

Precision medicine for mitochondrial diseases caused by pathogenic variants in the *SUCLG1* gene

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Introduction

Mitochondrial DNA Depletion Syndrome 9 (MTDPS9) is a severe, early-onset autosomal recessive disorder characterized by lactic acidosis, profound hypotonia, and progressive multiorgan failure. The disease is caused by biallelic mutations in the *SUCLG1* gene, which encodes the alpha subunit of succinate-CoA ligase (SUCL). SUCL is not only a critical enzyme in the tricarboxylic acid (Krebs) cycle, but it also plays a key role in the synthesis and maintenance of mitochondrial DNA (mtDNA). Consequently, impairment of SUCL disrupts the mitochondrial nucleoside salvage pathway, leading to severe mtDNA depletion and the subsequent failure of oxidative phosphorylation (OXPHOS).

Given the rarity and clinical heterogeneity of MTDPS9, standardizing effective treatments remains a formidable challenge. To address this, our research focuses on precision medicine by utilizing patient-derived primary fibroblasts. These personalized cellular models provide an accessible, physiologically relevant platform to investigate the precise molecular consequences of specific *SUCLG1* mutations and to perform targeted pharmacological screenings. Building upon this approach, we have observed that treatment with a customized cocktail, consisting of vitamins and mitochondrial enhancers, previously used as mtUPR activator exerts positive effects, successfully ameliorating the pathophysiological alterations studied in these patient-specific models.

Objectives

- To validate fibroblasts derived from patients harbouring mutations in *SUCLG1* gene as a suitable cellular model for studying pathophysiological features of MTDPS9.
- To identify potential therapeutic compounds for MTDPS9 through targeted pharmacological screenings in patient-derived fibroblasts.
- To obtain induced neurons (INs) by direct reprogramming, aiming to investigate the pathophysiological features in a cell type more severely affected by the disease.
- To test the therapeutic compounds that showed positive effects in fibroblasts on the newly generated neuronal models.

