



Araw Nerve

Two recently discovered genes involved in ALS could reveal the pathways that are central to the neurodegeneration caused by the disease. But as some researchers storm ahead suggesting treatments targeting these genes, others are questioning how relevant the reports are for nonheritable forms of the disease. **Virginia Hughes** investigates why the new findings seem to have struck a nerve in the ALS community.

At a walkathon one Saturday in September, nearly 5,000 people traced two miles of Chicago's lakefront to raise money for research into the progressive nerve disease that is thought to have killed baseball star Lou Gehrig. Janice Caliendo was there collecting blood samples from friends of those affected by the incurable disease to be used as controls in future genetic studies. Caliendo, a lab manager at Northwestern Memorial Hospital in the Streeterville neighborhood of the city, often attends these sorts of fundraisers, but this time she was getting more attention than usual.

Her lab, headed by Northwestern University neurologist Teepu Siddique, has been all over the news recently for a study published in August in *Nature* reporting a new gene associated with the disease formally known as amyotrophic lateral sclerosis (ALS)¹. "Breakthrough could lead to effective treatment for Lou Gehrig's disease," read the *LA Times's* headline; "Cause of ALS is found, Northwestern team says," wrote the *Chicago Tribune*. In honor of the study, in fact, the event's organizers asked

Siddique to lead the walkathon. Countless people approached Caliendo that day with the same questions: Does this mean there's a cure? Is there a blood test for ALS? Is there a drug to treat it?

The answer to all these inquiries was 'no'. "It's not a cure, but people read into it what they want to hear," Caliendo says. "I don't think they were disappointed, though, because it's still very good news. It's huge."

The study, some two decades in the making, was certainly newsworthy: it uncovered mutations in a gene called *UBQLN2* that seemed to cause ALS in a handful of individuals with hereditary forms of the disease. But, according to Siddique, that's not even the exciting part. In the new paper, his team analyzed postmortem spinal cord tissue from dozens of people with different forms of the disease, including those who developed ALS spontaneously and didn't carry *UBQLN2* mutations. To their surprise, Siddique and his colleagues found abnormal blobs of the ubiquilin-2 protein encoded by *UBQLN2* in the neurons of every single individual they looked at.

In Siddique's view, his study proves that all forms of ALS converge on a glitch in protein recycling that results in the accumulation of many types of proteins and the death of motor neurons. It's similar, he says, to the discovery decades ago that people with a genetic disease called familial hypercholesterolemia carry mutations in a receptor for 'bad' cholesterol. On the basis of those data, researchers designed drugs—statins—that are now taken not only by those affected by the rare disorder but also by the majority of people with all forms of heart disease in the developed world.

"What we're showing here is a direct functional mechanism that causes disease," Siddique says. "It's not just another cause; it's not just another pathology; it's a game changer."

However, many of Siddique's colleagues worry that his statements extend beyond what the data show. "To those of us who live in this world, it's great that there's another new gene," says Jeffrey Rothstein, director of the Robert Packard Center for ALS Research at Johns Hopkins University in Baltimore. "But it's been way overblown."

Because of the media frenzy, the study ended up overshadowing two papers published exactly one month later that many ALS researchers find more significant but that didn't receive as much press coverage. The studies, published back to back on 29 September in *Neuron*, reported mutations in a region of chromosome 9 called *C9ORF72* that crop up in as many as one-third of all familial cases of ALS^{2,3}. In contrast, mutations in all of the other ALS-associated genes combined—including *UBQLN2*—only account for about 25% of all familial cases.

The *Neuron* studies, one of which included Rothstein as an author, bring the number of genes linked to familial forms of ALS up to 18, depending on how you count them. Yet most experts say the field is still far from understanding how any of these genes cause the disease—let alone how these genes relate to sporadic forms of ALS, which make up about 90% of all diagnoses. Plus, none of the genetic culprits are easy targets for drug development.

"There is a lot of excitement about [these discoveries], and nobody is more excited than I am," says Lewis Rowland, a neurologist at Columbia University Medical Center in New York. "But then there is this horrible fact that knowing the genetics of the disease doesn't help you treat the patients—either the genetic ones or the sporadic ones. ALS is just brutal as hell."

The long hunt

Siddique has been searching for the genetic causes of ALS for more than 25 years. Together with his wife Nailah, a clinical nurse at Northwestern, he has traversed the US in search of families plagued by rare familial forms of ALS, and, today, his collection includes more than 900 such lineages. "I've been in more homes of patients with ALS than I have been in friends' homes," he says. The effort led Siddique to the first causal gene ever discovered for the disease, *SOD1*, reported in 1993⁴—and, also, to Joanne Saltzman.

