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## AUTISM GENES: Cracking the Code

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How reimagining the past shapes our memories, emotions and future

# What If...

**Sonic Surgery**

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# What Really Causes Autism

## The mystery is largely solved: autism is primarily a genetic disorder but a complex one that is slowly yielding its secrets

Seven actors stand around a circle of swirling colors—blue, gold and white painted in the middle of the stage. Interspersed among them are twice as many children. Most of the younger players look withdrawn. Many appear disabled, intellectually or physically. One girl, about 12 years old, sits quietly in an electric wheelchair. The professional cast take turns, enticing their young charges into the center of the colorful "island," where they play simple games—practicing facial expressions and chanting words—all based on emotional scenes from Shakespeare's play *The Tempest*.

By Simon Makin

Every child engages, even those who initially seem unreachable. Many react with unquestionable joy. It is possibly one of the most moving things I've ever witnessed. But this experience isn't really for the audience. My ticket reads: "*The Tempest,* Suitable for 8–24-year-olds with autism," and the production showcases a novel therapy for autism spectrum disorder (ASD). The approach—pioneered by Kelly Hunter of the Royal Shakespeare Company and developed in conjunction with psychologists at Ohio State University—is unproved. But the idea behind it is compelling: core abilities involved in drama match up strikingly well with what is often described as the main triad of impairments in ASD: problems with social interaction, communication and imagination. In short, the actors are gifted in the very things that are deficient in the young participants and able to reach powerfully across the divide of disability.

The diversity of the children onstage is a telling reflection of just how complicated autism is behind the scenes. An official diagnosis calls for the trio of difficulties described above, along with repetitive behaviors—typically hand flapping, rocking or head banging—before the age of three. That said, ASD sufferers exhibit a wide range of both physical and neurological symptoms. High-functioning people with autism, including those with Asperger's syndrome (a diagnosis that was recently cut from the American Psychiatric Association's manual of disorders), have normal and sometimes high IQs and often show only mild to moderate social deficits. At the other end of the spectrum, children with profound autism are often intellectually disabled and so socially detached that they seem locked in a world of their own.

*Illustration by* **ALEX WILLIAMSON**



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In this special production of *The Tempest,* actors from the Royal Shakespeare Company showcase a novel therapy for autism.

Complicating the clinical picture, the condition often coincides with other diagnoses, such as anxiety disorders, attention-deficit/hyperactivity disorder (ADHD), depression and epilepsy. According to the latest estimates, ASD affects one in 68 children. For decades researchers have debated its cause—an argument that grew increasingly urgent in the past 25 years as diagnostic rates soared. But recent studies have pretty well settled the question: autism is primarily genetic in origin, although it does not follow a simple hereditary pattern. Thanks to advances in DNA sequencing and collaborative efforts to pool data sets from laboratories around the world, scientists have found scores of genes that appear to be strongly linked to the disorder and many more that may also play supporting roles.

These new discoveries are offering important clues about the biology underlying ASD, insights that could eventually lead to targeted drug therapy. There is also a dawning realization that neurodevelopmental disorders in general—from autism to Down syndrome—may result not just from abnormal brain development but also from ongoing dysfunctional processes. The promise in that revelation is tremendous: although early interventions will remain vital in helping afflicted children reach their greatest potential, the hope is that even in adults, some aspects of ASD may one day be treatable.

FAST FACTS

**FIGURING THE ODDS**

1. Thanks to recent advances in DNA-sequencing techniques and large-scale collaborations among laboratories worldwide, scientists have now identified scores of genes strongly linked to autism spectrum disorder (ASD).
2. Identifying specific causes is difficult because an individual's risk often comes from some combination of common and rare variants, many of which are inherited but some of which can be spontaneous.
3. The identification of ASD-linked genes is helping scientists to understand the biological processes involved in causing autism, which could lead to novel, more targeted treatment options.

