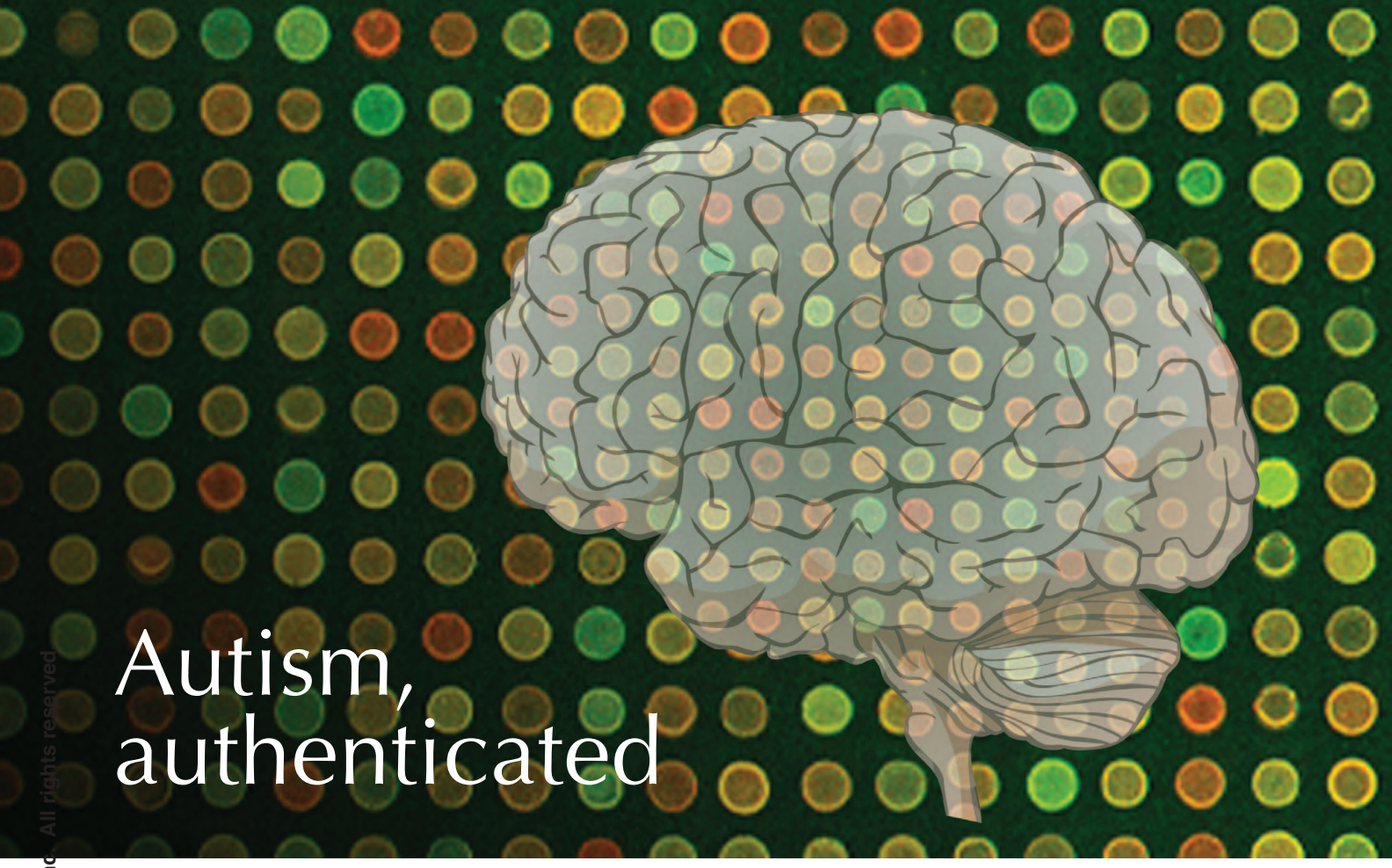

# Autism, authenticated



A burst of research into the genetics of autism has given scientists insight into the basis for the disorder. Now, some companies aim to capitalize on these findings by developing DNA screens that might one day provide a diagnosis at birth. **Hannah Waters** examines the genetic tests and explores what parents—and their autistic children—have to gain.

