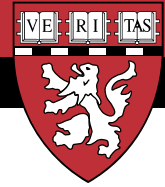


# ON THE BRAIN

THE HARVARD MAHONEY NEUROSCIENCE INSTITUTE LETTER



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## Genetic “Hot Spot” May Increase Susceptibility to Autism

**R**ESearchers from Harvard Medical School have identified a chromosomal abnormality, or genetic “hot spot,” that may increase a child’s susceptibility to autism. The study, which was published in the *The New England Journal of Medicine*, reveals that a small section of chromosome 16, which has genes linked to brain development and various developmental disorders, is either deleted or duplicated in about one percent of children with autism or related disorders.

The finding suggests that this chromosomal abnormality may double a child’s risk of developing autism. It also holds promise for discovering other genetic “hot spots” that may predispose children to the disorder.

“While epidemiologic studies indicate a very large genetic component to autism, little is known about how specific genes are involved,” said Mark J. Daly, PhD, of Massachusetts General Hospital’s Center for Human Genetic Research and the study’s senior author, in an MGH press release. “We’re still a long way from understanding how this chromosomal deletion or duplication increases the risk for autism, but this is a critical first step toward that knowledge.”

### Missing genetic material

Autism spectrum disorders (ASD) are characterized by impaired social interaction, problems with verbal and nonverbal communication, and unusual, repetitive, or severely limited activities and interests. The disorder varies widely in severity and may go unrecognized, especially in children who are only mildly affected. Today, according to the Centers for Disease Control, one out of every 150 children is diagnosed with autism.

Population studies indicate that there is a genetic component to 90 percent of cases of autism and autism spectrum disorders (ASD). However, only

about 10 percent of these cases can be attributed to known genetic or chromosomal syndromes. Since several of these conditions involve deletions or duplications of segments of chromosomes, the HMS researchers conducted complete genome scans of samples from the Autism Genome Research Exchange (AGRE), which contains DNA from families in which at least one child has an ASD or a related disorder. The findings were replicated using clinical samples from Children’s Hospital Boston and data obtained by deCODE Genetics of Iceland.

“We didn’t start out looking at chromosome 16,” says David T. Miller, MD, PhD, assistant director of the Genetics Diagnostic Laboratory at Children’s and a co-author of the study. “We took samples [of chromosomes] of patients with autism and looked for missing genetic material. This occurred more often on chromosome 16 with patients with autism.”

### “Too much, too little of one ingredient”

Researchers from the Autism Consortium, a collaboration involving leading universities, including HMS, and medical centers in the Boston area, scanned DNA samples from more than 3,000 children and families, nearly half of whom were diagnosed with ASD. Five of the individuals with ASD had a chromosome 16 deletion. The deCODE group found the same deletion in three of 299 people, and the Children’s team, using a high-resolution genomic technique designed by the hospital’s laboratory team for clinical use, found five more cases of the deletion among 512 patients referred for developmental delays or suspected ASD. The Children’s researchers also identified four patients with a duplication, rather than a deletion, of the specific region of chromosome 16. No deletions or duplications were identified in non-ASD participants.

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Mark J. Daly, PhD

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## Controlling Brain Receptors in the Fight Against Obesity

**D**ESPITE A NATIONAL health objective to reduce obesity in the United States, data suggests that the situation is worsening rather than improving. Today, nearly 33 percent of adults and more than 17 percent of children are obese, according to the National Health and Nutrition Examination Study.

This increase in obesity is leading to a number of growing—and dangerous—medical conditions, including high blood pressure, type 2 diabetes, coronary heart disease, stroke, osteoarthritis, and certain types of cancer.

Obesity is defined as the state of being above one's normal body weight. It is a label for ranges of weight that are greater than what is generally considered healthy for a given height. Because decisions about when to eat and how much to eat are voluntary acts, scientists at Harvard Medical School are trying to determine whether something goes wrong in the brain's wiring that prevents certain people from being able to control their food intake and thus their body weight.

