NEWS

Mouse study questions autism gene's link to head size

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Deleting KCTD13, a gene in the autism risk region 16p11.2, has little effect on brain or head size in mice, according to a new study[1].

The results contradict a prominent 2012 study suggesting that **KCTD13** levels control head and brain size in zebrafish[2].

“We were very surprised when we didn’t see changes in brain size or cell proliferation,” says lead investigator **Craig Powell**, Ed and Sue Rose Distinguished Professor in Neurology at the University of Texas Southwestern in Dallas.

However, the study reveals a new function for KCTD13: It limits the levels of an enzyme called RhoA, which controls the **construction of the cell skeleton**. Loss of KCTD13 increases RhoA, decreases the number of **synapses** — the connections between neurons — and stifles communication among the cells. Blocking RhoA with a drug restores neuronal communication. The results appeared 1 November in *Nature*.

The study also suggests it is unlikely that there is a **single gene** in the 16p11.2 chromosomal region that accounts for all of the traits seen in people with a deletion of the region.

“It’s probably not KCTD13 alone modulating all the phenotype of the 16p11.2 rearrangement,” says **Alexandre Reymond**, director of the Center for Integrative Genomics at the University de Lausanne in Switzerland, who was not involved in the study.

Heads up:

About **1 percent of people with autism** have a deletion or duplication of **16p11.2**. People with a

deletion of the region tend to have enlarged heads, whereas those with a duplication tend to have small heads.

This feature is thought to be largely due to KCTD13. The 2012 study found that suppressing KCTD13 expression leads to head enlargement in zebrafish, whereas enhancing the gene's expression results in unusually small heads. Suppressing KCTD13 also leads to excessive growth of new neurons in the brains of developing zebrafish, according to that study.

Powell and his colleagues sought to replicate these results in mice but wanted to mimic the gene's loss, rather than suppression, in people. So instead of suppressing the gene, they bred mice missing one or both copies of it.

At 12 weeks, the brains of these mice weigh the same as those of controls. Brain scans in 7-day-old and 12-week-old mice revealed no differences in the size of the brain overall or that of multiple brain regions. Analyses of brain tissue samples from embryonic, juvenile and adult mice showed no changes in how neurons form, multiply or migrate to their ultimate locations.

The discrepancy between these findings and those from the 2012 study may have to do with the different approaches used to eliminate KCTD13, says **Nicholas Katsanis**, director of the Center for Human Disease Modeling at Duke University in Durham, North Carolina, and lead investigator on the 2012 study. "The big question is, is this a technical difference or is this a biological difference?" says Katsanis. "I frankly think we do not know."

Mild effects:

To rule out any species differences, Powell and his colleagues also deleted KCTD13 from zebrafish. But once again, they saw nothing amiss.

Powell says it may be that the brain responds differently to transient suppression of KCTD13 than it does to permanent loss of the gene. Katsanis says **genetic background** could also explain the inconsistency: He says he tried suppressing KCTD13 in a different strain of zebrafish and found no effect on head size.

Katsanis saw only mild effects on head size with KCTD13 alone, and more pronounced ones when his team enhanced the expression of two other genes in the 16p11.2 region. Those genes are involved in a separate pathway mediated by protein called RAS.

"Maybe [this] pathway, in addition to the KCTD13/RhoA pathway, is required to get more dramatic phenotypes to head and body size," says **Lilia Iakoucheva**, associate professor of psychiatry at the University of California, San Diego, who was not involved in either of the studies.

Removing KCTD13 alone in mice has only a subtle effect on the animals' behavior. Apart from

hyperactivity, the animals behave much like controls do in terms of their grooming, motor skills and social behavior.

Skeleton crew:

The researchers also looked for effects of missing KCTD13 at a cellular level.

KCTD13 is part of a complex that degrades RhoA. By inactivating RhoA, it could boost neuronal connections by helping neurons to form or stabilize their signaling branches.

Powell and his team measured neuronal signaling in the hippocampus of mice lacking one or both copies of KCTD13. The electrical response in brain slices from the mutant mice is 50 percent lower than it is in controls, suggesting a dearth of synapses. Slices from the cerebral cortex, the brain's outer layer, give similar results.

The mutant mice have shorter and fewer dendrites, the neuronal branches that receive signals. They also show an increase in RhoA levels as early as 8 days after birth. Treating brain slices from these mice with either rhosin or C3 toxin, two compounds that block RhoA, normalizes the response to stimulation.

The findings suggest that the loss of KCTD13 alters neuronal connections through activation of RhoA. The results also hint that drugs may be able to compensate for the KCTD13's absence.

"That gives us one potential mechanism whereby KCTD13 alters brain function in 16p patients," Powell says. "Inhibiting that might be a strategy to correct brain function."

However, the results suggest the loss of KCTD13 has no effect on RhoA until after birth: That timing is at odds with the results of a 2015 study[3].

In that study, researchers found that CUL3, a key KCTD13 binding partner, is expressed midway through fetal development. By extension, KCTD13 loss should affect RhoA around the same time, says Iakoucheva, who led the 2015 study.

"Maybe mice are different from humans," Iakoucheva says. She and her colleagues are looking at the effects of KCTD13 loss in brain organoids derived from human cells.

In the meantime, Powell and his team plan to treat KCTD13 mice with rhosin to see if it normalizes the length and number of dendrites in the brain. They also plan to test the drug in mice missing a copy of 16p11.2.

REFERENCES:

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