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SHORT REPORT

Primary coenzyme Q_{10} deficiency presenting as fatal neonatal multiorgan failure

Maria Andrea Desbats[1,2], Annalisa Vetro[3], Ivan Limongelli[4,5], Giada Lunardi[1,2], Alberto Casarin[1,2], Mara Doimo[1,2], Marco Spinazzi[6], Corrado Angelini[6,7], Giovanna Cenacchi[8], Alberto Burlina[9], Maria Angeles Rodriguez Hernandez[10], Lino Chiandetti[11], Maurizio Clementi[1,2], Eva Trevisson[1,2], Placido Navas[10], Orsetta Zuffardi*[,5,12] and Leonardo Salviati*[,1,2]

Coenzyme Q_{10} deficiency is a clinically and genetically heterogeneous disorder, with manifestations that may range from fatal neonatal multisystem failure, to adult-onset encephalopathy. We report a patient who presented at birth with severe lactic acidosis, proteinuria, dicarboxylic aciduria, and hepatic insufficiency. She also had dilation of left ventricle on echocardiography. Her neurological condition rapidly worsened and despite aggressive care she died at 23 h of life. Muscle histology displayed lipid accumulation. Electron microscopy showed markedly swollen mitochondria with fragmented cristae. Respiratory-chain enzymatic assays showed a reduction of combined activities of complex I+III and II+III with normal activities of isolated complexes. The defect was confirmed in fibroblasts, where it could be rescued by supplementing the culture medium with 10 μM coenzyme Q_{10}. Coenzyme Q_{10} levels were reduced (28% of controls) in these cells. We performed exome sequencing and focused the analysis on genes involved in coenzyme Q_{10} biosynthesis. The patient harbored a homozygous c.545T>G, p.(Met182Arg) alteration in *COQ2*, which was validated by functional complementation in yeast. In this case the biochemical and morphological features were essential to direct the genetic diagnosis. The parents had another pregnancy after the biochemical diagnosis was established, but before the identification of the genetic defect. Because of the potentially high recurrence risk, and given the importance of early CoQ_{10} supplementation, we decided to treat with CoQ_{10} the newborn child pending the results of the biochemical assays. Clinicians should consider a similar management in siblings of patients with CoQ10 deficiency without a genetic diagnosis.

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INTRODUCTION

Coenzyme Q_{10} (CoQ_{10}) is a component of crucial cellular pathways, among which the mitochondrial respiratory chain (RC), and beta oxidation. CoQ_{10} biosynthesis is a still incompletely characterized pathway that involves at least 15 genes (COQ genes). Primary CoQ_{10} deficiency is a clinically and genetically heterogeneous disorder caused by pathogenic variants in COQ genes.[1] *COQ2* encodes the prenyl-transferase that catalyzes the condensation of the ring precusor to the polyprenyl tail of CoQ_{10}.[2] *COQ2* mutations have been associated to an extreme variety of phenotypes ranging from a fatal neonatal multisystem disorder to an senile-onset encephalopathy resembling multiple system atrophy.[1,3] The severe, neonatal-onset phenotype has been reported only in two kindreds[4,5] and is characterized by onset at birth or in the first days of life of multiorgan failure rapidly leading to death. *COQ2* patients usually respond well to oral CoQ_{10} supplementation,[6] although those with the neonatal onset usually are diagnosed post mortem.

CLINICAL REPORT AND METHODS

A girl, the third child of a consanguineous couple, was born at 38 weeks of gestation after an uneventful pregnancy. Apgar scores were reported 9 at 1′ and 10 at 5′. Weight, length, and head circumference were between 10th and 25th centile. Gas analysis on chord blood was normal. At 1 h of life she was admitted to the intensive care unit because of oxygen desaturation and hyporeactivity. She was intubated and mechanically ventilated. She had metabolic acidosis with marked lactic acidosis and was treated with inotropic agents because ecocardiography revealed a hypokinetic and dilated left ventricle. In the following hours metabolic acidosis persisted despite of aggressive treatment. There were no signs of infection, but there was evidence of hepatic insufficiency (low albumin and prolonged PT despite vitamin-K administration), there was also proteinuria with tubular normal function. Metabolic studies were relevant for revealed ketosis, mild dicarboxylic aciduria, and slightly elevated alanine. Relevant laboratory investigations are reported in Table 1. Muscle and skin biopsies were

