Study of the tropism of a new variant of TDP-43 (G376V-TDP-43) responsible for distal myopathy but not an ALS

Amyotrophic Lateral Sclerosis (ALS) and ALS associated with Fronto Temporal Lobar Degeneration (ALS-FTLD) are fatal neurodegenerative disorders characterized by progressive muscular paralysis reflecting degeneration of upper and lower motor neurons (MNs) in the primary motor cortex, corticospinal tracts, brainstem and spinal cord. The deterioration of the patient's conditions is irreversible and death occurs with 1-5 years after the onset for 70% of affected patients, often by respiratory failure. Approximately 10% of ALS cases are considered "familial" (fALS), or transmitted within families, while the remaining cases are considered "sporadic" (sALS), or presenting without a clear familial history. Although more than 30 potentially causative or disease-modifying genes have been identified, pathogenic variants in *SOD1*, *C9ORF72*, *FUS/TLS*, and *TARDBP*, account for over 50% of the familial cases, most frequently with disease causing variants in other genes being relatively uncommon. Many of the identified variant genes were found to be involved in RNA or protein homeostasis cellular processes.

Although less common than sALS, fALS has played an outsize role in our understanding of disease mechanisms through the discovery of ALS-causing mutations within families and subsequent experimental perturbations of these mutant genes. Because fALS occurs within the same family across multiple generations, genetic approaches can be used to pinpoint the mutated gene that tracks with people who developed ALS and away from those who did not. Like several neurodegenerative disorders, ALS and ALS-FTLD are associated with the accumulation of misfolded proteins both inside and outside of neuronal and glial cells. TDP-43 (reviewed in Smethurst *et al.*, Neuropathol&Appl Neurobiol 2015) is the major component of Tau an α-synuclein negative but Ubiquitin positive pathological inclusions found in the brains of patients with ALS and FTLD (Arai *et al*., BBRC 2006; Neumann *et al.*, Science 2006). TDP-43 pathological inclusions are observed in more than 95% of ALS and ALS-FTLD affected patients making this protein a key component in the pathology that it is essential to study and characterize.

We recently identified a new missense variant of TDP-43 (G376V-TDP-43) into the C-terminal prion-like domain of the protein in two French families affected by an autosomal dominant distal myopathy but not fulfilling diagnostic criteria for ALS (Zibold *et al.,* Brain 2023). Patients from both families presented with progressive weakness and atrophy of distal muscles, starting in their 5th-7th decade. Muscle biopsies revealed a degenerative myopathy characterized by accumulation of rimmed (autophagic) vacuoles, disruption of sarcomere integrity and severe myofibrillar disorganization. Variant pathogenicity was supported by functional studies. The G376V mutant increased the formation of cytoplasmic TDP-43 condensates in cell culture models, promoted assembly into high molecular weight oligomers and aggregates in vitro, and altered morphology of TDP-43 condensates arising from phase separation. Moreover, the variant led to the formation of cytoplasmic TDP-43 condensates in patient-derived myoblasts and induced abnormal mRNA splicing in patient muscle tissue. Strikingly, changing the same glycine residue into an aspartic acid (G376D) causes an ALS with a rapid progression. Comparisons of G376V and G376D in different cellular models as well as in vitro using recombinant proteins revealed that the G376V variant was more prone to form insoluble TDP-43 condensates compared to both the G376D variant and the wild type TDP-43, while the G376D variant had only a very minor effect.

This is the first time that two different substitutions altering the same amino acid position in TDP-43, primary drive an inherited disease in two separated directions ie muscular disorder versus a fatal ALS. In this context, we propose to investigate at the cellular and transcriptomic levels (mRNA expression and splicing variants characterizations) if the G376V TDP-43 variant displays a muscle tropism and can impact this specific tissue compared to the G736D variant. For this purpose, we will explore the functional consequences of the expression of both variants (G376V and G376D) into MNs and muscle cells differentiated from CRISPR Cas9 modified-iPSC but also from iPSC derived from patients to evaluate the imprinting impact of the familial genetic background. Interestingly, two additional French families suffering of distal myopathy (unpublished) were recently identified with the same G376V TDP-43 variant. Analyses will also be conducted in patient muscle biopsies from the different families to characterize the impact of this mutation.
Because we cannot exclude that additional modifier genes could attenuate the phenotype associated with the G376V-TDP-43 variant into our families, we also propose to determine through whole exome analyses, if candidate modifier genes can be highlighted in different patients from the different identified families.
Overall, this project will give important informations on molecular and cellular properties of TDP-43 through an original experimental approach that will consist in starting from patients with a distal myopathy associated with a TDP-43 mutation to better characterize the role of TDP-43 in the ALS pathology.
Understanding why the G376V-TDP-43 is associated with a less deleterious disease is of utmost importance to identify potential therapeutical targets to fight against ALS.

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