Mitochondrion 13 (2013) 337–341

Contents lists available at SciVerse ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

# Coenzyme $Q_{10}$ deficiency in mitochondrial DNA depletion syndromes

Raquel Montero [a,b], Manuela Grazina [c,d], Ester López-Gallardo [b,e], Julio Montoya [b,e], Paz Briones [b,f,g], Aleix Navarro-Sastre [b,f], John M. Land [h], Iain P. Hargreaves [h], Rafael Artuch [a,b,*] and Coenzyme $Q_{10}$ deficiency study group.

Coenzyme $Q_{10}$ deficiency study group: Maria del Mar O'Callaghan [a], Cristina Jou [a], Cecilia Jimenez [a], Nuria Buján [f], Mercè Pineda [a,b], Angels García-Cazorla [a,b], Andrés Nascimento [a], Belen Perez-Dueñas [a], Eduardo Ruiz-Pesini [e], Carl Fratter [i], Leonardo Salviati [j], Marta Simões [d], Cândida Mendes [d], Maria João Santos [d], Luisa Diogo [k], Paula Garcia [k], Plácido Navas [b,l]

[a] Clinical Chemistry, Pathology and Neurology Departments, Hospital Sant Joan de Déu, Barcelona, Spain
[b] Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Spain
[c] Faculty of Medicine, University of Coimbra, Portugal
[d] Center for Neuroscience and Cell Biology, University of Coimbra, Laboratory of Biochemical Genetics, Portugal
[e] Departamento de Bioquímica, Biología Molecular y Celular, Universidad de Zaragoza, Spain
[f] IBC-Secció d'Errors Congènits del Metabolisme, Servei de Bioquímica i Genètica Molecular, Hospital Clínic. Barcelona, Spain
[g] Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain
[h] Neurometabolic Unit, National Hospital for Neurology and Neurosurgery, London, UK
[i] Oxford Medical Genetics Laboratories, Churchill Hospital, Oxford, UK
[j] Servizio di Genetica Clinica ed Epidemiologica, Department of Pediatrics, University of Padova, Italy
[k] Hospital Pediátrico de Coimbra, Centro Hospitalar e Universitário de Coimbra, Portugal
[l] Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide-CSIC Sevilla, Spain

ARTICLE INFO

*Article history:*
Received 15 October 2012
Received in revised form 1 April 2013
Accepted 3 April 2013
Available online 11 April 2013

*Keywords:*
Mitochondrial DNA depletion syndrome
Coenzyme $Q_{10}$ deficiency
Mitochondrial disorders

ABSTRACT

We evaluated coenzyme $Q_{10}$ (CoQ) levels in patients studied under suspicion of mitochondrial DNA depletion syndromes (MDS) ($n = 39$). CoQ levels were quantified by HPLC, and the percentage of mtDNA depletion by quantitative real-time PCR. A high percentage of MDS patients presented with CoQ deficiency as compared to other mitochondrial patients (Mann–Whitney-U test: $p = 0.001$). Our findings suggest that MDS are frequently associated with CoQ deficiency, as a possible secondary consequence of disease pathophysiology. Assessment of muscle CoQ status seems advisable in MDS patients since the possibility of CoQ supplementation may then be considered as a candidate therapy.



## 1. Introduction

Mitochondrial DNA depletion syndromes (MDS) are a heterogeneous group of disorders characterized by low number of mitochondrial DNA (mtDNA) copies in different tissues (Spinazzola, 2011). These syndromes are frequently associated with severe infant and childhood mitochondrial respiratory chain (MRC) deficiencies and patients may present with different phenotypes. Myopathic (OMIM: 609560), encephalomyopathic (OMIM: 612073 and 612075) and hepatocerebral (OMIM 251880) are common forms, although a wide range of clinical presentations are being profiled (Nogueira et al., 2011; Rötig and Poulton, 2009 Suomalainen and Isohanni, 2010). MDS have been related to impaired mtDNA replication (mutations in *POLG1* and *C10orf2*/*PEO1* genes), to altered mitochondrial metabolism of deoxynucleotide pools (mutations in *TK2*, *DGUOK*, *MPV17*, *RRM2B*, *SUCLA2*, *SUCLG1* and *TYMP* genes) (Nogueira et al., 2011; Rötig and Poulton, 2009; Suomalainen and Isohanni, 2010). However, in a high percentage of the cases the aetiology of the disease remains to be elucidated (Alberio et al., 2007).