[1]Clinical Genetics Unit, Department of Woman and Child Health, University of Padova, Padova, Italy; [2]IRP Città della Speranza, Padova, Italy; [3]Biotechnology Research Laboratory, Foundation IRCCS, Policlinico San Matteo, Pavia, Italy; [4]Department of Industrial and Information Engineering, University of Pavia, Pavia, Italy; [5]Department of Molecular Medicine, University of Pavia, Pavia, Italy; [6]Neuromuscular Laboratory, Department of Neurosciences, University of Padova, Padova, Italy; [7]Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) San Camillo, Venice, Italy; [8]Department of Radiological and Histocytopathological Sciences, University of Bologna, Bologna, Italy; [9]Metabolic Disorders Unit, Azienda Ospedaliera di Padova, Padova, Italy; [10]Centro Andaluz de Biología del Desarrollo (CABD-CSIC), Universidad Pablo Olavide, and CIBERER, Instituto de Salud Carlos III, Carretera de Utrera Km. 1, Sevilla, Spain; [11]Neonatal Intensive Care Unit, Department of Woman and Child Health, University of Padova, Italy; [12]Mondino National Institute of Neurology Foundation, IRCCS, Pavia, Italy

*Correspondence: O Zuffardi, Department of Molecular Medicine, University of Pavia, Pavia 27100, Italy. Tel: +39 038 298 7733; Fax: +39 038 252 5030; E-mail: zuffardi@unipv.it or Professor L Salviati, Department of Woman and Child Health, University of Padova, via Giustiuniani 3, Padova 35128, Italy. Tel: +39 049 821 1402; Fax: +39 049 821 7619; E-mail: leonardo.salviati@unipd.it

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Table 1 Relevant laboratory results

	Birth	*1 h*	*6 h*	*16 h*	*20 h*
Blood Gas	pH 7.32; pCO_2 41.1 mm Hg, HCO_3^- 21.1 mEq/L; BE = 4.6 mmol/L (venous)	pH 6.74; pCO_2 47.7 mm Hg, HCO_3^- 6.4 mEq/L; BE = 29.7 mmol/L (arterial)	(other parameters not reported) HCO_3^- 9.8 mEq/L; (arterial)	(other parameters not reported) HCO_3^- 8.7 mEq/L; (arterial)	pH 7.06; pCO_2 46.4 mm Hg, HCO_3^- 10.1 mEq/L; BE = 18 mmol/L (arterial)
Lactic acid (mmol/L; normal <2.3)		10	25	28	45
Ammonia (μmol/L; normal <90)		177	102	112	
Aspartate aminotransferase (U/L; normal <150)		220	289	489	
Alanine aminotransferase (U/L; normal <40)		42	41	50	
C-reactive protein (mg/L; normal >6		2.5		2.9	
PT (INR, normal <1.3)			1.6	1.8	

Other analyses (performed at 6 h of life).
Complete blood count (at 1, 6, and 16 h): normal; Plasma amino acid, normal (except alanine 817 umol/l–normal <500); Acetylcarnitine profile on blood spot: normal; Albumin 17 g/l (normal >30).
Microbiology: blood cultures for bacteria, fungi, and chlamydia; serologies for TORCH, HSV1, 2 and 6, adenovirus, coxackievirus, and chlamydia; All normal.
Urinary: Protenuria (protein/creatinine ratio 500 mg/g–normal <80); Tubular function: normal; Mild dicarboxylic aciduria and ketonuria.

obtained at 16 h; neurological function progressively deteriorated, lactic acidosis worsened, and the patient died at 23 h of life.

All diagnostic procedures were carried out with the informed consent of the parents.

Muscle histology and histochemistry were performed using standard methods. Respiratory-chain enzyme activities were assayed spectrophotometrically as described.[7] CoQ_{10} content and biosyntetic rates in fibroblasts were measured as described.[8,9]

The unaffected 3-year-old brother had normal growth and psychomotor development, neurological examination, urine analysis, plasma lactic acid, and hearing function were normal.

Whole-exome capture, sequencing, and bioinformatic analysis were performed as reported.[10] The *COQ2* nomenclature follows that of Quinzii *et al.*[11] and is based on the sequence NM_015697.7.

