CHARACTERIZATION OF THE P.L145F AND P.S135N MUTATIONS IN SOD1: IMPACT ON THE METABOLISM OF FIBROBLASTS DERIVED FROM ALS PATIENTS

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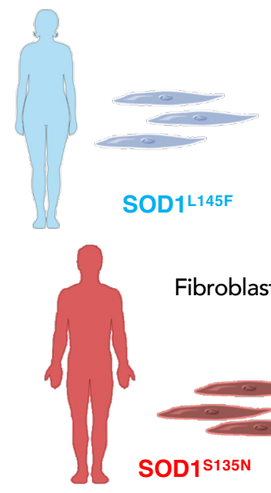
INTRODUCTION

With aging, the nervous system gradually undergoes degeneration. Increased oxidative stress, mitochondrial dysfunction, and protein aggregation are common pathophysiological mechanisms of various neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), which is characterized by the loss of upper and lower motor neurons[1]. The causes and mechanisms behind most NDs are still vague. A common hallmark of several NDs is the accumulation and aggregation of proteins. At least 25% of ALS patients carry a genetic mutation which is likely to be the cause of the disease[2]. SOD1 is the first studied gene among the ones related to ALS. Mutant SOD1 can adopt multiple misfolded conformation, lose the correct coordination of metal binding, decrease structural stability, and form aggregates[3].

AIMS AND EXPERIMENTAL PLAN

The use of cells derived from patients may help to identify impaired cellular functions and correlations with symptoms, genetic alterations and disease stage

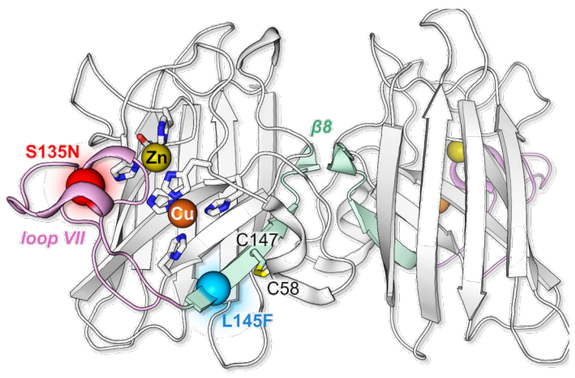


Characterization of the alterations of ALS-associated mutant SOD1

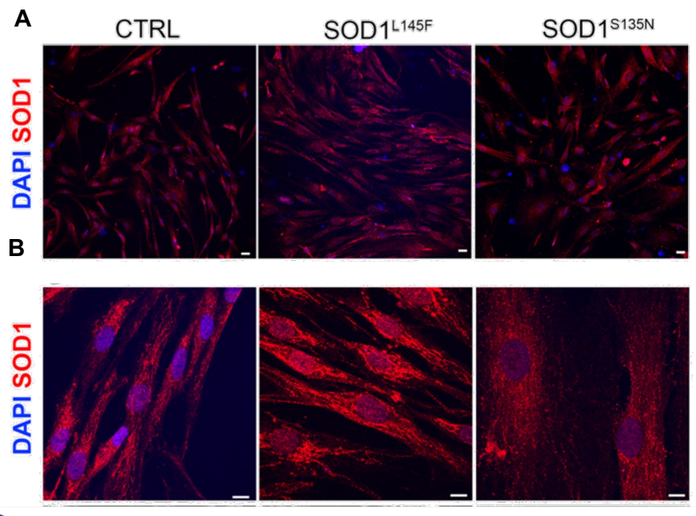
- Bioinformatic analysis
- Biochemical assays
- Seahorse analysis

RESULTS

SOD1 mutations alter protein stability and distribution

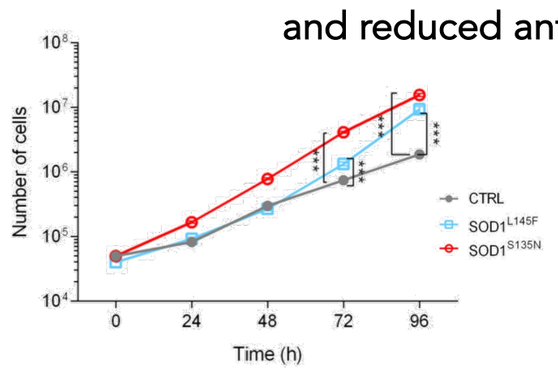


Bioinformatic analysis of Cu/Zn superoxide dismutase-1 shows that the p.L145F mutation lightly affects the enzymatic core of the protein, most likely impairing SOD1 ability to form dimers, while the p.S135N has a more destabilizing effect on the metal-binding core.

