Leigh Syndrome with Nephropathy and CoQ_{10} Deficiency Due to *decaprenyl diphosphate synthase subunit 2* (*PDSS2*) Mutations

Luis Carlos López, Markus Schuelke, Catarina M. Quinzii, Tomotake Kanki, Richard J. T. Rodenburg, Ali Naini, Salvatore DiMauro, and Michio Hirano

Coenzyme Q_{10} (CoQ_{10}) is a vital lipophilic molecule that transfers electrons from mitochondrial respiratory chain complexes I and II to complex III. Deficiency of CoQ_{10} has been associated with diverse clinical phenotypes, but, in most patients, the molecular cause is unknown. The first defect in a CoQ_{10} biosynthetic gene, *COQ2,* was identified in a child with encephalomyopathy and nephrotic syndrome and in a younger sibling with only nephropathy. Here, we describe an infant with severe Leigh syndrome, nephrotic syndrome, and CoQ_{10} deficiency in muscle and fibroblasts and compound heterozygous mutations in the *PDSS2* gene, which encodes a subunit of decaprenyl diphosphate synthase, the first enzyme of the CoQ_{10} biosynthetic pathway. Biochemical assays with radiolabeled substrates indicated a severe defect in decaprenyl diphosphate synthase in the patient's fibroblasts. This is the first description of pathogenic mutations in *PDSS2* and confirms the molecular and clinical heterogeneity of primary CoQ_{10} deficiency.

Coenzyme Q_{10} (CoQ_{10}), a lipophilic molecule present in all cell membranes, functions as an electron carrier in the mitochondrial respiratory chain, where it transports electrons from complexes I and II to complex III. In addition, CoQ_{10} is an antioxidant, a membrane stabilizer, and a regulator of mitochondrial permeability transition pores, and its oxidized form serves as a cofactor for uncoupling proteins in brown adipose tissue.[1,2]

CoQ_{10} is composed of a benzoquinone and a decaprenyl side chain (fig. 1). Whereas the quinone ring is derived from the amino acids tyrosine or phenylalanine, the isoprenoid side chain is produced by addition of isopentenyl diphosphate molecules to farnesyl diphosphate or geranylgeranyl diphosphate (derived from mevalonate pathway) in multiple steps catalyzed by decaprenyl diphosphate synthase (fig. 1). After *para*-hydroxybenzoate (PHB) and decaprenyl diphosphate are produced, at least seven enzymes catalyze condensation, methylation, decarboxylation, and hydroxylation reactions to synthesize CoQ_{10}.[1,2]

CoQ_{10} deficiency (MIM #607426) has been associated with autosomal recessive neurological disorders that are responsive to CoQ_{10} supplementation. Clinical phenotypes include: (1) a predominantly myopathic form characterized by recurrent myoglobinuria and CNS involvement with seizures, ataxia, or mental retardation[3–5]; (2) childhood-onset cerebellar ataxia, which is often associated with seizures, muscle weakness, mental retardation, pyramidal tract signs, and peripheral neuropathy[6–8]; (3) a multisystem infantile variant with encephalopathy, cardiomyopathy, ataxia, optic-nerve atrophy, deafness, and nephropathy[9–12]; (4) Leigh syndrome with growth retardation, ataxia, and deafness[13]; and (5) isolated childhood- or adult-onset myopathy.[14–15] Recently, in two siblings with the multisystem infantile form of CoQ_{10} deficiency, we identified the first mutation in a CoQ_{10} biosynthetic gene, *COQ2* (MIM #609825), which encodes PHB-polyprenyl transferase, the enzyme that catalyzes the second dedicated step in CoQ_{10} biosynthesis.[12] The absence of mutations in *COQ2* in other patients with CoQ_{10} deficiency suggests that mutations in other CoQ_{10} biosynthetic genes may exist. Identification of the molecular causes of the CoQ_{10} deficiency will allow early and accurate diagnosis, which is particularly critical because patients can respond to replacement therapy.

