Biochemical Assessment of Coenzyme $Q_{10}$ Deficiency

Juan Carlos Rodríguez-Aguilera $^{1,2}$, Ana Belén Cortés $^{1,2}$, Daniel J. M. Fernández-Ayala $^{2,3}$ and Plácido Navas $^{2,3,*}$

$^1$ Laboratorio de Fisiopatología Celular y Bioenergética, 41013 Sevilla, Spain; jcrdaguy@upo.es (J.C.R.-A.); abcorrod@upo.es (A.B.C.)
$^2$ Centro de Investigación Biomédica en Red de Enfermedades Raras, Instituto de Salud Carlos III, Universidad Pablo de Olavide-CISC, 41013 Sevilla, Spain; dmorfer@upo.es
$^3$ Centro Andaluz de Biología del Desarrollo, 41013 Sevilla, Spain
* Correspondence: pnavas@upo.es; Tel.: +34-954-349-385

Academic Editor: Iain P. Hargreaves
Received: 18 January 2017; Accepted: 28 February 2017; Published: 5 March 2017

Abstract: Coenzyme $Q_{10}$ ($CoQ_{10}$) deficiency syndrome includes clinically heterogeneous mitochondrial diseases that show a variety of severe and debilitating symptoms. A multiprotein complex encoded by nuclear genes carries out $CoQ_{10}$ biosynthesis. Mutations in any of these genes are responsible for the primary $CoQ_{10}$ deficiency, but there are also different conditions that induce secondary $CoQ_{10}$ deficiency including mitochondrial DNA (mtDNA) depletion and mutations in genes involved in the fatty acid $\beta$-oxidation pathway. The diagnosis of $CoQ_{10}$ deficiencies is determined by the decrease of its content in skeletal muscle and/or dermal skin fibroblasts. Dietary $CoQ_{10}$ supplementation is the only available treatment for these deficiencies that require a rapid and distinct diagnosis. Here we review methods for determining $CoQ_{10}$ content by HPLC separation and identification using alternative approaches including electrochemical detection and mass spectrometry. Also, we review procedures to determine the $CoQ_{10}$ biosynthesis rate using labeled precursors.

Keywords: coenzyme $Q_{10}$; $CoQ_{10}$ deficiency syndrome; $CoQ_{10}$ biosynthesis; mitochondria diseases

1. Introduction

The mitochondrial respiratory chain (MRC) generates most of the cellular ATP and is comprised of five multi-subunit enzyme complexes. Both the mitochondrial DNA (mtDNA) and the nuclear DNA (nDNA) encode for polypeptides of these complexes and also proteins involved in mitochondrial function. Besides MRC enzyme complexes, two electron carriers, coenzyme Q ($CoQ$) and cytochrome $c$, are vital for mitochondrial synthesis of ATP. Mutations in genes of either genome may cause mitochondrial diseases, which are common among inherited metabolic and neurological disorders [1].

$CoQ$ is a lipid-soluble component of virtually all cell membranes. It is composed of a benzoquinone ring with a polyprenyl side chain, the number of isoprene units being a characteristic of given specie, e.g., 10 in humans ($CoQ_{10}$). $CoQ_{10}$ transports electrons from MRC Complexes I and II to Complex III. These electrons come from either NADH or succinate [2] although $CoQ_{10}$ can be alternatively reduced with electrons provided by different redox reactions in mitochondria [3]. Consequently, $CoQ_{10}$ is essential for ATP production inside mitochondria, although it is also an indispensable antioxidant in extramitochondrial membranes and a key factor for pyrimidine nucleotide synthesis [4].

$CoQ$ biosynthesis depends on a pathway that involves at least 11 genes ($COQ$ genes), showing a high degree of conservation among species, and is carried out by a putative multi-subunit enzyme complex [5]. Most of the information about the $CoQ$ biosynthesis pathway comes from yeast, and
maintains a high homology with mammal gene components (Table 1) [6]. The CoQ_{10} biosynthesis pathway is highly regulated by transcription factors PPAR_{α} and NF_{κ}B [7–9]. HuR and hnRNP C1/C2 binding proteins stabilize COQ7 mRNA as another CoQ_{10} biosynthesis regulatory mechanism [10].

**Table 1.** Yeast COQ genes and their characterized human homologues.

| Yeast  | Human       | Function                    |
|--------|-------------|-----------------------------|
| COQ1   | PDSS1 */PDSS2 * | Synthesis of polyprenyl-diphosphate |
| COQ2   | COQ2 *       | pHB-prenyl-transferase       |
| COQ3   | COQ3 *       | Methyltransferase           |
| COQ4   | COQ4 *       | Organization of the multi-enzyme complex |
| COQ5   | COQ5         | Methyltransferase           |
| COQ6   | COQ6 *       | Mono-oxygenase              |
| COQ7   | COQ7 *       | Hydroxylase                 |
| COQ8   | ADCK3 */ADCK4 * | Unorthodox kinase (regulatory) |
| COQ9   | COQ9 *       | Lipid binding protein       |
| COQ10  | COQ10A/COQ10B | CoQ chaperone               |
| PTC7   | PPTC7        | Phosphatase (regulatory)    |

* These genes were mutated in human causing primary CoQ_{10} deficiency.