## Searching for the Cause

There is a saying common among people familiar with autism: "If you've met one person with autism, you've met *one* person with autism." This diversity is also proving true of the genetics behind the disorder. About one in 10 cases can be traced to mutations affecting a single gene. These so-called monogenic varieties generally occur as part of multifaceted syndromes that also cause physical malformations: fragile X, Phelan-McDermid, Rett and Timothy syndromes, as well as tuberous sclerosis and neurofibromatosis, to name a few.

Far more often ASD is considered idiopathic, meaning its root cause is unknown. Twin studies from as far back as the 1970s indicated that ASD was strongly heritable, but the subsequent rise in diagnoses led many researchers and parents to look for environmental influences. Currently experts believe that much of the increase stems from growing awareness among parents, pediatricians and educators and improved diagnostic criteria. Psychiatrist Terry Brugha of the University of Leicester in England and his colleagues found evidence in support of this idea in 2011, showing that a representative sample of previously undiagnosed adults had rates of ASD that were similar to recent estimates for children.

In recent years the evidence for genetic causes has

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advanced dramatically. A barrage of studies has produced a steady stream of genes strongly linked to autism. Some estimate that the number of associated genes may ultimately top 1,000. One especially important discovery is the role that so-called de novo mutations play. These glitches in the genetic code occur spontaneously in a sperm or egg cell and so are not inherited from either parent.

In 2007 molecular biologist Michael Wigler of Cold Spring Harbor Laboratory, geneticist Jonathan Sebat, now at the University of California, San Diego, and their colleagues noted some of the first de novo mutations linked to ASD in the form of copy-number variants—alterations in chromosomes that involve the deletion or duplication of whole chunks of DNA, which can affect multiple genes. Soon other scientists started to find autism-linked de novo point mutations (also referred to as single-nucleotide variants because they are one-letter changes in the DNA) implicating specific genes. Since then, a rash of studies has homed in on several de novo mutations (both copy-number variations and single-nucleotide variants) that substantially raise an individual's risk for ASD—sometimes 20-fold and, in rare cases, even 80-fold.

At the same time, multiple studies found that de novo mutations increase with paternal age. For instance, in 2012 Brian O'Roak, then working in geneticist Evan Eichler's lab at the University of Washington, and his colleagues discovered that 80 percent of spontaneous point mutations occur within sperm cells and that the number of mutations tends to increase with a father's age. The findings explain a small percentage of the known increase in risk for autism among children of older fathers.

Last November two studies published simultaneously in *Nature* upped the total number of genes linked to autism from around nine to more than 70. Both investigations used a technique called whole exome sequencing, which focuses exclusively on exons, regions of the genome containing code for building proteins. This approach lets researchers quickly and more affordably screen the 1 percent of the human genome we know the most about. (*Scientific American Mind* is part of Springer Nature.)

The first report, by Wigler, Eichler, Matthew State of the University of California, San Francisco, and their colleagues, analyzed the exomes of more than 2,500 families from the Simons Simplex Collection, a set of DNA samples from so-called simplex families who, by definition, have only one child with autism. By comparing each child's genome with their parents', the researchers estimated that de novo mutations contributed to around 30 percent of ASD diagnoses in these families and to 45 percent of diagnoses in girls. They also identified 27 genes strongly linked to ASD.

Drama-based interventions capitalize on the fact that actors possess gifts kids with autism often lack—strong communication, imagination and social skills. Psychologists are studying whether rehearsing these abilities helps to develop them.

NICK SPRATLING (*child with actor*)

The second study came from the Autism Sequencing Consortium (ASC), involving researchers from 37 different institutions, by neuroscientist Joseph D. Buxbaum of the Icahn School of Medicine at Mount Sinai and his colleagues. Buxbaum—together with State, geneticist Mark Daly of the Broad Institute in Cambridge, Mass., and statisticians Kathryn Roeder of Carnegie Mellon University and Bernie Devlin of the University of Pittsburgh—founded the consortium in 2010, with support from the National Institute of Mental Health, to share samples and data. Looking for both inherited and spontaneous mutations, the team analyzed 3,871 autism cases and 9,937 unaffected individuals. They identified 33 genes strongly linked to ASD and more than 70 additional candidates. The genes implicated in these two studies overlap somewhat. Roeder reports that, along with geneticist Stephan Sanders of U.C.S.F., she has produced an unpublished list that includes genes affected by de novo copy variants. The top 71 of these genes are 90 percent likely to be involved in autism.

