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# Whole-Exome Sequencing Study of Fibroblasts Derived From Patients With Cerebellar Ataxia Referred to Investigate CoQ10 Deficiency

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## Abstract

### Background and Objectives

Coenzyme $Q_{10}$ ($CoQ_{10}$)–deficient cerebellar ataxia can be due to pathogenic variants in genes encoding for $CoQ_{10}$ biosynthetic proteins or associated with defects in protein unrelated to its biosynthesis. Diagnosis is crucial because patients may respond favorably to $CoQ_{10}$ supplementation. The aim of this study was to identify through whole-exome sequencing (WES) the pathogenic variants, and assess $CoQ_{10}$ levels, in fibroblasts from patients with undiagnosed cerebellar ataxia referred to investigate $CoQ_{10}$ deficiency.

### Methods

WES was performed on genomic DNA extracted from 16 patients. Sequencing data were filtered using a virtual panel of genes associated with $CoQ_{10}$ deficiency and/or cerebellar ataxia. $CoQ_{10}$ levels were measured by high-performance liquid chromatography in 14 patient-derived fibroblasts.

### Results

A definite genetic etiology was identified in 8 samples of 16 (diagnostic yield = 50%). The identified genetic causes were pathogenic variants of the genes *COQ8A* (*ADCK3*) (n = 3 samples), *ATP1A3* (n = 2), *PLA2G6* (n = 1), *SPG7* (n = 1), and *MFSD8* (n = 1). Five novel mutations were found (*COQ8A* n = 3, *PLA2G6* n = 1, and *MFSD8* n = 1). $CoQ_{10}$ levels were significantly decreased in 3/14 fibroblast samples (21.4%), 1 carrying compound heterozygous *COQ8A* pathogenic variants, 1 harboring a homozygous pathogenic *SPG7* variant, and 1 with an unknown molecular defect.

### Discussion

This work confirms the importance of *COQ8A* gene mutations as a frequent genetic cause of cerebellar ataxia and $CoQ_{10}$ deficiency and suggests *SPG7* mutations as a novel cause of secondary $CoQ_{10}$ deficiency.

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## Glossary

**ACMG** = American College of Medical Genetics; **AF** = allele frequency; **atypical NAD** = atypical neuroaxonal dystrophy; **cDNA** = complementary DNA; **CLN7** = ceroid lipofuscinosis type 7; **$CoQ_{10}$** = Coenzyme $Q_{10}$; **IRB** = Institutional Review Board; **OxPhos** = oxidative phosphorylation; **PLAN** = PLA2G6-associated neurodegeneration; **SCA** = spinocerebellar ataxia; **WES** = whole-exome sequencing.

Coenzyme $Q_{10}$ ($CoQ_{10}$) deficiency is a clinically and genetically heterogeneous mitochondrial disorder,[1] defined by the low $CoQ_{10}$ levels in muscle, fibroblasts, or other tissues. $CoQ_{10}$ is a lipid molecule important for several biological processes including oxidative phosphorylation (OxPhos), reactive oxygen species detoxification, sulfide oxidation, and synthesis of pyrimidine nucleotides.[2] Cerebellar ataxia, variably associated with other neurologic and systemic symptoms, is one of the most common clinical presentations of $CoQ_{10}$ deficiency.[1] It was initially described in 6 patients in 2001.[3] In 2003, a study[4] reported that approximately 10% of patients with cerebellar ataxia of undefined etiology displayed low levels of $CoQ_{10}$ in muscle. Based on these findings, we collected biological samples from patients with genetically undiagnosed cerebellar ataxia referred to investigate $CoQ_{10}$ deficiency.

$CoQ_{10}$ deficiency in cerebellar ataxia can be primary as well as secondary to mutations in genes unrelated to its biosynthesis.[1,5] The most frequent cause of primary $CoQ_{10}$-deficient cerebellar ataxia is pathogenic variants in *COQ8A* gene (previously known as *ADCK3*),[6] while secondary $CoQ_{10}$ deficiency has been reported in association with mutations in *ANO10*,[7] *APTX*,[8-10] *SLC25A26*,[11] *GLUT1*,[12] and in several other OxPhos and non-OxPhos disorders.[12] Remarkably, $CoQ_{10}$ supplementation is partially effective in both primary and secondary cases.[13]

In this study, we report the results of the biochemical assessment of $CoQ_{10}$ content and whole-exome sequencing (WES) study in a cohort of fibroblasts from patients with undiagnosed cerebellar ataxia and referred to investigate $CoQ_{10}$ deficiency.

