

CABC1 Gene Mutations Cause Ubiquinone Deficiency with Cerebellar Ataxia and Seizures

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Coenzyme Q₁₀ (CoQ₁₀) plays a pivotal role in oxidative phosphorylation (OXPHOS) in that it distributes electrons between the various dehydrogenases and the cytochrome segments of the respiratory chain. Primary coenzyme Q₁₀ deficiency represents a clinically heterogeneous condition suggestive of genetic heterogeneity, and several disease genes have been previously identified. The *CABC1* gene, also called *COQ8* or *ADCK3*, is the human homolog of the yeast *ABC1/COQ8* gene, one of the numerous genes involved in the ubiquinone biosynthesis pathway. The exact function of the Abc1/Coq8 protein is as yet unknown, but this protein is classified as a putative protein kinase. We report here *CABC1* gene mutations in four ubiquinone-deficient patients in three distinct families. These patients presented a similar progressive neurological disorder with cerebellar atrophy and seizures. In all cases, enzymological studies pointed to ubiquinone deficiency. CoQ₁₀ deficiency was confirmed by decreased content of ubiquinone in muscle. Various missense mutations (R213W, G272V, G272D, and E551K) modifying highly conserved amino acids of the protein and a 1 bp frameshift insertion c.[1812_1813insG] were identified. The missense mutations were introduced into the yeast *ABC1/COQ8* gene and expressed in a *Saccharomyces cerevisiae* strain in which the *ABC1/COQ8* gene was deleted. All the missense mutations resulted in a respiratory phenotype with no or decreased growth on glycerol medium and a severe reduction in ubiquinone synthesis, demonstrating that these mutations alter the protein function.

Introduction

Coenzyme Q₁₀ (CoQ₁₀, ubiquinone), a lipophilic component located in the inner mitochondrial membrane, plays a pivotal role in oxidative phosphorylation (OXPHOS). CoQ₁₀ shuttles electrons from complexes I and II to complex III. CoQ₁₀ also plays a critical function in antioxidant defenses. CoQ₁₀ deficiency [MIM 607426] represents a clinically heterogeneous and largely under-diagnosed condition. This clinical heterogeneity is suggestive of a genetic heterogeneity, and several disease genes have been hitherto identified. The human *PDSS1* (MIM 607429) and *PDSS2* (MIM 610564) genes encode the two subunits of the prenyl diphosphate synthase (encoded by the *COQ1* gene in yeast), the enzyme elongating the prenyl side chain of ubiquinone. *PDSS1-2* mutations have been identified in patients presenting with either multi-organ involvement¹ or Leigh syndrome and nephropathy.² Similarly, the *COQ2* (MIM 609825) gene encodes the *para*-hydroxybenzoate-polyprenyltransferase that condensates the 4-OH benzoate ring with the prenyl side chain. *COQ2* mutations have been reported in a family with encephalomyopathy and nephropathy^{3,4} and in a family with fatal infantile multi-organ disease.¹ Yet, because of the small size of the families, the disease causing gene remains unknown in most cases of CoQ₁₀ deficiency.

On the other hand, the quinone biosynthesis pathway has been extensively studied in bacteria and in the yeast *Saccharomyces cerevisiae*. So far, ten genes encoding proteins involved in ubiquinone synthesis have been identified in yeast (*COQ1–COQ10*). All of them except *COQ1* and *COQ2* catalyze hydroxylation, *O*-methylation, decarboxylation, and *C*-methylation reactions and contribute to the synthesis of the fully substituted ubiquinone.⁵ On the basis of protein homology, homolog genes have been identified in humans. Despite their unknown exact function, they represent obvious candidate genes for quinone deficiency in humans.

The *CABC1* (MIM 606980) gene is the human homolog of the yeast *ABC1/COQ8* gene. *ABC1* (for Activity of bc1 complex) has been initially identified as a multicopy suppressor of a cytochrome *b* translation defect, and the Abc1p was believed to act as a chaperon-like protein of the bc1 complex.⁶ More recently, *ABC1* has been shown to complement the *coq8-1* mutant defective in ubiquinone synthesis.⁷ *ABC1* and *COQ8* therefore represent one single gene. The exact function of the Abc1/Coq8 protein is as yet unknown, but this protein is classified as a putative protein kinase based on the presence of kinase motifs in its amino acid sequence. The yeast *ABC1/COQ8* gene is the homolog of the *ubiB* and *aarF* genes required for the first mono-oxygenation step of ubiquinone synthesis in the

