

PRECISION MEDICINE FOR MITOCHONDRIAL DISEASES CAUSED BY PATHOGENIC VARIANTS IN THE *SPATA5* (*AFG2A*) GENE

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INTRODUCTION

Mitochondrial diseases encompass a clinically and genetically heterogeneous group of neuromuscular and neurodegenerative disorders that currently lack effective therapeutic options. Among these, autosomal recessive pathogenic variants in the *SPATA5* (*AFG2A*), and its paralog *SPATA5L1*, genes have been identified as the underlying cause of a severe neurodevelopmental syndrome (SPATA5-NDD or DEE29). This syndrome is characterized by progressive microcephaly, intellectual disability, sensorineural hearing loss, intractable epilepsy, and muscle tone alterations (1). At the molecular level, *SPATA5* encodes a specialized AAA+ ATPase that acts within a multiprotein complex (55LCC) and plays an essential role in ribosome biogenesis, specifically promoting maturation steps of the pre-60S subunit (2). Despite its primary cytosolic role, *SPATA5* deficiency secondarily induces severe mitochondrial dysfunction, leading to disruption of organellar proteostasis and bioenergetic failure. Based on previous findings of our lab, where modulation of mitochondrial unfolded protein response (UPRmt) yielded positive results (3), this study aims to evaluate UPRmt modulation as a therapeutic candidate to rescue *SPATA5*-associated cellular pathophysiology.

OBJECTIVES

The main objectives of this project are:

- 1. Pathophysiological characterization:** validate patient-derived primary fibroblasts as cellular models to study the cellular pathophysiology associated with pathogenic variants of *SPATA5* gene
- 2. Pharmacological screening:** evaluate the efficacy of UPRmt modulation through a panel of candidate therapeutic compounds
- 3. Direct reprogramming:** generate induced neurons (iNs) by direct reprogramming of patient-derived primary fibroblasts and confirm the metabolic rescue in a neuronal phenotype

METHODOLOGY

Primary dermal fibroblasts were established from skin biopsies of 8 patients carrying *SPATA5* and *SPATA5L1* pathogenic variants and maintained under standard culture conditions (37°C, 5% CO₂). The experimental strategy and the clinical genetic variants are summarized below.



Figure 1: Schematic workflow of primary dermal fibroblasts isolation from skin biopsy

Patient	Mutation (Allele1/Allele2)	Patient	Mutation (Allele1/Allele2)
P1	c.251G>A; p.Arg84Gln c.1064T>C; p.Ile355Thr	P5	c.251G>A; p.Arg84Gln c.1586G>A; p.Arg529Gln
P2	c.251G>A; p.Arg84Gln c.251G>A; p.Arg84Gln	P6	c.251G>A; p.Arg84Gln c.2130_2133del; p.Glu711Profs*21
P3	c.327_328del; p.Lys110fs c.1631T>G; p.Leu544Arg	P7	c.251G>A; p.Arg84Gln c.2174T>C; p.Phe725Ser
P4*	c.1235_1236del; p.Cys412Ter c.1190C>A; p.Ala397Glu	P8	c.989_991del; p.Thr330del c.1714+1G>A

*This patient harbours mutations of *SPATA5L1* gene

Table 1. Pathogenic genetic variants identified in the *SPATA5* and *SPATA5L1* patients