Here, we describe a male infant who presented with neonatal pneumonia and hypotonia. He was the second child of healthy, nonconsanguineous white parents, and his elder sister was healthy. At age 3 mo, he developed refractory left-sided seizures with secondary generalization, despite various combinations of antiepileptic drugs. He became progressively floppy (fig. 2*A*) and had difficulties feeding because of exhaustion. At age 7 mo, severe episodic vomiting prompted duodenal tube feeding, and he rapidly developed widespread edema. His low serum albumin of 4.3 g/liter (normal range 35–52) and massive proteinuria led to the diagnosis of nephrotic syndrome. His serum lactate was markedly elevated (7.5 mmol/liter; normal <2.0), and his lactate:pyruvate ratio was at the upper limit of normal (21; normal <20). Electroencephalogram (EEG) studies revealed focal spikes over the right hemisphere (fig. 2*C*), with occasional secondary generalization. The absence of purposeful gaze and visual evoked-potential responses, together with normal funduscopic findings, suggested cor-

From the Department of Neurology, Columbia University College of Physicians and Surgeons, New York (L.C.L.; C.M.Q.; T.K.; A.N.; S.D.; M.H.); Department of Neuropediatrics, Charité Virchow University Hospital, Berlin (M.S.); and Nijmegen Centre for Mitochondrial Disorders, Radboud University Nijmegen Medical Centre, Nijmengen, The Netherlands (R.J.T.R.)

Received September 15, 2006; accepted for publication October 6, 2006; electronically published October 27, 2006.

Address for correspondence and reprints: Dr. Michio Hirano, Department of Neurology, Columbia University College of Physicians and Surgeons, 630 W 168th Street, P&S 4-443, New York, NY 10032. E-mail: mh29@columbia.edu

Am. J. Hum. Genet. 2006;79:1125–1129. 0002-9297/2006/7906-0016$15.00

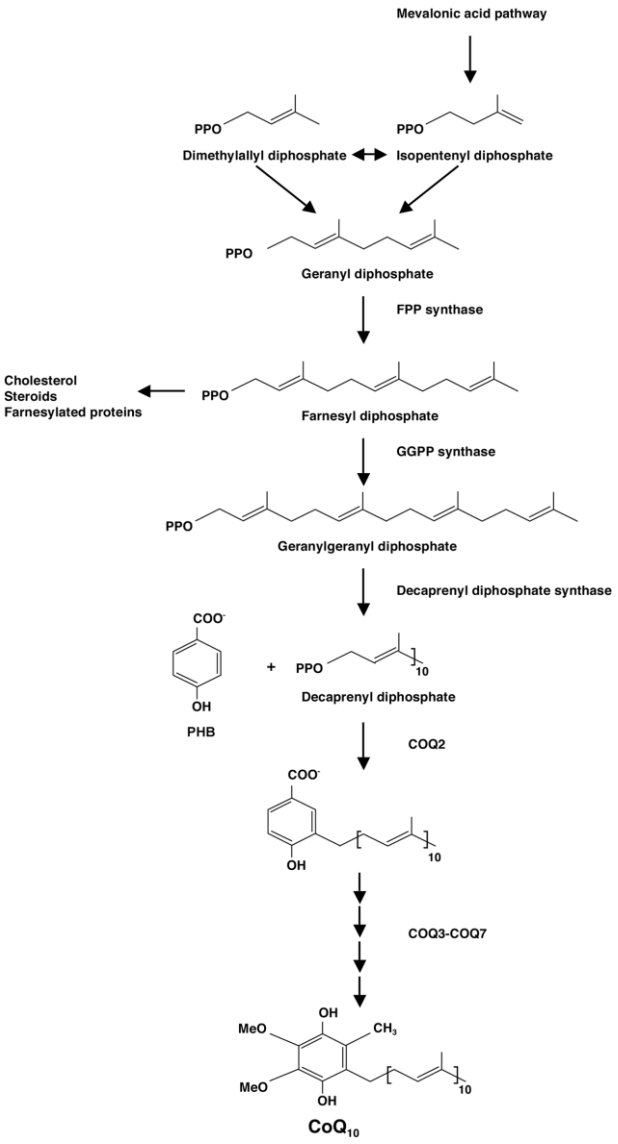

Figure 1. CoQ_{10} biosynthetic pathway. Isopentenyl diphosphates derived from the mevalonate pathway generate geranylgeranyl diphosphate. Additional isopentenyl diphosphates are added by decaprenyl diphosphate synthase, which, in humans is a heterotetramer composed of two prenyl diphosphate synthase subunit 1 and two prenyl diphosphate synthase subunit 2 molecules. PHB is derived from the amino acids tyrosine or phenylalanine. After PHB and decaprenyl diphosphate are produced, at least seven enzymes catalyze condensation, methylation, decarboxylation, and hydroxylation reactions to synthesize CoQ_{10}.[1,2]