Yeast media and growth conditions were previously reported.[12] The coq2 gene was inactivated by homologous recombination with a *KANMX4* cassette in a haploid W303 strain. The mutants were then transformed with a pCM189 plasmid containing the different *COQ2* sequences and a URA3 selection marker. The p.Met182Arg variant was introduced in the construct by site-specific mutagenesis as previously reported.[13]

RESULTS

Muscle studies

Histological analysis of frozen-muscle sections revealed an essentially normal pattern for COX, with some sparse mitochondrial granulations on SDH and NADH-TR (Figure 1a–c). ATPase stain showed prevalence of type-II fibers (75% of total) with hypotrophy of type-I fibers (Figure 1d), there were increased lipids with the oil-red O stain (Figure 1e), and fiber-size variability (Figure 1f).

Electron microscopy showed focal areas of loss of myofibrillar organization, and accumulation of granular-amorphous material in subsarcolemmal and intermyofibrillar regions. Mitochondria displayed degenerative features with swollen appearance, fragmented cristae and partial loss of inner matrix (Figure 1g and h).

Activities of RC complexes I,II,III, and IV, were normal, whereas those of I+III and II+III were reduced (Figure 2a).

Fibroblast studies

RC analysis was repeated on cultured skin fibroblast and confirmed muscle data. Interestingly, complex II+III activity could be rescued both by the addition of 10 μM CoQ_{10} to the culture medium, and by addition of decylubiquinone,[14] a short-chain analog of CoQ, to the reaction cuvette (Figure 2b). Because these data were highly suggestive of CoQ deficiency we measured CoQ_{10} levels in fibroblasts, and found that they were reduced to 28% of controls (33 ± 4 pmol/mg protein; controls 120 ± 8). Incorporation of ^{14}C-labeled 4-hydroxybenzoate, which reflects the CoQ_{10} biosynthetic rate, was also reduced to 19 ± 11% of controls.

Molecular studies

Because of the high number of genes involved in CoQ biosynthesis,[15] we performed whole-exome sequencing in the patient, her unaffected brother and the two parents. We obtained a mean-target coverage of 63x for the four samples (Supplementary Table S1). After initial filtering, we focused on the 22 038 variants non-synonymous or possibly affecting splicing. Among them we considered those homozygous in the proband but not in her healthy brother, and heterozygous in the parents ($n = 174$). This number was further reduced by filtering out those reported in our in-house database (100 unrelated exomes), or in dbSNP135 or ESP6500SI-V2 (http://evs.gs.washington.edu/EVS/) with a frequency above 1% (Supplementary Table S2). Ranking of variants on the basis of prediction tools results highlighted 11 candidates that were further stratified on the basis of their possible role in the mitochondrion (Supplementary Table S3). Because of the biochemical data *COQ2* appeared to be the most obvious.

The homozygous missense variant c.545T>G (p.Met182Arg) in *COQ2* was confirmed by Sanger sequencing (Figure 2c). The variant was absent in the 1000-genome database. Even though methionine 182 is conserved only in mammals, other species always display a hydrophobic residue in this position (Figure 2d). Data were submitted to the LOVD database (http://www.lovd.nl/COQ2; Patient ID 00010537).

No further variants were identified in genes involved in CoQ biosynthesis.

Yeast studies

To confirm the pathogenicity of this variant we employed functional complementation in yeast. We developed a novel system based on the pCM189 plasmid and the W303 strain. The deleted yeast (Δcoq2) cannot grow on non-fermentable media, but transformation with either the yeast or the human cDNA rescued the respiratory phenotype. Instead, strains transformed with the human p.Met182Arg variant displayed a reduction of growth in selective medium (Figure 2e) and of CII+III activity (which directly reflects CoQ_6 levels;[16] Figure 2f).

