Molecular Syndromology

Mol Syndromol 2014;5:163–169
DOI: 10.1159/000360494

Published online: March 18, 2014

Pathomechanisms in Coenzyme Q_{10}-Deficient Human Fibroblasts

Luis C. López[a, b] Marta Luna-Sánchez[a, b] Laura García-Corzo[a, b]
Catarina M. Quinzii[c] Michio Hirano[c]

[a]Department of Physiology, Faculty of Medicine, and [b]Institute of Biotechnology, Biomedical Research Center, University of Granada, Granada, Spain; [c]Department of Neurology, Columbia University Medical Center, New York, N.Y., USA

Key Words

Apoptosis · ATP · Coenzyme Q_{10} deficiency · Mitochondria · Mitophagy · Molecular basis of disease · mtDNA · Oxidative stress · Ubiquinone

Abstract

Primary coenzyme Q_{10} (CoQ_{10}) deficiency is a rare mitochondrial disorder associated with 5 major clinical phenotypes: (1) encephalomyopathy, (2) severe infantile multisystemic disease, (3) cerebellar ataxia, (4) isolated myopathy, and (5) steroid-resistant nephrotic syndrome. Growth retardation, deafness and hearing loss have also been described in CoQ_{10}-deficient patients. This heterogeneity in the clinical presentations suggests that multiple pathomechanisms may exist. To investigate the biochemical and molecular consequences of CoQ_{10} deficiency, different laboratories have studied cultures of skin fibroblasts from patients with CoQ_{10} deficiency. In this review, we summarize the results obtained in these studies over the last decade.

The first clinical report of coenzyme Q_{10} (CoQ_{10}) deficiency was published by Ogasahara et al. [1989], who described 2 sisters with recurrent rhabdomyolysis associated with seizures and mental retardation. Myopathy was the predominant clinical feature in these patients. In their muscle, the activities of mitochondrial respiratory complexes I+III and II+III were markedly reduced, and their CoQ_{10} levels were 3.7 and 5.4% of controls [Ogasahara et al., 1989]. From subsequent reports of patients with CoQ_{10} deficiency, striking clinical heterogeneity of the condition has emerged [Quinzii et al., 2008a]. To date, CoQ_{10} deficiency syndrome (MIM 607426) has been associated with 5 major clinical phenotypes: (1) encephalomyopathy, (2) severe infantile multisystemic disease, (3) cerebellar ataxia, (4) isolated myopathy, and (5) steroid-resistant nephrotic syndrome [Quinzii and Hirano, 2010]. Moreover, growth retardation, deafness and hearing loss have been described in some CoQ_{10}-deficient patients.

Since 2006, multiple molecular causes of ubiquinone deficiencies have been identified. Primary CoQ_{10} deficiencies are due to pathogenic mutations in genes involved in the biosynthesis of CoQ_{10}. To date, 33 patients have been identified with primary CoQ_{10} deficiency due to mutations in *COQ2* [Quinzii et al., 2006; Diomedi-Camassei et al., 2007; Mollet et al., 2007], *PDSS1* [Mollet et al., 2007], *PDSS2* [López et al., 2006], *COQ4* [Salviati et al., 2012], *COQ6* [Heeringa et al., 2011], *ADCK3* [Lagier-Tourenne et al., 2008; Mollet et al., 2008], and *COQ9*

KARGER

E-Mail karger@karger.com
www.karger.com/msy

1661–8769/14/0054–0163$39.50/0

Luis Carlos López, PhD
Institute of Biotechnology, Biomedical Research Center, Lab 139, University of Granada
Avenida del Conocimiento s/n
ES–18100 Armilla (Spain)
E-Mail luisca@ugr.es

[Duncan et al., 2009]. CoQ_{10} deficiencies may also result from pathogenic mutations in genes not directly related to CoQ_{10} biosynthesis, and such cases are referred to as secondary CoQ_{10} deficiencies [Turunen et al., 2004; Montero et al., 2005, 2009; Quinzii et al., 2005; Aeby et al., 2007; Gempel et al., 2007; Le Ber et al., 2007; Miles et al., 2008; Sacconi et al., 2010; Cótan et al., 2011; Quinzii and Hirano, 2011].

The definitive diagnosis of CoQ_{10} deficiency, in most patients, is based on measurements of CoQ_{10} levels in muscle. Additionally, patients with primary CoQ_{10} deficiency frequently show decreased CoQ_{10} levels in skin fibroblasts. Consequently, cultured skin fibroblasts from patients with primary CoQ_{10} deficiency have been utilized for biochemical characterization of CoQ_{10} biosynthetic defects, in vitro evaluation of pathogenic mechanisms and in vitro assessments of efficacy of CoQ analogs supplementation.

