

A child's hand is shown reaching out towards a large, translucent soap bubble that is suspended in the air. The bubble is the central focus of the image, and the child's hand is positioned to the right, with fingers slightly spread as if about to touch or pop the bubble. The background is a soft, out-of-focus light color.

The Early Origins of Autism

*New research into the causes
of this baffling disorder is focusing
on genes that control
the development of the brain*

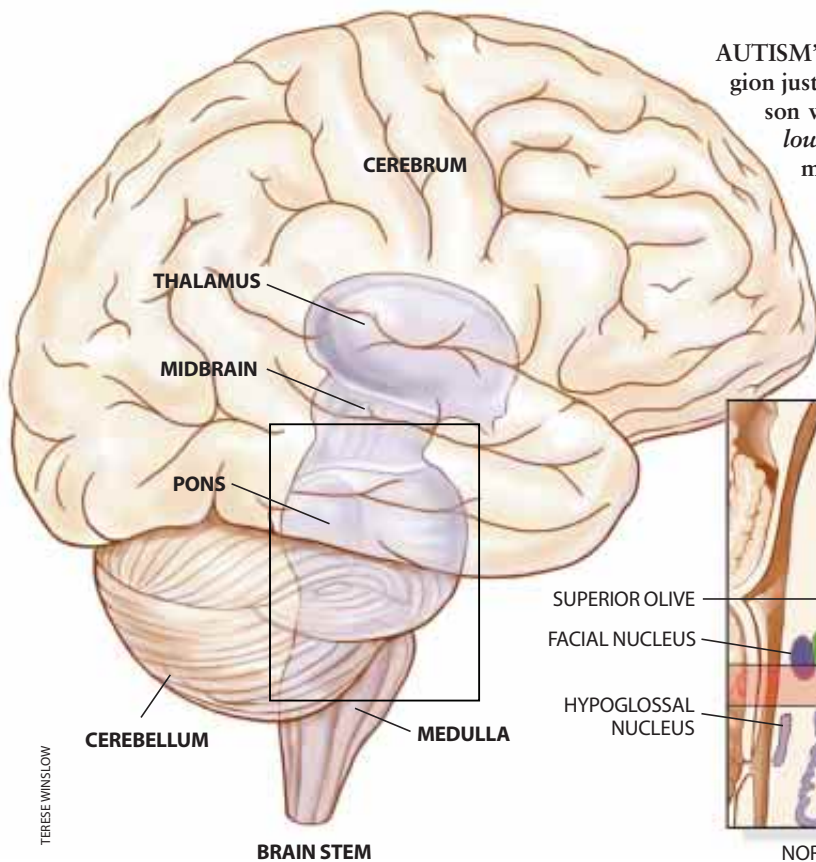
by Patricia M. Rodier

Autism has been mystifying scientists for more than half a century. The complex behavioral disorder encompasses a wide variety of symptoms, most of which usually appear before a child turns three. Children with autism are unable to interpret the emotional states of others, failing to recognize anger, sorrow or manipulative intent. Their language skills are often limited, and they find it difficult to initiate or sustain conversations. They also frequently exhibit an intense preoccupation with a single subject, activity or gesture.

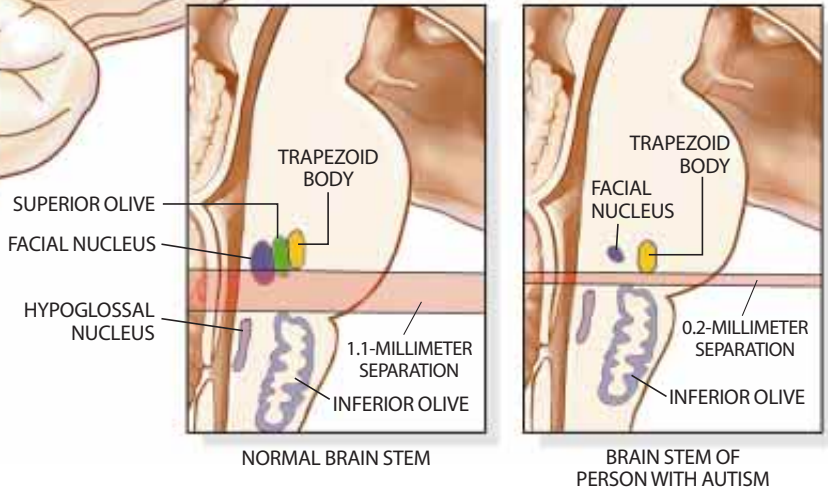
These behaviors can be incredibly debilitating. How can you be included in a typical classroom if you can't be dissuaded from banging your head on your desk? How can you make friends if your overriding interest is in calendars? When children with autism also suffer from mental retardation—as most of them do—the prognosis is even worse. Intensive behavioral therapy improves the outcome for many patients, but their symptoms can make it impossible for them to live independently, even if they have normal IQs.

