

Pushed by parent advocates, scientists are unearthing intriguing clues about what causes autism. Ongoing studies point to neuroanatomical and genetic defects

New Hints Into the Biological Basis of Autism

Last month, with the mayor of Sacramento and a crowd of some 3000 parents and supporters looking on, construction crews broke ground on a 1.4-hectare plot on the University of California, Davis's medical campus in Sacramento. The blueprints call for two new buildings that will provide 13,000 square meters designed to do something unprecedented: provide a state-of-the-art comprehensive clinic and research center to diagnose, treat, and study children with autism. This \$38.8 million facility, funded by the state of California, is a sign of the increasing research emphasis on autism, a mysterious disorder that keeps children from interacting socially and emotionally—and the power of parent advocates, who lobbied the state legislature to raise the funds.

Autism was long a poorly understood condition, rarely discussed. But that changed when advocacy groups began promoting research into its causes and possible treatments. In Hollywood, a movie mogul with an autistic son set up a tissue exchange bank. A New Hampshire mother of an autistic boy promoted a possible cure for autism (see sidebar on p. 37), triggering a media frenzy that prompted the National Institutes of Health (NIH) to jump-start clinical trials at record pace. In numerous congressional hearings, Representative Dan Burton (R-IN), who has an autistic grandson, has explored the largely discredited connection between childhood vaccinations and autism. Meanwhile, the rising numbers of parents requesting social services for autism has sparked fear—but few data—that the United States is experiencing a spreading epidemic of the disease (see sidebar on p. 35).

Researchers and funding agencies have responded. In 1997 NIH started a 5-year,

\$42 million network of collaborative programs of excellence for autism. Next month, the first large, interdisciplinary meeting of researchers interested in autism will be held in conjunction with the Society for Neuroscience meeting.

The political momentum isn't flagging either: February marked the formation of a congressional caucus for autism, currently boasting 120 members. "This is a period of mobilization for autism research," says David Amaral, director of the Medical Investigation of Neurodevelopmental Disorders (MIND) Institute, whose clinic is being expanded at UC Davis.

And this increased attention is paying off, Amaral says. After years of frustration because of autism's confusing array of

searchers believe, are perhaps as many as 20 genes that may interact with yet unknown environmental triggers.

Together, the evidence seems to point to problems with brain development before birth and through early childhood. Although genetic factors clearly play a major role, a number of other causes and potential cofactors have been postulated, including vaccines, exposure to toxins, infection, and immunologic and metabolic problems.

Whatever the causes, researchers hope to find ways to identify autistic children before or soon after birth, either with genetic tests or biomarkers such as blood-borne proteins, so that they can begin behavioral treatments sooner, when they seem more likely to succeed. A cure for autism, however, is a faraway prospect.



Socially isolated. One of autism's chief manifestations is an impaired ability to interact with other people.

manifestations—and confusing patterns of inheritance—researchers are beginning to get their first solid sense of its biological basis. Behavioral studies led the way, spelling out the social and cognitive deficits that mark the disorder. Neuroanatomists have begun to identify abnormalities in brain structure, and more recently, imaging studies have provided hints of faulty circuitry. Underlying many of these problems, re-

A world apart

First described in 1943, autism's primary manifestation is an impaired ability to relate socially with other people, although it almost always occurs with other debilitating symptoms. Autism is associated with language problems, and those who speak largely do so in a monotone. People with autism also seem to have trouble inferring what other people think and feel. "These people are very childlike," says Nancy Minshew, a neurologist at the University of Pittsburgh School of Medicine and director of

one of NIH's programs of excellence. "They can't deceive you; they are incapable of lying." They tend to be sober-faced, suggesting that they don't feel the normal range of emotions, and yet often react with toddler-like tantrums. Up to 60% of people with autism are also mentally retarded, with some 20% having an IQ less than 35; a small fraction are, however, gifted in some areas such as music, drawing, or calculations.

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Indeed, the symptoms of the disorder can vary so greatly, both in nature and severity and from person to person, that it is sometimes difficult to diagnose. The problems seen in autism seem to stem not from the senses, but from interpretation of the world. When healthy people view a laboratory film of moving triangles and a circle, for example, they infer social relationships among the objects. Autistic people usually see the shapes and movements as inanimate. They also have trouble interpreting faces. Work by Fred Volkmar and colleagues at Yale University and others has shown that when people with autism interact with someone, they generally spend most of their time looking at the mouth—not the eyes, as most people do. Likewise, Volkmar and his colleagues have shown that a part of the brain called the fusiform gyrus isn't activated when autistic people look at faces, as it is in people not affected by the condition.

