Coenzyme Q$_{10}$ defects may be associated with a deficiency of Q$_{10}$-independent mitochondrial respiratory chain complexes

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Abstract

Background: Coenzyme Q$_{10}$ (CoQ$_{10}$ or ubiquinone) deficiency can be due either to mutations in genes involved in CoQ$_{10}$ biosynthesis pathway, or to mutations in genes unrelated to CoQ$_{10}$ biosynthesis. CoQ$_{10}$ defect is the only oxidative phosphorylation disorder that can be clinically improved after oral CoQ$_{10}$ supplementation. Thus, early diagnosis, first evoked by mitochondrial respiratory chain (MRC) spectrophotometric analysis, then confirmed by direct measurement of CoQ$_{10}$ levels, is of critical importance to prevent irreversible damage in organs such as the kidney and the central nervous system. It is widely reported that CoQ$_{10}$ deficient patients present decreased quinone-dependent activities (segments I + III or G3P + III and II + III) while MRC activities of complexes I, II, III, IV and V are normal. We previously suggested that CoQ$_{10}$ defect may be associated with a deficiency of CoQ$_{10}$-independent MRC complexes. The aim of this study was to verify this hypothesis in order to improve the diagnosis of this disease.

Results: To determine whether CoQ$_{10}$ defect could be associated with MRC deficiency, we quantified CoQ$_{10}$ by LC-MSMS in a cohort of 18 patients presenting CoQ$_{10}$-dependent deficiency associated with MRC defect. We found decreased levels of CoQ$_{10}$ in eight patients out of 18 (45%), thus confirming CoQ$_{10}$ disease.

Conclusions: Our study shows that CoQ$_{10}$ defect can be associated with MRC deficiency. This could be of major importance in clinical practice for the diagnosis of a disease that can be improved by CoQ$_{10}$ supplementation.

Keywords: Mitochondrial disease, CoQ$_{10}$ deficiency, Respiratory chain, Spectrophotometry, LC-MSMS

Background

Coenzyme Q$_{10}$ (CoQ$_{10}$ or ubiquinone) is a lipid-soluble component of the mitochondrial inner membrane that plays a central role in mitochondrial respiratory chain (MRC) function, as electrons carrier from complexes I and II to complex III, thus participating in ATP production [1].

CoQ$_{10}$ deficiency encompasses several clinical phenotypes such as encephalomyopathy, severe infantile multisystemic disease, cerebellar ataxia, isolated myopathy or nephrotic syndrome [2]. CoQ$_{10}$ deficiency can be primary, due to mutations in genes involved in CoQ$_{10}$ biosynthesis or secondary, due to mutations in genes unrelated to CoQ$_{10}$ biosynthesis [3]. Secondary CoQ$_{10}$ deficiency has been described in patients with mitochondrial DNA (mtDNA) mutations or deletions, with mtDNA depletion syndrome (MDS) [4–6] and in patients with mutations in APTX [7], ETFDH [8, 9], BRAF [10], ACADVL or NPC genes [11]. CoQ$_{10}$ defect is the only oxidative phosphorylation (OXPHOS) disorder that can be clinically improved after oral CoQ$_{10}$ supplementation with limitation of neurological and renal manifestations, amelioration of muscular symptoms and attenuation of histological alterations. Early treatment is crucial to prevent irreversible damage in organs such as the kidney and the central nervous system [12–14]. Reduced activities of CoQ$_{10}$-dependent enzymes by spectrophotometric...
with multiple MRC enzymatic deficiency, we measured CoQ10 levels by liquid chromatography coupled with tandem mass spectrometry detection (LC-MSMS) in eight patients out of 10 in the first group and in three patients in the second group. CoQ10-deficient enzymatic deficiency was associated with MRC enzymatic defect. Secondary CoQ10 deficiency was likely involved in the development of heart complications in this child and we hypothesized that a CoQ10 deficiency may be associated with MRC deficiency [17]. The aim of this study was to verify this hypothesis in order to improve the diagnosis of this disease.

Over a 6-year period, we analyzed by spectrophotometry 700 tissue samples from 495 patients in whom a mitochondrial disease was suspected. Isolated CoQ10-dependent activity deficiency led to identification of CoQ10 disease in eight cases. Eighteen patients presented CoQ10-dependent enzymatic deficiency associated with MRC defect by spectrophotometry in muscle or in fibroblasts. In order to validate our original observation and to establish if CoQ10 quantitative defect may be associated with multiple MRC enzymatic deficiency, we measured CoQ10 in this group of 18 patients. We found decreased CoQ10 levels by liquid chromatography coupled with tandem mass spectrometry detection (LC-MSMS) in eight patients out of 18 (45%), thus confirming CoQ10 disease and its association with MRC enzymatic deficiency. Furthermore, CoQ10 disease cannot be ruled out in all other patients insofar as the quantitative assay could not always be performed in the affected tissue.