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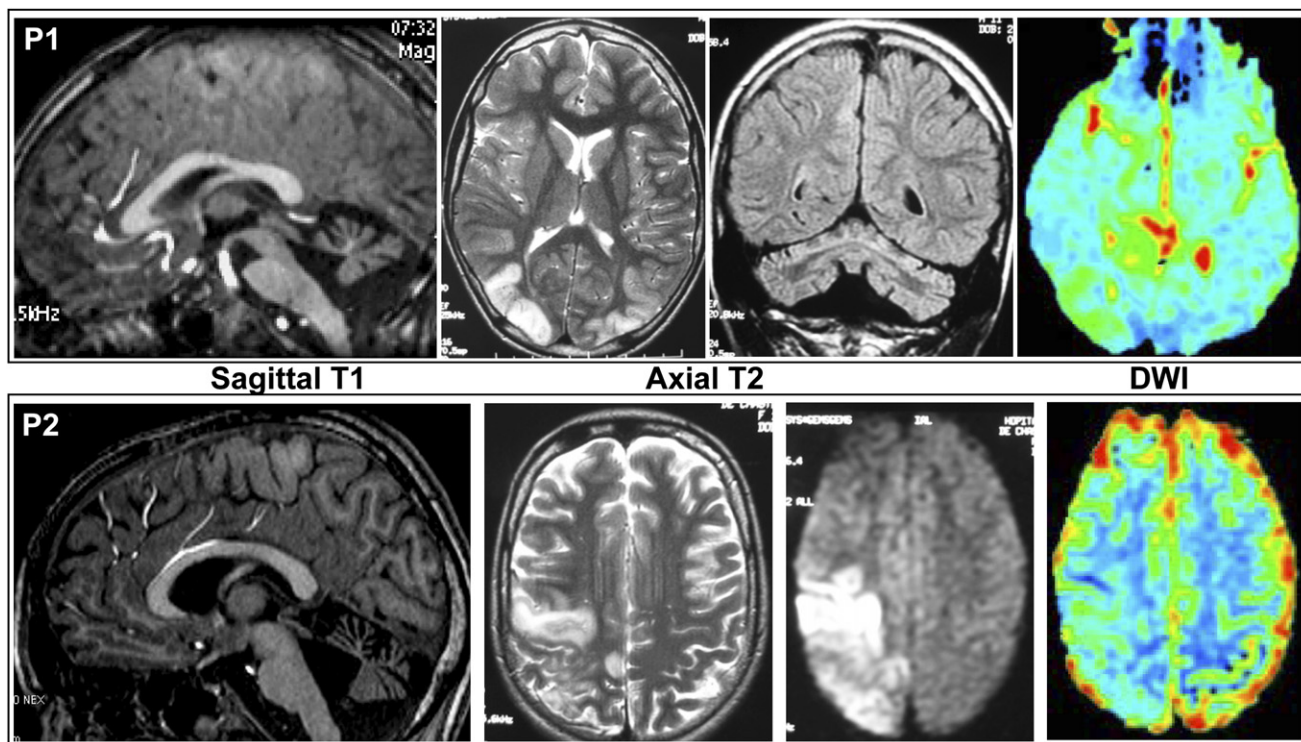


Figure 1. Brain MRI of Patients 1 and 2

The sagittal T1 showed an important cerebellar atrophy in both patients. The axial T2 also revealed abnormal hyperintensities in the posterior cerebral regions. The diffusion-weighted imaging (DWI) showed a normal apparent diffusion coefficient in these posterior regions. These abnormalities were interpreted as a stroke-like episode.

prokaryotes *E. coli* and *P. stuartii*, respectively.^{8,9} *CABC1* (Chaperone activity of bc1 complex), the human counterpart of the yeast *ABC1/COQ8* gene, is also called *COQ8* or *ADCK3*.

We report here on *CABC1* gene mutations in four ubiquinone-deficient patients belonging to three distinct families. These patients were considered to be respiratory-chain deficient because they presented with a progressive neurological disorder with cerebellar atrophy, developmental delay, and hyperlactatemia. Reduced amounts of ubiquinone in their muscle prompted us to systematically study the human genes involved in ubiquinone synthesis and to eventually identify various missense or frameshift *CABC1* mutations in these children.