Methodology

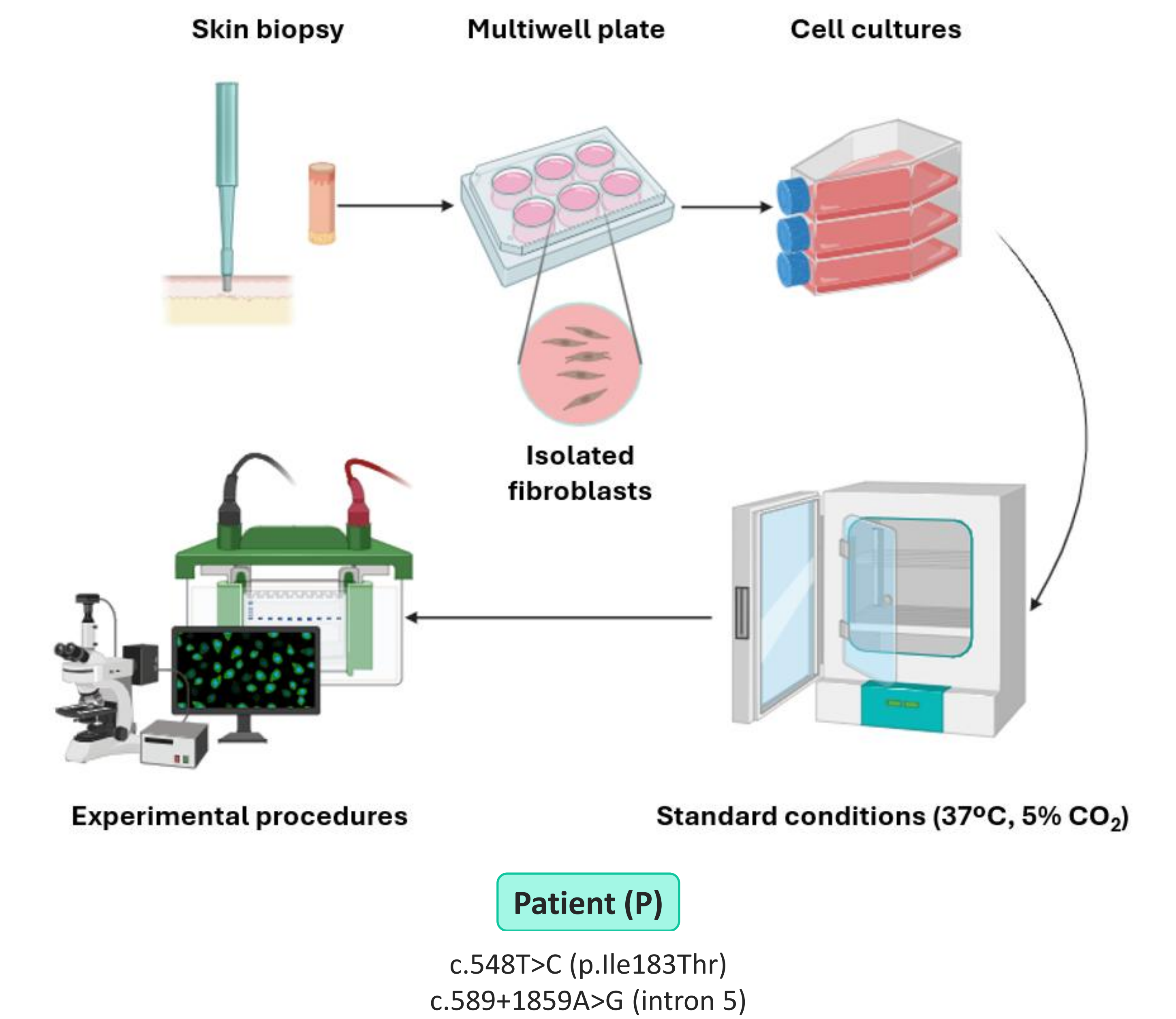


Figure 1. Schematic overview of the laboratory workflow following skin biopsy and cell lines derived from the patient currently comprising the study.

References

Chen YM, Chen W, Xu Y, Lu CS, Zhu MM, Sun RY, Wang Y, Chen Y, Shi J, Wang D. Novel compound heterozygous *SUCLG1* variants may contribute to mitochondrial DNA depletion syndrome-9. *Mol Genet Genomic Med*. 2022 Sep;10(9):e2010. doi: 10.1002/mgg3.2010. Epub 2022 Jun 28. PMID: 35762302; PMCID: PMC9482404.

Romero-Domínguez JM, Cilleros-Holgado P, Gómez-Fernández D, Piñero-Pérez R, Reche-López D, Romero-González A, Álvarez-Córdoba M, López-Cabrera A, De Oliveira MC, Rodríguez-Sacristán A, González-Granero S, García-Verdugo JM, Aroca A, Sánchez-Alcázar JA. Mitochondrial Unfolded Protein Response (mtUPR) Activation Improves Pathological Alterations in Cellular Models of Ethylmalonic Encephalopathy. *Antioxidants (Basel)*. 2025 Jun 16;14(6):741. doi: 10.3390/antiox14060741. PMID: 40563372; PMCID: PMC12189359.

Cilleros-Holgado P, Gómez-Fernández D, Piñero-Pérez R, Romero-Domínguez JM, Reche-López D, López-Cabrera A, Álvarez-Córdoba M, Munuera-Cabeza M, Talaverón-Rey M, Suárez-Carrillo A, Romero-González A, Sánchez-Alcázar JA. Mitochondrial Quality Control via Mitochondrial Unfolded Protein Response (mtUPR) in Ageing and Neurodegenerative Diseases. *Biomolecules*. 2023 Dec 13;13(12):1789. doi: 10.3390/biom13121789. PMID: 38136659; PMCID: PMC10741690.