Results
Description of patients involved in the study
We studied 18 patients, including 10 males and eight females, ranging in age from day 1 to 76 years. Clinical presentations were very heterogeneous (Table 1). The age at onset of the disease was highly variable, ranging from (i) neonatal forms (seven cases with severe phenotypes), (ii) onset before 1 year of age (four cases with either Leigh syndrome or epileptic encephalopathy), (iii) childhood-onset (four cases including two myopathic forms and two complex phenotypes) to (iv) adult-onset (three cases with two myopathic presentations and one cerebellar ataxia). The 18 patients were divided into two different groups according to molecular results.

The first group included 10 patients with identified mutations in responsible genes (Table 1). Patient P01 presented a severe neonatal multisystemic disease secondary to a homozygous missense mutation in the CoQ2 gene [18]. Spectrophotometric analysis in fibroblasts revealed a CoQ10-dependent activities defect (segments II + III and G3P + III reduction) associated with a complex IV deficiency (Table 2). Six patients (P02–P07) presented a mitochondrial disease or dysfunction secondary either to mtDNA abnormalities (P02 and P03) or to mutations in nuclear genes (P04–P07). Patient P02 had a large heteroplasmic mtDNA deletion responsible for Kearns–Sayre syndrome and patient P03 presented with a severe neonatal polyvisceral failure secondary to a heteroplasmic mtDNA mutation in the MT-CYB gene. Patients P04 and P05 presented with sensory ataxia neuropathy dysarthria and ophthalmoplegia (SANDO) phenotype associated with recessive mutations in POLG. Patient P06 had a neonatal encephalopathy with lactic acidosis and mild methylmalonic aciduria linked to mutations in the SLC25A1 gene. P07 had a diagnosis of multiple acyl-CoA dehydrogenation deficiency (MADD) with ETFDH mutation. The last three patients in the first group presented malignant migrating partial seizures with mutations in TBC1D24 (P08), CDG syndrome type Iq with SRDS5A3-CDG mutations (P09) and 1p36 deletion syndrome (P10). Patients P02–P10 had a CoQ10-dependent activities deficiency (segments I + III or G3P + III and II + III reduction) associated with a multiple MRC defect in muscle or in fibroblasts (Table 2).

The second group included eight patients suspected of CoQ10 deficiency with an absence of molecular diagnosis. Except for individual P11, who developed cerebellar ataxia during adulthood, all patients had an early-onset disease ranging from neonatal period to childhood. They presented severe neurological symptoms including two Leigh syndromes (P12 and P16) and one child had an unexplained severe respiratory failure at birth (P17). In the second group, all patients presented a CoQ10-dependent enzymatic deficiency associated with MRC defect in muscle or in fibroblasts (Table 2).

Confirmation of CoQ10 disease in eight patients by CoQ10 quantification
Quantitative analysis of CoQ10 in muscle or fibroblasts showed that eight patients presented CoQ10 content below normal values (Table 2). CoQ10 defect was found in five patients out of 10 in the first group and in three patients out of eight in the second group. CoQ10-deficient individuals were six males and two females, ranging in age from day 1 to 76 years. The age of onset was highly variable, ranging from neonatal forms to diseases appearing after 25 years of age, although six patients had childhood onset. One patient (P01) presented a polyvisceral failure at birth and all the others had neurological symptoms either isolated or combined with muscular
### Table 1: Clinical phenotypes of patients presenting CoQ10-dependent enzymatic deficiency associated with MRC defect