Another common symptom is the ability to understand facts but not concepts. "They have wonderful memory for facts, but they can't make sense of them," Minshew explains. "They don't see a room; they see every detail." Children with autism can learn a particular task that involves identifying shapes or colors, for instance, but they usually have trouble applying or generalizing that task to other situations. If asked what their father is like as a person, they are likely to say he's a man, he's tall, and he wears glasses, rather than saying he is kind and hard-working.

Minshew believes the conceptual problems stem from difficulty processing complex information, and she says that's true not just with objects and social cues but with the motor system as well. Autistic children have trouble kicking balls, writing, or tying shoes. Functional magnetic resonance imaging studies, by Minshew's colleagues John Sweeney and Marcel Just, support this interpretation. In research not yet published, they show that higher functioning individuals with autism have reduced connections between the brain regions that support higher order cognitive abilities compared to peers of their age and IQ.

Other anatomical differences have turned

Scant Evidence for an Epidemic of Autism

Between 1987 and 1998, the number of children being treated for autism in California jumped a whopping 273%, according to a 1999 report by the California Department of Developmental Services. Similar jumps appeared nationwide: The U.S. Department of Education reported a 556% increase from 1991 to 1997.

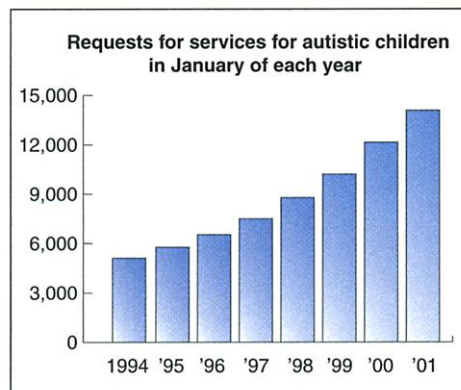
To many parents of autistic children and a few researchers, these startling numbers are evidence that the country is experiencing an epidemic of autism. This, in turn, bolsters suspicions that environmental factors, such as pesticides or childhood vaccines, may be to blame.

But hard data documenting an increase in actual cases are sorely lacking. "The grounds for an increase [are] completely nonexistent," says epidemiologist Eric Fombonne of McGill University in Montreal, although others are more tempered in their assessments. Fombonne and others suspect that the rise in demand for services can probably be traced to an increased awareness of the condition, more common referrals due to the availability of better services, and an ever-broadening definition of just what constitutes autism.

Even the prevalence of autism is hard to gauge. Large studies in the last 15 years have found an average of about 10 cases per 10,000 people. Including related conditions, the figure appears to be about 27 per 10,000. But "it's by no means what I would consider irrefutable," says Craig

Newschaffer, an epidemiologist at the Johns Hopkins University Bloomberg School of Public Health. "There's a real data void here." Indeed, some smaller studies have turned up a much higher rate. In 1998, the Centers for Disease Control and Prevention (CDC) in Atlanta investigated the town of Brick Township, New Jersey, where parents feared a high rate of autism. The prevalence was 40 per 10,000, and 67 per 10,000 for the spectrum of related conditions—the second highest rate ever seen. CDC has since begun surveillance programs in nine states to see whether that rate is typical.

Starting from such an uncertain baseline, "it makes no sense to try to interpret trends over time," Fombonne says. However difficult, other researchers, such as those at the University of California, Davis, are trying to determine whether the rise in reported referrals in California reflects an increasing risk of autism. "I'm still not convinced that there hasn't been an increase [in prevalence]," says David Amaral, who directs



On the rise. Demand for services has increased in California, but it's unclear whether the risk of autism is growing.

the Medical Investigation of Neurodevelopmental Disorders Institute there.

Whether or not the risk of autism has changed, the number of referrals for social services is clearly going up. "That need is real," says Newschaffer, who has an autistic child. Based on a conservative estimate of 20 cases of autism-spectrum disorders per 10,000, Fombonne points out, the number of reported cases in California so far is still an undercount.