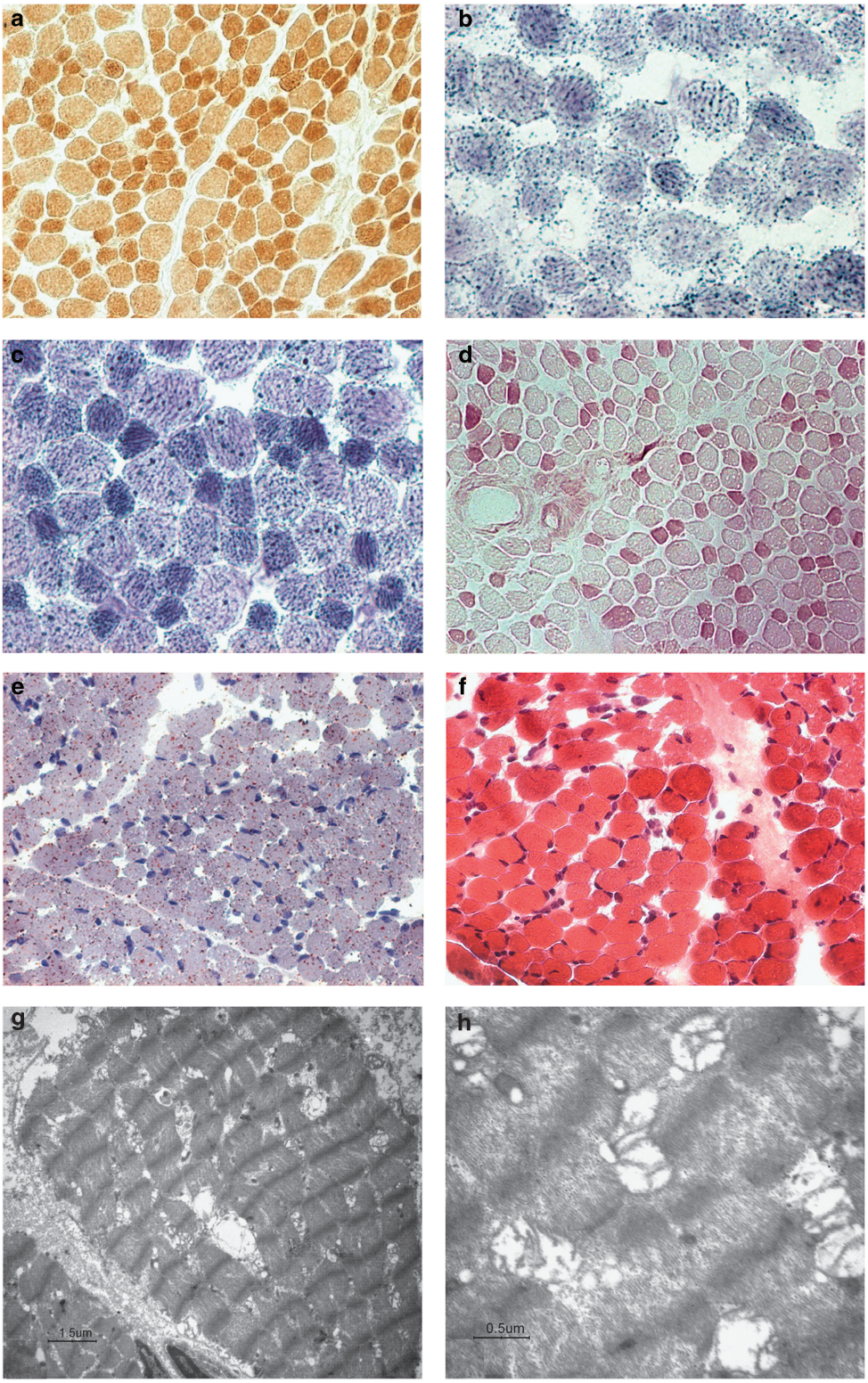

Figure 1 Histochemical and ultrastructural feature of muscle biopsy. Cross sections of quadriceps femoris-muscle biopsy stained for cytochrome C oxidase (**a**), Succinate dehydrogenase (**b**), NADH-TR reductase (**c**), acid ATPase (**d**), Oil-Red-O (**e**), Hematoxylin-eosin (**f**), showing hypotrophy of type-1 fibers (**a**, **d**), increased reaction of mitochondria that appear as distinct granules (**b**, **c**), increased triglyceride droplets (**e**) and variability of fiber size (**f**). Microscope magnification x200 (**a**, **d**–**f**) and x400 (**b**, **c**). Muscle transmission electron microscopy (**g**, **h**) showing focal areas of loss of myofibrillar organization, accumulation of granular-amorphous material in subsarcolemmal and intermyofibrillar region (**g**). The mitochondria show degenerative features with swallowed appearance, fragmented cristae, and partial loss of inner matrix (**h**). The enlargement is indicated by the internal bars.

