is also a scientist, to try to figure out what we could do."

There was solid research in psychology and behavioral treatments for autistic patients. But except for some progress in genetics, the biology of autism was virtually unknown, Dolmetsch says. "It was a little bit shocking that there was very little in the way of really good basic research."

Dolmetsch was in a unique position to do something about this gap. "I decided to shift the focus of my lab completely."

He is among a group of researchers at Stanford who are trying to untangle the root causes of autism. Some have been in the field for decades; others, like Dolmetsch, are newcomers, spurred by personal and professional motivations to understand a disorder that affects 1 in every 110 kids. (The rate is 1 in 70 for boys and 1 in 315 for girls.)

Autism is a spectrum of disorders that share three core features—language deficits, social deficits, and repetitive interests and movements. On one end of the spectrum are people with Asperger's syndrome, who are socially awkward but who often have above-average intelligence. Dolmetsch's son has this diagnosis: He was late to speak and when he did speak, he talked in a stilted and peculiar way—more like a little adult than a child; and he has problems socializing. The most serious autism cases involve severe mental disabilities (about 40 percent of children with autism have IQs below 70) and behavioral problems. Between these two extremes, children may have normal intelligence but pronounced language and social impairments.

J.C. Flores, '87, has 15-year-old identical twins with autism. Lomasi can speak, but she avoids talking and gives mostly one-word answers. Marielle can say only a few words and has no functional communication system. "They're sweet and they like to hang around people," Flores says. "But they don't really feel the need to communicate. They don't see why you have to."

In about 10 percent of cases, autism is secondary to a more pervasive genetic disorder, such as fragile-X syndrome or another chromosomal abnormality. But the vast majority of cases are unexplained, or idiopathic. Awareness about autism is at an all-time high, but public discussion has largely been dominated by questions such as: What accounts for the rapid increase in cases? Or is something in the environment causing autism? Though these questions are important (see sidebar), Dolmetsch and others at Stanford and elsewhere are trying to answer a more fundamental question: What is the underlying biology of autism? It's only by answering this question that they believe they can achieve real breakthroughs—the kinds that lead to cures.

A GENETIC PUZZLE: Flores's identical twin daughters both have autism but have different capabilities.

COURTESY JC FLORES

Autism is a CATCHALL DIAGNOSIS that likely includes a host of DISTINCT DISORDERS.

One of the biggest challenges is that autism is not one thing: It's a catchall diagnosis that likely includes a host of biologically distinct disorders. Though children with autism share a set of symptoms, these symptoms are quite varied and may have many, diverse biological origins. "Autism is incredibly heterogeneous. We've been lumping everyone together under this name autism, and unfortunately it makes it very difficult to study the biological features when we are treating multiple groups as one," says Sophia Colamarino, '90, vice president of research for Autism Speaks—a science and advocacy group—

and consulting associate professor of psychiatry and behavioral science. To get a toehold into the biology, researchers will need to identify unique subgroups of autism, she says. Researchers across the globe are defining subgroups based on genes, molecular pathways, or signatures in the brain and blood.

Another impediment: access to the brain. Scientists can slice cancer cells out of a tumor and study them directly, but they can't just scoop cells out of the brain. Stanford is on the forefront of solving this problem—in fact, Dolmetsch's solution seems straight out of a science fiction novel.

Although much remains unknown, these efforts are giving the world its first glimpses into the biology of autism.

JOACHIM HALLMAYER describes himself as a "grandfather in the field of autism." He's been chasing down the genes for the disorder for 20 years, beginning as a postdoctoral fellow in genetics at Stanford. At that time, the disease was considered rare (4 out of 10,000 kids). "I knew in a snap everybody in the Bay Area who ever diagnosed a child with autism," says Hallmayer, associate professor of psychiatry and behavioral science.

Hallmayer joined the field because of a parent: Carmen Pingree, the mother of an autistic boy in Utah, who approached his mentors at Stanford, urging them to study the disease. Hallmayer collaborated with Pingree, who used her ties in the Mormon community to assemble pedigrees for 146 families with multiple cases of autism. It was the largest genetic study of the disease at the time. The parents of children with autism continue to motivate him, he says.

Autism is particularly heartbreaking because it colors the most fundamental social bond—that between a parent and a child. Even children with milder forms of autism may have trouble displaying or returning affection. "Autistic children are as emotional as other children; I'm very convinced of this. But they don't show it," Hallmayer says. "They show it in a different way. And it's a very, very hard learning process for most parents."