—E.S.

up during autopsies and brain scans. The amygdala, part of the brain's limbic system that helps control emotions and social behaviors, tends to be smaller. The hippocampus, a key structure in memory and learning, is usually also smaller. Both show evidence of reduced connections with other brain regions. The autopsies also revealed that the cerebellum typically has a severe deficiency of Purkinje cells, which participate in circuits involved in many brain functions. How all these findings relate to each other, however, is not clear. "There's no consensus at this point about the typical brain pathology of autism," Amaral says. "We're decades behind the research on Alzheimer's or Parkinson's."

Brain biochemistry seems to be altered as well. A 1998 study of 30 controls and 29 autistic children showed that their blood contained on average significantly less oxytocin, a neuropeptide that regulates social

behavior in animals. Larry Young of Emory University last year created knockout mice that lacked oxytocin. They behaved normally, except that they couldn't learn to recognize other mice or recognize their mother's scent, even though their sense of smell was normal. A single dose of oxytocin into the brain, however, cured the mice. "That gives you hope that if autism is related to oxytocin, it's not permanent," Young says.

To see whether this approach might work in humans, Eric Hollander of the Mount Sinai School of Medicine in New York City and colleagues injected a synthetic form of oxytocin into the blood of adults with autism. In this preliminary study, not yet published, repetitive behaviors were significantly decreased as long as the drug was administered; a placebo had no effect, the researchers report. Hollander believes that if oxytocin could be delivered in a sustained manner, it

might ameliorate some symptoms. Unfortunately, there is no available compound that stimulates oxytocin receptors in a lasting manner. Indeed, it's highly unlikely that a single shot of anything will cure the disease, given the differences in brain structure and function among people with autism.

Short circuits

What creates these anatomical differences? Many researchers believe that the problems begin as neurons are finding their places in the brain, from before birth through the first 2 to 3 years of life. One hint comes from a study of neonatal blood samples archived by the California Birth Defects Monitoring Program. As child neurologist Karin Nelson of the National Institute of Neurological Disorders and Stroke in Bethesda, Maryland, and her colleagues reported in the May issue of *Annals of Neurology*, levels of several important regulators of early brain development, such as brain-derived neurotrophic factor, were elevated in children who were later diagnosed with autism. Nelson says it's difficult to know the specific effect of these elevated levels, although it may be related to another common symptom, enlarged heads during childhood.

Although normal size at birth, the heads of children with autism tend to grow disproportionately faster than normal during the first 3 years of life; by adulthood, they are normal size again. One hypothesis is that fewer neurons are pruned in the brains—a process that sculpts sophisticated neural circuitry.

Another perplexing feature is that 10% to 25% of children with autism seem to develop normally until symptoms of autism appear suddenly between 12 and 24 months of age. Because this is when children receive a suite of vaccinations, some parents have blamed the vaccines. Most researchers, however, don't think the evidence supports that charge. "A consistent body of epidemiological evidence shows no association at a population level" between the measles-mumps-rubella vaccine and autism, the Institute of Medicine concluded in an April report, although it could not exclude the possibility that the vaccine may contribute to autism in a few children.

Genetic puzzle

Twin studies have shown that autism clearly has a genetic component. But pinning down

the chromosomal regions that contain the genes that are involved—much less the genes themselves—has been a daunting task. More than a half-dozen research groups are scanning DNA samples to find markers that are inherited by more than one autistic sibling in the same family. If a marker consistently crops up, that region may contain genes related to autism. But usually when one group homes in on an area, the others can't replicate the finding.

The difficulty is probably due to extreme genetic diversity underlying the condition. Autism researchers estimate that between five and 20 genes contribute to the disorder,

er chromosomes—2q and 16p—as they reported in the September issue of the *American Journal of Human Genetics*. Other research suggests that chromosome 15 plays a role too.

But to nail down candidate genes reliably, the groups will need DNA samples from more families. IMGSAC hopes to have 250 by the end of the year, says Tony Monaco of the University of Oxford. Researchers are also trying to identify the most useful subgroups in which to search for genes, such as overall severity of symptoms, language ability, or head size.

More than 10 teams are racing to identify possible autism genes within the suspect chromosomal regions. Using sequence data from the draft human genome, they scan the region for genes that might be related to autism—say, because they are known to play a role in brain development. Then they search for mutations in DNA from affected individuals.

