Modelling a *TARDBP*-ALS Family in iPSC-Derived Neural Stem Cells Uncovers Metabolic and Proliferative Defects

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Keywords: ALS, TDP-43, hiNSCs, Proliferation, Metabolism, Oxidative Stress

Abstract:

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disorder that primarily affects upper and lower motor neurons leading to paralysis and death. Approximately 10% of ALS cases are familiar (fALS) arising from an inherited gene mutation. However, the cellular and molecular mechanisms underlying the disease remain poorly understood.

Growing evidence shows a pivotal role of abnormalities in energy metabolism and mitochondrial dysfunction in ALS, thus suggesting a strong association between mitochondria metabolism disorder and neurodegeneration. Moreover, emerging findings suggest that cellular alterations of stem cell population in central nervous system (CNS), may contribute to ALS.

To interrogate the presence of stem cell-fALS signatures, we focused on an Italian family carrying the c.1127G → A mutation in the *TARDBP* gene which encodes for the p.G376D variant of TAR-DNA-binding protein-43 (TDP-43^{G376D}). To explore this hypothesis, we leveraged the use of iPSC-derived Neural Stem Cells (hiNSCs) from family members belonging to the *TARDBP*-ALS family.

Our cohort includes cell lines obtained from a TDP-43^{G376D} patient at early and late stage of the disease, an asymptomatic mutation carrier, a healthy familial individual and healthy individuals unrelated to the family.

To uncover functional alterations associated with mutant TDP-43^{G376D}, we assessed proliferation dynamics, oxidative stress levels, energy metabolism and mitochondria functionality.

Preliminary findings revealed that hiNSCs carrying the p.G376D mutation displayed altered cellular morphology and a hyperproliferation phenotype accompanied by dysregulated cell cycle progression. Moreover, this hyperproliferative behaviour also induce a metabolic reframing. Indeed, hiNSCs derived from *TARDBP*-ALS family members exhibit shared pathological features including increased oxidative stress, altered mitochondrial functionality, reduced energetic profile, diminished mitochondrial membrane potential and lower ATP production. These alterations go along with a modest shift toward glycolysis and decreased reliance on fatty acid oxidation. Together, our findings reveal shared metabolic and cellular abnormalities across family members, suggesting the presence of previously unrecognized alterations that may contribute to ALS.

Collectively, our study supports the use of hiNSCs as a reliable cell model, providing a platform to unravel critical gaps in understanding disease onset and progression.