H1N1-triggered narcolepsy may stem from ‘molecular mimicry,’ Stanford study finds

STANFORD, Calif. — In genetically susceptible people, narcolepsy can sometimes be triggered by a similarity between a region of a protein called hypocretin and a portion of a protein from the pandemic H1N1 virus, according to a new study by researchers at the Stanford University School of Medicine.

The study provides some of the most compelling cellular and molecular evidence to date for a scientific concept known as “molecular mimicry.” Mimicry is the idea that the normal immune response to a pathogen, in this case the pandemic 2009 H1N1 influenza virus, can trigger autoimmunity — when the immune system mistakenly attacks healthy components of the body — because of similarity between a pathogen protein and a human protein.

In a 2009 study, Stanford researchers reported genetic evidence supporting the idea that narcolepsy, a debilitating disorder characterized by sudden, uncontrollable sleepiness and muscle weakness, occurs because the body’s immune system mistakenly destroys brain cells that make a “wakefulness” protein: hypocretin. The new study confirms that narcolepsy is an autoimmune disease.

“The relationship between H1N1 infection, vaccination and narcolepsy gave us some very interesting insight into possible causes of the condition,” said Emmanuel Mignot, MD, PhD, professor of psychiatry and behavioral sciences. “In particular, it strongly suggested to us that T cells of the immune system primed to attack H1N1 can occasionally also cross-react with hypocretin and somehow cause the destruction of hypocretin-producing neurons.”

The new study suggests new ways to try to intervene before complete destruction of the specialized brain cells. Their loss is the hallmark of the disease and leads to its dramatic symptoms. The study also could pave the way to a new blood test to diagnose narcolepsy. And it sheds light on a previously observed association between a pandemic H1N1 vaccine used in Europe in 2009 and an increase in narcolepsy cases in Scandinavia the subsequent year.
Mignot, a narcolepsy researcher and director of the Stanford Center for Sleep Sciences and Medicine, shares senior authorship of the research with Elizabeth Mellins, MD, an immunology researcher and professor of pediatrics at Stanford. The study will be published Dec. 18 in *Science Translational Medicine*. Postdoctoral scholars Alberto de la Herrán-Arita, MD, PhD, and Birgitte Kornum, PhD, share lead authorship of the study.

“This study will shape the next decade of research into narcolepsy. It will focus investigators on immune-mediated mechanisms of neuronal death, which ultimately may shed light on other autoimmune diseases, particularly of the brain,” Mellins said. “By giving us a new way to think about how neurons in these patients die, it also suggests new therapeutic approaches that we would not have considered if we hadn’t learned that this is an autoimmune disease.”

There are few effective treatments for narcolepsy, which affects about one in 3,000 people. Although the cause of the disorder has remained a mystery for many years, it has a genetic component: Nearly all people with narcolepsy express a particular subtype of immune-associated proteins called human leukocyte antigens. But relatively few people with this genetic signature, which is found in about 20 percent of the population, ever develop the disease.

This association with particular human leukocyte antigens, or HLA molecules, has caused researchers to wonder whether narcolepsy results from an autoimmune reaction to neurons in the brain that produce hypocretin — a small protein responsible for maintaining wakefulness in humans. These neurons (of which there are normally about 70,000) are missing in people with narcolepsy. But until now, no one has been able to demonstrate an immune response to the protein or the neurons that produce it.

The Stanford research also was influenced by unexpected increases in narcolepsy incidence. In 2010, a study in China showed an increase in sudden-onset narcolepsy in children with the narcolepsy-associated HLA signature who were living in areas in which
the then-novel pandemic 2009 H1N1 influenza virus had spread the previous year. At the same time, clinicians in Scandinavia noticed clusters of narcolepsy cases in children who had been vaccinated with Pandemrix, a newly developed anti-H1N1 vaccine (although the overall number of cases remained relatively small even among those with the susceptible HLA signature).

The Pandemrix vaccine mixed portions of viral proteins with a non-viral “adjuvant” to induce a stronger and presumably more effective immune response. All told, it may have precipitated narcolepsy in a few thousand cases in Europe. (About 31 million Europeans received the vaccination that year.) The Pandemrix vaccine was never used in the United States and is no longer used in humans. But the association gave the researchers some critical clues.