Results

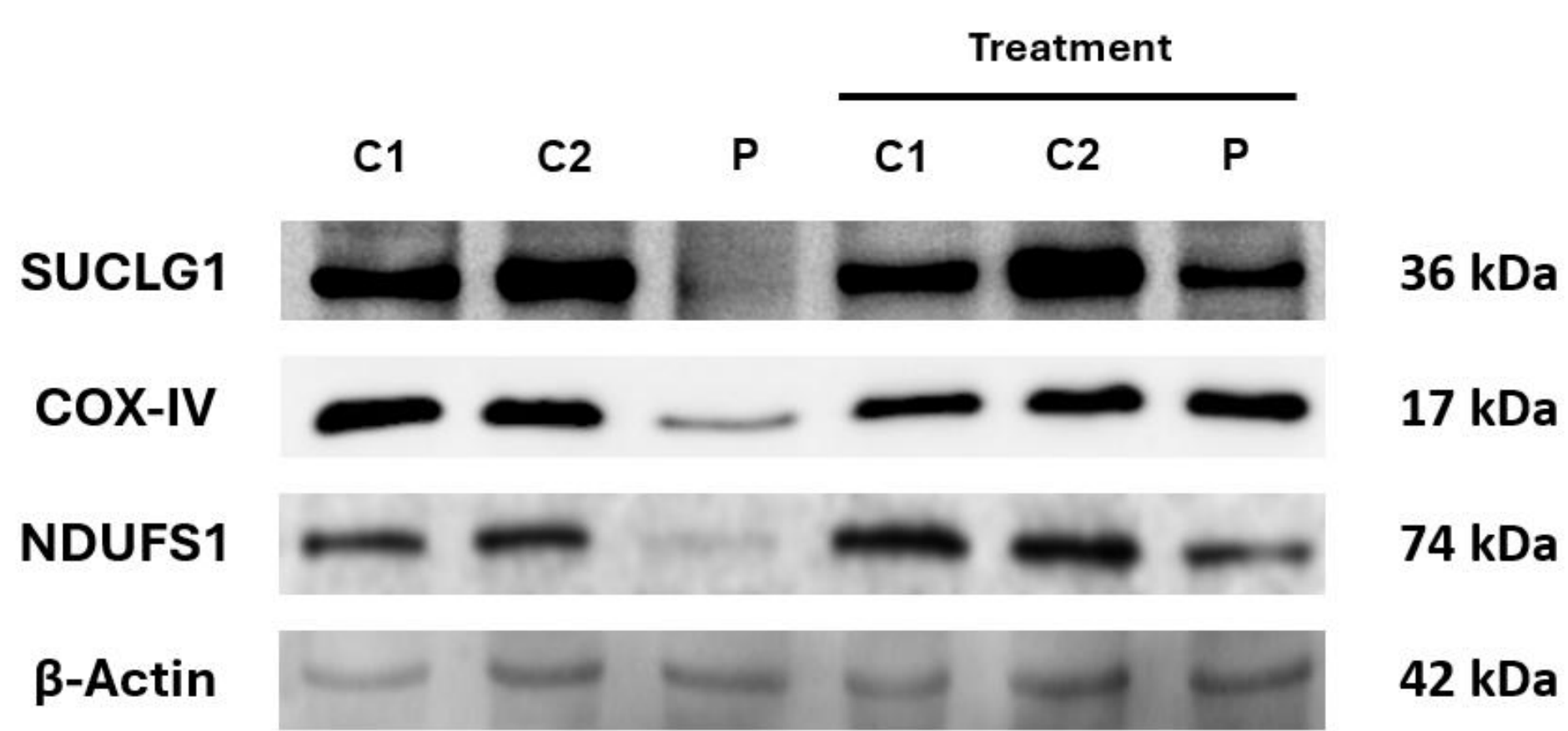


Figure 2. Protein expression levels of SUCLG1, COX-IV and NDUFS1 in control and fibroblasts derived from the patient affected with MTDPS9. Cell extracts from controls (C1 and C2) and the patient with mutations in the *SUCLG1* gene (P) were subjected to perform immunoblot analysis. Protein extracts (30 µg) were separated on an SDS-polyacrylamide gel and subsequently probed with antibodies against SUCLG1, COX-IV, NDUFS1 and β-actin (the latter used as a loading control).

