Medical Xpress

# Knocking out one key gene leads to autistic traits, mouse study shows

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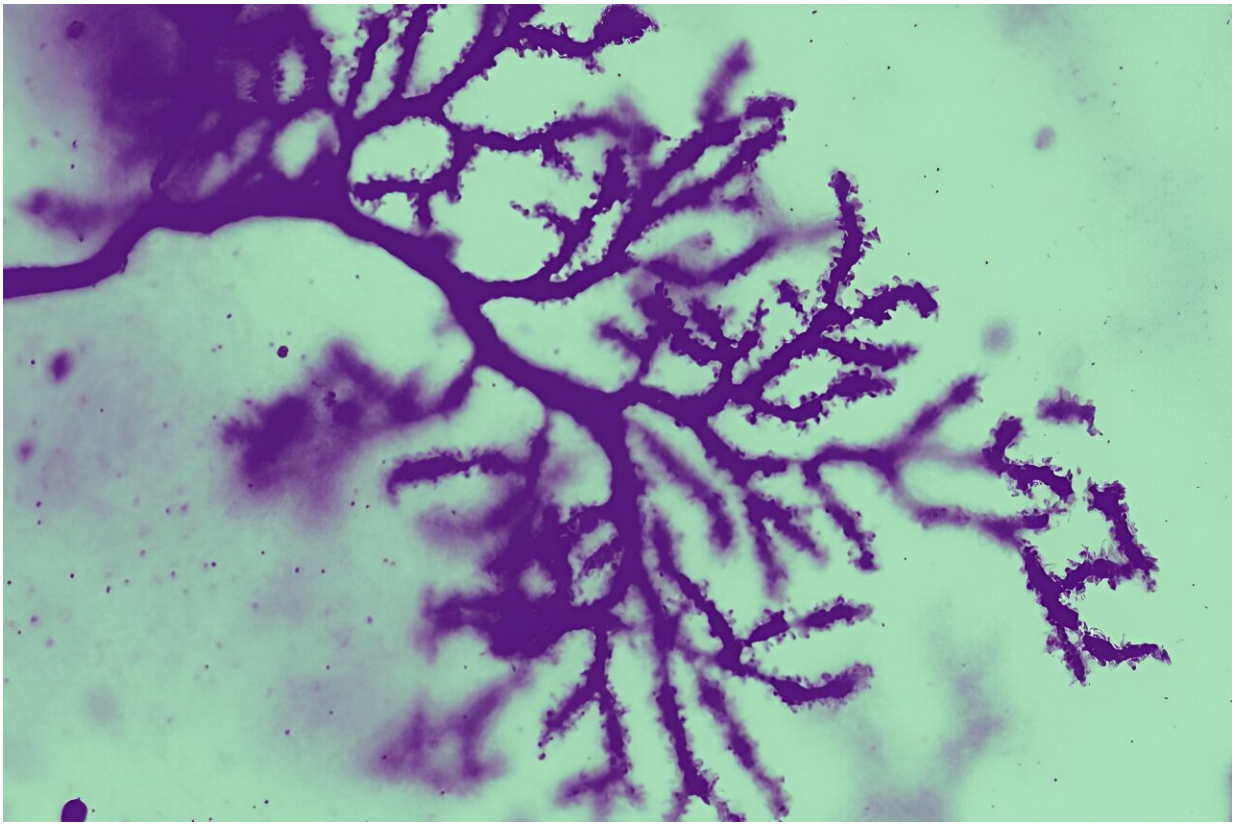


Purkinje cells in the cerebellum, stained and magnified 63 times, revealing fine details of the dendritic spines. Credit: Hatten Lab

More than 70 genes have been linked to autism spectrum disorder (ASD), a developmental condition in which differences in the brain lead

to a host of altered behaviors, including issues with language, social communication, hyperactivity, and repetitive movements. Scientists are attempting to tease out those specific associations gene by gene, neuron by neuron.

One such gene is Astrotactin 2 (ASTN2). In 2018, researchers from the Laboratory of Developmental Neurobiology at Rockefeller University discovered how defects in the protein produced by the gene disrupted circuitry in the cerebellum in children with neurodevelopmental conditions.

Now the same lab has found that knocking out the gene entirely leads to several hallmark behaviors of autism. As they describe in a new paper in *PNAS*, mice that lacked ASTN2 showed distinctly different behaviors from their wild-type nestmates in four key ways: they vocalized and socialized less but were more hyperactive and repetitive in their behavior.