| Patient | Tissue   | Sex | Age at biopsy | Age of onset | Heredity | Familial history | Neurological symptoms | Muscular symptoms | Other symptoms | Muscle histology | Enzymology | Diagnosis or molecular analyses |
|---------|----------|-----|---------------|--------------|----------|------------------|-----------------------|-------------------|----------------|----------------|------------|----------------------------------|
| P01<sup>a</sup> | Fibroblasts | M | D1 | Neonatal | Recessive | Affected brother | Neonatal polyvis­ceral failure | Not done | Cx IV deficiency; segments I + III and G3P + III reduction | COQ2: homozygous mutation (c.437G > A; p.Ser146Asn) |
| P02    | Muscle   | M | 54y | 25y | Sporadic | No | Brain MRI: mild atrophy and lacunar strokes | CPEO | T2DM, hepatic steatosis, dyslipidemia | RRF (5–10%) and Cox-fibers | Cxes I, II, IV and V deficiency; segments I + III and II + III reduction | Large-scale deletion of mtDNA |
| P03<sup>a</sup> | Fibroblasts | M | D1 | Neonatal | de novo | No | Hypotonia, epilepsy and diffuse brain lesions | Neonatal polyvisceral failure: respiratory distress, hepatic failure, hypertrophic CMP, lactic acidosis ++ | Not done | Cxes II and III deficiency; segments II + III and G3P + III reduction | MT‑CYB: heteroplasmic mtDNA mutation (m.15635T > C; p.Ser297Pro) |
| P04<sup>a</sup> | Fibroblasts | M | 15y | 11y | Recessive | No | Ataxic sensory axonal neuropathy | CPEO | RRF and Cox-fibers (40%) | Cxes I, II, III and IV deficiency; segments II + III and G3P + III reduction | Sando with multiple mtDNA deletions and compound heterozygous mutations in POLG: (c.911T > G, p.Leu304Arg) |
| P05    | Muscle   | F | 54y | 45y | Recessive | No | Ataxic sensory axonal neuropathy | CPEO | Lipid accumulation, RRF and Cox-fibers (20%) | Cxes II, III, IV and V deficiency; segments I + III and II + III reduction | Sando with multiple mtDNA deletions and compound heterozygous mutations in POLG: (c.752C > T, c.2452G > A; p.Thr251Ile/p.Gly848Ser) |
| P06<sup>a</sup> | Fibroblasts | M | D1 | Neonatal | Recessive | No | Encephalopathy and hypotonia | Severe lactic acidosis, methylmalonic aciduria | Not done | Cxes II, III and IV deficiency; segments II + III and G3P + III reduction | SUCLG1: compound heterozygous mutations (c.97 + 3G > C, c.2509C > G; p.Pro170Arg/p.Glu847Val) |
| P07    | Muscle   | F | 18y | 4y | Recessive | Blindness in paternal family | Bilateral ptosis, proximal myopathy, dysphonia, dysphagia, exercise intolerance | Retinitis pigmentosa, cyclic vomiting, hyper‑CKemia | Lipid accumulation | Cxes I and III deficiency; segments I + III and II + III reduction | MADD with mutations in ETFDH |
| Patient | Tissue | Sex | Age at biopsy | Age of onset | Heredity | Familial history | Neurological symptoms | Muscular symptoms | Other symptoms | Muscle histology | Enzymology | Diagnosis or molecular analyses |
|---------|--------|-----|---------------|-------------|----------|-----------------|---------------------|------------------|---------------|----------------|------------|--------------------------------|
| P08     | Muscle | F   | 4 m           | 4 m         | Recessive | Affected sister  | Encephalopathy with refractory migrating partial seizures | Lipid accumulation |             |               |             | Malignant migrating partial seizures with compound heterozygous mutations in TBC1D24: (c.1596C > A/c.662C > T, p.Gly156X/p.Phe229Ser) |
| P09     | Fibroblasts | M | D1 | Neonatal | Recessive | No | Hypotonia | Hypertrrophic CMP, dysmorphic, hepatic cytolysis, hypospadias | Glycogenic accumulation | Cxes II, III, IV and V deficiency; segments II + III and G3P + III reduction |              | CDG syndrome type Iq: homoyzygous mutation in SRD5A3: (c.620T > G, p.Met207Arg) |
| P10     | Fibroblasts | M | 3 m | Neonatal | de novo | No | Hypotonia, epilepsy, dysphagia | Glycogenic accumulation | Cxes III and IV deficiency; segments II + III and G3P + III reduction |              | 1p36 deletion syndrome |
|         |        |     |               |             |          |                 |                     |                  | Patients with no molecular diagnosis |
| P11     | Muscle | M | 76y | Adult | ? | No | Cerebellar ataxia | 2 RRF and Cox-fibers (20-30 %) | Cx IV deficiency; segments I + III and II + III reduction | Multiple mtDNA deletions |
| P12     | Fibroblasts | F | 7 m | 6 m | ? | No | Leigh syndrome | Not done | Cx II deficiency; segments I + III and G3P + III reduction | mtDNA depletion, absence of mtDNA and POLG, SUCLA2, TK2 mutation |
| P13     | Muscle | F | 41y | Childhood | Recessive | Consanguinity | Spastic tetraparesis, chorea, mental retardation | Myopathy | Glaucoma, cataract, lactic acidosis | RRF ++ | Absence of mtDNA and POLG, OMA1, OPA3 mutation |
| P14     | Muscle | F | 33y | Childhood | Recessive | No | Epilepsy, spastic diplegia, dystonia, dyskinesia, tremor | 1 Cox-fiber | Cxes III and V deficiency; segments I + III and II + III reduction | Absence of mtDNA and POLG, TTC19, DYT5 mutation |
| P15     | Muscle | F | 28y | Childhood | Recessive | Affected siblings | Encephalopathy, mental retardation | Normal | Cxes II, III and V deficiency; segments I + III and II + III reduction | Absence of mtDNA mutation |
Table 1 continued