## Adding Up the Risks

Most of the genes identified in the second study fall into three main categories. Some are involved in synaptic function—or how nerve cells in the brain communicate across the gaps, or synapses, between them. Some contribute to transcription, the process by which DNA is translated into proteins. And some play a role in remodeling chromatin—densely packed complexes of DNA and proteins whose changing structure determines which stretches of DNA are accessible for transcription. Because the latter two actually influence the activity of genes, they, too, may ultimately affect the growth and function of neurons and synapses.



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## A Genetics Glossary

**Copy-number variations**
Genes contain long series of "words" made up of the four DNA "letters" (adenine, cytosine, guanine and thymine), and sometimes these words—or entire paragraphs—are repeated once, twice or even 4,000 times. Such copy-number variations may be harmless or linked to specific diseases. Repeats of the three-letter sequence CGG on the X chromosome, for example, causes fragile X syndrome.

**De novo mutations**
Named for the Latin, "from the new," de novo mutations occur spontaneously in sperm, egg cells or fertilized eggs and are not inherited from either parent. The sperm of older fathers contains more de novo mutations, raising the risk of autism in offspring.

**Epigenetics**
Epigenetics refers to the study of gene-environment interactions, in which external factors—toxins, infections or even experiences—may change if and how certain genes are expressed. Once in place, these modifications can be passed down from one generation to the next.

**Exome**
The exome is that 1 percent of the genome containing exons, which are DNA sequences that carry the instructions for building proteins. The remaining 99 percent of the genome plays a regulatory role that influences how other genes function.

**Genetic background**
A gene's impact depends on interactions with other genes that make up a person's genetic background. This is why the same mutation can be benign in one person but cause disease in someone else.

**Mitochondrial DNA**
Inherited only through the maternal line, this DNA is found within mitochondria, the organelles that power our cells by converting chemical energy from food into a more usable form.

**Point mutations**
Also known as single-nucleotide variants, these mutations occur at a single DNA letter. They include one-letter swaps, deletions and insertions. Point mutations are implicated not only in autism but in cancer and color blindness, among other conditions.

**Somatic mutations**
These errors in the genetic code happen after conception during cell division. They can arise in any cell in the body except sperm and eggs. They multiply with cell division but are not inherited from one generation to the next.

**Spontaneous versus inherited mutations**
Inherited mutations are those that offspring share with their parents, but spontaneous mutations are unique to that individual. The genetic risk for autism appears to involve a mix of common and rare, inherited and spontaneous mutations.

These are mission-critical processes within and between cells, and so any disruptions during development could have far-reaching biological consequences. As such, these findings may explain evidence suggesting that ASD shares much of its genetic origins with other conditions, especially other developmental psychiatric disorders, such as schizophrenia, but also seemingly unrelated ones, such as congenital heart disorders. Researchers think that whether these genes actually trigger autism or something else or nothing at all may depend on the rest of a person's genetic makeup.

For instance, a specific high-risk mutation might potentially lead to a range of diseases or disabilities but will cause autism in those families whose inherited "genetic background" increases that particular risk. "There are some factors—not many—that increase risk enormously, some that increase it a lot, some that increase it some and a *lot* that increase risk a tiny bit," Buxbaum explains. "The manifestation of autism is the sum of all that risk in each individual, with some liability threshold, which differs for boys and girls."

More than four times as many boys are diagnosed with ASD as are girls. No one is exactly sure why, beyond assuming that girls are somehow protected from the effects of some mutations. A 2014 study by Sébastien Jacquemont, then at the University of Lausanne in Switzerland, and his colleagues found support for this idea, showing that affected girls tend to have more severe mutations. Also, these mutations were much more likely to have been inherited from the girls' mothers. One implication is that mothers can pass ASD-linked mutations on to their children without having the disorder themselves. These findings, together with the newly found importance of de novo mutations, may help explain why so many cases appear in families with no previous autism diagnoses.

