Primary coenzyme Q₁₀ deficiency presenting as fatal neonatal multiorgan failure

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Coenzyme Q₁₀ 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 Q₁₀. Coenzyme Q₁₀ levels were reduced (28% of controls) in these cells. We performed exome sequencing and focused the analysis on genes involved in coenzyme Q₁₀ 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 CoQ₁₀ supplementation, we decided to treat with CoQ₁₀ 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 Q₁₀ (CoQ₁₀) is a component of crucial cellular pathways, among which the mitochondrial respiratory chain (RC), and beta oxidation. CoQ₁₀ biosynthesis is a still incompletely characterized pathway that involves at least 15 genes (COQ genes). Primary CoQ₁₀ deficiency is a clinically and genetically heterogeneous disorder caused by pathogenic variants in COQ genes.¹ COQ2 encodes the prenyltransferase that catalyzes the condensation of the ring precursor to the polyisoprenyl tail of CoQ₁₀.² 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.¹³ The severe, neonatal-onset phenotype has been reported only in two kindreds,³,⁴ 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 CoQ₁₀ 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 of respiratory failure. 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

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Table 1 Relevant laboratory results

| Blood Gas               | Birth       | 1 h          | 6 h          | 16 h         | 20 h         |
|-------------------------|-------------|--------------|--------------|--------------|--------------|
| pH 7.32;                | pH 6.74;    | (other parameters not reported) | (other parameters not reported) | pH 7.06;    |
| pCO2 41.1 mm Hg;        | pCO2 47.7 mm Hg; | HCO3− 21.1 mEq/L; | HCO3− 9.8 mEq/L; | pCO2 46.4 mm Hg; |
| HCO3− = 4.6 mmol/L (venous) | HCO3− = 29.7 mmol/L (arterial) | (arterial) | (arterial) | HCO3− = 10.1 mEq/L; |
| BE = 18 mmol/L (arterial) | (arterial) | (arterial) | (arterial) | BE = 18 mmol/L (arterial) |

Lactic acid (mmol/L; normal <2.3) 10 25 28 45
Ammonia (µmol/L; normal <90) 177 289 489
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 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 B17 umol/l-normal <500); Acetyl carnitine 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: Proteinuria (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 assessed spectrophotometrically as described. CoQ10 content and biosynthetic rates in fibroblasts were measured as described.

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. The COQ2 nomenclature follows that of Quinzi et al. and is based on the sequence NM_015697.7.

Yeast media and growth conditions were previously reported. 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.

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 CoQ10 to the culture medium, and by addition of decylubiquinone, a short-chain analog of CoQ, to the reaction cuvette (Figure 2b). Because these data were highly suggestive of CoQ deficiency we measured CoQ10 levels in fibroblasts, and found that they were reduced to 28% of controls (33 ± 4 pmol/mg protein; controls 120 ± 8). Incorporation of 14C-labeled 4-hydroxybenzoate, which reflects the CoQ10 biosynthetic rate, was also reduced to 19 ± 11% of controls.

Molecular studies

Because of the high number of genes involved in CoQ biosynthesis, 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 homoygous 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/ESVS/) 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 homoygous 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 CoQ6 levels; 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.
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 CoQ10 deficiency. Therefore, immediately after delivery a skin biopsy was obtained to perform CoQ10 measurements and, pending the results, CoQ10 supplementation 30 mg/kg/day was started. After 4 weeks, CoQ10 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 CoQ10 deficiency.
deficiency. Severe lactic acidosis is not typical of CoQ10 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.15,19

Muscle morphology was rather aspecific, however there were signs of lipid accumulation, another common finding in COQ10 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 CoQ10 deficiency and correlates quite well with residual CoQ10 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 CoQ10 deficiency, whereas direct determination of CoQ10 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 CoQ10 determination.

Given the genetic heterogeneity of CoQ10 deficiency the optimal strategy for genetic testing is debated.20 We chose exome sequencing because it can also identify secondary causes of CoQ10 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 biochemical studies and direct CoQ10 determination. especially in neonates, there may not be enough sample for both biochemical studies and direct CoQ10 determination.

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 CoQ10 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 CoQ10 we decided to treat the child pending the results of the skin biopsy. Clinicians should consider a similar management in siblings of patients with CoQ10 deficiency without a genetic diagnosis. Future studies should be aimed at evaluating whether CoQ10 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 immensely 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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