| Patient | Tissue   | Sex | Age at biopsy | Age of onset | Heredity | Familial history | Neurological symptoms | Muscular symptoms | Other symptoms | Muscle histology | Enzymology | Diagnosis or molecular analyses |
|---------|----------|-----|---------------|--------------|----------|------------------|-----------------------|-------------------|---------------|-----------------|------------|----------------------------------|
| P16     | Fibroblasts | M   | 9y            | Infancy      | ?        | No               | Psychomotor retardation, behavior disorders, dystonia, dyspraxia, and basal ganglia involvement at brain MRI (Leigh) | Normal           | Absence of mtDNA mutation |
| P17     | Fibroblasts | F   | D3            | D2           | ?        | No               | Unexplained severe respiratory failure | Normal           | Cx II and III deficiency, segments II + III and G3P + III reduction | Absence of mtDNA mutation |
| P18     | Fibroblasts | M   | 2y            | D18          | ?        | No               | Encephalopathy with refractory epilepsy | Microcephaly      | Cx III and IV deficiency, segments II + III and G3P + III reduction | Absence of mtDNA mutation |

M male, F female, D day, m month, y year, CPK Creatine Phosphokinase, CPEO Chronic Progressive External Ophthalmoplegia, T2DM Type 2 Diabetes Mellitus, CMP Cardiomyopathy, RRF Ragged Red Fibers, Cox cytochrome c oxidase, cx complex, mtDNA mitochondrial DNA, SANDO Sensory Ataxia Neuropathy Dysarthria and Ophthalmoplegia, MADD Multiple Acyl-CoA Dehydrogenation Deficiency, CDG Carbohydrate-Deficient Oligosaccharides

Patient deceased
and/or other signs. In the first group, the very low CoQ10 level observed in the fibroblasts of patient P01 confirmed the primary CoQ10 defect associated with the c.437G > A homozygous missense mutation (p.Ser146Asn) in the CoQ2 gene, involved in CoQ10 biosynthesis [18]. In the four other patients in the same group, CoQ10 defect was clearly secondary because the responsible genes were unrelated to CoQ10 biosynthesis. Three patients had a mitochondrial disease linked to a large mtDNA deletion (patient P02) or to mutations in POLG (patient P04) or SUCLG1 (patient P06). Patient P08 alone did not have a mitochondrial disease, her encephalopathy with refractory malignant partial seizures being linked to mutations in the TBC1D24 gene. In the second group, low CoQ10 levels were found in three patients with no molecular diagnosis. Two patients were strongly suspected of having a mitochondrial disease: patient P11, who had a cerebellar ataxia with 20–30 % of COX-negative fibers and multiple mtDNA deletions in muscle, and patient P16 who presented with a Leigh syndrome. The last patient (P15) had an encephalopathy with intellectual disability but no histological sign of mitochondrial myopathy.

Discussion
While primary CoQ10 defects are rare, secondary defects have been observed in various pathologies. In a previous work, we suspected for the first time a secondary CoQ10 defect in a child with propionic acidemia, who succumbed to acute heart failure in the absence of decompensation of his metabolic condition [17]. CoQ10 deficiency was not evoked at the outset because CoQ10-dependent activities deficiency was associated with multiple MRC deficiency in the liver of the patient and it had been widely reported that enzymatic activities of MRC complexes are normal in CoQ10 disease [16]. However, it