Skin Fibroblasts for Diagnosis of Primary CoQ_{10} Deficiency

Once CoQ_{10} deficiency is confirmed in patients' muscle and skin fibroblasts, assessment of CoQ_{10} biosynthesis in cultured skin fibroblasts can be used to prove the defect in the CoQ_{10} biosynthetic pathway. These assays utilize radiolabeled substrates for the biosynthesis of CoQ_{10} [López et al., 2006; Quinzii et al., 2006; Mollet et al., 2007; Tekle et al., 2008]. Typically, [^{3}H]-mevalonate, which is the initial substrate used in the formation of the decaprenyl tail, and [^{14}C]-4-hydroxybenzoate (4HB), which is precursor of the benzoquinone ring of the ubiquinone, are employed in cell culture. In addition, [^{3}H]-decaprenyl-pyrophosphate ([^{3}H]-decaprenyl-PP), which is poorly cell penetrant due to high lipophilicity, is used in homogenized fibroblast extracts. The combined use of [^{14}C]-4HB and [^{3}H]-decaprenyl-PP may be useful to discriminate defects upstream or downstream of the reaction catalyzed by decaprenyl diphosphate synthase. For example, fibroblasts with defects of COQ2 (which condenses 4-HB with decaprenyl-PP) produce less radiolabeled CoQ_{10} with both [^{14}C]-4HB and [^{3}H]-decaprenyl-PP substrates [Quinzii et al., 2006], while, in contrast, *PDSS2* mutant fibroblasts synthesize less radiolabeled CoQ_{10} with [^{14}C]-4HB but a normal amount of CoQ_{10} with [^{3}H]-decaprenyl-PP [López et al., 2006]. Multiple steps in the CoQ_{10} biosynthetic pathway downstream of COQ2 cannot be distinguished with the available assays, but this may become possible in the future by the generation of new radiolabeled intermediates or by using tandem mass spectrometry methods to identify the accumulation of abnormal metabolites [Xie et al., 2012]. In fact, the accumulation of an abnormal metabolite was found in the *COQ9* mutant fibroblasts by the routine electrochemical-high-performance liquid chromatography methods [Duncan et al., 2009]. However, identification of the chemical structure of this metabolite is not possible with high-performance liquid chromatography techniques.

Bioenergetics Defects and Oxidative Stress

The disparate phenotypes of CoQ_{10}-deficient patients suggest divergent pathogenic mechanisms in this syndrome. To address this issue, research laboratories have studied various molecular and biochemical changes of cultured skin fibroblasts from patients with primary CoQ_{10} deficiency. Initially, effects of CoQ_{10} deficiency on mitochondrial bioenergetics were assessed because of its essential function for mitochondrial ATP synthesis as an electron carrier from mitochondrial complexes I and II to complex III [Geromel et al., 2001, 2002; Quinzii et al., 2008b, 2010]. In addition, inherited mitochondrial respiratory chain defects frequently result in increased oxidative stress [Kirkinezos and Moraes, 2001], and CoQ_{10} is recognized as an important endogenous antioxidant, which protects the cell both directly by preventing lipid peroxidation and indirectly by regenerating other antioxidants such as vitamins C and E [Mellors and Tappel, 1966; Turunen et al., 2004]; therefore, a second focus has been on the consequences of CoQ_{10} deficiency on the fibroblasts' oxidative status. Geromel et al. [2001, 2002], studying CoQ_{10}-deficient fibroblasts of unknown genetic etiology, showed mild defects of mitochondrial respiration and growth rate, but no signs of oxidative stress. López-Martín et al. [2007] also showed defects in the ubiquinone-dependent complexes activities, CI+III and CII+III, in CoQ_{10} deficiency fibroblasts with a *COQ2* mutation. Quinzii et al. [2008b] demonstrated that CoQ_{10}-deficient fibroblasts with *PDSS2* mutations and ~20% of residual CoQ_{10} showed a severe bioenergetics defect, but no signs of oxidative stress, whereas *COQ2* mutant fibroblasts with ~40% of residual CoQ_{10} showed less severe bioenergetics defects with striking signs of oxidative stress. Similar to the *PDSS2* mutant fibroblasts, *COQ9* mutant fibroblasts with ~15% residual CoQ_{10} showed bioenergetics defects without oxidative stress, whereas fibroblasts from an additional patient with

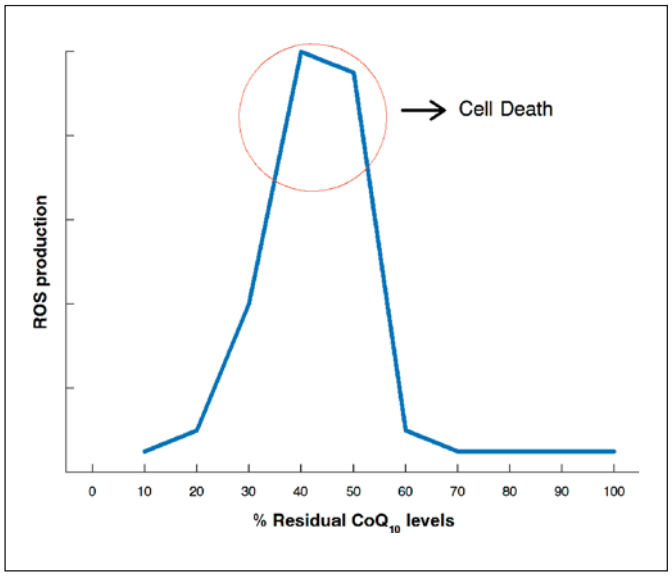

Fig. 1. ROS production in CoQ_{10}-deficient fibroblasts depends on the residual CoQ_{10} levels. 25–45% of residual CoQ_{10} levels induce maximal ROS production with increased cell death.