Studies of twins have shown that autism has a large genetic component, but it's not all genes. Identical twins have the same DNA, but sometimes only one twin gets autism. In other cases, as with Flores's daughters, Lomasi and Marielle, the severity of the disorder differs. The genetics are also complex. Different genes may be involved in different people; and, in

Why the Increase? (No, it's not vaccines.)

Twenty years ago, people diagnosed with autism were relatively rare, and the public's awareness of the disorder was chiefly limited to Hollywood depictions such as that in the 1988 Academy Award-winning movie *Rain Man*. Today, many people know a neighbor, friend or classmate who is dealing with autism—prevalence has risen about twenty-fold since 1990. But what's behind this startling increase? Scientists agree that some of it is due to changes in diagnosis and awareness, but just how much is hotly debated.

"The best papers I've seen suggest that there is a genuine increase," Ricardo Dolmetsch says. "But it's not as big as it seems. A big, big contribution is societal awareness and changes in diagnostic criteria."

There have been at least two shifts in diagnosis, Dolmetsch says. First, some kids who might have once been diagnosed as mentally retarded now are recognized as autistic. Second, children with autism display some of the same characteristics as those who have attention-deficit hyperactivity disorder (ADHD). When the diagnosis is ambiguous, doctors in California tend to diagnose autism rather than ADHD, because the state pays for services (such as behavioral therapy) for autism but not ADHD, Dolmetsch says.

Moreover, the medical establishment's thinking about what constitutes abnormal behavior has shifted, Joachim Hallmayer says. "There's no doubt that what we diagnose as autism is different than what we diagnosed 20 years ago." Autism has been on the rise globally, but the explosion of cases occurs at different times in different countries, which points to a large role for societal awareness, Hallmayer says.

Some of the increase is definitely real, though, according to Antonio Hardan. He cites two contributors. More premature babies are surviving, and preemies are at high risk of autism (about 10 percent will develop it), and more couples are having babies when they're older, which increases the risk of genetic errors that can lead to autism.

Could something in the environment also be a factor? Maybe, the researchers say, but there is little scientific evidence to back any such claim. Despite widespread concerns about a link between pediatric vaccinations and autism, vaccines have been soundly exonerated, they say.

Paula Gani, '91, says her 4-year-old son Marcus, who has a milder form of autism, "is one of those kids who would not have been diagnosed 20 years ago." But the diagnosis and early intervention he's received are likely rewriting his future. He made limited eye contact and didn't engage socially when he was diagnosed two years ago, but "yesterday, we were going out to pizza with another mom and her kid. And he took the other little boy's hand and started singing 'The more we get together, together, together . . . '," she recounts. "It's a huge difference."

Dolmetsch is using an astonishing NEW TISSUE MODEL. "We're making little pieces of BRAIN IN A DISH."

any given individual, the disease may arise from changes in one gene, changes in several genes, or a combination of genetic and environmental factors. (Though no specific environmental triggers have been identified, researchers are testing everything from exposure to heavy metals and pesticides to TV watching.) Tackling this complexity requires large studies.

Hallmayer has spearheaded several massive, multisite collaborations. He chairs the committee of senior investigators for the Autism Genome Project, an international consortium of more than 50 universities that has collected data on 2,000 families with multiple cases. He also has assembled a study of 220 pairs of twins from California in which at least one twin has autism.

In the past decade, geneticists have discovered a handful of genes that when mutated or missing can cause autism. Though rare, these genes have given scientists some of their best clues about the disease's biological underpinnings. "I think it's very exciting, even if it's rare cases. We can at least get a better understanding of one piece of the puzzle and then we can branch out from there," Hallmayer says. For example, several of these genes are involved in communication between neurons. In a 2010 paper in *Nature*, Hallmayer and his colleagues from the Autism Genome Project greatly expanded this list of genes—reporting hundreds of rare genetic events that may be involved in autism.

It's long been known that about 5 percent of autistic kids have a chromosomal abnormality that can be seen under a microscope—part of a chromosome is missing, duplicated or in the wrong place. Because these changes affect a large number of genes, the children often have many problems in addition to autism. What wasn't known until recently is that we all have slight imperfections in our chromosomes—small regions of DNA that are duplicated or deleted. When these stretches of DNA contain genes, people can end up with one or three copies of the genes instead of the standard two. Technological advances have made it possible to detect these "copy-number variants," or CNVs. And it turns out they're important in autism and some psychiatric disorders. For example, a region of chromosome 16—containing about 25 genes, some involved in brain function and development—is deleted or duplicated in 1 to 2 percent of people with autism (and some with schizophrenia). Scientists are beginning to study these patients as an autism subgroup.