| Table 2  Biochemical analysis of patient fibroblasts and muscle biopsies |
|-----------------------------------------------|
| OXPHOS activities (spectrophotometry) | I | II | III | IV | V | G3P + III | II + III | CS | CoQ10 quantity (LC-MSMS) |
|-----------------------------------------------|
| Fibroblast measurements | | | | | | | | | |
| Control values (nmole/min/mg of proteins) | 9.0–27.1 | 21.0–54.0 | 62.0–176.2 | 109.9–350.0 | 22.0–46.2 | 6.5–23.0 | 15.0–37.2 | 74.7–161.1 |
| P01 | 11.2 | 27.7 | 89.7 | 29.2 | 33.5 | 2.3 | 7.5 | 156.2 |
| P03 | 11.5 | 18.5 | 21.3 | 177.7 | 34.1 | 4.1 | 12.5 | 106.7 |
| P04 | 7.5 | 18.6 | 40.2 | 108.2 | 30.5 | 4.7 | 8.6 | 95.0 |
| P06 | 11.6 | 20.6 | 47.9 | 78.1 | 37.6 | 5.5 | 10.8 | 116.6 |
| P09 | 12.0 | 13.4 | 53.4 | 57.0 | 15.8 | 4.1 | 8.9 | 80.9 |
| P10 | 13.5 | 22.9 | 54.4 | 65.0 | 28.3 | 5.4 | 12.0 | 148.2 |
| P12 | 10.9 | 17.6 | 76.6 | 173.3 | 25.0 | 4.4 | 10.1 | 102.5 |
| P16 | 11.2 | 20.7 | 57.4 | 134.9 | 38.3 | 6.1 | 14.5 | 124.0 |
| P17 | 12.9 | 20.0 | 61.5 | 181.7 | 29.3 | 5.6 | 14.7 | 130.3 |
| P18 | 14.4 | 22.5 | 39.4 | 78.9 | 39.3 | 5.5 | 13.2 | 147.0 |
| Muscle biopsy measurements | | | | | | | | | |
| Control values (nmole/min/mg of proteins) | 11.0–32.0 | 22.0–65.0 | 109.0–236.0 | 93.0–347.0 | 40.0–89.0 | 14.0–50.0 | 20.0–50.0 | 82.0–234.0 |
| P02 | 6.7 | 21.5 | 130.4 | 59.9 | 32.5 | 9.4 | 10.8 | 113.4 |
| P05 | 5.7 | 21.2 | 29.4 | 59.4 | 12.7 | 10.9 | 15.2 | 122.2 |
| P07 | 4.2 | 28.6 | 108.8 | 170.4 | 50.0 | 10.5 | 16.7 | 272.4 |
| P08 | 10.9 | 14.1 | 102.5 | 92.8 | 63.3 | 10.8 | 17.0 | 116.5 |
| P11 | 25.7 | 29.7 | 157.6 | 80.2 | 45.0 | 2.6 | 10.8 | 109.9 |
| P13 | 7.6 | 28.9 | 112.7 | 212.7 | 58.4 | 9.4 | 13.4 | 192.6 |
| P14 | 15.5 | 26.6 | 31.6 | 154.5 | 32.8 | 13.7 | 13.2 | 100.5 |
| P15 | 16.2 | 20.0 | 92.3 | 191.7 | 39.8 | 11.9 | 17.5 | 86.1 |

Respiratory chain enzyme activities were measured spectrophotometrically. Results are expressed as absolute values for controls or patients (in nanomoles of substrate per minute per milligram of protein). CoQ10 quantity was measured by LC-MSMS. Results are expressed as absolute values for controls or patients (in picomoles per milligram of protein). Abnormal values are shown in italics.

OXPHOS oxidative phosphorylation; LC-MSMS liquid chromatography coupled with tandem mass spectrometry detection.
is likely that a secondary CoQ10 defect was involved in the development of heart complications leading to the child’s death and that oral CoQ10 supplementation would have been able to prevent cardiac failure if results had been obtained before acute clinical aggravation. This hypothesis is supported by a recent study, which describes a successful reversal of propionic acidemia-associated cardiomyopathy after treatment [19]. The child in this case presented with myocardial CoQ10 quantitative defect associated with signs of mitochondrial dysfunction such as enlarged mitochondria with atypical cristae and low MRC complex IV activity [19]. Several studies performed on cellular models of CoQ10 defect suggested a possible association with mitochondrial dysfunction: PDSS2 and COQ9 mutant fibroblasts presented a markedly reduced ATP synthesis and COQ2 mutant fibroblasts presented a partial defect in ATP synthesis, as well as significantly increased ROS production and oxidation of lipids and proteins [20, 21]. In 2013, Duberley and colleagues established the first pharmacologically-induced CoQ10 deficient cellular model in neuroblastoma-derived SH-SY5Y cells by using para-aminobenzoic acid (PABA). They showed that, after PABA treatment, SH-SY5Y cells presented a progressive decrease in the activities of CoQ10-dependent II + III segment but also a deficiency in MRC complexes I and IV. They also reported a concomitant decrease in the level of total ATP with an increase of mitochondrial oxidative stress [22]. Lastly, deficiency of complexes I, II, III and/or IV has also been previously reported in association with CoQ10 defect in the patient’s fibroblasts, muscle or kidney [8, 11, 18, 23].

