NEWS

Mouse models point to early troubles in tuberous sclerosis

BY **SARAH DEWEERDT**

28 NOVEMBER 2011

Giant result: Researchers have produced a mouse model of tuberous sclerosis (center panels) that has distinctive giant cells with (black arrows) and without (white arrows) vacuoles, similar to those seen in the brains of children with the disorder (right panels).

Giant result: Researchers have produced a mouse model of tuberous sclerosis (center panels) that has distinctive giant cells with (black arrows) and without (white arrows) vacuoles, similar to those seen in the brains of children with the disorder (right panels).

The brain abnormalities characteristic of tuberous sclerosis may begin early in development and involve malfunctioning of neuronal precursors, according to studies of two different mouse models of the disorder published online in October.

Tuberous sclerosis is caused by a mutation in either **TSC1** or **TSC2**, both of which help regulate the synthesis of a large number of proteins. Individuals with tuberous sclerosis have epilepsy, intellectual disability and benign tumors called tubers in their brains and elsewhere in the body. About **40 percent of these individuals also have autism**.

“I think tuberous sclerosis overall is either a stem cell disorder or a progenitor cell disorder,” says **Kevin Ess**, assistant professor of neurology and pediatrics at Vanderbilt University in Nashville, Tennessee, who led one of the new studies.

Both new mouse models involve genetic manipulations that are different from the mutations seen in people with tuberous sclerosis, notes **Alcino Silva**, professor of behavioral neuroscience and psychology at the University of California, Los Angeles, who was not involved in either study.

But that’s not a criticism, Silva says. “The point of models is you isolate one variable and you study it,” he says. “I think that they’re both valuable efforts that add to what we know about tuberous sclerosis.”

Necessary, but not sufficient:

In people, tuberous sclerosis usually occurs when one copy of either TSC gene is mutated. By contrast, the mouse model created by Ess’ team completely lacks TSC1, but only in one type of neurons — those that produce the chemical messenger gamma-aminobutyric acid (GABA).

About half of these mice die within a few days of birth, the researchers reported in *Cerebral Cortex*[1]. The mice that survive have fewer GABA-releasing cells in the cerebral cortex and in the hippocampus compared with controls.

GABA is an inhibitory **neurotransmitter** that dampens the firing of nerve cells. “There’s a long history of the potential involvement of [GABA-releasing] interneurons in psychiatric disorders,” Silva notes.

GABA-releasing cells have been implicated in both **epilepsy** and autism, which many scientists believe involves an **imbalance between excitatory and inhibitory signaling** in the brain.

There are also some hints that GABA plays an important role in tuber-induced seizures in people with tuberous sclerosis.

“Not all tubers are created equal,” Ess says. “Often we find that there is one, maybe two, of the tubers that are really the most aggressive seizing ones.” Unpublished data from Ess’ lab suggest that the tubers that are most involved in causing seizures have low levels of GABA activity.

The new mouse model data support an important role for GABA in tuberous sclerosis, “but it wasn’t a slam dunk,” Ess says. Mice lacking TSC1 in GABA-releasing cells don’t have spontaneous seizures. But flurothyl, a seizure-inducing drug, produces seizures more rapidly in these mutant mice than in controls.

Some other mouse models of tuberous sclerosis, in which TSC genes are disrupted in multiple

types of cells, do have spontaneous seizures. Together, these models suggest that “the GABAergic cells are playing an important role, but by themselves are not sufficient [to cause epilepsy],” Ess says.

Giant effects:

In the second new study, **David Kwiatkowski** and his colleagues created a mouse with an on-off switch for the TSC1 gene, enabling them to turn the gene off at different points in embryonic development.

Ess, who was not involved in this study, calls the approach “elegant,” because the researchers could “control both in space as well as in time the gene’s expression.”

The effects of TSC1 loss vary depending on exactly when the gene is switched off, the researchers reported in the *Proceedings of the National Academies of Sciences*[2].

When TSC1 is turned off on day 8 of embryonic development, for example, mice lose the protein in some cells of the thalamus, brainstem and spinal cord. By contrast, turning TSC1 off on day 13 or 16 results in the loss of TSC1 from about half of all cells in the cerebral cortex, and produces features intriguingly similar to those of tuberous sclerosis in people.

These mice have spontaneous seizures, for example. By the time they are 6 months old, their brains also contain giant cells similar to those seen in people with tuberous sclerosis. It’s the first time a mouse model has reproduced these characteristic cells.

“We also looked at those giant cells in a lot of detail and found that they had a number of abnormalities,” says Kwiatkowski, professor of medicine at Harvard Medical School.

Like those in people with tuberous sclerosis, the giant cells in the mice have abnormal mitochondria, the energy-producing structures in the cell, and far too many of them. The cells’ protein-transporting machinery, or endoplasmic reticulum, also appears to be under stress.

The mouse giant cells also contain large vacuoles. “There’s sort of an empty spot in the cell, which is not normal,” says Kwiatkowski. The researchers looked at tubers surgically removed from people with tuberous sclerosis, and found that some of them contain cells with similar vacuoles.

The precise sequence of events that lead to giant cells in mice and in humans isn’t yet clear, notes **David Sulzer**, associate professor of neuroscience at Columbia University in New York, who was not involved in the work. Still, Sulzer says, “I don't think you would come up with these giant cells just by accident. I think it’s a big step.”

The researchers also showed that treating these TSC1-deficient mice starting at about 1 week of

age with rapamycin prevents the formation of giant cells as well as seizures and other symptoms.

Rapamycin, a drug in clinical trials to treat people with tuberous sclerosis, inhibits a protein called mTOR. In turn, mTOR's activity is regulated by TSC1 and TSC2.

Ess' mouse model also adds weight to the view that mTOR is central to tuberous sclerosis: In those mice, GABA-releasing cells that lack TSC1 also show increased activity of one form of mTOR.

Both groups of researchers are conducting behavioral studies of their mice to determine whether they have characteristics related to autism.

This line of research may also illuminate autism not associated with tuberous sclerosis, Sulzer says. "A surprising number of [forms of autism] seem to be converging on mTOR-related pathways."

References:

1: Fu C. *et al. Cereb. Cortex* Epub ahead of print (2011) **PubMed**

2: Goto J. *et al. Proc. Natl. Acad. Sci. U. S. A.* **108**, E1070-E1079 (2011) **PubMed**