## Methods

### Standard Protocol Approvals, Registrations, and Patient Consents

The Ethics Committee of the IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy) and the Institutional Review Board (IRB) of Columbia University (New York) approved the study. Written informed consent for genetic analyses, anonymous publication of the patients' clinical features, and analyses of biological samples was obtained from all involved patients or their legal guardians/parents under Columbia University Medical Center IRB–approved protocol. Because most samples were sent from patients followed up by outside physicians, complete clinical data were not available for all of them (eTable 1, links.lww.com/NXG/A583). Fibroblasts were collected from 16 patients referred to investigate $CoQ_{10}$ deficiency based on the referring physicians' clinical suspicion and/or favorable response to $CoQ_{10}$ supplementation.

### CoQ10 Measurement

Levels of total $CoQ_{10}$, reduced (ubiquinol-10) and oxidized (ubiquinone-10), were measured by high-performance liquid chromatography in 14 patients (2 lines were not available), and 3 control cultured skin fibroblasts as previously described.[14] Cells were grown in triplicate, unless otherwise specified. $CoQ_{10}$ levels were compared with our laboratory's normal values (48.7 ± 9.6, n = 45). The Mann-Whitney nonparametric *U* test was used to compare gene expression and $CoQ_{10}$ levels between patient and control fibroblasts, and a $p$ value of <0.05 was considered statistically significant.

### Genetic Analyses

Genomic DNAs were extracted from 16 skin fibroblasts. Friedreich ataxia, spinocerebellar ataxia (SCA)1, SCA2, and SCA3 were excluded before undergoing subsequent analyses. WES was performed using the Nextera Exome Library protocol (Illumina) and an Illumina NextSeq500 sequencing platform. Variant calling and annotation were performed by GATK4 and ANNOVAR tools. Sequencing data were filtered using a virtual gene panel containing the genes associated with $CoQ_{10}$ deficiency and/or cerebellar ataxia, looking for rare variants (minor allele frequency <0.0002) with protein impact (eTable 2, links.lww.com/NXG/A584). Variants were confirmed by Sanger sequencing.

Total RNA was isolated from cultured skin fibroblast using NucleoSpin RNAII (Macherey-Nagel Supplier), and complementary DNA (cDNA) was synthesized with SuperScript1 VILO cDNA Synthesis Kit (Invitrogen). The mRNA levels of *COQ8A* and *PLA2G6* were measured by reverse transcription quantitative real-time PCR using an Applied Biosystems StepOne TM RealTime PCR System Thermal Cycling Block. Gene expression levels were determined in triplicate using TaqMan Fast Advanced Master Mix and normalized to *GAPDH*. Patients' haplotypes were resolved by cloning a single allele with TOPO-TA Cloning Kit for Sequencing (Invitrogen) and subsequent Sanger sequencing. In brief, cDNA was amplified by PCR with appropriate primer pairs depending on the set of mutations present. The alleles were cloned into pCR4-TOPO vector and transformed into OneShot competent cells. Bacteria were plated on lysogeny broth agar plate supplemented with 50 μL/mL of kanamicin; after overnight growth at 37°C, single colonies were picked and grown in lysogeny broth supplemented with 50 μg/mL of kanamicin for at least 3 hours at 37°C in agitation. Colony PCR was used to screen proper transformants for subsequent sequencing. PCR products were cleaned by Exonuclease I and FastAP treatment (Thermo Scientific) before amplification with BigDye terminator v3.1 Cycle Sequencing kit using as sequencing primer the former pair from the first amplification or