Today, in most diagnostic laboratories, a spectrophotometric deficiency in one or several MRC enzymes associated with a decrease in CoQ10-dependent activities is not considered to be a sign of a CoQ10 disease, leading to a possible under-estimation of the frequency of this disorder. With the aim of achieving a better diagnostic approach, we quantified CoQ10 by LC-MSMS in 18 patients presenting a CoQ10-dependent enzymatic deficiency associated with a MRC defect by spectrophotometry. CoQ10 quantitative analysis in muscle or in fibroblast cells confirmed CoQ10 disease in eight patients (45%). These data show that a primary CoQ10 defect can be associated with MRC enzymic deficiency because patient P01, who carried a deleterious homozygous mutation (c.437G > A; p.Ser146Asn) in the CoQ2 gene, also presented a complex IV deficiency in muscle. Our data also confirm that a secondary CoQ10 defect can be associated with mitochondrial disease. Indeed, three other patients with a low CoQ10 level presented a respiratory chain deficiency linked to mtDNA deletion (patient P02) or to mutations in POLG and SUCLG1 genes (patients P04 and P06). Secondary CoQ10 defect has already been reported in patients with mitochondrial diseases or dysfunctions including Kearns–Sayre syndrome [24], mtDNA depletion and PEO [5] or mutations in ETFDH coding for electrontransferring-flavoprotein dehydrogenase and causing MAD [8, 9]. Secondary CoQ10 defect has also been described in non-mitochondrial disorders linked to genes such as APTX coding for aprataxin and causing ataxia oculomotor-apraxia [7], BRAF coding for serine/threonine-protein kinase B-Raf and causing cardiofaciocutaneous syndrome [10], ACADVL causing very long-chain Acyl-CoA dehydrogenase deficiency or NPC causing Niemann-Pick-type C disease [11]. Here, we report for the first time a secondary CoQ10 defect associated with mutations in the TBC1D24 gene, leading to malignant migrating partial seizures (Patient P08). The mechanisms linking CoQ10 defect and decreased activity of MRC complexes are unknown. Studies in patients with metabolic diseases showed an increase in oxidative stress-markers and a decrease in antioxidant defences [25]. More specifically, ubiquinol depletion in patient tissues may lead to increased reactive oxygen species activity [26] and, since all enzymes of the MRC are susceptible to free radical induced oxidative damage [27], we can hypothesize that CoQ10-independent MRC dysfunction may result from a high level of mitochondrial oxidative stress creating an imbalance with the CoQ10 antioxidant capacity, as previously evoked [25]. In parallel, a possible reason for a secondary CoQ10 defect resulting from a primary MRC deficiency is that the enzymes involved in CoQ10 biosynthesis are found in a supercomplex in the inner mitochondrial membrane [28]. We hypothesize that the increased oxidative stress resulting from a primary MRC deficiency may inhibit these enzymes resulting in a secondary CoQ10 defect.

Conclusions

In conclusion, our work highlights the probability that, based on spectrophotometric analysis, the frequency of CoQ10 disease is underestimated in routine clinical practice. Several studies, which performed a systematic CoQ10 quantification on muscle biopsies from pediatric and adult populations presenting a wide range of clinical phenotypes, also reported an underestimation of CoQ10 defects and proposed a systematic evaluation of CoQ10 content in all muscle biopsies [5, 29, 30]. However, first-line CoQ10 quantification seems difficult to set up as a routine analysis in all diagnosis laboratories. Based on our observations, we suggest that CoQ10 quantification be performed in all tissues presenting a spectrophotometric deficiency of CoQ10-dependent enzymes, associated or not with MRC defect, regardless of the patient’s age, clinical presentation or molecular diagnosis. This could prove of great value in clinical practice for the
diagnosis of a disease that can be improved by CoQ_{10} supplementation.

**Methods**

**Patients**

All patients were explored in the Reference Centre for Mitochondrial Disease (CHU of Nice, France). Selection of the 18 patients was based on the following inclusion criteria: (1) availability of a muscle sample or fibroblast culture and (2) spectrophotometric deficiency of CoQ_{10}-dependent activities (reduction of segments I + III or G3P + III and II + III) associated with MRC defect in muscle or in fibroblasts. The following data were systematically collected: sex, age at biopsy, age of onset, heredity, familial history, clinical presentation, brain MRI, metabolic screening, mitochondrial enzymatic studies, histological and molecular analyses. The age of onset of clinical symptoms ranged from neonatal period to 45 years of age. Blood and tissue samples were obtained after adult patients and parents of affected children had given informed consent.

Patients were divided into two groups (Table 1), according to the results of molecular analysis: (1) individuals with a molecular diagnosis, carrying mutations in mtDNA or in nuclear genes and, (2) individuals with no molecular diagnosis.

**Cell culture**

Primary fibroblast cultures were obtained from patient skin punches, using standard procedures, in RPMI medium supplemented with 10 % Fetal Bovine Serum, 45 μg/ml uridine and 275 μg/ml sodium pyruvate. Cultures were incubated at 37 °C with 5 % CO_{2}.

**OXPHOS spectrophotometric measurements**

Enzymatic spectrophotometric measurements of the OXPHOS respiratory chain complexes and citrate synthase were performed at 37 °C on muscle crude homogenates or fibroblasts according to standard procedures [31]. Proteins were measured according to Bradford microassay [32] and results were expressed as nmole/min/mg of proteins.

