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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

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analysis (segments I + III or G3P + III and II + III) are evocative of CoQ_{10} deficiency but direct measurement of CoQ_{10} levels is the most reliable test for diagnosis [15]. It is widely reported in the literature that, in patients with CoQ_{10} deficiency, enzymatic activities of MRC complexes I, II, III, IV, V are normal [16]. In a previous report, we described an 11-year-old boy presenting with a propionic acidemia who succumbed to acute heart failure in the absence of decompensation of his metabolic condition. Spectrophotometric analysis in liver identified CoQ_{10}-dependent activities deficiency that was associated with MRC enzymatic defect. Secondary CoQ_{10} deficiency was likely involved in the development of heart complications in this child and we hypothesized that a CoQ_{10}-defect 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 CoQ_{10}-dependent activity deficiency led to identification of CoQ_{10} disease in eight cases. Eighteen patients presented CoQ_{10}-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 CoQ_{10} quantitative defect may be associated with multiple MRC enzymatic deficiency, we measured CoQ_{10} in this group of 18 patients. We found decreased CoQ_{10} levels by liquid chromatography coupled with tandem mass spectrometry detection (LC-MSMS) in eight patients out of 18 (45 %), thus confirming CoQ_{10} disease and its association with MRC enzymatic deficiency. Furthermore, CoQ_{10} 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 CoQ_{10}-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 *SUCLG1* 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 *SRD5A3-CDG* mutations (P09) and 1p36 deletion syndrome (P10). Patients P02–P10 had a CoQ_{10}-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 CoQ_{10} 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 CoQ_{10}-dependent enzymatic deficiency associated with MRC defect in muscle or in fibroblasts (Table 2).

Confirmation of CoQ_{10}disease in eight patients by CoQ_{10} quantification

Quantitative analysis of CoQ_{10} in muscle or fibroblasts showed that eight patients presented CoQ_{10} content below normal values (Table 2). CoQ_{10} defect was found in five patients out of 10 in the first group and in three patients out of eight in the second group. CoQ_{10}-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 CoQ_{10}-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
Patients with molecular diagnosis												
P01[a]	Fibroblasts	M	D1	Neonatal	Recessive	Affected brother			Neonatal polyvisceral failure	Not done	Cx IV deficiency; segments II + 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[a]	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[a]	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 homozygous mutation 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 I, 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[a]	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.509C > G (p.Pro170Arg)
P07	Muscle	F	18y	4y	Recessive	Blindness in paternal family		Bilateral ptosis, proximal myopathy, dysphonia, dysphagia, exercice intolerance	Retinitis pigmentosa, cyclic vomiting, hyperCPKemia	Lipid accumulation	Cxes I and III deficiency; segments I + III and II + III reduction	MADD with mutations in *ETFDH*

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
P08	Muscle	F	4 m	4 m	Recessive	Affected sister	Encephalopathy with refractory migrating partial seizures			Lipid accumulation	Cx I, II, III and IV deficiency; segments I + III and II + III reduction	Malignant migrating partial seizures with compound heterozygous mutations in *TBC1D24*: (c.468C > A/c.686C > T; p.Cys156X/p.Phe229Ser)
P09	Fibroblasts	M	D1	Neonatal	Recessive	No	Hypotonia		Hypertrophic CMP, dysmorphic, hepatic cytolysis, hypospadia	Glycogenic accumulation	Cxes II, III, IV and V deficiency; segments II + III and G3P + III reduction	CDG syndrome type Iq : homozygous mutation in *SRD5A3* : (c.620T > G; p.Met207Arg)
P10[a]	Fibroblasts	M	3 m	Neonatal	*de novo*	No	Hypotonia, epilepsy, dysphagia		Dilated CMP, aortic dilatation	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[a]	Fibroblasts	F	7 m	6 m	?	No	Leigh syndrome			Not done	Cx II deficiency; segments II + III and G3P + III reduction	mtDNA depletion, absence of mtDNA and *POLG, SUCLA2, TK2* mutation
P13[a]	Muscle	F	41y	Childhood	Recessive	Consanguinity	Spastic tetraparesis, chorea, mental retardation	Myopathy	Glaucoma, cataract, lactic acidosis	RRF ++	Cx I deficiency; segments I + III and II + III reduction	Absence of mtDNA and *POLG, OPA1, OPA3* mutation
P14	Muscle	F	33y	6 m	?	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	28 y	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	Cxes II and III deficiency; segments II + III and G3P + III reduction	Absence of mtDNA mutation
P17	Fibroblasts	F	D3	D2	?	No			Unexplained severe respiratory failure	Normal	Cxes II, III deficiency; segments II + III and G3P + III reduction	Absence of mtDNA mutation
P18	Fibroblasts	M	2y	D18	?	No	Encephalopathy with refractory epilepsy		Microcephaly	Normal	Cxes 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* oxydase, *cx* complex, *mtDNA* mitochondrial DNA, *SANDO* Sensory Ataxia Neuropathy Dysarthria and Ophthalmoplegia, *MADD* Multiple Acyl-CoA Dehydrogenation Deficiency, *CDG* Carbohydrate-Deficient Glycoprotein