tical blindness. Brain magnetic resonance imaging (MRI) showed bilateral symmetric areas of increased T_2- and decreased T_1-signal intensity in the basal ganglia, consistent with Leigh syndrome (fig. 2*B*). Urinary excretion of amino acids and the acylcarnitine profile in blood were at normal levels.

Modified Gomori trichrome and cytochrome *c* oxidase (COX) stains of muscle revealed increased proportions (4%–7%) of fibers with excessive subsarcolemmal mitochondrial aggregates (fig. 2*D*), but no COX-negative fibers were present. Measurement of respiratory-chain enzyme activities in muscle and cultured fibroblasts showed decreased complex II+III activities (muscle: 20 mU/U citrate synthase, control range 37–285; fibroblasts: 84 mU/U COX, control range 160–440). Complex I activity was mildly reduced in fibroblasts (88 mU/U COX, normal range 110–260) but normal in muscle. Isolated activities of complexes II, III, and IV were normal. After addition of decylubiquinone to the reaction mixtures, activity of complex II+III increased 8.9-fold in the patient's muscle and 2.2-fold in control muscle. Similarly, decylubiquinone addition increased complex II+III activity 4.7-fold in the patient's fibroblasts and 3.6-fold in control fibroblasts.

CoQ_{10} in muscle was extracted, mixing 50 μl of muscle homogenate and 950 μl of 1-propanol, and CoQ_{10} in fibroblasts was extracted in hexane and was measured by high-performance liquid chromatography with electrochemical detection (HPLC-EQ) by use of a reverse-phase column and isocratic mobile phase.[16] The results showed that CoQ_{10} concentration was severely reduced in muscle (4.6 μg/g tissue; mean $\pm$ SD in 185 controls 32.1 $\pm$ 6.8) and fibroblasts (patient 6.7 $\pm$ 2.6 ng/mg protein; mean in 10 controls 52.2 $\pm$ 9.1). By use of the Stanbio cholesterol Liquicor assay kit (Stanbio Laboratory), levels of cholesterol were similar in fibroblasts from the patient and from controls (patient 5.6 μg/mg protein; mean of three controls 6.7 $\pm$ 2.1), thus excluding a defect in shared biosynthetic pathway of cholesterol and CoQ_{10}.

Daily oral therapy with 500 mg L-carnitine, 5 mg biotin, 20 mg riboflavin, 50 mg thiamine, and, beginning at age 3 mo, 50 mg CoQ_{10} did not lead to clinical improvement. The child died at age 8 mo because of severe refractory focal status epilepticus. The lack of clinical improvement may have been due to poor CNS penetration of CoQ_{10}, the severity of brain damage prior to oral supplementation, or both.

We sequenced 11 known human genes (*PDSS1* [MIM #607429], *PDSS2, COQ2, COQ3* [MIM *605196], *COQ4, COQ5, COQ6, COQ7* [MIM #601683], *CABC1* [MIM #606980], *COQ9,* and *ADCK2*) encoding COQ_{10} biosynthetic proteins.[2] We also sequenced *COQ10A* and *COQ10B,* which encode proteins required for CoQ_{10} function in the respiratory chain.[17] Primer sequences and PCR conditions for amplification of candidate genes are listed in table 1.

We found two nonsynonymous nucleotide changes in *PDSS2,* which encodes decaprenyl diphosphate synthase subunit 2[18,19]: a heterozygous C→T transition at nucleotide 964, changing amino acid 322 from glutamine to a stop codon and a heterozygous C→T transition at nucleotide 1145, changing amino acid 382 from serine to leucine in the seventh conserved domain in trans-prenyl diphosphate synthase.[19] The first mutation was present in the father and sister but not in the mother. The second transition was present in the mother but not in the father and sister. Both transitions were absent in DNA from 210 chromo-

somes tested by RFLP with use of *Hpy*188III and *Hpy*188I restriction endonucleases (80% power to exclude 0.01 polymorphism frequency at $\alpha = 0.05$).[20] No mutations were found in the other CoQ_{10} biosynthetic genes.