De novo mutations seem to tip the balance in only about 14 percent of cases. Such severe mutations are rare precisely because they have a big effect and so reduce the likelihood of their carriers having children. "The usual reason a variant is rare is that it's brand-new in the population," Roeder says. Still, they offer a promising research avenue in that damaging genetic rarities often reveal more about the biological mechanisms of a disease than common, less harmful variants can. "We've learned a tremendous amount about cancer, hyperlipidemia, neurodegenerative disorders, and more through rare variations that account for a fleetingly small proportion of the population risk but have opened up compelling and widely applicable insights into biology," State observes. (For example, it was a gene for a rare, familial form of Parkinson's disease that led scientists to appreciate the role of the protein alpha-synuclein in all forms of the disorder.)

To date, researchers have had less luck nailing down the common genetic variations linked to ASD. Common variants collectively account for more of the risk of autism than rare ones do, but they individually confer such small increases that they are hard to identify. "At this point we have not pinpointed what specific common variants are relevant," says geneticist Benjamin Neale of the Broad Institute, who worked


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Autism shares much of its genetic origins with other conditions, especially other developmental psychiatric disorders, such as schizophrenia, but also seemingly unrelated ones, such as congenital heart disorders.

on the ASC study. "But there are multiple reports that common variation has a substantial influence."

The interplay among different kinds of variation—rare, common, inherited and spontaneous—is key to understanding the genetics of autism, scientists say. This past February geneticist Stephen Scherer of the University of Toronto and his colleagues published the results of a study in which they sequenced the entire genomes of 85 so-called quartet families—two parents and two ASD siblings. It turned out that nearly 70 percent of these affected brothers and sisters had *different* rare variants previously linked with autism. Buxbaum speculates that these families may have different underlying risks because of common variation, which when combined with rarer, possibly spontaneous, variants push individual children over the ASD threshold. "I think that's what's going on," he says. "The family has increased risk, and then the two siblings have different final causes."

To try to parse the relative contributions of common and rare variations, Roeder and Devlin have developed statistical tools to extend the methods for estimating the heritability of a trait. Together with Buxbaum and his colleagues in the Population-Based Autism Genetics and Environment Study Consortium (PAGES), they evaluated more than 3,000 people from Sweden's universal health registry, including more than 450 with ASD. After their analysis, they estimated that 49 percent of the total risk for ASD stems from common variants, whereas only 6 percent is from rare mutations (3 percent inherited, 3 percent de novo). Other studies have shown that another 4 percent can be credited to things such as recessive genes. But that still leaves 41 percent unexplained.

## From Genes to Biology

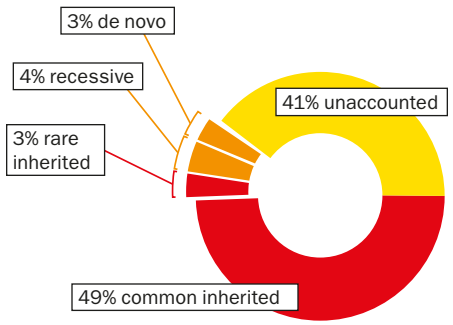
Some of this missing risk could reflect environmental factors—perhaps infections, or certain drugs or toxins in the mother's system during pregnancy, or birth complications—any of which might permanently alter the expression of genes (a gene-environment interaction known as epigenetics) or increase risk in other ways. But additional phenomena are involved, from random chance to somatic mutations, which are not present in the egg or sperm but arise in cells as they divide during development. In rare cases, autism has been associated with mutations in mitochondrial DNA, inherited exclusively through the maternal line. And the gut microbiome might be implicated. Some people with autism appear to harbor unusual communities of bacteria in the digestive tract that can produce waste that harms the brain.