Coq7p is post-translationally regulated in yeast that involves mitochondrial phosphatase Ptc7 [11,12]. Ptc7 human orthologue (PPTC7) is related to cellular bioenergetics and stress resistance [13]. Coq7p activity is a key regulator of the CoQ biosynthesis complex [6,14], which may depend on the interaction with Coq9p contributing to the stabilization of the biosynthesis complex [15–18]. The level of CoQ is highly regulated inside cells and tissues but its concentration is different in each tissue and organ, and depends on dietary conditions and age [19,20]. CoQ also varies greatly in human diseases such as Alzheimer’s disease, cardiomyopathy, Niemann-Pick and diabetes.

2. **CoQ_{10} Deficiency Syndrome**

CoQ_{10} deficiency syndrome includes diverse inherited pathological diseases defined by the decrease of CoQ_{10} content in muscle and/or cultured skin fibroblasts. CoQ_{10} deficiency impairs oxidative phosphorylation and causes clinically heterogeneous mitochondrial diseases [21,22]. When the decrease in CoQ_{10} content is due to mutations in genes encoding proteins of the CoQ biosynthesis pathway or its regulation (COQ genes), it causes primary CoQ_{10} deficiency [23,24]. Secondary CoQ_{10} deficiencies may be due to defects in genes unrelated to the CoQ_{10} biosynthetic pathway. Secondary CoQ_{10} deficiency is a common finding in oxidative phosphorylation (OXPHOS) and non-OXPHOS disorders [25]. A low mitochondrial CoQ_{10} content is described in mtDNA depletion [26], mutations in the DNA repairing aprataxin [27], mutations of the enzyme EF_5DH of the β-oxidation of fatty acids [28], recurrent food intolerance and allergies [29], methylmalonic aciduria [30], myalgic encephalomyelitis chronic fatigue syndrome [31], and propionic acidemia [32]. We propose that cases of secondary CoQ_{10} deficiency associated with OXPHOS defects could be adaptive mechanisms to maintain a balanced OXPHOS which is required to keep cells alive, although the mechanisms explaining these deficiencies and the pathophysiological role in the disease are unknown.

The clinical phenotypes of primary CoQ_{10}-deficient patients are broader than initially reported in 1989 [33], including (i) a multisystem disorder with steroid-resistant nephrotic syndrome as the main clinical manifestation (COQ1-PDSS2) [34], (COQ2) [35], (COQ6) [36] and (ADCK4) [37]; (ii) a multisystem disorder without nephrotic syndrome (COQ1-PDSS1) [38], (COQ9) [39] and (COQ7) [40]; (iii) cerebellar ataxia (COQ8-ADCK3) [41–47]; and (iv) myopathy and encephalopathy (COQ4) [48–50].
3. Primary CoQ\textsubscript{10} Deficiency Therapy

Primary CoQ\textsubscript{10} deficiency is unique among mitochondrial diseases because an effective therapy is available for patients, which is the supplementation of CoQ\textsubscript{10}. Ubiquinol, the reduced form of CoQ\textsubscript{10}, was recently approved as an orphan drug for primary CoQ\textsubscript{10} deficiency [51].

While this approach is quite successful in some patients, with a clear improvement of the pathological phenotype [52], some cases do not show any clinical relief as would be expected [53], probably because they are suffering secondary CoQ\textsubscript{10} deficiency. High-dose oral CoQ\textsubscript{10} supplementation can stop the progression of the encephalopathy and allows the recovery of renal damage [52]. High-dose CoQ\textsubscript{10} supplementation was also able to prevent the onset of renal symptoms in PDSS2-deficient mice [54]. Furthermore, CoQ\textsubscript{10} but not other quinones can restore mitochondrial function in deficient human fibroblasts [55]. Due to the therapeutic possibility of CoQ\textsubscript{10} supplementation for these patients, a rapid and unequivocal diagnosis of the deficiency is essential.

4. CoQ\textsubscript{10} Determination in Cells and Tissues

Content of CoQ\textsubscript{10} has been determined in plasma, white blood cells, skin fibroblasts and skeletal muscle biopsies to assess a deficiency diagnosis [56–58], and recently useful determination in the urine of pediatric patients was demonstrated [59]. Although CoQ can be measured in plasma and white blood cells, you cannot use it for the diagnosis of mitochondrial diseases since CoQ content in plasma and white blood cells is often not decreased in these conditions.

CoQ\textsubscript{10} content is mainly analyzed by the injection of lipid extracts in HPLC and detected by either electrochemical and/or UV-vis detectors, or mass spectrometry. Electrochemical detection has significant advantages compared to UV-vis detection; these include higher sensitivity and also the ability to measure oxidized and reduced forms of CoQ, either separately or combined, according to differential positioning of the conditioning cell (before or after the injector valve, respectively).

CoQ\textsubscript{10} extraction from biological samples (0.5 mg protein) requires the disruption of hydrophobic elements (lipid bilayers and lipoproteins) by adding SDS (1% final concentration). Lipids are dispersed with an alcohol cocktail (2-propanol 5% in ethanol) mixed with the disrupted biological sample (ratio 1:2 v/v), and they undergo subsequent triplicated hexane extraction (dispersed sample:hexane ratio 3:5 v/v). Hexane fractions are mixed and dried under vacuum, and then reconstituted in ethanol prior to HPLC analysis. To estimate CoQ\textsubscript{10} recovery, 100 pmol CoQ\textsubscript{9} was included in the alcohol cocktail (2-propanol 5% in ethanol). Trace amounts of CoQ\textsubscript{9} may have eventually been found in human tissues (probably from dietary uptake), but this does not interfere with the significant amount of internal standard added.

For convenience in high-throughput analysis, volumes are scaled down for extraction and vortex in 1.5 mL polypropylene tubes or 2 mL cryo vials.

