NEWS

Mice made with CRISPR usher in new era of autism research

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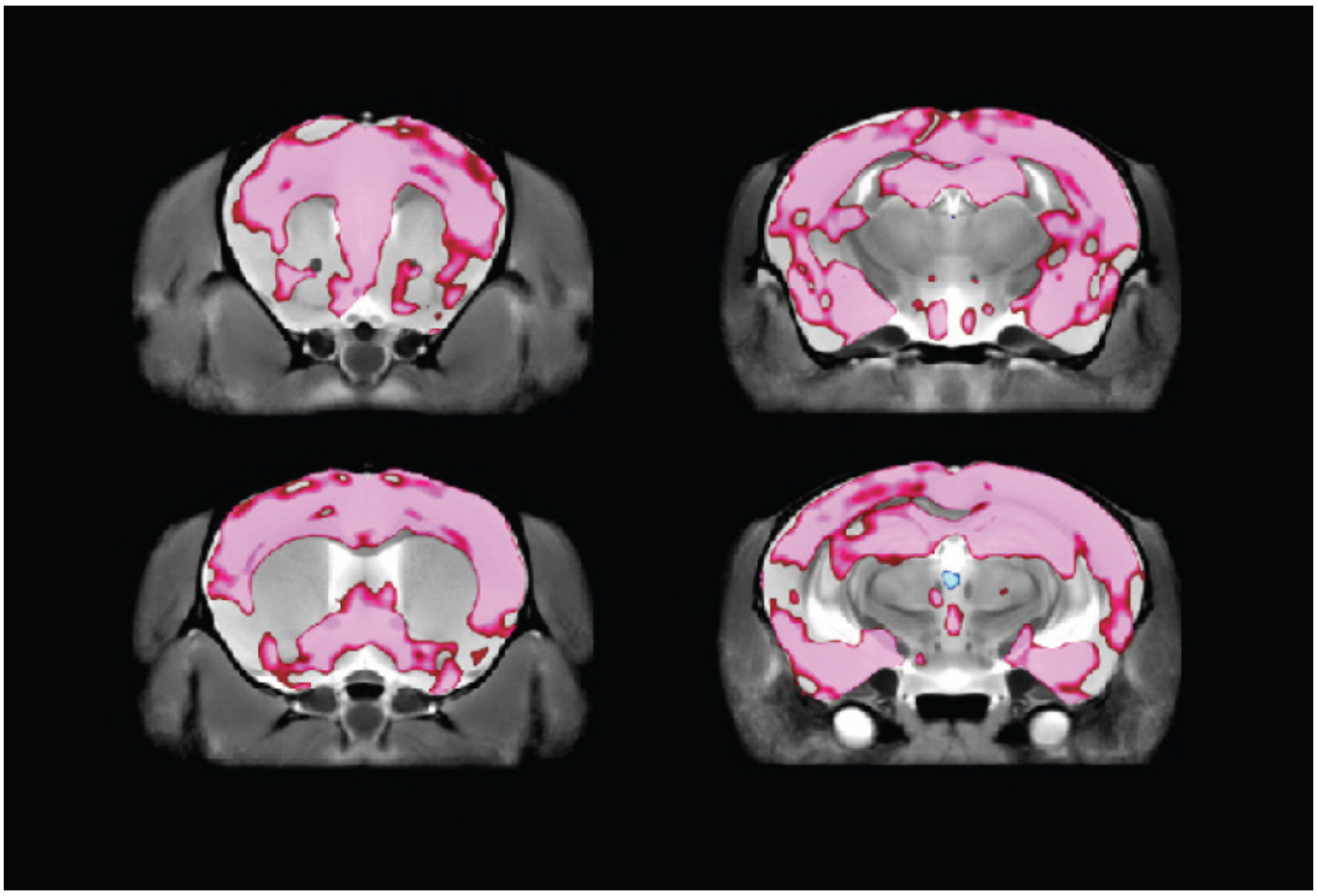

Researchers have debuted two mouse models of autism made using the gene-editing tool CRISPR. Both strains lack one functional copy of **CHD8**, a gene with strong ties to autism[1,2].

CRISPR allows researchers to quickly and efficiently insert specific mutations into single-cell mouse embryos. Several teams have used the method to make mouse models for other conditions, including Rett syndrome, an autism-related condition. The new mice represent the first use of the method to make models expressly for autism.

CHD8, **a top autism candidate**, was an obvious choice for this first foray: Almost all individuals with a harmful CHD8 mutation also have autism. They also have a **characteristic syndrome** that includes an enlarged head, gut problems and intellectual disability.

The two new strains of mice, along with **three others** made with conventional techniques, recapitulate some features seen in people with a CHD8 mutation. But they differ slightly from each other in their brain and behavioral features.

