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Short communication

Cerebellar ataxia with coenzyme Q_{10} deficiency: Diagnosis and follow-up after coenzyme Q_{10} supplementation

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Abstract

Our aim was to report a new case with cerebellar ataxia associated with coenzyme Q_{10} (CoQ) deficiency, the biochemical findings caused by this deficiency and the response to CoQ supplementation.

Patient: A 12-year-old girl presenting ataxia and cerebellar atrophy.

Biochemical studies: Coenzyme Q_{10} in muscle was analysed by HPLC with electrochemical detection and mitochondrial respiratory chain (MRC) enzyme activities by spectrophotometric methods. CoQ biosynthesis in fibroblasts was assayed by studying the incorporation of radiolabeled 4-hydroxy[U-^{14}C] benzoic acid by HPLC with radiometric detection.

Results: Mitochondrial respiratory chain enzyme analysis showed a decrease in complex I+III and complex II+III activities. CoQ concentration in muscle was decreased (56 nmol/g of protein: reference values: 157–488 nmol/g protein). A reduced incorporation of radiolabeled 4-hydroxy[U-^{14}C] benzoic acid was observed in the patient (19% of incorporation respect to the median control values). After 16 months of CoQ supplementation, the patient is now able to walk unaided and cerebellar signs have disappeared.

Conclusions: Cerebellar ataxia associated with CoQ deficiency in our case might be allocated in the transprenylation pathway or in the metabolic steps after condensation of 4-hydroxybenzoate and the prenyl side chain of CoQ. Clinical improvement after CoQ supplementation was remarkable, supporting the importance of an early diagnosis of this kind of disorders.
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1. Introduction

Coenzyme Q_{10} (CoQ) is a lipid-soluble component of cell membranes, which transports electrons from complexes I and II to complex III of the mitochondrial respiratory chain (MRC). It also has a key role as a free radical scavenger, regenerating other antioxidants in other cellular membranes such as plasma membrane [1]. Coenzyme Q_{10} is composed of a benzoquinone ring, synthesized from tyrosine through

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4-hydroxybenzoate, and a polyprenyl side-chain, generated from acetyl-CoA through the mevalonate pathway, which is common for the synthesis of other compounds, such as cholesterol and dolichol-phosphate [2].

Since the first description of a mitochondrial encephalomyopathy associated with CoQ deficiency [3], several patients have been reported. This is an autosomal recessive disorder with a clinical spectrum that encompasses several main phenotypes [4]: a myopathic form, with myoglobinuria, epilepsy and ataxia [3,5]; a severe infantile syndrome with encephalopathy and renal disease [6]; and an ataxic form presenting with cerebellar atrophy [7]. However, other phenotypes have been reported, such as a neonatal presentation with fatal evolution [8], Leigh syndrome in adulthood [9], and liver failure plus Leigh syndrome [10]. A common feature in these cases is the presence of a variable degree of CoQ deficiency in muscle and/or fibroblasts causing decreased activities of NADH:cit c oxidoreductase and succinate:cit c oxidoreductase of the MRC. Interestingly, treatment response has been remarkable in most cases, highlighting the importance of an early diagnosis of these disorders.

Here we present the diagnosis of a new case of cerebellar ataxia associated with CoQ deficiency. We also report the biochemical findings caused by this deficiency and the clinical response after CoQ supplementation.

2. Materials and methods

2.1. Case report

A 12-year-old girl, born after a term pregnancy and normal delivery of non-consanguineous parents. Her elder brother and her mother both have long Q-T syndrome. Her father (45 years old) described a history of clumsiness, frequent falls, tremor and nistagmus from the early years of life. After a normal newborn period and early motor development, at 15 months of age it was noticed that the patient was clumsy fell frequently when walking unaided. At 5 years of age, general physical examination was normal. Neurological exam showed slight dysarthria with slurring speech, rapid alternating movements and finger–nose testing with oscillating movement, without decomposition. Finger–finger test evidenced mild instability for the action with no action tremor. On knee–tibia test, movement was decomposed in several phases and was abnormally slow with no tremor. Pronation–supination alternating movements were slow and irregularly performed. Ocular pursuit was saccadic. Balance evidenced slight oscillations with feet together, but she was unable to walk with feet in tandem position. Gait speed was slightly reduced with broad base. Limb tone and tendon reflexes were decreased. No muscle weakness was observed. Plantar response was flexor. Normal oculi fundi and cognitive level were present. Electrocardiogram, motor, sensory nerve conduction and electromyography were normal. Brain MRI showed severe cerebellar atrophy (Fig. 1A). The patient's father also showed such cerebellar atrophy (Fig. 1B). Clinical evolution remained stable until 12 years of age.

