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Electrical brainstorms traced to genetic mutations

Researchers from Seattle Children's and Novartis shed light on developmental brain disorders that lead to intractable epilepsy.

By Alyssa Kneller | May 16, 2016

Electrical signals pulse through the gray matter of your brain, allowing you to read and understand this sentence. The cerebral cortex—home to your gray matter—is packed with more than 20 billion neurons, which are organized into circuits.[1] Collectively, these circuits are the seat of human cognition. And the results can be dire when they don't form properly.

Take children with a disease called focal cortical dysplasia (FCD). Born with an enlarged, disorganized area of the cortex, these patients often experience seizures, brainstorms of uncontrolled electrical activity that can lead to developmental delays and disabilities. In fact, FCD is the most common cause of intractable epilepsy in children.[2] For years, researchers speculated about the underlying mechanisms of the disease. Could a virus be triggering the brain overgrowth? Or maybe the tissue had been bumped and bruised during pregnancy?

Scientists now believe that many cases of FCD have genetic roots. Collaborators from Seattle Children's Research Institute, the Novartis Institutes for BioMedical Research and other organizations recently traced four cases of FCD to genetic mutations, publishing their findings online in JAMA Neurology. Specifically, the team identified mutations in a molecular pathway called mTOR, which plays an essential role in regulating cell growth. The researchers also found MTOR mutations in six patients with more widespread, diffuse brain overgrowth. The discovery bolsters a growing body of evidence that such diseases can be genetic and suggests new treatment approaches.

"We found that there are genetic changes in the brain tissue of these patients and showed that the changes are related to the structural abnormalities that occur in the brain," says first author Ghayda Mirzaa, a physician-scientist at Seattle Children's Research Institute. "Now we have a chance to test molecularly-targeted therapies in epilepsy."

Clues in patient tissue

Mirzaa's colleague William Dobyns, last author on the new study, began building a registry of patients with FCD and other brain overgrowth disorders in 1990. The goal was to learn more about them and identify new therapeutic approaches.

By 2012, the research team at Seattle Children's Hospital, in collaboration with the neurosurgical team, led by Jeff Ojemann, had gathered tantalizing clues by studying brain tissue from the patients. Some children with the disorders undergo epilepsy surgery, a treatment of last resort and a source of invaluable samples. If patients fail to respond to anti-epilepsy medication, then neurosurgeons may remove the portion of the cortex that's generating abnormal electrical activity in an attempt to block further seizures. This precious tissue gives scientists a window into the disorders.

Biochemical tests indicated that the mTOR pathway was overactive in many of the samples. DNA sequencing revealed mutations in key components of the pathway, but only in patients with diffuse brain

overgrowth. The mutations didn't show up in any patients with FCD.

Mirzaa and Dobyns, who are also clinical geneticists at Seattle Children's Hospital, suspected that the mutations were simply hiding due to a quirk of biology. High school students learn that an individual's DNA is determined when sperm and egg meet. But this is an oversimplification. While DNA is generally replicated faithfully as cells divide in a developing embryo, there are some exceptions. As a result, two or more populations of cells with different DNA can exist in the same organism, a phenomenon known as mosaicism.

The Seattle Children's researchers wondered if there was a small population of neurons with mTOR mutations in FCD patients. Perhaps the population was so small that the mutations weren't registering with standard DNA sequencing techniques.

Uncovering hidden mutations

Luckily, Wendy Winckler's next-generation sequencing group in Oncology at Novartis had the tools to test the hypothesis.

"Being a cancer sequencing lab, we specialize in finding mutations that only occur in a small fraction of cells," says Winckler. "A tumor is a mix of normal cells, immune cells and cancer cells, so we have to be able to detect low-level mutations in samples."

Zeroing in on protein-coding genes, her group performed deep sequencing on the samples, reading tens of thousands of cells in each one. The team also sequenced tissue from the patients' parents and from the periphery of the patients (blood, saliva or skin) so that they had a basis for comparison. Bioinformatician Katie Campbell then analyzed the data.

"We've tuned our software to catch mutations that occur in less than 5 percent of the cells," explains Campbell. "We also have experience finding mTOR mutations because they're relatively common in tumors, given that the pathway regulates cell growth."

Campbell analyzed samples from eight patients with FCD and their parents. She identified mTOR pathway mutations—including genetic lesions identical to those seen in cancer patients—at a low level in four of the FCD patients. Researchers at Seattle Children's Hospital and the University of Washington Genome Sciences Center used targeted sequencing and deep sequencing to screen 93 additional children with unexplained FCD or diffuse brain overgrowth. They found mTOR pathway mutations in six of the patients with diffuse brain overgrowth, suggesting that it's related to FCD.

In parallel, Novartis scientists within the Developmental & Molecular Pathways and Neuroscience groups set out to determine exactly how the mutations affect brain cells. Carleton Goold, Sue Menon and their teams introduced them into rat neurons, which proceeded to grow very large. The researchers also tested mTOR pathway activity in the neurons and confirmed that it was elevated.

The final step was to rescue the swollen cells. When the team applied an mTOR inhibitor to the mutant neurons, the cells shrank to a healthy size, pointing toward a potential therapeutic strategy for patients.

"This pathway is extremely well known in the cancer space, but now it's coming up as an important target in neuroscience," says Leon Murphy, who led the validation effort at Novartis. "I think that we're going to see mTOR popping up in other areas as well. It might be possible to repurpose cancer drugs for these diseases based on preclinical data and potentially provide patients with more options at some point."

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Researchers introduced an mTOR mutation identified from focal cortical dysplasia patients into these rat neurons. The neurons are enlarged, similar to what is seen in brain tissue from the patients. Image by Jonathan Biag/Novartis

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1. Pelvig, D. P., Pakkenberg, H., Stark, A K., and Pakkenberg, B. (2008). Neocortical glial cell numbers in human brains. Neurobiol. Aging 29, 1754–1762.
2. Kabat, J. and Krol, P. (2012). Focal cortical dysplasia – review. Pol J Radiol. Apr-Jun; 77(2): 35–43.