**Table 1** Clinical and Genetic Summary of the Samples in Which the Pathogenic Variants Were Identified

| Sample | Gene | Variants (cDNA) | Variants (AA) | gnomAD allele frequency | ACMG criteria | ACMG classification | Phenotype | Age at evaluation (y) | $CoQ_{10}$ deficiency |
|---|---|---|---|---|---|---|---|---|---|
| **CU19001** | *COQ8A* | c.827A>G | p.Lys276Arg | 0.000014 | PM1, PM2, PP5, PP3 | Pathogenic | Ataxia, impaired vertical gaze, dysphagia | 9 | Muscle: + |
| | | c.1799_1800insC | p.Val600fs*127 | Absent | PVS1, PM2, and PP3 | Pathogenic | | | Fibroblasts: + |
| **CU19004** | *ATP1A3* | c.2452G>A | p.Glu818Lys | Absent | PS3, PP5, PM1, PP3, PM2 | Pathogenic | Ataxia (CAPOS) | 12 | Muscle: unk |
| | | | | | | | | | Fibroblasts: − |
| **CU19008** | *SPG7* | c.1715C>T | p.Ala572Val (hom) | 0.0000358 | PP5, PP3, PM2 | Pathogenic | Ataxia, spastic paraplegia, cognitive impairment | 3 | Muscle: unk |
| | | | | | | | | | Fibroblasts + |
| **CU19010** | *PLA2G6* | c.2222G>A | p.Arg741Gln | 0.0000903 | PP5, PS3, PM5, PM1, PM2, | Pathogenic | Ataxia, pyramidal signs, cognitive impairment | Childhood | Muscle: unk |
| | | c.1186C>T | p.Leu396Phe | Absent | PVS1, PM2, PM3 | Pathogenic | | | Fibroblasts: − |
| **CU19012** | *ATP1A3* | c.2452G>A | p.Glu818Lys | Absent | PS3, PP5, PM1, PP3, PM2 | Pathogenic | Ataxia (clinical data not available) | Unk | Muscle: unk |
| | | | | | | | | | Fibroblasts: − |
| **CU19013** | *MFSD8* | c.753A>G | p.Glu251= (hom) Splice-disruptive | 0.000014 | PVS1, PM2, PP3 | Pathogenic | Ataxia, myoclonus, visual impairment | Childhood | Muscle: unk |
| | | | | | | | | | Fibroblasts: − |
| **CU19014** | *COQ8A* | c.127_128delinsA | Leu43Serfs*166 | Absent | PVS1, PM2, PP3 | Pathogenic | Ataxia, seizures, neuropathy, myoclonus, muscle weakness | 1 | Muscle: + |
| | | c.895C>T | p.Arg299Trp | 0.0000279 | PP5, PS3, PM1, PP3, PM2 | Pathogenic | | | Fibroblasts: − |
| **CU19015** | *COQ8A* | c.827A>G | p.Lys276Arg | 0.000014 | PM1, PM2, PP5, PP3 | Pathogenic | Ataxia, seizures, exercise intolerance, ptosis, PEO | 63 | Muscle: + |
| | | c.1748delC | p.Thr584Profs*7 | Absent | PVS1, PM2, PP3 | Pathogenic | | | Fibroblasts: − |

Abbreviations: AA = Amino Acid; ACMG = American College of Medical Genetics; $CoQ_{10}$ = Coenzyme $Q_{10}$; PEO = progressive external ophthalmoplegia; PM = pathogenic moderate; PVS = pathogenic very strong; Unk = Unknown.

the pair M13F and T3. Sequences were precipitated by acetate/ethanol procedure and sequenced on 3130xl GeneticAnalyzer (ABI Prism). The biallelic status of mutations was confirmed if at least 1 allele sequenced over the positions of both mutations was found to carry only one of them. Data not provided in the article because of space limitations may be shared (anonymized) at the request of any qualified investigator for purposes of replicating procedures and results.

## Results

A definite genetic etiology was identified in 8 samples of 16 (diagnostic yield = 50%). The identified genetic causes were pathogenic variants affecting *COQ8A* (*ADCK3*) (n = 3 samples), *ATP1A3* (n = 2), *PLA2G6* (n = 1), *SPG7* (n = 1), and *MFSD8* (n = 1). The genetic results and available clinical data are summarized in Table 1. Causative genetic mutations were found in the following samples: CU19001, CU19004, CU19008, CU19010, CU19012, CU19013, CU19014, and CU19015. Conversely, a definitive genetic diagnosis could not be obtained in samples CU19002, CU19003, CU19005, CU19006, CU19009, CU19011, CU19016, and CU19017.