Moreover, ASD genes do not act in isolation but interact with one another, the environment and other biological processes in complex ways we are only beginning to understand. All these additional factors help to explain why identical twins—who have nearly exactly the same DNA—are only somewhere between 80 to 90 percent likely to share an ASD diagnosis. (When they do not both have autism, the twin without it will often have another psychiatric diagnosis, such as ADHD.)

To find out where, and *when,* in the brain genes linked to autism interact and begin to cause problems, scientists are turning to cutting-edge projects such as the BrainSpan Atlas of the Developing Human Brain, developed by the Allen Institute for Brain Science in Seattle in collaboration with several universities. This dynamic atlas charts the activation of genes in the brain throughout development, from a fetus to an adult. Several recent studies have combined these data with genetic findings. In doing so,

### What Are the Chances?

**Scientists estimate that about 49 percent of the risk for autism comes from common genetic variants, another 3 percent from rare inherited mutations, 3 percent more from rare de novo mutations and 4 percent from recessive genes. That leaves 41 percent unexplained.**




SOURCE: "MOST GENETIC RISK FOR AUTISM RESIDES WITH COMMON VARIATION," BY TRENT GAUGLER, ET AL., IN *NATURE GENETICS*, VOL. 46, NO. 8; AUGUST 2014

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Even though identical twins inherit nearly exactly the same DNA from their parents, they are only somewhere between 80 and 90 percent likely to share an autism diagnosis.

researchers can map networks of genes that are expressed together in specific brain regions and cell types at the same time.

These investigations have revealed that many ASD-linked genes appear to function together in parts of the cortex during the mid- to late-fetal period, roughly five months after conception. Some studies specifically implicate what are known as projection neurons—cells responsible for forging long-range connections from one part of the brain to another. The finding bolsters some prominent theories that trace autism symptoms to abnormalities in how the brain is wired. Among those theories: there is an excess of local connections and insufficient long-distance ones.

Other scientists have considered not only where and when ASD genes are active in the brain but also how the proteins they produce interact. For instance, this past February systems biologist Lilia Iakoucheva of U.C.S.D. and her colleagues published findings from their investigation of an autism-linked copy-number variation known as 16p11.2. This stretch of chromosome 16 includes 29 different genes. Deletions increase the risk for autism; duplications increase the risk for both autism and schizophrenia.

Focusing on the genes found in this region, the team built up a related network of protein interactions. The researchers found that the protein produced by one 16p11.2 gene—called *KCTD13*—forms a structure with another protein, Cul3, during mid-fetal development. The *Cul3* gene lies in a different part of the genome but has been previously linked to autism in the form of de novo point mutations. Together these proteins control the levels of a third protein, RhoA, which is involved in choreographing the migration of cells in a developing brain.

The findings fit strikingly well with what was already known about how this mutation affects head size. When 16p11.2 regions are deleted, head size increases, whereas duplications decrease head size. (Larger than average head size is common among individuals with autism.) Iakoucheva says they were surprised to then find that mutations in a completely different gene, *CACNA1C,* which causes the rare form of autism called Timothy syndrome, have also been tied to this same RhoA mechanism. This convergence of three different mutations on the same biological process—one that might disrupt cell migration during fetal brain development—typifies much current thinking in the field: namely the suspicion that many of the 1,000 or more mutations that may be involved will ultimately converge on a limited number of underlying mechanisms.

## The Path Ahead

Understanding exactly how ASD arises can only ease the anguish many parents have felt as they struggle to understand why the lightning bolt of severe autism happened to strike their family and worry that it will strike again. Scientists now have a set of genes they know will put a developing child

CHARLY FRANKLIN *Getty Images*, FOR ILLUSTRATION PURPOSES ONLY



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## As genetic testing improves, parents with one affected child will be able to gauge the risk to their subsequent children.

at high risk for ASD, a list that can only grow. These findings will eventually transform diagnosis and facilitate earlier interventions. As genetic testing for autism expands and improves, parents with one affected child will be able to determine the risks that subsequent children may face. If the dominant cause of ASD in a firstborn is a de novo mutation, it might suggest little or no increased risk. Inherited mutations, on the other hand, could up the odds to something as high as 50 percent. Prenatal testing may also eventually become available.