COQ2 mutations and ~35% residual CoQ_{10} manifested increased oxidative stress markers [Quinzii et al., 2010]. The study was also extended to fibroblasts from 3 different CoQ_{10}-deficient patients due to *ADCK3* mutations and >50% of residual CoQ_{10}. In these cases, very mild defects in ATP levels were observed, and no signs of oxidative stress were detected. Taken together, the results indicated that the levels of CoQ_{10} correlate with reactive oxygen species (ROS) production and oxidative damage: 10–20% and >50% residual CoQ_{10} are not associated with significant oxidative stress, whereas 25–45% residual CoQ_{10} is accompanied by increased ROS production and oxidative damage (fig. 1). Nevertheless, because the increase of oxidative stress was only limited to *COQ2* mutant fibroblasts, it was possible that defective 4-parahydroxybenzoate:polyprenyl transferase (COQ2) protein specifically produced ROS. For this reason, Quinzii et al. [2012] evaluated mitochondrial bioenergetics and oxidative stress in cultured fibroblasts with CoQ_{10} deficiency induced by pharmacological inhibition of CoQ_{10} biosynthesis. The drug used to inhibit CoQ_{10} biosynthesis, 4-nitrobenzoate (4-NB), competes with 4-HB in the reaction catalyzed by COQ2 [Forsman et al., 2010]. Pharmacological inhibition of CoQ_{10} biosynthesis led to 55–70% decreases of the endogenous CoQ_{10} (30–45% residual CoQ_{10} relative to normal) in both control and *ADCK3* mutant fibroblasts. As a consequence of this, there were decreases in ATP levels and increases in ROS production and oxidative damage [Quinzii et al., 2012]. These results confirmed that a range of 30–45% of residual CoQ_{10} is the key factor in generating ROS (fig. 1). However, the fundamental mechanisms of ROS generation associated with CoQ_{10} deficiency in human skin fibroblasts remain to be elucidated. In this regard, 2 possible mechanisms operating in mitochondria may be responsible: (1) reduction of CoQ-dependent CI+III activity may increase the NADH/NAD+ ratio in the matrix, leading to an increase of O_2 formation through the FMN group of the CI [Murphy, 2009]; or (2) increased proton motive force (e.g. increased mitochondrial membrane potential) without concomitant enhanced ATP synthesis, leading to increased ROS production [Korshunov et al., 1997; Murphy, 2009]. This second possibility is particularly relevant because hyperpolarization of mitochondria and increased ROS production have been observed in fibroblasts with 30–45% of residual CoQ_{10} [Quinzii et al., 2010, 2012]. The reason for the increased mitochondrial membrane potential may apply to other respiratory chain defects because the F_1F_0-ATPase (complex V) activity is regulated by 2 factors: free energy of the adenosine phosphates and proton electrochemical potential. Thus, in the setting of impaired respiration, membrane potential is initially decreased, but if the cells have sufficient ATP supply, e.g. by the upregulation of glycolysis, complex V may operate as a proton-translocating ATPase, increasing the membrane potential by pumping protons in reverse [Scott and Nicholls, 1980; McKenzie et al., 2007]. Nevertheless, this mechanism does not explain how differences in mitochondrial membrane potential increase ROS generation in fibroblasts with 20–45% residual CoQ_{10}.

From a bioenergetics point of view, another interesting observation is the reduced levels of subunits of complex III and IV with normal levels of complex I subunits in immunoblots of extracts of CoQ_{10}-deficient fibroblasts with *COQ2* mutations or other unknown etiological defects [Rodríguez-Hernández et al., 2009]. The authors suggested that CoQ_{10} deficiency may affect the activity, organization and assembly of complex III as it has been reported in yeast [Santos-Ocaña et al., 2002], with a secondary effect on complex IV. Further experiments are needed to confirm these results in CoQ_{10}-deficient fibroblasts with different molecular defects and whether the destabilization of complex III is the cause or the effect of increased ROS production associated with CoQ_{10} deficiency.

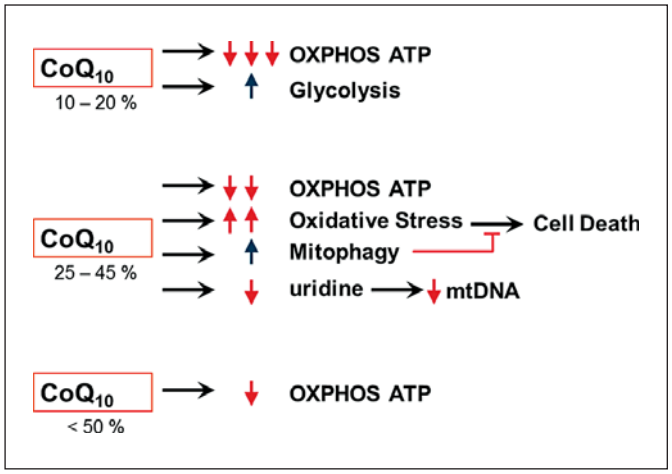

Fig. 2. Schematic diagram summarizing the pathomechanisms identified in CoQ_{10}-deficient fibroblasts. OXPHOS = Oxidative phosphorylation.

