ASD-like phenotypes and brain abnormalities in *Usp15* KO mice

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Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disease defined by significant social, communication, and behavior challenges. Accumulating evidence suggests that genetic defect is the major cause of ASD. Ubiquitin carboxy-terminal hydrolase 15 (*Usp15*) is one of the candidate genes identified by the whole-exome sequencing of ASD patients. Previous studies have found that *Usp15* regulates autistic behavior and balance of excitatory/inhibitory (E/I) synapses. However, the mechanism of how the absence of *Usp15* leads to autistic behavior and abnormal brain structure remains unknown. In this study, I reported the differences in brain development and behavioral patterns between wild-type (WT) and *Usp15* knockout (KO) mice. I found that *Usp15* KO mice exhibit morphological abnormalities in the cerebral cortex and hippocampus, altered microglial state, and ASD-like behavior. These findings suggest that a defect in the *Usp15* gene is implicated in early brain development and the manifestation of behavioral patterns associated with ASD.

Material & Methods

1. Mice

Male and female WT and *Usp15* KO mice at 4-5 weeks of age were used for behavior experiments. *Usp15*$^{+/+}$, CX3CR1 GFP $^{+/-}$ mice and *Usp15* $^{-/-}$, CX3CR1 GFP $^{+/-}$ mice were generated by crossing *Usp15*$^{+/-}$ mice and CX3CR1 GFP $^{+/-}$ or CX3CR1 GFP $^{+/+}$ mice for microglial quantification. All mice used in this study were backcrossed with the C57BL/6J background.

2. Behavior tests

For the marble burying test, a total of twenty marbles were evenly arranged on 5cm-thick bedding in the plastic cage and 4-week-old mice were placed in the cage for 30 minutes. The number of marbles buried more than 75% were counted. For the nest-building test, the same size and weight of nesting material were placed in the cage with 4-week-old mice for 24h. Nests were scored based on the weight of intact nesting material and the shape of a nest.

3. Immunohistochemistry

The brains were perfused with PBS and fixed by 4% PFA/PBS overnight. After 30% sucrose infiltration, the samples were embedded in OTC compound and sliced at a 40-µm thickness. The brain sections were washed in PBS and permeabilized with 0.4% TritonX-100 in PBS, then blocked with 5% bovine serum albumin (BSA) and 0.4% Triton X-100 in PBS for 1 hour. Sections were incubated with primary antibodies diluted in a blocking solution at 4°C overnight. Sections were washed in PBS containing 0.4% TritonX-100 and incubated with secondary antibodies diluted in a blocking solution at room temperature for 2 hours. Fluorescence images were obtained using a fluorescence microscope (Leica Microsystems THUNDER 3D Cell Culture) and analyzed by FIJI ImageJ software.

Results & Discussion

Previously we identified that *Usp15* KO mice show autistic behavior by a three-chamber test. To assess detailed ASD-like behavior in *Usp15* KO mice, I performed additional behavior tests. In the marble burying test, *Usp15* KO mice buried significantly fewer marbles than WT mice. In the nest-building test, none of the *Usp15* KO mice could build a proper nest resulting in significantly lower nesting scores in *Usp15*KO mice compared to the WT mice. These results indicate that *Usp15*KO mice exhibit additional ASD-like behavior else than impaired sociability. Then I focused on the structural change of the brain from one of the most common autistic phenotypes, cortex thinning. Quantification of cortex thickness in the WT and *Usp15* KO mice at postnatal (P) 21 and P28 with the brain sections immunostained by DAPI showed that the cerebral cortex is significantly thinner in *Usp15*KO mice brains at P28.

Next, I focused on the hippocampus which is known to be highly involved in ASD. I found that *Usp15* KO mice exhibit abnormal dentate gyrus (DG) structures, which were significantly shorter in *Usp15*KO mice compared to WT at P28. This result is in line with previous studies that have reported abnormal DG development is involved in the expression of ASD-like behavior. These data demonstrate that a loss of the *Usp15* gene causes abnormal brain formation.

As it has been reported that microglia are associated with ASD and our finding supports the idea that impaired microglia are involved in the mechanism of ASD-like behavior and brain abnormalities. I therefore next quantified the number of microglia to identify potential molecular mechanisms. Quantification of the GFP signal at the cerebral cortex and hippocampus in the *Usp15*$^{+/+}$, CX3CR1 GFP$^{+/-}$ mice and *Usp15* $^{-/-}$, CX3CR1 GFP$^{+/-}$ mice at P28 showed that the density of microglia is significantly decreased in *Usp15*$^{-/-}$, CX3CR1 GFP$^{+/-}$. However, the expression level of Iba1, which is a marker of reactive microglia, was higher in *Usp15* KO mice. Thus, these results indicate that *Usp15* KO mice exhibit a decreased number of microglia in the cerebral cortex and hippocampus and impaired microglial states, which may contribute to imbalanced synapses and cortex thinning. Taken together, my results suggest that the absence of *Usp15* induces a decreased number of microglia and impaired microglial states that lead to ASD-like behavior and significant differences in brain morphology in *Usp15*KO mice.