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Research Article

Coenzyme Q_{10} (CoQ_{10}) plays a pivotal role in oxidative phosphorylation (OXPHOS), as it distributes electrons among the various dehydrogenases and the cytochrome segments of the respiratory chain. We have identified 2 novel inborn errors of CoQ_{10} biosynthesis in 2 distinct families. In both cases, enzymologic studies showed that quinone-dependent OXPHOS activities were in the range of the lowest control values, while OXPHOS enzyme activities were normal. CoQ_{10} deficiency was confirmed by restoration of normal OXPHOS activities after addition of quinone. A genome-wide search for homozygosity in family 1 identified a region of chromosome 10 encompassing the gene *prenyldiphosphate synthase, subunit 1* (*PDSS1*), which encodes the human ortholog of the yeast*COQ1* gene, a key enzyme of CoQ_{10} synthesis. Sequencing of *PDSS1* identified a homozygous nucleotide substitution modifying a conserved amino acid of the protein (D308E). In the second family, direct sequencing of *OH-benzoate polyprenyltransferase* (*COQ2*), the human ortholog of the yeast *COQ2* gene, identified a single base pair frameshift deletion resulting in a premature stop codon (c.1198delT, N401fsX415). Transformation of yeast Δ*coq1* and Δ*coq2* strains by mutant yeast *COQ1* and mutant human *COQ2* genes, respectively, resulted in defective growth on respiratory medium, indicating that these mutations are indeed the cause of OXPHOS deficiency.

Prenyldiphosphate synthase, subunit 1 (*PDSS1*) and *OH-benzoate polyprenyltransferase* (*COQ2*) mutations in ubiquinone deficiency and oxidative phosphorylation disorders

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Coenzyme Q_{10} (CoQ_{10}) plays a pivotal role in oxidative phosphorylation (OXPHOS), as it distributes electrons among the various dehydrogenases and the cytochrome segments of the respiratory chain. We have identified 2 novel inborn errors of CoQ_{10} biosynthesis in 2 distinct families. In both cases, enzymologic studies showed that quinone-dependent OXPHOS activities were in the range of the lowest control values, while OXPHOS enzyme activities were normal. CoQ_{10} deficiency was confirmed by restoration of normal OXPHOS activities after addition of quinone. A genome-wide search for homozygosity in family 1 identified a region of chromosome 10 encompassing the gene *prenyldiphosphate synthase, subunit 1* (*PDSS1*), which encodes the human ortholog of the yeast *COQ1* gene, a key enzyme of CoQ_{10} synthesis. Sequencing of *PDSS1* identified a homozygous nucleotide substitution modifying a conserved amino acid of the protein (D308E). In the second family, direct sequencing of *OH-benzoate polyprenyltransferase* (*COQ2*), the human ortholog of the yeast *COQ2* gene, identified a single base pair frameshift deletion resulting in a premature stop codon (c.1198delT, N401fsX415). Transformation of yeast Δ*coq1* and Δ*coq2* strains by mutant yeast *COQ1* and mutant human *COQ2* genes, respectively, resulted in defective growth on respiratory medium, indicating that these mutations are indeed the cause of OXPHOS deficiency.

Introduction

Oxidative phosphorylation (OXPHOS) deficiency constitutes a clinically and genetically heterogeneous group of inherited diseases. One of these diseases, coenzyme Q_{10} (CoQ_{10}, ubiquinone) deficiency, is a recently identified entity of particular theoretical and practical importance. CoQ_{10} transfers reducing equivalents from various dehydrogenases to complex III (ubiquinone cytochrome *c* reductase) and acts as a transmembrane hydrogen carrier. CoQ_{10} also plays a critical role in antioxidant defenses (1). Primary CoQ_{10} deficiency is a rare, but possibly treatable, autosomal recessive condition with 3 major clinical presentations: (a) an encephalomyopathic form, characterized by exercise intolerance, mitochondrial myopathy, myoglobinuria, epilepsy, and ataxia (2–5); (b) a generalized infantile variant with severe encephalopathy and renal disease (6–8); and (c) an ataxic form, dominated by ataxia, seizures, cerebral atrophy, and/or anomalies of the basal ganglia (9, 10). Recurrence of the disease and/or consanguinity of the parents in some reported families suggest an autosomal recessive mode of inheritance. This has been demonstrated by recent reports of *OH-benzoate polyprenyltransferase* (*COQ2*) and *prenyldiphosphate synthase, subunit 2* (*PDSS2*) mutation in 2 independent families (11, 12).

Little is known about CoQ_{10} biosynthesis in humans, but several genes have been identified in the human genome by homology with other organisms, especially the *Saccharomyces cerevisiae* yeast (Figure 1). In the present study, in an inbred family with CoQ_{10} deficiency manifesting as a multisystem disease with early-onset deafness, encephaloneuropathy, obesity, livedo reticularis, and valvulopathy, homozygosity mapping allowed the disease to be attributed to a homozygous missense mutation in *PDSS1*, the enzyme that elongates the prenyl side chain of coenzyme Q. Moreover, direct sequencing of various genes involved in ubiquinone biosynthesis in an unrelated patient with fatal infantile multiorgan disease detected a homozygous single base pair frameshift deletion in *COQ2*. This study extends our understanding of this newly recognized and possibly treatable cause of OXPHOS deficiency in humans.

Results

Enzymologic studies and quinone quantification. Assessment of individual OXPHOS enzyme activities in cultured skin fibroblasts of patient 1 revealed normal activity of complex II, complex III, and complex IV (Table 1). However, quinone-dependent activities

Nonstandard abbreviations used: *COQ2*, *OH-benzoate polyprenyltransferase*; CoQ_{10}, coenzyme Q_{10}; DQ, decylubiquinone; FPP, farnesylpyrophosphate; G3PDH, glycerol 3 phosphate dehydrogenase; GPP, geranylpyrophosphate; OXPHOS, oxidative phosphorylation; *PDSS1*, *prenyldiphosphate synthase, subunit 1*; PP, pyrophosphate.