Apoptosis, Necrosis and Mitophagy

Increased oxidative stress and ATP depletion have been described as initial events promoting mitochondrial-mediated apoptotic cell death. In studies of CoQ_{10}-deficient skin fibroblasts, Quinzii et al. [2010] found that only the *COQ2* mutant fibroblasts showed increased apoptotic cell death. Because these cells also showed increased ROS production, the authors hypothesized that the induction of apoptosis was related to increased oxidative stress. Whether the oxidative stress-induced cell death was specific to *COQ2* defects or due to the severity of CoQ_{10} deficiency was assessed by the pharmacological inhibition of CoQ_{10} biosynthesis [Quinzii et al., 2012]. Once again, skin fibroblasts with 30–45% of residual CoQ_{10} were associated with cell death as a consequence of the increased oxidative stress and independent of the molecular defect [Quinzii et al., 2012]. Another interesting observation of these studies is that, contrary to the initial expectations, *PDSS2* and *COQ9* mutant fibroblasts did not show increased cell death, despite the cells' severe depletion of ATP (fig. 2) [Quinzii et al., 2010]. One possible explanation is that cells with <20% of residual CoQ_{10} acquire resistance to mitochondrial stress-induced apoptosis, compared to those with partial blockage of respiratory chain electron flux associated with a heightened sensitivity to cell death, as has been previously reported [Dey and Moraes, 2000; Park et al., 2004; Quinzii et al., 2010]. In support of this notion, studies have indicated that mitophagy can selectively degrade dysfunctional mitochondria preventing the mitochondrial-dependent apoptotic cell death. In CoQ_{10}-deficient fibroblasts, Rodríguez-Hernández et al. [2009] observed increased levels of lysosomal markers and enhanced expression of transcriptional and translational levels of autophagic genes in cell lines carrying *COQ2* mutations and in 2 other cell lines from patients with CoQ_{10} deficiency and unknown molecular defects. Because inhibition of autophagy resulted in apoptotic cell death, the authors suggested that autophagy is a protective mechanism involved in the degradation of dysfunctional mitochondria (fig. 2) [Rodríguez-Hernández et al., 2009]. Thus, *PDSS2* and *COQ9* mutant fibroblasts may have increased mitophagy to remove dysfunctional mitochondria. Additionally, compensatory production of ATP via upregulation of glycolysis may also protect cells by decreasing oxidative stress and associated cell death [Quinzii et al., 2008b].

Pyrimidine Metabolism

In addition to its bioenergetics and antioxidant role, CoQ_{10} is also a cofactor in the reaction catalyzed by the mitochondrial protein dihydroorotate dehydrogenase [Rawls et al., 2000]. This enzyme catalyzes the oxidation of dihydroorotate to orotate at the FMN group, which is reduced to $FMNH_2$. $FMNH_2$ is then re-oxidized by reaction with ubiquinone, which is reduced to ubiquinol [Rawls et al., 2000]. The product of the reaction, orotate, is an intermediate of the de novo synthesis of the pyrimidines by the conversion to the ribonucleotide UMP, which can be converted through subsequent reactions in pyrimidine ribonucleotides and deoxyribonucleotides [Löffler et al., 1997; Rawls et al., 2000]. The conversion of ribonucleotides into deoxyribonucleotides is catalyzed by the ribonucleotide reductases [Nordlund and Reichard, 2006]. Until recently, it was thought that mtDNA replication and repair in quiescent cells was dependent on salvage synthesis of deoxynucleotide triphosphates [Ferraro et al., 2005], but the scenerio changed suddenly with the identification of mutations in a p53 inducible small ribonucleotide reductase subunit, called p53R2 [Tanaka et al., 2000] in patients with mtDNA depletion [Bourdon et al., 2007]. Subsequent experiments confirmed that the de novo supply of deoxynucleotide triphosphates is required for replication and repair of mtDNA in cells and mice [Bourdon et al., 2007; Pontarin et al., 2008]. As a consequence of these findings, it has been postulated that the mtDNA depletion identified in the muscle of a patient with CoQ_{10} deficiency may be the result of the reduction

in the deoxynucleotide triphosphates supply [Montero et al., 2009]. mtDNA depletion has also been described in skin fibroblasts with *COQ2* mutations [Quinzii et al., 2010], and the slow rates of growth observed in these fibroblasts were restored by addition of uridine in the culture medium [López-Martín et al., 2007] (fig. 2). Alternatively, it is possible that the uridine effect in the growth rate of the *COQ2* mutant fibroblasts may be due to the role of uridine metabolism in the gycolysis through the catabolism of galactose [Petry and Reichardt, 1998].

