Clinical phenotype, in silico and biomedical analyses, and intervention for an East Asian population-specific c.370G>A (p.G124S) *COQ4* mutation in a Chinese family with CoQ10 deficiency-associated Leigh syndrome

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Received: 5 October 2018 / Revised: 19 December 2018 / Accepted: 4 January 2019 / Published online: 18 January 2019
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Abstract

COQ4 mutations have recently been shown to cause a broad spectrum of mitochondrial disorders in association with CoQ10 deficiency. Herein, we report the clinical phenotype, in silico and biochemical analyses, and intervention for a novel c.370 G>A (p.G124S) *COQ4* mutation in a Chinese family. This mutation is exclusively present in the East Asian population (allele frequency of ~0.001). The homozygous mutation caused CoQ10 deficiency-associated Leigh syndrome with an onset at 1–2 months of age, presenting as respiratory distress, lactic acidosis, dystonia, seizures, failure to thrive, and detectable lesions in the midbrain and basal ganglia. No renal impairment was involved. The levels of CoQ10 and mitochondrial respiratory chain complex (C) II + III activity were clearly lower in cultured fibroblasts derived from the patient than in those from unaffected carriers; the decreased CII + III activity could be increased by CoQ10 treatment. Follow-up studies suggested that our patient benefitted from the oral supplementation of CoQ10, which allowed her to maintain a relatively stable health status. Based on the genetic testing, preimplantation and prenatal diagnoses were performed, confirming that the next offspring of this family was unaffected. Our cases expand the phenotypic spectrum of *COQ4* mutations and the genotypic spectrum of Leigh syndrome.

Introduction

Human coenzyme Q (CoQ), also known as CoQ10 or ubiquinone, is a 1,4-benzoquinone with a tail of ten

isoprenyl units. CoQ10 plays an important role in the mitochondrial respiratory chain (MRC) by shuttling electrons from complex I and II to complex III [1]. In addition, CoQ is involved in pyrimidine biosynthesis and fatty acid β -oxidation, and may be involved in apoptosis [2–4].

Thus far, more than ten genes have been shown to be involved in CoQ10 biosynthesis [5]. Mutations in these genes can induce primary CoQ10 deficiency, a group of rare, clinically heterogeneous autosomal recessive disorders presenting as multisystem manifestations, including progressive encephalomyopathy and nephropathy [6, 7].

COQ4 encodes a ubiquitously expressed 265-aa protein localized to the matrix side of the mitochondrial inner membrane [8]. *COQ4* is involved in CoQ biosynthesis; however, its exact function is unknown. A study on the yeast ortholog of *COQ4* suggested its crucial structural role in the stabilization of a multi-heteromeric complex including several CoQ biosynthetic enzymes [9]. A decade ago, human *COQ4* was shown to efficiently restore the growth and CoQ content of *COQ4*^{null} yeast strains, and it was proposed as a candidate gene for patients with isolated

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CoQ10 deficiency [8]. Thus far, 15 *COQ4* mutations have been reported in 14 patients in association with primary CoQ10 deficiency, confirming these previous viewpoints [4, 10–13]. Notably, a founder *COQ4* mutation was also identified in the Ashkenazi Jewish population [4]. In this study, we report a novel CoQ deficiency-associated *COQ4* mutation in the East Asian population, along with its clinical presentation, in silico and biochemical analyses, and corresponding interventions.

Materials and methods

Samples and DNA extraction

Peripheral blood and amniotic fluid samples were collected from members of the patients' family. Genomic DNA was extracted from these samples using the Super/HF16 plus DNA Extraction System (MagCore, Xiamen, China), according to the manufacturer's protocol. DNA samples from 1500 healthy Chinese adults were also collected from a sample database at the Xiamen Maternal and Child Health Hospital.