"It's clear that the brain controls what we eat," says Bradford Lowell, MD, PhD, a researcher at Beth Israel Deaconess Medical Center's Department of Endocrinology, Diabetes and Metabolism and a professor of medicine at HMS. "The brain controls our emotional state and the homeostatic mechanisms that sense when we should eat and how much we should eat. After receiving signals from the periphery about fuel and hormone levels in the body, the brain takes the information, integrates it, and determines how much and when we eat."

Based on that set of criteria, the brain tells us to eat when we are hungry and to stop eating when we are full and have a sufficient amount of calories for the energy we need. However, the big question, Lowell adds, is what circuitry in the brain is involved in this activity and what goes awry in people who are obese.

### Complex neurocircuitry behind weight control

Three years ago, Lowell published a paper in the journal *Cell* that demonstrated for the first time that the neuronal pathways that help to keep body weight stable diverge at what is called the melanocortin-4 receptor, or MC4R, to regulate either food intake or energy expenditure. This discovery has helped scientists understand the complex neurocircuitry behind body weight control.

"Maintaining a stable body weight is a delicate balancing act between the amount of food eaten versus the number of calories burned," said Lowell when the study was released in November 2005. "The brain controls both food intake and calories expended with the purpose of keeping body weight stable. When something goes wrong with this process, obesity results."

Researchers had previously found that MC4R play a critical role in helping the brain make appropriate adjustments in food intake and energy expenditure in order to prevent obesity. Studies have shown that when all MC4R are removed from genetically engineered mice, the animals become morbidly obese. Defects in these receptors also cause obesity in humans.

In the *Cell* study, Lowell and his team focused on two specific areas of the brain that control food intake: the paraventricular hypothalamus (which regulates certain metabolic processes such as hunger and thirst) and a subpopulation of neurons in the amygdala (which controls the body's emotional responses). When MC4R were activated in these two regions, obesity did not occur in 60 percent of the sample. This suggests that these receptors play a key role in the regulation of body weight. In addition, they found that food intake and energy expenditure are regulated separately by MC4R in different areas of the brain.

"Ultimately," the scientists wrote, "these new findings help to refine our understanding of the neuronal logic behind body weight."

### POMC neurons link brain with obesity, type 2 diabetes

Last year, Lowell and a team of scientists from HMS, the University of Texas Southwestern Medical Center, and Oregon Health and Science University found that a gene active in certain neurons in the brain, called pro-opiomelanocortin (POMC) neurons, interacts with fat and glucose. This interaction suggests a link between the brain, obesity and type 2 diabetes, a disorder in which obesity is a strong contributing factor.

Type 2 diabetes is a disorder in which the body's cells inappropriately regulate blood glucose levels. This results from the improper functioning of pancreatic beta cells and impairment of insulin's actions on certain tissues in the body, including those in the liver, fat and muscles. Lowell's group identified a third irregularity linking type 2 diabetes



with obesity, a previously unrecognized role for glucose-sensing neurons in the onset of the disease.

Knowing that POMC neurons regulate body weight in both mice and humans, Lowell and his colleagues looked at the electrical properties of these cells in animal models and found that POMC neurons become electrically excited by a rise in glucose, similar to what would happen after someone eats a meal. They then disrupted the glucose-sensing ability of these neurons, confirming that they play

a critical role in regulating blood-glucose levels in mice. Further, they found that POMC neurons' glucose-sensing ability was defective in mice with obesity-induced type 2 diabetes.

"These findings add to our understanding of type 2 diabetes at a critically important time," says Lowell in a BIDMC press release. "The incidence of the disease has risen to epidemic proportions, and

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## Genetic "Hot Spot" May Increase Susceptibility to Autism

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Miller says that genes need to be "expressed at exactly the right level" to work properly. He compares this to a recipe in which too much or too little of one ingredient changes the taste of the dish. In fact, chromosome 16 abnormalities are implicated in a number of disorders, including a neuropathic condition called Charcot-Marie-Tooth disease, polycystic kidney disease, inflammatory bowel disease, and Crohn's disease, and now, it seems, ASD.

