Development of a method for mitochondrial network analysis by confocal microscopy in fibroblasts from patients with a *DNM1L* mutation

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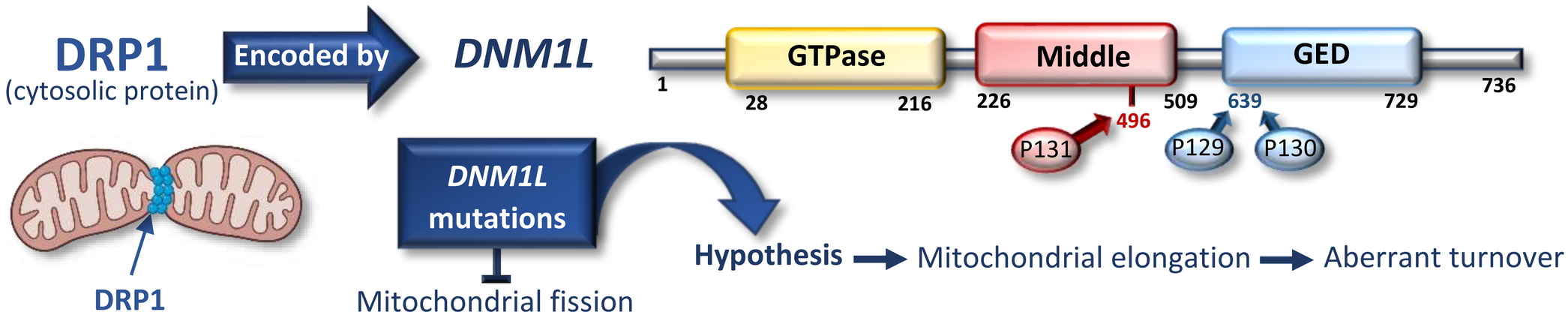
DRP1 is an essential GTPase in mitochondrial cleavage, trafficking, and distribution encoded by Dynamin1-like gene (DNM1L). This protein is produced in the cytosol as a dimer, but it must be activated and recruited to the outer mitochondrial membrane for mitochondrial fission to take place. Mutations in this gene involve imbalances in mitochondrial function produced by alterations in mitochondrial fission. To date, a small number of patients with mutations in DRP1 have been described. They show a variable and complex phenotype, ranging from hypotonia, cognitive development, developmental delay, and epilepsy to lethal encephalopathy in neonates. Due to the wide variety of symptoms observed in affected individuals, it is important to characterize how *DNM1L* mutations can alter mitochondrial physiology.

In this study, we present the case of a mother and her 11-year-old son, both with a variant in the DNM1L gene, c.1916G>A; p.Arg639Gln, in heterozygosis, and a child (P131) with a variant in the DNM1L gene: c.1486_1487delinsGA; p.Leu496Asp in heterozygosis de novo. This project aims to characterize the structure, morphology, and mitochondrial functions in fibroblasts derived from patients with mutations in DNM1L.



Mitochondrial Structure

- Immunostaining with Tomm20
- Confocal Microscopy
- Image analysis with ImageJ/Fiji

→ Morphology, Mitochondrial dinamics

Number | Volume | Sphericity | Network characteristics

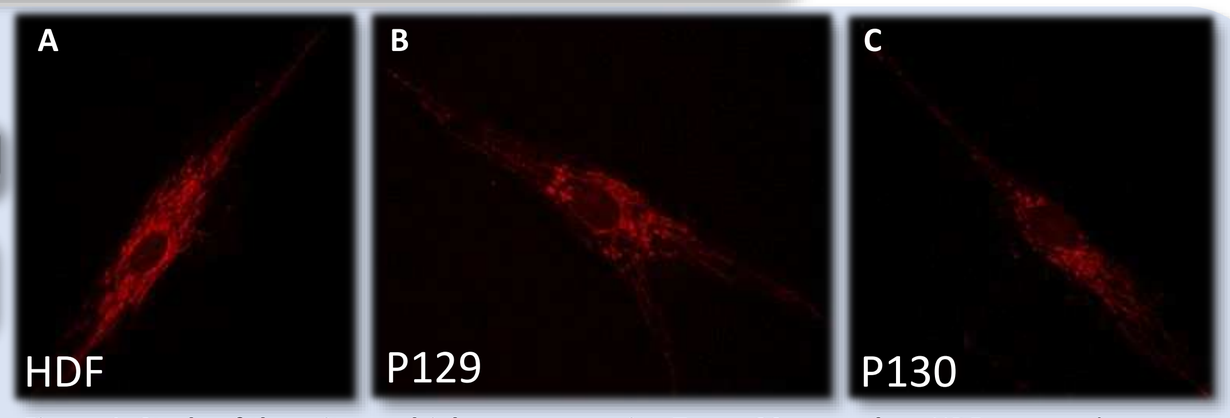
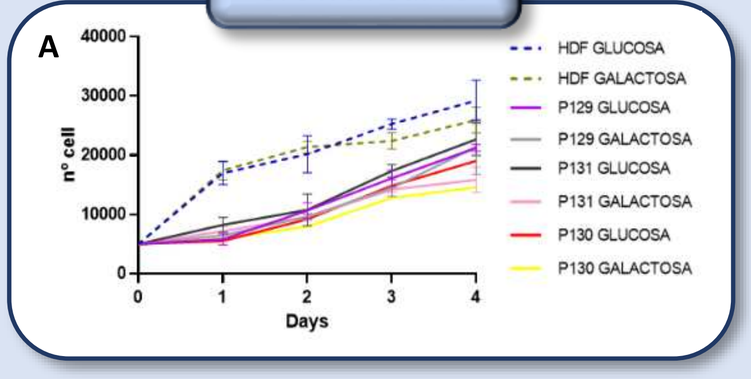