Hallmayer and his colleagues scanned the genomes of thousands of people with autism and 2,000 healthy individuals looking for rare CNVs. They found that children with autism had more rare CNVs that overlapped genes, including genes previously implicated in autism. Some CNVs were inherited from a parent, but some arose spontaneously in the child, likely due to a genetic error in the sperm or egg. They identified disruptions in hundreds of genes that occurred only in autism cases, never in controls. Not all of the genes will turn out to be relevant to autism, but the ones that are could explain maybe 10 to 15 percent of cases, Hallmayer says.

DOLMETSCH IS STUDYING several subtypes of autism defined by genes, including Timothy syndrome and the chromosome 16 CNV, using an astonishing new tissue model. Essentially, he says, "We're making little pieces of brain in a dish."

In 2007, scientists in Japan figured out how to genetically reprogram adult cells into pluripotent stem cells—which, like embryonic stem cells, can give rise to any other cell type in the body. Using this technology, Dolmetsch's team recreates human neural development. They take skin cells from patients with autism, turn them into stem cells, and then, using a cocktail of proteins, coax these cells to form a neural tube. A neural tube is the earliest neural structure to form and later grows into the brain at one end and the spinal cord on the other. The researchers then take slices of cells from the neural tube and direct them to become specific parts of the brain, such as the cortex. "It sounds like science fiction. It's kind of incredible this even works," Dolmetsch says. "Nobody had ever actually made neurons from autistic people before."

Timothy syndrome is caused by a defect in calcium channels, which play a critical role in electrical activity in the brain and heart. Because Timothy patients often have life-threatening heart arrhythmias, Dolmetsch's team is also making "little pieces of heart."

Sitting in his office on the second floor of the Fairchild Research Building, Dolmetsch shows movies of the hearts on his computer. The ball of cells looks like one chamber of a fetal heart beating on an ultrasound. Those laboratory hearts—

FROM TOP: PHOTOS COURTESY STANFORD SCHOOL OF MEDICINE (4); JENNY PEGG

developed from the cells of a healthy subject—beat just like the real thing, 60 times per minute. When grown from the cells of Timothy patients, however, the hearts beat erratically—they miss a beat, or have a double beat. Dolmetsch's team has already used the models in a practical way: They screened 20 different drugs that correct heart arrhythmias and found the one that works best for Timothy patients. "You can't give a child 50 drugs and see which one works, but you can do that with these cells," Dolmetsch says.

He also shows movies of thousands of lab-created neurons firing. When given an electric pulse, the blue-colored cells light up in a sudden flash of green, like fireworks going off, and then they flicker and shimmer in a fallout of electrical activity. He is studying these neurons to figure out what is different in the autistic brain, starting with Timothy patients. The results so far: In addition to abnormal calcium signaling, kids with Timothy syndrome have too many cells that produce the neurotransmitters dopamine and norepinephrine. They also have too few cells that form long-distance connections and too many that form local connections.

"This may help to explain why children with autism often have problems integrating lots of different classes of information, but they can often perform quite well in one domain," Dolmetsch says. (In other words, the wiring that impairs, say, the ability to discern symbolism in Shakespeare, might enable an extraordinary ability to memorize a scene from *Hamlet*.) "This is the first time that anybody has ever seen any cellular defects associated with any psychiatric disease, much less autism." A group from UC-San Diego also has adopted this technology—they recently reported specific defects in neurons created from patients with Rett syndrome, another genetic anomaly that can cause autism.

Dolmetsch's team has grown brain cells from 20 patients with Timothy syndrome or other known mutations. Their ultimate goal, however, is to grow brain cells from patients with autism who have no known genetic defect and then to classify these patients according to their cellular and molecular defects—such as problems with neural communication or problems with calcium channels.

Dolmetsch plans to use these models to screen for treatments that can reverse or overcome the biological defects. He also could use them to screen potential environmental contributors to autism. Some environmental agents may injure developing neurons in ways that mimic known genetic hits, he says.

LIKE DOLMETSCH, Thomas Südhof was a basic biologist working on fundamental questions about the brain when he was drawn into autism research a few years ago. Südhof is an expert in synapses, the junctions between neurons. He played an instrumental role in working out the molecular details of how messages travel across synapses—work for which he shared the $1 million Kavli Prize in neuroscience in 2010. Serendipity brought Südhof, professor of molecular and cellular physiology, to autism research. A number of autism cases were traced to rare mutations in synapse pro-

Solution Set?

