Coenzyme Q deficiency and cerebellar ataxia associated with an aprataxin mutation

C. M. Quinzii, A. G. Kattah, A. Naini, H. O. Akman, V. K. Mootha, S. DiMauro and M. Hirano

Neurology 2005;64;539-541
DOI: 10.1212/01.WNL.0000150588.75281.58

Coenzyme Q deficiency and cerebellar ataxia associated with an *aprataxin* mutation

Abstract—Primary muscle coenzyme Q10 (CoQ10) deficiency is an apparently autosomal recessive condition with heterogeneous clinical presentations. Patients with these disorders improve with CoQ10 supplementation. In a family with ataxia and CoQ10 deficiency, analysis of genome-wide microsatellite markers suggested linkage of the disease to chromosome 9p13 and led to identification of an *aprataxin* gene (*APTX*) mutation that causes ataxia oculomotor apraxia (AOA1 [MIM606350]). The authors' observations indicate that CoQ10 deficiency may contribute to the pathogenesis of AOA1.

NEUROLOGY 2005;64:539–541

C.M. Quinzii, MD; A.G. Kattah, BS; A. Naini, PhD; H.O. Akman, PhD; V.K. Mootha, MD; S. DiMauro, MD; and M. Hirano, MD

Coenzyme Q10 (CoQ10), or ubiquinone, is a lipophilic component of the electron-transport chain, which transfers electrons to complex III (ubiquinol–cytochrome *c* reductase) from complex I (nicotinamide adenine dinucleotide [reduced form]–CoQ reductase), complex II (succinate-CoQ10 reductase), and from fatty acids and branched-chain amino acids via flavin-linked dehydrogenase. CoQ10 is also an antioxidant, a membrane stabilizer, and an essential cofactor in the proton pumping activity of uncoupling proteins.[1]

A 1989 study reported a disorder characterized by recurrent myoglobinuria, brain involvement, and ragged red fibers associated with CoQ10 deficiency in muscle.[2] Three additional patients with identical clinical presentations have been reported.[3,4] Two other clinical variants of CoQ10 deficiency are a severe infantile encephalomyopathy with renal disease[5] and Leigh syndrome.[6]

We reported 23 patients with cerebellar ataxia and CoQ10 deficiency responsive to CoQ10 supplementation.[7,8] Because this syndrome appeared to be inherited as an autosomal recessive trait and because most known causes of cerebellar ataxia had been excluded, we suggested that this was a new form of primary CoQ10 deficiency. To identify the genetic defect, we performed linkage analysis and candidate gene screening in a family with this syndrome.

Methods. We studied four affected individuals in a family with the ataxic form of CoQ10 deficiency (figure). The siblings were previously reported as Patients 3 through 5.[7] Two other siblings and the parents of the proband individuals are healthy. The Sicilian-American father and the German-American mother are not consanguineous. The affected cousin has a 9-year-old sister and a 7.5-month-old brother who are healthy. Clinical features and CoQ levels in muscle of the patients are summarized in table 1.

All three affected siblings were wheelchair bound, with alternating esotropia, severe limb ataxia with the slightest purposeful movement, peripheral neuropathies, and scoliosis. One had generalized seizures, and another had dystonia. Serum cholesterol was elevated in the proband and his affected sister but normal in his affected brother. Serum albumin was low only in the proband (see table 1). Molecular genetic screening of the proband excluded Friedreich ataxia and known causes of spinocerebellar ataxias. Muscle biopsy revealed nonspecific myopathic changes in all patients.

After CoQ10 deficiency was demonstrated, the proband began CoQ10 supplementation at age 20 years. His strength and ataxia improved, and he became able to walk a few steps. His siblings showed similar improvements. Seizures in the affected sister also disappeared with CoQ10 therapy, and her anticonvulsant medication was discontinued.

The fourth patient had cerebellar ataxia beginning at approximately age 6 to 7 months, as well as oculomotor apraxia. Brain MRI at age 5 years showed cerebellar atrophy. The referring physician reported that the following studies were normal: complete blood count, serum electrolytes, liver profile, serum cholesterol, serum albumin, urine organic acids, thyroid function, serum amino acids, alpha fetoprotein, and EKG. After CoQ10 muscle deficiency was demonstrated, the patient was started on 200 mg CoQ10 daily, and his parents noted an improvement in his energy level; the dose was increased to 600 mg daily.

Among the 13 patients with low muscle CoQ10 levels screened for *APTX* mutations, two are described in Musumeci et al.[7] (Patients 1 and 6), and four are described in Lamperti et al.[8] (Patients 1, 6, 7, and 11); the other seven have ataxia; one has developmental delay, mental retardation, and ophthalmoparesis; and two have peripheral neuropathy. Cholesterol levels were normal in both patients tested.

Blood samples from the family were collected under a Columbia University Institutional Review Board protocol. To identify the chromosomal locus of the disease, we performed molecular genetic linkage studies using leukocyte DNA from 18 family members (14 unaffected and 4 affected individuals). For genotyping, we used fluorescently labeled microsatellite markers in the ABI Prism Linkage Mapping Set-MD10 (Applied Biosystems, Foster City, CA). We performed two-point lod score analysis using the MLINK option of FASTLINK 4.0 (disease penetrance of 90% and a disease frequency of 1 in 100,000). To narrow the chromosomal region, we analyzed additional ^{32}P-radiolabeled PCR-amplified microsatellite markers. We subsequently screened eight candidate genes for mutations.