To confirm that the patients had a defect of CoQ_{10} biosynthesis, we measured the incorporation of two radiolabeled substrates, ^{14}C-PHB (50 Ci/mol) and ^{3}H-decaprenyl-PP (20 Ci/mmol) in cultured fibroblasts, as reported.[12] In the first assay, cells were incubated with only ^{14}C-PHB substrate, whereas, in the second assay, fibroblasts were incubated with two substrates (PHB and ^{3}H-decaprenyl-PP). In both assays, radiolabeled CoQ_{10} was isolated by HPLC with a C18 reversed-phase column and was collected and quantified in a scintillation counter.[12] In the first assay, the patient's fibroblasts incubated with ^{14}C-PHB showed decreased CoQ_{10} synthesis (patient, 402 ± 83 decays per min/mg protein/d; control mean 3,324 ± 526 decays per min/mg protein/d, $n = 5$) (table 2). However, in the second assay, the patient's fibroblasts incubated with PHB and ^{3}H-decaprenyl-PP synthesized CoQ_{10} at rates similar to control fibroblasts (patient = 11.5 ± 0.5 pmol/mg protein/h; control mean = 14.29 ± 2.3, $n = 5$) (table

Table 1. Primers Used for PCR Amplification and Sequencing of CoQ_{10} Biosynthetic Genes