RICARDO DOLMETSCH
Uses human tissue to simulate a developing autistic brain in lab-grown models.

JOACHIM HALLMAYER
Helps oversee the Autism Genome Project, collecting data on thousands of families.

THOMAS SÜDHOF
Rare mutations in brain proteins offer insights about autism's effects on synapses.

ANTONIO HARDAN
MRIs of children with autism show differences in neural connections.

KAREN PARKER
Defects in the hormone oxytocin may play a role in social deficiencies.

FROM TOP: PHOTOS COURTESY STANFORD SCHOOL OF MEDICINE (4); JENNY PEGG

teins, including two that Südhof discovered in the 1990s—neurexins and neuroligins.

These proteins bind to each other across the synapse to help neurons connect and communicate; they also are believed to play a role in "synaptic plasticity"—changes in the ability of the synapse's chemistry and structure that underlie learning and memory. What's interesting about autism, Südhof says, is that the brain is not globally impaired—for example, kids who are unable to speak have normal motor skills. "You really need a pretty good brain for that," he says. Thus, autism is likely due to subtle changes in the brain's wiring, such as how neurons connect physically, or how they communicate.

In a 2007 paper in *Science*, Südhof and his colleagues reported the first mouse model of idiopathic autism. They took a mutated form of the neuroligin gene that was discovered in two brothers, one with autism and the other Asperger's, and inserted it into mice. The mice showed some features of both autism and Asperger's. (They were less inclined to hang out with other mice, but faster at learning a water maze.) In 2009, Südhof engineered mice to have a neurexin deletion that, in humans, is associated with 1 in 200 autism cases. The mice had increased repetitive behaviors, such as excessive self-grooming, but no obvious changes in social behavior. The neurons of both sets of mice had altered synaptic signaling.

The mice aren't a perfect replica of human autism (animal models are inherently limited—after all, mice don't have language), but they have yielded important insights. Südhof's team has begun testing many other genes that have been implicated in autism in mouse models to see if they also affect synapse function. "One thing is clear: Anything in the brain involves the synapse, so the synapse is a good bet," he says.

Once you pinpoint molecular pathways that underlie autism, such as problems with calcium channels or problems with synapses, then you can develop targeted treatments. Recent advances in fragile-X syndrome are a perfect example, Colamarino says. Patients with fragile-X (about one-third of whom also have autism) are missing a protein that helps regulate synaptic function. Without this protein, there is an excess of synaptic signaling, which scientists believe could lead to intellectual disabilities. Several companies have begun testing drugs that dampen the noise. In mice with fragile-X, the drugs can reverse some neurological symptoms. It's too early to say if the drugs can reverse mental disabilities in people with fragile-X, but they have eased behavioral symptoms in short-term trials of affected adults.

No one thought it was possible to reverse symptoms of a neurodevelopmental disorder in adulthood, Colamarino says. "The idea that you can do this has completely reinvigorated the field of autism treatment research."

AT 10 P.M. on a Friday night at the Lucas Center, postdoctoral fellow Kari Berquist, doctoral student Grace Lee and senior research assistant Sweta Patnaik are waiting in a darkened MRI control room. The silhouettes on the other side of the glass window finally appear motionless. There's a moment of breathless anticipation as the father sneaks away from his sleeping son on the MRI table. Then 4-year-old Joshua (patients' names have been changed) wakes up again. They won't be able to get the scan done tonight. The father emerges from the MRI room; Joshua, who cannot yet speak, cries, kicks and clings to his dad, making *bbb* and *mmm* sounds. Joshua's mother buckles her other son—a healthy 11-month-old who already says

The 'cuddle hormone' may yield important clues

Oxytocin is the hormone that promotes social bonding, so it makes sense that defects in oxytocin might play a role in some cases of autism. Karen Parker is exploring whether changes in oxytocin levels in the blood or cerebrospinal fluid could be a marker of the disorder. "My work is thinking about the biology of social functioning," says Parker, an assistant professor of psychiatry and behavioral sciences. Oxytocin is sometimes called the cuddle hormone or the love hormone, because it's released during physical contact and sex; it also plays a role in social interaction, trust and empathy, as well as monogamy in certain animals. A behavioral neuroscientist, Parker studied the role of oxytocin in voles and monkeys before moving to autism research.