Separation in C18 RP-HPLC columns (5 \(\mu\)m, 150 \(\times\) 4.6 mm) requires 20 mM AcNH\textsubscript{4} pH 4.4 in methanol (solvent A) and 20 mM AcNH\textsubscript{4} pH 4.4 in propanol (solvent B). A gradient method with a 85:15 solvent mixture (A:B ratio), and a flow rate of 1.2 mL/min, is regularly used as the starting conditions. The mobile phase turns to a 50:50 A:B ratio starting in minute 6 and completed in minute 8, as the flow rate decreases to 1.1 mL/min. After 20 min (run time) at 40 °C, the columns are re-equilibrated to the initial conditions for three additional minutes.

The detection of total CoQ\textsubscript{10} can be achieved either by UV-vis (set to 275 nm) or electrochemical (ECD) detectors (channel 1 set to −700 mV and channel 2 set to +500 mV, conditioning guard cell after injection valve). For complex samples including many peaks, the CoQ\textsubscript{10} peak is confirmed by spectral information (UV-vis) or by the redox area ratio (ECD detector, −700/+500 area ratio), compared to pure CoQ\textsubscript{10}. Figure 1 illustrates two chromatograms that correspond to normal age-matched human dermal fibroblasts (black plot) compared to patient dermal fibroblasts with CoQ\textsubscript{10} deficiency (red plot).
were incubated with 4.5 nM \( \text{CoQ}_{10} \) in parallel with either electrochemical or UV-vis detectors. Lipid extraction is done as we described in Arias et al. 

Previously, cultures of human skin fibroblasts, and murine embryonic fibroblast and stem cells [10,61]. Previously, cultures of human skin fibroblasts, and murine embryonic fibroblast and stem cells [10,61].

 biosynthesis by the level of incorporation of labeled of \( \text{CoQ}_{10} \) has been quantified in any type of cell culture, such as cancer cells, human skin fibroblasts, and murine embryonic fibroblast and stem cells [10,61]. Previously, cultures were incubated with 4.5 nM \( \text{CoQ}_{10} \) for one to three days, depending on the cell-specific rate of growth. The \( \text{CoQ}_{10} \)-HB was chemically synthesized in our laboratory from \(^{14}C\)-thyrosine [61]. Labeled-\( \text{CoQ}_{10} \) content is analyzed by lipid extract injection in HPLC and detected by the radio-flow detector LB 509 with a solid cell YG 150 Al-U4D (Berthold Technologies, Bad Wildbad, Germany) in parallel with either electrochemical or UV-vis detectors. Lipid extraction is done as we described above for \( \text{CoQ}_{10} \) determination in cells and tissues, but isocratic HPLC analysis lipid separation is performed with methanol:propanol (65:35) plus 20 mM AcNH\(_4\) pH 4.4 at a constant flow rate of 1 mL/min (Figure 2).

Figure 1. HPLC elution profile of lipid extracts from human skeletal muscular tissue. Patient pathological profile (red plot) shows that \( \text{CoQ}_{10} \) is clearly diminished compared to healthy control volunteers (black plot). \( \text{CoQ}_{9} \) is used as internal standard for normalization.

5. Analysis of \( \text{CoQ}_{10} \) Biosynthesis

Another important approach to assess \( \text{CoQ}_{10} \) deficiency in cells is to determine the rate of biosynthesis by the level of incorporation of labeled of \( \text{CoQ}_{10} \) precursors such as para-hydroxybenzoate (\( p\)-HB) labeled with either \( ^{13}C\)-\( p\)-HB or \( ^{14}C\)-\( p\)-HB, which is the precursor of the benzoquinone ring, or \( ^2\)H-mevalonate, which is the precursor of the isoprenyl side chain [10,60].

Polyisoprenyl-\( p\)-HB transferase activity was assayed by measuring the incorporation of \( ^{14}C\)-\( p\)-HB into nonaprenyl-4-hydroxybenzoate [35]. Isolated mitochondria (0.1–1 mg protein) were mixed with assay buffer (50 mM phosphate buffer, pH 7.5, 10 mM MgCl\(_2\), 5 mM EGTA containing 1 mM PMSF, 20 \( \mu \)g/mL each of the protease inhibitors chymostatin, leupeptin, antipain, and pepstatin A, 5 \( \mu \)M solanesyl pyrophosphate solubilized in detergent solution (1% in water), and \( 10^5 \) DPM of \( ^{14}C\)-\( p\)-HB). A sufficient volume of a 10% detergent stock solution was also added to the reaction medium to achieve a final detergent concentration of 1%. The following detergents were tested: Triton X-100, Chaps, sodium cholate, sodium deoxycholate, lysophosphatidyl choline, and octylglucoside. After incubation for 30 min at 37°C with gentle stirring, the reaction was stopped by chilling samples to 4°C. Prenylated \( ^{14}C\)-\( p\)-HB was separated by organic extraction with hexane and then measured using a liquid scintillation counter. Specific activity was expressed as disintegrations per minute (DPM) min\(^{-1}\)·mg-protein\(^{-1}\).