Routine laboratory tests including complete blood count, blood chemistry and thyroid function were normal. Laboratory tests for screening of inborn errors of metabolism in blood and urine (including analysis of sialotransferrin and urine organic acids) were all normal. No hyperlactacidemia or hyperalaninemia were observed during the evolution of the patient. Plasma CoQ concentration (0.57 μmol/L) was also within the reference ranges (0.41–1.15 μmol/L). Inherited causes of ataxia were also ruled out (ataxia–telangiectasia, Friedreich ataxia, ataxia with vitamin E deficiency and abetalipoproteinemia). Muscle and skin biopsies were taken at 12 years of age, and studies for diagnosis of a mitochondrial disorder were started. Muscle and skin biopsies from the father were also studied.

Fig. 1. Cranial MRI from the index case at 12 years of age (A) and her father at 35 years of age (B). Cerebellar atrophy was observed.

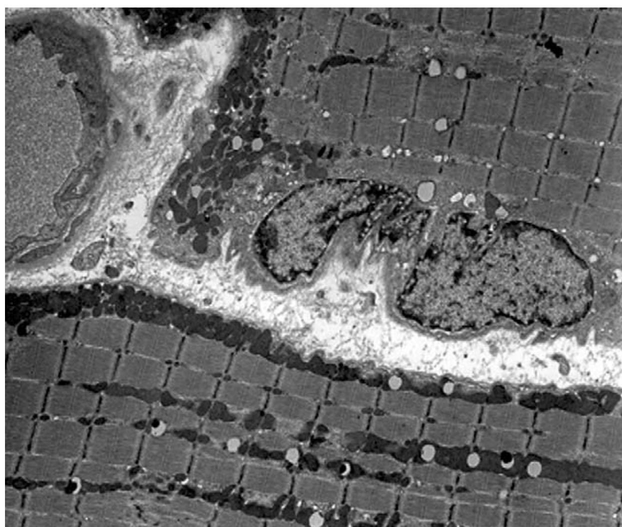

Fig. 2. Electron microscopy examination of muscle biopsy, which showed the presence of an important subsarcolemmic mitochondrial accumulation.

Samples from patients were obtained after written permission. The Ethics Committee of the Hospital Sant Joan de Déu approved the study.

2.2. Measurements

Neurological assessment of ataxia was performed by means of the International Cooperative Ataxia Rating Scale (ICARS) [11].

2.2.1. Histological studies

Both optic (trichromic stain and inmunohistochemistry of respiratory chain complexes) and electronic microscopy analysis were performed on muscle biopsies.

2.2.2. Biochemical studies

Coenzyme Q_{10} analysis in serum, muscle and fibroblasts were performed by reverse phase HPLC with electrochemical detection (Coulochem II, ESA, USA), according to previously reported procedure [12]. Briefly, CoQ from serum and muscle and fibroblast homogenates was extracted with *n*-hexane, evaporated under nitrogen stream and dissolved in ethanol. 50 μL was injected onto the HPLC, and CoQ (ubiquinone and ubiquinol) was separated in a nucleosil C-18 column (25 cm, Teknokroma) with a mobile phase consisting of 55:45 methanol/ethanol (v/v) plus 20 mmol/L of lithium perchlorate). Activities of NADH:cytochrome c oxidoreductase (complex I+CoQ+complex III), succinate:cytochrome c reductase (complex II+CoQ+complex III), succinate dehydrogenase (complex II), decylubiquinol:cytochrome c oxidoreductase (complex III), cytochrome c oxidase (complex IV), and citrate synthase were determined according to described spectrophotometric methods: [13,14]. Protein was determined according to Lowry et al. [15].

To study CoQ biosynthesis in fibroblasts, incorporation of radiolabeled 4-hydroxy[U-^{14}C] benzoic acid (4-[U-^{14}C]HB), a precursor of the quinone ring in CoQ_{10}, was assayed. After incubation of fibroblasts, the CoQ generated was analysed by HPLC with radiometric detection.

Mitochondrial function on fibroblasts was studied by membrane potential assessment (MitoTracker Red; Molecular Probes, Eugene, OR) and cytochrome c immunostaining. Oxidative stress in fibroblasts was assayed by incubating cells for 30 min with 1 μmol/L dihydrorodhamine. The fluorescence produced was measured by flow cytometry.