We found significantly decreased levels of $CoQ_{10}$ in 3 of 14 cell lines (21.4%) (i.e., CU19001, CU19002, and CU19008) (eTable 1, links.lww.com/NXG/A583). $CoQ_{10}$ levels were decreased also in CU19003 (eTable 1, links.lww.com/NXG/A583); however, the decrease was not statistically significant because we could measure $CoQ_{10}$ only in 1 biological replicate due to a severe growth defect of this fibroblast line.

### *COQ8A* (*ADCK3*)

Sample CU19001 carried 2 heterozygous *COQ8A* variants (National Center for Biotechnology Information transcript identifier: NM_020247.5): c.827A > G (p.Lys276Arg) and c.1799_1800insC (p.Val600fs*127), which were demonstrated to be in trans. The c.827A > G is a known (rs1223030341) but extremely rare missense variant (gnomAD allele frequency [AF] = 0.000014). It is predicted to be pathogenic by all the in-silico

prediction tools tested (i.e., CADD, DANN, MutationTaster, Polyphen2, and SIFT). This variant has been reported already as a likely pathogenic variant in a single report[15] and was identified in another patient of our cohort (i.e., CU19015). It meets the following pathogenicity criteria of the American College of Medical Genetics (ACMG): PM1, PM2, PP5, and PP3 (pathogenic). The second variant (c.1799_1800insC) is a novel frameshift mutation absent from population databases (e.g., gnomAD and ExAC). It is a null variant predicted as pathogenic by all in silico tools. It meets the following ACMG criteria: PVS1, PM2, and PP3 (pathogenic). Unsurprisingly, low levels of $CoQ_{10}$ were found in sample CU19001.

Three heterozygous *COQ8A* variants were called by bioinformatic tools in sample CU19014: c.125delC (p.Leu43Cysfs*166), c.128T > A (p.Leu43Gln), and c.895C > T (p.Arg299Trp). However, the c.125delC and the c.128T > A were associated in cis as demonstrated by their co-occurrence in the same sequencing reads; therefore, the correct nomenclature of the mutation was c.127_128delinsA (Leu43Serfs*166). Hence, the patient carried only 2 heterozygous variants (c.127_128delinsA and c.895C > T), which were proved to be associated in trans. The first variant is a novel frameshift mutation absent from population databases. It is a null variant predicted as pathogenic by in silico tools. It meets the PVS1, PM2, and PP3 ACMG criteria (pathogenic). The second variant (c.895C > T) is a known pathogenic variant already associated with recessive cerebellar ataxia and reported in genetic databases (e.g., ClinVar).

Sample CU19015 was found to carry 2 compound heterozygous *COQ8A* variants: c.827A > G (p.Lys276Arg) and c.1748delC (p.Thr584Profs*7). The first variant was already characterized for sample CU19001. The second variant is a novel frameshift mutation absent from population databases and predicted to be pathogenic by in silico prediction tools. Regarding ACMG criteria, it meets the following: PVS1, PM2, and PP3 (pathogenic). The 3 patients from which the fibroblasts CU19001, CU19014, and CU19015 were derived had low levels of $CoQ_{10}$ in muscle, but only CU19001 displayed decreased levels of $CoQ_{10}$ in fibroblasts (eTable 1, links.lww.com/NXG/A583).

A single heterozygous c.1751C > T (p.Thr584Ile) *COQ8A* variant was identified in sample CU19006. It is a known (rs779649324) but extremely rare genetic variation (gnomAD AF = 0.000008). It is predicted pathogenic by most in silico tools; however, a single heterozygous mutation is not sufficient to cause the disease. No reduction of *COQ8A* mRNA levels in patient fibroblasts compared with controls was observed, making less likely the hypothesis of a highly deleterious cryptic variant involving the other allele. In the absence of another causative *COQ8A* mutation, at the moment, the c.1751C > T should be considered a variant of unknown significance.