Parker has collected blood from children with autism, their healthy siblings (who sometimes show more subtle social deficits) and normal controls to try to detect differences in their oxytocin levels. She also is setting up one of the first studies in the cerebrospinal fluid (CSF). Studies in monkeys show that blood oxytocin may be normal even when brain levels, reflected in the CSF, are low. It's difficult to obtain CSF because it requires a spinal tap. Her team is investigating genetic variation in the oxytocin receptor and other genes involved in the oxytocin pathway. If Parker's and Hardan's studies show an oxytocin deficiency, patients could receive replacement oxytocin.

It's promising research because low oxytocin is treatable, Parker says. In a recent study, when 13 autistic adults were given an oxytocin nasal spray (which delivers the drug straight to the brain), they had significant improvements in reading social cues and in making eye contact. "I think it's actually fascinating that you can apply oxytocin once and see these pretty acute social changes," Parker says. "Oxytocin is the only drug that's been shown to alter social functioning. There's nothing else that touches the social deficits."

The wiring that impairs, say, the ability to DISCERN SYMBOLISM in Shakespeare, might enable an extraordinary ability to MEMORIZE A SCENE FROM *HAMLET*

"bubble" and "mama"—into his car seat for the ride home.

MRI machines are noisy and require the patient to lie perfectly still for several minutes at a time, so most children—especially those with autism—cannot tolerate them while awake. So the idea is to bring them in already sleeping. However, the next study participant also arrives awake. But 4-year-old Padma, who has autism but is high-functioning, surprises everyone by agreeing to be scanned, enticed by the promise that she will get pictures of her brain. It's a partial success: The team gets one usable scan before they finally give up at 11:30 p.m. Padma proudly tells the researchers that she is going to post her brain pictures on Facebook.

Antonio Hardan is studying these brain pictures looking for signatures of autism and of subgroups of autism. Unlike others on campus who are working from the genes up, Hardan is working backward from the brain to the underlying biology. Hardan, who sees patients in addition to conducting research, has been involved with autism since he began volunteering to work with autistic patients in medical school. "And 27 years later, that's my life in a way, my research life," says Hardan, associate professor of psychiatry and behavioral science. "When you connect with something emotionally, then that's what you want to do."

It's long been known that children with autism have larger-than-normal brains early in their development. Hardan has refined this observation, showing that some (but not all) children with autism have an increase in the thickness of the cortex—the part of the brain responsible for complex functions such as language and social behavior—that disappears as they grow. It might be possible to link this back to genetics, says Hardan, who frequently collaborates with Hallmayer. We know some of the genes that contribute to cortical thickness, he says.

Hardan is exploring new brain imaging technologies that offer an unprecedented level of detail. For example, diffusion tensor imaging (DTI) shows the individual axons (the elongated parts of neurons) that connect different parts of the brain. And MRI spectroscopy measures the levels of specific chemicals in various parts of the brain. DTI studies from several universities suggest that autistic children have abnormal long-distance brain connections, an observation that dovetails with Dolmetsch's studies in neurons. Using MRI spectroscopy, Hardan also has detected specific chemical imbalances in the brains of children with autism. He's looking for treatments that can normalize these imbalances.

Hardan's team is involved in about 15 different clinical studies. Whenever possible, he tries to link treatment responses to changes in brain images and to a subgroup of people with autism. For example, Joshua and Padma were being re-scanned after they'd received a behavioral intervention that can improve language. These images may give clues as to how the treatment is working in the brain and what subgroups of patients are likely to respond. Hardan also is collaborating with Karen Parker, who is looking for biomarkers of autism in the blood and cerebraospinal fluid. (See sidebar, page 52.)

The commitment of parents keeps him going, Hardan says. "It's amazing what they would do for their kids. My admiration of these parents fuels what I do."

WHILE AUTISM remains largely a mystery, scientists are making inroads, bit by bit.

As a parent, Dolmetsch says he's been very fortunate. When faced with his son's diagnosis, there was something concrete he could do. Thanks in part to behavioral therapy, his son, now 8, is doing well. "He's at the edge between character and dysfunction. And some days it's very dysfunctional; other days it's just character."

Having a child with autism has changed the way he thinks about science. "You always want to publish findings that are correct, but the bar is higher when your own child is involved, he says. "You don't want to do anything to mislead the field when they are trying to find a cure for your kid."

He also cares less about doing things first. "I just want somebody to do it," he says. "I just want somebody to come up with some plausible biological explanations, so we can convince people that it's worth developing treatments." ■

KRISTIN SAINANI, MS '94, PhD '02, *is a freelance writer and a clinical assistant professor in the department of health research and policy.*