Material and Methods

Patients

Patient 1 is the second child of healthy, unrelated parents originating from the Reunion Island. An older sister is healthy. Patient 1 was able to walk unaided at 1 year, but he fell frequently. This was at first ascribed to proximal muscle weakness at 18 months. Cerebellar ataxia and strabismus were diagnosed 6 months later. At 2.5 years of age, he presented generalized tonic seizures with high CSF lactate (4 mmol/liter, $n < 2$ mmol/liter). He was given rivotril and valproate (675 mg/kg/day) orally, and these controlled seizures for the following five years. At 3 years of age, he still presented trunk hypotonia and a slight intellectual regression. He

received oral CoQ₁₀ (5 mg/kg/day) from age 3 years, 8 months to age 4, and then he was put on higher doses (10 mg/kg/day) from age 4 to age 7, with no clinical benefits. Because of his unresponsiveness to oral CoQ₁₀, he was put on a short-chain quinone, idebenone (10 mg/kg/day), which not only failed to improve his condition but also worsened the course of the disease and was therefore discontinued after 7 months. His neurological condition worsened at 12 years of age, and 1 year later he was unable to walk or speak, and the number of seizures increased. Brain MRI performed at 12 years of age revealed severe cerebellar atrophy and stroke-like anomalies with hyperintensity on T2 and FLAIR (Figure 1). At 16 years, he has *epilepsia partialis continua* and consistently elevated plasma lactate (3 mmol/liter, $n = 1.0$ – 1.55 mmol/liter) as well as an elevated L/P ratio (18, $n = 6$ – 14). A histological study of his skeletal muscle at 6 years revealed mitochondrial accumulation and lipid droplets in 10%–20% of the fibers. No ragged-red fibers were observed.

Patient 2 is the third child of healthy, unrelated parents of French origin. Two older siblings are healthy (Figure 2). Patient 2 was normal at birth but presented mild hypotonia in the first months of life. She could sit unaided at 9 months and walk at 2 years but presented *talus valgus*. Developmental delay was first noted at 18 months. At 8 years, she presented seizures controlled by oral sodium valproate. Then she developed cerebellar ataxia and ptosis with brain MRI evidence of vermis hypoplasia. At 14 years of age, she had *epilepsia partialis continua*. She has been on oral CoQ₁₀ (20 mg/kg/day) since then with no clinical benefit. Brain MRI at that age revealed a severe cerebellar atrophy with stroke-like anomalies and hyperintensities on T2 and FLAIR (Figure 1).

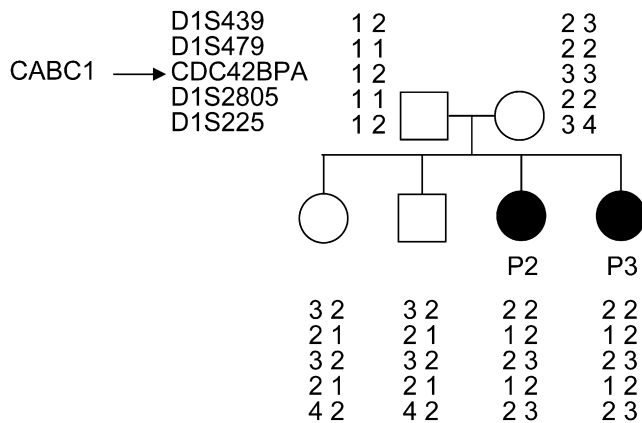


Figure 2. Pedigree and Haplotype Analysis in a Multiplex Family: Patients 2 and 3

Haplotypes are given (top to bottom) for loci D1S439, D1S479, CDC42BPA, D1S2805, and D1S225.

Patient 3 is the younger sister of patient 2. Her clinical course is similar to that of her sister. She is now 13 years old and presents a cerebellar ataxia.

Patient 4 has been previously reported.¹⁰ An early intolerance to exercise was followed by progressive cerebellar syndrome and cerebellar atrophy.

All procedures were approved by the review board of the Necker Hospital. We obtained informed consent from parents of all patients and siblings before collecting blood for DNA extraction or performing tissue biopsies.

Enzyme Studies and Analysis of CoQ₁₀ Content

Polarographic tests and/or spectrophotometric respiratory-chain enzyme assays were carried out on skeletal-muscle mitochondria, muscle homogenate, or cultured skin fibroblasts as previously described.¹¹ Quinones from frozen muscle (3–10 mg), cultured skin fibroblasts, and yeast were extracted and quantified as previously described.¹ All solvents (Merck) were HPLC grade. The various yeast strains were grown in 5 ml of glucose minimum medium for 60 hr and then in YPGal for 12 hr. Total yeast extracts were obtained after spheroblasting the cells according to Mamnun et al.,¹² Quinones were extracted and quantified as previously described.¹ Quinone content in yeast was expressed as the ratio CoQ₆/CoQ₁₀, where CoQ₁₀ (10 μM) was used as an internal standard.

Mutation Screening

The exons and exon-intron boundaries of the human *CABC1* gene were PCR amplified with specific intronic primers (Table 1). Amplification products were directly sequenced with the PRISM Ready Reaction Sequencing Kit (Perkin-Elmer) on an automatic sequencer (Applied Biosystems).