Biosynthesis of \( ^{14}C\)-\( \text{CoQ}_{10} \) has been quantified in any type of cell culture, such as cancer cells, human skin fibroblasts, and murine embryonic fibroblast and stem cells [10,61]. Previously, cultures were incubated with 4.5 nM \( ^{14}C\)-\( p\)-HB for one to three days, depending on the cell-specific rate of growth. The \( ^{14}C\)-\( p\)-HB was chemically synthesized in our laboratory from \( ^{14}C\)-thyrosine [61]. Labeled-\( \text{CoQ}_{10} \) content is analyzed by lipid extract injection in HPLC and detected by the radio-flow detector LB 509 with a solid cell YG 150 Al-U4D (Berthold Technologies, Bad Wildbad, Germany) in parallel with either electrochemical or UV-vis detectors. Lipid extraction is done as we described above for \( \text{CoQ}_{10} \) determination in cells and tissues, but isocratic HPLC analysis lipid separation is performed with methanol:propanol (65:35) plus 20 mM AcNH\(_4\) pH 4.4 at a constant flow rate of 1 mL/min (Figure 2).
COQ deficiencies are components of the biosynthesis pathway or its regulation. CoQ deficiency is important in tissues and organs. Primary deficiency is caused by defects in proteins encoded by COQ genes, which are components of the biosynthesis pathway or its regulation. CoQ supplement is the current treatment of primary CoQ deficiency, which highly improves symptoms. A rapid and distinct characterization of the deficiency is important, and it is mainly determined in skeletal muscle and/or skin dermal fibroblasts. The main approach is to analyze the total content of CoQ in lipid extracts by HPLC and UV and/or electrochemical detection. Alternatively, a non-radioactive protocol to analyze CoQ biosynthesis was developed using either 2H-mevalonate or 13C-phydroxybenzoate as CoQ precursors as described by Buján et al. (2014) [60]. Human fibroblasts at 60%–70% were incubated with these precursors for 24–72 h at different concentrations. After incubation, cells were trypsinized and washed twice with isotonic sucrose, 2 mmol/L EDTA, 10 mmol/L Tris and 100 UI/mL heparin, pH 7.4, and sonicated twice for 5 s. These homogenates were used to determine CoQ biosynthesis measuring by HPLC-MS/MS, as described in Arias et al. (2012) [62]. Briefly, HPLC separation was as indicated above and extracted peaks were analyzed by MS/MS in a Micromass Quattro micro™ (Waters/Micromass, Manchester, UK). The MS/MS was operated in the electrospray positive ion mode with a cone voltage (CV), and collision energy (CE) of 15 V and 20 eV, respectively. The following multiple-reaction monitoring transitions were selected: m/z 900 > 203 and 897>197 for 13C-CoQ, or 2H-CoQ, respectively, 894 > 197 for the physiological CoQ and 826 > 197 for CoQ (internal standard). The dwell time for each transition was 200 ms and the run-time was 16 min. Nitrogen (at a flow rate of 50 L/h) and argon (adjusted to obtain a vacuum of $3^{-10^{-3}}$ bar) were used as the nebulizing and collision gas, respectively.

6. Concluding Remarks

Coenzyme Q deficiency syndrome includes a group of mitochondrial diseases showing diverse inherited pathological phenotypes. The common aspect of them is the lower content of CoQ in tissues and organs. Primary deficiency is caused by defects in proteins encoded by COQ genes, which are components of the biosynthesis pathway or its regulation. CoQ supplementation is the current treatment of primary CoQ deficiency, which highly improves symptoms. A rapid and distinct characterization of the deficiency is important, and it is mainly determined in skeletal muscle and/or skin dermal fibroblasts. The main approach is to analyze the total content of CoQ in lipid extracts by
HPLC and UV and/or electrochemical detection. Alternatively, the CoQ10 biosynthesis rate in cultured cells can be determined by incubation with radiolabeled precursors.

**Acknowledgments:** This work has been funded by the Instituto de Salud Carlos III FIS PI14-01962 grant. Authors were also funded by the Junta de Andalucía BIO177 research group.