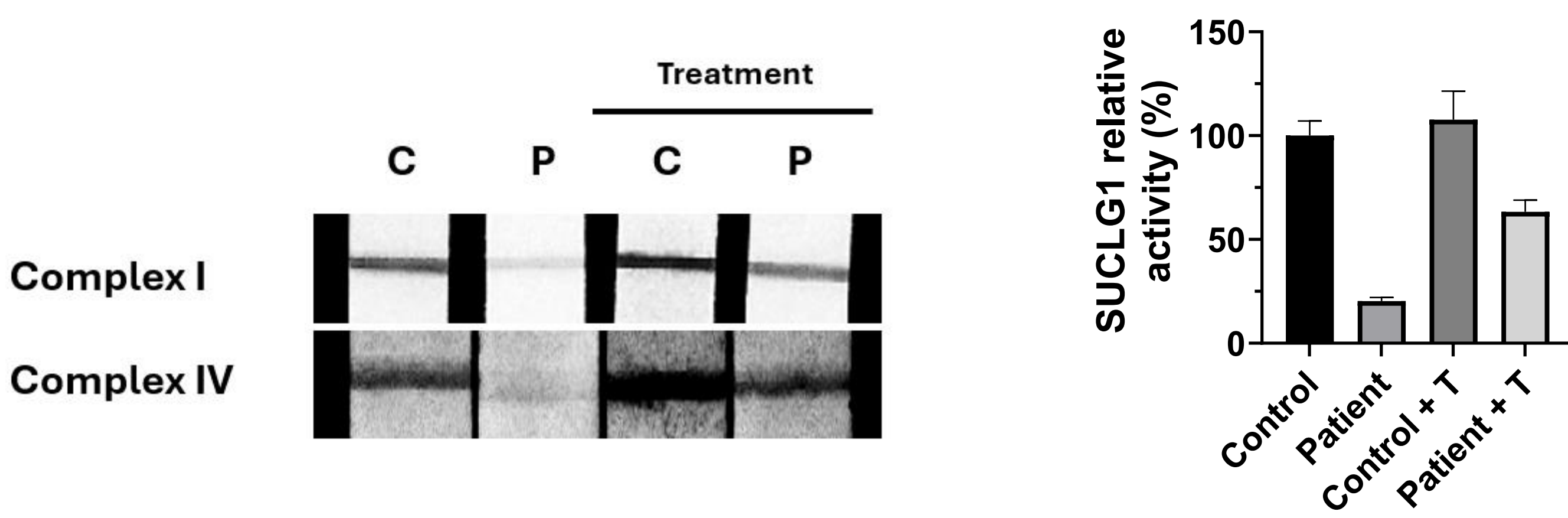


Figure 3. Complex I and complex IV activities through dipstick assays of control and fibroblasts derived from the patient affected with MTDPS9. Cell extracts from a pool of controls (C) and the patient with mutations in the *SUCLG1* gene (P) cells were used to analyze the activity of complexes I and IV through Enzyme Activity Dipstick Assay Kits.