Whole-exome sequencing

DNA samples from the two patients and their parents were analyzed by commercial whole-exome sequencing (WES; Novogene, Beijing, China). The exome sequences from 1.0 µg of genomic DNA were enriched using a liquid capture system (Agilent SureSelect Human All Exon V5; Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's protocol, and sequenced on the HiSeq4000 platform (Illumina, San Diego, CA, USA) to generate 150-bp paired-end reads. The mean exome coverage was 100×, allowing for the examination of the selected region with sufficient depth to accurately match with 99% of the targeted exome.

In silico analysis

The sequence conservation of *COQ4* was examined using the HomoloGene database, the functional importance of the mutation was predicted using SIFT, PolyPhen-2, and Mutation Taster databases, and the allele frequency of the mutation was examined using the Exome Aggregation Consortium (ExAC) Browser.

Allele-specific PCR

A duplex allele-specific PCR assay was designed for the population-based screening of the mutation in 1500 healthy Chinese adults. This assay was also used for the prenatal

diagnosis of the mutation. The sequences of primers are 5'-CGACATCCACCCTCGACCT-3' and 5'-CCAGATACA CAGGAGCTGAA-3'. The sequences of the hydrolysis probes are 5'-FAM-CTCCCTCGGTGCGA-BHQ1-3' for the wild-type allele and 5'-HEX-TCGCGACCGAGGG AG-BHQ1-3' for the mutant allele. The total volume of the reaction mixture was 25 µL; the reaction mixture contained 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1 U of TaqHS (Takara, Dalian, China), 2.0 mmol/L Mg²⁺, 0.2 mmol/L of each deoxynucleoside triphosphate, and 0.2 µmol/L of each primer and probe. The cycling conditions were as follows: 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 20 s, and 72 °C for 20 s. Fluorescence data from the FAM and HEX channels were collected at the end of the annealing step on a LightCycler 480 II thermocycler (Roche Diagnostics, Roche Instrument Center AG, Rotkreuz, Switzerland).

Biochemical analysis of cultured fibroblasts

Fibroblasts were obtained from patient II-2 and her parents via skin biopsies and were cultured in Dulbecco's modified eagle medium containing 20% fetal calf serum. The CoQ10 content of the cultured fibroblasts was assessed using the Human coenzyme Q10 (CoQ10) ELISA Kit (CUSABIO, Wuhan, China), according to the manufacturer's protocol. Mitochondria were isolated from the cultured fibroblasts, and the activities of the MRC enzymes were assessed using MitoCheck® series kits (Cayman Chemical, Ann Arbor, MI, USA), according to the manufacturer's instructions.

Informed consent

Written informed consent to conduct the genetic tests and publish the research data was obtained from the patients' guardians. The study protocol was approved by the ethics committee of the Xiamen Maternal and Child Health Hospital.

Results

Report of cases

Patient II-1 was a Chinese boy who was born at 39 weeks of gestation via spontaneous vaginal delivery after an uneventful pregnancy. According to his parents, the patient showed a failure to thrive, progressive motor deterioration, weak responsiveness, hearing impairment, feeding difficulty, and dystonia after birth. Phenobarbital and levetiracetam were administered when complex partial seizures were noted at 2 months of age. However, there was no clinical response. A magnetic resonance imaging (MRI) of

Fig. 1 Imaging of patients II-1 and II-2. **a–f** Symmetrical lesions in the midbrain were noted in the axial diffusion-weighted imaging of patient II-1 at the age of 5 months and 18 days. **g** No evident brain abnormality was noted in the axial T1-weighted imaging of patient II-2 at the age of 1 month. Axial diffusion-weighted imaging of patient II-2 at the age of 4 months indicated lesions in the midbrain (**h–j**) and basal

ganglia (**k–m**). Multiple-voxel proton MR spectroscopy (TE = 144 ms) of the basal ganglia displayed an elevated lactate (lower arrow) and a reduced N-acetyl-aspartate peak (upper arrow, in **n**). The axial computed tomography (CT) scan of patient II-2 at the age of 3 years and 8 months indicated symmetrical, patchy, low-density shadows in the bilateral basal ganglia (**o**). Diffuse brain atrophy was also noted (**p**)

the brain was performed at another hospital, and no abnormality was noted except for slightly widened bilateral frontal and temporal lobes (no image was obtained). He was sent to our hospital at the age of 5 months and 18 days with respiratory distress, vomiting, and tachycardia. He received continuous positive airway pressure after hospitalization, but rapidly deteriorated and required invasive ventilation due to severe respiratory distress; he eventually fell into a coma. He displayed severe lactic acidosis