**Author Contributions:** J.C.R.-A., A.B.C., D.J.M.F.-A., and P.N. have contributed to writing and editing the review and figures.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Gorman, G.S.; Chinnery, P.F.; DiMauro, S.; Hirano, M.; Koga, Y.; McFarland, R.; Suomalainen, A.; Thorburn, D.R.; Zeviani, M.; Turnbull, D.M. Mitochondrial diseases, Nature reviews. *Dis. Prim.* 2016, 2, 16080. [CrossRef] [PubMed]
2. Lapuente-Brun, E.; Moreno-Loshuertos, R.; Acin-Perez, R.; Latorre-Pellicer, A.; Colas, C.; Balsa, E.; Perales-Clemente, E.; Quiros, P.M.; Calvo, E.; Rodriguez-Hernandez, M.A.; et al. Supercomplex assembly determines electron flux in the mitochondrial electron transport chain. *Science* 2013, 340, 1567–1570. [CrossRef] [PubMed]
3. Alcazar-Fabra, M.; Navas, P.; Brea-Calvo, G. Coenzyme Q biosynthesis and its role in the respiratory chain structure. *Biochim. Biophys. Acta* 2016, 1857, 1073–1078. [CrossRef] [PubMed]
4. Lopez-Lluch, G.; Rodriguez-Agullera, J.C.; Santos-Ocana, C.; Navas, P. Is coenzyme Q a key factor in aging? *Mech. Ageing Dev.* 2010, 131, 225–235. [CrossRef] [PubMed]
5. Bentinger, M.; Tekle, M.; Dallner, G. Coenzyme Q—Biosynthesis and functions. *Biochem. Biophys. Res. Commun.* 2010, 396, 74–79. [CrossRef] [PubMed]
6. Gonzalez-Mariscal, I.; Garcia-Teston, E.; Padilla, S.; Martin-Montalvo, A.; Vici, T.P.; Vazquez-Fonseca, L.; Dominguez, P.G.; Santos-Ocana, C. The regulation of coenzyme q biosynthesis in eukaryotic cells: All that yeast can tell us. *Mol. Syndromol.* 2014, 5, 107–118. [CrossRef] [PubMed]
7. Turunen, M.; Peters, J.M.; Gonzalez, F.J.; Schedin, S.; Dallner, G. Influence of peroxisome proliferator-activated receptor alpha on ubiquinone biosynthesis. *J. Mol. Biol.* 2000, 297, 607–614. [CrossRef] [PubMed]
8. Bentinger, M.; Tekle, M.; Dallner, G. Stimulation of coenzyme Q synthesis. *Biofactors* 2008, 32, 99–111. [CrossRef] [PubMed]
9. Brea-Calvo, G.; Siendones, E.; Sanchez-Alcazar, J.A.; de Cabo, R.; Navas, P. Cell survival from chemotherapy depends on NF-kappaB transcriptional up-regulation of coenzyme Q biosynthesis. *PLoS ONE* 2009, 4, e5301. [CrossRef] [PubMed]
10. Cascajo, M.V.; Abdelmohsen, K.; Noh, J.H.; Fernandez-Ayala, D.J.; Willers, I.M.; Brea, G.; Lopez-Lluch, G.; Valenzuela-Villatoro, M.; Cuezva, J.M.; Gorospe, M.; et al. RNA-binding proteins regulate cell respiration and coenzyme Q biosynthesis by post-transcriptional regulation of COQ7. *RNA Biol.* 2016, 13, 622–634. [CrossRef] [PubMed]
11. Martin-Montalvo, A.; Gonzalez-Mariscal, I.; Padilla, S.; Ballesteros, M.; Brautigan, D.L.; Navas, P.; Santos-Ocana, C. Respiratory-induced coenzyme Q biosynthesis is regulated by a phosphorylation cycle of Cat5p/Coq7p. *Biochem. J.* 2011, 440, 107–114. [CrossRef] [PubMed]
12. Martin-Montalvo, A.; Gonzalez-Mariscal, I.; Pomares-Viciana, T.; Padilla-Lopez, S.; Ballesteros, M.; Vazquez-Fonseca, L.; Gandolfi, P.; Brautigan, D.L.; Navas, P.; Santos-Ocana, C. The phosphatase Ptc7 induces coenzyme Q biosynthesis by activating the hydrolase Coq7 in yeast. *J. Biol. Chem.* 2013, 288, 28126–28137. [CrossRef] [PubMed]
13. Lanning, N.J.; Looyenga, B.D.; Kaufman, A.L.; Niemi, N.M.; Sudderth, J.; DeBerardinis, R.J.; MacKeigan, J.P. A mitochondrial RNAi screen defines cellular bioenergetic determinants and identifies an adenylate kinase as a key regulator of ATP levels. *Cell Rep.* 2014, 7, 907–917. [CrossRef] [PubMed]
14. Gonzalez-Mariscal, I.; Garcia-Teston, E.; Padilla, S.; Martin-Montalvo, A.; Pomares-Viciana, T.; Vazquez-Fonseca, L.; Gandolfi-Dominguez, P.; Santos-Ocana, C. Regulation of coenzyme Q biosynthesis in yeast: A new complex in the block. *JUBMB Life* 2014, 66, 63–70. [CrossRef] [PubMed]
15. Padilla, S.; Tran, U.C.; Jimenez-Hidalgo, M.; Lopez-Martin, J.M.; Martin-Montalvo, A.; Clarke, C.F.; Navas, P.; Santos-Ocana, C. Hydroxylation of demethoxy-Q6 constitutes a control point in yeast coenzyme Q6 biosynthesis. *Cell. Mol. Life Sci.* 2009, **66**, 173–186. [CrossRef] [PubMed]

16. Lohman, D.C.; Forouhar, F.; Beebe, E.T.; Stefely, J.A.; Minogue, C.E.; Ulbrich, A.; Stefely, J.A.; Sukumar, S.; Luna-Sanchez, M.; Jochem, A.; et al. Mitochondrial COQ9 is a lipid-binding protein that associates with COQ7 to enable coenzyme Q biosynthesis. *Proc. Natl. Acad. Sci. USA* 2014, **111**, E4697–E4705. [CrossRef] [PubMed]

17. Stefely, J.A.; Licitra, F.; Laredj, L.; Reidenbach, A.G.; Kemmerer, Z.A.; Grangeray, A.; Jaeg-Ehret, T.; Minogue, C.E.; Ulbrich, A.; Hutchins, P.D.; et al. Cerebellar Ataxia and Coenzyme Q Deficiency through Loss of Unorthodox Kinase Activity. *Mol. Cell* 2016, **63**, 608–620. [CrossRef] [PubMed]

18. Stefely, J.A.; Reidenbach, A.G.; Ulbrich, A.; Oruganty, K.; Floyd, B.J.; Jochem, A.; Saunders, J.M.; Johnson, I.E.; Minogue, C.E.; Wrobel, R.L.; et al. Mitochondrial ADCK3 Employs an Atypical Protein Kinase-like Fold to Enable Coenzyme Q Biosynthesis. *Mol. Cell* 2015, **57**, 83–94. [CrossRef] [PubMed]

19. Parrado-Fernandez, C.; Lopez-Lluch, G.; Rodriguez-Bies, E.; Santa-Cruz, S.; Navas, P.; Ramsey, J.J.; Villalba, J.M. Calorie restriction modifies ubiquinone and COQ transcript levels in mouse tissues. *Free Radic. Biol. Med.* 2011, **50**, 1728–1736. [CrossRef] [PubMed]