Treatment Evaluation in CoQ_{10}-Deficient Skin Fibroblasts

The only therapeutic option currently available for CoQ_{10} deficiency syndrome is exogenous CoQ_{10} supplementation. However, of patients with identified mutations, only 20% improved after exogenous CoQ_{10} supplementation [Rahman et al., 2001; López et al., 2006; Diomedi-Camassei et al., 2007; Mollet et al., 2007, 2008; Lagier-Tourenne et al., 2008; Montini et al., 2008; Heeringa et al., 2011]. In order to understand the low percentage of successful treatments, studies have evaluated the effects of CoQ_{10} supplementation in CoQ_{10}-deficient fibroblasts. We attempted to counteract the bioenergetics defect and oxidative stress of the CoQ_{10}-deficient fibroblasts by adding 5 μM of CoQ_{10} in culture cells [López et al., 2010]. The results showed that CoQ_{10} supplementation for 1 week, but not for 24 h, improved ATP levels and ATP/ADP ratio. Similar to these results, yeast *coq* mutants showed an inefficient uptake of exogenous CoQ_6 to the mitochondrial inner membrane, which was reflected in a low succinate cytochrome *c* reductase activity after 2–15 μM CoQ_6 supplementation for 48 h [Do et al., 2001; Santos-Ocaña et al., 2002]. The complete rescue of growth of the yeast *coq* mutants supplemented with 15 μM CoQ_6 was only possible after 6–8 days [Do et al., 2001; Jonassen et al., 2002; Santos-Ocaña et al., 2002]. In contrast to these results, López-Martín et al. [2007] noted normalization of mitochondrial complexes I+III and II+III activities in *COQ2* mutant fibroblasts after 24 h of 10 μM CoQ_{10} supplementation. Paradoxically, the same authors, using the same *COQ2* mutant cells and other genetically undefined CoQ_{10}-deficient fibroblasts, found that the activities of complex II+III increased only slightly and remained below control values after 72 h of 100 μM CoQ_{10} supplementation [Rodríguez-Hernández et al., 2009]. This discrepancy may be due to the fact that in the second study, but not in the first, respiratory chain enzyme activities were normalized to activity of citrate synthase [Rodríguez-Hernández et al., 2009], a marker of mitochondrial mass [Kirby et al., 2007]. The discrepant results may be also explained by different methodologies used to evaluate mitochondrial bioenergetics after CoQ_{10} supplementation: López et al. [2010] measured cellular ATP levels and ATP/ADP ratio; in the other 2 studies spectrophotometric methods were used to measure activities of CoQ-dependent complexes. The enzymatic activity assay may produce artifacts due to sonication, freeze-thaw, or detergents that fracture mitochondria and allow access of the CoQ_{10} accumulated in the cellular membrane to the inner mitochondrial membrane. Additionally, these manipulations disrupt the mitochondrial supercomplexes, minimizing the differences between the experimental groups [Kruse et al., 2008]. Finally, the different sources of the CoQ_{10}, its different formulations and the different solubilization methods may also influence the experimental results.

Because in vitro studies suggested that high lipophilicity of CoQ_{10} is the major cause of delayed effects of CoQ_{10} supplementation, the effects of less lipophilic ubiquinone analogs with shorter isoprenoid tail were also tested. However, the 2 short-tail ubiquinone analogs, CoQ_2 and idebenone, did not correct bioenergetics defects [López et al., 2010], which highlights the importance of the decaprenyl tail. Despite the lack of correction of the bioenergetics defect in *COQ2* mutant fibroblasts, all of the CoQ analog tested decreased superoxide anion production and oxidative stress-induced cell death [López et al., 2010]. Thus, the in vitro study of CoQ_{10}-deficient fibroblasts revealed important insights regarding CoQ_{10} supplementation therapy: (1) the prolonged pharmacokinetics of CoQ_{10} in restoring respiratory chain activity in CoQ_{10}-deficient cells [Bentinger et al., 2003], a factor that may contribute to the late clinical response to the oral supplementation of CoQ_{10} [Montini et al., 2008] and suggest that high doses of CoQ_{10} must be administered; (2) short-tail ubiquinone analogs do not substitute for CoQ_{10} in the mitochondrial respiratory chain revealing the importance of the decaprenyl tail; and (3) oxidative stress and cell death can be ameliorated by the administration of lipophilic and hydrophilic antioxidants [López et al., 2010].

Concluding Remarks

Studies of CoQ_{10}-deficient fibroblasts indicate that primary CoQ_{10} deficiencies cause variable defects on ATP synthesis, oxidative stress and cell death, which appear to be related to the specific molecular defect, residual level

of CoQ_{10}, or both (fig. 2). Therapy leads to the amelioration of these effects. Mitochondrial apoptosis has been observed in CoQ_{10}-deficient cells; however, the pathways involved in the induction of this pathway remain to be elucidated. The studies in CoQ_{10}-deficient fibroblasts have been limited by the small number of available cell lines. The clinical heterogeneity and tissue-specificity of CoQ_{10} deficiency syndrome suggest that differentiated cells may respond to the deficit in ubiquinone differently compared to fibroblasts. In support of this premise, a recent study in a human neuronal model of CoQ_{10} deficiency has shown different degrees of mitochondrial respiratory chain dysfunction and oxidative stress relative to CoQ_{10}-deficient fibroblasts [Duberly et al., 2013]. Future studies on additional cellular and animal models will help us understand the clinical heterogeneity of CoQ_{10} deficiency syndrome and may contribute to the development of more effective therapies.