SEVEN-YEAR-OLD WITH AUTISM reaches for a soap bubble during playtime at the Eden Institute, a school for children with autism in Princeton, N.J.





AUTISM'S EFFECTS include changes to the brain stem, the region just above the spinal cord (*left*). The brain stem of a person with autism is shorter than a normal brain stem (*below*): the structures at the junction of the pons and the medulla (such as the facial nucleus and the trapezoid body) are closer to the structures of the lower medulla (the hypoglossal nucleus and the inferior olive). It is as though a band of tissue were missing. The brain stem of a person with autism also lacks the superior olive and has a smaller-than-normal facial nucleus. Such changes could occur only in early gestation.



I became involved in the search for autism's causes relatively recently—and almost by accident. As an embryologist, I previously focused on various birth defects of the brain. In 1994 I attended a remarkable presentation at a scientific conference on research into birth defects. Two pediatric ophthalmologists, Marilyn T. Miller of the University of Illinois at Chicago and Kerstin Strömmland of Göteborg University in Sweden, described a surprising outcome from a study investigating eye motility problems in victims of thalidomide, the morning-sickness drug that caused an epidemic of birth defects in the 1960s. The study's subjects were adults who had been exposed to the drug while still in the womb. After examining these people, Miller and Strömmland made an observation that had somehow eluded previous researchers: about 5 percent of the thalidomide victims had autism, which is about 30 times higher than the rate among the general population.

When I heard these results, I felt a shock of recognition, a feeling so powerful that I actually became dizzy and began to hyperventilate. In the effort to identify autism's causes, researchers had

long sought to pinpoint exactly when the disorder begins. Previous speculation had focused on late gestation or early postnatal life as the time of origin, but there was no evidence to back up either hypothesis. The connection with thalidomide suddenly threw a brilliant new light on the subject. It suggested that

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autism originates in the early weeks of pregnancy, when the embryo's brain and the rest of its nervous system are just beginning to develop. Indeed, Miller and Strömmland's work convinced me that the mystery of autism could soon be solved.

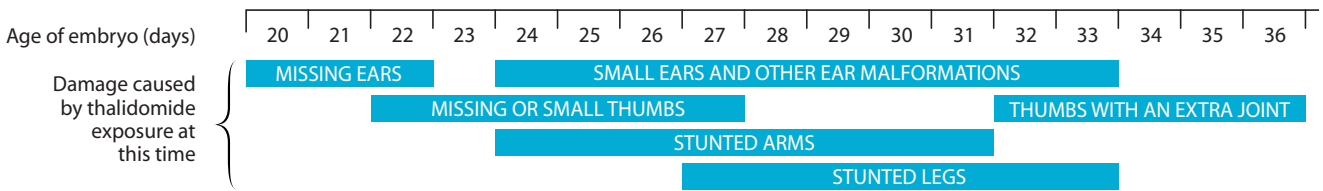
Genetic Factors

At least 16 of every 10,000 babies is born with autism or one of its related disorders [see box on page 60]. Since autism was first identified in 1943, scientists have made great strides

in describing its symptoms. The biological basis for autism, however, has been elusive—an unfortunate circumstance, because such an understanding could enable researchers to identify the leading risk factors for autism and possibly to design new treatments for the condition.

