

Editor's note: The following article appeared in the 28 April 2006 issue of Science magazine (www.sciencemag.org). We are grateful for permission to reprint it here. The article also included a sidebar (see page 26) on recent drug-based research to address symptoms of FXS being conducted by Dr. Stephen Warren at Emory University and Dr. Mark Bear of MIT. Drs. Randi Hagerman, Paul Hagerman, Stephen Warren, Ben Oostra and Elizabeth Berry-Kravis, all mentioned in the article, are either current or former members of the NFXF's Scientific and Clinical Advisory Committee, recipients of various NFXF awards, and/or recipients of NFXF research grants. In addition, Dr. Emily Osterweil, a member of Dr. Bear's lab, currently receives NFXF research funding.

Fragile X's

By Greg Miller

Unwelcome Relative



Randi (top) and Paul Hagerman

By studying the grandfathers of children with fragile X syndrome, scientists have found a surprisingly common neurological disorder that may be due to abnormal RNA

Randi Hagerman may be the only pediatrician to discover a disease that strikes in old age. Hagerman specializes in treating children with fragile X syndrome (FXS), the most common inherited form of mental retardation. Several years ago, she began to notice something odd when she chatted with her patients' parents. "Typically, the moms would bring the children in to see Randi, and in the course of the discussion, the moms would say, 'I'm concerned about my father. He's falling down a lot,'" says

molecular biologist Paul Hagerman, Randi's husband and research collaborator. "This was a pattern she would hear over and over."

At first, the Hagermans suspected this was nothing more than a few isolated cases of ataxia, or coordination problems. That changed in 2000, when Randi presented neurological workups of a small group of her patients' grandfathers at a Fragile X conference for researchers and parents. At the end of her talk, she asked if anyone in the audience had seen similar problems. "Of the mothers in the room, I would say a third of the hands went up," Randi says. "It was an epiphany of sorts," Paul recalls.

Follow-up studies by the Hagermans, now at the University of California (UC), Davis, and collaborators have

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recently documented a suite of symptoms that strike the relatives—most often the maternal grandfathers—of children with fragile X syndrome. These men are typically healthy early in life and have average to above-average IQ's. But in their 50s and 60s, many begin to experience tremors and movement difficulties that grow progressively worse. Studies have turned up cognitive and psychiatric problems in these men as well.

The symptoms are far more disabling than the general decline people experience with age, and they can lead to death.

The newly identified disorder, called fragile X-associated tremor/ataxia syndrome (FXTAS), may turn out to be one of the most common inherited forms of neurodegenerative disease. Work by the Hagermans and others has linked FXTAS to the same gene responsible for fragile X syndrome—even though the two disorders are drastically different. Researchers are now studying postmortem brain tissue from FXTAS patients and creating genetically altered fruit flies and mice in hopes of unraveling the disorder's underlying biology. Physicians are also documenting the clinical progression of

FXTAS, work that should help neurologists avoid misdiagnosing it—as happens often.

“At first, no one was quite sure this was real,” says Stephen Warren, a geneticist at Emory University in Atlanta, Georgia, and a co-discoverer of the genetic mutation that causes FXS. Doctors had always told relatives of children with the syndrome that they had no reason to expect health problems themselves and that their only risk was passing on a bad gene to the next generation. Now, says Warren, it’s clear that this counsel was misguided.

A PUZZLING PREMUTATION

Fragile X syndrome earned its name from the brittle appearance of the X chromosome in people with the disorder: Under a microscope, part of the chromosome looks as if it’s dangling by a thread. In 1991, researchers identified a mutated gene that resides in that part of the chromosome. A genetic stutter gives the gene, called FMR1, 200 or more repeats of the same sequence of three nucleotides: a cytosine followed by two guanines, or CGG. People without FXS have about 30 CGG repeats in FMR1, but 200-plus repeats disables the gene, and its protein, called FMRP, doesn’t get made. How the lack of FMRP causes mental retardation and other FXS symptoms isn’t clear, but researchers have recently gotten excited about a theory linking the deficit to aberrations of neural plasticity (see sidebar, page 26).

In some ways, the inheritance pattern of FXS sticks to the script every student learns in Genetics 101. Because a boy’s X chromosome always comes from his mother, he can only get a bad FMR1 gene from mom. And because they have only one X chromosome, boys who inherit the Fragile X mutation have no other way to make FMRP. But girls are complicated. Despite having a backup copy of FMR1 on their second X chromosome, girls can also develop Fragile X, although they tend to have less mental retardation.

Another puzzle about the genetics is that most mothers of FXS sons have fewer than 200 CGG repeats themselves. Instead, they carry a “premutation” with an intermediate number of repeats ranging from 55 to 200. Through some still-mysterious process, the number of repeats expands into the full mutation that causes fragile X syndrome when passed from mother to offspring. Men can also carry a premutation and pass it on to their daughters. (Only daughters inherit dad’s X chromosome.)