Complementation of a Yeast *coq8* Null Mutant

The R77W, G130V, G130D, and E409K mutations, corresponding to the human R213W, G272V, G272D, and E551K mutations, respectively, were introduced into the wild-type yeast *COQ8* gene cloned in pFL44 (gift from G. Dujardin) with the site-specific mutagenesis kit (Stratagene) and specific primers (Table 1; mutations are shown in brackets). The yeast $\Delta coq8$ strain (gift from G. Dujardin; *ade2, leu2, trp1, his3, ura3, coq8::LEU2*) was transformed according to Gietz et al.,¹³ and the resulting clones were grown

Table 1. Primers

Human <i>CABC1</i> Intronic Primers	
Exon 2F	5'-GTTGGTAGTGTGGGTTTGGC-3'
Exon 2R	5'-AGAGCTCCAGGTCCCACC-3'
Exon 3F	5'-GACAGGCAGGGAGGAGC-3'
Exon 3R	5'-CATCACTGTGTGGTGG-3'
Exon 4F	5'-CTGAAGGTTGGTCTGTGCC-3'
Exon 4R	5'-GGTCCCACCTCCATGC-3'
Exon 5F	5'-GGTGTCTGTGGTGTCTGC-3'
Exon 5R	5'-CTGACACTCCAGAGCACAG-3'
Exon 6F	5'-CTTGCCATCCCCTCCC-3'
Exon 6R	5'-CGGCCCTGGAAGTTGAC-3'
Exon 7-8F	5'-CTGGGCGCACACTTAAATC-3'
Exon 7-8R	5'-GTGTAGAGGCTCCATGAGC-3'
Exon 9-10F	5'-CCAGGACACAGCTGGGAAG-3'
Exon 9-10R	5'-CTGAACATGGTCCCTTCTGC-3'
Exon 11F	5'-TGCCTGGGGTGAAGGAG-3'
Exon 11R	5'-GTCACCAAACAGCCACACAG-3'
Exon 12-13F	5'-CTTCTGTGGCCCCAGTCTC-3'
Exon 12-13R	5'-CATCCTGTCCCAGCCTCAG-3'
Exon 14F	5'-CTCGGGTGTGGCACTTG-3'
Exon 14R	5'-CCCTGTGCTTTCATCCATTC-3'
Exon 15F	5'-CTGGTGGAGGGCTCTGG-3'
Exon 15R	5'-CACCACGACCTCGTACTG-3'
Yeast <i>ABC1/COQ8</i> Primers for Site-Directed Mutagenesis	
Yeast-R77W-F	5'-ACTTACGAATATCG(T)G(G)TTGTTCCATTATGGC-3'
Yeast-R77W-R	5'-GCCATAATGGAACAA(C)C(A)CGATATTCGTGAAGT-3'
Yeast-G130V-F	5'-TCTAAAATGAGAG(T)TGTTGCCCTTAAAATAGGG-3'
Yeast-G130V-R	5'-CCCTATTTTAAAGGGCAACA(A)CTCTCATTTTAGA-3'
Yeast-G130D-F	5'-TCTAAAATGAGAG(A)TGTTGCCCTTAAAATAGGG-3'
Yeast-G130D-R	5'-CCCTATTTTAAAGGGCAACA(T)CTCTCATTTTAGA-3'
Yeast-E409K-F	5'-TATCTCACAGGCTTA(A)AATCGCAGTCCATGAAA-3'
Yeast-E409K-R	5'-TTTCATGGACTGCGATT(T)TAAGCCTGTGAGATA-3'

Mutations are shown in parentheses.

on WO plates supplemented with appropriate amino acids and replicated on a glycerol-rich medium for 3–4 days at 30°C.

Results

Enzymologic Studies and Quinone Quantification

A severe impairment of malate+pyruvate and succinate oxidation was observed in muscle mitochondria of patient 1 (Table 2). Assessment of individual OXPHOS enzyme activities in this tissue revealed normal CIV activity but severely reduced CII+III activity. Respiratory-chain enzyme activities in his cultured skin fibroblasts were normal, but a slight increase in the CIV/CII+III ratio was observed. In patient 2, muscle mitochondria displayed high individual enzyme activities, but CIV/CII+III and CII+III/CII activity ratios were also elevated, indicating quinone deficiency. Similarly, high respiratory-chain activities and a slight increase of CIV/CII+III were noted in her cultured skin fibroblast. Enzyme activities in muscle mitochondria were substantially increased by the addition of decylubiquinone (DQ, an exogenous ubiquinone analog, Table 2), again suggesting quinone deficiency. Addition of DQ resulted in a 40% induction of CII+III activity (276 and 396 nmol/min/mg protein before and after DQ addition, respectively).