These variations may be due to differences in the mice's genetic background, says **Alex Nord**, assistant professor of neuroscience at the University of California, Davis, who made one of the new CRISPR models.

Because the genetic background of people is also widely variable, the ability to make multiple mouse models of the same syndrome inexpensively is an advantage.

"The continued accumulation of these data, models and reagents is going to be enormously important for the field," says **Michael Talkowski**, associate professor of neurology at Harvard University, who was not involved in making the mice.

Mouse model 2.0:

Traditional methods for disabling a gene involve breeding mice for several generations in order to generate animals that carry the mutation in each of their cells. CRISPR, by contrast, allows researchers to alter the genome directly in a single-cell mouse embryo, speeding up the process and lowering costs substantially.

"Since CRISPR/CAS9 has come out, making the mouse is no longer the longest part of the process," Nord says. CRISPR has shortened the timeline for engineering a mouse model from roughly two years to about six months, researchers say.

CRISPR also makes it possible to introduce mutations in mice that are otherwise genetically identical to controls. Researchers can tweak the gene in any way they like, inserting specific mutations rather than deleting genes. (Some studies have suggested that CRISPR can **introduce unintended mutations**, however.)

Nord and his colleagues made mice with a mutation that shuts down one copy of CHD8. The animals forget having seen an object before and fail to associate a location or sound with a shock —

features suggestive of memory and learning problems. They do not have social deficits or **repetitive behaviors**, both of which are hallmarks of autism. The researchers **presented the mice** at the **2016 International Meeting for Autism Research**, and published their findings 26 June in *Nature Neuroscience.*

The brains of the Nord mice are larger than those of controls across several regions, including the cortex, hippocampus and amygdala. The mice with the biggest differences have the most trouble with learning and memory.

The researchers measured gene expression in brain tissue from the mice during gestation, at birth and in adulthood. They found hundreds of genes expressed at lower levels than in control mice. These include many genes that influence how genetic messages are spliced, or edited, into their final protein-coding sequences. Using a statistical model, the researchers concluded that splicing is altered in the mutant mice.

The researchers also tracked the expression of 141 genes associated with autism; they found 37 of these are expressed at unusually low levels in the mutant mice.

Social mice:

The other set of CHD8 mice made using CRISPR come from researchers at the Massachusetts Institute of Technology. These mice are just as likely as controls to approach and interact with another mouse. But unlike controls, they do not spend extra time with a mouse they've never met before.

This could be a sign of social deficits, but it could also indicate a problem with memory, says co-lead investigator **Guoping Feng**, professor of brain and cognitive sciences.

The mice show no repetitive behaviors, but they show clear signs of anxiety, and avoid open spaces. They are also better than controls at learning how to balance on a rotating rod. This feature is seen in mice missing copies of other genes linked to autism, such as **PTEN** and **NLGN3**.

Overall, the findings in the Feng mice seem to match those in the Nord mice and most **other CHD8 models**, says **Jill Silverman**, assistant professor of psychiatry and behavioral sciences at the University of California, Davis. Silverman led the behavioral analysis of the Nord mice.

It "was really reassuring, we saw all the same things as [the Feng team] with regards to autism-relevant behavior," Silverman says.

The Feng mice show atypical expression of genes that regulate the light-dark cycle and protein processing, among others. This is consistent with results in other CHD8 models, including the Nord mice.

Rewarding experiment:

Feng's team found that levels of genes involved in **WNT signaling**, a signaling pathway important for development, are significantly altered in the nucleus accumbens. This region, nestled deep in the brain, plays a role in sensing reward.

The researchers measured the strength of electrical currents in the nucleus accumbens in brain slices from the mutant mice, and found that excitatory signaling in the region is enhanced compared with controls. Dampening CHD8 levels specifically in the nucleus accumbens improves the mice's motor learning, but not their anxiety. The study appeared 11 April in *Cell Reports*.

The findings implicate a reward brain region in autism, says Silverman. "People with autism may not be finding social interactions as rewarding, so it's a really interesting approach in that way," she says.

Feng and his team plan to use CRISPR to make mice that lack both copies of CHD8, but only in the brain. (Missing both copies throughout the body is lethal). This approach would enhance the mutation's effects and make it easier to pin down CHD8's role in the brain, Feng says. His team also intends to make mice that carry each of the CHD8 mutations seen in people with autism.

Nord and his colleagues also plan to make mice with individual mutations linked to autism. These include a mutation in a genomic region that may control CHD8's expression. Because CRISPR mice are relatively inexpensive to make, Nord says, the researchers can take the risk that the mutation, and others like it, in fact do nothing.

REFERENCES:

1. Gompers A.L. *et al. Nat. Neurosci.* **20**, 1062-1073 (2017) **PubMed**
2. Platt R.J. *et al. Cell Rep.* **19**, 335-350 (2017) **PubMed**