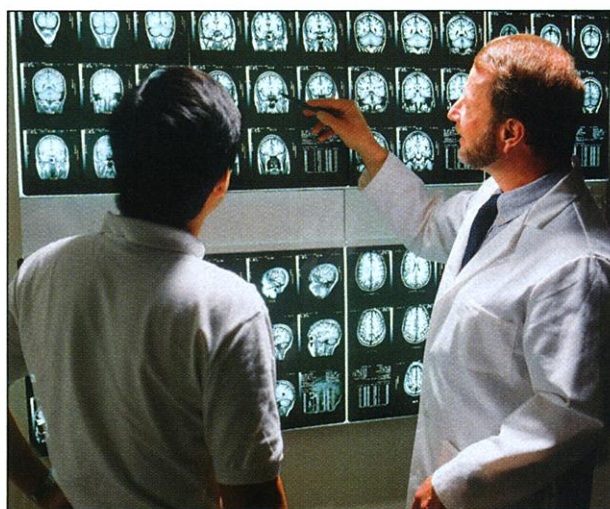
This tactic has already led to the identification of several tentative candidates, although none of the findings has been replicated. A team led by Thomas Wassink, a psychiatric geneticist at the University of Iowa, Iowa City, has been studying *WNT2*, a developmental gene in the 7q region. Intriguingly, a knockout mouse that lacks part of the WNT pathway displays social abnormalities, such as not huddling with other mice during sleep. And variant DNA sequence adjacent to *WNT2* is 50% more likely to occur in children with autism,

Wassink's team reported in the July issue of the *American Journal of Medical Genetics*.

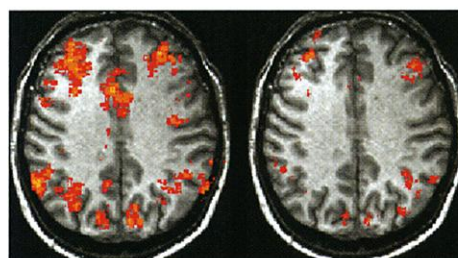
Similar clues implicate the *reelin* gene, which is also on 7q. This gene codes for a protein

that is thought to help neurons find their proper location. When the gene is knocked out in mice, they have defects in the cortical layers and cerebellum that resemble those found in the brains of autistics. A variant of this gene raises the risk of autism 3.5-fold, reported Flavio Keller, a biochemist and cell biologist at the Campus Bio-Medico University in Rome, Italy, and colleagues in the March issue of *Molecular Psychiatry*.

A third gene, on chromosome 7p, *Hoxa1*, is known from knockout mice to be important in the development of the hindbrain. These mice also have features that resemble those of autism, such as misshapen ears. That led Patricia Rodier of the University of Rochester School of Medicine and Dentistry in New York state and her colleagues to search for variants of the gene in 57 autistic individuals. Last December, they reported in



Less connected. Brain scans suggest that some circuits aren't as efficient in people with autism (bottom, right).



with some involved in certain patients but not in others. "It's the equivalent of lumping type 1 and type 2 diabetes; that's how heterogeneous this syndrome is," says Edwin Cook of the University of Chicago. Compounding the problem, most genome scans have looked at fewer than 100 families, whereas many more than 200 may be needed to reliably find a gene in a complex disease, Cook says.

But at least one chromosome region does seem firm. Last month, a group of 21 institutions, called the International Molecular Genetic Study of Autism Consortium (IMGSAC), published the strongest support yet for a susceptibility locus on chromosome 7q in a study of 153 families, one of the largest to date. Other groups have replicated the finding to varying degrees. The 7q region is interesting because a putative language gene maps there. Other regions are beginning to look strong as well. IMGSAC found additional support for loci on two oth-

Desperate Parents Spark Search for New Treatment

The story of Victoria Beck is dramatic: a *Lorenzo's Oil* of autism. Beck's belief that the drug secretin radically improved her son's symptoms—and the media storm that resulted—raised the hopes of many parents and galvanized public and private research. But the weight of evidence so far suggests that secretin doesn't work for the core symptoms of autism, at least for most children.

In 1996, Beck's young autistic son had a routine gastrointestinal exam—many autistic children have GI problems—that involved a diagnostic injection of secretin, a drug that stimulates the pancreas. Soon afterward, his symptoms lessened dramatically, and he began speaking again, sleeping well, and eating normally for 3 months. Convinced that secretin had led to this turnaround, Beck doggedly tried to get doctors to prescribe the drug, but to no avail.