"All of these traits have parallels in people with ASD," says Michalina Hanzel, first author of the paper. "Alongside these behaviors, we also found structural and physiological changes in the cerebellum."

"It's a big finding in the field of neuroscience," says lab lead Mary E. Hatten, whose work has focused on this brain region for decades. "It also underscores this emerging story that the cerebellum has cognitive functions that are quite independent of its motor functions."

## An unexpected role

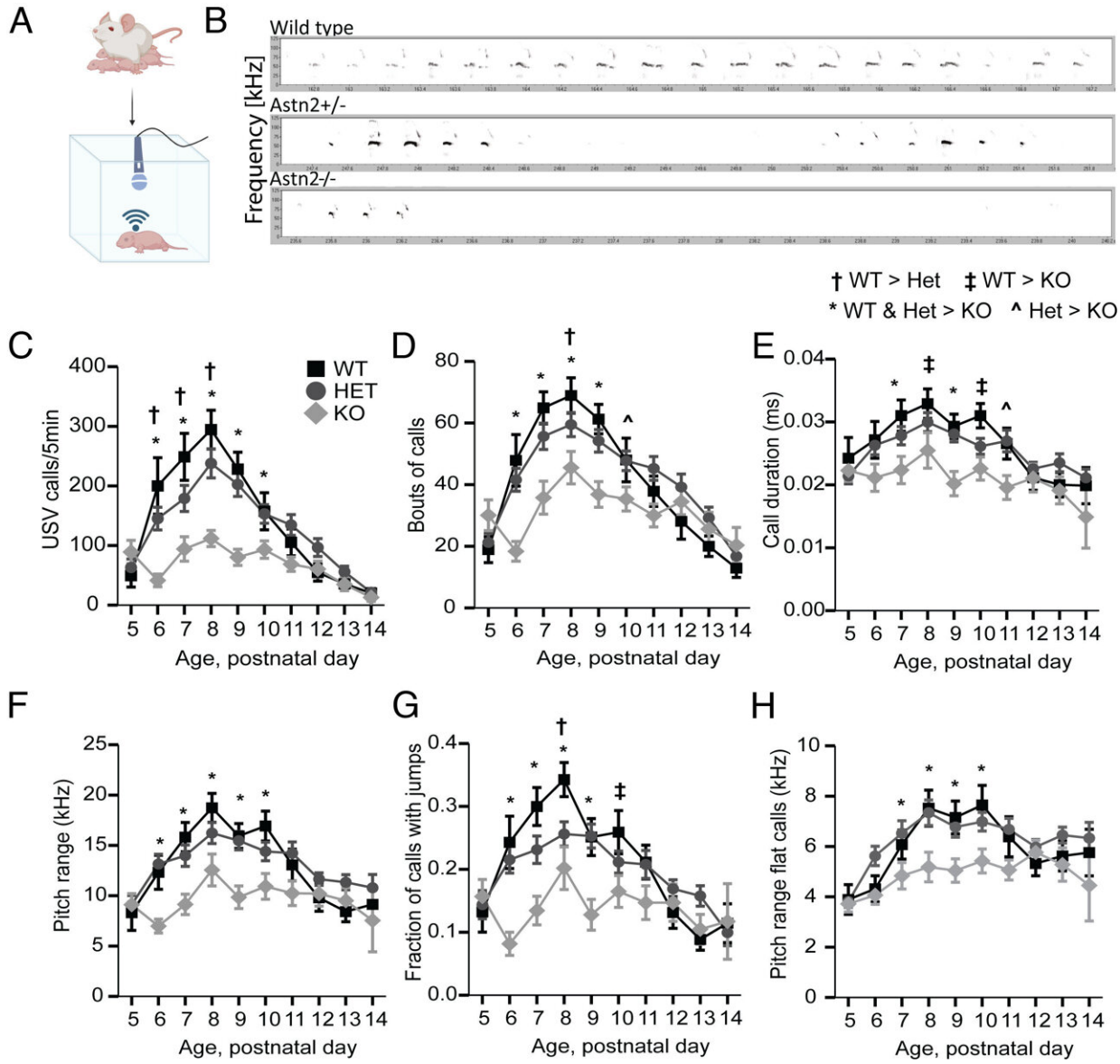
In 2010, Hatten's lab discovered that proteins produced by the ASTN2 gene help guide neurons as they migrate during the development of cerebellum and form its structure. In the 2018 study, they examined a family in which three children had both neurodevelopmental disorders

and ASTN2 mutations. They found that in a developed brain, the proteins have a similar guiding role: they keep the chemical conversation between neurons going by ushering receptors off the neural surfaces to make room for new receptors to rotate in.

