Poster

Functional validation of CoQ deficiency fibroblast model with COQ7 mutations

Torralba Carnerero, Manuel (1), Cascajo Almenara, María V. (1,2), y Santos Ocaña, Carlos (1,2,*)

(1) Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide, 41013 Sevilla, Spain.

(2) Centre for Biomedical Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain

Tutor académico: Manuel Ángel Ballesteros Simarro

Keywords: Coenzyme Q10 deficiency; COQ7; COQ proteins; molecular diagnosis.

ABSTRACT

Motivation: Coenzyme Q10 (CoQ10) deficiency syndrome comprises a heterogeneous group of mitochondrial disorders characterized by a decrease in CoQ10 content in cells and tissues. Primary CoQ deficiencies are rare genetic conditions caused by mutations in COQ genes, whose encoded proteins are directly linked to the final biochemical pathway of CoQ biosynthesis. Early diagnosis is both essential and one of the most challenging issues of this disease, mainly due to the variety of associated clinical manifestations. Here we present four clinical cases of primary CoQ10 deficiency, which is presumably caused by COQ7 mutations. The motivation for this work is to validate it in a cellular model based on primary cultures from patients' skin fibroblasts, in order to complete the previously started molecular diagnosis by whole-exome sequencing.

Methods: Cultured patients fibroblasts was the biological starting material of our study. CoQ10 levels were measured by HPLC-ECD. In order to study mitochondrial function and respiration, oxygen consumption rate (OCR) was analysed using Seahorse technology. In addition, COQ7, several COQ proteins and other mitochondrial proteins expression were analysed by Western Blotting.

Results: Patients' fibroblasts showed a basal level of CoQ10 lower than control fibroblasts (HDF). Moreover, the chromatogram revealed a peak corresponding to DMQ10, which was not seen in HDF. OCR showed mitochondrial respiration was affected in terms of maximal respiration and spare capacity with respect to control cells. Western blot analysis revealed the absence of COQ7 protein in patients' fibroblasts. Moreover, several COQ proteins, which are involved in the CoQ10 biosynthetic pathway, presented a moderate decreased expression. However, several mitochondrial related proteins maintained their physiological levels, such as VDAC, NDUFA9, UQCRCII or mtCOII.

Conclusions: CoQ10 deficiency was confirmed in patients' fibroblasts. Since DMQ10 is the substrate of the reaction catalyzed by COQ7 protein, DMQ10 accumulation indicates that the COQ7 reaction is impaired. These results reveal that COQ7 mutation identified in patients' fibroblasts affects protein expression, CoQ10 levels and mitochondrial respiration. Finally, our data support the previous diagnosis obtained by exome analysis, proving that in these clinical cases, the CoQ10 deficiency is being produced by the absence of COQ7 protein.

REFERENCES

Alcázar-Fabra, M., Navas, P., and Brea-Calvo, G. (2016) Coenzyme Q biosynthesis and its role in the respiratory chain structure. Biochim. Biophys. Acta - Bioenerg. 1857, 1073–1078

Salviati, L., Trevisson, E., Doimo, M., & Navas, P. (2017). Primary Coenzyme Q10 Deficiency. In M. P. Adam (Eds.) et. al., GeneReviews®. University of Washington, Seattle.

Delia Yubero, Raquel Montero, Carlos Santos-Ocaña, Leonardo Salviati, Placido Navas & Rafael Artuch (2018) Molecular diagnosis of coenzyme Q10 deficiency: an update, Expert Review of Molecular Diagnostics, 18:6, 491-498, DOI: 10.1080/14737159.2018.1478290

El número de confirmación de la tarea es 2786a112-7db0-495b-ab57-3a3c9622ee90