**Coenzyme Q_9 quantification**

Total coenzyme Q_{10} was extracted from tissues and analyzed by reverse phase liquid chromatography separation (column C18 symmetry 150 × 2.1 mm, 3.5 μm, Waters, France) as previously described [33]. Detection and quantification were done by mass spectrometry using an API 3000 tandem mass spectrometer (ABSciex, France) equipped with an APCI source. CoQ_{10} and CoQ_{9} were analyzed in the positive mode using the following m/z 864 → 197 and 796 → 197 transitions. CoQ_{9} was used as internal standard for quantification. External calibrations were performed using CoQ_{10} solutions. A stock solution was prepared by dissolving 10 mg of CoQ_{10} in 4 ml of methanol/chloroform (98:2 v/v). This solution was stable for 3 months at −80 °C. The working solutions were prepared daily by diluting the stock solution into methanol to provide a range of 0.05–1 μmol/L. The intra-assay and inter-assay CV’s were, respectively, 5.7 and 6.3 % for a CoQ_{10} concentration of 0.25 μmol/L.

**Author’s contributions**

Study conception and design: KF, AC, VP-F. LC-MSMS experiments: JFB. Molecular analysis: SA, SB, CR. Biochemical explorations: KF, CC. Data collection and analysis: KF, AC, JFB, SA, SB, CR, VP-F. Manuscript drafting: KF, AC, VP-F. Study supervision: VP-F. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

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**References**

1. Turunen M, Olsson J, Dallner G. Metabolism and function of coenzyme Q. Biochim Biophys Acta. 2004;1660:171–99.

2. Quinzii CM, Hirano M. Coenzyme Q and mitochondrial disease. Dev Disabil Res Rev. 2010;16:183–8.

3. Desbats MA, Lunardi G, Doimo M, Trevisson E, Salviati L. Genetic bases and clinical manifestations of coenzyme Q10 (CoQ 10) deficiency. J Inherit Metab Dis. 1991;14:443–7.

4. Matsuoka T, Maeda H, Goto Y, Nonaka I. Muscle coenzyme Q10 in mitochondrial encephalomyopathies. Neuromuscul Disord. 1991;1:443–7.

5. Sacconi S, Trevisson E, Salviati L, Ayme S, Rigal O, Redondo AG, et al. Coenzyme Q10 is frequently reduced in muscle of patients with mitochondrial myopathy. Neuromuscul Disord. 2010;20:444–8.

6. Montero R, Grazina M, Lopez-Gallardo E, Montoya J, Briones P, Navarro-Sastre A, et al. Coenzyme Q_{10} deficiency in mitochondrial DNA depletion syndromes. Mitochondrion. 2013;13:337–41.

7. Quinzii CM, Kattah AG, Naini A, Akman HO, Moottha VK, DiMauro S, et al. Coenzymes Q_{9} deficiency and cerebellar ataxia associated with an aprataxin mutation. Neurology. 2005;64:339–41.

8. Gempel K, Topaloglu H, Talim B, Schneider P, Schoer BS, Hans VH, et al. The myopathic form of coenzyme Q10 deficiency is caused by mutations in the electron-transferring-flavoprotein dehydrogenase (ETFDH) gene. Brain. 2007;130:2037–44.

9. Liang WC, Ohkuma A, Hayashi YK, Lopez LC, Hirano M, Nonaka I, et al. ETFDH mutations, CoQ10 levels, and respiratory chain activities in patients with riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency. Neuromuscul Disord. 2009;19:212–6.

10. Aeby A, Sznajer Y, Cave H, Rebuffat E, Van Coster R, Rigal O, et al. Cardiomyocutaneous (CFC) syndrome associated with muscular coenzyme Q10 deficiency. J Inherit Metab Dis. 2007;30:827.

11. Buján N, Arias A, Montero R, García-Villoria J, Lissens W, Seneca S, et al. Characterization of CoQ_{9} biosynthesis in fibroblasts of patients.
with primary and secondary CoQ10 deficiency. J Inherit Metab Dis. 2014;37:53–62.

12. Geromel V, Darin N, Chétien D, Bénit P, Delonlay P, Rötig A, et al. Coenzyme Q10 and idebenone in the therapy of respiratory chain diseases: rationale and comparative benefits. Mol Genet Metab. 2002;77:21–30.

13. Trevisson E, DiMauro S, Navas P, Salvatì L. Coenzyme Q deficiency in muscle. Curr Opin Neurol. 2011;24:449–56.

14. García-Corzo L, Luna-Sánchez M, Doerrier C, Ortiz F, Escames G, Acuña-Castroviejo D, et al. Ubiquinol-10 ameliorates mitochondrial encephalopathy associated with CoQ deficiency. Biochim Biophys Acta. 2014;1842:893–901.

15. Rustin P, Munnich A, Rötig A. Mitochondrial respiratory chain dysfunction caused by coenzyme Q deficiency. Methods Enzymol. 2004;382:81–8.

