Mitochondrial membrane Protein-Associated Neurodegeneration (MPAN): Pathophysiological characterization and pharmacological screening for potential therapies

Cilleros Holgado, P., Munuera Cabeza, M., Sánchez Alcázar, J.A.

Centro Andaluz de Biología del Desarrollo (CABD) Crta. De Utrera Km 1. Universidad Pablo de Olavide, Sevilla

Introduction

Neurodegeneration with Brain Iron Accumulation (NBIA) is a group of different rare disorders in which iron is accumulated in the brain, especially in the basal ganglia and substantia nigra. Prevalent syntomps are dystonia, spasticity and neuropsychiatric disorders.

In this study, we focused on Mitochondrial membrane Protein-Asocciated Neurodegeneration (MPAN) which is a subtype of NBIA caused by mutations in the C19orf12 gene. This gene encodes a protein located in the external membrane of the mitochondrial of unknown function. However, it is proposed that C19orf12 could be implicated in fatty acid biosynthesis, calcium exchange, coenzyme A biosynthesis or mitophagy.

On the other hand, in recent years there has been a possitive advance in the molecular genetics area, however, it is known that from the appearance of the first symptoms until a precise diagnosis is obtained, an average of five years elapse and most of the rare diseases included MPAN do not have a curative treatment, only a palliative one, so further studies and researches are needed in this field.

Methods

We studied the pathophysiological alterations using fibroblasts derived from three confirmed patients identified as MPAN1, MPANH and MPANB, the first two having the same mutation (p.Gly69Arg) and MPANB with a different one (p.Gly58Arg) but all of them with a C19orf12 truncated protein. The three patients were from three different countries.

In order to achieve the aim, pathophysiological mechanisms will be studied examining protein expression levels of several cellular pathways by using Western blot analysis and iron accumulation by Prussian Blue Staining.

To identify potential therapies, fibroblasts are treated with different cocktails or compounds for a week, and, later, they are analyzed by Western Blotting and Prussian Blue Staining to confirm their effectiveness.

Stastically significant differences are determined in all the experiments by Student´s t test with a statically significant p valor of <0.05.

Results

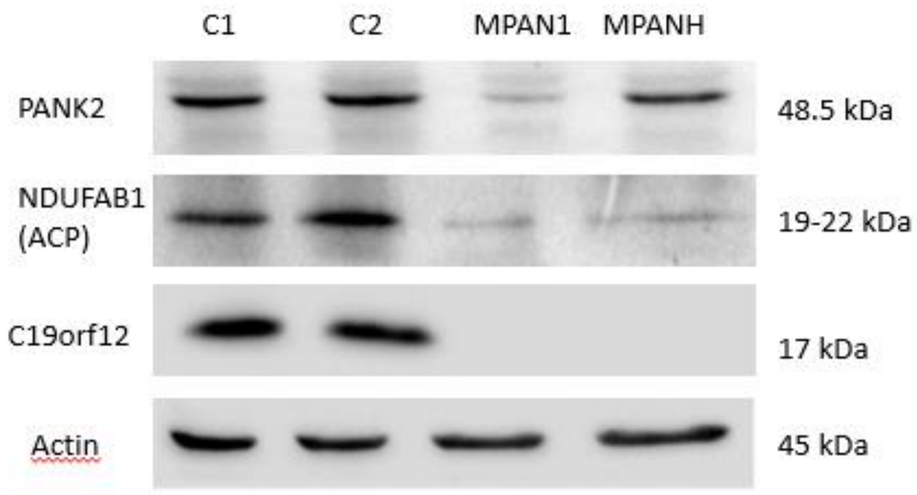

Fig 1. Western blot of PANK2, NDUFAB1 (ACP), C19orf12 and Actin in two controls and two patients cultured fibroblasts. It is observed reduced levels of PANK2 and ACP, both implicated in the CoA metabolism, and the absence of C19orf12. Actin acts as a charge control.

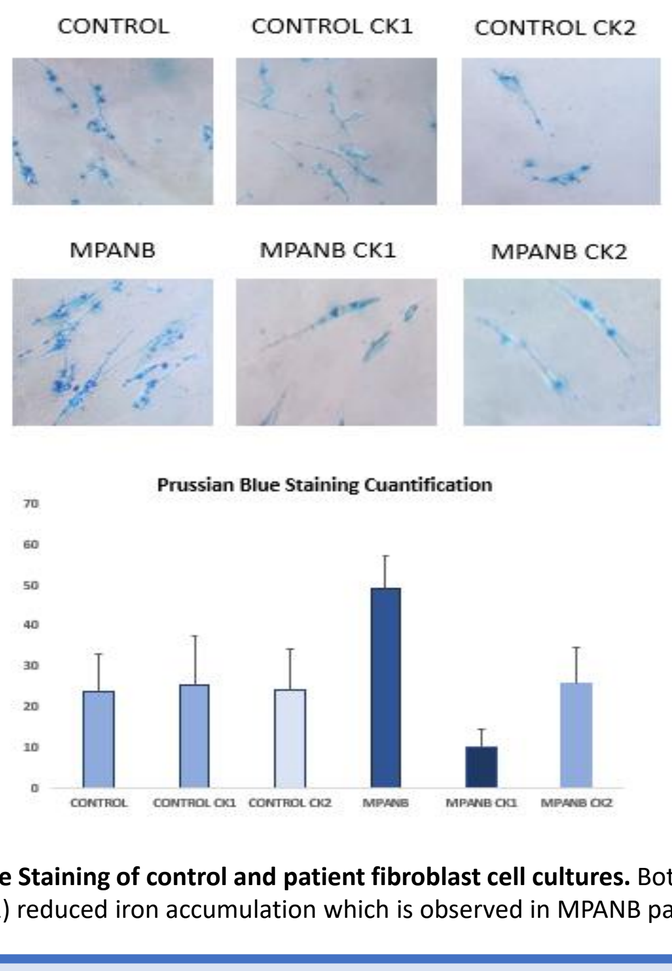

Fig 2. Prussian Blue Staining of control and patient fibroblast cell cultures. Both pharmacological cocktails (CK1, CK2) reduced iron accumulation which is observed in MPANB patient.

Conclusions

Fibroblast cell cultures derived from patients harboring C19orf12 mutation are interesting cellular models for both disease modelling and pharmacological screenings.

Pharmacological cocktails could be a good alternative to treat the patients which could improve their quality of life and reduce the pathological phenotype.

However, further studies are needed to elucidate the mechanism of action of pharmacological cocktails and to confirm our preliminary results.

References

Alvarez-Cordoba M, Villanueva-Paz M, Villalón-García I, Povea-Cabello S, Suárez-Rivero JM, Talaverón-Rey M, et al. Precision medicine in pantothenate kinase-associated neurodegeneration. Neural Regen Res 2019;14:1177-85.

Hartig MB, Iuso A, Haack T, Kmiec T, Jurkiewicz E, Heim K, et al. Absence of an orphan mitochondrial protein, C19orf12, causes a distinct clinical subtype of neurodegeneration with brain iron accumulation. Am J Hum Genet 2011;89:543-50.

Venco P, Bonora M, Giorgi C, Papaleo E, Iuso A, Prokisch H, et al. Mutations of C19orf12, coding for a transmembrane glycine zipper containing mitochondrial protein, cause mis-localization of the protein, inability to respond to oxidative stress and increased mitochondrial Ca2+. Front Genet 2015;6:1-14.

Centro Andaluz de Biología del Desarrollo