Ultimately the goal is to develop effective treatments. One reason the American Psychiatric Association replaced Asperger's syndrome and other subtypes with the generic term "autism spectrum disorder" is that biological evidence for the old subtypes was lacking. But as genetic findings help researchers to uncover the biological mechanisms involved, it may lead to more individualized approaches to treatment, as is already happening in other areas of medicine. It may be that one day the diversity of the kids I watched participate in *The Tempest* will be matched by a similarly diverse array of therapeutic options.

"Gene discovery is the thing we're trying to get done as quickly as possible before we get down to the real work of understanding the biology and pathogenesis of the disorder and where we can usefully intervene," Buxbaum says. The mechanism identified by Iakoucheva and her colleagues, for example, offers one possible treatment target. She and her collaborators plan to use stem cell technology to investigate whether an existing drug called Rhosin, which alters RhoA protein levels in nerve cells, might be helpful. If it works, researchers will still face the challenge of how to deliver the drug to fetal brains because Rhosin cannot cross the blood-brain barrier.

Researchers have also made great strides in understanding the molecular biology of monogenic syndromes such as fragile X and Rett syndromes and have developed interventions that show promise in animal models of these conditions, which were previously thought to be completely irreversible. "Finding out that, at least in animal models, you can erase many of the consequences, even in adulthood, is tremendously exciting," State says.

The discoveries have led to preliminary drug trials. Buxbaum and his colleagues recently published preliminary findings from an early-stage clinical trial of insulinlike growth factor-1 (IGF-1) in nine children with Phelan-McDermid syndrome, which is caused by mutations in *SHANK3*, one of the highest-risk ASD genes. In all the children, who ranged in age from five to 15, the growth factor—which enhances the maturity of synapses—improved social functioning and lessened repetitive behaviors, such as rocking.

Additional studies show that IGF-1 may also help children with Rett syndrome, but whether it will benefit more genetically complex forms of ASD remains an open question. Buxbaum cautions that these preliminary results need to be replicated in larger samples, but it is worth noting that IGF-1 crosses the blood-brain barrier. Other researchers are testing substances found to reverse deficits in fragile X syndrome and tuberous sclerosis to see if they might work in genetically complex cases.

At the moment there are many ways forward for autism researchers. Larger samples and better study designs will enable new variants with smaller effects to be found, and whole genome sequencing will make it possible for scientists to identify risky mutations in the large parts of the genome they have not yet fully explored. As the resolution of BrainSpan and similar resources improves, they may reveal more about how these genes shape the developing brain. Over the long term, this will lead to new interventions for a condition for which effective treatment has been elusive. "That's really what we're trying to do," Buxbaum says. "Everything else is just steps toward that goal." **M**

MORE TO EXPLORE


- **Most Genetic Risk for Autism Resides with Common Variation.** Trent Gaugler et al. in *Nature Genetics,* Vol. 46, No. 8, pages 881–885; August 2014.
- **The Contribution of De Novo Coding Mutations to Autism Spectrum Disorder.** Ivan Iossifov et al. in *Nature,* Vol. 515, pages 216–221; November 13, 2014.
- **Synaptic, Transcriptional and Chromatin Genes Disrupted in Autism.** Silvia De Rubeis et al. in *Nature,* Vol. 515, pages 209–215; November 13, 2014.
- **Autism Spectrum Disorders: From Genes to Neurobiology.** A. Jeremy Willsey and Matthew W. State in *Current Opinion in Neurobiology,* Vol. 30, pages 92–99; February 2015.
- **Spatiotemporal 16p11.2 Protein Network Implicates Cortical Late Mid-Fetal Brain Development and KCTD13-Cul3-RhoA Pathway in Psychiatric Diseases.** Guan Ning Lin et al. in *Neuron,* Vol. 85, No. 4, pages 742–754; February 18, 2015.

*From Our Archives*

- **Autism Grown Up.** Jennifer Richler; January/February 2015.




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