

## Leigh Syndrome with Nephropathy and CoQ<sub>10</sub> Deficiency Due to decaprenyl diphosphate synthase subunit 2 (PDSS2) Mutations

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Coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) is a vital lipophilic molecule that transfers electrons from mitochondrial respiratory chain complexes I and II to complex III. Deficiency of CoQ<sub>10</sub> has been associated with diverse clinical phenotypes, but, in most patients, the molecular cause is unknown. The first defect in a CoQ<sub>10</sub> 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<sub>10</sub> 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<sub>10</sub> 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<sub>10</sub> deficiency.

Coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>), 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<sub>10</sub> 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.<sup>1,2</sup>

CoQ<sub>10</sub> 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<sub>10</sub>.<sup>1,2</sup>

CoQ<sub>10</sub> deficiency (MIM #607426) has been associated with autosomal recessive neurological disorders that are responsive to CoQ<sub>10</sub> supplementation. Clinical phenotypes include: (1) a predominantly myopathic form characterized by recurrent myoglobinuria and CNS involvement with seizures, ataxia, or mental retardation<sup>3-5</sup>; (2) childhood-onset cerebellar ataxia, which is often associated with seizures, muscle weakness, mental retardation, pyramidal tract signs, and peripheral neuropathy<sup>6-8</sup>; (3) a multisystem infantile variant with encephalopathy, cardiomyopathy, ataxia, optic-nerve atrophy, deafness, and nephropathy<sup>9-12</sup>; (4) Leigh syndrome with growth retarda-

tion, ataxia, and deafness<sup>13</sup>; and (5) isolated childhood- or adult-onset myopathy.<sup>14-15</sup> Recently, in two siblings with the multisystem infantile form of CoQ<sub>10</sub> deficiency, we identified the first mutation in a CoQ<sub>10</sub> biosynthetic gene, *COQ2* (MIM #609825), which encodes PHB-polyprenyl transferase, the enzyme that catalyzes the second dedicated step in CoQ<sub>10</sub> biosynthesis.<sup>12</sup> The absence of mutations in *COQ2* in other patients with CoQ<sub>10</sub> deficiency suggests that mutations in other CoQ<sub>10</sub> biosynthetic genes may exist. Identification of the molecular causes of the CoQ<sub>10</sub> deficiency will allow early and accurate diagnosis, which is particularly critical because patients can respond to replacement therapy.

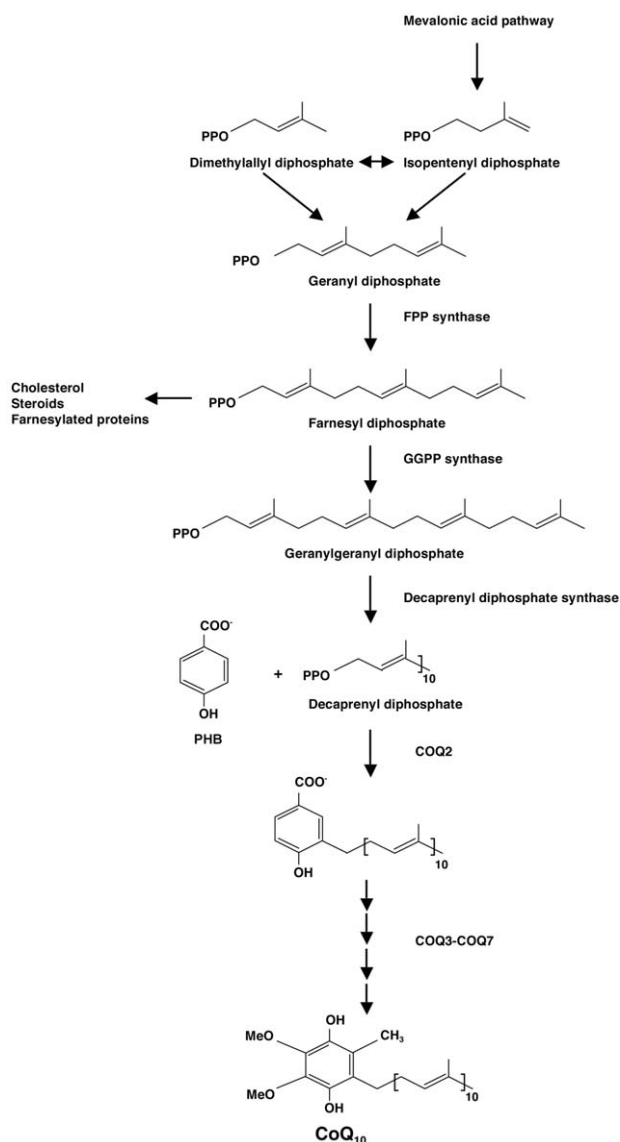
Here, we describe a male infant who presented with neonatal pneumonia and hypotonia. He was the second child of healthy, nonconsanguineous white parents, and his elder sister was healthy. At age 3 mo, he developed refractory left-sided seizures with secondary generalization, despite various combinations of antiepileptic drugs. He became progressively floppy (fig. 2A) 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. 2C), with occasional secondary generalization. The absence of purposeful gaze and visual evoked-potential responses, together with normal funduscopic findings, suggested cor-

