Poster

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

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ABSTRACT

Motivation: DRP1 is an essential GTPase in mitochondrial cleavage, trafficking, and distribution, and is encoded by the Dynamin1-like gene (DNM1L). This protein is produced in the cytosol as a dimer, but requires binding to the outer mitochondrial membrane to activated and initiate the mitochondrial fission. This recruitment stimulates the assembly of the protein, which oligomerizes into ring structures, drives membrane constriction, and promotes division following GTP hydrolysis. DRP1 consists of three domains: a middle domain involved in DRP1 oligomerization, a GTPase domain responsible for membrane constriction, and a GED domain that stimulates GTPase activity. Mutations in this gene involve imbalances in mitochondrial function produced by alterations in mitochondrial fission. To date, few patients with mutations in DNM1L have been described. They show a variable and complex phenotype, ranging from hypotonia, cognitive development, developmental delay, and epilepsy to lethal encephalopathy in neonates. The pathogenic variants described in DNM1L are related to the defect in mitochondrial fission resulting from alterations in the DRP1 protein. Due to the wide variety of symptoms observed in these patients, it is important to characterize how DNM1L mutations can alter mitochondrial physiology. In this study, we aim to characterize the structure, morphology, and mitochondrial dynamics in fibroblasts derived from a mother with a speech disorder and pes cavus and her 11-year-old son with global developmental delay, equinus gait, and epilepsy, both with a variant in the DNM1L gene, c.1916G>A; p.Arg639Gln, in heterozygosis of maternal origin.

Methods: Using fibroblasts derived from patients with a variant in the DNM1L as a study model, we have developed a protocol of immunostaining and confocal microscopy using Tomm20 as a mitochondrial marker. Tomm20 is a component of the receptor complex that directs and translocates proteins from the cytosol to the mitochondria, and it is localized in the mitochondrial outer membrane, making the anti-Tomm20 antibody a good mitochondrial marker. After staining, imaging is performed on a Leica SPE/baby confocal microscope. For the morphometric study, the ImageJ/Fiji image analysis platform compares the mitochondrial morphology, structure and dynamics of patients versus controls by using different parameters, such as area, circularity, and branching the mitochondrial network, among others.

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