Acknowledgements

This work was partially supported by grants from the Marie Curie International Reintegration Grant Programme (COQMIT-MEL-266691) within the 7th European Community Framework Programme, from Ministerio de Economía y Competitividad, Spain (SAF2009-08315), from the Consejería de Economía, Innovación, Ciencia y Empleo, Junta de Andalucía (P10-CTS-6133) and from the 'CEIBioTic' (20F12/1) and 'Incent' Programs from the Universidad de Granada. L.C.L. is supported by the 'Ramón y Cajal' National Programme, Ministerio de Economía y Competitividad, Spain. M.L.-S. is a predoctoral fellow from the Consejería de Economía, Innovación, Ciencia y Empleo, Junta de Andalucía. C.M.Q. is supported by K23HD065871 from the Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD). M.H. is supported by NIH grants 1R01HD057543, R01HD056103 and 1RC1NS070232, a Muscular Dystrophy Association grant, and by the Marriott Mitochondrial Disorder Clinical Research Fund (MMDCRF).

References

Aeby A, Sznajer Y, Cavé H, Rebuffat E, Van Coster R, et al: Cardiofaciocutaneous (CFC) syndrome associated with muscular coenzyme Q_{10} deficiency. J Inherit Metab Dis 30: 827 (2007).

Bentinger M, Dallner G, Chojnacki T, Swiezewska E: Distribution and breakdown of labeled coenzyme Q_{10} in rat. Free Radic Biol Med 34: 563–575 (2003).

Bourdon A, Minai L, Serre V, Jais JP, Sarzi E, et al: Mutation of *RRM2B*, encoding p53-controlled ribonucleotide reductase (p53R2), causes severe mitochondrial DNA depletion. Nat Genet 39:776–780 (2007).

Cotán D, Cordero MD, Garrido-Maraver J, Oropesa-Ávila M, Rodríguez-Hernández A, et al: Secondary coenzyme Q_{10} deficiency triggers mitochondria degradation by mitophagy in MELAS fibroblasts. FASEB J 25:2669–2687 (2011).

Dey R, Moraes CT: Lack of oxidative phosphorylation and low mitochondrial membrane potential decrease susceptibility to apoptosis and do not modulate the protective effect of Bcl-x_L in osteosarcoma cells. J Biol Chem 275: 7087–7094 (2000).

Diomedi-Camassei F, Di Giandomenico S, Santorelli FM, Caridi G, Piemonte F, et al: *COQ2* nephropathy: a newly described inherited mitochondriopathy with primary renal involvement. J Am Soc Nephrol 18:2773–2780 (2007).

Do TQ, Hsu AY, Jonassen T, Lee PT, Clarke CF: A defect in coenzyme Q biosynthesis is responsible for the respiratory deficiency in *Saccharomyces cerevisiae abc1* mutants. J Biol Chem 276:18161–18168 (2001).

Duberley KE, Abramov AY, Chalasani A, Heales SJ, Rahman S, Hargreaves IP: Human neuronal coenzyme Q_{10} deficiency results in global loss of mitochondrial respiratory chain activity, increased mitochondrial oxidative stress and reversal of ATP synthase activity: implications for pathogenesis and treatment. J Inherit Metab Dis 36:63–73 (2013).

Duncan AJ, Bitner-Glindzicz M, Meunier B, Costello H, Hargreaves IP, et al: A nonsense mutation in *COQ9* causes autosomal-recessive neonatal-onset primary coenzyme Q_{10} deficiency: a potentially treatable form of mitochondrial disease. Am J Hum Genet 84: 558–566 (2009).

Ferraro P, Pontarin G, Crocco L, Fabris S, Reichard P, Bianchi V: Mitochondrial deoxynucleotide pools in quiescent fibroblasts: a possible model for mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). J Biol Chem 280:24472–24480 (2005).

Forsman U, Sjöberg M, Turunen M, Sindelar PJ: 4-Nitrobenzoate inhibits coenzyme Q biosynthesis in mammalian cell cultures. Nat Chem Biol 6:515–517 (2010).

Gempel K, Topaloglu H, Talim B, Schneiderat P, Schoser BG, et al: The myopathic form of coenzyme Q_{10} deficiency is caused by mutations in the electron-transferring-flavoprotein dehydrogenase *(ETFDH)* gene. Brain 130:2037–2044 (2007).

Geromel V, Kadhom N, Ceballos-Picot I, Chrétien D, Munnich A, et al: Human cultured skin fibroblasts survive profound inherited ubiquinone depletion. Free Radic Res 35:11–21 (2001).

