Otud7a Knockout Mice Recapitulate Many Neurological Features of 15q13.3 Microdeletion Syndrome

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15q13.3 microdeletion syndrome is characterized by a wide spectrum of neurodevelopmental disorders, including developmental delay, intellectual disability, epilepsy, language impairment, abnormal behaviors, neuropsychiatric disorders, and hypotonia. This syndrome is caused by a deletion on chromosome 15q, which typically encompasses six genes. Here, through studies on OTU deubiquitinase 7A (Otud7a) knockout mice, we identify OTUD7A as a critical gene responsible for many of the cardinal phenotypes associated with 15q13.3 microdeletion syndrome. Otud7a-null mice show reduced body weight, developmental delay, abnormal electroencephalography patterns and seizures, reduced ultrasonic vocalizations, decreased grip strength, impaired motor learning/motor coordination, and reduced acoustic startle. We show that OTUD7A localizes to dendritic spines and that Otud7a-null mice have decreased dendritic spine density compared to their wild-type littermates. Furthermore, frequency of miniature excitatory postsynaptic currents (mEPSCs) is reduced in the frontal cortex of Otud7a-null mice, suggesting a role of Otud7a in regulation of dendritic spine density and glutamatergic synaptic transmission. Taken together, our results suggest decreased OTUD7A dosage as a major contributor to the neurodevelopmental phenotypes associated with 15q13.3 microdeletion syndrome, through the misregulation of dendritic spine density and activity.

Introduction

The proximal part of chromosome 15 long (q) arm is one of the most unstable regions of the human genome, largely due to six low copy repeat (LCR) elements, which are likely to mediate non-allelic homologous recombination (NAHR). These LCR elements often become breakpoints in chromosomal rearrangements and are named breakpoints BP1–BP6.

Chromosomal deletions and duplications are frequently associated with disease. 15q13.3 microdeletions were first described in 2008 as a rare condition associated with intellectual disability and seizures.1 Current estimate of the frequency of 15q13.3 microdeletion in the general population is 1 in 30,000 to 40,000 individuals, with some sources reporting an even higher frequency.1–4 Further assessment of clinical implications of 15q13.3 microdeletion suggests that some individuals who carry the deletion may be unaffected, whereas others manifest a wide spectrum of neurodevelopmental disorders, including developmental delay, abnormal behaviors, neuropsychiatric disorders, and hypotonia. This syndrome is caused by a deletion on chromosome 15q, which typically encompasses six genes. Here, through studies on OTU deubiquitinase 7A (Otud7a) knockout mice, we identify OTUD7A as a critical gene responsible for many of the cardinal phenotypes associated with 15q13.3 microdeletion syndrome. Otud7a-null mice show reduced body weight, developmental delay, abnormal electroencephalography patterns and seizures, reduced ultrasonic vocalizations, decreased grip strength, impaired motor learning/motor coordination, and reduced acoustic startle. We show that OTUD7A localizes to dendritic spines and that Otud7a-null mice have decreased dendritic spine density compared to their wild-type littermates. Furthermore, frequency of miniature excitatory postsynaptic currents (mEPSCs) is reduced in the frontal cortex of Otud7a-null mice, suggesting a role of Otud7a in regulation of dendritic spine density and glutamatergic synaptic transmission. Taken together, our results suggest decreased OTUD7A dosage as a major contributor to the neurodevelopmental phenotypes associated with 15q13.3 microdeletion syndrome, through the misregulation of dendritic spine density and activity.

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Material and Methods

sgRNA and Cas9 Preparation
Donor DNAs expressing sgRNAs under the T7 promoter were prepared by PCR, using Phusion High-Fidelity polymerase (New England Biolabs), pX330 as a template, and a combination of reverse primer with one of two forward primers: forward primer for exon 4 of *OTUD7A* 5'-TTAATACGACTCATATAGGGTACGAGGCGGCAATGTAAGTTTACAGTGAAGAATAGC-3', forward primer for exon 6 of *OTUD7A* 5'-TTAATACGACTCACTATAGGGAAAGTTGTTGCGAGGTCGCTGTTTTAGCTGAGAAATAGC-3', reverse primer 5'-AAAAAGCACCAGCTGGTGGCC-3'.

The resulting PCR products were subjected to RNA transcription, using the MEGAscript T7 Transcription kit (Thermo Fisher Scientific) with manufacturer's protocol. The sgRNAs were purified by the MEGAscript Transcriptase Clean-Up kit (Thermo Fisher Scientific).

Cas9 was ordered from PNA TECHNOLOGY and stored at −80°C. On the day of microinjection, final concentrations of 30 ng/μL Cas9 and 20 ng/μL sgRNA (total) were mixed into a 150 μL injection buffer (10 mM Tris [pH 7.5], 0.25 mM EDTA) and heated at 37°C for 5 min. After centrifuging at 20,000 × g for 10 min at 4°C, the top 2/3 of supernatant was used for cytoplasmic microinjection.

Cytoplasmic Microinjection
C57BL/6j female mice were superovulated and mated with C57BL/6j males, and fertilized eggs were collected from the oviduct. The pronuclear-stage eggs were injected with Cas9 and sgRNAs at the indicated concentrations. The eggs were cultured by PCR, using Phusion High-Fidelity polymerase (New England Biolabs), pX330 as a template, and a combination of reverse primer with one of two forward primers: forward primer for wild-type 5’-TCATGAAACCGCTCCCTT-3’, reverse primer for wild-type 5’-GCTAGGATGCGAGGTGTCATT-3’, forward primer for mutant 5’-TGTCACGAGGTGTCATT-3’, reverse primer for mutant 5’-GCTAGGATGCGAGGTGTCATT-3’. To genotype off-target candidates, primers in Table S1 were used.