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Back in 1999, when Randi Hagerman started growing concerned about the maternal grandfathers of her patients, she consulted neurologist Maureen Leehey, a movement specialist at the University of Colorado Medical Center in Denver, where the Hagermans worked at the time. Several of the men had been told they had Parkinson’s disease, which involves degeneration of the basal ganglia, a brain region that helps execute movements. But Leehey’s neurological tests pointed to problems in a different brain region. The men did poorly, for example, on a test called the tandem gait test—the toe-to-heel walk police use to assess the sobriety of suspected drunk drivers. Parkinson’s patients do surprisingly well on this test, Leehey says, but the grandfathers could barely stand with one foot in front of the other, let alone walk in a straight line. That suggested a problem in the cerebellum, a structure at the back of the brain that’s important for balance and coordination.

Subsequent brain scan studies have confirmed this hunch, revealing shrinkage in the middle cerebellar peduncle, a major communication link between the cerebellum and brain stem. These studies have also found signs throughout the brain of degenerated white matter, the axons carrying signals from neuron to neuron.

What could cause axons to wither? The FMR1 gene isn’t silenced in FXTAS patients as it is in people with FXS; in fact, levels of the gene’s product, FMRP, appear to be nearly normal. That casts suspicion on the mRNA that translates the gene’s instructions into protein, says Paul Hagerman. In people with the premutation, FMR1 mRNA bears an unusually high number of CGG repeats just as the gene itself does. Unexpectedly, however, people with the premutation make five to 10 times more FMR1 mRNA than do those without it, Hagerman has found. “It’s a puzzle,” he says. “You’d expect it to go down, not up.”

In a 2002 paper in *Brain*, the Hagermans and colleagues reported that the brains of four men who died with FXTAS were riddled with tiny blobs of protein and other material. These “inclusions” clustered inside the nuclei of neurons and astrocytes, a type of support cell, and contained high concentrations of FMR1 mRNA. The team has now analyzed

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a total of 11 brains from FXTAS patients and found that those patients who had more CGG repeats in FMR1 had more inclusions and died at a younger age than did men with fewer repeats. The findings appeared in the January issue of *Brain*.

In a second study reported in the same issue, the Hagermans' team identified more than 20 proteins inside the inclusions. One, lamin A/C, is especially interesting, says Paul Hagerman. Lamin A/C is a filamentlike protein that among other duties supports the membrane forming the nucleus of a cell. Hagerman suspects that the CGG repeats make the FMR1 mRNA an unusually attractive binding target for various proteins, including lamin A/C. According to this theory, the mRNA sops up the proteins, preventing them from doing their usual chores inside the cell.

Indeed, adding FMR1 mRNA with extra CGG repeats to cultured human neural cells disrupts lamin, Hagerman and colleagues reported in the 1 December 2005 issue of *Human Molecular Genetics*. "Normally, you see a beautiful ring around the nuclear membrane when you stain for lamin," Hagerman says. "But when you express the repeats, the ring breaks down and just forms clumps." Hagerman says it's too early to say how lamin A/C disruptions might cause axon degeneration, but he notes that lamin irregularities have been implicated in another neurodegenerative disorder, Charcot-Marie-Tooth disease.

Other researchers agree that the sticky mRNA scenario is plausible. Many see a parallel with an inherited muscle disorder called myotonic dystrophy. In the most common form, the problem stems from a mutant gene whose mRNA bears abnormal repetition of the nucleotide sequence CTG. Various proteins glom onto the mRNA's repeat region and neglect their usual duties, causing the cells to malfunction. Although many inherited disorders are caused by a mutation that silences a gene (as in FXS) or results in a malformed, toxic protein (as in Huntington's disease), myotonic dystrophy is the only disorder known to be caused by abnormal RNA.

"The concept of RNA toxicity is really just emerging," says Emory geneticist Peng Jin. Like the Hagermans, Jin suspects that such toxicity is the root cause of FXTAS. In collaboration with Warren and others, he published a paper in *Neuron* in 2003 showing that expanded CGG repeats in FMR1 mRNA causes neurodegeneration in fruit flies. The flies also had inclusions in brain cells similar to those seen in FXTAS patients.

At the same time, researchers have begun studying the effects of FMR1 premutations in animals with nervous systems more closely resembling our own. Last year, Ben Oostra and colleagues at Erasmus University in Rotterdam, the Netherlands, described FXTAS-like symptoms in male mice with 98 CGG repeats in the gene. "If you look at the mice when they're young, there's no difference" between the mutants and their normal brethren, says Oostra. But by 1 year—middle age for a mouse—the mice with the premutation develop symptoms of ataxia, Oostra says. The Dutch researchers also reported in the 30 July 2005 issue of *Behavioral Brain Research* that these mice become unusually skittish and have memory deficits that grow worse with age—both features that have been described in people with FXTAS.

MISSED DIAGNOSIS

Physicians are still clarifying the symptoms of FXTAS in people. Recent studies have found that memory and cognitive problems often follow the ataxia and tremor, says Randi Hagerman. Some patients act as if they have frontosubcortical dementia, she and colleagues reported in the January issue of the *Journal of Clinical Psychiatry*. This type of dementia is characterized by difficulty controlling mental processes, and


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patients often have trouble formulating plans, focusing their attention, or knowing what's appropriate behavior. "We had one guy [with FXTAS] whose family told us when they went out for dinner, he went to the bathroom and came back with the toilet seat on his head as a joke," Hagerman says.