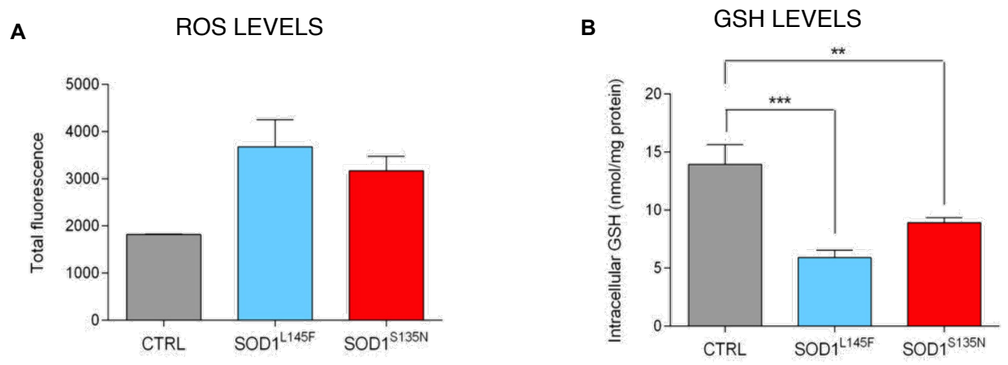


Immunofluoresscence shows that SOD1 protein in $SOD1^{L145F}$ fibroblasts appears more concentrated in the cytoplasmic region aroud the nucleus, respect to healthy and $SOD1^{S135N}$cells.
Scale bars: in A 25 μm, in B 10 μm

SOD1 mutant fibroblasts have a higher proliferation rate and reduced anti-oxidant potential



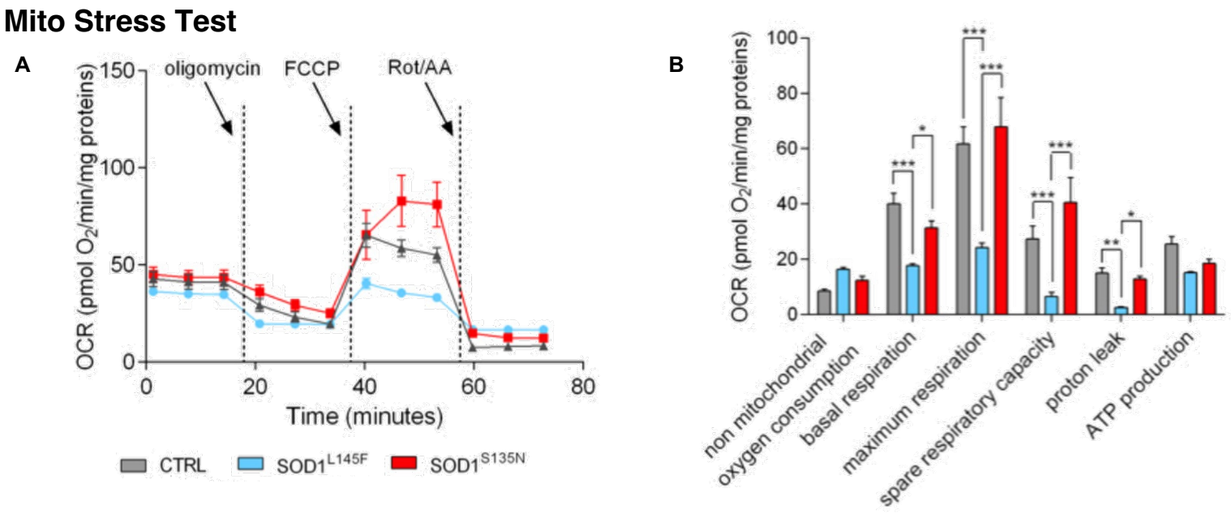
Growht curves show that both the SOD1 mutations are associated with increased proliferation rates compared to the control fibroblasts. Statistically significant differences were observed at the two timepoints considered (72 and 96 h).