Genotyping
The last 1–2 mm of mouse tails were cut into a 1.5 mL Eppendorf tube containing 135 μL 50 mM NaOH and incubated overnight on a 55°C shaker. DNAs were extracted by adding 15 μL Tris-HCL at pH 6.8, and centrifuging at 20,000 × g for 1 min.

1 μL of DNA was used for each PCR reaction using Econo taq DNA polymerase (Lucigen, WI). Forward primer for wild-type 5’-TCATGAAACCGCTCCCTT-3’, reverse primer for wild-type 5’-GCTAGGATGCGAGGTGTCATT-3’, forward primer for mutant 5’-TGTCACGAGGTGTCATT-3’, reverse primer for mutant 5’-GCTAGGATGCGAGGTGTCATT-3’. To genotype off-target candidates, primers in Table S1 were used.

Plasmid Preparation
To construct pCMV3xFLAG-OTUD7A plasmid, human OTUD7A coding regions were PCR amplified and placed into the pCMV10-3xFLAG plasmid between multicloning sites HindIII and XbaI. To construct a pCMV6-OTUD7A-MYC-FLAG, human OTUD7A coding regions together with MYC were PCR amplified from a Myc-DDK-tagged OTUD7A plasmid (Origene Ca. #RC213015) and placed into a pCMV6-FLAG plasmid.

Animals
Mice were maintained on a 14 hr light/10 hr dark cycle, with access to regular mouse chow and water *ad libitum*. For behavioral assessments, heterozygous founders from CRISPR/Cas9 microinjection were backcrossed to wild-type mice (C57BL/6j) for two generations to obtain heterozygous mice for breeding pairs. All of the *Otud7a* homozygous (KO) and heterozygous (HET) mutant mice as well as the wild-type (WT) mice used in our experiments were derived from HET breeding pairs. Mice were randomly assigned and group-housed, with two to five animals per cage, immediately after weaning. Cohort one, consisting of 21 WT, 27 HET, and 15 KO mice, with mixed sex, were monitored for developmental phenotypes. Mice for ultrasonic vocalization recordings (cohort two) consist of 16 WT, 29 HET, and 19 KO mice, with mixed sex. Cohort three, consisting of 14 WT (♂), 20 HET (♂, 14 KO (♂), 14 WT (♀), 20 HET (♀), and 16 KO (♀) mice, went through a series of assays, including elevated plus maze, open field activity, light-dark activity, self-grooming, holeboard exploration, rotarod test, three-chamber test, partition test, forced swimming test, prepulse inhibition, conditioned fear, and nest building, all of which started at 10 weeks of age. Cohort four, consisting of 13 WT (♂), 19 HET (♂), 14 KO (♂, 19 WT (♀), 18 HET (♀), and 13 KO (♀) mice, were monitored for body weight and tested in grip strength and novel object recognition. Experiments were performed during the light cycle, and mice were given inter-test intervals of 1–2 days between each test. All behavioral tests were performed at 700–750 lux illumination and background white noise at approximately 60 dB, with the exception of the partition test, in which there was no background white noise.

For all assays, the experimenter remained blind to the genotypes. All research and animal care procedures were approved by the Baylor College of Medicine Animal Care and Use Committee and were performed in accordance with the relevant guidelines and regulations. The *Otud7a-null* mouse line will be available from The Jackson Laboratory as JAX#031294.

Developmental Assessment
Neurobehavioral development was examined during the preweaning period, using a battery of tests that evaluated sensorial and motor responses reflecting the maturation of the CNS, as described previously. The day of appearance of developmental landmarks, including negative geotaxis, cliff aversion, incisor eruption and growth, eye lid opening, and ear opening, was recorded. To evaluate negative geotaxis, pups were placed facedown on a paper box that was tilted 30° from the horizon line. The day when the pup avoided turning around to face upward was recorded. To evaluate cliff aversion, pups were placed with their legs just within the edge of the box. The day when the pup avoided falling off the edge was recorded. Incisor growth was evaluated by the appearance of shiny spots at the tip of the incisors.

Ultrasonic Vocalization
Ultrasonic vocalization was recorded on postnatal days 2, 4, 6, 8, and 10. All recordings were performed between 10:00 AM and 4:00 PM. On the first day of recording, the subjects were marked for identification by Sharpie pen on tails or backs, immediately after recording. On day 10, subjects were ear tagged and the last 1–2 mm of tail was clipped following the recording. On the following days, subjects were re-marked in the same way immediately after recording. On day 2, the subjects were ear tagged and the last 1–2 mm of tail was clipped following the recording. On the following days, subjects were re-marked in the same way immediately after recording. On day 2, the subjects were ear tagged and the last 1–2 mm of tail was clipped following the recording. On the following days, subjects were re-marked in the same way immediately after recording. On day 2, the subjects were ear tagged and the last 1–2 mm of tail was clipped following the recording. On the following days, subjects were re-marked in the same way immediately after recording. On day 2, the subjects were ear tagged and the last 1–2 mm of tail was clipped following the recording. On the following days, subjects were re-marked in the same way immediately after recording.
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