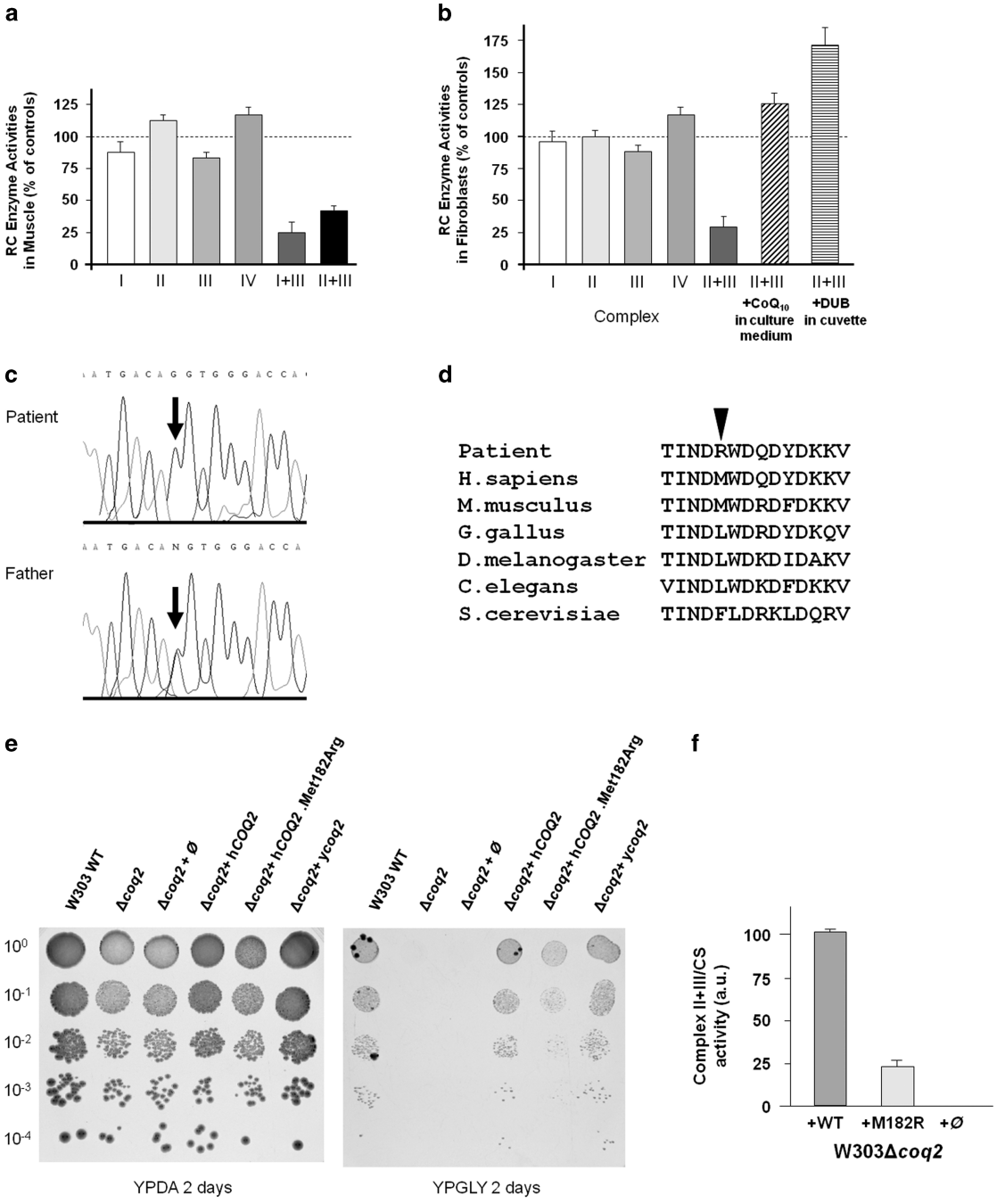

Figure 2 Biochemical and genetic studies. (**a**) Spectrophotometric analysis of RC enzyme activities in frozen muscle and (**b**) in cultured skin fibroblasts of the patient. (**c**) Sequence of the COQ2 gene in the patient and in the father with the homozygous or heterozygous c.545T>G (p.Met182Arg) variant. (**d**) Sequence conservation of methionine 182 of COQ2 in different species. (**e**) Functional complementation in yeast. Serial dilution of wild-type W303 or Δcoq2 yeast transformed with the empty vector (Ø), the wild-type human gene (*hCOQ2*), the mutant (*hCOQ2* p.Met182Arg), or the wild-type yeast gene (ycoq2) were plated in glucose medium (YPDA) or non-fermentable glycerol medium (YPGLY) and grown for 2 days at 28 °C. (**f**) Complex II+III activity in isolated mitochondria of Δcoq2 yeast transformed with wild type or mutant human *COQ2*.

Management of the younger sister of the patient

The patient's mother was pregnant again two months after the patient's death. In the absence of a molecular defect we could not perform prenatal diagnosis. However, by the time the baby was born we had established the biochemical diagnosis of CoQ_{10} deficiency. Therefore, immediately after delivery a skin biopsy was obtained to perform CoQ_{10} measurements and, pending the results, CoQ_{10} supplementation 30 mg/kg/day was started. After 4 weeks, CoQ_{10} levels and complex II+III activity in fibroblasts were found normal and supplementation was suspended. She was later found to be a heterozygous carrier of the variant.

DISCUSSION

Several metabolic disorders may manifest at birth with fatal multiorgan failure and represent a major diagnostic challenge. In this patient several features pointed to a RC defect, particularly to CoQ_{10}

deficiency. Severe lactic acidosis is not typical of CoQ_{10} deficiency, but it is reported in neonatal cases.[4,5] The patient had proteinuria, a hallmark of the disease,[17] whereas renal tubular function was apparently preserved. There were signs of hepatic dysfunction (prolonged PT and hypoalbuminemia) without evidence of cytolysis. The heart was also affected, although there were no clear signs of hypertrophic cardiomyopathy, which was described in some infantile *COQ2* cases.[18,19]

Muscle morphology was rather aspecific, however there were signs of lipid accumulation, another common finding in CoQ_{10} deficiency.[15] The markedly altered aspect of mitochondria on electron microscopy was also another important clue for the diagnosis.

