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ORIGINAL PAPER

Primary Coenzyme Q deficiency Due to Novel ADCK3 Variants, Studies in Fibroblasts and Review of Literature

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Abstract

Primary deficiency of coenzyme Q10 (CoQ10 ubiquinone), is classified as a mitochondrial respiratory chain disorder with phenotypic variability. The clinical manifestation may involve one or multiple tissue with variable severity and presentation may range from infancy to late onset. *ADCK3* gene mutations are responsible for the most frequent form of hereditary CoQ10 deficiency (Q10 deficiency-4 OMIM #612016) which is mainly associated with autosomal recessive spinocerebellar ataxia (ARCA2, SCAR9). Here we provide the clinical, biochemical and genetic investigation for unrelated three nuclear families presenting an autosomal form of Spino-Cerebellar Ataxia due to novel mutations in the *ADCK3* gene. Using next generation sequence technology we identified a homozygous Gln343Ter mutation in one family with severe, early onset of the disease and compound heterozygous mutations of Gln343Ter and Ser608Phe in two other families with variable manifestations. Biochemical investigation in fibroblasts showed decreased activity of the CoQ dependent mitochondrial respiratory chain enzyme succinate cytochrome c reductase (complex II + III). Exogenous CoQ slightly improved enzymatic activity, ATP production and decreased oxygen free radicals in some of the patient's cells. Our results are presented in comparison to previously reported mutations and expanding the clinical, molecular and biochemical spectrum of *ADCK3* related CoQ10 deficiencies.

Keywords Spinocerebellar ataxia · Q10 deficiency-4 · SCAR9 · Coenzyme Q · ADCK3 · Mitochondrial disease

Special issue in honor of Professor Vera Adam-Vizi.

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Introduction

Primary deficiency of coenzyme Q10 (CoQ10, ubiquinone), is classified as a mitochondrial respiratory chain disorder [1, 2]. There are no formal criteria for 'primary coenzyme Q10 deficiency' diagnosis however, it refers to the group of conditions characterized by a reduction of CoQ10 levels in tissues or cultured cells associated with biallelic mutations of one of the nine genes (*COQ2*, *COQ4*, *COQ6*, *COQ7*, *COQ8A*, *COQ8B*, *COQ9*, *PDSS1* and *PDSS2*) involved in the biosynthesis of CoQ10 (collectively called 'COQ genes') [1–4]. The primary coenzyme Q10 deficiency is associated with diverse tissues, range and age of clinical expression, since, it may be early onset multisystem or late onset and isolated tissue involvement. Moreover, the diversity of the clinical picture presented by neurologic abnormalities (encephalopathy, seizures, intellectual disability, peripheral neuropathy, cerebellar ataxia, dystonia, spasticity), metabolic (Leigh syndrome), muscular (exercise intolerance, muscle weakness, myopathy), cardiac (hypertrophic cardiomyopathy), renal (tubulopathy and steroid-resistant nephrotic syndrome), and neurosensory (sensorineural hearing loss, retinopathy and optic atrophy) [5–10].

The pathophysiology of primary CoQ10 deficiency presented by a wide spectrum of clinical abnormalities may be explained by the fact that, beside of being an essential component of the mitochondrial electron transport chain to enable oxidative phosphorylation, CoQ10 contributes to the membrane structure and functioning as a lipophilic antioxidant [7]. One form of hereditary CoQ deficiency, is the Autosomal Recessive Cerebellar Ataxia 2 (ARCA2, SCAR9), which is a rare ataxia caused by mutations in aarF-domain-containing kinase 3 (*ADCK3*) gene, an ortholog of yeast *coq8* [5, 6]. ARCA2 is characterized by slowly progressive gait abnormality, cerebellar atrophy. More features can be found include exercise intolerance, epilepsy, and intellectual disability [7].

Here we provide the clinical, biochemical and genetic investigation for unrelated three nuclear families presenting an autosomal form of Spino-Cerebellar Ataxia due to novel mutations in the *ADCK3* gene.

Methods

Written Informed consent was obtained from all individual participants (or their parents if under 18y) included in the study.

Whole Exome Sequencing

DNA was extracted from peripheral leukocytes following standard protocols. We performed whole-exome sequencing (WES) in probands from the two Arab families (with informed consent).

Exonic and adjacent intronic regions were enriched from genomic DNA derived from peripheral blood via the SureSelect All Exon V5 target enrichment kits from Agilent Technologies, and paired-end sequencing was performed on Illumina HiSeq 4000 platforms. Raw data was uploaded to Emedgene Technologies LTD. platform called WELLS, and were aligned to the human reference genome (hg19(with BWA MEM mapping algorithm) [11], after mapping and realignment nucleotide variants have been identified with multiple variant callers including SAM tools [12], FREEBAYES and GATK4 [13]. The variants were annotated using VEP [14] and additional local annotations. WELLS platform was used to filter common variants and to identify the pathogenic mutation in the family, after analysis and examination of the results in different inheritance modes. Due to the high number of sequenced samples in the family, there were no other potential candidates found in the analysis.

Sanger Sequencing

Dual ABI 3730XL instruments and custom primers were used for high-quality capillary-based fluorescent sequencing of DNA.