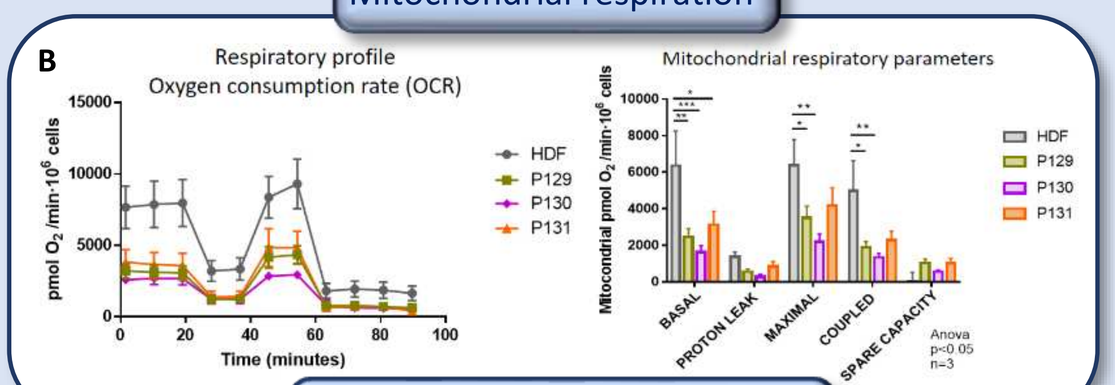
Figure 1. Study of the mitocondrial structure ussing Tomm20 as marker. HDF was used as a control (A) to compare with patients (B y C).

Mitochondrial Functions

Growth curve



Mitochondrial respiration



Expression level of DRP1 protein

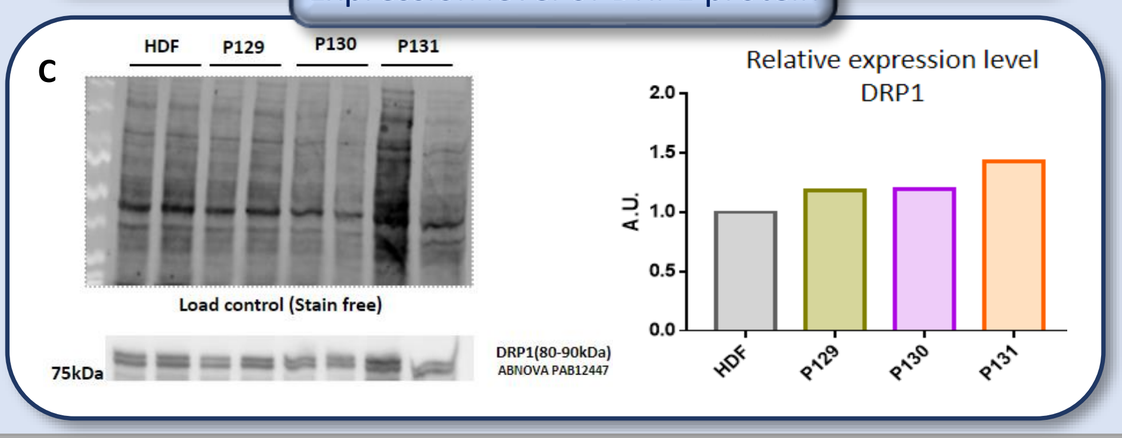


Figure 2. Study of mitocondrial functions. A) Growth curve using different carbon sources. Fibroblasts of patients with mitocondrial diseases show lower growth in galactose-cultured. B) Analysis mitocondrial respiration flux profile indicating respiratory control parameters. The results were expressed as means and standard error of the mean (SEM). * =p<0,05. N=3. C) Expression level and quantification of DRP1 protein in patients with a *DNM1L* mutation.

Conclusion:

- Patients with mutations in DNM1L present mitochondrial dysfunction compatible with DRP1 protein modifications.

REFERENCES

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