This past spring, Christian Schaaf sat back and watched seven-year-old Lily play in his office at the Baylor College of Medicine in Houston. She looked just like any other girl her age, he recalls, but she didn't seek interaction or even eye contact in the way a child normally would. Instead, she communed with a corner of the room, excitably hopping and flapping her arms as if that spot held a treat too great to bear. Without peering into the file in front of him, Schaaf knew what afflicted Lily. "I've seen enough children that when I see someone with autism, I have a high suspicion for it," he says.

Lily (not her real name) and her mother didn't come to Schaaf's office that day for a diagnosis; a psychiatrist had already detected autism after her fourth birthday. They visited Schaaf, a clinical geneticist, to search her genome using a chromosomal microarray. The technology can find duplications or deletions of small segments of DNA, known as copy-number variants (CNVs), to pinpoint the genetic aberration that might have caused the disorder. Lily's parents hoped that a genetic diagnosis would help them better understand and treat her specific form of autism—and, ultimately, help her get the services she needs to have the best chance at adult independence.

Such genetic tests for autism have only become available in the last few years. But, owing to high demand, autism testing has expanded from research centers to private companies. In the US, six companies now offer laboratory-developed tests to doctors that specifically target the developmental disorder, searching the genome for either irregular CNVs or single-nucleotide polymorphisms (SNPs) that could explain the symptoms. And these tests aren't cheap: a microarray costs, on average, $1,500, and that's without the bells and whistles such as doctor visits and additional gene sequencing. Although the tests themselves aren't therapeutic, they represent the leading edge of a deeper genetic understanding of autism that could lead to targeted therapies—a market that UK-based research publisher Global Data expects to top $5 billion in the US in 2018, according to an October report.

For the most part, the diagnosis of autism remains the domain of psychiatrists, who do so on the basis of a range of symptoms, including delayed speech, repetitive behaviors and social withdrawal. These abnormalities remain difficult to detect until a child is around four years of age or older, which is unfortunate because receiving therapy from age two can improve outcomes for youngsters with developmental disabilities. "The earlier the diagnosis, the earlier you can start some type of interventional therapy," says Stephen Scherer, director of the Centre for Applied Genomics at Toronto's Hospital for Sick Children.

The diagnostic holy grail is a molecular test that can pinpoint the disorder at birth to hook children into therapies straightaway. Just a few decades ago, this suggestion would have sounded ludicrous. From the 1950s through the 1970s, doctors thought autism resulted from poor parenting and social conditioning by 'refrigerator mothers', so called for parents supposedly being cold with their kids. "Now there has been a paradigm shift," says Schaaf. "We think that 80–90% of what causes autism is really the genetics.

But pinning down the genetic cause of autism has been difficult. Known mutations comprise fewer than 20% of all cases of

autism spectrum disorder (ASD), a diagnosis that encompasses many disabilities that superficially look the same but have a multitude of different causes.

When Scherer was first developing his research program in the late 1990s, he noticed several patients with autism with seemingly unique genetic anomalies—"karyotypic abnormalities, break points in genes and things"—which prompted him to do more in-depth genetic screening. To better scrutinize the mutations, a decade ago he started running chromosomal microarrays on blood samples taken from children with autism, probing the DNA for common and rare variants. Rather than seeing sweeping patterns, he found a number of CNVs, each linked to one or two cases of autism.

Lily's microarray revealed one of these rare variants: a deletion in the 16p11.2 region on the short arm of chromosome 16 found in less than 1% of individuals with ASD. But this mutation alone isn't the final word on an autistic diagnosis. Some people with the 16p11.2 deletion or duplication have no or few autistic symptoms. And many rare variants associated with autism present schizophrenic, epileptic or bipolar symptoms instead, says Peter Szatmari, vice-chair of psychiatric and behavioral neuroscience research at McMaster University in Hamilton, Ontario. "There's a degree of complexity that none of us dreamed about when we walked into this 10, 15, 20 years ago," he says.

## An array of possibilities

A microarray screening for CNVs pinpointed the probable genetic basis for Lily's autism, but few patients are so lucky. Despite extensive data collection and genomic screening, these microarrays can only identify an associated genetic abnormality in 8–25% of known clinical cases of autism, depending on whom you ask. "We hoped when the microarray came around that we'd find something in maybe 50% of the kids tested," says Judith Miles, a medical geneticist at the University of Missouri Children's Hospital in Columbia. "But we're still left with 75% of kids who still have an unknown cause."