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**Figure 1.** CoQ<sub>10</sub> 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<sub>10</sub>.<sup>1,2</sup>

tical blindness. Brain magnetic resonance imaging (MRI) showed bilateral symmetric areas of increased T<sub>2</sub>- and decreased T<sub>1</sub>-signal intensity in the basal ganglia, consistent with Leigh syndrome (fig. 2B). 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. 2D), 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<sub>10</sub> in muscle was extracted, mixing 50 μl of muscle homogenate and 950 μl of 1-propanol, and CoQ<sub>10</sub> 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.<sup>16</sup> The results showed that CoQ<sub>10</sub> concentration was severely reduced in muscle (4.6 μg/g tissue; mean ± SD in 185 controls 32.1 ± 6.8) and fibroblasts (patient 6.7 ± 2.6 ng/mg protein; mean in 10 controls 52.2 ± 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 ± 2.1), thus excluding a defect in shared biosynthetic pathway of cholesterol and CoQ<sub>10</sub>.

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<sub>10</sub> 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<sub>10</sub>, 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<sub>10</sub> biosynthetic proteins.<sup>2</sup> We also sequenced *COQ10A* and *COQ10B*, which encode proteins required for CoQ<sub>10</sub> function in the respiratory chain.<sup>17</sup> 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<sup>18,19</sup>: 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.<sup>19</sup> 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-

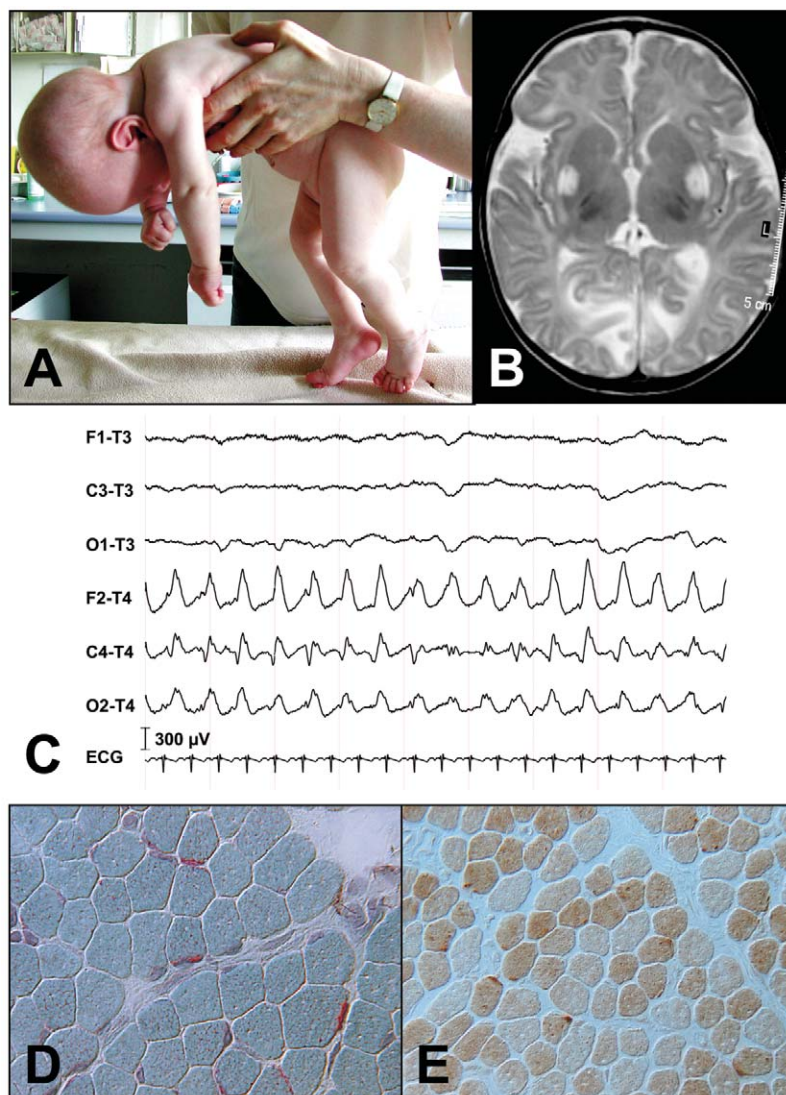
somes tested by RFLP with use of *Hpy*188III and *Hpy*188I restriction endonucleases (80% power to exclude 0.01 polymorphism frequency at  $\alpha = 0.05$ ).<sup>20</sup> No mutations were found in the other CoQ<sub>10</sub> biosynthetic genes.