20. Lopez-Lluch, G.; Santos-Ocana, C.; Sanchez-Alcazar, J.A.; Fernandez-Ayalas, D.J.; Asencio-Salcedo, C.; Rodriguez-Aguilera, J.C.; Navas, P. Mitochondrial responsibility in ageing process: Innocent, suspect or guilty. *Biogerontology* 2015, **16**, 599–620. [CrossRef] [PubMed]

21. Schon, E.A.; DiMauro, S.; Hirano, M.; Gilkerson, R.W. Therapeutic prospects for mitochondrial disease. *Trends Mol. Med.* 2010, **16**, 268–276. [CrossRef] [PubMed]

22. Quinzii, C.M.; Emmanuele, V.; Hirano, M. Clinical presentations of coenzyme q10 deficiency syndrome. *Mol. Syndromol.* 2014, **5**, 141–146. [CrossRef] [PubMed]

23. Doimo, M.; Desbats, M.A.; Cerqua, C.; Cassina, M.; Trevisson, E.; Salvati, L. Genetics of coenzyme Q10 deficiency. *Mol. Syndromol.* 2014, **5**, 156–162. [CrossRef] [PubMed]

24. Desbats, M.A.; Lunardi, G.; Doimo, M.; Trevisson, E.; Salvati, L. Genetic bases and clinical manifestations of coenzyme Q10 (CoQ 10) deficiency. *J. Inherit. Metab. Dis.* 2015, **38**, 145–156. [CrossRef] [PubMed]

25. Yubero, D.; Montero, R.; Martin, M.A.; Montoya, J.; Ribes, A.; Grazina, M.; Trevisson, E.; Rodriguez-Aguilera, J.C.; Hargreaves, I.P.; Salvati, L.; et al. Secondary coenzyme Q10 deficiencies in oxidative phosphorylation (OXPHOS) and non-OXPHOS disorders. *Mitochondrion* 2016, **30**, 51–58. [CrossRef] [PubMed]

26. Montero, R.; Sanchez-Alcazar, J.A.; Briones, P.; Navarro-Sastre, A.; Gallardo, E.; Bornstein, B.; Herrero-Martin, D.; Rivera, H.; Martin, M.A.; Marti, R.; et al. Coenzyme Q10 deficiency associated with a mitochondrial DNA depletion syndrome: A case report. *Clin. Biochem.* 2009, **42**, 742–745. [CrossRef] [PubMed]

27. Quinzii, C.M.; Kattah, A.G.; Naini, A.; Akman, H.O.; Mootha, V.K.; DiMauro, S.; Hirano, M. Coenzyme Q deficiency and cerebellar ataxia associated with an aprataxin mutation. *Neurology* 2005, **64**, 539–541. [CrossRef] [PubMed]

28. Gempel, K.; Topaloglu, H.; Talim, B.; Schneiderat, P.; Schoser, B.G.; Hans, V.H.; Palmafgy, B.; Kale, G.; Tokatli, A.; Quinzii, C.; et al. The myopathic form of coenzyme Q10 deficiency is caused by mutations in the electron-transferring-flavoprotein dehydrogenase (ETFDH) gene. *Brain* 2007, **130**, 2037–2044. [CrossRef] [PubMed]

29. Miles, M.V.; Putnam, P.E.; Miles, L.; Tang, P.H.; DeGrauw, A.J.; Wong, B.L.; Horn, P.S.; Foote, H.L.; Rothenberg, M.E. Acquired coenzyme Q10 deficiency in children with recurrent food intolerance and allergies. *Mitochondrion* 2011, **11**, 127–135. [CrossRef] [PubMed]

30. Haas, D.; Niklowitz, P.; Horster, F.; Baumgartner, E.R.; Prasad, C.; Rodenburg, R.J.; Hoffmann, G.F.; Menke, T.; Okun, J.G. Coenzyme Q(10) is decreased in fibroblasts of patients with methylmalonic aciduria but not in mevalonic aciduria. *J. Inherit. Metab. Dis.* 2009, **32**, 570–575. [CrossRef] [PubMed]

31. Maes, M.; Mihaylova, I.; Kubera, M.; Uytterhoeven, M.; Vrydags, N.; Bosmans, E. Coenzyme Q10 deficiency in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is related to fatigue, autonomic and neurocognitive symptoms and is another risk factor explaining the early mortality in ME/CFS due to cardiovascular disorder. *Neuro Endocrinol. Lett.* 2009, **30**, 470–476. [PubMed]
32. Fragaki, K.; Cano, A.; Benoist, J.F.; Rigal, O.; Chaussenot, A.; Rouzier, C.; Banwarth, S.; Caruba, C.; Chabrol, B.; Paquis-Flucklinger, V. Fatal heart failure associated with CoQ10 and multiple OXPHOS deficiency in a child with propionic academia. *Mitochondrion* 2011, 11, 533–536. [CrossRef] [PubMed]

33. Ogasahara, S.; Engel, A.G.; Frens, D.; Mack, D. Muscle coenzyme Q deficiency in familial mitochondrial encephalomyopathy. *Proc. Natl. Acad. Sci. USA* 1989, 86, 2379–2382. [CrossRef]

34. Lopez, L.C.; Schuelke, M.; Quinzii, C.M.; Kanki, T.; Rodenburg, R.J.; Naini, A.; Dimauro, S.; Hirano, M. Leigh syndrome with nephropathy and CoQ10 deficiency due to decaprenyl diphosphate synthase subunit 2 (PDSS2) mutations. *Am. J. Hum. Genet.* 2006, 79, 1125–1129. [CrossRef]