Saltzman's only memories of her father, Elmer, come from the few times she visited him in the late 1940s at the Mount Alto Veterans Hospital in Washington, DC. "He was not able to speak, he could only make noises and he was in a wheelchair," recalls Saltzman, now 72 and a retired office manager in Annapolis, Maryland. Elmer died at the age of 39 when Saltzman was just 9 years old of what doctors thought was multiple sclerosis. Saltzman had never heard of ALS until decades later when her sister started getting sick. The family contacted a genetic foundation, and, many years later, one of



Walk the walk: Teepu Siddique (left), shown here leading the ALS Walk4Life in September, has a bold new theory of the neurodegenerative disease.

Saltzman's close relatives put her in touch with Siddique.

From the get-go, Saltzman and her family have eagerly participated in research, donating blood, skin and postmortem tissue samples to Siddique and his colleagues. "It's been quite a worry for me to think about the rest of my family," she says. Saltzman's sister died three years after getting diagnosed, at age 39. Just over a year ago, ALS took her 51-year-old son, and, in February, it took her 52-year-old niece. She hopes that Siddique's research might one day help provide a treatment for her family's genetic curse. "I have six grandchildren and three other children," she says, "and you just never know when it's going to hit."

Tracking down medical history for bygone generations is tough, but Siddique got lucky with Saltzman's father. The US Armed Forces Institute of Pathology in Washington, DC had Elmer's autopsy records and slides of his brain and spinal cord tissue, which allowed Siddique's team to confirm an ALS diagnosis. By poring through death and medical records, Siddique traced the disease back to Elmer's maternal grandmother and filled in disease status for four generations of her descendants. The family tree holds at least 19 members with ALS. Because the pedigree had no examples of father-to-son transmission, Siddique suspected that the problem was on the X chromosome. His team eventually struck upon *UBQLN2* in July 2000 after sequencing

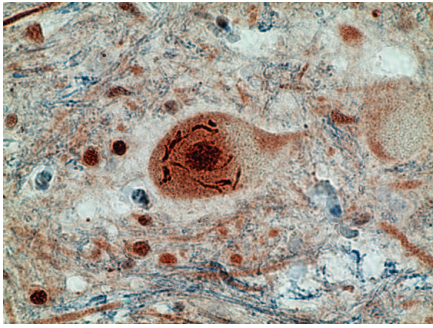
a total of 41 suspected X-chromosome genes.

The researchers knew that the mutation was highly causal in ALS-affected families: Saltzman, for example, is one of only two in her extended family who carry a *UBQLN2* mutation but do not have ALS. What Siddique didn't know at the time—and what he spent the next ten years investigating before publishing his results—was how relevant the gene might be to all forms of the disease.

ALS is characterized by coiled globs of protein in spinal cord motor neurons that have no known function. Siddique's team analyzed 47 postmortem spinal cord samples from people with several forms on ALS and healthy controls and showed that in all of the ALS cases, every protein aggregate tested positive for ubiquitin-2, a protein that helps move damaged proteins out of a cell. Ubiquitin-2 "seemed to be a common focal point downstream, no matter what the causation," Siddique says.

Working in mouse cell lines, Siddique's study also showed that *UBQLN2* mutations directly impair the cells' ability to clear away unwanted proteins. "This clinched it," he says. He's also made a mouse carrying a *UBQLN2* mutation that has "representative pathology and representative cognitive disturbances," he says. These data remain unpublished.

Siddique calls his study a "paradigm shift" that is bound to move the field toward more work on the protein degradation pathway. Yet



Han-Xiang Deng / *Nature* 477, 211–215 (2011).

Strike a cord: A spinal section with ubiquitin-2.

few other researchers take as bold a view of his findings. “Whether this protein is going to be ultimately found as *the* cause I think remains at the moment uncertain,” says Raymond Roos, a neurologist at the University of Chicago. “We really only have one paper.”

Many experts point out that protein recycling was implicated in ALS long before Siddique’s paper came out. In December, for example, a team led by Bryan Traynor, head of neuromuscular research at the US National Institute on Aging in Bethesda, Maryland, fingered a gene, encoding valosin-containing protein, that aids in the maturation of autophagosomes, cellular organelles that collect and destroy unneeded proteins⁵. According to Traynor, however, it’s unlikely that all of ALS will boil down to one pathway. “A motor neuron either works or it doesn’t,” he says. “The clinical picture might look very similar from case to case, even if the underlying cause was different.”

Traynor led one of the recent *Neuron* studies that connected *C9ORF72* to ALS. His team screened hundreds of cases of familial ALS and found that more than a third of the study subjects carried a six-letter string of DNA that repeated hundreds of times in this chromosomal region. The second *Neuron* study, led by Rosa Rademakers at the Mayo Clinic in Jacksonville, Florida, identified the same repeat in around a quarter of individuals with familial ALS, as well as in about 10% of people with a form of hereditary dementia. Traynor suspects that when the repeated stretch of DNA gets expressed, it results in loads of “toxic” RNA tangles. “These inclusions are very sticky, and they could bind up other RNAs, and other RNA-binding proteins, and basically muck up transcription that goes on within the cell,” he says.