(blood lactate = 15.1 mM, reference range < 2 mM), hyperglycemia (blood glucose = 27.4 mM, reference range = 2.8–6.2 mM), and hyperammonemia (blood ammonia = 79 μ M, reference range = 20–45 μ M). Bilateral, symmetrical lesions in the midbrain were noted on the MRI (Fig. 1a–f). Mild cardiomegaly (cardiothoracic ratio = 0.63) was indicated on the chest X-ray; however, the ultrasonic cardiogram examination was not performed due to the rapid demise of the patient. There were no significant findings in

Fig. 2 Pedigree and conservation analysis of the mutation. **a** The black circle and square represent the affected female and male patients, respectively, and the open circles and square represent unaffected

females and male, respectively. **b** The mutated position is highly conserved across species

the karyotyping, mitochondrial genome sequencing, and analyses of metabolic profiles, including the levels of urinary and plasmatic amino acids, organic acids, and acylcarnitines, and no evidence of renal impairment was observed. He was diagnosed with mitochondrial encephalopathy. Although he was admitted to the pediatric intensive care unit and received treatment, his condition continued to deteriorate. Eventually, with the agreement of his parents, ventilator support was withdrawn, and he died at the age of 5 months and 20 days.

Patient II-2 is the sister of patient II-1, and she was also born at term via spontaneous vaginal delivery. The pregnancy was uncomplicated, with normal serology and ultrasounds. The Apgar scores were also normal. Like her brother, she presented with a failure to thrive, progressive motor deterioration, weak responsiveness, feeding difficulty, and dystonia after birth. There were no significant findings on the brain MRI at the age of 1 month (Fig. 1g). At 2 months of age, she displayed nystagmus, respiratory distress, severe lactic acidosis (blood lactate = 17.6 mM), hyperglycemia (blood glucose = 22.9 mM), and hyperammonemia (blood ammonia = 122 μ M). The electroencephalograph showed generally normal background activity with occasional spikes in the left parietal region during rapid eyes movement sleep. At the age of 4 months, screaming and seizures were observed. At this time point, lesions were noted in the midbrain (Fig. 1h–j) and basal ganglia (Fig. 1k–n). There were no significant findings in the karyotyping, mitochondrial genome sequencing, and metabolic profiles, and no evidence of cardiac or renal impairment was observed. She was continuously treated with levetiracetam and phenobarbital for seizure management. At the same time, CoQ10 (5 mg/day) and carnitine (50 mg/kg/day) were administered to empirically treat the presentations of mitochondrial disease. After medication, the seizures, screaming, and respiratory distress improved. However, there were no

evident improvements in nystagmus, dystonia, psychomotor development, or ambulation.

At 12 months of age, patient II-2 underwent genetic testing by WES. DNA samples from patient II-1 and her parents were also tested. A homozygous c.370 G > A (p. G124S) mutation in *COQ4* was detected in patients II-1 and II-2 by WES, while their parents were heterozygous for this mutation (Fig. 2a). This mutation was confirmed by Sanger sequencing. After genetic testing, biochemical analyses were performed with the cultured fibroblasts of patient II-2 and her parents.

In silico analysis of the mutation

Analysis using the HomoloGene database showed that the Gly124 in *COQ4* is highly conserved among species (Fig. 2b). Based on the SIFT (score: 0.000) and PolyPhen-2 (score: 0.999 for HumDiv model and 0.910 for HumVar model, respectively) results, the p.G124S mutation was predicted to probably be damaging to the structure/function of the protein. Similarly, based on the Mutation Taster results (probability: 1.000), this mutation was predicted to be disease causing. Analysis using the ExAC database showed that this mutation was exclusively found in the East Asian population, with an allele frequency of 0.001272 (11/8650, accessed on 8/8/2018). We then screened 1500 healthy Chinese adults for this mutation and found three carriers, which indicated a carrier frequency of 0.002 and an allele frequency of 0.001.