By examining the inheritance of the disorder, researchers have shown that autism runs in families, though not in a clear-cut way. Siblings of people with autism have a 3 to 8 percent chance of being diagnosed with the same disorder. This is much greater than the 0.16 percent risk in the general population but much less than the 50 percent chance that would characterize a genetic disease caused by a single dominant mutation (in which one faulty gene inherited from one parent is sufficient to cause the disorder) or the 25 percent chance that would characterize a single recessive mutation (in which a copy of the faulty gene must be inherited from each parent). The results fit best with models in which variants of several genes contribute to the outcome. To complicate matters further, relatives of people with autism may fail to meet all the criteria for the disorder but still have some of its symptoms. Although these relatives may have some of the gene variants linked to autism—what-

Thalidomide Timeline



BIRTH DEFECTS caused by thalidomide vary depending on when the mother was exposed to the drug (*above*). A 1994 study showed that thalidomide victims with autism had ear anomalies and normal limbs, suggesting that the drug triggered the disorder 20 to 24 days after conception, when the embryo's nervous system is starting to form (*right*).

ever they may be—for some reason the genetic factors are not fully expressed in these individuals.

Studies of twins in the U.K. confirm that autism has a heritable component but suggest that environmental influences play a role as well. For example, if genetic factors alone were involved, monozygotic (identical) twins, who share the same genes, should have a 100 percent chance of sharing the same diagnosis. Instead, when one twin has autism, the second twin has only a 60 percent chance of being diagnosed with the same disorder. That twin also has an 86 percent chance of having some of autism's symptoms. These figures indicate that other factors must modify the genetic predisposition to the disorder.

The Embryology of Autism

Several environmental risk factors are already known. In utero exposure to rubella (German measles) or to birth defect-causing substances such as ethanol and valproic acid increases the chances that autism will develop. People with certain genetic diseases, such as phenylketonuria and tuberous sclerosis, also have a greater chance of developing autism. None of these factors, however, is present frequently enough to be responsible for many cases. Furthermore, most exposures to diseases or hazardous substances would be likely to affect both members of a pair of twins rather than just one. Some of the environmental influences must be more subtle than those identified so far. Researchers do not know how the multiple factors combine to make some people display symptoms while allowing others to escape them. This variation makes the search for autism's causes especially difficult.

In their 1994 study Miller and Ström-land added another environmental contributor to autism: thalidomide expo-

sure in utero. All their subjects—Swedish adults born in the late 1950s and early 1960s—exhibited some of the malformations for which thalidomide is infamous: stunted arms and legs, misshapen or missing ears and thumbs, and neurological dysfunctions of the eye and facial muscles. Because scientists know which organs of the embryo are developing at each stage of pregnancy, they can pinpoint the exact days when a malformation can be induced: the thumb is affected as early as day 22 after conception, the ears from days 20 to 33, and the arms and legs from days 25 to 35. What made the new study so exciting for me was Miller and Ström-land's discovery that most of the thalidomide victims with autism had anomalies in the external part of their ears but no malformations of the arms or legs. This pattern indicated that the subjects had been injured very early in gestation—20 to 24 days after conception—before many women even know they are pregnant.

For embryologists, nothing tells us so much about *what* happened to an em-



JOHNNY JOHNSON

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bryo as knowing *when* it happened. In the case of thalidomide-induced autism, the critical period is much earlier than many investigators would have guessed. Very few neurons form as early as the fourth week of gestation, and most are motor neurons of the cranial nerves, the ones that operate the muscles of the eyes, ears, face, jaw, throat and tongue. The cell bodies of these neurons are located in the brain stem, the region between the spinal cord and the rest of the brain. Because these motor neurons develop at the same time as the external ears, one might predict that the thalidomide victims with autism would also suffer from dysfunctions of the cranial nerves. Miller and Ström-land confirmed this prediction—they found that all the subjects with autism had abnormalities of eye movement or facial expression, or both.