To sequence *APTX*, we designed 7 primer pairs flanking the exons and 10 additional primer pairs to amplify the promoter region, the 3'-UTR region, and the full-length cDNA of the long and short isoforms of *APTX*.[9,10] We also sequenced the *APTX* cDNA of Patient 4. Real-time quantitative PCR (RT Q-PCR) was performed on fibroblast RNA using TaqMan assays (Applied Biosystems) for *APTX* and *RNase P*. We also sequenced *APTX* in 13 unrelated patients with CoQ10 deficiency and cerebellar ataxia.

From the Department of Neurology (Drs. Quinzii, Naini, Akman, DiMauro, and Hirano, A.G. Kattah), Columbia University, College of Physicians & Surgeons, New York, NY; and Whitehead Institute/MIT Center for Genome Research (Dr. Mootha), Cambridge, MA.

Supported by Telethon-Italy (Fellowship No. GFP03009), NIH grants (P01NS11766 and R01HD37529 to M.H.), and a Muscular Dystrophy Association grant.

Received June 15, 2004. Accepted in final form October 1, 2004.

Address correspondence and reprint requests to Dr. Michio Hirano, Columbia University College of Physicians & Surgeons, 630 West 168th Street, P&S 4-443, New York, NY 10032; e-mail: mh29@columbia.edu

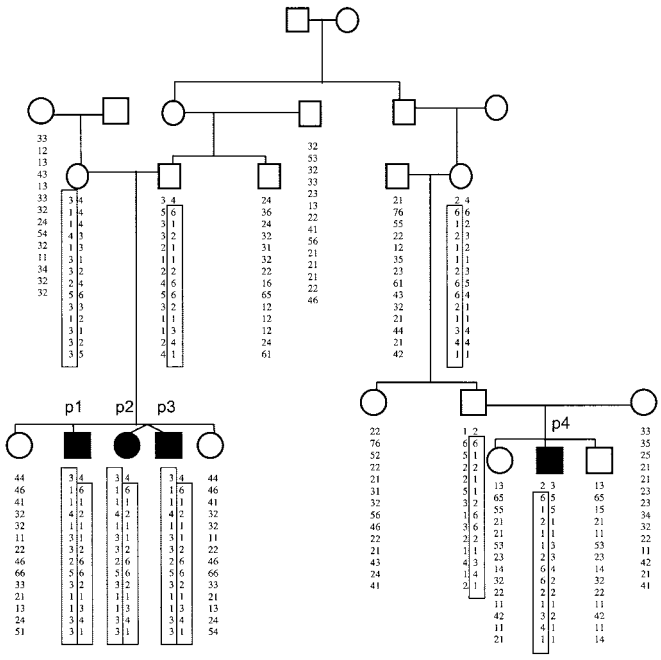

Figure. Pedigree of the family with chromosome 9p13 microsatellite marker alleles under individuals studied. Blackened symbols indicate affected individuals. Haplotypes shared among the affected individuals and obligate carriers are boxed.

Primer sequences and PCR conditions of candidate genes' amplifications are available from the author on request.

Results. Analysis of microsatellite markers revealed a 56.1-centimorgan region of chromosome 9p13 with potential linkage to the disease. The maximum two-point lod score was 2.94 with marker D9S1874 at theta = 0 (table 2).

The gene encoding frataxin (*FRDA*), mapping to chromosome 9q13-q21.1, was an obvious candidate gene. However, the presence of the GAA triplet expansion had been previously excluded, and we found no mutations by sequencing all of the exons and flanking regions of the gene and six other candidate genes. The human *coq4* gene required for CoQ biosynthesis has been mapped to chromosome 9p34.11 near a marker with a lod score of 1.11 (D9S1690). We sequenced this gene, although it is outside our critical region, but again we found no mutation. We did not analyze other coq genes because none are located at 9p13.

In the three index patients of the family, we identified a previously reported nonsense homozygous mutation (W279X) in exon 6 of the *APTX*;[9] Patient 4 is heterozygous for the same mutation, but we did not identify a second mutation despite sequencing all exons, exon-flanking intronic regions, promoter and untranslated regions, and the entire cDNA of the long isoform of *APTX*. RT Q-PCR showed reduced expression of *APTX* in this patient's fibroblasts; his *APTX*/*RNase P* transcript ratio was 0.07, whereas the ratios were 19.8, 17.9, and 21 in three control subjects. We did not identify any mutation in the *APTX* gene in 13 other patients with CoQ10 deficiency and cerebellar ataxia.

