«METABOTYPING OF ALS PATIENTS: *SOD1* AND *TARDBP* MUTATED FIBROBLASTS DISPLAY AN ENHANCED METABOLIC ACTIVITY AND GLYCOLYTIC STATE

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Background

Among the 150 genes associated with ALS, *TARDBP* and *SOD1* play an important role.

Mutant SOD1 protein induce toxicity through a gain-of-function mediated by the adoption of misfolded conformations and formation of protein aggregates. While mutant TDP-43 protein is mis-localized from the nucleus and aggregates in the cytoplasm, thus losing its RNA metabolism-related activity.

Interplay between oxidative stress and metabolic dysfunctions has been associated with ALS.

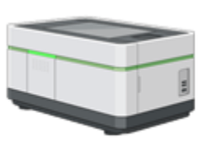
Indeed, SOD1 and TDP-43 mutations affect the electron transport chain complexes activity, thus initiating a "vicious-cycle" that sustain metabolic stress[1].

Experimental approach

1 Primary fibroblasts from ALS patients

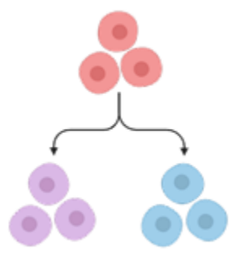
Agilent Seahorse XF Analyzer

Operetta High Content Imaging System



Metabotyping

2 hiPSCs



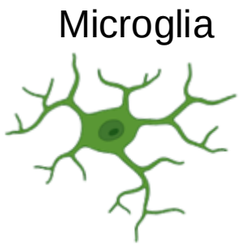
Definition of fully human, patient tailored models

3 Complex *in vitro* models

Neuroprogenitor cells

Neurons

Microglia

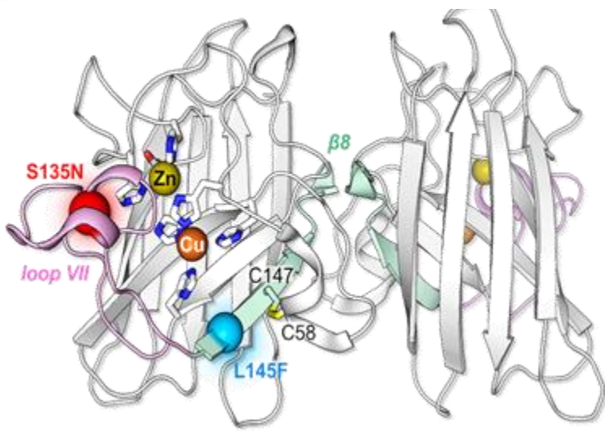


Astrocytes

Finding a link between **genetic background** and **metabolic manifestations** and correlation with disease features (susceptibility, age of onset and progression rate/survival).

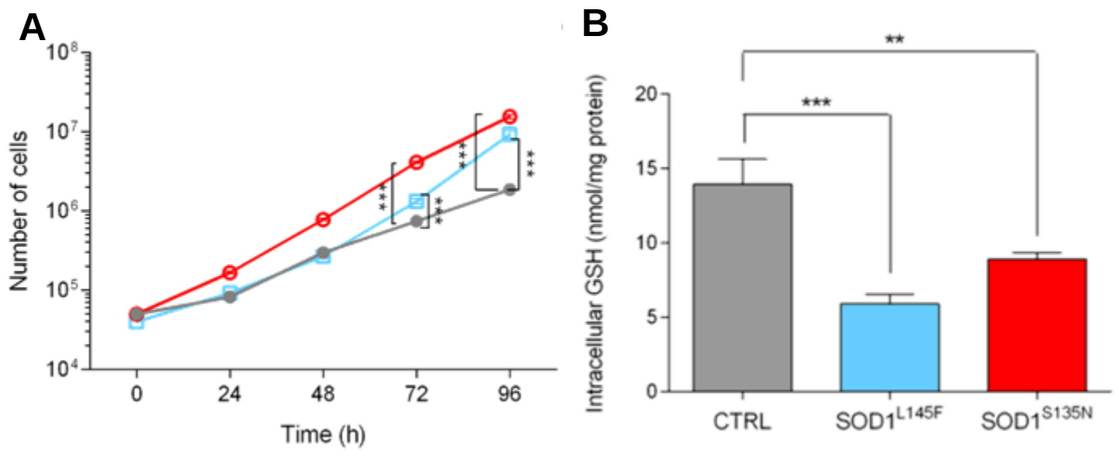
Results

SOD1



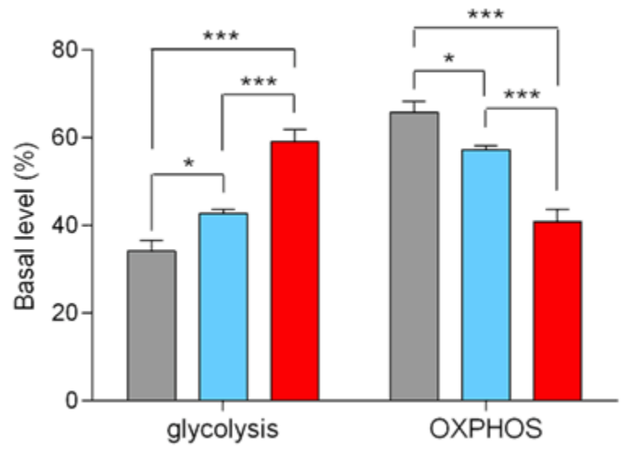
Bioinformatic analysis revealed that p.S135N results in greater destabilization as compared to p.L145F