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

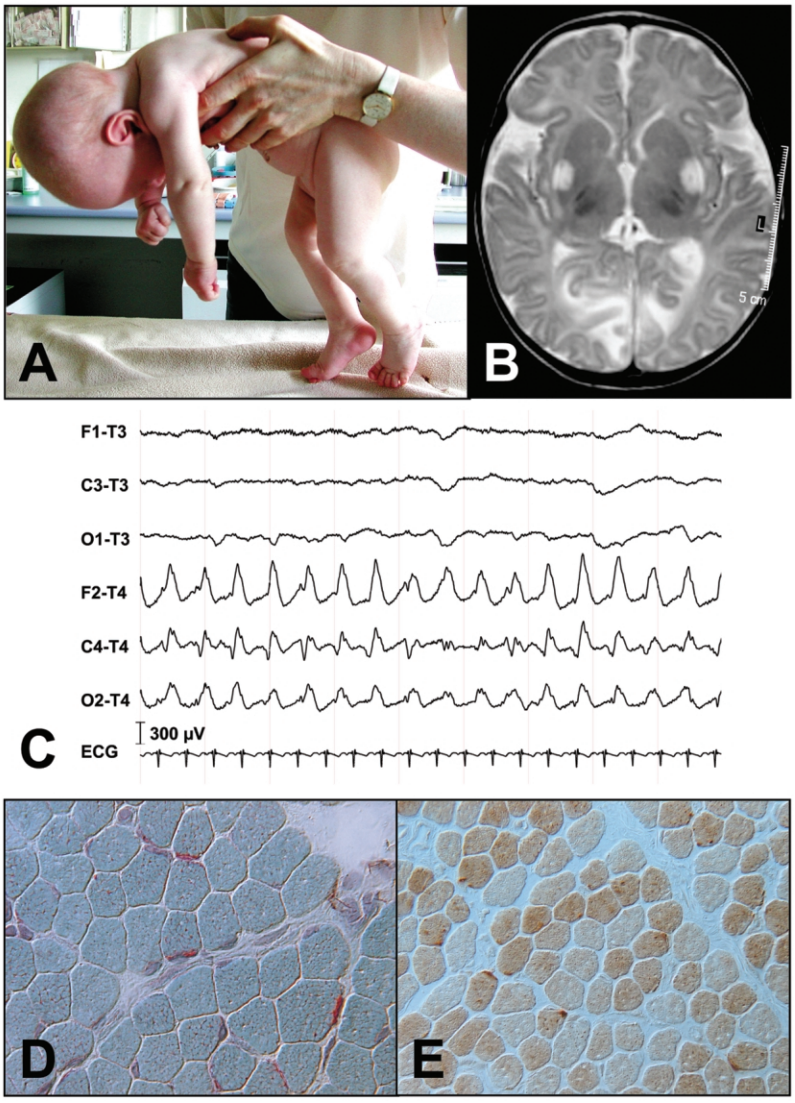

Figure 2. *A,* At age 5 mo, the child was severely hypotonic. *B,* T_2-weighted MR image of the brain at age 3 mo, showing bilateral increased signal intensities at the putamen and globus pallidus, as well as increased white matter signal in the parieto-occipital regions and atrophy in the occipital lobe and peri-insular region. *C,* EEG during right-sided focal status epilepticus, showing continuous high-voltage 2-hz sharp waves over the entire right hemisphere. *D,* Gomori trichrome stain, demonstrating several muscle fibers with abnormal subsarcolemmal mitochondrial aggregates (500 ×). *E,* COX stain, showing a similar pattern of increased subsarcolemmal stain (400 ×).

Table 2. Biochemical Assays for COQ_{10} Biosynthesis

Fibroblasts	CoQ10 Biosynthesis[a]	% of Control Mean
Assay 1[b]:		
Control	3,324 ± 526 DPM/mg protein/d	100
Patient	402 ± 83 DPM/mg protein/d	12
Assay 2[c]:		
Control	14.29 ± 2.3 pmol/mg protein/h	100
Patient	11.5 ± 0.5 pmol/mg protein/h	81

[a] In the three assays, radiolabeled COQ_{10} was isolated by HPLC and was quantitated in a scintillation counter. Controls are measured as means ± SDs ($n = 5$). Patient measurements were realized per duplicated and the results are shown as means ± SDs. DPM = decays per minute.

[b] Cultured cells were incubated for 48 h with 0.1 μCi ^{14}C-PHB (50 Ci/mol specific activity).

[c] Fibroblast homogenates were incubated with ^{3}H-decaprenyl-PP and PHB for 1 h.

2), indicating that the CoQ_{10} biosynthetic pathway after decaprenyl diphosphophosphate synthase is intact (fig. 1). This result, combined with the defect of CoQ_{10} biosynthesis detected with ^{14}C-PHB, indicates that endogenous levels of decaprenyl diphosphate are reduced in the patient's fibroblasts and support the pathogenicity of the nucleotide changes in *PDSS2*.

PDSS2 encodes the second subunit of decaprenyl diphosphate synthase,[18,19] which is considered one of the rate-limiting enzymes in CoQ_{10} biosynthesis.[2,18,19] Saiki et al.[19] demonstrated that, in *Schizosaccharomyces pombe,* mice, and humans, decaprenyl diphosphate synthases are heterotetrameric enzymes, formed by two protein subunits encoded by *PDSS1* (previously designated as "*TPRT*") and two protein subunits encoded by *PDSS2*. In the absence of *PDSS1* or *PDSS2* (originally reported as "*DPS1*" and "*DLP1*"),[18,19] the enzyme is not functional and does not produce CoQ_{10}. Moreover, in mice and humans, two different *PDSS2* transcripts have been identified: one encoded by eight exons and the other by four exons.[19] Both transcripts share the first three exons. Saiki et al. demonstrated that the first transcript—but not the second—encodes a functional subunit of decaprenyl diphosphate synthase.[19] Consistent with the notion that the long transcript of *PDSS2* is functionally important, both mutations in our patient are localized in exon 6 and exon 8, which are present only in the first transcript.

In summary, this study reports the first pathogenic mutations in *PDSS2* causing primary CoQ_{10} deficiency in an infant with fatal Leigh syndrome and nephrotic syndrome. Pathogenicity of these mutations is supported by (1) the substitution of a stop codon for a glutamine and a hydrophobic leucine for a polar uncharged serine in two conserved domains of decaprenyl diphosphate synthases; (2) the absence of these mutations in 210 control chromosomes; (3) the defect of CoQ_{10} synthesis in the patient's fibroblasts when incubated with PHB contrasting with normal synthesis in fibroblasts incubated with PHB and decaprenyl-PP. These assays confirm that the biosynthetic defect is impaired formation of decaprenyl diphosphate, catalyzed by decaprenyl diphosphate synthase. Farnesyl diphosphate and geranyl diphosphate are essential substrates for decaprenyl diphosphate synthase and are generated by farnesyl diphosphate synthase and geranyl diphosphate synthase (fig. 1). However, farnesyl diphosphate and geranyl diphosphate are also necessary substrates for cholesterol and dolichol synthesis.[2] Since the cholesterol level was normal in our patient's fibroblasts, defects in farnesyl diphosphate synthase and geranyl diphosphate synthase are unlikely.