Biochemical analysis of the mutation

As shown in Fig. 3, the CS-normalized CoQ10 level and mitochondrial respiratory chain complex (C) II + III activity were clearly decreased in cultured fibroblasts from patient II-2, while no evident decrease in CI, CII, and CIV activities was detected. Moreover, when the fibroblasts of patient II-2

Fig. 3 Biochemical analysis of cultured fibroblasts derived from affected patient (II-2) and her parents (I-1 and I-2). **a** CoQ10 content. **b–f** Mitochondrial respiratory chain enzyme activities in cultured fibroblasts with or without CoQ10 supplement

were cultured with 10 mM CoQ10, a clear increase in CII + III activity was observed (Fig. 3d).

Treatment with coenzyme Q10

Immediately after the mutation was identified by WES in patient II-2 at the age of 12 months, she began taking the following oral supplementations: CoQ10, 50 mg/kg/day and thiamine, 30 mg/day, divided into three doses per day. Since starting the supplementation, no respiratory distress, screaming, or seizures were observed, and nystagmus occurred infrequently. In addition, her responsiveness and feeding difficulties had improved. However, there was no evident improvement in dystonia, ambulation, or speech. At the age of three years and 8 months, she underwent a physical examination. She was 100 cm tall and weighed 13 kg. She was unable to speak or ambulate, but was able to make some spontaneous sounds and laugh. Due to her noncooperation, brain CT was performed instead of an MRI. Symmetrical, patchy, low-density shadows were observed in the bilateral basal ganglia, and diffuse brain atrophy was noted (Fig. 1o, p).

Preimplantation and prenatal diagnosis of the *COQ4* mutation

After the in silico and biochemical analyses, the parents of the patient underwent assisted reproduction, and preimplantation genetic diagnosis was performed at the

Reproductive and Genetic Hospital of CITIC-Xiangya. According to the medical records, an embryo with a heterozygous c.370 G > A mutation was implanted. At 17 weeks of gestation, amniocentesis was performed in our hospital, and the prenatal diagnosis was confirmed by the fetal genotype. In June 2018, a healthy baby (II-3) was labored at the gestational age of 39 weeks and 4 days.

Discussion

As shown in Table 1, phenotypic heterogeneity in clinical presentations and biochemical findings is evident with *COQ4* mutations [4, 10–13]. The exact mechanism underlying this phenotypic heterogeneity is unclear. It could be related to the different *COQ4* mutations and/or other genetic variants present elsewhere in the genome that cause variable levels of CoQ10 deficiency and various adaptive responses to the reduced CoQ10 availability. Unlike most cases in previous studies, in our cases, dystonia was observed instead of hypotonia, and the midbrain and basal ganglia were the major affected brain regions observed by MRI. In addition, the onset of notable presentations, such as seizures, respiratory distress, lactic acidosis, and MRI-detectable brain lesions in our cases occurred at later time point than in the other early-onset cases. Therefore, our cases expand the phenotypic spectrum of *COQ4* mutations. Although phenotypic heterogeneity exists, studies of the characteristic phenotypes of