Miller says the researchers "don't know the mechanism for the deletion or duplication of the chromosome. We merely observed the event and found that the missing or extra chromosome 16 is more common in those with autism or developmental delays with features of autism."

### Rare deletion, strong risk factor

The chromosome 16 deletion or duplication accounts for about one percent of ASD cases. About 10 percent of ASD are diagnosed through genetic testing, so this one percent, represents a relatively large proportion.

"These chromosomal deletions are rare," says Daly, "so finding precisely the same deletion in such a significant proportion of patients suggests that this is a very strong risk factor of autism. We're now pursuing more detailed genetic studies to figure out which genes in this region are responsible for this effect in order to gain a better understanding of the underlying biology and potential clues to therapeutic approaches."

In a majority of cases, the chromosome abnormality was not inherited from a parent. Miller, a clinical geneticist, says the researchers think the deletion or duplication occurred prior to embryonic

development, during the time chromosomal information is split and copied in egg and sperm cells. This suggests that the chances of another child in the family having an ASD when a sibling has this deletion or duplication are as small as perhaps five percent compared to 50 percent if the trait is inherited from a parent.

### High-tech diagnostic testing

The discovery of the chromosome 16 abnormality was made possible by new, highly sensitive chromosome scanning technology from Affymetrix and Agilent Technologies. This equipment allows researchers to conduct high-resolution microarrays to look for small, missing, or extra pieces of DNA material.

Children who are newly diagnosed with an ASD or other developmental disorders can now be tested for this chromosome 16 defect. The tests, however, are costly. Children's Hospital has developed a simple, rapid, cost-effective test that will facilitate detection of this deletion or duplication in children with ASD or developmental delays.

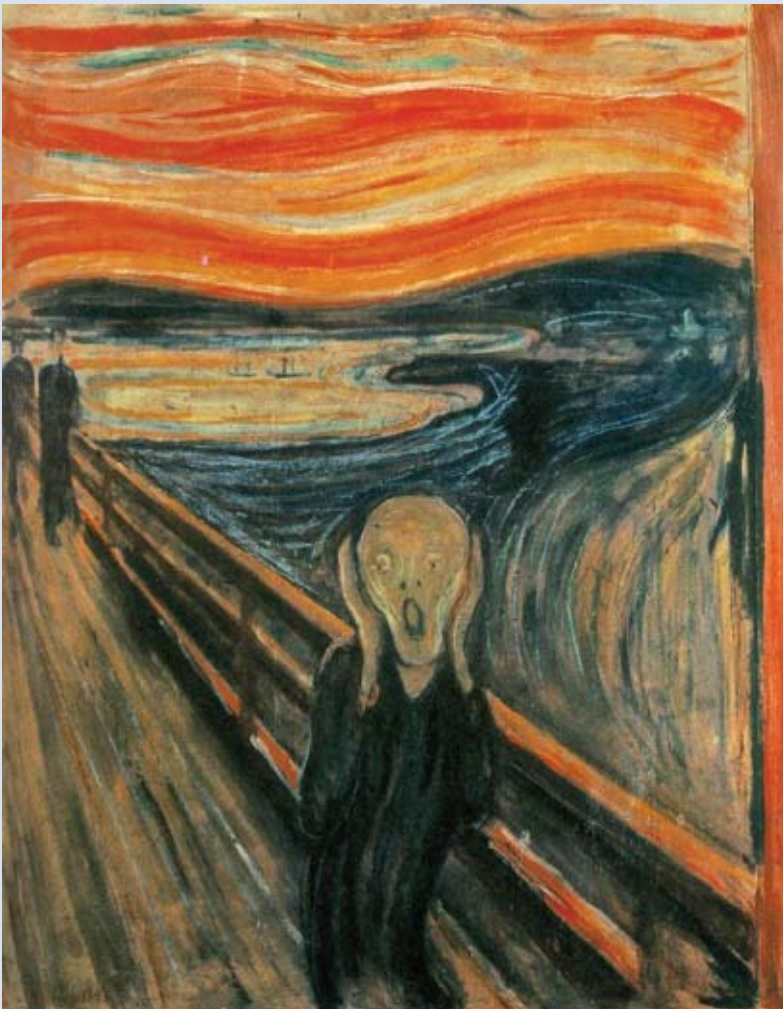
"We are gratified that our research observations have jumped the gap to the clinic and become part of the diagnostic testing we offer to patients," says Bai-Lin Wu, PhD, Director of the Genetics Diagnostic Laboratory at Children's and another senior author on the study.