Regulatory oversight

The two new *Neuron* studies aren’t the first to tie ALS to RNA regulation, though. In fact, this process has taken center stage in the field since 2006, when researchers

discovered that a protein called TDP-43 accumulates in the brain and spinal cord in most forms of the disease⁶. Later, researchers discovered mutations in *TARDBP*, the gene encoding TDP-43, in some families with ALS⁷. TDP-43’s function is murky, but one of its roles is to help transcribe DNA into RNA in the nucleus. “Right now in the field, the common belief is that ALS is likely a disorder of RNA metabolism,” says Michael Strong, a neurologist at the University of Western Ontario’s Schulich School of Medicine & Dentistry in London, Canada. And unpublished data from his lab suggest that this phenomenon goes well beyond TDP-43.

Last month at the International Congress of Human Genetics in Montreal, Strong reported that three different RNA-binding proteins in addition to TDP-43 form aggregates together in spinal cord motor neurons of people with both familial and sporadic forms of ALS. Thus, Strong proposes that these abnormal RNA regulatory proteins are the underlying cause of the neurodegeneration seen in people with ALS and that the misfolded proteins seen by Siddique and others could be a downstream consequence of RNA processing gone astray.

Siddique, however, maintains that the fundamental problem lies in the protein recycling system, which then could lead to the build up of various RNA-binding proteins such as TDP-43—not the other way around. He points out that in ALS samples from his *Nature* study all of the protein aggregates tested positive for ubiquitin-2, whereas only some also tested positive for TDP-43. “It illustrates the fact that there may be many etiologies, but they all seem to converge on ubiquitin-2 pathology as predicted,” Siddique says.

Some scientists—spurred on by Siddique’s paper—are investigating how TDP-43 and ubiquitin-2 may be working in tandem in ALS. “We’re all reductionists at heart. We all want to identify a simple linear pathway that causes motor neuron death, but that is almost certainly not the case,” says Randal Tibbetts of the University of Wisconsin–Madison. “When you express a mutant protein like TDP-43 or ubiquitin-2 for 40 or 50 years in a motor neuron, a lot of things can go awry.”

Unfortunately, most experts say that neither protein makes for an easy target for drug development. RNA regulation is crucial for all kinds of cellular workings, and researchers don’t yet understand which

parts of the process, if any, are specific to ALS. Protein degradation, meanwhile, involves more than 800 proteins in one way or another, and scientists don’t know much about the function of half of them, including ubiquitin-2. Worse, the field doesn’t agree on whether protein aggregation is a protective mechanism or the cause of disease. “So are you supposed to upregulate the proteasome system, or are you supposed to downregulate it?” asks Steven Perrin, chief executive of the ALS Therapy Development Institute, a nonprofit biotech in Cambridge, Massachusetts dedicated to finding treatments for the disease. “We can’t even answer that basic question.”

For the most part, these sobering realities haven’t found their way into the newspapers. “We’ve had to talk folks down to earth,” Perrin

says. Bob Baloh, a neurologist at Washington University’s Neuromuscular Disease Center in St. Louis, says that after Siddique’s paper came out he received calls from someone asking to enroll in a “clinical trial of ubiquitin-2” (confusing the gene with a new treatment) and from another ALS-affected individual who was reduced to tears when told that there is no ubiquitin-2 cure. “This

is a great discovery,” Baloh says. “But at the same time, it’s important to communicate it in a way that doesn’t give patients unrealistic expectations.”

But Siddique says that now is the time for optimism. With his new findings, “for the first time, we have a scientific and rational approach to treatment,” he says. “People who are pessimistic are those who have barked up the wrong tree.”

As for Saltzman, she realizes that a cure isn’t necessary imminent. But after watching so many of her relatives die from the disease, Siddique’s study has given her hope. “Thank goodness for Dr. Siddique, because at least he’s found something that maybe they can work on to eliminate this disease,” she says. “I don’t think it’ll happen in my lifetime, but maybe in my grandchildren’s.”

Virginia Hughes is a science reporter in Brooklyn, New York.

1. Deng, H.X. *et al. Nature* 477, 211–215 (2011).
2. DeJesus-Hernandez, M. *et al. Neuron* 72, 245–256 (2011).
3. Renton, A.E. *et al. Neuron* 72, 257–268 (2011).
4. Rosen, D.R. *et al. Nature* 362, 59–62 (1993).
5. Johnson, J.O. *et al. Neuron* 68, 857–864 (2010).
6. Neumann, M. *et al. Science* 314, 130–133 (2006).
7. Sreedharan, J. *et al. Science* 319, 1668–1672 (2008).