Conflict of interest: The authors have declared that no conflict of interest exists.

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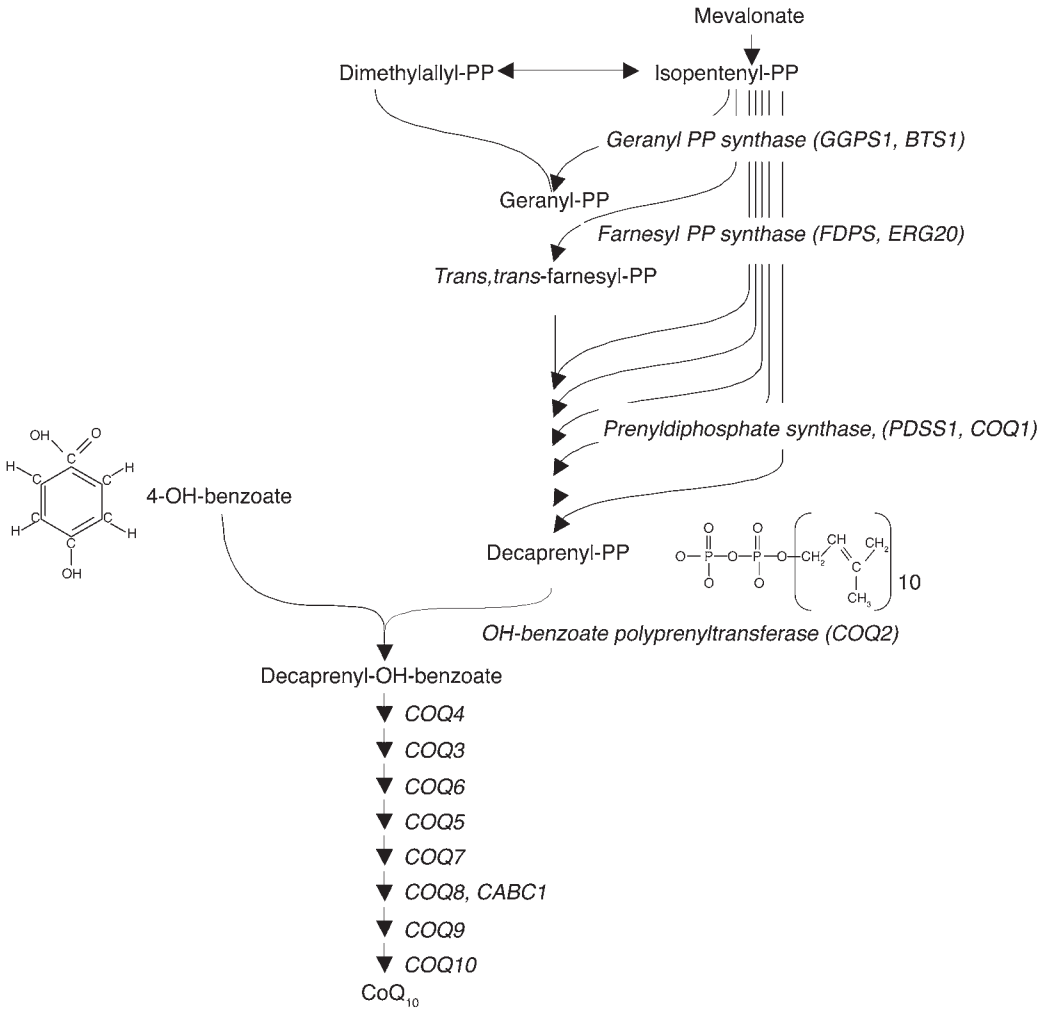

Figure 1
CoQ_{10} biosynthesis pathway. Enzyme and human gene symbols are shown in italics. The yeast gene symbol is indicated when different from the human gene.

(CII+CIII, glycerol 3 phosphate dehydrogenase [G3PDH]+CIII) were in the range of the lowest control values, and activity ratios (CIV/CII+CIII, CII+CIII/G3PDH+CIII), which optimally detect unbalanced respiratory chain enzyme functions, were markedly altered compared with controls, suggesting quinone deficiency. Muscle mitochondria of patient 1 showed high absolute activity values for all complexes (Table 1) but abnormal activity ratios (CI+CIII/CI, CIII/CI+CIII), also indicating quinone deficiency. The hypothesis of ubiquinone deficiency was further supported by the dramatic effect of decylubiquinone (DQ, an exogenous ubiquinone analog) on succinate oxidation of fibroblasts from patient 1, as addition of DQ during succinate oxidation measurement restored normal activity (8 and 16 nmol/min/mg protein before and after DQ addition, respectively; normal values: 9.8–20.5 nmol/min/mg protein) in the patient's permeabilized fibroblasts (Figure 2A). Consistently, addition of DQ during measurement of succinate–cytochrome *c* reductase activity restored normal activity of cultured skin fibroblasts (19 and 44 nmol/min/mg protein before and after DQ addition, respectively; normal values: 22–47 nmol/min/mg protein; Figure 2B). Finally, respiratory chain enzyme analysis in the liver of patient 3 revealed normal absolute enzyme activities but increased CIV/CII+CIII and CIV/CI+CIII activity ratios, also suggesting quinone deficiency (Table 1).