In a mutated gene, the proteins fail to act and the receptors pile up, resulting in a traffic jam that hinders neuronal connections and communication. This impact could be seen in the children's afflictions, which included intellectual disability, language delays, ADHD, and autism.

The find was part of a growing body of evidence that the cerebellum—the oldest cortical structure in the brain—is important not just for motor control but also for language, cognition, and social behavior.

For the current study, Hanzel wanted to see what effects a total absence of the ASTN2 gene might have on cerebellar structure and on behavior. Collaborating with study co-authors Zachi Horn, a former postdoc in the Hatten lab, and with assistance from Shiaoching Gong, of Weill Cornell Medicine, Hanzel spent two years creating a knockout mouse that lacked ASTN2, and then studied the brains and activity of both infant and adult mice.




Loss of Astn2 results in fewer and less dynamic USVs in the mouse pups. Credit: *Proceedings of the National Academy of Sciences* (2024). DOI: 10.1073/pnas.2405901121

## Behavioral parallels

The knockout mice participated in several noninvasive behavioral

experiments to see how they compared to their wild-type nestmates. The knockout mice showed distinctly different characteristics in all of them.

In one study, the researchers briefly isolated baby mice, then measured how frequently they called out for their mothers using ultrasonic vocalizations. These sounds are a key part of a mouse's social behavior and communication, and they're one of the best proxies researchers have for assessing parallels to human language skills.

The wild-type pups were quick to call for their mothers using complex, pitch-shifting sounds, while the knockout pups gave fewer, shorter calls within a limited pitch range.

Similar communication issues are common in people with ASD, Hanzel says. "It's one of the most telling characteristics, but it exists along a spectrum," she says. "Some autistic people don't understand metaphor, while others echo language they've overheard, and still others do not speak at all."

In another experiment, the researchers tested how ASTN2 mice interacted with both familiar and unfamiliar mice. They preferred to interact with a mouse they knew rather than one they didn't. In contrast, wild-type mice always choose the social novelty of a new face.

This, too, has parallels in human ASD behavior, with a reluctance towards unfamiliar environments and people being common, Hanzel adds. "That's a very important result, because it shows that mice with the knockout mutation do not like social novelty and prefer to spend time with mice they know, which corresponds to people with ASD, who tend to like new social interactions less than familiar ones."

In a third experiment, both types of mice were given free rein to explore an open space for an hour. The ASTN2 mice traveled a significantly

longer distance than the other mice, and engaged in repetitive behaviors, such as circling in place, 40% more. Both hyperactivity and repetitive behaviors are well-known hallmarks of ASD.

## Miscommunication between brain regions

When they analyzed the brains of the ASTN2 mice, they found a few small but apparently potent structural and physiological changes in the cerebellum. One was that large neurons called Purkinje cells had a higher density of dendritic spines, structures that are spotted with the synapses that send neural signals. But they only detected this change in distinct areas of the cerebellum. "For example, we found the biggest difference in the posterior vermis region, where repetitive and inflexible behaviors are controlled," Hanzel says.

The scientists also found a decrease in the number of immature dendritic spines known as filopodia and the volume of Bergmann glial fibers, which help with cell migration.

"The differences are quite subtle, but they are clearly affecting how the mice are behaving," Hatten says. "The changes are probably altering the communication between the cerebellum and the rest of the brain."

In the future, the researchers plan to study human cerebellar cells, which they've been developing for a half-dozen years from stem cells, as well as cells with ASTN2 mutations that were donated by the family in the 2018 study.

"We'd like to see if we can find parallel differences to what we found in mice in human cells," Hatten says.

She continues, "We also want to look at the detailed biology of other genes that are associated with autism. There are dozens of them, but

there's no agreed-upon commonality that binds them together. We're very excited that we've been able to show in detail what ASTN2 does, but there are a lot more genes to investigate."

**More information:** Michalina Hanzel et al, Mice lacking Astn2 have ASD-like behaviors and altered cerebellar circuit properties, *Proceedings of the National Academy of Sciences* (2024). DOI: 10.1073/pnas.2405901121

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