“This intersection of genetically susceptible people with a particular environmental trigger, in the form of the H1N1 virus or the Pandemrix vaccine, gave us a powerful scientific opportunity to begin to understand the molecular basis of narcolepsy,” Mellins said.

The researchers emphasize that there are still many, as-yet-unknown steps to developing narcolepsy. Around 20 percent of the general population shares the genetic predisposition to narcolepsy conferred by the unique HLA signature, but far fewer ever develop the condition. Furthermore, it was exceedingly rare for a child to develop the condition after receiving the vaccine (only about one in every 16,000 recipients).

The researchers homed in on T cells because of their association with the HLA signature found in nearly all narcolepsy patients. HLA molecules sit on the surface of specialized antigen-presenting cells and display small bits of proteins gathered from the cells’ environment. When these proteins are foreign, they are recognized by T cells, which begin to divide and patrol throughout the body to eradicate the invader. But sometimes they make mistakes.

“When we saw that the portion of the hypocretin that seemed to be recognized by the
immune system in narcolepsy patients was similar to a part of the pandemic 2009 H1N1 influenza hemagglutinin molecule, we were very hopeful that we were on the right track," Mellins said.

Specifically, the researchers found that a short, 13-amino-acid portion of the H1N1 hemagglutinin protein closely resembles two equally short segments of the hypocretin protein. T cells from narcolepsy patients, but not those from people without the condition, reacted strongly to the hypocretin protein segments. Furthermore, presenting the small portion of hemagglutinin to T cells from narcolepsy patients grown in a laboratory dish increased the proportion of hypocretin-reactive cells.

"Surprisingly, we also found hypocretin cross-reactive T cells in blood samples from narcolepsy patients collected before 2009, which is when H1N1 first began to circulate in humans," Mignot said. "This discovery implies that other viruses or pathogens could occasionally cause a similar confusion among the T cells. Indeed, there is a growing appreciation that cross-reactivity of immune T cell recognition may not be as uncommon as once thought. Although this cross-reactivity may make the immune system more adaptable to new infections, it may also increase the chance of mistakes that could result in autoimmune diseases."

Conversely, it's possible that vaccination with most flu vaccines or prior flu infections may provide a protective effect by giving the body the means to fight off an actual infection before cross-reactivity occurs.

Mignot, Mellins and their team plan to continue their studies to determine how T cell cross-reactivity to hypocretin can destroy the hypocretin neurons, and whether this process could be blocked to potentially prevent narcolepsy. They're also eager to know whether other HLA-associated brain disorders, such as schizophrenia, are linked to autoimmunity.

"People have long thought that the brain is somewhat immune to autoimmune diseases," Mignot said, "but we're learning this is wrong. Fortunately, narcolepsy seems to be a very
simple disorder to use as a model. There is one HLA molecule involved, and there may be only one target, hypocretin. It will allow us to learn so much more about human autoimmune disorders.”

Other Stanford authors include postdoctoral scholars Joshua Mahlios, PhD, Wei Jiang, PhD, Tieying Hou, MD, PhD, and Evan Newell, PhD; research associate Claudia Macaubas, PhD; research assistant Mali Einen; senior research scientist Ling Lin, PhD; and professor of microbiology and immunology Mark Davis, PhD.

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GlaxoSmithKline was the maker of the Pandemrix vaccine; Jazz Pharmaceutical is the maker of a narcolepsy treatment called sodium oxybate. Mignot has been a paid consultant for Jazz Pharmaceutical and GlaxoSmithKline. He, Mellins and Kornum are inventors on a patent to use the hypocretin epitopes for narcolepsy diagnosis and to modify the pHA1 2009 epitope in influenza vaccines. Stanford owns the intellectual property rights for narcolepsy diagnosis, and GlaxoSmithKline owns the rights for vaccine improvements.

Information about Stanford’s Department of Psychiatry and Behavioral Sciences, which also supported the work, is available at http://psychiatry.stanford.edu.