Table 1 Clinical presentations and biochemical findings in *COQ4* mutation cases

Item	Our study	J Med Genet. 49, 187–191 (2012)	Am J Hum Genet. 96, 309–317 (2015)	J Med Genet. 52, 627–635 (2015)	Mol Genet Metab Rep. 12, 23–27 (2017)	Mol Genet Metab Rep. 17, 19–21 (2018)	Total or % of total
Number of patients	2	1	5	6	1	2	17
Female to male	1:1	0:1	3:2	6:0	0:1	1:1	11:6
Age at presentation	1–2 months	NA	Birth–10 months	Birth–1 day	1 day	4–9 years	Birth–9 years
Age of death ^a	~6 months	NA	Birth–10 days	36h–19 min	4 months	NA	88%; ^b 100% ^b
Respiratory distress	2/2	1/1	4/4	6/6	1/1	0/2	75%; ^b 89% ^b
Cerebellar atrophy	2/2	NA	3/3	4/4	0/1	0/2	71%; ^b 86% ^b
Lactic acidosis and/or elevated cerebrospinal fluid (CSF) lactate	2/2	0/1	4/5	5/6	1/1	0/2	65%
Seizures	2/2	0/1	3/5	3/6	1/1	2/2	63%
Hypotonia	0/2	1/1	1/5	5/5	1/1	2/2	59%
Cardiomyopathy	1/2	1/1	2/5	5/6	1/1	0/2	12%
Dystonia	2/2	0/1	0/5	0/6	0/1	0/2	0
Nephropathy	0/2	0/1	0/5	0/6	0/1	NA	0
Location of brain lesions	Basal ganglia; midbrain	NA	Olivopontocerebellar and thalamic areas; white matter; occipital-cortical, and juxtacortical areas.	White matter; occipital lobes; brain stem.	Cortex	Left occipital lobe	
Respiratory chain complexes (C) abnormality							
CI	0/1	0/1	4/5	NA	0/1	NA	50%; 0 ^c
CII	0/1	0/1	1/4	NA	0/1	NA	14%; 0 ^c
CI/CIII	NA	NA	1/3	NA	1/1	NA	50%
CII/CIII	1/1	1/1	4/5	NA	1/1	NA	88%; 100% ^c
CIII	NA	0/1	3/5	NA	NA	NA	50%
CIV	0/1	0/1	3/5	NA	0/1	NA	38%; 0 ^c
CV	NA	NA	NA	NA	0/1	NA	0
CS	0/1	0/1	2/5	NA	1/1	NA	38%; 33% ^c

^aStatistic data derived from demise cases^bStatistic data derived from early-onset cases^cStatistic data derived from cultured fibroblasts

COQ4-related CoQ10 deficiency may promote the possibility of a timely diagnosis and treatment (e.g., supplementation of CoQ10), which is important for the management of this early-onset (for most cases), life-threatening disease. According to existing reports, respiratory distress, cerebellar atrophy, and lactic acidosis are the most frequent clinical presentations in cases of *COQ4* mutation, especially in early-onset cases, suggesting that these presentations are important as the first clues to the presence of a *COQ4* mutation. Moreover, consistent with previous *COQ4* mutation cases, renal impairment was not involved in our cases; this suggests that the pathogenesis of *COQ4* mutation is different from that of the primary CoQ10 deficiencies caused by other COQ genes, and may also be a useful diagnostic indicator [14] (Table 1). In terms of biochemical analyses, apart from the CoQ10 content, CII + III activity was characteristically decreased in most cases since the shuttling of electrons from CI and II to CIII was most likely affected by the CoQ10 deficiency [1]. It should be noted that some results of the biochemical analyses were derived from muscle biopsy samples [10], while others were derived from cultured fibroblast samples [11, 13]; this could partially be responsible for the heterogeneity of the MRC complex activities. Biochemical changes in cases with CoQ10 deficiencies were suggested to be more specific in fibroblasts than in muscle samples [15]. Similarly, with regards to the fibroblasts, the biochemical results of our case were highly consistent with those of two previous cases, in which CII + III was the major MRC complex affected in the *COQ4* mutation events (Table 1). Noticeably, the clinical manifestations were more severe in the male patient than in his sister, suggesting that the *COQ4* mutation is more fatal to males than females, which is in accordance with the observation of a preponderance of female patients [4].