RESULTS

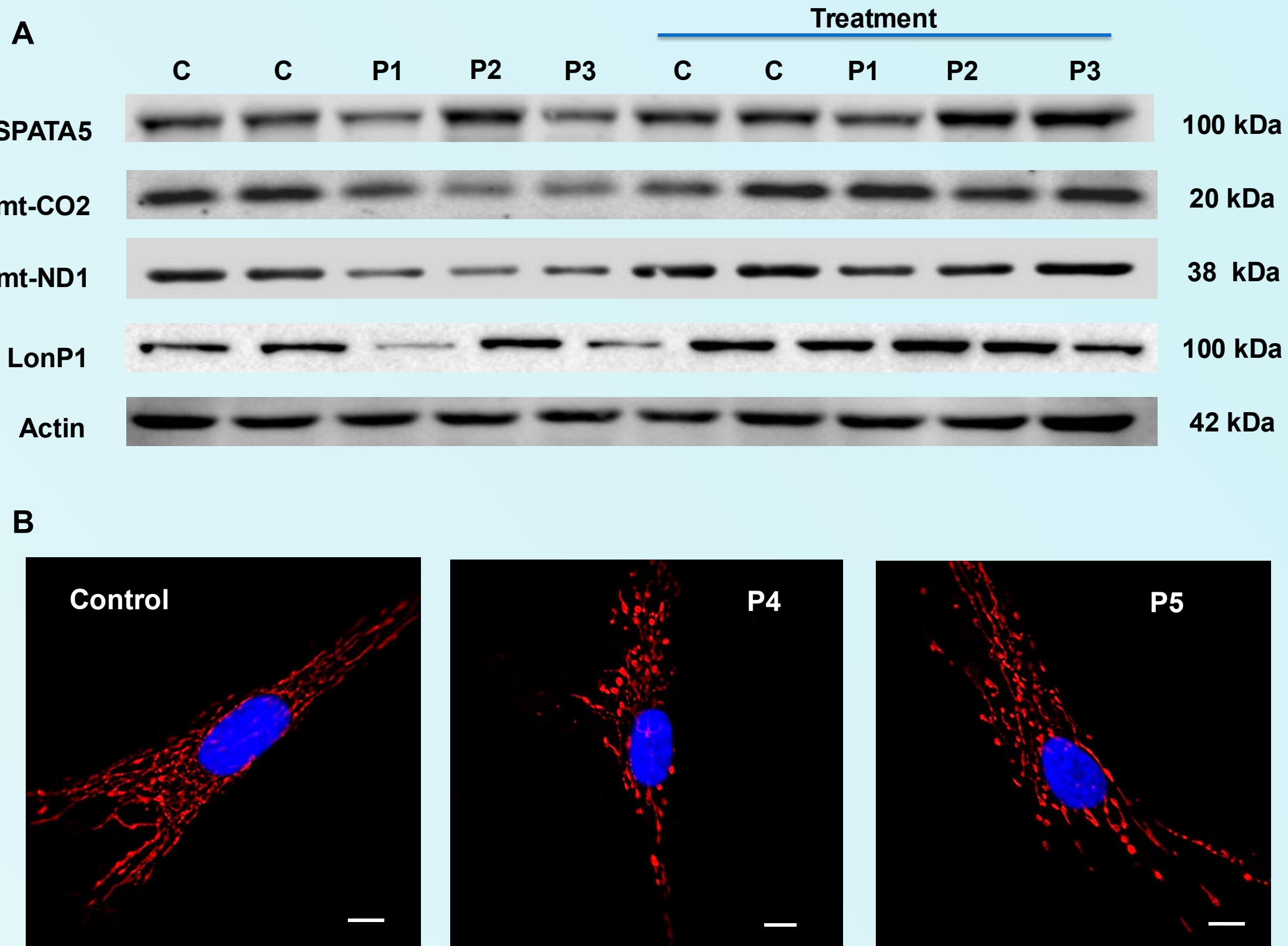


Figure 2. Protein expression and mitochondrial network morphology. A. Western blot analysis of SPATA5, mt-CO2, mt-ND1 and LonP1 levels in both untreated and treated control (C) and patient (P1, P2, P3) fibroblasts. Cells were treated for 7 days. Actin was used as a loading control. B. Representative immunofluorescence images showing mitochondrial morphology (red, MitoTracker CMXRos) and nuclei (blue, DAPI) in control and patient (P4, P5) fibroblasts. Scale bar: 15 um.

