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NEWS

The OTUD7A-Ankyrin pathway: a newly identified disease mechanism for the 15q13.3 microdeletion disorder

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No specific treatments are available for many neurodevelopmental disorders (NDDs), including the 15q13.3 microdeletions disorder, mostly due to unknown disease mechanisms. A recent study by Unda et al. [1] in Molecular Psychiatry reveals a new critical mechanism for neurodevelopment, which is impaired in the 15q13.3 microdeletion disorder.

Genomic copy number variations (CNVs) that involve deletions and/or duplications of segments of DNA are often associated with NDDs [2]. The 15q13.3 microdeletion disorder is a recurrent CNV with a highly heterogeneous range of clinical manifestations due to incomplete penetrance and expressivity, including intellectual disability (ID), autism spectrum disorder (ASD), epilepsy, and schizophrenia [3]. The 15q13.3 microdeletion contains several candidate protein-coding genes that potentially underly the clinical phenotype, including the Ovarian tumor domain containing protein 7 A (*OTUD7A*). Interestingly, a previous study using heterozygous 15q13.3 microdeletions and *Otud7a* knockout mouse models demonstrated that *OTUD7A* is critical for the regulation of cortical neuron dendritic and spine development [4]. However, the molecular mechanisms underlying the *OTUD7A*-driven neuronal abnormalities observed in the 15q13.3 microdeletion syndrome remained elusive.

The recent study by Unda et al. [1] for the first time identified the interaction between OTUD7A and Ankyrins which regulate important aspects of dendritic spine and axon initial segment (AIS) formation. While impaired dendritic branching and spines formation in cortical neurons of Df(h15q13)/+ mice have been previously described, in this paper they provide cross-species evidence for a role of OTUD7A in this phenotype [4–6]. They found that the heterozygous 15q13.3 microdeletion in both mouse and iPSC-derived neuronal (iNeuron) models lead to decreased dendritic arborization of cortical neurons. Of note, iNeurons derived from a patient carrying the OTUD7A$^{L233F/L233F}$ mutation also showed the same morphological phenotype. Consistently, the overexpression of WT OTUD7A, but not OTUD7A$^{L233F/L233F}$, rescued the dendritic morphology in both the mouse and iNeuron model of the 15q13.3 microdeletion disorder.

The authors then examined the OTUD7A protein-protein interaction (PPI) network using BioID2, a discovery-based proximity-labeling proteomics technique. BioID2 has been widely used in mammalian cells to identify PPI networks, but here they developed a neuron-specific BioID2 pipeline to target *OTUD7A* in primary mouse cortical neurons. They identified the OTDU7A PPI network which included synaptic, cytoskeletal and axon initial segment (AIS) proteins and was enriched for ASD and epilepsy-associated proteins, including Ankyrin-B and Ankyrin-G. Interestingly, to identify the ASD and epilepsy-associated pathways, the authors compared the PPI networks of OTUD7A to the OTUD7A N492_K494del (ASD) and OTUD7A L233F (epileptic encephalopathy) mutations. In the PPI networks of both variants the functional enrichment for most cellular compartments, most notably the synapse, was lost. Remarkably, in the epilepsy-associated *OTUD7A* L233F variant interactions with putative AIS proteins, including Ankyrin-G (*Ank3*) and Ankyrin-B (*Ank2*) were disrupted. This BioID2 results confirmed previous studies identifying OTUD7A at dendritic spines [4], but also for the first time identified OTUD7A localization at the axon and AIS and a significant impact of the OTUD7A L233F variant on AIS localization or binding to AIS proteins.

