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Oxidative stress and mitochondrial health in neurodevelopmental disease Rett syndrome: the role of mitochondrial nutrients.

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INTRODUCTION

Mitochondria are fundamental intracellular organelles for the production of ATP that is necessary for nearly all cellular functions. In particular, neurons strictly depend on mitochondrial energy metabolism. Therefore mitochondrial alterations can be critical for the pathogenesis of neurodevelopmental desease[1]. Rett syndrome is a neurodevelopmental disease that affect almost exclusively females with a frequency of 1: 10,000. In most of the cases it is caused by mutations in X-linked methyl-CpG binding protein-2 gene (MECP2) and it is characterized by an early neurological regression, followed by loss of acquired cognitive, social and motor skills[2]. There are a lot of emerging evidences that indicates an oxidative imbalance and a complex mitochondrial alteration that could have a central role in the pathogenesis of the syndrome[3]. **The aim of the study** is the investigation of the possible role of mitochondrial nutrient as Coenzyme Q_{10} (CoQ_{10}), a lipophilic endogenous cofactor with bioenergetic and antioxidant property and other nutrients, in ameliorating the mitochondrial dysfunction that can characterizes Rett patients.

METHODS

The **Fibroblasts** were cultivated in DMEM Low Glucose (10% FBS, 1% glutamine, 1% penicillin-streptomycin) at 37°C and 5% CO_2. At confluence **cells were treated with 5 µg/mL of exogenous ubiquinol** ($CoQ_{10}H_2$) for 24h. **CoQ_{10} levels** and oxidative status were quantified by HPLC with electrochemical detector (Shiseido), provided by concentrant, reducent and analyitical column. **Oxidative stress markers** were analyzed by flow cytometry (Guava, Millipore) and two fluorescent probes (CM-H_2DCFDA, MitoSox red). The expression of **Mitochondrial quality control markers** were assessed with Western Blot assay.

PATIENT	TYPE	GENE	MUTATION	FENOTYPE	BIOPSY
CONTROL	/	/	/	/	SKIN
RETT 1	TIPICAL	MECP2	R133C	MILD	SKIN
RETT 2	TIPICAL	MECP2	T158M	SEVERE	SKIN
RETT 3	TIPICAL	MECP2	C-TERMINAL DELETION	MILD	SKIN

RESULTS

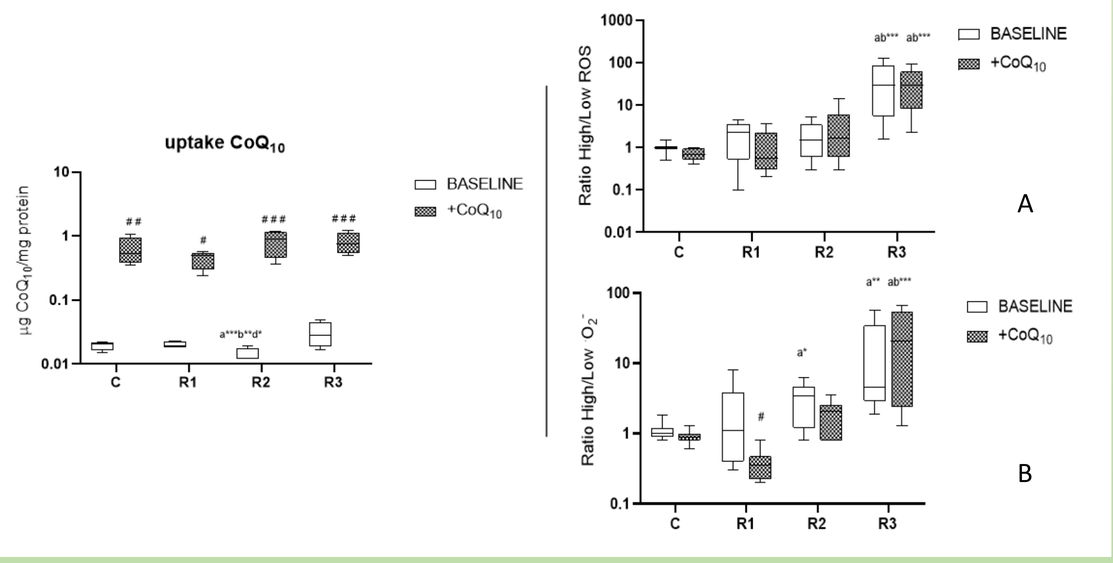
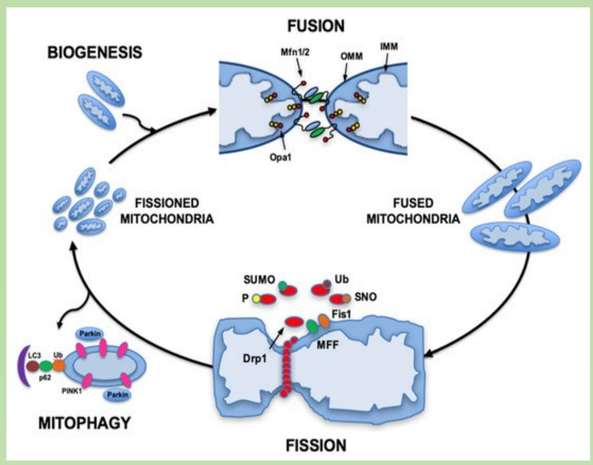


FIGURE 1. Total cellular Coenzyme Q_{10} (CoQ_{10}) level after 24 hours of treatment with 5 µg/mL of Ubiquinol ($CoQ_{10}H_2$) expressed as µg of CoQ_{10} normalized on milligram (mg) of cellular protein content.