Table 2. Respiratory-Chain Enzyme Activities in Muscle Mitochondria and Cultured Skin Fibroblasts

	Muscle Mitochondria		Muscle Mitochondria		Muscle Homogenate		Fibroblasts		
	P1	Control	P2	Control	P4 ^a	C ^a	P1	P2	Control
Activities (nmol/min/mg protein)									
malate+glutamate oxidase	<8*	80 ± 30	-	-	-	-	-	-	-
malate+pyruvate oxidase	<8*	80 ± 30	-	-	-	-	-	-	-
succinate oxidase	<8*	91 ± 26	85	59.5 ± 12.2	-	-	-	-	-
glycerol-3-P oxidase	-	-	19.9	11.9 ± 2.3	-	-	-	-	-
CI	-	-	143	73 ± 15	76	22 ± 7	60	88	41 ± 5
CII	-	-	162	98 ± 20	71	31 ± 8	98	139	74 ± 8
CIII	-	-	2030	1458 ± 257	337	107 ± 26	1052	1197	762 ± 103
CIV	2334	3151 ± 760	1281	740 ± 146	99	56 ± 17	538	798	384 ± 44
CV	-	-	372	335 ± 68	-	-	140	132	110 ± 14
CI+III	-	-	-	-	4*	17 ± 10	-	-	-
CII+III	48*	728 ± 122	276	233 ± 48	6*	18 ± 7	155	279	138 ± 16
CII+III activation by DQ	-	-	43%*	0%	-	-	-	0%	0%
CS	2415	2624 ± 694	-	-	956	153 ± 35	255	247	238.5 ± 25.6
Activity Ratios									
CIV/CS	1.8	1.3 ± 0.3	-	-	-	-	2.1	3.2*	1.7 ± 0.2
CII+III/CS	0.016*	0.33 ± 0.08	-	-	-	-	0.6	1.13*	0.58 ± 0.1
CIV/CII+III	48*	4 ± 1.3	4.6*	3.2 ± 0.3	-	-	3.4*	2.9*	2.0 ± 0.2
CII+III/CII	-	-	1.7*	2.5 ± 0.2	-	-	-	2.0	2.1 ± 0.2

Enzyme activities on muscle mitochondria have been determined in two different laboratories for patients 1 and 2. Abnormal values are indicated with an asterisk.

^a Previously published by Aure et al., 2004.¹⁰

Direct evidence of quinone deficiency was eventually provided by quantification of CoQ₁₀ in the patients' muscle. Indeed, the muscle CoQ₁₀ content was markedly decreased in patient 1 and in the lower control values in patient 2 (Table 3). The quinone content was normal in cultured skin fibroblasts from patients 1 and 4.

Molecular Studies

The genes known to be involved in ubiquinone biosynthesis were systematically studied by direct sequencing in patients 1–4. Patient 1 carried a homozygous G-to-A transition at nucleotide 1655 in exon 14 of the *CABC1* gene. This transition resulted in the change of a highly conserved glutamic acid into a lysine (E551K, Figure 3). Both parents were heterozygous for this mutation. Patients 2 and 3 showed haploidentity for markers D1S439, D1S479, D1S2805, and D1S225 surrounding *CABC1*

Table 3. Quinone Content in Muscle and Cultured Skin Fibroblasts

	P1	P2	Control	P4 ^a	Control ^a
Muscle (pmol/mg muscle)	2.6*	9.4*	32.2 ± 9.8 (n = 42)	<1 µg/g*	19–30 µg/g
Fibroblasts (pmol/mg protein)	125	-	120 ± 32 (n = 13)	normal	

Quinone content was determined in two different laboratories as pmol/mg muscle for patients 1 and 2 and as µg/g for patient 4. Abnormal values are indicated with an asterisk.

^a Previously published by Aure et al., 2004.¹⁰

gene, as well as for the intragenic CDC42BPA marker, whereas their healthy siblings presented distinct haplotypes (Figure 2). They were compound heterozygotes for a C-to-T transition inherited from the father at nucleotide 636 (R213W) and a G-to-T transversion inherited from the mother at nucleotide 815 (G272V, Figure 3). Both mutations resulted in the modification of highly conserved residues in the protein. The two healthy siblings were heterozygous carriers for the R213W mutation. Finally, patient 4 was a compound heterozygote for one missense G-to-A transition inherited from the father at nucleotide 815 (G272D) and one base pair insertion inherited from the mother c.[1812_1813insG] (Figure 3). When RT-PCR amplification of fibroblast RNAs was performed with primers in the last exon, it showed that the abnormal transcript was still present in patient 4 (not shown). This finding suggests that a resulting mutant protein might be synthesized in this patient (728 instead of 647 amino acids). None of the c.[1655G > A] or c.[815G > A] mutations were found in more than 460 chromosomes. However, heterozygosity for the c.[636C > T] and c.[815G > T] mutations were found in 2/440 (0.45%) and 3/470 (0.64%) controls, respectively.