Geromel V, Darin N, Chrétien D, Bénit P, DeLonlay P, et al: Coenzyme Q_{10} and idebenone in the therapy of respiratory chain diseases: rationale and comparative benefits. Mol Genet Metab 77:21–30 (2002).

Heeringa SF, Chernin G, Chaki M, Zhou W, Sloan AJ, et al: *COQ6* mutations in human patients produce nephrotic syndrome with sensorineural deafness. J Clin Invest 121:2013–2024 (2011).

Jonassen T, Marbois BN, Faull KF, Clarke CF, Larsen PL: Development and fertility in *Caenorhabditis elegans clk-1* mutants depend upon transport of dietary coenzyme Q_8 to mitochondria. J Biol Chem 277:45020–45027 (2002).

Kirby DM, Thorburn DR, Turnbull DM, Taylor RW: Biochemical assays of respiratory chain complex activity. Methods Cell Biol 80:93–119 (2007).

Kirkinezos IG, Moraes CT: Reactive oxygen species and mitochondrial diseases. Semin Cell Dev Biol 12:449–457 (2001).

Korshunov SS, Skulachev VP, Starkov AA: High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. FEBS Lett 416:15–18 (1997).

Kruse SE, Watt WC, Marcinek DJ, Kapur RP, Schenkman KA, Palmiter RD: Mice with mitochondrial complex I deficiency develop a fatal encephalomyopathy. Cell Metab 7:312–320 (2008).

Lagier-Tourenne C, Tazir M, López LC, Quinzii CM, Assoum M, et al: ADCK3, an ancestral kinase, is mutated in a form of recessive ataxia associated with coenzyme Q_{10} deficiency. Am J Hum Genet 82:661–672 (2008).

Le Ber I, Dubourg O, Benoist JF, Jardel C, Mochel F, et al: Muscle coenzyme Q_{10} deficiencies in ataxia with oculomotor apraxia 1. Neurology 68:295–297 (2007).

Löffler M, Jöckel J, Schuster G, Becker C: Dihydroorotat-ubiquinone oxidoreductase links mitochondria in the biosynthesis of pyrimidine nucleotides. Mol Cell Biochem 174: 125–129 (1997).

López LC, Schuelke M, Quinzii CM, Kanki T, Rodenburg RJ, et al: Leigh syndrome with nephropathy and CoQ_{10} deficiency due to decaprenyl diphosphate synthase subunit 2 *(PDSS2)* mutations. Am J Hum Genet 79: 1125–1129 (2006).

López LC, Quinzii CM, Area E, Naini A, Rahman S, et al: Treatment of CoQ_{10} deficient fibroblasts with ubiquinone, CoQ analogs, and vitamin C: time- and compound-dependent effects. PLoS One 5:e11897 (2010).

López-Martín JM, Salviati L, Trevisson E, Montini G, DiMauro S, et al: Missense mutation of the *COQ2* gene causes defects of bioenergetics and de novo pyrimidine synthesis. Hum Mol Genet 16:1091–1097 (2007).

McKenzie M, Liolitsa D, Akinshina N, Campanella M, Sisodiya S, et al: Mitochondrial ND_5 gene variation associated with encephalomyopathy and mitochondrial ATP consumption. J Biol Chem 282:36845–36852 (2007).

Mellors A, Tappel AL: The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol. J Biol Chem 241:4353–4356 (1966).

Miles MV, Miles L, Tang PH, Horn PS, Steele PE, et al: Systematic evaluation of muscle coenzyme Q_{10} content in children with mitochondrial respiratory chain enzyme deficiencies. Mitochondrion 8:170–180 (2008).

Mollet J, Giurgea I, Schlemmer D, Dallner G, Chrétien D, et al: Prenyldiphosphate synthase, subunit 1 *(PDSS1)* and OH-benzoate polyprenyltransferase *(COQ2)* mutations in ubiquinone deficiency and oxidative phosphorylation disorders. J Clin Invest 117:765–772 (2007).

Mollet J, Delahodde A, Serre V, Chrétien D, Schlemmer D, et al: *CABC1* gene mutations cause ubiquinone deficiency with cerebellar ataxia and seizures. Am J Hum Genet 82:623–630 (2008).

Montero R, Artuch R, Briones P, Nascimento A, García-Cazorla A, et al: Muscle coenzyme Q_{10} concentrations in patients with probable and definite diagnosis of respiratory chain disorders. Biofactors 25:109–115 (2005).

Montero R, Sánchez-Alcázar JA, Briones P, Navarro-Sastre A, Gallardo E, et al: Coenzyme Q_{10} deficiency associated with a mitochondrial DNA depletion syndrome: a case report. Clin Biochem 42:742–745 (2009).

Montini G, Malaventura C, Salviati L: Early coenzyme Q_{10} supplementation in primary coenzyme Q_{10} deficiency. N Engl J Med 358:2849–2850 (2008).

Murphy MP: How mitochondria produce reactive oxygen species. Biochem J 417:1–13 (2009).