The authors further used co-immunoprecipitation assays on HEK293 and P20 C57BL/6–3XFlag-Otud7a mouse cortex to establish binding and a possible functional interaction between OTUD7A and Ankyrin-G, as well as singling out the catalytic domain of OTUD7A as the critical domain for binding to the Ankyrin-G spectrin binding domain. Utilizing structured illumination microscopy (SIM) to analyze the subcellular localization of Ankyrin-G, Unda *et al*. also showed reduced level of Ankyrin-G in dendrites, mushroom spines, and the AIS of both Df(h15q13)/+ mouse cortical neurons and patient-derived iNeurons carrying either the 15q13.3 microdeletion or OTUD7A$^{L233F/L233F}$ mutation. Interestingly, while the authors observed increased ubiquitination of Ankyrin-G in both patient-derived iNeurons, they only observed reduced Ankyrin-G stability in neurons carrying the 15q13.3 microdeletion and not in the OTUD7A$^{L233F/L233F}$ iNeurons. These results suggest that Ankyrin-G is poly-ubiquitinated and may be targeted for degradation, but is not always processed by the proteasome leaving the protein stability unchanged in the OTUD7A$^{L233F/L233F}$ iNeurons. Ankyrin-G overexpression in the Df(h15q13)/+ mouse cortical neurons, similar to WT OTUD7A overexpression, was sufficient to rescue the impairments in dendrite and dendritic spine morphology observed in the microdeletion model, further establishing the involvement of Ankyrin-G in the disease pathogenesis of the 15q13.3 microdeletion disorder. However, it has yet to be determined whether similar mechanisms are present in neurons carrying the OTUD7A$^{L233F/L233F}$ patient mutation.

Considering the importance of Ankyrin-G in regulating different aspects of the AIS and neuronal excitability, loss of an important

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interactor of Ankyrin-G likely impairs axonal growth and overall neuronal functionality. Indeed, iNeurons from multiple families carrying the 15q13.3 microdeletion showed reduced axonal length. However, the effect on neuronal excitability appears to be less consistent, with only some probands showing an increased rheobase, reduced AP threshold, or increased repetitive firing. It is also important to note that the iNeurons carrying the OTUD7A$^{L233F/L233F}$ patient mutation did not display most of the axonal phenotypes seen in the probands with the15q13.3 microdeletion. Similarly, the multi-electrode array (MEA) recordings of the iNeurons provided in this paper, whilst extensive, displayed a large range of phenotypes between probands. This might indicate that patient-specific genomic background can influence the neuronal phenotype and potential affect disease mechanism.

To summarize, this study provides important novel insights on the disease mechanisms of the 15q13.3 microdeletion disorder and its genetic driver OTUD7A. First, using a unique and neuronal-specific proximity labeling proteomic approach the authors showed that OTUD7A has a rich protein-protein interaction network spanning synaptic, axonal and cytoskeletal proteins and shares protein interactors with Ankyrin-G. Second, they showed that the 15q13.3 microdeletion patients and mouse neurons have reduced levels of Ankyrin-G, which might be a result of increased protein instability. Finally, they showed that Ankyrin-G over-expression can rescue the dendritic and spine abnormalities associated with 15q13.3 microdeletion.

Outstanding questions however still remain. While OTUD7A is an important driving gene of the 15q13.3 microdeletion disorder, the question remains how the loss of other driving genes might also contribute to the neuronal phenotypes observed and if this might be different among different individuals [6–10]. This is also supported by the fact that OTUD7A $^{L233F/L233F}$ neurons do not replicate the complete phenotype of the 15q13.3 microdeletion neuronal model. Possible future research can determine the disease pathology of the OTUD7A L233F variant in comparison to OTUD7A loss of function. In addition, other studies reported that even in individuals with small 15q13.3 microdeletions, only encompassing the gene CHRNA7, the first exon of OTUD7A is always affected [6]. It would be interesting to measure OTUD7A expression in neurons derived from patients with small 15q13.3 microdeletions. Finally, while the expression of OTUD7A is higher in excitatory neurons, it might be worthwhile to investigate the role of OTUD7A in other cell types [5].

Unda et al. [1] provide novel insights in the involvement of the OTUD7A-Ankyrin pathway in neuronal functioning. These findings highlight the use of targeting CNV genes through cell-type specific proteomics to identify shared disease mechanisms in NDDs and potentially design therapeutic strategies to reverse neuronal deficits.

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AUTHOR CONTRIBUTIONS

NS, UC, EJHvH and NNK wrote the manuscript and edited the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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