Functional-Complementation Studies

To confirm the deleterious nature of the various *CABC1* mutations, we tested mutant genes for their ability to complement the respiratory-chain defect in the *Saccharomyces cerevisiae* strain deleted for the *ABC1/COQ8* gene. We introduced the various missense mutations into the yeast

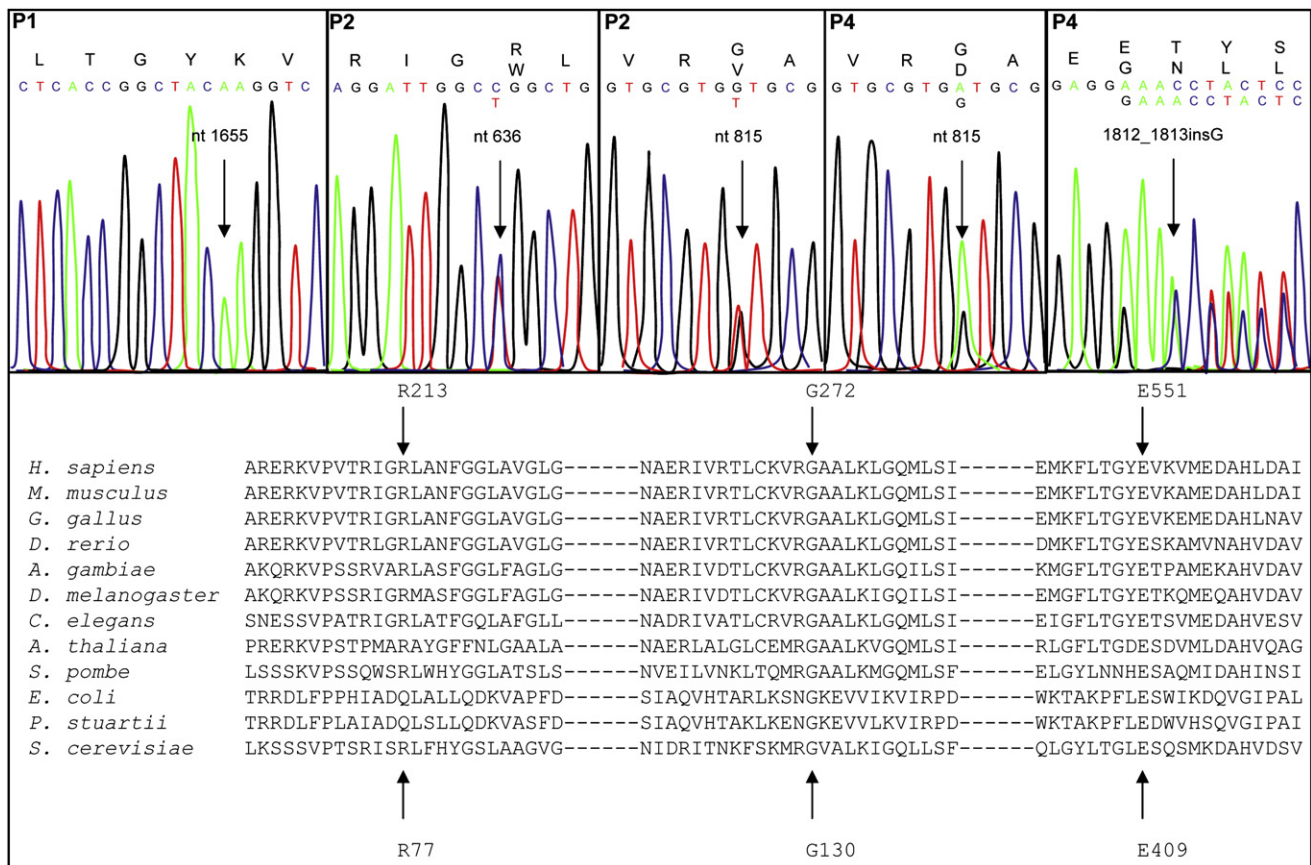


Figure 3. Sequence analysis of the *CABC1* gene in patients 1–4 (upper panel) and sequence alignment of the *CABC1* proteins from human and non-human sources (lower panel). The arrows indicate the various mutations

ABC1/COQ8 gene cloned in the pFL44 high-copy plasmid by site-directed mutagenesis. We used the wild-type and the four different mutant plasmids to transform a yeast