COURTESY OF SUSAN L. HYMAN

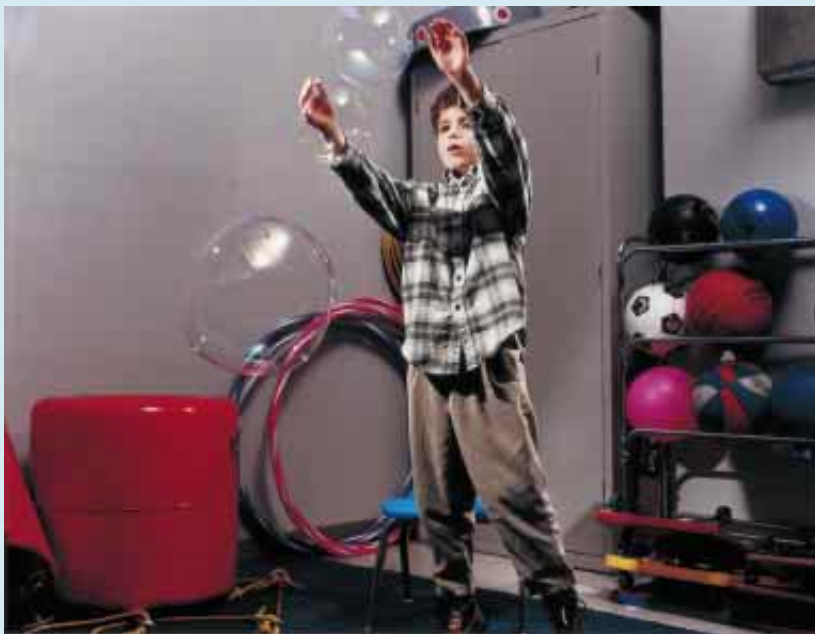
CHILD WITH AUTISM is normal in appearance, at least to the untrained eye. But he has a few physical anomalies characteristic of the disorder. The corners of his mouth are low compared with the center of his upper lip, and the tops of his ears flop over (*left*). His ears are a bit lower than normal and have an almost square shape (*right*).

The Spectrum of Autism Disorders

A diagnosis of autism requires that the patient exhibit abnormal behaviors in three categories [see list at right] and have especially notable deficits in the category of social interaction. In addition, clinicians have identified several related disorders that share some of the behavioral features of autism but have different emphases or additional symptoms. For example, Pervasive Development Disorder, Not Otherwise Specified (PDD-NOS) denotes patients who miss fulfilling the autism criteria in one of the three categories. As is true of autism, PDD-NOS includes patients with the whole range of IQs. Asperger syndrome is used to describe patients with normal IQs and no evidence of language delay. Two much rarer diagnoses are Childhood Disintegrative Disorder, in which normal early devel-

opment is followed by regression to severe disability, and Rett syndrome, a progressive neurological disorder that occurs only in females.

Although many scientists have long known that autism is an inherited disease, recent family studies by Peter Szatmari's group at McMaster University in Ontario suggest that it is the spectrum of symptoms that runs in families rather than a single diagnosis. For example, a child with autism may have a brother with Asperger syndrome, or a woman with autism may have a nephew with PDD-NOS. These family studies strongly suggest that at least three of the diagnoses—autism, PDD-NOS and Asperger syndrome—arise from some of the same inherited factors. —P.M.R.



PHOTOGRAPHS BY JUSTINE PARSONS; SOURCE FOR "DIAGNOSTIC CATEGORIES": DIAGNOSTIC AND STATISTICAL MANUAL OF MENTAL DISORDERS (DSM-IV)

The next logical question was, "Are the cases of autism after thalidomide exposure similar to cases of unknown cause, or are they different?" Aside from their behavioral symptoms, people with autism have often been described not only as normal in appearance but as unusually attractive. They are certainly normal in stature, with normal-to-large heads. The few studies that have tested nonbehavioral features of people with autism, however, have concluded that there are indeed minor physical and neurological anomalies in many cases, and they are the same ones noted in thalidomide-induced autism. For example, minor malformations of the external ears—notably posterior rotation, in which the top of the ear is tilt-

ed backward more than 15 degrees—are more common in children with autism than in typically developing children, children with mental retardation or siblings of children with autism. Dysfunctions of eye movement had been associated with autism before the thalidomide study, and lack of facial expression is one of the behaviors used to diagnose the condition.