Once Beck appeared on *Dateline NBC* in October 1998, however, word spread like wildfire over the Web, and other parents clamored to know more. "The next day I had hundreds of e-mails," recalls Marie Bristol Power, special assistant for autism at the National Institute of Child Health and Human Development in Bethesda, Maryland. "The rapidity with which news spreads through the community is astounding."

Based on this report and the countless newspaper articles that followed, several thousand parents across the country found doctors who would give their children multiple doses of secretin—an "off-license" use for which the drug was not approved. Researchers became alarmed. "This was not an insignificant treatment," says

Manny DiCicco-Bloom, who studies secretinlike peptides at the University of Medicine and Dentistry of New Jersey in New Brunswick. Among the risks, he says, are inflammatory bowel disease and anaphylactic shock.

Worried, the National Institutes of Health (NIH) sprang into action, setting up clinical trials within a few months. Meanwhile, Repligen, a biotech company in Needham, Massachusetts, raised \$9 million in venture funding to investigate the drug.

Then, bad news. In the 9 December 1999 issue of *The New England Journal of Medicine*, scientists reported that the first trial showed no difference in 16 measures of behavior between 27 autistic children injected with a single dose of secretin and 29 who received a placebo. Since then, four other trials also have found no difference.

But Repligen hasn't given up, modifying NIH's methodology by testing multiple doses. In July, Repligen researchers presented unpublished data at the Autism Society of America's annual meeting in San Diego. The company's phase II clinical trial showed a statistically significant improvement in some measures of social function in children receiving multiple doses of secretin compared to a placebo. Repligen plans to forge ahead with more trials.

Ed Cook, who collaborated in one of the negative trials, isn't optimistic about secretin and doesn't think physicians should prescribe it for autism until it's been shown to work. —E.S.

"The rapidity with which news spreads through the community is astounding."

Teratology that about 40% carried a variant of the gene, compared to roughly 25% of relatives and controls.

All three genes are reasonable candidates for shifting normal development toward autism, researchers say, but the evidence isn't compelling for any of them. "None are really strong this-is-it genes," says Susan Folstein of Tufts University School of Medicine/New England Medical Center in Boston, who heads the Collaborative Linkage Study of Autism. Moreover, the three genes don't fit together into a neat story. Not until that happens can researchers begin to think about prenatal tests that might identify children for early intervention.

Treatment

For the moment, autism is usually diagnosed at 2 to 3 years of age. Although a few drugs can reduce some associated symptoms, none relieve the core problems. The treatment with the most scientific support is behavioral training. The most intensive therapy can take up to 40 hours a week and cost tens of thousands of dollars a year,

putting it well out of reach for many families. Many studies have shown that children who receive this treatment tend to follow instructions better, learn how to imitate, and have enriched vocabularies; the largest and quickest gains, not surprisingly, occur with children who were higher functioning to begin with.

But few data exist to evaluate which of the different approaches to behavioral training work best or how they compare to other interventions. Also unknown is whether some treatments are more suitable to particular sub-

groups of children. "It's very frustrating," says Catherine Lord of the University of Michigan, Ann Arbor. More troubling still, says a June National Research Council report, is the limited amount of interventions available at most schools and a lack of trained teachers.

Improving treatment through a better understanding of autism's causes is one of the goals of UC Davis's MIND Institute. "There's not going to be major progress until you start doing more comprehensive assessments of more kids," Amaral says. Researchers there will evaluate different treatment therapies in a clinic designed to handle 1000 patients a month. Another new building will house lab space for biomarker research to aid in early diagnosis, supported with \$4 million in annual state funding. A team of what will eventually be 20 researchers aims to tie genetic and biochemical findings with data from neuroimaging, psychological assessment, and computer-aided education. At the parents' insistence, all the comprehensive data gained at the MIND Institute will be shared with autism researchers throughout the world. That's because the parents charged the MIND researchers to rise to a new level of cooperation, Amaral says. "It doesn't matter who solves the problem," he says the parents told him, "just how soon it gets solved."

—ERIK STOKSTAD



Eye-spy. Special cameras help reveal how people with autism watch social situations.

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