A novel ALS-associated DCTN1 variant promotes TDP-43 aggregation in vitro

Veronica Bettoni[1], Matteo Bordoni[2], Eveljn Scarian[2], Camilla Viola[1,2], Orietta Pansarasa[2], Luca Diamanti[3]
1 Department of Brain and Behavioural Sciences, University of Pavia, Pavia, Italy ,
2 Cellular Models and Neuroepigenetics Section, IRCCS Mondino Foundation, Pavia, Italy ,
3 Neuroncology Unit, IRCCS Mondino Foundation, Pavia, Pavia, Italy

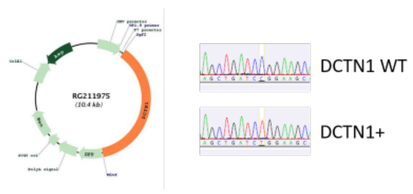
OBJECTIVE

Since the discovery of pathogenic mutations in SOD1 in 1993, numerous other gene variants have been found to be linked to Amyotrophic Lateral Sclerosis (ALS). Among these, mutations in the human dynactin subunit 1 (DCTN1) gene have been described as rare causes of ALS[(1,2)]. Here, we describe and functionally characterize, for the first time, the DCTN1 c.1867C>T variant in a neuronal cell model.

PATIENTS AND METHODS

A 63-year-old woman presented with hyposthenia in her lower limbs and tetra-hyperreflexia. A family history of motor neuron disease was reported (her mother had ALS, with bulbar onset and rapid progression), and a previous C9orf72 molecular analysis was negative. The EMG study showed diffuse denervation activity, more pronounced in the bulbar region. Neuropsychological tests revealed an ALS-ci-compatible profile, characterized by impairment in executive functions.

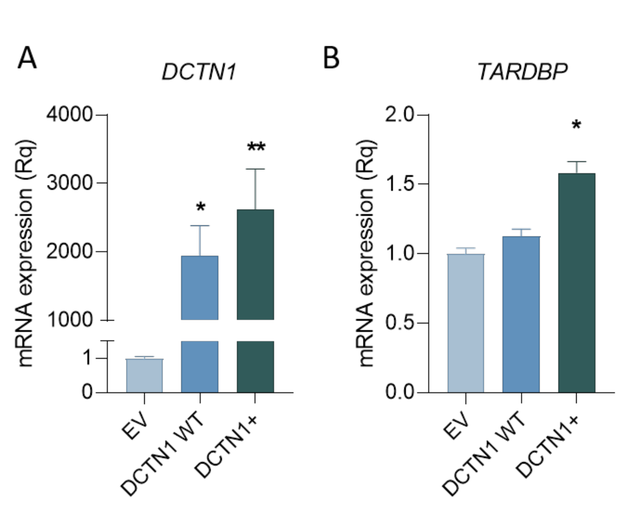
The search for genetic mutations revealed a c.1867C>T variant in DCTN1. This variant was initially identified by next-generation sequencing and subsequently confirmed by Sanger sequencing. For functional analyses, SH-SY5Y cells were transfected with a plasmid expressing the mutant DCTN1 (DCTN1+). Control conditions included cells transfected with an empty vector (EV) and cells transfected with a plasmid expressing wild-type DCTN1 (DCTN1 WT). Molecular analyses were performed using standard techniques, including quantitative PCR (qPCR) and western blotting.



Structure of plasmid RG211975 containing DCTN1 WT, used for the experiments, and sequencing electropherogram showing the successful introduction of the mutation

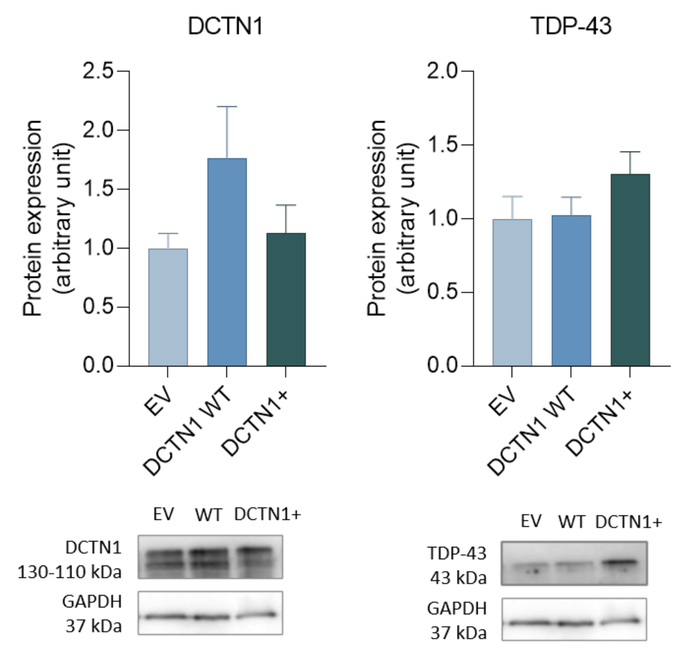
RESULTS

qPCR and western blot analyses revealed a significant increase in TDP-43 expression in DCTN1+ cells, and a slight increase in DCTN1 WT cells, compared to EV controls. These results suggest that DCTN1 expression modulates TDP-43 levels, and that the c.1867C>T mutation, in particular, leads to a substantial accumulation of TDP-43. The genetic analysis of the patient's brothers did not reveal the presence of the mutation.