Direct evidence of quinone deficiency was finally provided by quantification of CoQ_{10} in the patients' fibroblasts, as the CoQ_{10} content of the 3 patients' fibroblasts was markedly decreased compared with normal values (Table 2). Interestingly, a normal CoQ_9 level was found in patients 1 and 2. The markedly decreased CoQ_{10}/CoQ_9 ratio in patients 1 and 2 (0.3; controls: 13 ± 3) suggested a defective addition of the tenth prenyl to the polyprenyl chain. Fibroblasts from controls and patient 3 were grown in the presence of [^{3}H]mevalonate to estimate the ability of these cells to synthesize CoQ_{10}. Substantial incorporation of [^{3}H]mevalonate into cholesterol, squalene, and dolichol was detected in controls and patient 3, but no [^{3}H]CoQ_{10} was detected in cultured skin fibroblasts from patient 3 (Figure 2C). In these experiments, all lipid extracts were treated with acid phosphatase to dephosphorylate accumulated intermediates. In patient 3, a peak with a retention time of 18.5 minutes was observed and was identi-

Table 1
Respiratory chain enzyme activities in muscle mitochondria and cultured skin fibroblasts

Enzyme	Patient 1 Cultured skin fibroblasts	Patient 1 Muscle mitochondria	Patient 3 Liver homogenate
		Activities (nmol/min/mg protein)	
CI	–	279 (65.2 ± 16.6)	33 (28.6 ± 6.3)
CII	14 (20.1 ± 3.5)	–	202 (158.5 ± 28.1)
CIII	**123** (169.6 ± 37.5)	3,838 (1,258.8 ± 327.5)	327 (250.8 ± 40.6)
CIV	93 (107.1 ± 25.6)	1,827 (730.3 ± 233.4)	364 (270.6 ± 48.3)
CV	–	–	114 (96.2 ± 32.1)
CI+CIII	–	414 (199 ± 56.4)	**25** (67.5 ± 16.5)
CII+CIII	**19** (33.4 ± 6.7)	596 (228.8 ± 61.8)	**25** (69.5 ± 15.4)
G3PDH	13 (14.4 ± 2.5)	–	–
G3PDH+CIII	**5** (18.2 ± 3.5)	43 (38.6 ± 11.6)	–
CS	71 (77 ± 20.7)	139 (324.1 ± 92.9)	74 (102.5 ± 15.9)
		Activity ratios	
CIV/CI	–	4.4 (3.5 ± 0.9)	11 (8.8 ± 1.8)
CIV/CII	6.6 (5 ± 0.5)	–	1.8 (1.5 ± 0.4)
CIV/CIII	0.8 (0.6 ± 0.01)	0.5 (0.5 ± 0.04)	–
CIV/CV	–	2.3 (2.5 ± 0.3)	3.2 (2.0 ± 0.4)
CIV/CII+CIII	**4.8** (3 ± 0.2)	3.1 (3.2 ± 0.3)	**14.6** (2.8 ± 0.6)
CIV/CI+CIII	–	4.4 (3.4 ± 0.9)	**14.6** (3.6 ± 0.9)
CI+CIII/CI	–	**1.5** (3.3 ± 0.7)	–
CII+CIII/G3PDH+CIII	**3.7** (1.4 ± 0.1)	**13.6** (5.5 ± 1.3)	–
CIII/CI+CIII	–	**9.3** (6.5 ± 1.6)	**13.08** (3.01 ± 0.52)
CI/CS	–	–	0.44 (0.38 ± 0.07)
CIV/CS	1.3 (1.37 ± 0.21)	–	**4.9** (3.1 ± 0.39)
CII+CIII/CS	**0.26** (0.47 ± 0.07)	–	**0.33** (1.07 ± 0.16)

Abnormal values are shown in bold; control values are shown in parentheses; and ratio values are presented as mean ± 1 SD. CI, complex I; CS, citrate synthase.

fied as decaprenol (Figure 2C). Decaprenyl-pyrophosphate (PP) accumulation reflected a deficiency in COQ2, which conjugates decaprenyl-PP with the benzoquinone ring.

Molecular studies. A genome-wide search for homozygosity was performed in the family of patients 1 and 2 using 400 polymorphic markers. This study identified 6 chromosomal regions of homozygosity on chromosomes 1 (D1S2762–D1S431, 3.9 cM), 7 (D7S2426–D7S2462, 9.4 cM), 10 (D10S1714–D10S593, 9.6 cM), 12 (D12S1608–D12S1685, 5 cM, and D12S1725–D12S77, 11.3 cM), and 13 (D13S280–D13S286, 8.4 cM). The 10p12.2–p12.1 region (Figure 3) encompasses a candidate gene, *PDSS1*, encoding the prenyldiphosphate synthase, subunit 1, which elongates the prenyl side chain of coenzyme Q in the quinone biosynthesis pathway (1, 13) (Figure 1). A mutation search was therefore undertaken in the family by direct sequencing of the 12 exons of the *PDSS1* gene. A homozygous T→G transversion at nucleotide 977 was found in exon 10 (Figure 4A). This transversion resulted in the change of a highly conserved aspartic acid into a glutamic acid (D308E). This aspartic acid is also conserved in eukaryote and prokaryote farnesylpyrophosphate (FPP) synthases and geranylpyrophosphate (GPP) synthases (13–15). Moreover, this aspartic acid lies in the signature pattern of polyprenyl synthase ([LIVMFY]-G-x(2)-[FYL]-Q-[LIVM]-x-D-D-[LIVMFY]-x-[DNG]), an aspartic acid–rich region of the protein that could be involved in the catalytic mechanism and/or binding of the substrates (Figure 4E). Both parents were heterozygous for the T→G transversion (Figure 4B), and unaffected children were either heterozygous or homozygous for the wild-type allele. The T→G transversion created an MnlI restriction site. Exon 10 was amplified, and the PCR product was then digested by the restriction enzyme MnlI. This generated 2 fragments in controls (393 and 180 bp) and 3 fragments in patients (352, 180, and 41 bp; Figure 4D). This mutation was absent from 100 controls of the same ethnic origin.