### ATP1A3

Samples CU19004 and CU19012 carried the known pathogenic c.2452G > A (p.Glu818Lys) *ATP1A3* mutation (NM_152,296.5). Heterozygous *ATP1A3* mutations are associated with at least 3 phenotypes: rapid-onset dystonia parkinsonism, alternating hemiplegia of childhood, and cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS syndrome).[16] So far, the p.Glu818Lys is the only mutation associated with CAPOS syndrome.[16] The clinical presentation of the patient from which CU19004 sample derived was compatible with CAPOS syndrome. He was seen at the Columbia Neuromuscular Center at the age of 12 years, presenting with obvious hearing loss, mild distal weakness, moderate cerebellar dysarthria, oculomotor dyspraxia with broken pursuit, and bilateral dysmetria of upper limbs. Reflexes were absent. Romberg sign was present. He had an ataxic gait and was unable to walk tandem. The neurologic abnormalities started at the age of 2 years, when a febrile illness caused the patient to be weak and flaccid and led to admission to an intensive care unit. Strength gradually improved partially over the following years. A second similar episode of generalized weakness occurred at the age of 5 years in association with another febrile illness, probably due to a Salmonella infection. After this second event, hearing loss and a slurred speech were first documented, and he progressively developed the aforementioned clinical picture, predominated by cerebellar ataxia, areflexia, and sensorineural hearing loss. No clinical information was available for sample CU19012. Patients' muscle biopsies were not available. $CoQ_{10}$ levels in fibroblasts CU19004 and CU19012 were normal.

### SPG7

Sample CU19008 harbored a homozygous c.1715C > T (p.Ala572Val) *SPG7* variant (NM_003119.4), which is a known pathogenic mutation reported in genetic databases (e.g., ClinVar) and already associated with an ataxic presentation of spastic paraplegia 7.[17] Muscle biopsy was not available, but fibroblasts showed significantly decreased $CoQ_{10}$ levels (36.9 ± 5.1, 76%) (eTable 1, links.lww.com/NXG/A583).

### PLA2G6

In sample CU19010, 2 *PLA2G6* variants were found (NM_003560.4): c.2222G > A (p.Arg741Gln) and c.1186C > T (p.Leu396Phe). Biallelic *PLA2G6* mutations are the genetic cause of PLA2G6-associated neurodegeneration (PLAN), which comprises a clinical continuum of 3 phenotypes: infantile neuroaxonal dystrophy, atypical neuroaxonal dystrophy (atypical NAD), and PLA2G6-related dystonia-parkinsonism.[18] Cerebellar ataxia and cerebellar atrophy can be predominant clinical features in PLAN, especially in atypical NAD. The c.2222G > A is a known *PLA2G6* pathogenic variant already associated with PLAN and reported in genetic databases (e.g., ClinVar).[19] The c.1186C > T is a novel missense variant absent from population databases (transcript NM_003560.4). Of interest it represents a truncating variant (p.Gln396Ter) in the *PLA2G6* NM_001199562 transcript, which is predominant in brain, particularly in the cerebellum (Figure). Transcripts analyses demonstrated nonsense-mediated RNA decay of the allele carrying this variant and in-trans association with the

c.2222G > A. The variant meets the following ACMG criteria: PVS1, PM2, and PM3 (pathogenic). The clinical presentation of the patient from which the CU19010 sample was derived resembled atypical NAD, being characterized by early-onset cerebellar ataxia with unsteady gait and pyramidal signs followed by progressive psychomotor and cognitive decline. Muscle biopsy of this patient was not available, and $CoQ_{10}$ levels in fibroblasts were normal.

### *MFSD8*

A very rare homozygous c.753A > G (p.Glu251 = ) variant of *MFSD8* gene was found in sample CU19013 (NM_152,778). Biallelic *MFSD8* mutations cause neuronal ceroid lipofuscinosis type 7 (CLN7). The phenotype of CLN7 is characterized by infantile-onset psychomotor developmental regression, cerebellar ataxia, seizures, visual impairment, and myoclonus.[20,21] The synonymous c.753A > G is reported at an extremely low frequency in population databases (GnomAD AF = 0.000014), but it is absent from clinical databases, and it has never been associated with human disease. It is located at the splice site, and it is expected to impair the normal transcript splicing. All the in-silico tools for splicing defect prediction (e.g., SpliceAI, VarSeak, and AdaBoost) indicate this variant to be splice disruptive (i.e., splicing donor loss and exon skipping). The patient presented clinically with cerebellar ataxia, myoclonus, and visual defect. Muscle biopsy was not available, and $CoQ_{10}$ levels in fibroblasts were normal.