RT-qPCR analysis of DCTN1 and TARDBP expression levels in SH-SY5Y cells following transfection with EV, DCTN1 WT, and DCTN1+ plasmids.
A) A statistically significant increase in DCTN1 levels was observed in cells transfected with DCTN1 WT and DCTN1+ plasmids (*p < 0.05 and **p < 0.01, respectively). No significant changes in DCTN1 levels were detected in cells transfected with the EV plasmid. B) TARDBP levels were significantly increased (*p < 0.05) upon transfection with the DCTN1+ plasmid. No significant changes in TARDBP levels were observed in cells transfected with EV or DCTN1 WT plasmids.

Data were analyzed using Student's t-test followed by Mann-Whitney test and are presented as mean ± SEM. N=3.A



Western blot (WB) analysis of DCTN1 and TDP-43 levels in SH-SY5Y cells following transfection with EV, DCTN1 WT, and DCTN1+ plasmids.
A) WB analysis showed a non-significant trend toward increased DCTN1 expression in cells transfected with the DCTN1 WT plasmid compared to cells transfected with EV and DCTN1+ plasmids.
B) WB analysis revealed a non-significant trend toward increased TDP-43 expression in cells transfected with the DCTN1+ plasmid compared to cells transfected with EV and DCTN1 WT plasmids. *Representative immunoblot images of DCTN1 and TDP-43 in cells transfected with EV, DCTN1 WT, and DCTN1+ plasmids are shown; GAPDH was used as a loading control.*

DISCUSSION AND CONCLUSION

DCTN1 variants have been found to be implicated in multiple neurodegenerative diseases, such as Perry syndrome, Progressive supranuclear palsy (PSP)-like syndromes, and ALS. The dynactin protein complex plays a major role in axonal transport, and DCTN1 mutations have been shown to alter microtubule function, causing cytoplasmic mislocalization and aggregation of the nuclear RNA-binding protein TDP-43[(3)] Our findings support the hypothesis that the DCTN1 c.1867C>T variant promotes the accumulation and aggregation of TDP-43 in neuronal cells, potentially leading to neuronal toxicity, and may contribute to ALS pathogenesis, as previously proposed by Liu et al. [(4)]

Understanding the interaction between DCTN1 mutations, TDP-43 aggregation, and neurodegeneration could play an important role in understanding ALS pathogenesis. Further studies are needed to confirm our findings, including functional assays on patient's fibroblasts-derived iPSCs.

References

1. Münch C, Sedlmeier R, Meyer T, Homberg V, Sperfeld AD, Kurt A, Prudlo J, Peraus G, Hanemann CO, Stumm G, Ludolph AC. Point mutations of the p150 subunit of dynactin (DCTN1) gene in ALS. Neurology. 2004 Aug 24;63(4):724-6. doi: 10.1212/01.wnl.0000134608.83927.b1. PMID: 15326253.
2. Konno T, Ross OA, Teive HAG, Sławek J, Dickson DW, Wszolek ZK. DCTN1-related neurodegeneration: Perry syndrome and beyond. Parkinsonism Relat Disord. 2017 Aug;41:14-24. doi: 10.1016/j.parkreldis.2017.06.004. Epub 2017 Jun 12. PMID: 28625595; PMCID: PMC5546300.
3. Deshimaru M, Kinoshita-Kawada M, Kubota K, Watanabe T, Tanaka Y, Hirano S, Ishidate F, Hiramoto M, Ishikawa M, Uehara Y, Okano H, Hirose S, Fujioka S, Iwasaki K, Yuasa-Kawada J, Mishima T, Tsuboi Y. DCTN1 Binds to TDP-43 and Regulates TDP-43 Aggregation. Int J Mol Sci. 2021 Apr 13;22(8):3985. doi: 10.3390/ijms22083985. PMID: 33924373; PMCID: PMC8070438.
4. Liu X, Yang L, Tang L, Chen L, Liu X, Fan D. DCTN1 gene analysis in Chinese patients with sporadic amyotrophic lateral sclerosis. PLoS One. 2017 Aug 8;12(8):e0182572. doi: 10.1371/journal.pone.0182572. PMID: 28792508; PMCID: PMC5549744.