Table 2 *Two-point lod score at theta = 0 of chromosome 9p13 markers*

Marker	Position (Mb)	Lod
D9S259	25.9	−2.68
D9S169	27.1	−6.98
D9S1817	33.7	2.83
D9S1805	34.0	2.52
D9S1804	35.8	0.83
D9S1791	36.2	1.19
D9S1859	36.5	0.82
D9S1874	37.1	2.94
D9S1862	66.5	0.91
D9S1787	66.8	0.70
D9S1800	67.3	1.60
D9S273	67.8	−2.89
D9S1799	68.7	2.55
D9S175	73.3	−5.07

Discussion. Ataxia oculomotor apraxia (AOA1) is a common autosomal recessive form of ataxia characterized by early-onset ataxia, oculomotor apraxia, cerebellar atrophy, axonal sensorimotor neuropathy, hypoalbuminemia, and hypercholesterolemia.[9,10] The gene causing AOA1 has been mapped to chromosome 9p13 and encodes aprataxin, a member of the histidine triad superfamily, which may be involved in nuclear DNA single strand break repair.[9,10] Our results show that AOA1 and a form of ataxia associated with muscle CoQ10 deficiency are the same disease. Three affected individuals are homozygous for the reported W279X mutation, whereas Patient 4

Table 1 *Clinical features of the four patients*

Patient no.	Age at onset/ examination	Ataxia	Oculomotor apraxia	Peripheral neuropathy	Muscle weakness	Mental retardation	Seizures	Chorea/ dystonia	Cerebellar atrophy	CoQ10 levels in skeletal muscle, μg/g	Serum albumin, mg/dL	Serum cholesterol, mg/dL
1	4y/25y	+	−	+	+	+	−	−	+	7.1	3.1	225
2	4y/24y	+	−	+	+	−	+	−	+	7.1	3.4–3.8	294–320
3	4y/24y	+	−	+	+	−	−	+	+	8.2	3.6	173
4	6mo/6.5y	+	+	+	+	−	−	+	+	−	−	−
Normal range										27.9 ± 8.9*	3.4–5.5	100–200

* Values expressed as mean ± SD.

is heterozygous for the same mutation. We hypothesize that the patient harbors a second mutation in a noncoding region because he has AOA1 (see table 1) and reduced expression of the gene. The absence of *APTX* gene mutations in 13 other patients with cerebellar ataxia and muscle CoQ10 deficiency indicates that this disorder is a syndrome rather than a single disease.

The clinical features of our patients reflect the phenotypic variability of AOA1.

Our findings, coupled with the clinical improvement of patients after CoQ10 supplementation, suggest that CoQ10 deficiency plays a pathogenic role in AOA1. Intriguingly, CoQ10 and cholesterol share a common biosynthetic pathway;[1] therefore, in AOA1, altered levels of these molecules could be caused by aberrant biosynthesis. Nevertheless, there is no obvious link between aprataxin and regulation of CoQ10 synthesis or catabolism. Further studies are needed to confirm CoQ10 deficiency in muscle from additional patients with AOA1 and to verify that CoQ10 supplementation can retard or reverse the progression of symptoms.

Acknowledgment

The authors thank the patients and their relatives for their participation.

References

1. Crane FL. Biochemical functions of coenzyme Q10. J Am Coll Nutr 2001;20:591–598.
2. Ogasahara S, Engel AG, Frens D, Mack D. Muscle coenzyme Q deficiency in familial mitochondrial encephalomyopathy. Proc Natl Acad Sci USA 1989;86:2379–2382.
3. Sobreira C, Hirano M, Shanske S, et al. Mitochondrial encephalomyopathy with coenzyme Q10 deficiency. Neurology 1997;48:1238–1243.
4. Di Giovanni S, Mirabella M, Spinazzola A, et al. Coenzyme Q10 reverses pathological phenotype and reduces apoptosis in familial CoQ10 deficiency. Neurology 2001;57:515–518.
5. Rötig A, Appelkvist EL, Geromel V, et al. Quinone-responsive multiple respiratory-chain dysfunction due to widespread coenzyme Q10 deficiency. Lancet 2000;356:391–395.
6. Van Maldergem L, Trijbels F, DiMauro S, et al. Coenzyme Q-responsive Leigh's encephalopathy in two sisters. Ann Neurol 2002;52:750–754.
7. Musumeci O, Naini A, Slonim AE, et al. Familial cerebellar ataxia with muscle coenzyme Q10 deficiency. Neurology 2001;56:849–855.
8. Lamperti C, Naini A, Hirano M, et al. Cerebellar ataxia and coenzyme Q10 deficiency. Neurology 2003;60:1206–1208.
9. Date H, Onodera O, Tanaka H, et al. Early-onset ataxia with ocular motor apraxia and hypoalbuminemia is caused by mutations in a new HIT superfamily gene. Nat Genet 2001;29:184–188.
10. Moreira MC, Barbot C, Tachi N, et al. The gene mutated in ataxia-ocular apraxia 1 encodes the new HIT/Zn-finger protein aprataxin. Nat Genet 2001;29:189–193.

Coenzyme Q deficiency and cerebellar ataxia associated with an aprataxin mutation

C. M. Quinzii, A. G. Kattah, A. Naini, H. O. Akman, V. K. Mootha, S. DiMauro and M. Hirano

Neurology 2005;64;539-541
DOI: 10.1212/01.WNL.0000150588.75281.58