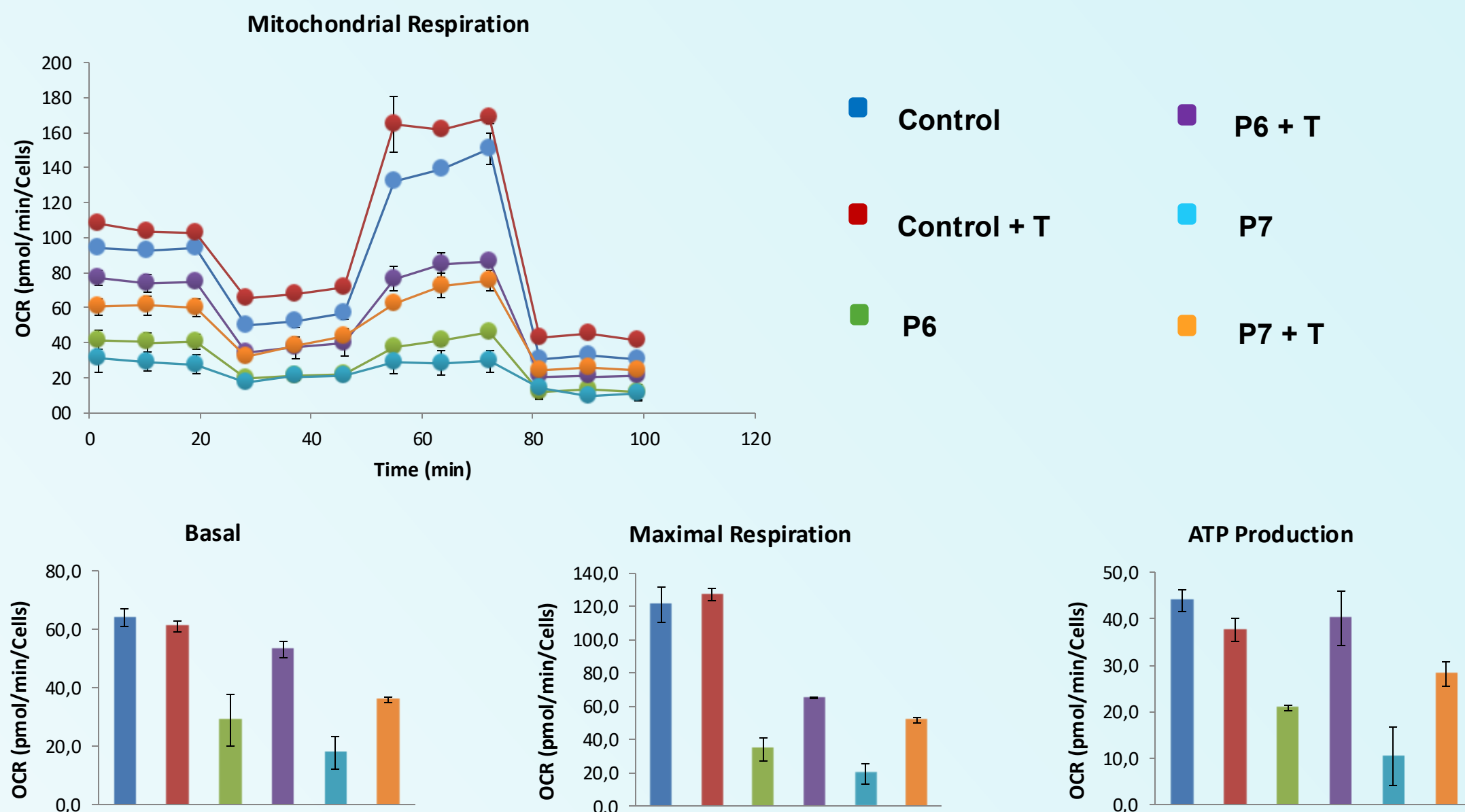


Figure 3. Mitochondrial respiration profile. Oxygen consumption rate (OCR) was measured by Seahorse XFe Analyzer in both untreated and treated control and patient (P6, P7) fibroblasts. Cells were treated for 7 days. Preliminary data, statistical analysis was not performed.

CONCLUSIONS

- Patient-derived primary fibroblasts represent a promising cellular model reproducing the cellular pathophysiology associated with *SPATA5/SPATA5L1* variants
- Protein expression analysis by western blotting reveals a downregulation of key mitochondrial proteins alongside altered UPRmt markers in most patient cells
- The Seahorse Mitostress Test assay demonstrates a notable bioenergetic defect in patient cells, which exhibits a trend toward functional rescue by the candidate treatment
- Pharmacological modulation of UPRmt appears to alleviate mitochondrial dysfunction, emerging as a promising therapeutic target for *SPATA5/SPATA5L1* deficiency
- Further experiments are required to validate these preliminary findings and extend their evaluation to induced neurons (iNs)

REFERENCES

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