A. *The measurement of DCF fluorescence shows higher levels of ROS in both cell lines carrying the SOD1 mutations compared to control. However, the differences are not statistically significant.* ***B.*** *GSH is the main non-enzymatic antioxidant scavenger. Fibroblasts derived from the healthy volunteer showed significantly higher total intracellular GSH levels compared to SOD1 mutants. These findings suggest that affected individuals may lack GSH protective activity.*

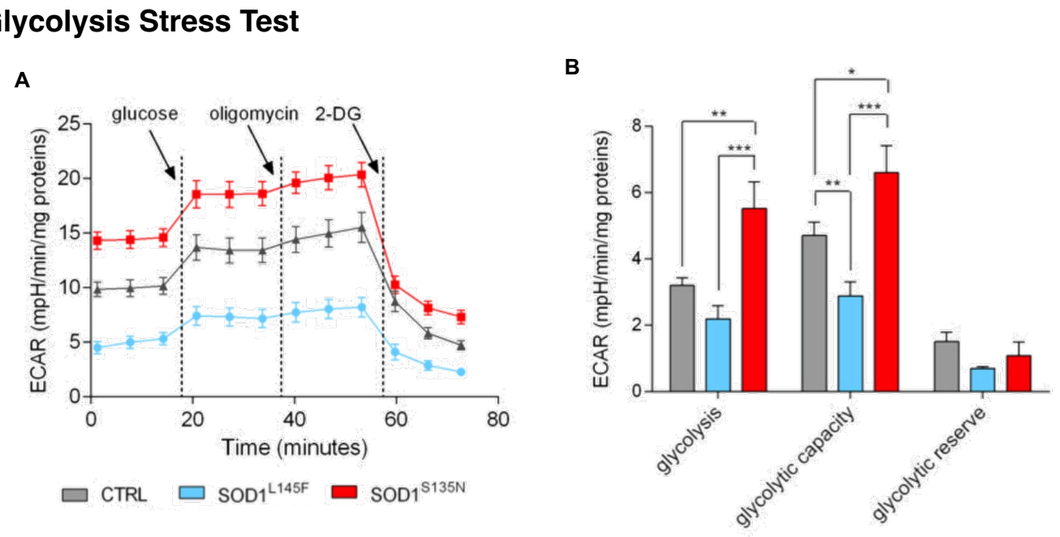
Bioenergetic alterations in $SOD1^{L145F}$ and $SOD1^{S135N}$ fibroblasts