[a] Patient deceased

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)	CoQ10
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*	*Control values (pmole/mg of proteins)*	*43.0–120.8*
P01	11.2	27.7	89.7	*29.2*	33.5	*2.3*	*7.5*	156.2	P01	*1.4*
P03	11.5	*18.5*	*21.3*	177.7	34.1	*4.1*	*12.5*	106.7	P03	65.4
P04	*7.5*	*18.6*	*40.2*	*108.2*	30.5	*4.7*	*8.6*	95.0	P04	*9.7*
P06	11.6	*20.6*	*47.9*	*78.1*	37.6	*5.5*	*10.8*	116.6	P06	*5.9*
P09	12.0	*13.4*	*53.4*	*57.0*	*15.8*	*4.1*	*8.9*	80.9	P09	62.0
P10	13.5	22.9	*54.4*	*65.0*	28.3	*5.4*	*12.0*	148.2	P10	55.9
P12	10.9	*17.6*	76.6	173.3	25.0	*4.4*	*10.1*	102.5	P12	62.1
P16	11.2	*20.7*	*57.4*	134.9	38.3	*6.1*	*14.5*	124.0	P16	*5.7*
P17	12.9	*20.0*	*61.5*	181.7	29.3	*5.6*	*14.7*	130.3	P17	58.1
P18	14.4	22.5	*39.4*	*78.9*	39.3	*5.5*	*13.2*	147.0	P18	58.2
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*	*Control values (pmole/mg of proteins)*	*17.8 –22.2*
P02	*6.7*	*21.5*	130.4	*56.9*	*32.5*	*7.4*	*10.8*	113.4	P02	*16.0*
P05	*5.7*	*21.2*	*28.9*	*59.4*	*12.7*	*10.9*	*15.2*	122.2	P05	35.5
P07	*4.2*	28.6	*108.8*	170.4	50.0	*10.5*	*16.7*	272.4	P07	25.9
P08	*10.9*	*14.1*	*102.5*	*92.8*	63.3	*10.8*	*17.0*	116.5	P08	*5.7*
P11	25.7	29.7	157.6	*80.2*	45.0	*13.6*	*19.6*	109.9	P11	*6.2*
P13	*7.6*	28.9	112.7	212.7	58.4	*9.4*	*13.4*	192.6	P13	22.4
P14	15.5	26.6	*31.6*	154.5	*32.8*	*13.7*	*13.2*	100.5	P14	22.2
P15	16.2	*20.0*	*92.3*	191.7	*39.8*	*11.9*	*17.5*	86.1	P15	*7.1*

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). CoQ_{10} 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

and/or other signs. In the first group, the very low CoQ_{10} level observed in the fibroblasts of patient P01 confirmed the primary CoQ_{10} defect associated with the c.437G > A homozygous missense mutation (p.Ser146Asn) in the *CoQ2* gene, involved in CoQ_{10} biosynthesis [18]. In the four other patients in the same group, CoQ_{10} defect was clearly secondary because the responsible genes were unrelated to CoQ_{10} 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 migrating partial seizures being linked to mutations in the *TBC1D24* gene. In the second group, low CoQ_{10} 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 CoQ_{10} defects are rare, secondary defects have been observed in various pathologies. In a previous work, we suspected for the first time a secondary CoQ_{10} defect in a child with propionic acidemia, who succumbed to acute heart failure in the absence of decompensation of his metabolic condition [17]. CoQ_{10} deficiency was not evoked at the outset because CoQ_{10}-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 CoQ_{10} disease [16]. However, it

is likely that a secondary CoQ_{10} defect was involved in the development of heart complications leading to the child's death and that oral CoQ_{10} 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 CoQ_{10} 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 CoQ_{10} 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 CoQ_{10} 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 CoQ_{10}-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 cellular 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 CoQ_{10} 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 CoQ_{10}-dependent activities is not considered to be a sign of a CoQ_{10} disease, leading to a possible under-estimation of the frequency of this disorder. With the aim of achieving a better diagnostic approach, we quantified CoQ_{10} by LC-MSMS in 18 patients presenting a CoQ_{10}-dependent enzymatic deficiency associated with a MRC defect by spectrophotometry. CoQ_{10} quantitative analysis in muscle or in fibroblast cells confirmed CoQ_{10} disease in eight patients (45 %). These data show that a primary CoQ_{10} defect can be associated with MRC enzymatic 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 CoQ_{10} defect can be associated with mitochondrial disease. Indeed, three other patients with a low CoQ_{10} 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 CoQ_{10} 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 MADD [8, 9]. Secondary CoQ_{10} defect has also been described in non-mitochondrial disorders linked to genes such as *APTX* coding for aprataxin and causing ataxia occulomotor-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 CoQ_{10} defect associated with mutations in the *TBC1D24* gene, leading to malignant migrating partial seizures (Patient P08). The mechanisms linking CoQ_{10} 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 CoQ_{10}-independent MRC dysfunction may result from a high level of mitochondrial oxidative stress creating an imbalance with the CoQ_{10} antioxidant capacity, as previously evoked [25]. In parallel, a possible reason for a secondary CoQ_{10} defect resulting from a primary MRC deficiency is that the enzymes involved in CoQ_{10} 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 CoQ_{10} defect.

Conclusions

In conclusion, our work highlights the probability that, based on spectrophotometric analysis, the frequency of CoQ_{10} disease is underestimated in routine clinical practice. Several studies, which performed a systematic CoQ_{10} quantification on muscle biopsies from pediatric and adult populations presenting a wide range of clinical phenotypes, also reported an underestimation of CoQ_{10} defects and proposed a systematic evaluation of CoQ_{10} content in all muscle biopsies [5, 29, 30]. However, first-line CoQ_{10} quantification seems difficult to set up as a routine analysis in all diagnosis laboratories. Based on our observations, we suggest that CoQ_{10} quantification be performed in all tissues presenting a spectrophotometric deficiency of CoQ_{10}-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_{10} 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 calibration was 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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