FIGURE 2. Cytoplasmic (A) and mitochondrial reactive oxygen species (B) production expressed as ratio of the percentage of cells with high and low content of ROS at the baseline and after 24 hours of treatment with 5 µg/mL of ubiquinol ($CoQ_{10}H_2$).

DISCUSSION AND PERSPECTIVES

The **preliminary results** of this project indicate the existence of **huge variability** of typical Rett syndrome. The data show that **where there is a mitochondrial disfunction** (a huge augmentation of production of reactive oxygen species) as in R3 (**p=0.004) **also the mitochondrial quality control is altered** (*p<0.05 MFN2 e **p<0.01 MFN1) leading to an accumulation of damaged mitochondria[4] and amplifying the production of mitochondrial superoxide anion.



In this context, **Ubiquinol supplementation could have a positive effect in mild to moderate oxidative stress conditions** as R2 and R1 where there isn't also a severe alteration of mitochondrial dynamics. To **better understand the mechanism** behind the alterations of the mitochondrial dynamics, **the levels of the eukaryotic initiation factor 5A (eIF5A) and spermidine** will be analyzed, in order to investigate the possible role in the mitochondrial quality control[5]. Subsequently, the exogenous spermidine will be supplemented to evaluate the effects on mitochondria health.

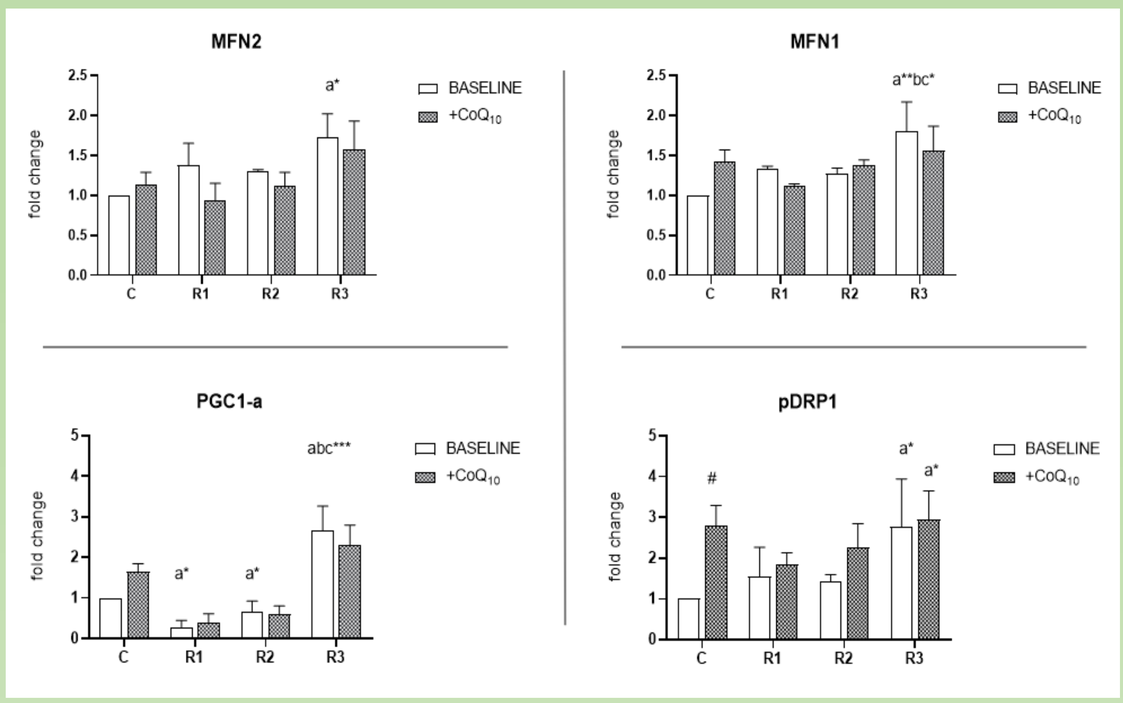


FIGURE 3. Mitochondrial quality control markers at baseline and after 24 hours of treatment with 5 µg/mL of ubiquinol ($CoQ_{10}H_2$) as MNF2 and MNF1 (fusion), pDRP1 (fission), PGC1-a (biogenesis) are expressed as fold change respect to the control p value *< 0.05; **<0.01; ***<0.001

REFERENCES

1) Daniela Valenti et *al.* Neurosci. Biobehav. Rev. (2014), http://dx.doi.org/10.1016/j.neubiorev.2014.01.012 **2)** Wendy A. Gold et al. ACS Chem Neurosci. 2018 Feb 21;9(2):167-176. doi: 10.1021/acschemneuro.7b00346 **3)** Natalya Shulyakova *et al.* Front. Cell. Neurosci. 11:58. doi: 10.3389/fncel.2017.00058 **4)** Ilaria Crivellari et *al.* Archives of Biochemistry and Biophysics 700 (2021) 108790 https://doi.org/10.1016/j.abb.2021.108790 5) Frank Madeo et *al. Science 359, eaan2788 (2018). DOI: 10.1126/science.aan2788* 6) picture https://doi.org/10.3390/biomedicines10061375