Δabc1/coq8 null strain. Growth of the mutant on glycerol-rich medium was restored by the wild-type yeast *ABC1/COQ8* gene (Figure 4A). However, the yeast G130D

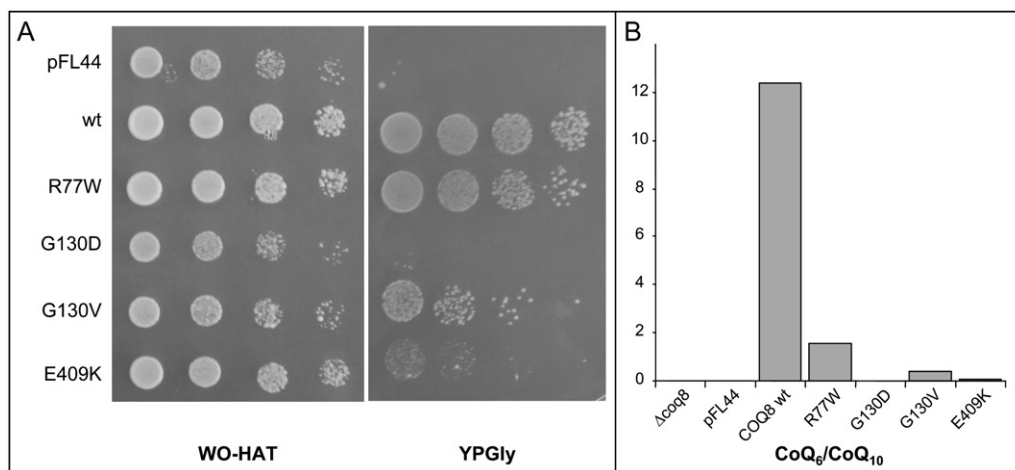


Figure 4. Functional Analysis of *CABC1* Mutations

(A) Functional complementation of the yeast *abc1/coq8* null mutant. Growth of *abc1/coq8* null mutant on either glucose (WO-HAT 20 g/liter glucose) or glycerol medium was compared (YPgly, 20 g/liter glycerol). The yeast *Δabc1/coq8* null mutant was transformed with the wild-type (wt), R77W, G130V, G130D, and E409K mutant *ABC1/COQ8* yeast genes cloned in pFL44 plasmid. The four spots for each experiment correspond to decreasing dilutions of transformed yeasts.

(B) Quinone content in transformed *Δabc1/coq8* null mutants. CoQ₆ content was measured by mass spectrometry and expressed as CoQ₆/CoQ₁₀. CoQ₁₀ was used as an internal standard.

protein corresponding to the human G272D protein completely failed to complement the yeast mutant (Figure 4A). The yeast E409K and G130V proteins corresponding to the human E551K and G272V proteins presented a very weak growth on glycerol medium (Figure 4A). Finally, the R77W mutation corresponding to the human R213W mutation resulted in slightly decreased growth on glycerol medium (Figure 4A). Because yeast synthesizes CoQ₆ as ubiquinone, we then measured the CoQ₆ amount in the various transformed *Δcoq8* null strains and observed a severe reduction of ubiquinone in these mutant strains. A more severe reduction was observed for the G130D (0% of wild-type ubiquinone content), whereas the R77W mutation displayed only 12% of control ubiquinone values (Figure 4B). These results demonstrate that the various tested mutations altered the protein function.

Discussion

This study first reports the identification of deleterious *CABC1* mutations in three unrelated families of various origins. These mutations were identified in children with respiratory-chain deficiency and reduced ubiquinone in muscle tissue. The clinical presentation of the patients was relatively similar. Indeed, the patients presented with muscle weakness and abnormal exercise fatigability between 18 months and 3 years. Then, they all developed cerebellar ataxia and MRI evidence of severe cerebellar atrophy. Seizures were characteristic of *epilepsia partialis continua*. Finally, no *CABC1* mutations could be identified in a series of seven patients with ubiquinone deficiency and various clinical presentations such as cardiomyopathy, Leigh syndrome, and multisystemic involvement. Several disease gene mutations have been hitherto reported in patients with ubiquinone deficiency and various clinical presentations. The small number of families unfortunately hampers genotype-phenotype correlation. Our present results suggest that *CABC1* mutations form a homogenous group of ubiquinone deficiency. Finally, secondary ubiquinone deficiency in patients with myopathy due to *ETFDH* mutations has been described and seems to represent a clinically and genetically homogenous entity.¹⁴

Despite clinical homogeneity, patients carrying *CABC1* mutations displayed various degrees of respiratory-chain impairment and ubiquinone deficiency. Indeed, patients 1 and 4 showed severely decreased muscle ubiquinone content, resulting in low CII+III and/or CI+III activities, whereas ubiquinone content in the muscle of patient 2 was in the lower control values. In this last case, only stimulation of CII+III activity by DQ suggested a ubiquinone deficiency. Interestingly, patient 2 carried the less severe mutations (R213W and G272V), as shown when these were expressed in the *Δcoq8* null yeast strain. One can hypothesize that *CABC1* mutations cause a more severe ubiquinone defect in CNS than in muscle, although muscle is the only available tissue. For this reason, we suggest systematically looking at DQ stimulation when perform-

ing CII+III assays. It should be mentioned that normal ubiquinone amounts were detected in fibroblasts of patients 1 and 4 and revealed a tissue-specific ubiquinone deficiency.