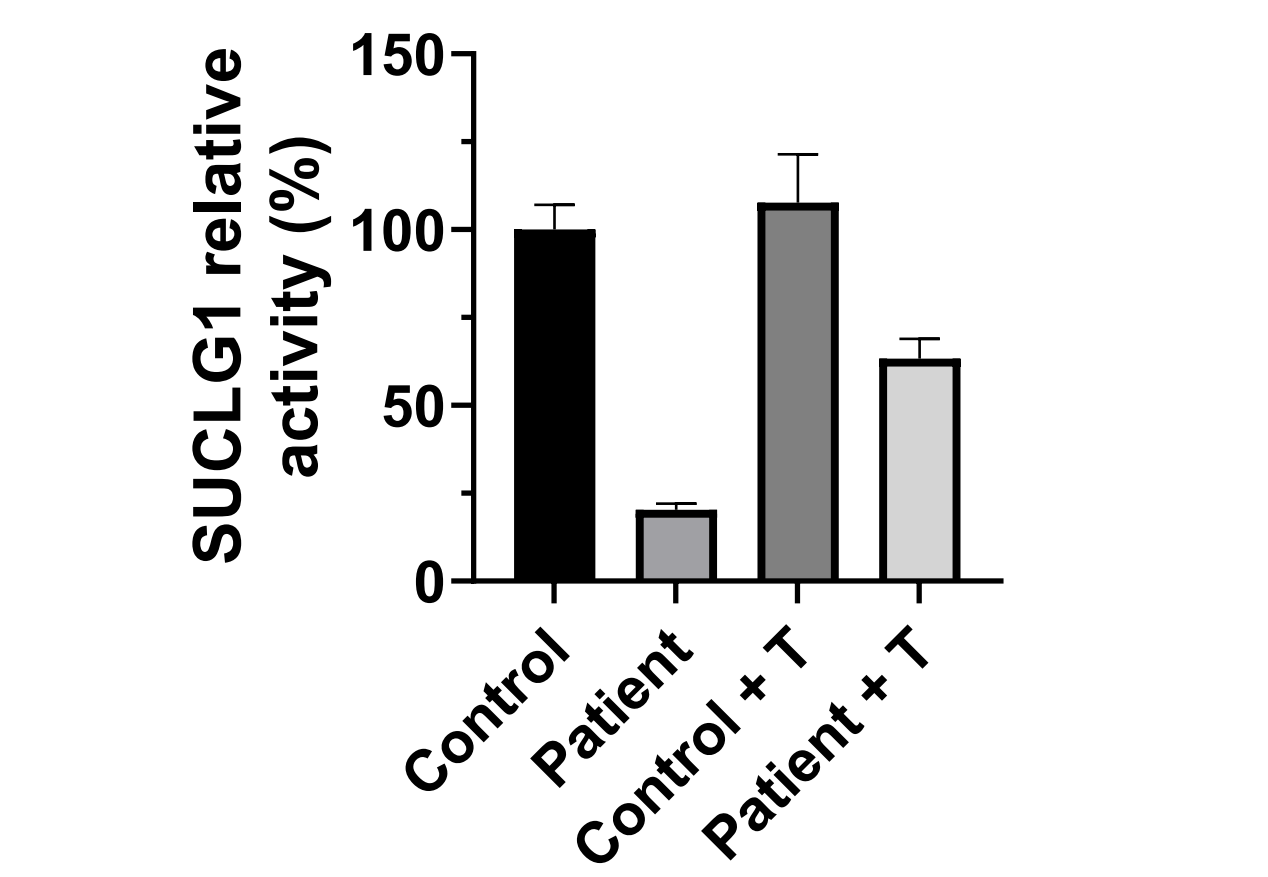


Figure 4. Activity of SUCLG1 protein in control and fibroblasts derived from the affected with MTDPS9. Cell extracts from a pool of controls and the patient with mutations in the *SUCLG1* gene cells were used to quantify relative activity of SUCLG1 activity through assay kit.