Finally, direct sequencing of the genes known to be involved in ubiquinone biosynthesis was systematically performed in patient 3 and identified a homozygous base pair deletion in exon 7 of the OH-benzoate polyprenyltransferase gene (*COQ2*, c.1198delT, N401fsX415; Figure 5A) resulting in a premature stop codon. Both parents were heterozygous for this single nucleotide deletion (Figure 5B), and the deletion was absent in 100 controls of the same ethnic origin. RT-PCR amplification on fibroblast RNAs showed that the abnormal transcript was still present in patient 3 (Figure 5C). This result suggests that a shortened mutant protein (364 instead of 371 amino acids with 14 C-proximal residues differing from the

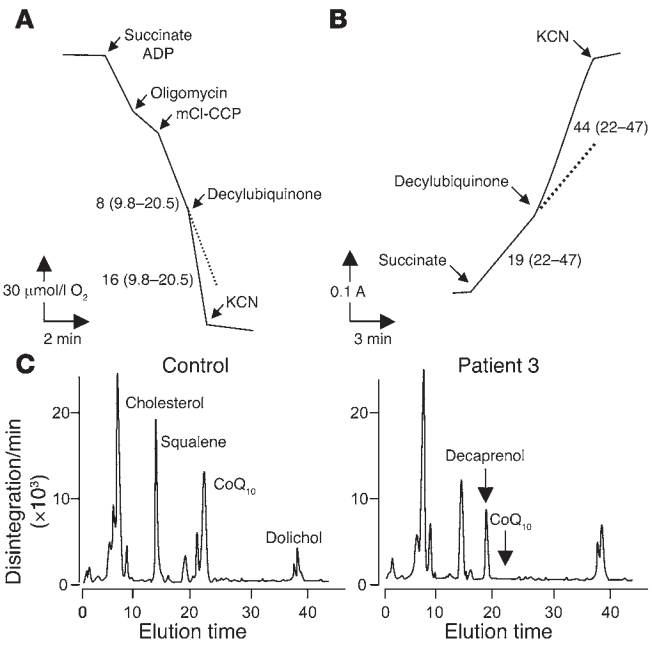

Figure 2
Effect of an exogenous quinone analog (DQ, 80 μM) on succinate oxidation (**A**) and succinate cytochrome *c* reductase activity of cultured skin fibroblasts from patient 1 (**B**). Numbers along the traces represent nmol/min/mg protein. Numbers in brackets are normal values. A, absorbance. (**C**) Elution profiles of lipid extracts from control and patient 3 skin fibroblasts incubated with [^{3}H]mevalonate and treated with acid phosphatase.

Table 2
Quinone content in cultured skin fibroblasts

	Patient 1	Patient 2	Patient 3	Control (n = 13)
CoQ_{10} (nmol/g protein)	4	4	29	120 ± 32
CoQ_9 (nmol/g protein)	12	11	3	10 ± 2
CoQ_{10}/CoQ_9	0.33	0.36	9.66	13 ± 3

Abnormal values are shown in bold.

normal protein) is synthesized in this patient. Unfortunately, no anti-human Coq2 antibody was available to test this hypothesis. Moreover, the global charge of the C-proximal part of the mutant protein was also modified (Figure 5D).

Functional complementation. To confirm the deleterious nature of the D308E *PDSS1* genotype, the human wild-type and mutant genes were tested for their ability to complement the OXPHOS defect in the *Saccharomyces cerevisiae* strain deleted for the *COQ1* gene. Wild-type and mutant *PDSS1* cDNAs as well as the yeast *COQ1* gene were cloned in pYES2.1. The 3 different plasmids were used to transform a yeast Δ*coq1*-null strain. Growth of the mutant on glycerol-rich medium was restored by the yeast *COQ1* gene but not by wild-type or mutant human *PDSS1* cDNAs, demonstrating that human prenyldiphosphate synthase does not complement the yeast Δ*coq1*-null mutation (Figure 6A). The mutation was then introduced into the yeast *COQ1* gene by site-directed mutagenesis. The yeast D365E mutant protein (corresponding to the human D308E protein) failed to complement the yeast mutant, whereas the normal protein did, demonstrating that the mutation affects protein function (Figure 6A).

To assess the deleterious nature of the OH-benzoate polyprenyltransferase gene mutation, the yeast Δ*coq2*-null strain was transformed with either normal or mutant human cDNAs cloned in pYES2.1. The human wild-type cDNA restored growth on glycerol-rich medium, whereas the mutant protein did not, showing that the mutation is indeed the cause of the deficiency (Figure 6B).

Discussion

This study reports on the identification of deleterious mutations in 2 different genes of the ubiquinone biosynthesis pathway (*PDSS1* and *COQ2*), which caused severe ubiquinone deficiency in 2 unrelated families. In the first consanguineous family, a genome scan analysis identified a homozygous mutation in the *PDSS1* gene encoding prenyldiphosphate synthase, one of the key enzymes of the ubiquinone biosynthesis pathway. This enzyme catalyzes elongation of GPP or FPP with several isopentenylpyrophosphate (IPP) groups, in *trans* configuration, to form the isoprenoid chain (6, 13). The mutation reported here changed a highly conserved aspartic acid into a glutamic acid (D308E). Human wild-type *PDSS1* cDNA failed to complement the OXPHOS defect of the Δ*coq1*-null yeast strain, while the PDSS1 and Coq1p proteins share 35% homology. However, growth of the Δ*coq1* yeast strain on a nonfermentable carbon source was restored by the wild-type but not the mutant yeast gene (D365E, corresponding to human D308E). We therefore conclude that the D308E mutation in our patient clearly induced prenyldiphosphate synthase deficiency and a profound quinone biosynthesis defect. In *Saccharomyces cerevisiae*, Coq1p (hexaprenylsynthase) elongates GPP or FPP (diprenyl biosynthesis intermediaries) to form hexaprenylpyrophosphate. The exact function of the PDSS1 protein in humans is not well known, but it can be hypothesized, by homology with yeast, that the PDSS1 enzyme also elongates geranyl pyrophosphate to form decaprenyl pyrophosphate. However, the normal CoQ_9 but very low CoQ_{10} levels detected in patients 1 and 2 suggest that the D308E PDSS1 mutation only impairs addition of the tenth prenyl to the polyprenyl chain. It is worth noting that quinone-dependent OXPHOS activities were only slightly decreased in patients 1 and 2, despite profound CoQ_{10} depletion in fibroblasts. It can be hypothesized that the mutant PDSS1 retains a low but significant residual activity, allowing the synthesis of trace amounts of CoQ_{10} and/or normal amounts of CoQ_9 sufficient to maintain a residual respiratory chain function compatible with life.