Coenzyme $Q_{10}$ (CoQ) is a mobile molecule that acts as an electron carrier in the MRC transferring electrons from complex I and complex II to complex III (Ernster and Dallner, 1995). It is also a cofactor for several mitochondrial dehydrogenases, including dihydroorotate dehydrogenase (EC 1.3.3.1), an enzyme involved in pyrimidine biosynthesis. A link between CoQ deficiency and impaired pyrimidine biosynthesis has previously been demonstrated (López-Martín et al., 2007), although

*Abbreviations:* CoQ, Coenzyme $Q_{10}$; MDS, mitochondrial DNA depletion syndromes; HPLC, high pressure liquid chromatography; mtDNA, mitochondrial DNA; MRC, mitochondrial respiratory chain; CS, citrate synthase.

* Corresponding author at: Clinical Biochemistry Department, Hospital Sant Joan de Déu, Passeig Sant Joan de Déu, 2, 08950 Esplugues, Barcelona, Spain. Tel.: +34 932806169; Fax: +34 932803626.
*E-mail address:* rartuch@hsjdbcn.org (R. Artuch).

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http://dx.doi.org/10.1016/j.mito.2013.04.001

only anecdotic reports have studied CoQ status in MDS patients (Montero et al., 2009). Mutations in 6 genes involved in CoQ biosynthetic pathway have been reported in association with a range of clinical phenotypes: mutations in *COQ2*, *PDSS1*, *PDSS2* and *COQ4* have been reported in cases of severe infantile multisystemic disease (Quinzii and Hirano, 2010; Sacconi et al., 2010; Salviati et al., 2012), mutations in the *ADCK3* gene have been associated with cerebellar ataxic form of CoQ deficiency (Lagier-Tourenne et al., 2008) and a mutation in the *COQ6* gene that causes nephrotic syndrome with sensorineural deafness (Heeringa et al., 2011). Interestingly, several studies have reported the presence of a secondary CoQ deficiency in diseases such as ataxia with oculomotor apraxia (Quinzii et al., 2005) and glutaric aciduria type II (Gempel et al., 2007). CoQ deficiency has also been frequently detected in mitochondrial disorders (DiMauro et al., 2007; Emmanuele et al., 2012; Miles et al., 2008, Quinzii and Hirano, 2011, Sacconi et al., 2010). In our global experience, CoQ deficiency is present in a similar percentage of patients with mitochondrial disorder to that reported in the study by Montero et al. (2005). Furthermore, secondary CoQ deficiency may be very difficult to differentiate from that of primary deficiency, since in most cases the molecular basis of the disease remains elusive (Emmanuele et al., 2012).

The aim of the present study was to evaluate muscle and liver CoQ levels in a cohort of patients with a clinical phenotype suggestive of MDS.

## 2. Methods

### 2.1. Patients

During the last 3 years, we have recruited 39 patients (age range: 1 month–24 years; average 2.6 years) with the following inclusion criteria: clinical phenotype suggestive of MDS; no CoQ supplementation therapy at the time of the biopsy; and biochemical, histopathological and/or genetic evidence of a mitochondrial disease according to previously established criteria for diagnosis of mitochondrial disease (Bernier et al., 2002). 12 patients were from Coimbra, 4 patients from London and 23 patients from Barcelona. The patients were classified in 2 groups.

Group 1: 14 patients (age range: 1 month–2 years; average 0.5 years) with the diagnosis of MDS (percentage of mtDNA depletion greater than 70%): 8 patients presented with decreased mtDNA copy number in muscle, 5 patients in liver and 1 patient in brain.

Group 2: 25 patients (range age: 1 month–24 years; average 3.1 years) with a clinical suspicion of a MDS (they presented with hepatocerebral involvement (n = 5), encephalopathy (n = 11) and myopathy plus encephalopathy (n = 8)) who did not show mtDNA depletion in the tissues studied (muscle or liver). The main laboratory findings of both groups of patients are stated in Tables 1 and 2, and the clinical details of the MDS patient group are outlined in Table 2. From the 39 patients, we investigated 30 muscle biopsies, 8 liver biopsies and 1 brain tissue collected after necropsy.

### 2.2. Laboratory studies

MRC and citrate synthase (CS) enzyme activities were determined by spectrophotometric enzyme assays in muscle biopsies as previously reported (Grazina, 2012; Rustin et al., 1994). We assessed MRC enzyme activities corrected for citrate synthase activity, and MRC deficiencies were considered as described by Grazina (2012) and Bernier et al. (2002).