A three-center study, led by Paul Hagerman at UC Davis, Leehey in Denver, and Elizabeth Berry-Kravis at Rush University Medical College in Chicago, Illinois, will help nail down the symptoms of the disorder and describe how it progresses. A major goal, says Berry-Kravis, is to determine whether the number of CGG repeats predicts the severity and type of symptoms.

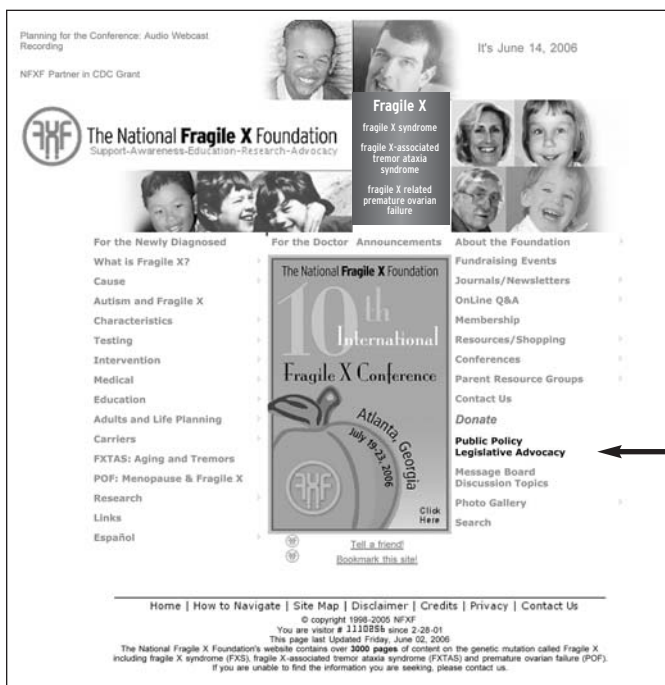
For men with the premutation, the prevalence of FXTAS increases sharply with age, from 17 percent of those in their 50s to 38 percent of those in their 60s to 75 percent of those 80 or older, the Hagermans and others reported in the *Journal of the American Medical Association* (JAMA) in 2004. The researchers estimated that the disorder will strike one in 3,000 men in the general population. (FXTAS appears to be very rare among women, although women with the premutation are susceptible to premature menopause for reasons that aren't understood.) If these calculations hold, FXTAS would be one of the most common neurodegenerative disorders linked to a specific gene, says Berry-Kravis, one of the authors of the JAMA study. Huntington's disease, which has been considered relatively common for this type of disorder, only strikes about one in 10,000 people, for example. Other disorders that have a genetic component but aren't tied to a single gene are far more common. Parkinson's disease falls into this category and affects about one in 100 people.

Misdiagnosing FXTAS as Parkinson's disease or another illness can lead to treatments that are futile or worse, notes Paul Hagerman. "I know of four cases where people had neurosurgery to implant shunts," he says. The patients were diagnosed with hydrocephaly because their brains had atrophied, making the fluid-filled ventricles deep in the brain look abnormally large.

The other reason patients need to know if they have FXTAS is the implications for genetic counseling, says Randi Hagerman. As awareness of FXTAS has grown, neurologists have begun to identify the disorder in men whose families include no one with fragile X syndrome, she says. Some of these men have daughters who may be thinking about starting families, Hagerman notes, and the pattern of inheritance means that all these women carry the premutation: "They didn't know they were carriers, and that's very important information for them." 

See related sidebar on page 26.

WHAT YOU CAN FIND AT www.FragileX.org



The screenshot shows the homepage of the National Fragile X Foundation. At the top, there is a banner for a conference recording from June 14, 2006. Below the banner is a navigation menu with several categories: 'For the Newly Diagnosed', 'For the Doctor', 'Announcements', and 'About the Foundation'. The 'Public Policy-Legislative Advocacy' option is highlighted with a black arrow pointing to it. The website footer includes copyright information for 1998-2005 and visitor statistics.

Our home page features "pop up" menus and submenus to help you navigate to the topic you are interested in learning more about.

Public Policy-Legislative Advocacy

Can single individuals make a difference with how the government thinks about and funds activities related to Fragile X? You bet they can, especially when they use The National Fragile X Foundation's "Public Policy-Legislative Advocacy" web pages accessed through our home page. It is no accident that both the National Institutes of Health and the Centers for Disease Control are providing funds for Fragile X research. Countless individuals, touched in some way by Fragile X, have made their concern known to their federal legislators. While some have been able to actually meet their legislators on Capitol Hill or in their home district offices, most have spoken up through the written word. That's where our website comes in. You will truly be amazed when you see how easy it is to identify the appropriate person or persons to write to and what you might say to them. You will also be able to read highly understandable descriptions of current and past legislation.

Exercise your rights as an American: Let your legislators in Washington, D.C. know what you want by visiting www.FragileX.org and selecting Public Policy-Legislative Advocacy. 