Nordlund P, Reichard P: Ribonucleotide reductases. Annu Rev Biochem 75:681–706 (2006).

Ogasahara S, Engel AG, Frens D, Mack D: Muscle coenzyme Q deficiency in familial mitochondrial encephalomyopathy. Proc Natl Acad Sci USA 86:2379–2382 (1989).

Park SY, Chang I, Kim JY, Kang SW, Park SH, et al: Resistance of mitochondrial DNA-depleted cells against cell death: role of mitochondrial superoxide dismutase. J Biol Chem 279: 7512–7520 (2004).

Petry KG, Reichardt JK: The fundamental importance of human galactose metabolism: lessons from genetics and biochemistry. Trends Genet 14:98–102 (1998).

Pontarin G, Fijolek A, Pizzo P, Ferraro P, Rampazzo C, et al: Ribonucleotide reduction is a cytosolic process in mammalian cells independently of DNA damage. Proc Natl Acad Sci USA 105:17801–17806 (2008).

Quinzii C, Naini A, Salviati L, Trevisson E, Navas P, et al: A mutation in para-hydroxybenzoate-polyprenyl transferase *(COQ2)* causes primary coenzyme Q_{10} deficiency. Am J Hum Genet 78:345–349 (2006).

Quinzii CM, Hirano M: Coenzyme Q and mitochondrial disease. Dev Disabil Res Rev 16: 183–188 (2010).

Quinzii CM, Hirano M: Primary and secondary CoQ_{10} deficiencies in humans. Biofactors 37: 361–365 (2011).

Quinzii CM, Kattah AG, Naini A, Akman HO, Mootha VK, et al: Coenzyme Q deficiency and cerebellar ataxia associated with an aprataxin mutation. Neurology 64:539–541 (2005).

Quinzii CM, López LC, Naini A, DiMauro S, Hirano M: Human CoQ_{10} deficiencies. Biofactors 32:113–118 (2008a).

Quinzii CM, López LC, Von-Moltke J, Naini A, Krishna S, et al: Respiratory chain dysfunction and oxidative stress correlate with severity of primary CoQ_{10} deficiency. FASEB J 22: 1874–1885 (2008b).

Quinzii CM, López LC, Gilkerson RW, Dorado B, Coku J, et al: Reactive oxygen species, oxidative stress, and cell death correlate with level of CoQ_{10} deficiency. FASEB J 24:3733–3743 (2010).

Quinzii CM, Tadesse S, Naini A, Hirano M: Effects of inhibiting CoQ_{10} biosynthesis with 4-nitrobenzoate in human fibroblasts. PLoS One 7:e30606 (2012).

Rahman S, Hargreaves I, Clayton P, Heales S: Neonatal presentation of coenzyme Q_{10} deficiency. J Pediatr 139:456–458 (2001).

Rawls J, Knecht W, Diekert K, Lill R, Löffler M: Requirements for the mitochondrial import and localization of dihydroorotate dehydrogenase. Eur J Biochem 267:2079–2087 (2000).

Rodríguez-Hernández A, Cordero MD, Salviati L, Artuch R, Pineda M, et al: Coenzyme Q deficiency triggers mitochondria degradation by mitophagy. Autophagy 5:19–32 (2009).

Sacconi S, Trevisson E, Salviati L, Aymé S, Rigal O, et al: Coenzyme Q_{10} is frequently reduced in muscle of patients with mitochondrial myopathy. Neuromuscul Disord 20:44–48 (2010).

Salviati L, Trevisson E, Rodríguez Hernández MA, Casarin A, Pertegato V, et al: Haploinsufficiency of *COQ4* causes coenzyme Q_{10} deficiency. J Med Genet 49:187–191 (2012).

Santos-Ocaña C, Do TQ, Padilla S, Navas P, Clarke CF: Uptake of exogenous coenzyme Q and transport to mitochondria is required for bc1 complex stability in yeast *coq* mutants. J Biol Chem 277:10973–10981 (2002).

Scott ID, Nicholls DG: Energy transduction in intact synaptosomes. Influence of plasma-membrane depolarization on the respiration and membrane potential of internal mitochondria determined in situ. Biochem J 186: 21–33 (1980).

Tanaka H, Arakawa H, Yamaguchi T, Shiraishi K, Fukuda S, et al: A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. Nature 404:42–49 (2000).

Tekle M, Turunen M, Dallner G, Chojnacki T, Swiezewska E: Investigation of coenzyme Q biosynthesis in human fibroblast and HepG2 cells. J Biochem Biophys Methods 70:909–917 (2008).

Turunen M, Olsson J, Dallner G: Metabolism and function of coenzyme Q. Biochim Biophys Acta 1660:171–199 (2004).

Xie LX, Ozeir M, Tang JY, Chen JY, Kieffer-Jaquinod S, et al: Over-expression of the Coq8 kinase in *Saccharomyces cerevisiae coq* null mutants allows for accumulation of diagnostic intermediates of the coenzyme Q_6 biosynthetic pathway. J Biol Chem 287:2357–23581 (2012).