The various *CABC1* mutations reported here affect highly conserved amino acids of the protein. The exact function of the *CABC1* protein in humans and other organisms is still unknown. It has been suggested that *Abc1* in yeast could act as a putative protein kinase, but none of the mutations identified here lie in any kinase domain. All the missense mutations, when introduced in the yeast *ABC1/COQ8* gene, resulted in a respiratory phenotype with no or decreased growth on glycerol medium and a severe reduction in ubiquinone synthesis. We observed a clear correlation between the growth defect on glycerol and the residual CoQ₆ content; the lower CoQ₆ content was observed for the more severe growth defects. Interestingly, the R77W yeast protein resulted in a mild growth defect, whereas the CoQ₆ content was significantly reduced. It appears therefore that a low residual CoQ₆ content allows a significant growth on selective medium. Finally, the frameshift mutation found in patient 4 is supposed to modify a highly conserved domain of the protein. It has been shown in yeast that *ABC1/COQ8* can suppress *coq9* mutants, suggesting that the two genes and proteins are functionally related.¹⁵ Stable physical interaction between the two proteins could not be observed in yeast; nevertheless, transient interaction cannot be excluded. One can hypothesize that the abnormal C-terminal end of the mutant protein resulting from the c.[1812_1813insG] insertion could alter a putative interaction or regulation between the *Coq8* and *Coq9* proteins.

On the basis of evidence of ubiquinone deficiency, the patients were given oral ubiquinone supplementation. This resulted in a slight improvement of patient 4, but a progressive cerebellar ataxia appeared 2 years later. Patients 1–3 did not significantly improve upon ubiquinone administration. Yet, the parents observed a slight improvement after one year. Activation of CII+III activity in muscle mitochondria and the rescue of yeast *Δabc1/coq8* mutant growth by Q₆ supplementation⁷ suggest that the respiratory-chain deficiency caused by *CABC1* mutations in humans (or in the yeast *abc1/coq8* null mutant) is rapidly corrected by exogenous quinone. Therefore, the absence of spectacular improvement after oral quinone supplementation suggests an insufficient uptake of CoQ₁₀ across the blood-brain barrier in our patients.

Genes involved in ubiquinone biosynthesis in humans, including *CABC1*, are ubiquitously expressed. Why mutations in this gene as well as in other ubiquinone-synthesis genes resulted in different clinical presentations and tissue-specific ubiquinone deficiency is intriguing. One can hypothesize that different organs have different ubiquinone requirements. According to another hypothesis, the expression level of the various proteins involved in ubiquinone synthesis could be different in various tissues. It has been shown in yeast that *coq3–coq9* mutants accumulate the same intermediate hexaprenylhydroxybenzoate¹⁶

and that Coq3, Coq4, Coq6, and Coq7 proteins form a complex.¹⁷ Coq8 protein does not seem to be involved in this complex but should be coordinately regulated with the proteins of this complex. Considering the high homology between yeast and human proteins involved in ubiquinone synthesis, it is tempting to speculate that such a complex also exists in humans. This would obviously require a coordinated and possibly tissue-specific regulation of the corresponding genes. Therefore, dysfunction in any of these proteins could result in changes in the other protein levels, perhaps with a tissue-specific response.

CABC1 is a target of the p53 tumor suppressor gene and is therefore the third p53-regulated gene in mitochondrial disorders.¹⁸ Indeed, *SCO2* mutations resulted in cardioencephalomyopathy and cytochrome oxidase deficiency,¹⁹ and it has been recently shown that aerobic respiration is regulated by p53 via *SCO2* expression.²⁰ Finally, we recently identified *p53R2*, encoding the p53-controlled small ribonucleotide reductase, as a new gene of mitochondrial DNA depletion.²¹ None of the patients with *CABC1*, *SCO2*, or *p53R2* mutations show major evidence of increased apoptosis or tumor susceptibility, suggesting that p53 not only is involved in the suppression of tumor development but could also play an as-yet-unknown function (including mitochondrial control) during cell life.

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Web Resources

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim>

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