Mito Stress Test



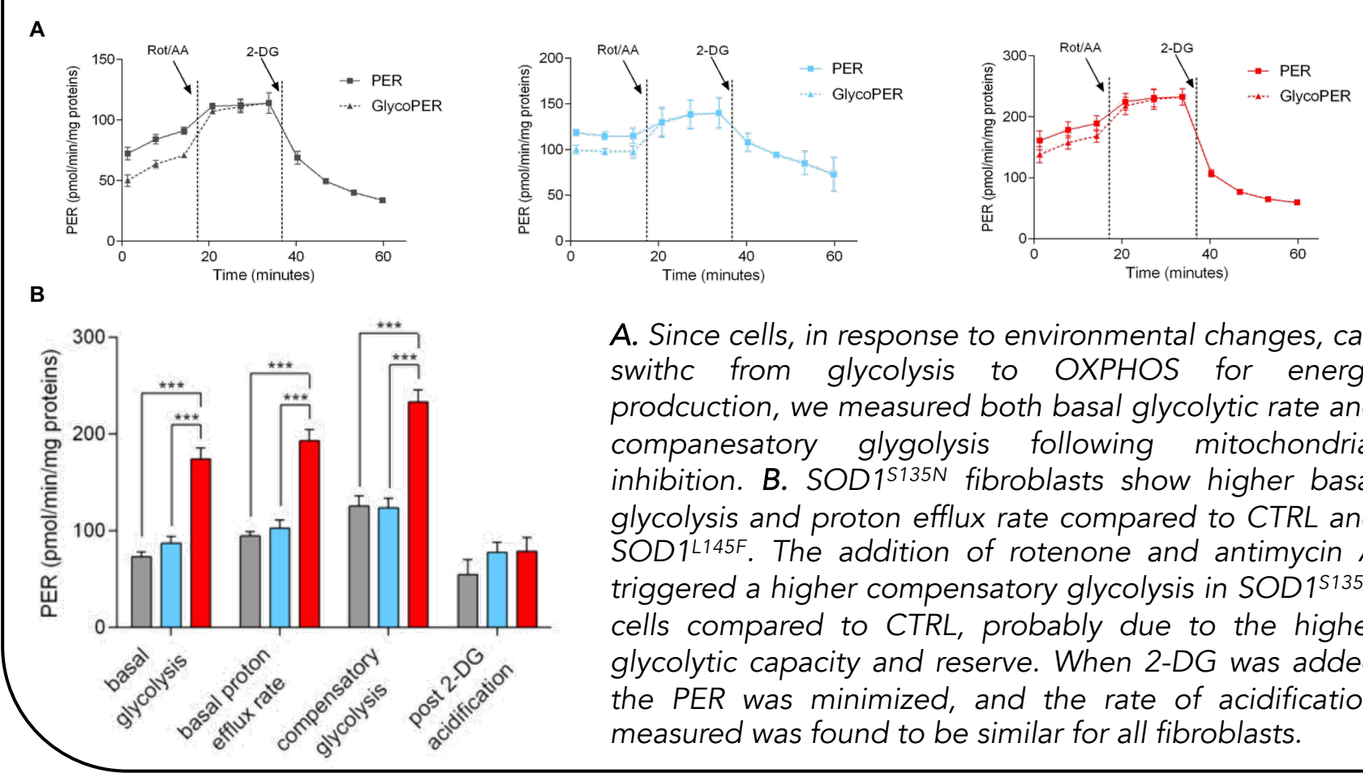
A major goal of current bioenergetic research is to study cellular and molecular pocesses in models of physiological relevance, while avoiding many of the artifacts associated with mitochondrial isolation and cell permeabilization. ***A.*** *Both CTRL and $SOD1^{S135N}$ fibroblasts have significantly higher basal and maximum respiration rated compared to $SOD1^{L145F}$ fibroblasts Since this was the maximum rate of respiration that the cell could achieve under a "physiological energy demand", this suggests that the $SOD1^{L145F}$ fibroblasts had already reached their highest respiratory rate and were not able to increase any further.* ***B.*** *The spare respiratory capacity, obtained by subtracting basal respiration to the maximum respiration rate, was significantly lower in $SOD1^{L145F}$ cells, a further sign that these cells were already operating close to their bioenergetic limit.*

Glycolysis Stress Test



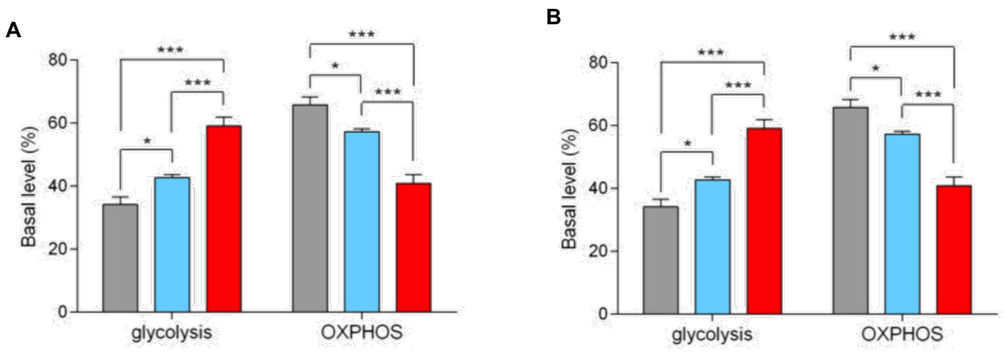
A. *The ECAR profile shows that $SOD1^{S135N}$ fibroblasts have a higher proton extrusion both at the basal level and after the injection of saturating glucose concentration.* ***B.*** *$SOD1^{S135N}$ cells show a significant increase in glycolysis, as well as in glycolytic capacity, while $SOD1^{L145F}$ show the lowest values for all the parameters.*