Muscle and liver CoQ levels were determined by reverse-phase high pressure liquid chromatography (HPLC, Waters, MA, USA) with electrochemical detection (ED; Coulochem II, ESA, MA, USA) (Montero et al., 2008). Briefly, CoQ was separated on a nucleosil C-18 column (5 μm, 25 × 0.4 cm, Teknokroma, Barcelona, Spain). Mobile phase consisted of 20 mmol/L of lithium perchlorate in ethanol/methanol (40/60; v/v). ED cells were attached at −600 mV (conditioning cell, Model 5021) and +600 mV (analytical cell, Model 5010). The muscle CoQ reference interval was established from 37 paediatric patients with no clinical presentation of muscle disease (age range 2–16 years; average 9.2 years). Details of the establishment of this reference interval have been previously reported (Montero et al., 2008). The muscle CoQ status of patients from the UK was determined by HPLC with UV detection at 275 nm (Jasco UV 975 detector, Jasco, UK) (Duncan et al., 2005). CoQ was separated on a (Techsphere ODS 5 μ, 150 × 4.6 mm) HPLC column. The mobile phase consists of ethanol:methanol:60% (v/v) perchloric acid; 700:300:1.2 (v:v) to which 7 g of sodium perchlorate is added. For the HPLC-UV procedure, the muscle CoQ reference interval was established from 20 patients with no clinical/biochemical evidence of muscle disease or MRC dysfunction (age range 0.6–18 years; average 5.6 years), as previously reported in the study by Duncan et al. (2005).

To establish the liver CoQ reference interval liver samples were collected following necropsy from 7 paediatric patients (age range 1 day–1 year, average 3 months) with no clinical/biochemical evidence of MRC disorder and analyzed by HPLC with ED. The results of CoQ determinations were expressed as nmol/g of total protein content as measured by Lowry et al. (1951). All HPLC determinations were undertaken with internal quality controls to ensure continuity between analyses. Recently, we have initiated a pilot quality control scheme between the London and Barcelona laboratories, with a good agreement in the determination of CoQ levels being reported (results available on request).

The mtDNA content was measured by quantitative real-time PCR according to the method of Marcuello et al. (2005), Ashley et al. (2008) and Navarro-Sastre et al. (2012). Briefly, the analysis was performed in a Step One Plus real-time PCR system (qRTPCR; PE7500 real-time PCR instrument; Applied Biosystems, Foster City, CA, USA). This method is based on the amplification of the mitochondrial 12S rRNA and m.3130–3301 and the quantity of mtDNA was corrected by simultaneous measurements of a single copy of nuclear RNAseP and APP genes. PCR was performed in a final reaction volume of 20 μL with 2 mM of $MgCl_2$, 0.5 μM each of the mitochondrial probes (5′-[6FAM]tgccagccaccgcg[BHQ1]-3′), forward and reverse primers (forward primer: 5′-ccacgggaaacagcatgtatt-3′, reverse primer: 5′-ctattgacttgggttaatcgtgtga-3′), 1 μL of TaqMan® RNAseP, 25 ng of DNA and 10 μL of TaqMan® Gene Expression Master Mix (Applied Biosystems).

**Table 1**
Biochemical and molecular data of the 39 patients under the suspicion of MDS. Data are expressed as range, average (SD), and median.

| | | % mtDNA depletion | CoQ (nmol/g) | Patients with impaired MRC |
|---|---|---|---|---|
| Muscle | Group 1: mtDNA depletion (n = 8) | 75–99<br>85 (10), 86 | 4–151<br>75 (47), 69 | ↓ CI + III (n = 1)<br>↓ CII + III (n = 2)<br>↓ CII (n = 2)<br>↓ CIII (n = 3)<br>↓ CIV (n = 5) |
| | Group 2: no mtDNA depletion (n = 22) | 4.1–66<br>37 (21), 36 | 74–319<br>165 (65), 148 | ↓ CI + III (n = 5)<br>↓ CII + III (n = 11)<br>↓ CII (n = 7)<br>↓ CIII (n = 10)<br>↓ CIV (n = 9) |
| | Reference values | <70% | 121–451[a]<br>229 (105)<br>140–580[b]<br>241 (95) | n.a. |
| Liver | Group 1: depletion (n = 5) | 74–96<br>88 (8), 90 | 223–617<br>419 (19), 418 | n.a |
| | Group 2: no depletion (n = 3) | 6.1–38%<br>29 (19), 38 | 312–464<br>398 (78), 420 | n.a. |
| | Reference values | <70% | 220–920[a]<br>614 (228) | n.a |

Case 13, patient with pathologic % mtDNA depletion in brain, was excluded of this table.
[a] Reference interval for muscle CoQ determined by HPLC-electrochemical detection.
[b] Reference interval for muscle CoQ determined HPLC-UV detection.