2.2.3. Genetic studies

DNA was isolated from peripheral white blood cells. Mutation screening of the aprataxin gene was performed by direct sequencing of purified PCR products (Qiage, Hilden, Germany), according to a previously reported procedure [16]. Spinocerebellar ataxia (SCA1, 2, 3, 6, 7, 8, 12 and 17) genes were investigated. To determine the individual's SCA genotypes, primer sequences and polymerase chain reaction (PCR) conditions were used for known CAG repeats of SCA genes as previously described [17]. PCR products were analysed using an ABI Prism 3100 sequencer (PE Applied Biosystems, Foster City, CA, USA).

3. Results

3.1. Histological and biochemical studies in muscle

Optic microscopy studies did not reveal pathological changes (data not shown). Electron microscopy examination of muscle biopsy showed the presence of an important subsarcolemmic mitochondrial accumulation (Fig. 2). Biochemical analysis of MRC enzyme activities showed a clear decrease in both NADH:cytochrome c oxidoreductase and succinate:cytochrome c reductase activities, with normal results for the other MRC complexes (Table 1), suggesting CoQ deficiency. These results led us to measure CoQ concentration in muscle, showing a clear decrement (Table 1). However, the father did not show any abnormalities, including normal muscle MRC enzyme activities and CoQ

Table 1
Results of muscle mitochondrial respiratory chain enzyme and CoQ analysis of a patient with CoQ deficiency

	Case 1	Reference values
Complex I+CoQ+complex III	103	107–560
Complex II+CoQ+complex III	51	75–149
Complex II	67	33–69
Complex III	1128	610–1760
Complex IV	641	590–1300
CoQ	56	157–488

Activities are reported related to citrate synthase (mU/U CS) and CoQ in nmol/g protein.

content (211 nmol/g protein). Histological analysis did neither reveal pathological changes.

3.2. Biochemical studies in fibroblasts

Coenzyme Q_{10} deficiency was confirmed in fibroblasts (44 nmol/g protein; control values: 124–277 nmol/g). Furthermore, a clearly decreased incorporation of radio-labeled 4-[U-^{14}C]HB measured as cpm/mg of protein was observed (19% of incorporation respect to the median control values (n=4)). A decreased incorporation of radio-labeled 4-[U-^{14}C]HB (24% of incorporation with respect to the median control values) was also observed in fibroblasts of the father.

Mitochondrial membrane potential assessment (Mito-Tracker Red) and cytochrome c immunostaining results are reported in Fig. 3. A clear decrease of both parameters was observed when comparing the patient with a control analysed simultaneously. Furthermore, fibroblast incubation with dihydrorodhamine showed an increased fluorescence (3.5 a.u.) compared with control values (1.3–1.6; median=1.5), suggesting increase in ROS production.

3.3. Genetic studies

No mutations were observed neither in the aprataxin gene after screening for each coding exon and flanking intronic sequences nor in any of the SCA studied genes.

3.4. CoQ supplementation and follow-up

Oral CoQ supplementation was started (2500 mg/day), divided in three doses. The doses were decreased every 3 months according to plasma CoQ monitoring data (Fig. 4). After 3 months of supplementation, ICARS scale evaluation showed a decreased score in fine motor and kinetic functions, ocular disorders and dysarthria (Fig. 4). After 16 months of treatment (current doses are 1000 mg/day) the patient is now able to walk unaided and cerebellar signs have disappeared. Monitoring of CoQ treatment was performed, and high plasma concentrations were observed compared with reference values over the duration of treatment (Fig. 4).

4. Discussion

The clinical features of our patient were similar to those previously reported [7,18], and the main signs are probably related to the cerebellar involvement. Biochemical and histological investigation results in muscle biopsy of the index case also showed impaired results, but still without muscle clinical involvement. These observations suggest that the clinical phenotypes of this disorder may present a continuous spectrum of symptoms and signs depending on the different degrees of CoQ deficiency in muscle or brain [7].

In our patient, ataxia was associated with a partial CoQ deficiency in both muscle and fibroblasts, affecting MRC enzyme activities as previously reported [7]. It has been suggested that myopathic forms of CoQ deficiency would only show decreased CoQ values in muscle, while patients with predominant brain involvement would show decreased CoQ values both in muscle and fibroblasts [7]. Partial deficiencies causing mild phenotypes would only affect the cerebellum, since antioxidant defenses and CoQ content are very limited in this brain area [19].