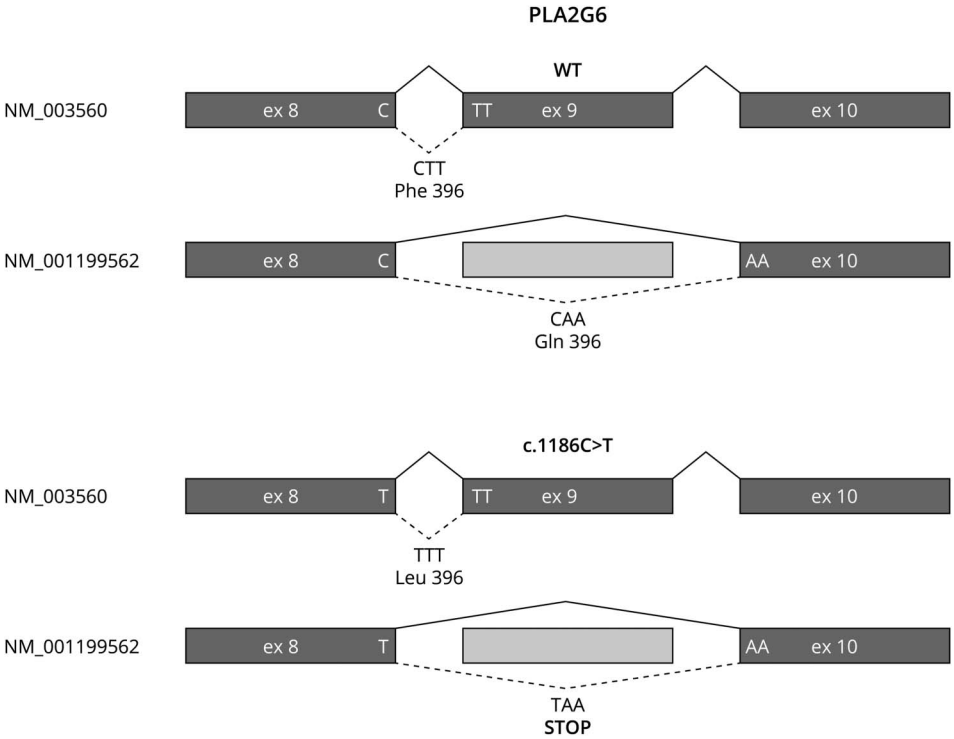
## Discussion

In this study, using WES, we genetically characterized a collection of fibroblasts derived from patients with cerebellar ataxia referred to investigate $CoQ_{10}$ deficiency. We identified the genetic cause of ataxia in 8 of 16 patients. The results of the genetic analysis were unexpected because only 3 patients had genetic mutations in a gene (i.e., *COQ8A*) known to cause cerebellar ataxia and $CoQ_{10}$ deficiency. The remaining genetically diagnosed samples carried mutations in ataxia-causative genes never previously associated with $CoQ_{10}$ deficiency.

Low levels of $CoQ_{10}$ were found in 21.4% of fibroblasts, confirming the results of the previous screening by Lamperti et al.[4] who measured $CoQ_{10}$ concentration in muscle biopsies from 135 patients with genetically undefined cerebellar ataxia and found it decreased in approximately 10%. The higher proportion of $CoQ_{10}$ deficiency in our cohort was expected because it was composed of fibroblasts of patients with cerebellar ataxia specifically referred for the investigation of $CoQ_{10}$ deficiency.

Regarding *COQ8A* mutations, the results of this study appear to be in line with the previous literature, confirming their role as the most frequent cause of primary $CoQ_{10}$ deficiency.[5] *COQ8A* gene is located on chromosome 1q42.13 and comprises 15 exons. It is ubiquitously expressed, with greater abundance in the heart, skeletal muscle, and cerebellum.[22] The encoded COQ8A protein is an atypical kinase

**Figure** The Different Effect on Protein Product of the PLA2G6 c.1186C > T Variant on 2 Different Transcripts (i.e., NM_003560 and NM_001199562)




The upper panel represents wild-type transcripts while the lower panel displays mutated transcripts. On transcript NM_003560, the variant is predicted to cause a missense change (p.Leu396Phe) while on transcript NM_001199562, it generates a premature stop codon causing (p.Gln396Ter) nonsense-mediated RNA decay (NMD) of the allele carrying it in fibroblasts.