16. Rötig A, Mollet J, Rio M, Munnich A. Infantile and pediatric quinone deficiency diseases. Mitochondrion. 2007;7(Suppl):S112–21.

17. Fragaki K, Cano A, Benoist JF, Rigal O, Chaussonet A, Rouzier C, et al. Fatal heart failure associated with CoQ10 and multiple OXPHOS deficiency in a child with propionic acidemia. Mitochondrion. 2011;11:533–6.

18. Diomedi-Camassei F, Di Giandomenico S, Santorelli FM, Caridi G, Piemonte F, Montini G, et al. COQ2 nephropathy: a newly described inherited mitochondrialopathy with primary renal involvement. J Am Soc Nephrol. 2007;18:2773–80.

19. Baruteau J, Hargreaves I, Krywawych S, Chalasani A, Land JM, Davison JE, et al. Successful reversal of propionic acidemia associated cardiomyopathy: evidence for low myocardial coenzyme Q10 status and secondary mitochondrial dysfunction as an underlying pathophysiological mechanism. Mitochondrion. 2014;17:150–60.

20. Quinzii CM, López LC, Vom-Molitke J, Nanni A, Krishna S, Schuëlle M, et al. Respiratory chain dysfunction and oxidative stress correlate with severity of primary CoQ10 deficiency. FASEB J. 2008;22:1874–85.

21. Quinzii CM, López LC, Gillerson RW, Dorado B, Coku J, Nanni AB, et al. Reactive oxygen species, oxidative stress, and cell death correlate with level of CoQ10 deficiency. FASEB J. 2010;24:3733–43.

22. Duberley KE, Abramow AM, Chalasani A, Heales SJ, Rahman S, Hargreaves IP. Human neuronal coenzyme Q10 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. 2013;36:663–73.

23. Lalani SR, Vladutiu GD, Plunkett K, Lotze TE, Adesina AM, Scaglia F. Isolated mitochondrial myopathy associated with muscle coenzyme Q10 deficiency. Arch Neurol. 2005;62:317–20.

24. Zierz S, Jahns G, Jerusalem F. Coenzyme Q in serum and muscle of 5 patients with Kearns-Sayre syndrome and 12 patients with ophthalmoplegia plus. J Neurol. 1989;236:97–101.

25. Mc Guire PL, Parkh A, Diaz GA. Profiling of oxidative stress in patients with inborn errors of metabolism. Mol Genet Metab. 2009;98:173–80.

26. Genova ML, Pich MM, Berreri A, Bernacchia A, Falasca A, Boivin C, et al. Mitochondrial production of oxygen radical species and the role of Coenzyme Q as an antioxidant. Exp Biol Med (Maywood). 2003;228:506–13.

27. Zhang Y, Marcillat O, Giulivi C, Ernster L, Davies KJ. The oxidative inactivation of mitochondrial electron transport chain components and ATPase. J Biol Chem. 1990;265:16330–6.

28. Hsieh EJ, Grin P, Gulmezian M, Tran UC, Saiki R, Marbois BN, et al. Saccharomyces cerevisiae Coq9 polypeptide is a subunit of the mitochondrial coenzyme Q biosynthetic complex. Arch Biochem Biophys. 2007;463:19–26.

29. Montero R, Artuch R, Briones P, Nascimento A, Garcia-Cazorla A, Vilaseca MA, et al. Muscle coenzyme Q10 concentrations in patients with probable and definite diagnosis of respiratory chain disorders. BioFactors. 2005;25:109–15.

30. Miles MV, Miles L, Tang PH, Horn PS, Steele PE, DeGrauw AJ, et al. Systematic evaluation of muscle coenzyme Q10 content in children with mitochondrial respiratory chain enzyme deficiencies. Mitochondrion. 2008;8:170–80.

31. Rustin P, Chretien D, Bourgeron T, Gerard B, Rötig A, Saudubray JM, et al. Biochemical and molecular investigations in respiratory chain deficiencies. Mitochondrion. 2008;8:170–80.

32. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248–54.

33. Benoist JF, Rigal O, Nivoche Y, Martin C, Biou D, Lombès A. Differences in mitochondrial coenzyme Q biosynthetic complex. Arch Biochem Biophys. 2004;382:81–8.

34. Mc Guire PL, Parkh A, Diaz GA. Profiling of oxidative stress in patients with inborn errors of metabolism. Mol Genet Metab. 2009;98:173–80.

35. Mc Guire PL, Parkh A, Diaz GA. Profiling of oxidative stress in patients with inborn errors of metabolism. Mol Genet Metab. 2009;98:173–80.

36. Mc Guire PL, Parkh A, Diaz GA. Profiling of oxidative stress in patients with inborn errors of metabolism. Mol Genet Metab. 2009;98:173–80.