Our cases expand the genotypic spectrum of Leigh syndrome (LS). LS is a clinical heterogeneous mitochondrial encephalomyopathy induced by abnormal energy metabolism with a defect in oxidative phosphorylation or pyruvate dehydrogenase complex activity [16, 17]. Typical presentations of LS are symmetrical lesions in the basal ganglia and/or brain stem observed by MRI, and a clinical course with rapid deterioration of cognitive and motor functions [16]. To date, more than 75 pathogenic genes have been identified in LS patients [17]. However, genetic diagnosis for many LS patients is still lacking, suggesting that more pathogenic genes may be identified in the future [18]. In this study, *COQ4* represents a newly identified pathogenic gene of LS; with regards to this gene, the presentations of our cases typically fulfill the general and even the stringent diagnostic criteria for LS [16, 17], and the overall clinical pictures of previously reported *COQ4*

mutation cases were also indicative of LS (Table 1). In fact, CoQ10 deficiency has been reported as a rare cause of LS [19, 20], and a pathogenic gene, *PDSS2*, has been identified [20]. According to existing clues [7], it could be reasonably speculated that the genes involved in CoQ10 biosynthesis could be the candidate causative genes of LS or Leigh-like syndrome.

Next-generation sequencing (NGS) is one of the most valuable tools for the clinical analysis of rare Mendelian diseases. In the past few years, NGS has identified more than 30 causative genes of LS and almost all the reported *COQ4* mutations [4, 10–12, 17]. In addition to transforming our ability to understand the genetic basis and genotype–phenotype correlation of diseases, NGS also enables the access to accurate and timely genetic counseling and intervention, e.g., medication, preimplantation, and prenatal diagnosis in our case. Therefore, with the rapid decrease of cost and turn-around time, NGS would become a first-line laboratory test for LS. However, the MRC enzyme testing of muscle tissue and/or cultured fibroblast samples, which provides essential information in the pathogenicity of identified gene variants, remains indispensable for LS diagnosis and study [18].

To date, there is no clear consensus or set of guidelines supporting the use of any interventions in LS [16]. However, CoQ10 deficiency is generally considered treatable [21]. Treatment with CoQ10 or its derivatives has been shown to be effective in previously reported cases of CoQ10 deficiency-associated LS and *COQ4* mutation [4, 10, 12, 13, 19, 22]. As seen in our case, oral supplementation with CoQ10 was also effective; patient II-2 was able to achieve and maintain a relatively stable health status, and was able to be cared for at home. However, although the treatment is beneficial for stopping or slowing down the progression of the clinical manifestations, it cannot reverse the existing damage. Therefore, some of the clinical manifestations due to brain damage, such as the loss of ambulation and speech, did not respond to CoQ10 treatment, which emphasizes the importance of early diagnosis and treatment. In general, early high-dose treatment with CoQ10 or its derivatives is recommended for the *COQ4* mutation cases. In addition to CoQ10, other drug and dietary administrations should be considered when it is necessary to deal with corresponding symptoms, e.g., acute aggravation or secondary mitochondrial dysfunction, etc [16, 22]. Noticeably, studies have revealed a broader cellular dysfunction other than the energy generation deficiency in LS, suggesting a different disease pathogenesis [23–25]. Therefore, investigation of the precise molecular mechanisms underlying the disease pathology could facilitate the development of new therapies [26, 27].

It should be noted that the current evaluation of the *COQ4* mutations was based on the limited clinical and laboratory data. Therefore, more clinical, biochemical, and

comprehensive functional studies, including more cases of *COQ4* mutations, are required to further delineate this subgroup of LS and develop therapeutic options.

In conclusion, we reported the clinical phenotype, in silico and biochemical analyses, and intervention for a c.370 G>A (p.G124S) *COQ4* mutation in a Chinese family. This mutation is exclusively present in the East Asian population, with an allele frequency of ~0.001. The homozygous mutation results in CoQ10 deficiency-associated LS, with an onset at 1–2 months of age. The patient with this mutation benefitted from the oral supplementation of CoQ10, achieving and maintaining a relatively stable health status. Our cases expand the phenotypic spectrum of *COQ4* mutations and the genotypic spectrum of LS.

Acknowledgements This work was supported by the National Natural Science Foundation of China (81572084 [to YZ]), and Xiamen Science and Technology Major Project (3502ZZ20171006 [to YZ]).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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