RUBEN SALAZAR JR

When doctors couldn't explain to Tracy Dixon-Salazar why her daughter had developed epilepsy she decided to find out for herself.

GENETICS

Complex expressions

Epilepsy is one of the most common neurological disorders to affect the human brain. Many genetic aspects of the disease have been identified, but mechanisms remains elusive.

BY CHARVY NARAIN

Some kinds of epilepsy are rooted in physical trauma — a brain injury at birth, perhaps. Others seem to show up like bolts from the blue, with no clear event to explain them. Yet one thing is clear: many cases of idiopathic epilepsy — epilepsy without an obvious physical cause — run in families, implicating heredity in their genesis. Indeed, studies[1] suggest that genetic variations in ion channels on the surface of neurons — electrically excitable cells in the nervous system — might lie at the heart of many cases of idiopathic epilepsy, and presumably cause the firing of these cells to get out of control.

But there is still much that scientists do not know about the genetics of idiopathic epilepsy. Currently, a gene variant can only be associated with 10–40% of patients, according to Holger Lerche, who researches the genetics of neurological disorders at the Centre for Integrative Neuroscience in Tübingen, Germany. "But actually, almost all idiopathic epilepsy is likely to be genetic, so this figure should be closer to 80–100%," he says. "The problem is that we haven't identified all disease-associated genes just yet — nor how they interact."

Adding to the complexity of the puzzle are the facts that two-thirds of healthy individuals carry a gene variant associated with epilepsy[2], that many genes pinpointed by genetic analyses are also implicated in other disorders, and that epilepsy often co-occurs with other diseases — 30% of children with autism also have epilepsy, for example. This makes it hard to connect a given genetic variant in a patient to one specific disease. Epilepsy is a complex genetic disorder involving interplay between many genes, often in unexpected ways.

When Tracy Dixon-Salazar's two-year-old daughter Savannah had her first seizure she thought that her child was choking. "When the paramedics told us that Savannah's symptoms sounded like a seizure, I didn't know what that was," she says. By the time she was three, Savannah had epileptic seizures every day, and by the age of five she was having hundreds. Her physical and mental development regressed dramatically, and she needed round-the-clock care. "Throughout it all," Dixon-Salazar says, "I really wanted to know why. Nobody could give us an explanation for how a child could go from being a healthy two-year-old to being completely taken over by epilepsy."

Dixon-Salazar's hunt for an explanation took her from being a stay-at-home mother, who had left full-time education after high school, to getting a PhD in neurobiology from the University of California, San Diego.

Along the way, "I fell in love with genetics," she says. As part of her postdoctoral work, she sequenced Savannah's exome — the part of the genome that encodes proteins — and finally found something approaching an explanation, hidden within an ion channel.

CHASING CHANNELS

As the name suggests, ion channels are conduits; they sit on the largely impenetrable surface of a neuron and selectively let in ions — charged particles that trigger a cascade of events that lead to the neuron producing a burst of electricity, called an action potential.

Different ion channels let in different kinds of ions, and mutations in the genes that carry instructions for making these channels have been correlated with epilepsy. These faulty channels then let in too many or too few ions, disturbing the way that the action potential is generated and how it travels down the neuron. Inheriting these faulty-channel genes leads to abnormalities in brain-cell firing.

Although Savannah Dixon-Salazar had no family history of the disease, an analysis of her genome turned up 25 ion-channel variants that mostly — based on the channels they affected, and how — would probably cause too many calcium ions to be let into the cell. And so, working with Savannah's doctors, her parents decided to try treating her with verapamil, a drug approved by the US Food and Drug Administration for treating heart arrhythmia, not epilepsy, and which disrupts the functioning of an ion channel that lets in calcium.

"The effects were startling and immediate," says Tracy Dixon-Salazar. "Within 30 days, Savannah's seizures reduced from 300 a month to around 50, and currently average around 20 to 25 a month. They now happen only at night, so she is much less likely to hurt herself." She adds that her daughter's intellectual development has come along greatly, and "she has gone from struggling to speak a word to speaking 10- to 15-word sentences easily".