Mitochondrial respiration

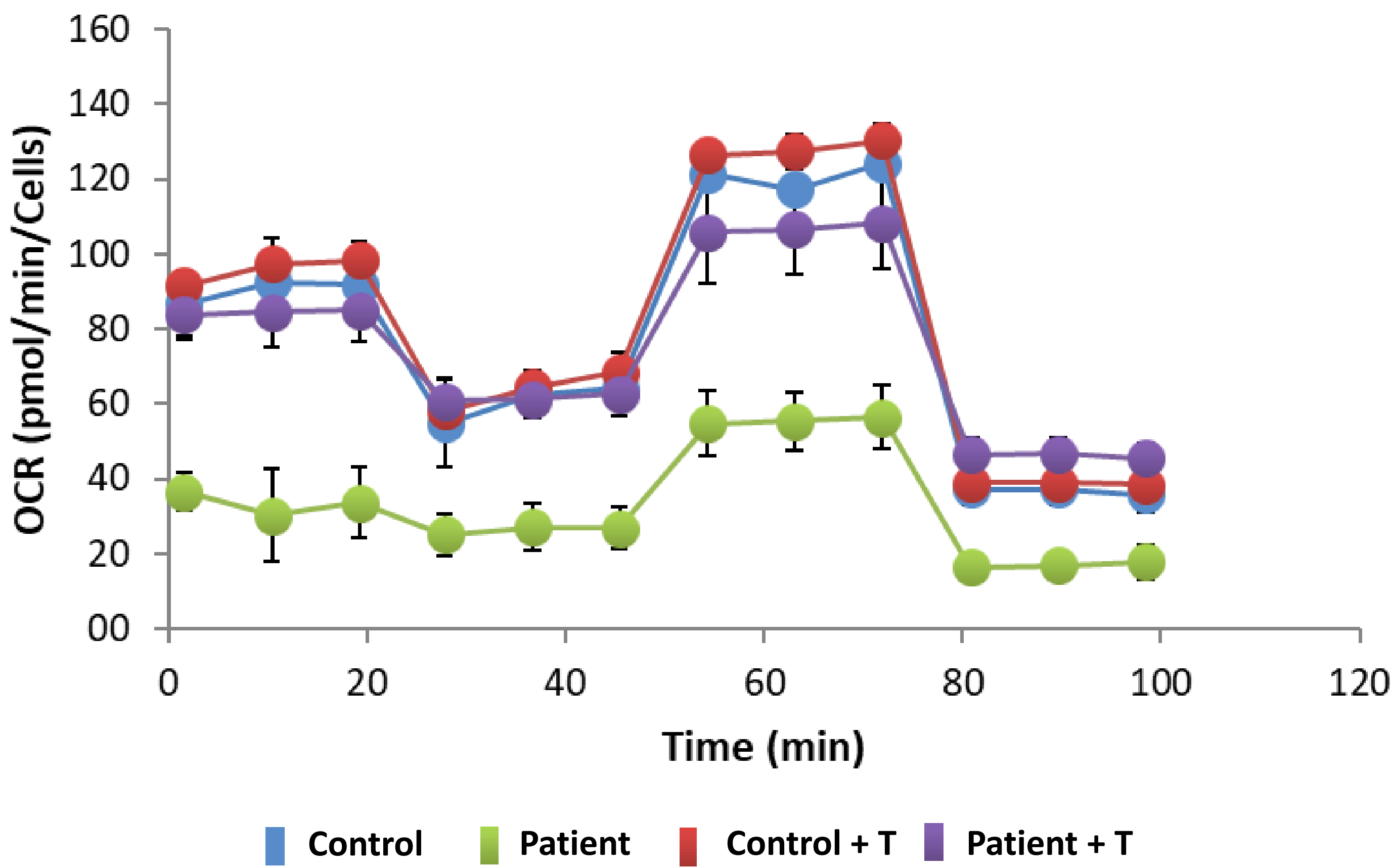


Figure 5. Mitochondrial bioenergetics of control and fibroblasts derived from the affected with MTDPS9. Mitostress bioenergetic assay in control and EE mutant cell lines. The mitochondrial respiration profile was measured with a Seahorse XFe24 analyser.

Conclusions

- MTDPS9 patient-derived fibroblasts represent a suitable model for studying the pathophysiological hallmarks of the disease.
- MTDPS9 patient-derived fibroblasts exhibit altered expression levels of *SUCLG1*, as well as of mitochondrial proteins belonging to complexes I and IV of the electron transport chain.
- MTDPS9 patient-derived fibroblasts display mitochondrial bioenergetic dysfunction.
- MTDPS9 patient-derived fibroblasts show reduced *SUCLG1* protein activity.
- The aforementioned alterations improve upon treating MTDPS9 patient-derived fibroblasts with a cocktail of vitamins and mitochondrial enhancers.
- These results are preliminary; therefore, further experiments are required to corroborate these findings and elucidate the underlying mechanisms driving the improvement of the observed pathophysiological alterations.



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