Miller says knowledge about this genetic flaw and its role in autism susceptibility may lead to discoveries of the molecular pathway of autism that offer hope for treatment. When this pathway is identified and better understood, scientists may be able to design drugs that target chemicals in the brain in order to treat or prevent autism.

## Fear...or Fear Not

**P**ICTURE YOURSELF on a dimly lit city street. You pass by an alleyway and hear a sound—something coming toward you. Your heart races, your pace quickens, your palms get sweaty. Terrified, you start to run, only to turn around to see a cat come out of the alley. What made you react as if your life was in danger?

In a word: fear. Fear is a chain reaction in the brain that begins with a stressful stimulus and ends with the release of stress hormones, including adrenaline, that cause the “fight-or-flight” response.



First described in 1915 by Harvard physiologist Walter Cannon, the fight-or-flight response is designed to protect us from danger and is critical for our survival. When we react to a threat, our sympathetic nervous system primes us to either flee from danger or fight in self-defense. This includes physical reactions like increased heart and respiratory rates, intensified awareness, and quickened impulses. In addition, blood is directed toward the muscles in our limbs, which require extra energy to flee or fight. We become prepared—physically and psychologically—to either confront the threat, or to run away from it.

But, what is fear and what causes such an intense reaction, even when the situation is not dangerous? And how can this fear response be alleviated?

### The ‘hub of fear’

“The amygdala,” says Mohammed Milad, PhD, an assistant professor of psychiatry at Harvard Medical School and Massachusetts General Hospital, “is the hub of fear. All fear expression is generated by the amygdala, whether that fear is learned or innate.”

An almond-shaped cluster of nerve cells located deep within the brain, the amygdala is part of the limbic system, the brain’s center for emotions. The amygdala performs a primary role in the processing and memory of emotional reactions, including fear. The amygdala is connected to the ventromedial prefrontal cortex (vmPFC), which is involved in cognitive processes such as decision-making and the inhibition of fear.

Milad says there is a distinction between innate fear and acquired fear. “Some fear is genetically hardwired,” he says, “like a fear of snakes or spiders. It’s just part of your system.” Fear of snakes, for example, has been found in people who have never been exposed to snakes. This is, perhaps, an evolutionary instinct passed down genetically from a time when snakes were a greater danger to the human population.

On the other hand, acquired, or conditioned, fear is a method by which animals, including humans, learn to fear a new stimulus. It is a form of learning in which fear is associated with a particular neutral context. In the laboratory, this is done by pairing a non-threatening stimulus (a certain sound) with an aversive stimulus, such as an electrical shock.

“The amygdala forms an association with the neutral stimulus,” says Milad, “and attaches an emotion to it, in this case fear. When an animal is exposed to that stimulus, the fear response returns. The amygdala tags the association and triggers an emotional response, including increased heart rate, sweating, and muscle tension.”

Joseph LeDoux of New York University described fear as a two-part process, one taking a “low road” and the other taking a “high road.” The low road is a “shoot first, ask questions later” approach that initiates the fight-or-flight response. The high road is a more thoughtful process that allows you to consider all of the possible options for what a stimulus might be. That’s why you had a moment of terror before you realized it was only a cat coming out of the alleyway.

### **Studies explore fear mechanism**

Milad, who specializes in the vmPFC and fear extinction, and his colleagues have conducted numerous studies on fear and the human brain. In a 2005 study that was published in the *Proceedings of the National Academy of Sciences*, he and his colleagues found that the vmPFC is thicker in people who are better able to react to fearful situations.