Glycolysis Rate Assay



A. *Since cells, in response to environmental changes, can swithc from glycolysis to OXPHOS for energy prodcuction, we measured both basal glycolytic rate and companesatory glygolysis following mitochondrial inhibition.* ***B.*** *$SOD1^{S135N}$ fibroblasts show higher basal glycolysis and proton efflux rate compared to CTRL and $SOD1^{L145F}$. The addition of rotenone and antimycin A triggered a higher compensatory glycolysis in $SOD1^{S135N}$ cells compared to CTRL, probably due to the higher glycolytic capacity and reserve. When 2-DG was added the PER was minimized, and the rate of acidification measured was found to be similar for all fibroblasts.*

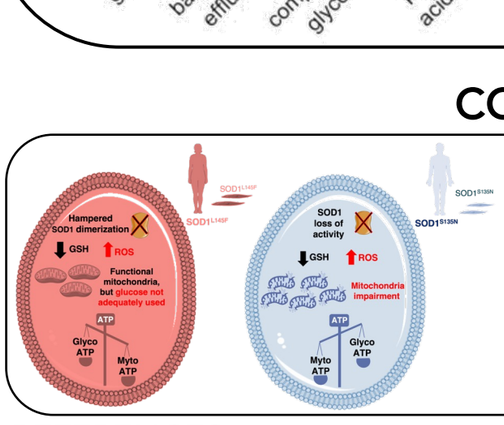
ATP production



The ATP levels were measured to assess the relative contributions of glycolysis and OXPHOS to the overall ATP production; the ATP symthase inhibitors and Rot/AA , and inhibitor of complexes I and III were added in sequence.
A. *No significant differences were detected in the overall levels of ATP between the cells carrying mutated SOD1 and control fibroblasts of the SOD1 mutations determined a higher basal glycolytic level than the control.* ***B.*** *Both of the SOD1 mutations determined a higher basal glycolytic level than the control cells; however, while both $SOD1^{L145F}$ and control fibroblasts still produce most of their ATP through OXPHOS, only $SOD1^{S135N}$ fibroblasts produced the majority of their ATP through glycolysis, showing a shift towards the Warburg effect.*

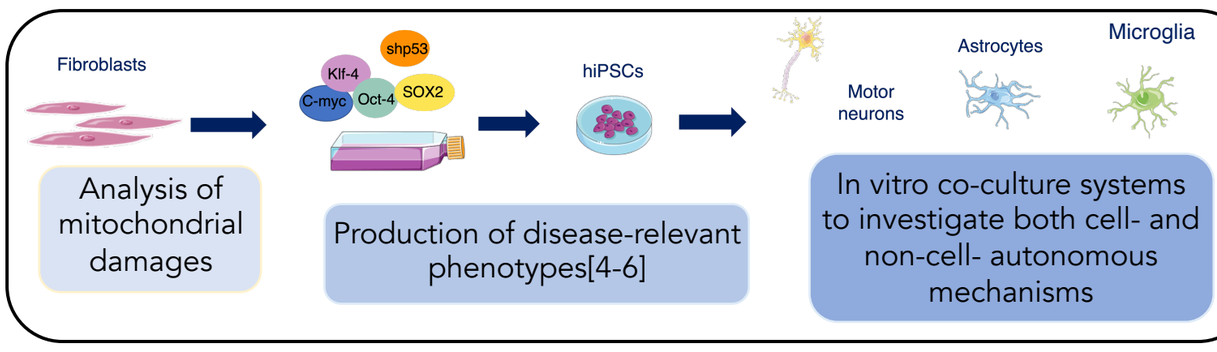
Statistical significance:
$p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***)

CONCLUSIONS



In both the mutated cell lines analyzed, metabolic rearrangements were detected. But each mutation affects the protein structure differently, matching its impact on the morphological, functional and energetic properties of patients-derived fibroblasts.

FUTURE PERSPECTIVES



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