35. Quinzii, C.; Naini, A.; Salvati, L.; Trevisson, E.; Navas, P.; Dimauro, S.; Hirano, M. A mutation in para-hydroxybenzoate-polyprenyl transferase (COQ2) causes primary coenzyme Q10 deficiency. *Am. J. Hum. Genet.* 2006, 78, 345–349. [CrossRef] [PubMed]

36. Heeringa, S.F.; Chernin, G.; Chaki, M.; Zhou, W.; Sloan, A.J.; Ji, Z.; Xie, L.X.; Salvati, L.; Hurd, T.W.; Vega-Warner, V.; et al. COQ6 mutations in human patients produce nephrotic syndrome with sensorineural deafness. *J. Clin. Invest.* 2011, 121, 2013–2024. [CrossRef] [PubMed]

37. Ashraf, S.; Gee, H.Y.; Woerner, S.; Xie, L.X.; Vega-Warner, V.; Lovric, S.; Fang, H.; Song, X.; Cattran, D.C.; Avila-Casado, C.; et al. ADCK4 mutations promote steroid-resistant nephrotic syndrome through CoQ10 biosynthesis disruption. *J. Clin. Invest.* 2013, 123, 5179–5189. [CrossRef] [PubMed]

38. Mollet, J.; Giurgea, I.; Schlemmer, D.; Dallner, G.; Chretien, D.; Delahodde, A.; Bacq, D.; de Lonlay, P.; Munnich, A.; Rotig, A. Prenyldiphosphate synthase, subunit 1 (PDSS1) and OH-benzoate polyprenyltransferase (COQ2) mutations in ubiquinone deficiency and oxidative phosphorylation disorders. *J. Clin. Invest.* 2007, 117, 765–772. [CrossRef] [PubMed]

39. Duncan, A.J.; Bitner-Glindzicz, M.; Meunier, B.; Costello, H.; Hargreaves, I.P.; Lopez, L.C.; Hirano, M.; Quinzii, C.M.; Sadowski, M.I.; Hardy, J.; et al. A nonsense mutation in COQ9 causes autosomal-recessive neonatal-onset primary coenzyme Q10 deficiency: A potentially treatable form of mitochondrial disease. *Am. J. Hum. Genet.* 2009, 84, 558–566. [CrossRef] [PubMed]

40. Freyer, C.; Stranneheim, H.; Naess, K.; Mourier, A.; Felser, A.; Maffezzini, C.; Lesko, N.; Bruhn, H.; Engvall, M.; Wibom, R.; et al. Rescue of primary ubiquinone deficiency due to a novel COQ7 defect using 2,4-dihydroxybensoic acid. *J. Med. Genet.* 2015, 52, 779–783. [CrossRef]

41. Mollet, J.; Delahodde, A.; Serre, S.; Chretien, D.; Schlemmer, D.; Lombes, A.; Boddaert, N.; Desguerre, I.; de Lonlay, P.; de Bauny, H.O.; et al. CABC1 gene mutations cause cerebellar ataxia and seizures. *Am. J. Hum. Genet.* 2008, 82, 623–630. [CrossRef] [PubMed]

42. Lagier-Tourenne, C.; Tazir, M.; Lopez, L.C.; Quinzii, C.M.; Assoum, M.; Drouot, N.; Busso, C.; Makri, S.; Ali-Pacha, L.; Benhassine, T.; et al. ADCK3, an ancestral kinase, is mutated in a form of recessive cerebellar ataxia associated with coenzyme Q10 deficiency. *Am. J. Hum. Genet.* 2008, 82, 661–672. [CrossRef] [PubMed]

43. Horvath, R.; Czermin, B.; Gulati, S.; Demuth, S.; Hargraves, I.P.; Lopez, L.C.; Hirano, M.; Quinzii, C.M.; Sadowski, M.I.; Hardy, J.; et al. A nonsense mutation in COQ9 causes autosomal-recessive neonatal-onset primary coenzyme Q10 deficiency: A potentially treatable form of mitochondrial disease. *Am. J. Hum. Genet.* 2008, 82, 661–672. [CrossRef] [PubMed]

44. Mignot, C.; Apartis, E.; Durr, A.; Lourenco, C.M.; Charles, P.; Devos, D.; Moreau, C.; de Lonlay, P.; Drouot, N.; Burglen, L.; et al. Phenotypic variability in ARCA2 and identification of a core ataxic phenotype with slow progression. *Orphanet J. Rare Dis.* 2013, 8, 173. [CrossRef]

45. Liu, Y.T.; Hersheson, J.; Plagnol, V.; Fawcett, K.; Duberly, K.E.; Preza, E.; Hargraves, I.P.; Chalasani, A.; Laura, M.; Wood, N.W.; et al. Autosomal-recessive cerebellar ataxia caused by a novel ADCK3 mutation that elongates the protein: Clinical, genetic and biochemical characterisation. *J. Neurol. Neurosurg. Psychiatry* 2012, 83, 174–178. [CrossRef] [PubMed]

46. Blumkin, L.; Leshinsky-Silver, E.; Zerem, A.; Yosovich, K.; Lerman-Sagie, T.; Lev, D. Heterozygous Mutations in the ADCK3 Gene in Siblings with Cerebellar Atrophy and Extreme Phenotypic Variability. *JIMD Rep.* 2014, 12, 103–107. [PubMed]