Coenzyme Q_{10} biosynthesis is controlled by nuclear genes [20]. However, to our knowledge, the molecular

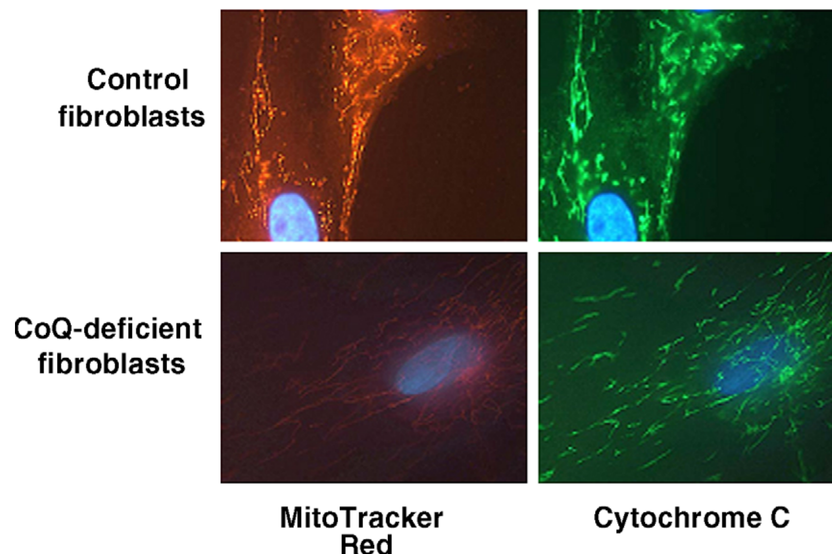

Fig. 3. Cultured fibroblasts from a healthy control (upper panels) and a patient with cerebellar ataxia and CoQ_{10}-deficiency (lower panels). Membrane potential was assessed using MitoTracker Red (Molecular Probes, Eugene, OR) in the left panels. Cytochrome c was visualized by immunostaining in the right panels. A clear decrement of both parameters was observed in the patient fibroblasts.