Despite the relatively low diagnostic yield, CNV-probing chromosomal microarrays are the recommended genetic test for autism by the American Society of Human Genetics and the Boston-based Autism Consortium (*Am. J. Hum. Genet.* **86**, 749–764, 2010; *Pediatrics* **125**, e727–e735, 2010). Thus, the real challenge presented to laboratories is to build the best microarray. A microarray can probe for tens of thousands of CNVs at a time, so the first step is careful selection of what regions are screened.

The microarrays available to physicians thus far are lab-developed tests, which are run by certified facilities at private companies or research centers that analyze doctor-submitted blood samples from patients. The US Food and Drug Administration currently does not require approval of lab-developed tests, since companies do not sell them directly to consumers.

Laboratories at research hospitals have designed their own microarrays based on autism-associated variants recently published in the literature, but getting an appointment at one of these big centers can take more than a year. Some private companies that already offered genetic screening are taking advantage of this demand and have redesigned microarrays specifically for autism and made them available to doctors. GeneDx, a genetic diagnostic company for rare diseases based in Gaithersburg, Maryland, has offered AutismDx since 2008, which bundles its standard microarray with sequencing of symptom-specific variants such as the macrocephaly-associated genes *MECP2*, *PTEN* and *CDKL5*. The microarray alone costs more than $1,500, and additional sequencing will add $500 to $5,800 onto that price tag depending on the gene.

Meanwhile, Signature Genomics, based in Spokane, Washington, was one of the first to handpick autism-specific genes from published papers for its microarray, first offered in 2004. The microarray now targets more than 150 autism-related genetic regions and costs $1,650. And, at the start of 2011, Salt Lake City, Utah–based Lineagen launched FirstStepDx, a $4,300 microarray packaged with sequencing of *FMR1*, the cause of the common ASD fragile X syndrome.

Population Diagnostics, based in Long Island, New York, is casting an even wider net to understand human genetic variation. Before finalizing its microarray test for autism-specific rare variants, their researchers performed microarrays on normal people to get a sense of normal human genetic diversity. "Once you have a true understanding of apparently healthy people, you can then go to a disease cohort as we've done with autism and do a highly confident comparison," says president and co-founder Jim Chinitz. He founded the company in 2006 and, even after five years of development, wouldn't speculate as to a release date, but he noted that he's "hoping that we'll be reaching milestones within the next 9–12 months." However, he did concede that, when the microarray is released, he plans to market it as a newborn diagnostic.

A genetic test that could accurately diagnose autism at birth would be truly novel. None of the microarrays currently in use in the US are actually marketed to the public as diagnostic tools. These laboratories only take referrals from doctors, like Schaaf, who rarely order the test unless the child already has a psychiatric diagnosis of autism.

IntegraGen

**SNP off the old block:** An IntegraGen scientist loads patient DNA into a genotyping chip.

**Laboratory-developed tests for autism by private companies**

| Company | Location | Tests offered |
|---|---|---|
| GeneDx | Gaithersburg, Maryland | CNV microarray; selective gene sequencing |
| Athena Diagnostics | Worcester, Massachusetts | CNV microarray; selective gene sequencing |
| Ambry Genetics | Aliso Viejo, California | CNV microarray; selective gene sequencing |
| Lineagen | Salt Lake City, Utah | CNV microarray; selective gene sequencing |
| Signature Genomics | Spokane, Washington | CNV microarray |
| Combimatrix | Irvine, California | CNV microarray |
| Population Diagnostics* | Long Island, New York | CNV screening |
| IntegraGen* | Cambridge, Massachusetts | SNP panel |

*test under development


Christian Schaaf

**Gene interpreter:** Baylor's Christian Schaaf.

The hefty price tag for genetic screening is one source of resistance among doctors. Insurance coverage varies, and, overall, insurance companies have not yet embraced genetic testing generally, much less for autism. "Insurance companies don't want to pay for the microarrays, and there's no rationale," says Miles. For Antonio Hardan, a psychiatrist and neurobiologist at Stanford University School of Medicine in California, the high price prevents him from ordering the tests. "If the test is easy, readily available and cheap, probably I would do it," he says. But he knows that not all families can afford to drop the thousands of dollars to confirm a diagnosis without even the prospect of benefitting from mutation-specific therapeutics. "When you play on the fear factor, parents might pay," Hardan says, "but you have to have value from that."