47. Hikmat, O.; Tzoulis, C.; Knappskog, P.M.; Johansson, S.; Boman, H.; Sztromwasser, P.; Lien, E.; Brodtkorb, E.; Ghezzi, D.; Bindoff, L.; Leshinsky-Silver, E.; Zerem, A.; Yosovich, K.; Lerman-Sagie, T.; Lev, D. ADCK3 mutations with epilepsy, stroke-like episodes and ataxia. *A POLG mimic? Eur. J. Neurol.* 2016, 23, 1188–1194. [CrossRef] [PubMed]

48. Salvati, L.; Trevisson, E.; Hernandez, M.A.R.; Casarin, A.; Pertegato, V.; Doimo, M.; Cassina, M.; Agosto, C.; Desbats, M.A.; Sartori, G.; et al. Haploinsufficiency of COQ4 causes coenzyme Q10 deficiency. *J. Med. Genet.* 2012, 49, 187–191. [CrossRef] [PubMed]
49. Brea-Calvo, G.; Haack, T.B.; Karall, D.; Ohtake, A.; Invernizzi, F.; Carrozzo, R.; Kremer, L.; Dusi, S.; Fauth, C.; Scholl-Burgi, S.; et al. COQ4 Mutations Cause a Broad Spectrum of Mitochondrial Disorders Associated with CoQ10 Deficiency. *Am. J. Hum. Genet.* 2015, 96, 309–317. [CrossRef] [PubMed]

50. Chung, W.K.; Martin, K.; Jalas, C.; Braddock, S.R.; Juusola, J.; Monaghan, K.G.; Warner, B.; Franks, S.; Yudkoff, M.; Lulis, L.; et al. Mutations in COQ4, an essential component of coenzyme Q biosynthesis, cause lethal neonatal mitochondrial encephalomyopathy. *J. Med. Genet.* 2015, 52, 627–635. [CrossRef] [PubMed]

51. Public Health. Community Register of Orphan Medicinal Products. Available online: http://ec.europa.eu/health/documents/community-register/html/o1765.htm (accessed on 4 March 2017).

52. Montini, G.; Malaventura, C.; Salvati, L. Early coenzyme Q10 supplementation in primary coenzyme Q10 deficiency. *N. Engl. J. Med.* 2008, 358, 2849–2850. [CrossRef] [PubMed]

53. Pineda, M.; Montero, R.; Aracil, A.; O’Callaghan, M.M.; Mas, A.; Espinos, C.; Martinez-Rubio, D.; Palau, F.; Navas, P.; Briones, P.; et al. Coenzyme Q(10)-responsive ataxia: 2-year-treatment follow-up. *Mov. Disord.* 2010, 25, 1262–1268. [CrossRef] [PubMed]

54. Saiki, R.; Lunceford, A.L.; Shi, Y.; Marbois, B.; King, R.; Pachuski, J.; Kawamukai, M.; Gasser, D.L.; Clarke, C.F. Coenzyme Q10 supplementation rescues renal disease in Pdss2kd/ki mice with mutations in prenyl diphosphate synthase subunit 2, American journal of physiology. *Ren. Physiol.* 2008, 295, F1535–F1544. [CrossRef] [PubMed]

55. Lopez, L.C.; Quinzii, C.M.; Area, E.; Naini, A.; Rahman, S.; Schuelke, M.; Salvati, L.; Dimauro, S.; Hirano, M. Treatment of CoQ(10) deficient fibroblasts with ubiquinone, CoQ analogs, and vitamin C: Time- and compound-dependent effects. *PLoS ONE* 2010, 5, e11897. [CrossRef] [PubMed]

56. Yubero, D.; Montero, R.; Armstron, J.; Espinos, C.; Palau, F.; Santos-Osca, C.; Salvati, L.; Navas, P.; Artuch, R. Molecular diagnosis of coenzyme Q10 deficiency. *Expert Rev. Mol. Diagn.* 2015, 15, 1049–1059. [CrossRef] [PubMed]

57. Yubero, D.; Montero, R.; Artuch, R.; Land, J.M.; Heales, S.J.; Hargreaves, I.P. Biochemical diagnosis of coenzyme q10 deficiency. *Mol. Syndromol.* 2014, 5, 147–155.

58. Trevisson, E.; DiMauro, S.; Navas, P.; Salvati, L. Coenzyme Q deficiency in muscle. *Curr. Opin. Neurol.* 2011, 24, 449–456. [CrossRef] [PubMed]

59. Yubero, D.; Montero, R.; Artuch, R.; Land, J.M.; Heales, S.J.; Hargreaves, I.P. Biochemical diagnosis of coenzyme q10 deficiency. *Mol. Syndromol.* 2014, 5, 147–155.

60. Trevisson, E.; DiMauro, S.; Navas, P.; Salvati, L. Coenzyme Q deficiency in muscle. *Curr. Opin. Neurol.* 2011, 24, 449–456. [CrossRef] [PubMed]

61. Fernandez-Ayala, D.J.; Lopez-Lluch, G.; Garcia-Valdes, M.; Arroyo, A.; Navas, P. Specificity of coenzyme Q10 for a balanced function of respiratory chain and endogenous ubiquinone biosynthesis in human cells. *Biochim. Biophys. Acta* 2005, 1706, 174–183. [CrossRef] [PubMed]

62. Arias, A.; Garcia-Villoria, J.; Rojo, A.; Bujan, N.; Briones, P.; Ribes, A. Analysis of coenzyme Q(10) in lymphocytes by HPLC-MS/MS. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 2012, 908, 23–26. [CrossRef] [PubMed]