Oxidative stress



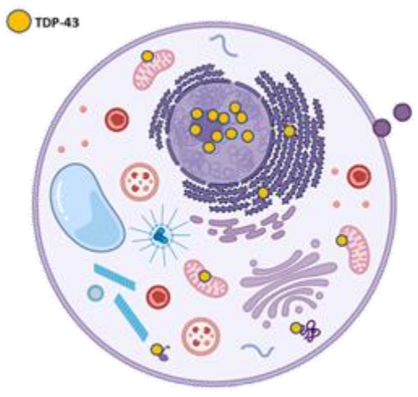
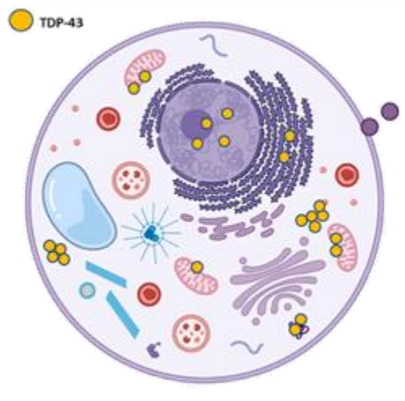
ALS-fibroblasts showed an increased proliferation rate (**A**) and significantly lower total intracellular glutathione levels compared to healthy control cells. These findings suggest that affected individuals may lack GSH protective activity (**B**).

Metabolic reframing

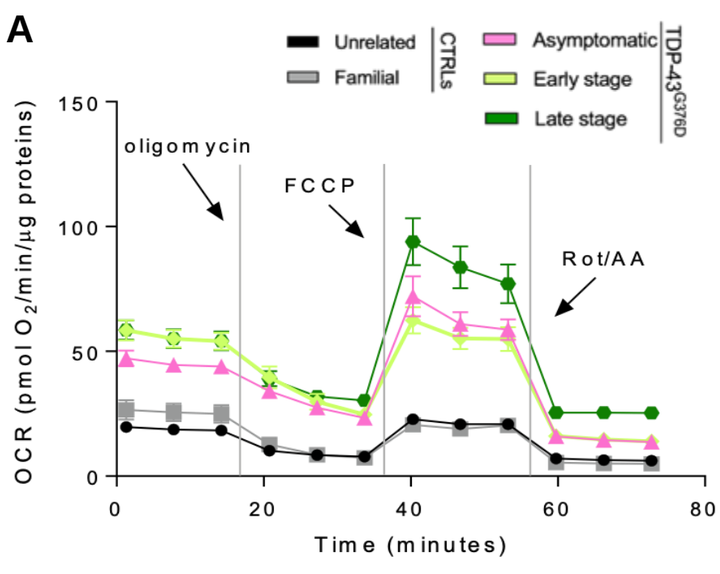


SOD1^{S135N} fibroblasts produced the majority of ATP through glycolysis, showing a shift towards Warburg effect.

TDP-43

HEALTH	DISEASE
	
Most of TDP-43 is found in the **nucleus**	TDP-43 **mislocalization** in the cytoplasm, ER and mitochondria

Hypermetabolism



TDP-43^{G376D} fibroblasts possess a higher basal and maximal mitochondrial activity compared to controls (**A**). No differences in their ability to upregulate the mitochondrial respiration to meet changing energy demand; yet, the TDP-43^{G376D} patient's fibroblasts increased the glycolytic activity significantly more (**B**).

Conclusions

Both *SOD1* and *TARDBP* mutant fibroblasts display metabolic rearrangements («**hypermetabolism**»[2] and progressive reliance in **glycolysis**[3]). This bioenergetic rearrangement might be hampered in neural cells that have a reduced ability to switch to glycolysis upon OXPHOS limitations. So, our following step is the development of "complex" in vitro models to study the interactions among neural, and non-neural cells.

Future perspectives

1. **Study pathological mechanisms**
2. **Cell therapy with neural stem cells[4]**
3. **Search of therapeutic targets**

References

1. Carrera-Juliá, et al., 2020. Antioxidant Alternatives in the Treatment of Amyotrophic Lateral Sclerosis: A Comprehensive Review. Frontiers in Physiology;
2. Ferri, A., Coccurello, R., 2017. What is "Hyper" in the ALS Hypermetabolism? Mediators of Inflammation 2017, e7821672.
3. Wang Y, Patti GJ. The Warburg effect: a signature of mitochondrial overload. Trends Cell Biol. 2023 Dec;33(12):1014-1020.
4. Leone MA, et al. Phase I clinical trial of intracerebroventricular transplantation of allogeneic neural stem cells in people with progressive multiple sclerosis. Cell Stem Cell. 2023 Dec 7;30(12):1597-1609.e8.