Over a two-day period, 14 study participants sat in front of computer monitors, with electrodes attached to their fingers. On the screen, they viewed a picture of a room with either a red or blue light. A non-painful but annoying shock was administered when they saw the blue light. The next day, they viewed the same pictures, but without the electric shock. To determine anxiety and fear, the amount of perspiration on the palm of the hand was measured while the volunteers viewed the pictures. The researchers also used brain scans to measure vmPFC thickness. The participants who appeared to be less anxious upon viewing the blue light also had thicker vmPFC.

“That was the only area of the brain correlated with extinction memory,” says Milad. “So, these results suggest that a thicker vmPFC may be protective against anxiety disorders or that a thinner one may be a predisposing factor. But exactly how that might work, we just don’t know.”

A 2003 study by HMS researcher Scott Rauch, MD, president and psychiatrist-in-chief at McLean Hospital, found that people suffering from post-traumatic stress disorder, or PTSD, had smaller vmPFC, suggesting a link between a small vmPFC

and certain anxiety disorders. Milad takes this research further to show that people with PTSD have a dysfunction in fear extinction.

### **Therapeutic approaches to inhibiting fear response**

While scientists are still learning about the molecular mechanisms of fear, several steps are being investigated to inhibit the fear response in humans. At Emory University in Atlanta, scientists have discovered that a certain chemical reaction in the amygdala plays a crucial role in overcoming fear. When that chemical reaction is deactivated in mice, they are unable to counter their fears. The researchers found that D-cycloserine (DCS), a drug used to treat tuberculosis, strengthens this chemical reaction.

DCS also increases the activity of cells upon which fear extinction is dependent. Milad says studies show promising results using DCS in combination with exposure therapy, which involves reliving a traumatic experience in a controlled environment, to treat fear of heights or other phobias.

Scientists are also looking at ways to stimulate the activity of the prefrontal cortex, as they have done in rats, to counteract fear. Physicians have used deep brain stimulation to alleviate anxiety disorders like depression and obsessive-compulsive disorders, but Milad says this is a “very invasive procedure” that requires strict parameters for use.

In the long run, however, Milad says any new anti-fear approaches need to be combined with psychotherapy to improve outcomes. “At this time, I don’t think you can replace therapy,” he says, “but you can make the outcomes better [with newer techniques.] The aim is to accelerate the therapy and make it more effective for a longer period of time.”

## Sound Training May Help Dyslexic Children Before Reading Begins

COMPARED TO NORMAL readers, many children with dyslexia have difficulty processing fast-changing sounds. This prevents them from properly learning syllables when they first hear language and can lead to reading difficulties as the children develop. Scientists at Harvard Medical School recently found that sound training with computer exercises can rewire children's brains to correct this sound-processing problem and improve reading.

Children with developmental dyslexia have otherwise normal intelligence but have problems sounding out words. In the 1970s, scientists first introduced the concept that children with dyslexia may have difficulty processing sound.

In 2005, Nadine Gaab, PhD, of the Developmental Medicine Center Laboratory at Children's Hospital Boston, was part of a Stanford University research team that found that people who learned to play musical instruments as children can detect more subtle changes in language. The Stanford scientists suggested that musical training may help the brain distinguish between rapidly changing sounds, an ability that is key to understanding and using language, and sounds that are slow-changing.

"Studies show that musicians are much better at processing rapidly changing sounds than people without musical training," says Gaab, an assistant professor of pediatrics at HMS. "If musicians are so much better at these abilities and you need these abilities to read, why not try musical training with dyslexic children and see if that improves their reading?"

### Scanning for fast-changing sounds

In order to learn language, infants must be able to properly process fast-changing sounds like "ba" and "da." If they are unable to identify this pattern, their brains may process these sounds incorrectly. For example, they may hear a mixture of "ba," "ka," "ga," and "da" when someone says "ba."