The mutated amino acid residue (D308) is situated in the second aspartic acid–rich region of the protein, which is common to all prenylsynthases reported to date. This family of proteins also includes FPP synthase and GPP synthase, 2 other enzymes of the ubiquinone synthesis pathway (13–15). Prenylsynthases are also involved in the biosynthesis of terpenoids in plants (16) and carotenoids in fungi (17). These enzymes are also involved in protein farnesylation (18). The aspartic acid–rich prenylsynthase signatures are supposedly involved in binding of the substrate, isopentenyl pyrophosphate, and elongating dimethylallyl pyrophosphate, by forming magnesium salt bridges between the substrate and the catalytic site (13). Site-directed mutagenesis of the second aspartate-rich motif has been performed in FPP synthase from *Saccharomyces cerevisiae* (19) and *Bacillus stearothermophilus* (20). These data show that the FPP synthase aspartate residue

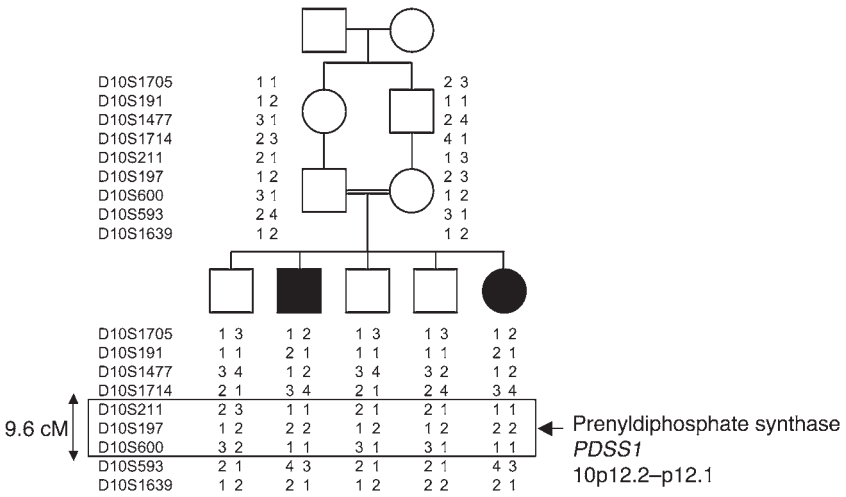

Figure 3
Pedigree and haplotype analysis of the family of patients 1 and 2. Haplotypes are given (top to bottom) for loci D10S1705, D10S191, D10S1477, D10S1714, D10S211, D10S197, D10S600, D10S593, and D10S1639. The haplotypes in the upper part are from the parents.

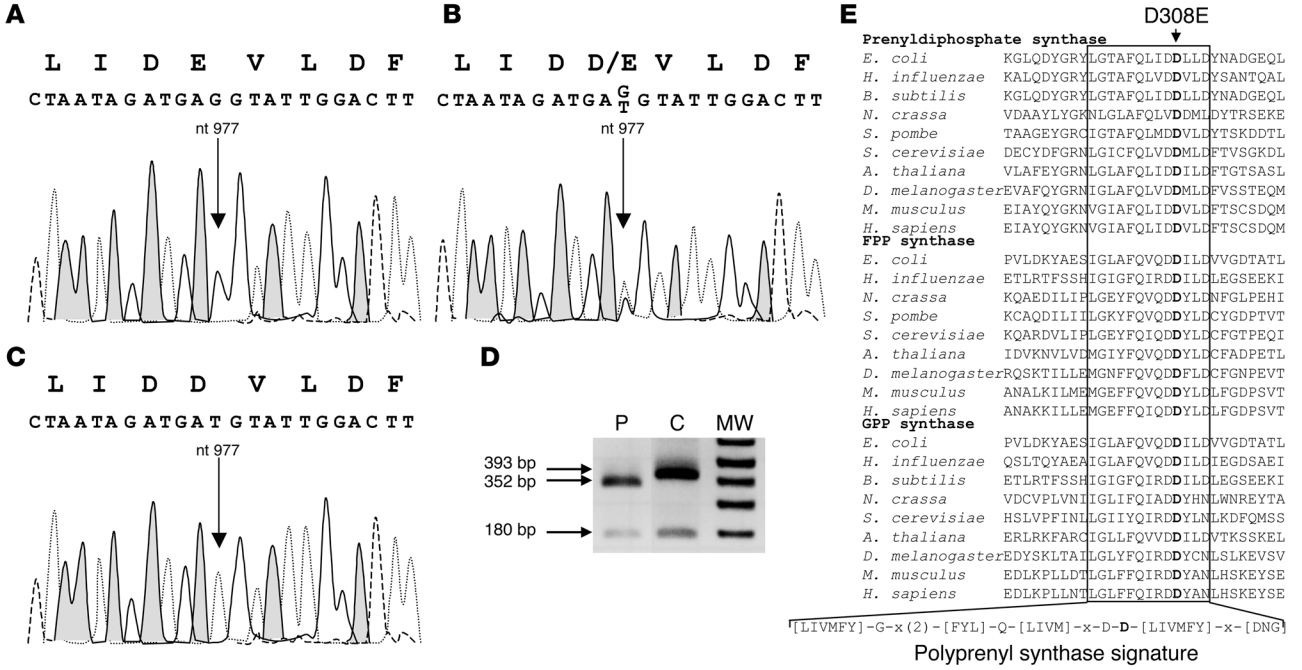

Figure 4
Molecular analysis of the *PDSS1* gene. Sequence analysis of *PDSS1* gene in patient 1 (**A**), parents (**B**), and controls (**C**). (**D**) MnlI restriction analysis of the T→G mutation at nt 977. MnlI restriction generated 2 fragments of 393 and 180 bp in controls and 3 fragments of 352, 180, and 41 bp in the patient. P, patient; C, controls; MW, molecular weight marker (DNA molecular weight marker VIII; Roche Applied Science). (**E**) Sequence alignment of the prenyldiphosphate synthase, FPP synthase, and GPP synthase from human and nonhuman sources. The box shows the polyprenyl synthase signature.

corresponding to the human PDSS1 residue D308 is essential for catalytic activity. Moreover, the mutagenesis of aspartate to glutamate at position 244 of the rat FPP synthase (D244E), equivalent to the D308E in PDSS1, resulted in a 7-fold reduction in the V_{max} value of the enzyme (21). The D308E mutation identified in our patients with ubiquinone deficiency therefore confirms the functional importance of this particular residue for the prenyl-synthase activity of the protein.

