EXPERIMENTAL MODEL

Gene-edited *SHANK-3* mutant macaques display autism behavior

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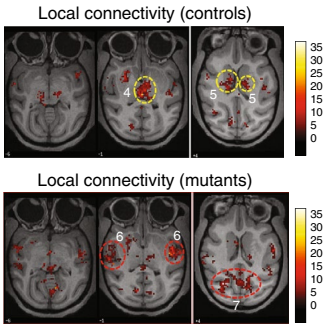
Mutations in *SHANK-3* — a gene encoding a synaptic scaffolding protein involved in the formation and maturation of excitatory synapses — are a major cause of Phelan-McDermid syndrome (PMS), an autism spectrum disorder (ASD) with symptoms that include intellectual disability, autistic behaviors, low muscle tone, and delayed or absent speech. *SHANK-3* mutations have also been identified in patients with other forms of ASD, further supporting the link between *SHANK-3* and ASD pathophysiology. Previous studies have reported autism-like behaviors in mice with *Shank-3* mutations, but the behavioral phenotype of the mutants did not model all the features of ASD, limiting the use of these small animal models for ASD research. In a new study published in *Nature*, investigators from the Chinese Academy of Sciences and Massachusetts Institute of Technology used a CRISPR-Cas9 gene editing strategy to engineer macaques with mutations in *SHANK-3*; these animals displayed atypical brain connectivity and behavioral abnormalities similar to that observed in individuals with ASD. *SHANK-3* mutant macaques might therefore become a useful model to better understand the biological mechanisms of ASD and discover treatment options.

The investigators used a CRISPR-Cas9 gene editing strategy to specifically create mutations in the *SHANK-3* gene of macaque embryos that were subsequently transferred into recipient females. Four males and one female (referred to as M1 to M5) were born and genotyping confirmed the presence of *SHANK*-3 mutations in all of them. At the end of the study, western blot analysis of brain tissue from the macaques showed that the level of SH3 and multiple ankyrin repeat domains protein 3 (SHANK-3) protein was decreased in all five mutants compared with age- and sex-matched control macaques from the same colony, which confirmed the successful disruption of the *SHANK-3* gene. To investigate if *SHANK-3* mutation could be passed to the next generation, sperm from macaque M2 was injected into wild-type oocytes: genotyping confirmed that all fertilized embryos carried the mutation, indicating that the mutation was germline-transmissible.



Axial views of local connectivity in controls (top) and mutants (bottom). Clusters with robust alterations of connectivity are highlighted. Reprinted with permission from Zhou et al. (2019) Springer Nature.

The investigators used several tests to study the behavioral phenotype of the *SHANK-3* mutant macaques. Data analysis of the activity recorded with an actigraphy device revealed a reduced overall activity level in mutant monkeys compared with controls. In addition, mutant macaques showed a longer latency to sleep and an increased frequency of waking, which indicated a reduction in sleep efficiency, a feature also observed in individuals with ASD. Next, video-recording of daily behavior revealed that mutant macaques displayed an increase in stereotyped or repetitive behaviors, such as back flipping, finger licking and biting of cage bars, compared with controls.

To evaluate potential changes in social behavior, the investigators designed a paired social interaction assay: after a habituation session to allow the animals to familiarize themselves with the new cage environment, control or mutant macaques were paired with wild-type monkeys. A video camera was used to record the habituation and social interaction sessions. During habituation, mutant macaques spent less time exploring and vocalized less than controls; during the five first minutes of the social interaction test, the mutants spent less time on aggregate social behaviors, such as chasing, following, and playing. The results of the behavioral tests therefore showed that *SHANK-3* mutants displayed an autism-like behavior and impaired social interaction, two typical features of ASD.

Next, the team used a video-based eye tracking assay designed in the lab to investigate the differences in gaze between the two groups; image analysis revealed an increased latency of pupillary reflex to the onset of luminance in the mutants, indicative of an alteration of gaze properties. Cognitive performance was assessed with a test that includes a visual discrimination task, a reversal task, and a Hamilton search task. M2 and M5 failed to participate to test. M1, M3 and M4 performed similarly to controls in the black and white discrimination task but were less engaged in the more difficult Hamilton task, suggesting learning impairment in the mutants.

Human studies have shown structural and functional changes in the brain of individuals with ASD. Similarly, functional connectivity analysis of MRI data from the macaques revealed a dysregulated global and local connectivity in *SHANK-3* mutants with hypoconnectivity in putative default mode networks, including the posterior cingulate cortex, medial prefrontal region, and motor regions and local hyperconnectivity in somatosensory cortex, extrastriate cortical areas and posterior cingulate cortex. "Our discovery in a non-human primate model of atypical connectivity in local and long range circuits—especially in the cingulate, frontal, thalamic and striatal regions—suggests a path for further studies to identify circuit abnormalities and potential biomarkers for treatment studies," say the investigators.

In conclusion, in addition to features also observed in the *Shank-3* mutant rodents such as reduced mobility, increased repetitive behaviors, and impaired sociability, *SHANK-3* mutant macaques also displayed sleep disturbance, altered social behaviors and stereotopies, as well as hypotonia. By recapitulating most of the phenotype observed in individual with ASD, *SHANK-3* mutant macaques might be useful models to better understand and treat ASD.

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