Although >30 patients have been described with CoQ_{10} deficiency, the molecular defects have been identified in only two other families. In the first family, three siblings and a cousin with cerebellar ataxia and CoQ_{10} deficiency had a mutation in the aprataxin gene, *APTX* (MIM #606350), which is known to cause ataxia and oculomotor apraxia 1 (AOA1 [MIM #208920]).[21] In the second family, the proband with infantile encephalomyopathy and nephrotic syndrome and his younger sibling with nephrotic syndrome had a homozygous missense mutation in *COQ2,* which encodes PHB-polyprenyl transferase.[11,12] Whereas there is no obvious link between aprataxin and CoQ_{10} metabolism, PHB-polyprenyl transferase catalyzes the second step in CoQ_{10} biosynthesis, indicating that CoQ_{10} deficiency can be primary or secondary.

Mutations in *PDSS2* should be considered as potential causes of CoQ_{10} deficiency in other patients with similar phenotypes, including two sisters with Leigh syndrome and CoQ_{10} deficiency[13] and two siblings with severe encephalomyopathy, renal failure, and CoQ_{10} deficiency in whom biochemical assays showed a defect in decaprenyl diphosphate synthase but no pathogenic mutation in *PDSS1*.[9]

Further studies of our patients and others with primary CoQ_{10} deficiencies should provide insights into pathogenic mechanisms and genotype–phenotype relationships. Moreover, the availability of genetic testing will allow early definitive diagnosis and intervention for one of the few treatable forms of mitochondrial encephalomyopathies. On the basis of the phenotypes of our patients with *COQ2* and *PDSS2* mutations, children with infantile-onset encephalopathies with nephrotic syndrome should be screened for primary CoQ_{10} deficiencies.

Acknowledgments

This work was supported by National Institutes of Health grants NS11766 and HD32062, by a grant from the Muscular Dystrophy Association, by the Marriott Mitochondrial Disorder Clinical Research Fund (MMDCRF), and by Deutsche Forschungsgemeinschaft grant SFB 577 TP B4 (Genetic Variability of Mitochondrial Disorders). L.C.L. is a postdoctoral fellow from the Ministerio de Educacion y Ciencia, Spain. C.M.Q. is supported by Telethon Italia.

Web Resource

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi

.nlm.nih.gov/Omim/ (for coenzyme Q_{10} deficiency, *COQ2, PDSS1, COQ3, COQ7, CABC1, APTX,* and AOA1)