The OH-benzoate transpolyprenyltransferase (*COQ2*) gene encodes the enzyme involved in the second step of ubiquinone biosynthesis (13, 22). In a second family with ubiquinone deficiency, a single base pair deletion was identified in the *COQ2* gene, resulting in a premature stop codon and modifying the last 21 amino acids of the protein. The normal but not the mutant protein rescued the growth defect of the Δ*coq2* yeast strain on a nonfermentable carbon source, therefore demonstrating the deleterious consequences of the mutation. Finally, the abnormal accumulation of decaprenyl-PP, the substrate of OH-benzoate polyprenyltransferase in cultured skin fibroblasts of the patient, also supports the relevance of this mutation in ubiquinone deficiency. The mutated domain of the protein is not well conserved across species, but the mutation modifies the global charge at the C-terminal end of the protein, possibly altering its interactions with other proteins. Several enzymes involved in ubiquinone biosynthesis in yeast have been shown to form a multisubunit complex, as Coq1p, Coq4p, Coq5p, and Coq6p have been shown to form a high-molecular-weight complex (23, 24). It is not yet known whether Coq2p is also involved in this complex in yeast and whether a similar complex exists in humans. Nevertheless, the deleterious effect of this human mutant protein in yeast suggests that various interactions between the proteins of the 2 species are still retained despite the absence of sequence homology between human and yeast Coq2p at the C-terminal ends of the proteins.

Primary ubiquinone deficiency is a rare cause of mitochondrial disorders, and the genetic bases of these disorders have rarely been identified, as *COQ2* or *PDSS2* mutations have only been reported twice in patients with encephalomyopathy and kidney involvement (11, 12). In this article, we report what we believe to be the first molecular/functional characterization of *PDSS1* and *COQ2* mutations and demonstrate that both mutations are disease causing.

It is noteworthy that optic atrophy, deafness, obesity, pancytopenia, and cardiac disease have not been previously reported in primary ubiquinone deficiency. While optic atrophy, deafness, and pancytopenia are occasionally reported in respiratory chain deficiency, it remains unclear whether obesity and valvulopathy are directly related to ubiquinone deficiency. Similarly, early and severe forms of ubiquinone deficiency, fatal in the early neonatal period, have seldom been reported (7). Why profound ubiquinone deficiency in cultured fibroblasts was associated with either neonatal death or long survival also remains unexplained (patients 1 and 2 were 22 and 14 years old, respectively). The clinical heterogeneity of ubiquinone deficiency is suggestive of a genetic heterogeneity that could be related to the large number of enzymes and corresponding genes involved in ubiquinone biosynthesis. It is hoped that identification of disease-causing genes in other families will help to elucidate the clinical variability of these rare conditions.

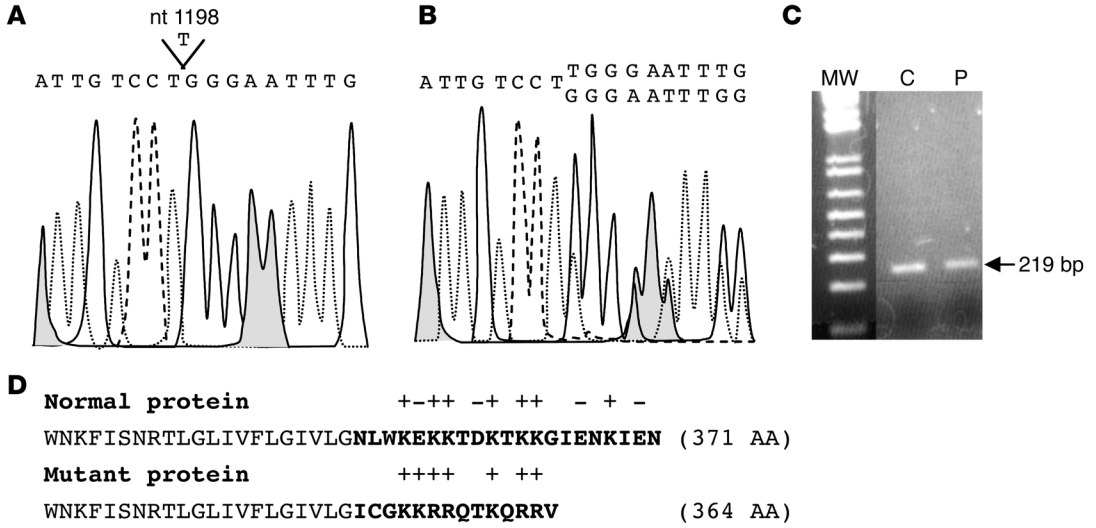

Figure 5
Molecular analysis of the *COQ2* gene. Sequence analysis of the *COQ2* gene in patient 3 (**A**) and her parents (**B**). (**C**) RT-PCR analysis of COQ2 transcript. (**D**) Normal and mutant coq2 proteins.