Dixon-Salazar acknowledges that these results are anecdotal and that verapamil is unlikely to be a general epilepsy treatment. But it could help some. And there is a lesson there, says Ivan Soltesz, a neurobiologist and epilepsy researcher at the University of California, Irvine. "These results highlight the fact that even if you don't know exactly what is wrong, and the exact pathway involved, genetic analysis can still help with treatment," he says.

INTRICATE INTERACTIONS

DNA variants affecting the calcium ion channel seemed to lead to Savannah's epilepsy. Lerche's work has implicated a different ion channel, which he does not want to describe before publishing his research. His team has found that when two variations of the same channel gene — one known to occur in the normal population and one novel variant found only in an epilepsy patient — came together, the channel's function changed. Individually, the mutations did not alter the channel's function. "This suggests that the patient's epileptic seizures are due to the occurrence of both variants occurring simultaneously," Lerche says.

Sometimes, however, adding epilepsy-related gene variants together does the opposite of cause disease — it dampens it. In a 2007 paper[3], a group led by epilepsy researcher Jeffrey Noebels of the Baylor College of Medicine in Houston, Texas, expressed two different epilepsy-associated ion-channel gene variants in the same mouse. What they got were animals with no, or reduced, epilepsy symptoms. The mutations had opposite effects on how likely a neuron is to fire an action potential. Disruption of the *Kcna1* gene, which encodes a particular class of potassium ion channels, normally leads to large increases in neuronal electrical excitability and subsequent epilepsy; on the flip side, malfunctions in the calcium ion channel encoded by the *Cacna1a* gene result in reduced neurotransmitter release and absence seizures (when the affected individuals 'blank out'). When expressed together, the two genes seemed to compensate for each other, reducing the serious seizures and death that the *Kcna1* mutants experience and masking the absence epilepsy of animals with *Cacna1a* mutations.

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Savannah (left) has around 20 seizures a month.

"Savannah has gone from struggling to speak a word to speaking ten-word sentences easily."

Noebels' studies also show that gene variants associated with epilepsy are common in the general population — and that the cause of the disorder is far less simple than the total number one has. In a study published in 2013, his group compared ion-channel genes between healthy people and people suffering from idiopathic epilepsy — and found that nearly 67% of the healthy group had mutations in at least one gene variant known to be linked to a familial form of epilepsy.[2] What's more, they found no gene variants that turned up only in the people with epilepsy. One of the healthy people in their study even had seven mutations in genes associated with epilepsy — just two less than the nine mutations detected in the most extreme epilepsy patient in the group. Having many gene mutations, it seems, is not a predictor of whether someone will be affected by the condition.

MODELLING MECHANISMS

Some researchers think that bioinformatics analyses — using computer simulations to look at the intricate interplay between epilepsy-associated genes — might reveal the complex genetic communication that determines who gets the disease and who doesn't. "The aim is to build a cellular map of epilepsy, including as many known epilepsy-associated genes as possible," Lerche says. "We can then use simulations to track how interactions between genes are happening."

For example, ion-channel expert Steven Petrou at the Florey Institute of Neuroscience and Mental Health in Parkville, Australia, used computational modelling to study the effect of sodium-channel mutations in a theoretical network of neurons mimicking epilepsy[4]. The results suggest that ion channel mutations may be more severe in their effects in a brain that is already abnormal. Epilepsy may initially be triggered by an external event such as a head injury or stroke — or, in a double whammy, ion-channel mutations may trigger the disease and once it is established, have an even greater effect in the altered brain than they did to begin with. It is a point not missed by Dixon-Salazar. "Savannah seized for 16 years before we found a drug that would make an impact on her seizures," she says. "The brain she had before the seizures is totally different from the one that she had afterwards. Perhaps if we had given the drug earlier, she might now be entirely seizure-free."

Noebels hopes that efforts to build computer simulations of the entire mammalian brain will help researchers to understand the complex network of interactions in the abnormal brain. "It takes a village to cause epilepsy," he says — myriad cellular factors must come together to result in disease. Eventually, the conversations between the villagers might be best tracked inside a computer. ■

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