The Neurobiology of Autism

Is it possible that all the symptoms of autism arise from changes in the function of the cranial nerves? Probably not. It is more likely that the nerve dysfunctions in people with autism reflect an early brain injury that not only af-

fects the cranial nerves but also has secondary effects on later brain development. That is, the injury to the brain stem might somehow interfere with the proper development or wiring of other brain regions, including those involved in higher-level functions such as speech, resulting in the behavioral symptoms of autism. Or perhaps the ear malformations and cranial nerve dysfunctions are only side effects of an injury that we don't understand. Whatever the true situation may be, the anomalies in patients with autism of unknown cause were much the same as the anomalies in the thalidomide victims with autism. The conclusion was clear: many cases of autism, if not all, are initiated very early in gestation.

Diagnostic Categories

Impairment of Social Interaction: Failure to use eye contact, facial expression or gestures to regulate social interaction; failure to seek comfort; failure to develop relationships with peers.

Impairment of Communication: Failure to use spoken language, without compensating by gesture; deficit in initiating or sustaining a conversation, despite adequate speech; aberrant language (for example, repeating a question instead of replying).

Restricted and Repetitive Interests and Behaviors: Abnormally intense preoccupation with one subject or activity; distress over change; insistence on routines or rituals with no purpose; repetitive movements, such as hand flapping.

BEHAVIORAL THERAPY for children with autism can help them lead happier lives as adults. Instructors at the Eden Institute school carefully evaluate the symptoms of each child to draw up an appropriate intervention plan. They often engage the children in stimulating play activities (*far left*). The institute also provides supervised housing for adults with autism (*left*). The 37-year-old man pictured here used videocassette spools to make the curtain behind his bed; his intense interest in these objects is a characteristic behavior of autism.



The region of the brain implicated by the thalidomide study—the brain stem—is one that has rarely been considered in studies of autism or in studies of other kinds of congenital brain damage, for that matter. On a simplistic level, neurobiologists associate the brain stem with the most basic functions: breathing, eating, balance, motor coordination and so forth. Many of the behaviors disturbed in autism, such as language, planning and interpretation of social cues, are believed to be controlled by higher-level regions of the brain, such as the cerebral cortex and the hippocampus in the forebrain.

Yet some symptoms common in autism—lack of facial expression, hypersensitivity to touch and sound, and sleep

disturbances—do sound like ones more likely to originate in the brain regions associated with basic functions. Furthermore, the most consistently observed abnormality in the brains of people with autism is not a change in the forebrain but a reduction in the number of neurons in the cerebellum, a large processing center of the hindbrain that has long been known to have critical functions in the control of muscle movement.

One reason for scientists' confusion about the brain regions involved in autism may be that our assumptions about where functions are controlled are shaky. For example, the laboratory group led by Eric Courchesne of the University of California at San Diego has shown that parts of the cerebellum are activated during certain tasks requiring high-level cognitive processing. Another difficulty is that the symptoms of autism are so complex. If simpler behavioral abnormalities could be shown to be diagnostic of the disorder, researchers might have a better chance of identifying their source in the nervous system [see box on next page].

In 1995 our research team had the opportunity to follow up on the thalidomide study by examining the brain stem of a person with autism. The tissue samples came from the autopsy of a young woman who had suffered from autism of unknown cause; she had died in the 1970s, but fortunately the samples of her brain tissue had been preserved. When we examined the woman's brain stem, we were struck by the near absence of two structures: the facial nucleus, which controls the muscles of facial expression, and the superior olive, which is a relay station for auditory information. Both structures arise from the same segment of the embryo's neural tube, the organ that develops into the central nervous system. Counts of the facial neurons in the woman's brain showed only about 400 cells, whereas counts of facial neurons in a control brain showed 9,000.

Overall, the woman's brain was normal in size; in fact, it was slightly heavier than the average brain. I hypothesized that the brain stem was lacking only the specific neurons already identified—those in the facial nucleus and the superior olive—and to test that idea I decided to measure the distances be-

tween a number of neuroanatomical landmarks. I was surprised to discover that my hypothesis was absolutely wrong. Although the side-to-side measures were indeed normal, the front-to-back measures were astonishingly reduced in the brain stem of the woman with autism. It was as though a band of tissue had been cut out of the brain stem, and the two remaining pieces had been knit back together with no seam where the tissue was missing.