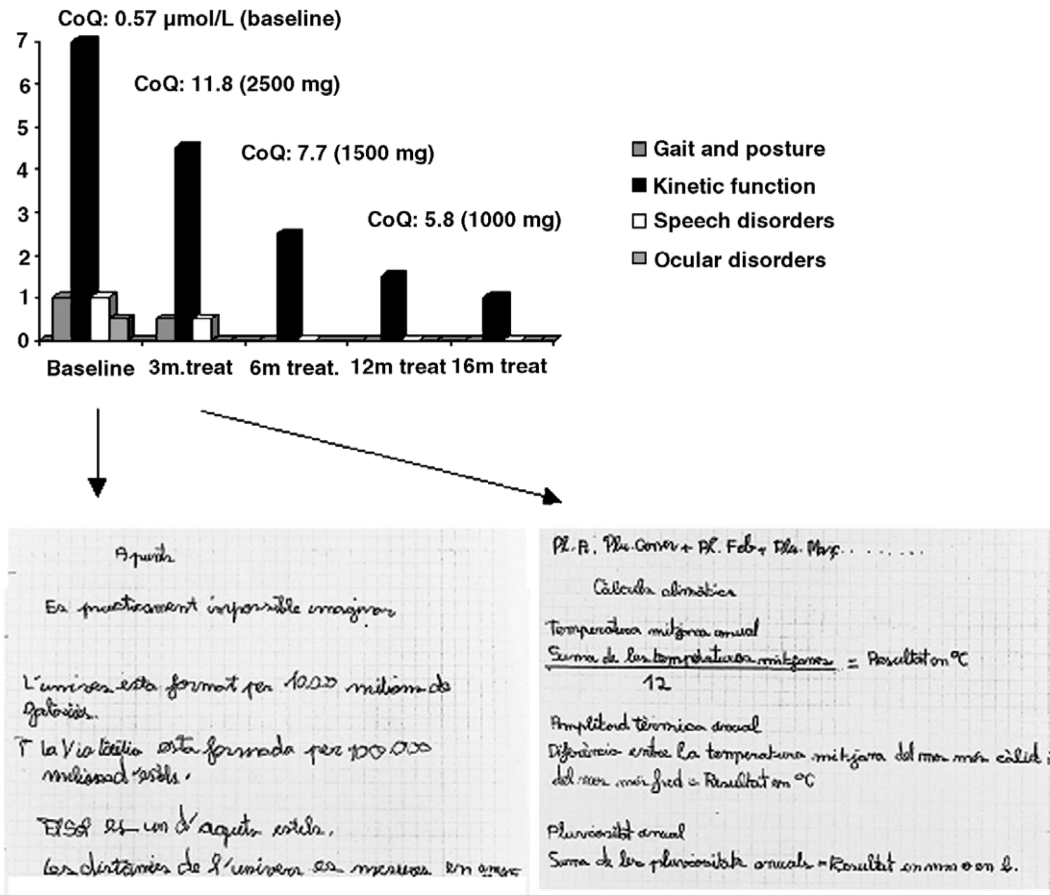

Fig. 4. Effect of CoQ supplementation and evaluation of ICARS scale and plasma CoQ concentration over 16 months of follow-up in case 1. Above columns, plasma CoQ concentrations (µmol/L) and doses applied (mg/day in brackets) are stated. A write test was also performed, showing a clear improvement after CoQ supplementation.

defects causing this disorder are still unknown. Only Rotig et al. [6], by incubating fibroblasts with 3H-labelled mevalonate, hypothesized that the defect might be allocated in the transprenylation part of the CoQ biosynthethic pathway. Similar conclusions were obtained by Rahman et al. [8], who showed normal organic acid profile and glycosylation of proteins, ruling out defects in the mevalonate pathway and in the transformation of tyrosine to 4-hydroxybenzoate. Furthermore, the possibility of an increased destruction of CoQ as responsible of CoQ deficiency has been ruled out by Boitier et al., since normal expression of CoQ-binding protein was observed by these authors [21]. Recently, it has been reported an association between cerebellar ataxia and CoQ deficiency with aprataxin mutations [22]. Our case did neither reveal abnormalities in the organic acid profile or in the glycosylation status of transferrin, suggesting that an impaired mevalonate pathway (which would cause organic acid accumulation and altered *N*-glycosylation of transferrin) or biosynthesis of 4-hydroxybenzoate is unlikely. Moreover, no mutations were found in the aprataxin gene, and autosomal dominat cerebellar ataxias were ruled out. Furthermore, the experiments with incorporation of radiolabeled 4-[U-^{14}C]HB confirmed these preliminary findings, supporting the hypothesis that the molecular defect might be allocated in the genes controlling the transprenylation pathway or in the metabolic steps after condensation of 4-hydroxybenzoate and the prenyl side chain of CoQ.

Concerning the physiopathological mechanisms involved in CoQ deficiency, it seems clear that this deficiency reduces the flux of electrons from both complex I and II to complex III, and therefore, causes a mitochondrial dysfunction [7]. These findings were further supported both by the histological changes observed in muscle biopsy and mitotrack and by the cytochrome c staining analysis in fibroblasts, suggesting a mitochondrial oxidative phosphorylation dysfunction, and probably, a reduction in ATP synthesis. Furthermore, in vitro measurement of free radical generation also showed higher values than those observed in controls, supporting the potential involvement of increased oxidative stress in this disorder. However, there are few controversial, reports concerning oxidative damage in CoQ deficiency [4,23] and this aspect deserves further investigations.

Patients with CoQ deficiency may benefit from CoQ supplementation [7,18], although the more severe phenotypes did not show such clear improvement in the clinical manifestations [8]. Our case showed a very good CoQ

supplementation response, with the main symptoms related with cerebellar dysfunction disappearing, and the ICARS scores decreasing after 16 months of supplementation. This improvement has not been observed in all patients with ataxia and CoQ deficiency previously reported [7,18], and the higher CoQ doses administered to our patient (1000 mg/day compared to 200–900 mg/day), may explain these differences. Although plasma CoQ concentrations of our patient were much higher than our reference ranges, a further reduction of the CoQ dose should be cautiously evaluated.

Concerning the father of our patient, muscle biopsy studies did not reveal an impaired MRC function or a decreased CoQ values. Furthermore, fibroblasts analysis did not clearly showed low CoQ concentration. However, this case presented cerebellar atrophy and some neurological signs and symptoms (and dysarthria and tremor improved after CoQ supplementation), and a decreased incorporation of radiolabeled 4-[U-^{14}C]HB in fibroblasts was also observed. These data would suggest that probably both cases have the same disease, although only the molecular identification of the disease will elucidate this question.

In conclusion, cerebellar ataxia associated with CoQ deficiency in our case might be allocated in the trans-prenylation pathway or in the metabolic steps after condensation of 4-hydroxybenzoate and the prenyl side chain of CoQ. Fibroblast analysis of CoQ biosynthesis provided useful information for the diagnosis of these patients. Clinical improvement after CoQ supplementation was remarkable, supporting the importance of an early diagnosis of this kind of disorders.

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