To confirm that the patients had a defect of CoQ<sub>10</sub> biosynthesis, we measured the incorporation of two radiolabeled substrates, <sup>14</sup>C-PHB (50 Ci/mol) and <sup>3</sup>H-decaprenyl-PP (20 Ci/mmol) in cultured fibroblasts, as reported.<sup>12</sup> In the first assay, cells were incubated with only <sup>14</sup>C-PHB substrate, whereas, in the second assay, fibroblasts were incubated with two substrates (PHB and <sup>3</sup>H-decaprenyl-PP). In both assays, radiolabeled CoQ<sub>10</sub> was isolated by HPLC with a C18 reversed-phase column and was collected and quantified in a scintillation counter.<sup>12</sup> In the

**Table 1. Primers Used for PCR Amplification and Sequencing of CoQ<sub>10</sub> Biosynthetic Genes**

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

first assay, the patient's fibroblasts incubated with <sup>14</sup>C-PHB showed decreased CoQ<sub>10</sub> 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 <sup>3</sup>H-decaprenyl-PP synthesized CoQ<sub>10</sub> at rates similar to control fibroblasts (patient = 11.5 ± 0.5 pmol/mg protein/h; control mean = 14.29 ± 2.3, *n* = 5) (table



**Figure 2.** *A*, At age 5 mo, the child was severely hypotonic. *B*, T<sub>2</sub>-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<sub>10</sub> Biosynthesis**

Fibroblasts	CoQ <sub>10</sub> Biosynthesis <sup>a</sup>	% of Control Mean
Assay 1 <sup>b</sup> :		
Control	3,324 ± 526 DPM/mg protein/d	100
Patient	402 ± 83 DPM/mg protein/d	12
Assay 2 <sup>c</sup> :		
Control	14.29 ± 2.3 pmol/mg protein/h	100
Patient	11.5 ± 0.5 pmol/mg protein/h	81

<sup>a</sup> In the three assays, radiolabeled CoQ<sub>10</sub> 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.

<sup>b</sup> Cultured cells were incubated for 48 h with 0.1 μCi <sup>14</sup>C-PHB (50 Ci/mol specific activity).

<sup>c</sup> Fibroblast homogenates were incubated with <sup>3</sup>H-decaprenyl-PP and PHB for 1 h.

2), indicating that the CoQ<sub>10</sub> biosynthetic pathway after decaprenyl diphosphosphate synthase is intact (fig. 1). This result, combined with the defect of CoQ<sub>10</sub> biosynthesis detected with <sup>14</sup>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,<sup>18,19</sup> which is considered one of the rate-limiting enzymes in CoQ<sub>10</sub> biosynthesis.<sup>2,18,19</sup> Saiki et al.<sup>19</sup> 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"),<sup>18,19</sup> the enzyme is not functional and does not produce CoQ<sub>10</sub>. Moreover, in mice and humans, two different *PDSS2* transcripts have been identified: one encoded by eight exons and the other by four exons.<sup>19</sup> 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.<sup>19</sup> 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<sub>10</sub> 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<sub>10</sub> 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.<sup>2</sup> 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<sub>10</sub> 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<sub>10</sub> deficiency had a mutation in the aprataxin gene, *APTX* (MIM #606350), which is known to cause ataxia and oculomotor apraxia 1 (AOA1 [MIM #208920]).<sup>21</sup> 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.<sup>11,12</sup> Whereas there is no obvious link between aprataxin and CoQ<sub>10</sub> metabolism, PHB-polyprenyl transferase catalyzes the second step in CoQ<sub>10</sub> biosynthesis, indicating that CoQ<sub>10</sub> deficiency can be primary or secondary.

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

Further studies of our patients and others with primary CoQ<sub>10</sub> 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<sub>10</sub> deficiencies.

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### 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<sub>10</sub> deficiency, *COQ2*, *PDSS1*, *COQ3*, *COQ7*, *CABC1*, *APTX*, and *AOA1*)

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