For the second time in my life, I felt a powerful shock of recognition. I heard a roaring in my ears, my vision dimmed, and I felt as though my head might explode. The shock was not generated by the unexpected result but by the realization that I had seen this pattern of shortening before, in a paper that showed pictures of abnormal mouse brains.

Many cases of autism, if not all, are initiated very early in gestation.

When I retrieved the article from the stacks of papers on my office floor, I found that the correspondence between the brain I had been studying and the mouse brains described in the article was even more striking than I had remembered. Both cases exhibited shortening of the brain stem, a smaller-than-normal facial nucleus and the absence of a superior olive. Additional features of the mice were clearly related to other anomalies associated with autism: they had ear malformations and lacked one of the brain structures controlling eye movement.

What had altered the brains of these mice? It was not exposure to thalidomide or any of the other environmental factors associated with autism but the elimination of the function of a gene. These were transgenic "knockout" mice, engineered to lack the expression of the gene known as *Hoxa1* so that researchers could study the gene's role in early development. The obvious question was, "Could this be one of the genes involved in autism?"

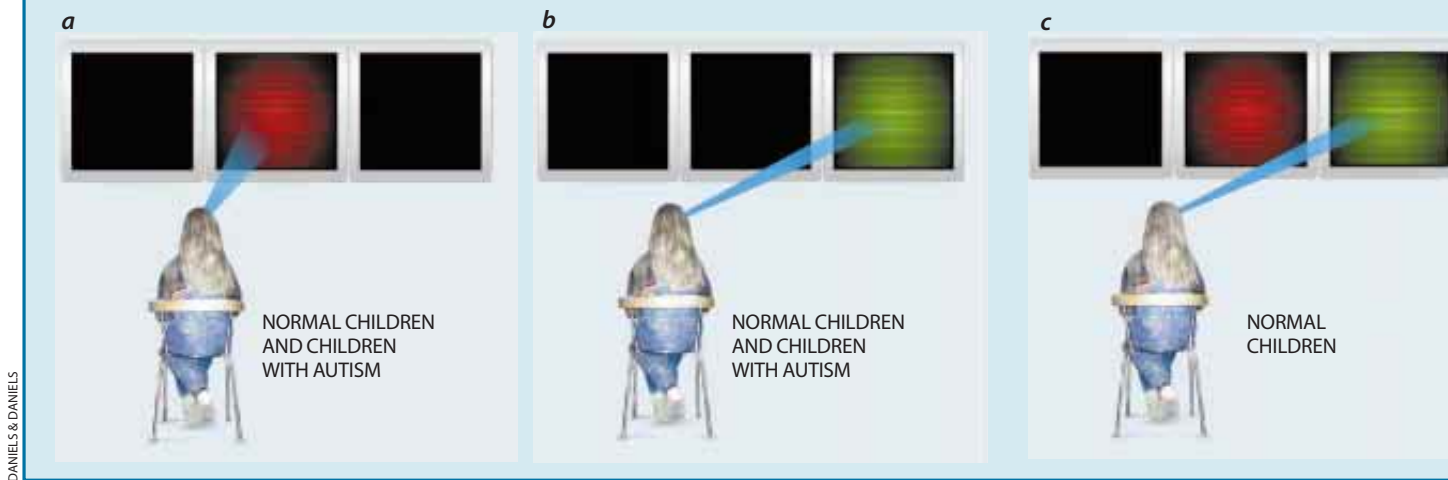
The literature supported the idea that *Hoxa1* was an excellent candidate for autism research. The studies of knockout mice showed that *Hoxa1* plays a central role in development of the brain stem. Groups in Salt Lake City and

A Simpler Symptom of Autism

Scientists at York University and the Hospital for Sick Children in Toronto have recently identified an autism-related behavior that is much simpler than the array of behaviors that have traditionally been used to diagnose the condition. Susan Bryson and her doctoral student Reginald Landry have found that children with autism respond abnormally to a task involving their reactions to visual stimuli. Because this mental activity is probably mediated by a primitive part of the brain—most likely the brain stem or the cerebellum, or both—the discovery has important implications for the neurobiology of autism. Bryson and Landry’s work could also help clinicians develop a simpler way to test children for the disorder.