involved in ubiquinone biosynthesis. It probably acts as a lipid kinase that phosphorylates a prenyl lipid intermediate in the $CoQ_{10}$ biosynthesis pathway.[23] More than 20 *COQ8A* pathogenic variants have been reported, including missense variants, null mutations, and a large deletion (from exon 3 to exon 15). All the pathogenic variants are private, and no founder effect has been identified so far.[5] In this study, we provide 3 novel pathogenic *COQ8A* mutations: c.1799_1800insC (p.Val600fs*127), c.127_128delinsA (Leu43-Serfs*166), and c.1748delC (p.Thr584Profs*7).

A single sample carried a homozygous pathogenic *SPG7* mutation, known to cause spastic paraplegia 7. Surprisingly, $CoQ_{10}$ levels were significantly decreased in the fibroblasts of this patient. SPG7 protein is an inner mitochondrial membrane transmembrane protein, which is part of the m-AAA metalloproteinase complex that plays a role in mitochondrial protein quality control.[24,25] Muscle biopsy performed in patients with unexplained ataxia, who were later found to have mutations in *SPG7*, showed cytochrome c oxidase–negative fibers, multiple mtDNA deletions, and, interestingly, $CoQ_{10}$ deficiency in a single patient.[26] Therefore, a pathogenic link between *SPG7* mutations and $CoQ_{10}$ deficiency can be hypothesized.

Two samples carried a pathogenic *ATP1A3* mutation (e.g., p.Glu818Lys) previously associated with CAPOS syndrome, a very rare inherited ataxia form. Another sample carried compound heterozygous mutations of *PLA2G6*, the causative gene of PLAN. One of the identified mutations was novel (i.e., c.1186C > T, p.Leu396Phe). Finally, a novel homozygous *MFSD8* mutation was found in 1 sample (i.e., c.753A > G, p.Glu251 = ). Biallelic pathogenic variants of this gene are associated with CLN7, a rare form of ceroid neuronal lipofuscinosis. In these cases, $CoQ_{10}$ deficiency was suspected based on clinical suspicion and/or a favorable response to oral $CoQ_{10}$ supplementation, but no biochemical evidence of $CoQ_{10}$ deficiency was present in any of these cases. Therefore, links between mutations in these genes (i.e., *ATP1A3*, *PLA2G6,* and *MFSD8*) and $CoQ_{10}$ deficiency are not supported by our findings.

CoQ10 measurement in skin fibroblasts as a diagnostic tool to identify ataxic patients with $CoQ_{10}$ deficiency is less sensitive than the same measurement in muscle. Indeed, 2 patients with COQ8A mutations displayed normal $CoQ_{10}$ values in fibroblasts while showing reduced $CoQ_{10}$ levels in muscle. Given the invasiveness of this procedure, the first-line approach for the diagnosis of inherited cerebellar ataxias with suspected $CoQ_{10}$ deficiency, in the absence of other specific clinical manifestations such as myopathic signs, should be the same as for other undiagnosed inherited ataxias, namely direct genetic testing, especially with a next-generation sequencing approach due to its low cost, the relatively high diagnostic yield, and the minimal invasiveness. Molecular diagnosis of a known $CoQ_{10}$-deficient cerebellar ataxia represents a strong indication for $CoQ_{10}$ supplementation.

In conclusion, this study describes 5 novel pathogenic mutations (*COQ8A* n = 3, *PLA2G6* n = 1, *MFSD8* n = 1) and confirms the importance and prevalence of *COQ8A* (*ADCK3*) gene mutations as a genetic cause of cerebellar ataxia and $CoQ_{10}$ deficiency and further suggests that *SPG7* gene mutations are a possible novel genetic determinant of secondary $CoQ_{10}$ deficiency. Although further studies are necessary to understand the causes and role of secondary $CoQ_{10}$ deficiency in cerebellar ataxias, diagnosis of $CoQ_{10}$ deficiency is important because patients with primary and secondary $CoQ_{10}$-deficient cerebellar ataxia may clinically respond to $CoQ_{10}$ supplementation.[6-8,13,27]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## Disclosure

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## Appendix *(continued)*

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