Currently, however, the benefit of knowing the genetic basis—especially if it can't be determined until after a psychiatric diagnosis—isn't clinically well founded. Lily's parents, knowing her genetic abnormality, can find other parents raising children with the 16p11.2 deletion to receive guidance on what her development may be like and what services she'll need. Researchers hope that, eventually, there may be drugs or behavioral therapies developed to treat the distinct genetic flavors of autism. But they aren't available yet, and many experts forewarn overselling the benefits of microarrays, which cost a few thousand dollars a pop.

"The caution about these tests should be that, because of the state of knowledge at this point, they provide limited value to most of the families," says Andy Shih, vice president of scientific research at the New York-based fundraising and advocacy organization Autism Speaks.

## It's all in the family

Most experts agree that the greatest currently applicable benefit of genetic testing is family planning. Most of the time, mutations associated with autism are formed *de novo* in the autistic person. But if the variant is inherited from a parent, future siblings are at an increased risk: a recent study found that 18% of children with a sibling with ASD also had the disorder (*Pediatrics* doi:10.1542/peds.2010-2825, 2011). "Particularly if there's an older child with the disorder, you might do genetic testing at birth," says Szatmari. "And you might follow that younger child more carefully, especially if that child has a significant variant."

One company is banking on this sibling re-occurrence. In 2010, the French biotech company IntegraGen, which currently offers sequencing services to researchers, opened an office in Cambridge, Massachusetts dedicated to the development of a genetic risk assessment test targeted at children whose older sibling has autism. Their approach goes against the grain: instead of running microarrays to screen for rare CNVs, they identify SNPs shared by people with autism to assess susceptibility for the disorder. "The idea of SNPs being a risk prediction thing is not new, but it's new to autism," says vice president of US operations Larry Yost. "If you talk to people who are involved with [SNPs and CNVs], there is a potential role for both."

"The idea of SNPs being a risk prediction thing is not new, but it's new to autism."

IntegraGen researchers have currently identified 31 SNPs, split into two separate tests for boys and girls, that increase the risk of autism in a sibling, and on 19 September they presented their results at the Society for Developmental and Behavioral Pediatrics meeting in San Antonio, Texas. In a retrospective screen of autistic and nonautistic genomes from a database, half of the males and just under 25% of females that their test diagnosed did, in fact, have the disorder. Once they finalize the panel of genes, IntegraGen plans to make the test available to US doctors in early 2012. And to further validate the test, the researchers plan to perform larger prospective clinical studies in infants who show early signs of autism at an age before a psychiatric diagnosis is possible.

With any of these tests, there will always be misdiagnoses: some children will come up positive for autism-associated genetic variations that don't develop ASD, and some will come up negative because of the limits of science. "Even at the end of the day, the complete genome sequences of 10,000 autistic diagnoses still won't be able to predict definitively what the detailed clinical outcome will or won't be," says Scherer. For that reason, experts emphasize the necessary role of clinical geneticists, like Schaaf, to help interpret the data and make it clear that genes aren't the be-all and end-all. And the companies are catching on: both IntegraGen and Lineagen have clinical geneticists on staff to help customers, and researchers, better understand how to put the data in context.

Their role is especially crucial because the results can have implications beyond the child tested. After Lily's test identified a genetic variant, Schaaf ran microarrays on her parents to evaluate whether their future children could have the disorder. He gathered Lily's entire family in his office to divulge the results: Lily's father also had the 16p11.2 deletion, though he does not have autism. The words had hardly left Schaaf's mouth when Lily's paternal grandmother broke down crying. There was always something abnormal about him, she told Schaaf, and people in their small town blamed her. "She said that it was such a relief for her to hear that it's nothing that she really did to him," he recalled.

For many parents, this is the real value of the test: they just want to know why. There are many circulating theories about the cause of autism, and the refrigerator-mothers idea holds tight in many minds. "Testing can help facilitate information for the families of why the autism in that child came about, so that they understand that they may not have done anything wrong," says Scherer. That answer lifts their guilt—and they know definitively to invest in the years of extensive (and expensive) therapy to help their child.

"It changes the way parents deal with their child's condition," says Shih. "For many parents, it's not only clarifying, it empowers them."

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