In their study Bryson and Landry observed the reactions of two groups of children, those with autism and those without it, as they

watched lights flashing on video screens [see illustration below]. The children ranged in age from four to seven. In the first test, each child was placed in front of a three-screen panel, and a flashing light appeared on the middle screen. This stimulus prompted all the children to focus their eyes on the flashes (a). Then the middle screen went blank, and a flashing light appeared on the far-right or far-left screen of the panel. Both groups of children shifted their eyes to that screen (b). In the second test, however, the lights on the middle screen kept flashing while the lights appeared on the other screen. The children without autism shifted their eyes to focus on the new stimulus (c), but the children with autism remained “stuck” on the first stimulus and failed to turn their eyes to the new one (d). The two tests were repeated many times for each child.



London had studied different knockout strains with similar results. They found that the gene is active in the brain stem when the first neurons are forming—the same period that Miller and Strömblad had identified as the time when thalidomide caused autism. *Hoxa1* produces a type of protein called a transcription factor, which modulates the activity of other genes. What is more, *Hoxa1* is not active in any tissue after early embryogenesis. If a gene is active throughout life, as many are, altered function of that gene usually leads to problems that increase with age. A gene active only during development is a better candidate to explain a congenital disability like autism, which seems to be stable after childhood.

Hoxa1 is what geneticists call a “highly conserved” gene, meaning that the sequence of nucleotides that make up its DNA has changed little over the course of evolution. We assume that this is a characteristic of genes that are critical to survival: they suffer mutations as other

genes do, but most changes are likely to be fatal, so they are rarely passed on to subsequent generations. Although many other genes appear in several forms—for example, the genes that encode eye color or blood type—highly conserved genes are not commonly found in multiple versions (also known as polymorphic alleles, or allelic variants). The fact that no one had ever discovered a variant of *Hoxa1* in any mammalian species suggested that my colleagues and I might have trouble finding one in cases of autism. On the other hand, it seemed likely that if a variant allele could be found, it might well be one of the triggers for the development of the disorder.

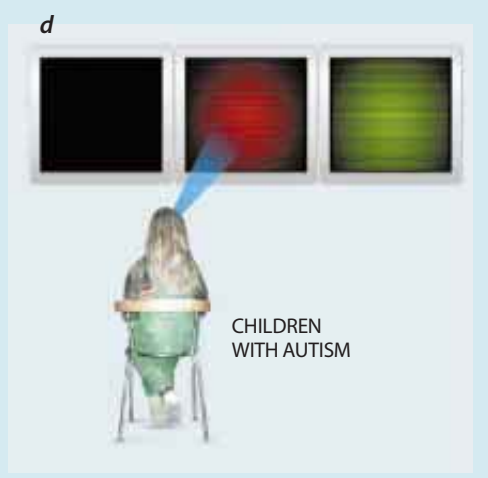
Zeroing in on *HOXA1*

The human version of the gene, labeled as *HOXA1*, resides on chromosome 7 and is relatively small. It contains just two protein-coding regions, or exons, along with regions that regulate the level of protein production

or do nothing at all. Deviations from the normal sequence in any part of a gene can affect its performance, but the vast majority of disease-causing variations are in the protein-coding regions. Thus, we began the search for variant alleles by focusing on the exons of *HOXA1*. Using blood samples from people with autism and from subjects in a control group, we extracted the DNA and looked for deviations from the normal sequence of nucleotides.

The good news is that we have identified two variant alleles of *HOXA1*. One has a minor deviation in the sequence of one of the gene’s exons, meaning that the protein encoded by the variant gene is slightly different from the protein encoded by the normal gene. We have studied this newly discovered allele in detail, measuring its prevalence among various groups of people to determine if it plays a role in causing autism. (The other variant allele is more difficult to investigate because it involves a change in the physical structure of the gene’s DNA.)