References

1. Kawamukai M (2002) Biosynthesis, bioproduction and novel roles of ubiquinone. J Biosci Bioeng 94:511–517
2. Turunen M, Olsson J, Dallner G (2004) Metabolism and function of coenzyme Q. Biochim Biophys Acta 1660:171–199
3. Ogasahara S, Engel AG, Frens D, Mack D (1989) Muscle coenzyme Q deficiency in familial mitochondrial encephalomyopathy. Proc Natl Acad Sci USA 86:2379–2382
4. Sobreira C, Hirano M, Shanske S, Keller RK, Haller RG, Davidson E, Santorelli FM, Miranda AF, Bonilla E, Mojon DS, Barreira AA, King MP, DiMauro S (1997) Mitochondrial encephalomyopathy with coenzyme Q10 deficiency. Neurology 48:1238–1243
5. Boitier E, Degoul F, Desguerre I, Charpentier C, Francois D, Ponsot G, Diry M, Rustin P, Marsac C (1998) A case of mitochondrial encephalomyopathy associated with a muscle coenzyme Q10 deficiency. J Neurol Sci 156:41–46
6. Musumeci O, Naini A, Slonim AE, Skavin N, Hadjigeorgiou GL, Kravwiecki N Weissman BM, Tsao CY, Mendell JR, Shanske S, De Vivo DC, Hirano M, DiMauro S (2001) Familial cerebellar ataxia with muscle coenzyme Q10 deficiency. Neurology 56:849–855
7. Lamperti C, Naini A, Hirano M, De Vivo DC, Bertini E, Servidei S, Valeriani M, Lynch D, Banwell B, Berg M, Dubrovsky T, Chiriboga C, Angelini C, Pegoraro E, DiMauro S (2003) Cerebellar ataxia and coenzyme Q10 deficiency. Neurology 60:1206–1208
8. Artuch R, Brea-Calvo G, Briones P, Aracil A, Galvan M, Espinos C, Corral J, Volpini V, Ribes A, Andreu AL, Palau F, Sanchez-Alcazar JA, Navas P, Pineda M (2006) Cerebellar ataxia with coenzyme Q(10) deficiency: Diagnosis and follow-up alter coenzyme Q(10) supplementation. J Neurol Sci 246:153–158
9. Rotig A, Appelkvist EL, Geromel V, Chretien D, Kadhom N, Edery P, Lebideau M, Dallner G, Munnich A, Ernster L, Rustin P (2000) Quinone-responsive multiple respiratory-chain dysfunction due to widespread coenzyme Q10 deficiency. Lancet 356:391–395
10. Rahman S, Hargreaves I, Clayton P, Heales S (2001) Neonatal presentation of coenzyme Q10 deficiency. J Pediatr 139:456–458
11. Salviati L, Sacconi S, Murer L, Zacchello G, Franceschini L, Laverda AM, Basso G, Quinzii C, Angelini C, Hirano M, Naini AB, Navas P, DiMauro S, Montini G (2005) Infantile encephalomyopathy and nephropathy with CoQ10 deficiency: a CoQ10-responsive condition. Neurology 65:606–608
12. Quinzii C, Naini A, Salviati L, Trevisson E, Navas P, DiMauro S, Hirano M (2006) A mutation in *para*-hydroxybenzoate-polyprenyl transferase (*COQ2*) causes primary coenzyme Q10 deficiency. Am J Hum Genet 78:345–349
13. Van Maldergem L, Trijbels F, DiMauro S, Sindelar PJ, Musumeci O, Janssen A, Delberghe X, Martin JJ, Gillerot Y (2002) Coenzyme Q-responsive Leigh's encephalopathy in two sisters. Ann Neurol 52:750–754
14. Lalani SR, Vladutiu GD, Plunkett K, Lotze TE, Adesina AM, Scaglia F (2005) Isolated mitochondrial myopathy associated with muscle coenzyme Q10 deficiency. Arch Neurol 62:317–320
15. Horvath R, Schneiderat P, Schoser BG, Gempel K, Neuen-Jacob E, Ploger H, Muller-Hocker J, Pongratz DE, Naini A, DiMauro S, Lochmuller L (2006) Coenzyme Q deficiency and isolated myopathy. Neurology 66:253–255
16. Naini A, Lewis VJ, Hirano M, DiMauro S (2003) Primary coenzyme Q10 deficiency and the brain. Biofactors 18:145–152
17. Barros MH, Johnson A, Gin P, Marbois BN, Clarke CF, Tzagoloff A (2005) The *Saccharomyces cerevisiae COQ10* gene encodes a START domain protein required for function of coenzyme Q in respiration. J Biol Chem 280:42627–42635
18. Saiki R, Nagata A, Uchida N, Kainou T, Matsuda H, Kawamukai M (2003) Fission yeast decaprenyl diphosphate synthase consists of Dps1 and the newly characterized Dlp1 protein in a novel heterotetrameric structure. Eur J Biochem 270: 4113–4121
19. Saiki R, Nagata A, Kainou T, Matsuda H, Kawamukai M (2005) Characterization of solanesyl and decaprenyl diphosphate synthases in mice and humans. FEBS J 272:5606–5622
20. Collins JE, Schwartz CE (2002) Detecting polymorphisms and mutations in candidate genes. Am J Hum Genet 71:1251–1252
21. Quinzii CM, Kattah AG, Naini A, Akman HO, Mootha VK, DiMauro S, Hirano M (2005) Coenzyme Q deficiency and cerebellar ataxia associated with an aprataxin mutation. Neurology 64:539–541