Methods

Patients. We obtained informed consent from parents and all probands and siblings before collecting blood for DNA extraction or performing tissue biopsies. Patient 1, a boy, was born to first-cousin healthy Moroccan parents. He had 3 unaffected brothers and 1 affected sister. He was normal at birth (weight, 2.7 kg; height, 47 cm; head circumference, 35 cm). Deafness was detected at 1 year of age, and he developed bulimia, obesity, livedo reticularis, and optic atrophy at 3 years. Valvulopathy characterized by mild aortic and mitral regurgitation with moderate pulmonary artery hypertension was detected at the age of 15. At the time of publication, he was 22 years old, with mild mental retardation, and, unlike his healthy siblings, he presented overweight (weight, 73 kg + 1.5 SD; height, 1.58 m – 2 SD) and macrocephaly (head circumference, 57 cm + 1.5 SD). He also has a peripheral neuropathy with absent deep tendon reflexes. Mildly elevated plasma lactate (2.1 mmol/l; N, 1.0–1.55) and lactate/pyruvate molar ratios (16; N, 6–14) were observed, and muscle biopsy showed mitochondrial aggregates.

Patient 2, his sister, was normal at birth (weight, 2.9 kg; height, 46 cm; head circumference, 34 cm). She also developed deafness in her second year and valvulopathy at the age of 9 years. At the time of publication, she was 14 years old and presented obesity (weight, 75 kg + 4 SD; height, 133 cm), macrocephaly (head circumference, 57 cm + 3 SD), optic atrophy, periph-

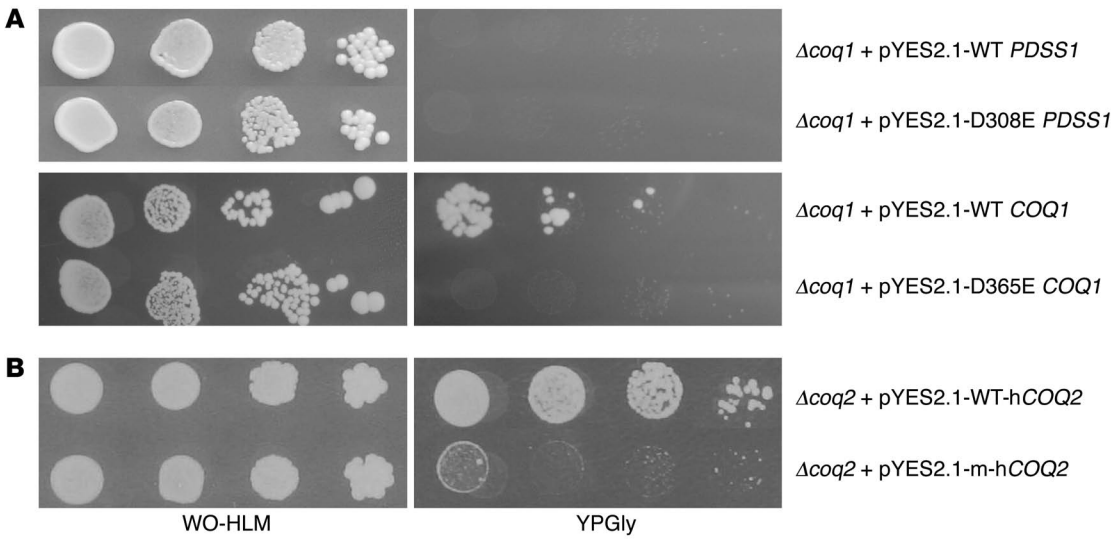

Figure 6
Functional complementation of yeast *coq1*- and *coq2*-null mutant. Growth of *coq1*- and *coq2*-null mutants on glucose (without amino acids, supplemented with histidine, leucine, and methionine, 20 g/l glucose) or glycerol medium (YPGly, 20 g/l glycerol) was compared. (**A**) The yeast Δ*coq1*-null mutant transformed with the wild-type and mutant (D308E) human PDSS1 cDNAs and the wild-type and mutant (D365E) yeast *COQ1* genes on the pYES2.1 plasmid. (**B**) The yeast Δ*coq2*-null mutant transformed with the wild-type and mutant (m) human *COQ2* genes on the pYES2.1 plasmid. The 4 spots for each experiment correspond to successive dilutions of transformed yeasts (1, 0.1, 0.01, and 0.001).

Table 3
Primers used for PCR amplification, sequencing, and cloning

Name of primer	Primer sequence
Human *PDSS1* intronic primers	
1F	GCAAGCGAGGAAGAGCGAAC
1R	TCCCATTCATTCCGCCCACTG
2F	TGAGGCCTGTAAGCCCCTTC
2R	CCCCTTGCACCTCCCAAATC
3F	GCAATCTACCTGTAAATGGG
3R	CTCCCCATCGCTACAAAAAA
4F	GGCTGAATTTTCCGTATCTG
4R	AACAGAGTGAGACTCCAGCT
5F	CAACAAGAGCGAAACTCCGT
5R	CCCAGCCGGTATATGCTAAT
6F	CCGATGTCCAGTTTTCACAA
6R	TCTGAGAAACAAATGCGGTC
7-8F	CCTTCCAGTCAGTTTCATCA
7-8R	CTCTTGGGATTTTTGGGTAC
9-10F	AAGGAATTCCTTCAGCCAGG
9-10R	GCAATCTTCCCATCAGCTGT
11F	CCCACATCATCTAGACACTT
11R	GTCACATCAAGAGTTCACCA
12F	GCAGGAAGTTAAAACGCTGA
12R	GACTTCAATGCAGACTTCAC
PDSS1 cDNA primers	
PDSS1-F	ATGGCCTCGCGCTGGTGG
PDSS1-R	TCATTTATCTCTTGTGAG
Yeast *COQ1* primers	
COQ1-mutF	TTCCAACTCGTAGATGA(G)ATGCTTGATTTTACTGT
COQ1-mutR	ACAGTAAAATCAAGCAT(C)TCATCTACGAGTTGGAA
COQ1-1	ATGTTTCAAAGGTCTGGCGCT
COQ1-1419	CTTTCTTCTTCTTAGTATACT
Human *COQ2* intronic primers	
1F	CGGGTHCCACTGCGCATGCCT
1R	GGAGCCGACTCGGAGGCTGCT
2F	TAACTGACTTAAATTTGTACC
2R	CACTATGTTATTCTGCAGAAT
3F	TGTTTAAATAAATGATTGAGTCA
3R	CCTATTTTCTCCATTTCAAAG
4F	ATTTCGTGGTTTCTAAGAGGT
4R	CTCCATAAAAGTGTAGTTTGC
5F	CCCTTTAGGATTCTAAAGTA
5R	ATTTGGTTCTTTAAAAACAGCA
6F	GAATTTTATGGTGTGTGAACT
6R	TTATACCAAGGAAATATGAGG
7F	TTTGTTATGACCCAAAGTCCA
7R	GTATCAGATTTTGTATTCAAATC
COQ2 cDNA primers	
COQ2-1	ATGACCCCAATTTCACAAGTA
COQ2-1114	ATTTTCTATTTTATTCTCTA
COQ1-mutF	TTTAGGGATTGTCC()TGGGAATTTGTGGAAA
COQ1-mutR	TTTCCACAAATTCCCA()GGACAATCCCTAAA