Infants use sound processing to capture sounds from their native language to create a sound map that gets imprinted in their brain. This sound map may become confused if children cannot process fast-changing sounds. Thus, cognitive scientists believe, these children may develop reading difficulties when they first see printed letters

because their brains wire their internal sound map to the letters they see on the page. Linking normal letters to confused sounds may lead to syllable-confused reading, a hallmark of dyslexia.

In her laboratory, Gaab took this research a step further and, for the first time, used functional magnetic resonance imaging (fMRI) scans to examine how the brains of normal readers and children with dyslexia respond to sounds. She first tested how the children's brains responded to both fast-changing and slow-changing sounds. Fast-changing sounds are those that change in pitch or other acoustic qualities over tens of milliseconds, as in normal speech, while slow-changing sounds change over hundreds of milliseconds.

The fMRI scans showed that 11 areas of the brain, including an area in the left prefrontal cortex (PFC), of the normal readers were activated when listening to fast-changing sounds. In the children with dyslexia, however, fast-changing sounds did not trigger activity in the PFC. Instead, the sounds were processed as if they were slow-changing. The prefrontal cortex is, among other things, involved in language processing.

"We predicted that the typical-readers would have already developed the necessary neural network involved in the processing of rapid auditory stimuli," wrote Gaab and her colleagues in the journal *Restorative Neurology and Neuroscience*, "and that disruption of this neural response to rapid auditory stimuli would be seen in children with developmental dyslexia."

### Software improves dyslexics' reading skills

Gaab's research team used educational software called "Fast ForWord Language" to determine how the brains of 9- to 12-year-old children, including both normal readers and children with developmental dyslexia, respond to sounds both before and after using the software. Fast ForWord is a reading intervention product from Scientific Learning Corporation that helps children with learning disabilities build foundational reading and language skills. It helps to develop critical brain processing, including "strengthening auditory and linguistic processing rates so that students can distinguish sounds quickly enough to discriminate individual phonemes [the smallest units of sound from which words are constructed] and understand

words and sentences,” according to the company’s Web site. Paula Tallal, PhD, of Rutgers University, who co-authored the study with Gaab, helped design the software.

Rather than reading, the children listened only to sounds, including simple, changing noises that increased in pitch. The children responded by clicking their mouse when the pitch increased or decreased. Initially, the sounds were played slowly, an easy-to-distinguish task for the children with dyslexia, and then gradually sped up, producing more of a challenge.

After eight weeks of daily sessions (about 60 hours of training in all), Gaab and her colleagues found that the brains of children with dyslexia were more like those of normal readers when processing fast-changing sounds. In essence, she says, the training rewired the children’s brains, and their reading and certain language skills improved. However, the researchers do not know yet if this training produces long-term results or if it is only temporary.

Gaab’s findings suggest that effective remediation can foster neural plasticity that enhances how the

brain responds to fast-changing sounds and can improve reading and language skills, as well.

### **Catching dyslexia before reading starts**

Earlier studies have shown that acoustical training can help young children with reading difficulties by helping them pick out fast-changing sounds in syllables. Others have shown that musical training alters the brain’s language areas that process pitch and timing changes that are common to both words and music.

Gaab is now using fMRI studies to detect sound-processing difficulties at an earlier stage, hoping to catch dyslexia even before children learn to read. That way, clinicians can use sound training, similar to what was done in her study, to prevent future reading difficulties. She is also developing auditory and musical training programs (either singing or playing a musical instrument) to determine the relationship between these programs and language or reading development and whether musical training can improve reading in children with dyslexia.





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## Controlling Brain Receptors in the Fight Against Obesity

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obesity is a big risk factor for the disease. The discovery that defects in glucose-sensing by the brain may also contribute to type 2 diabetes could help lead to new therapeutic strategies for this widespread problem.”

Lowell says that POMC neurons produce certain molecules that trigger MC4R activity to suppress food intake. While drugs that stimulate MC4R exist, he adds, they produce side effects—in addition to weight loss—that limit the possible use of such medications.

“That’s where the basic science is trying to figure out what controls these neurocircuits,” he says. “The hope is to be able to develop new drug targets; however, at this time, there is no magic bullet that produces marked weight loss.”

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