However, the crucial findings were the reduced activity of complex II+III (and I+III) in the presence of normal activities of the isolated complexes. This result is highly suggestive of CoQ_{10} deficiency and correlates quite well with residual CoQ_{10} levels.[14,16] We stress on the importance of including measurement of complex II+III when assaying the RC, because it is a simple and reliable assay which allows the indirect diagnosis of CoQ_{10} deficiency, whereas direct determination of CoQ_{10} is performed only in a few selected centers.[15] Moreover, especially in neonates, there may not be enough sample for both biochemical studies and direct CoQ_{10} determination.

Given the genetic heterogeneity of CoQ_{10} deficiency the optimal strategy for genetic testing is debated.[20] We chose exome sequencing because it can also identify secondary causes of CoQ_{10} deficiency, which in principle cannot be ruled out.

Yeast was essential for validating the *COQ2* variant. Compared with the previous system we used,[9] this has higher sensitivity to detect residual enzymatic activity, the plasmid has a stable copy number, and expression is not driven by galactose, therefore it is ideal for studying genotype–phenotype correlations.

The importance of a genetic diagnosis is obvious. Nevertheless, a correct biochemical diagnosis also has critical implications for counseling and for the management of future pregnancies. The newborn sister had a 25% probability of being affected too. The lack of clinical manifestations at birth did not, in principle, rule CoQ_{10} deficiency because of the clinical variability (onset of manifestations may occur also some time after birth).[4] Moreover, early treatment has the highest probability of success.[6] Given the safety and tolerability of CoQ_{10} we decided to treat the child pending the results of the skin biopsy. Clinicians should consider a similar management in siblings of patients with CoQ_{10} deficiency without a genetic diagnosis. Future studies should be aimed at evaluating whether CoQ_{10} supplementation to pregnant mothers could represent a therapeutic option for these families.

This case underscores the importance, even in the era of genomic sequencing, of collecting adequate biological samples in patients with these catastrophic neonatal diseases. In fact a precise biochemical diagnosis facilitates immensly the interpretation of genomic data, which at present is still a major obstacle to the implementation of exome sequencing for routine diagnosis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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