Empty parentheses indicate the position of deleted nucleotide.

eral neuropathy, and mental retardation similar to that of her brother. She also has livedo reticularis and mildly elevated plasma lactate levels (3.2 nmol/l; N, 1.0–1.55) and lactate/pyruvate ratios (21; N, 6–14).

Patient 3, a girl, was born to healthy parents of French origin. Both parents were from the same geographical region and shared the same patronymic, but they were not aware of any consanguinity. She presented neurologic distress at birth. Liver failure and nephrotic syndrome were diagnosed at 2 days of age. At 6 days, she developed anemia, pancytopenia, insulin-dependent diabetes, cytolysis, and seizures and presented hyperlactatemia (22.7 mmol/l) and high lactate/pyruvate molar ratios. She died at 12 days of age from multiorgan failure. Her older brother experienced collapse with hyperglycemia (15 mmol/l) and hyperlactatemia shortly after birth (16 mmol/l). He also had severe anemia, liver failure, and renal insufficiency and died at the age of 1 day.

Enzyme studies. Polarographic tests and/or spectrophotometric OXPHOS enzyme assays were carried out on skeletal muscle mitochondria, muscle homogenate, liver homogenate, cultured skin fibroblasts, or circulating lymphocytes, as previously described (25).

Genotyping. Fluorescent microsatellite markers of the Généthon database were used (26). The purified dye-labeled fragments were separated according to size on Amersham Biosciences MegaBACE 1000 96-capillary sequencers.

Analysis of CoQ_{10} content and biosynthesis in skin fibroblasts. All solvents (Merck) were HPLC grade. Quinones were extracted from freeze-thawed fibroblasts (10^6 cells in 1 ml of water) according to the method of Folch et al. (27), with 3 ml of chloroform/methanol (2:1) and 5 pmol of CoQ_6 (Sigma-Aldrich) as internal standard. After shaking and centrifugation (2,000 *g*; 5 minutes), the organic layer was evaporated under a nitrogen stream at 60°C and resuspended in 100 μl of methanol. CoQ_{10} was quantified by liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry using a triple quadruple tandem mass spectrometer (API3000; Applied Biosystems) coupled with a liquid chromatographic system (HP 1100 series; Agilent Technologies). Lipid extracts (20 μl) were injected into an Uptisphere ODB column (33 × 2 mm, 3 μm; Interchim) and separated by a linear gradient from 40% solvent A (methanol/water, 9:1) to 90% solvent B (chloroform/methanol, 1:1) at 500 μl/min for 2 minutes for a total run time of 5 minutes (column oven maintained at 35°C). Multiple-reaction monitoring transitions were 863.9–197.2 and 591.5–197.2 for CoQ_{10} and CoQ_6, respectively. CoQ_{10} content was expressed with reference to the protein content of the cell extracts. Cells were labeled with [^{3}H]mevalonate, and extraction of lipids and HPLC analyses were performed as previously described (6). Since decaprenyl-PP cannot be separated by reverse-phase HPLC, both phases of lipid extracts (petroleum ether and methanol) were combined, dried, and subjected to treatment with wheat germ acid phosphatase (28). Decaprenol was identified on the HPLC chromatogram by a reference sample and mass spectrometry.

Mutation screening. The exons and exon-intron boundaries of the human prenyldiphosphate synthase (*PDSS1*) and OH-benzoate polyprenyltransferase (*COQ2*) genes were PCR amplified using specific intronic primers (Table 3). Amplification products were directly sequenced using the PRISM Ready Reaction Sequencing Kit (Applied Biosystems) on an automatic sequencer (Applied Biosystems).

Complementation of a yeast coq1- and coq2-null mutant. The *COQ1* gene was PCR amplified using oligonucleotide primers COQ1-1 and COQ1-1419 and cloned in a pYES2.1 plasmid according to the manufacturer's recommendations (Invitrogen). The D365E mutation was introduced into the wild-type yeast *COQ1* gene cloned in pYES2.1 using the QuikChange XL site-directed mutagenesis kit (Stratagene) and COQ1-mutF and COQ1-mutR primers (Table 3; the mutation is shown in parentheses). Human wild-type and mutant PDSS1 cDNAs were RT-PCR amplified from control and patient fibroblast RNA using PDSS1-F and PDSS1-R primers (Table 3) and cloned in a pYES2.1 plasmid. Human wild-type COQ2 cDNA previously cloned in a pBV134.nuc vector (gift from I. Climent, Biovitrum, Stockholm, Sweden) was PCR amplified using COQ2-1 and COQ2-1114 primers and cloned in pYES2.1 plasmid. The c.1198delT, N401fsX415 mutation was introduced into the wild-type COQ2

cDNA using the site-specific mutagenesis kit (Stratagene) and COQ2-mutF and COQ2-mutR primers (Table 3; the deletion is shown in parentheses).

The yeast Δ*coq1* and Δ*coq2* strains (his$^-$, ura$^-$, leu$^-$, met$^-$) were transformed according to ref. 29, and the resulting clones were grown on yeast base plates without amino acids supplemented with histidine, leucine, and methioine and replicated on a glycerol-rich medium for 3–4 days at 30°C.

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