Bryson and Landry found that children with other kinds of brain damage are perfectly normal in their ability to disengage from one stimulus and focus on another. Children with autism, however, repeatedly fail to disengage from the first stimulus, even if they are highly intelligent. Researchers suspect that this ability is a low-level brain function because it typically appears in infants—as early as three to four months after birth—and in children with low IQs. Animals also orient themselves toward new stimuli, so scientists could conceivably use a similar test in animal studies to verify whether genetic manipulations or toxicologic exposures have produced this symptom of autism. —P.M.R.



We found that the rate of the variant allele among people with autism was significantly higher than the rate among their family members who do not have the disorder and the rate among unrelated individuals without the disorder. The differences were much greater than would be expected by chance.

The bad news is that, just as the family studies had predicted, *HOXA1* is only one of many genes involved in the

spectrum of autism disorders. Furthermore, the allele that we have studied in detail is variably expressed—its presence does not guarantee that autism will arise. Preliminary data indicate that the variant allele occurs in about 20 percent of the people who do not have autism and in about 40 percent of those who do. The allele approximately doubles the risk of developing the condition. But in about 60 percent of people with autism, the allele is not present, meaning that other genetic factors must be contributing to the disorder.

To pin down those factors, we must continue searching for other variants in *HOXA1*, because most genetic disorders result from many different deviant alleles of the same gene. Variations in other genes involved in early development may also predispose their carriers to autism. We have already discovered a variant allele of *HOXB1*, a gene on chromosome 17 that is derived from the same ancestral source as *HOXA1* and has similar functions in the development of the brain stem, but its effect in autism appears to be minor. Other investigators are scrutinizing candidate regions on chromosome 15 and on another part of chromosome 7. Although researchers are focusing on alleles that increase the risk of autism, other alleles may decrease the risk. These could help explain the variable expression of the spectrum of autism-related disorders.

Even a minimal understanding of the genetic basis of autism would be of great value. For example, researchers could transfer the alleles associated with autism from humans to mice, engineering them to be genetically susceptible to the disorder. By exposing these mice to substances suspected of increasing the risk of autism, we would be able to study the interaction of environmental factors with genetic background and perhaps compile an expanded list of

substances that women need to avoid during early pregnancy. What is more, by examining the development of these genetically engineered mice, we could learn more about the brain damage that underlies autism. If researchers can determine exactly what is wrong with the brains of people with autism, they may be able to suggest drug therapies or other treatments that could ameliorate the effects of the damage.

Devising a genetic test for autism—similar to the current tests for cystic fibrosis, sickle cell anemia and other diseases—would be a much more difficult task. Because so many genes appear to be involved in the disorder, one cannot accurately predict the odds of having a child with autism by simply testing for one or two variant alleles in the parents. Tests might be developed, however, for the siblings of people with autism, who often fear that their own children will inherit the disorder. Clinicians could look for a set of well-established genetic risk factors in both the family member with autism and the unaffected sibling. If the person with autism has several high-risk alleles, whereas the sibling does not, the sibling would at least be reassured that his or her offspring would not be subject to the known risks within his or her family.

Nothing will make the search for autism's causes simple. But every risk factor that we are able to identify takes away some of the mystery. More important, new data spawn new hypotheses. Just as the thalidomide results drew attention to the brain stem and to the *HOXA1* gene, new data from developmental genetics, behavioral studies, brain imaging and many other sources can be expected to produce more welcome shocks of recognition for investigators of autism. In time, their work may help alleviate the terrible suffering caused by the disorder. SA

The Author

PATRICIA M. RODIER is professor of obstetrics and gynecology at the University of Rochester. She has studied injuries to the developing nervous system since she was a postdoctoral fellow in embryology at the University of Virginia, but she began to investigate autism only after hearing the results of the thalidomide study. Rodier has assembled a group of scientists from many disciplines at six institutions to study the genetic and environmental causes of the disorder and says that working with experts from other fields is rejuvenating.

Further Information

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THINKING IN PICTURES: AND OTHER REPORTS FROM MY LIFE WITH AUTISM. Temple Grandin. Vintage Books, 1996.

More information on autism is available at the Web page of the National Alliance for Autism Research at www.naar.org