Tissue Cultures

Skin fibroblast from skin biopsies (taken with informed consent), were cultured in a permissive high-glucose–DMEM (Biological Industries Beit Hemek, Israel) medium containing 4.5 g/L glucose supplemented with 15% fetal calf serum (FCS), penicillin–streptomycin, L-glutamine, 110 µg/mL pyruvate, and 50 µg/mL uridine at 37 °C in 5% CO_2. Passages 3–4 were used for the experiments. For enzymatic assays 24 h prior to analysis the medium was changed to 10% dialyzed FCS or cultured in the presence of 1 µM Coenzyme Q_{10} (Sigma-Aldrich, Merck Chemicals), diluted from a 10 mM stock prepared in 1-propanol or vehicle for 72 h. Cells were washed in phosphate buffered saline harvested by trypsinization and stored as pellet frozen − 70 °C until enzymatic assays.

Enzymatic Assays

Thenoyltrifuoroacetone sensitive Succinate cytochrome c reductase (mitochondrial respiratory chain complexes II + III) was assayed in the presence or absence of 50 µM CoQ1 (a soluble CoQ analogue) in the reaction monitoring the reduction of cytochrome c at 550 nm as we have previously described [15]. Succinate dehydrogenase (SDH) was measured as succinate-mediated phenazine methosulfate reduction of dichloroindophenol at 600 nm. Citrate synthase (CS), a mitochondrial Krebs cycle enzyme, was measured

in the presence of acetyl-CoA and oxaloacetate by monitoring the liberation of CoASH coupled to 5′,5′-dithiobis (2-nitrobenzoic) acid at 412 nm [16]. These assays were also performed after CoQ10 supplementation in the growth medium. Measurements were carried out using a double beam spectrophotometer (UVIKON 930, Secomam, France).

ATP and ROS Production

3000 Cells were seeded in triplicate on three identical 96-well microtiter plates. The following day, medium was replaced with fresh medium containing dialyzed FCS in the presence or absence of 1 µM Coenzyme Q_{10}. Cell growth was estimated after 72 h by measuring cell content by a colorimetric method using methylene blue (MB) measuring absorbance was measured at 620 nm and intracellular ROS production was monitored by H_2DCFDA as we have previously described and normalized to cell content, as we have previously described [17]. Mitochondrial ATP production was measured in digitonin-permeabilized cells and incubated with ADP in the presence of glutamate and malate for 30 min at 37 °C as we have previously described [18, 19]. Subsequently, ATP formed was measured by luciferin–luciferase using the ATPlite™ (PerkinElmer,Waltham, MA, USA) luminescence assay system according to the manufacturer's instructions. Microtiter plate measurements were performed with a Synergy HT microplate reader instrument (BioTek, Winooski, VT, USA).

Results

Clinical Description

Herein we summarize the clinical data of three, apparently, unrelated Arab families living in the same village in the north of Israel.

Family-1

This family has three siblings whom all are affected with SCA. The parents are healthy first degree cousins.

Case-1 (F1-P1)

The oldest girl is 11 years old. She was born after normal pregnancy and delivery. At the age of 2 years she was diagnosed with strabismus and bilateral esotropia and needed a surgical repair at the age of 3 years. Her hearing test was normal. She had a moderate motor development delay; she walked at 18 months old. She has difficulties in running and climbing stairs and maintaining balance, she is still unable to jump on one leg. She has learning difficulties, mostly with mathematics and languages.

Brain MRI showed symmetric cerebellar and vermis atrophy with expansion of the vermis' sulci and enlargement of the 4th ventricle. At the age of 11 years her walk is clumsy and she has a mild ataxia. She also has difficulties in climbing stairs and in tandem gait. Romberg test is positive. Her cranial nerves test was normal apart from bilateral esotropia, without nystagmus. Metabolic evaluation, (urine organic acid, blood amino acids and lactate) was unremarkable.

Case-2 (F1-P2)

The second sibling is 9.5 years old. She was born after normal pregnancy and delivery. She has asthma till the age of 3 years, otherwise she is healthy. She often chokes while drinking fluids. At the age of 18 months, she had a tremor, an EEG was performed and was normal, the tremor resolved spontaneously.

She had a moderate motor and language development delay. She crawled at 18 months old, walked at 24 months old. She had difficulties in running and climbing stairs and maintaining balance, she is unable to jump on one leg and never tried to ride a bicycle. She started using sentences after the age of 2 years. She talks slowly and "stretching" words. Metabolic evaluation (urine organic acid, blood amino acids and lactate) within normal range. Brain MRI showed severe symmetric cerebellar and vermis atrophy, the hemispheres volume seems to be small with expansion of the CSF. She was treated with Q10 without any improvement.

At the age of 9.5 years, she has scanning speech and seems to have learning difficulties. Her walk is very clumsy and there is ataxia with almost falling down during rounds. Her walk is on a wide base. She has dysmetria and intention tremor. Romberg test is positive. Her cranial nerves were normal apart from bilateral esotropia, saccadic pursuit, without nystagmus. She is studying in a special education center with her brother and sister and she is diagnosed with moderate intellectual disability.

Case 3 (F1-P3)

The youngest sibling is 7.5 years old boy. He was also born after normal pregnancy and delivery. He is generally healthy. He had a motor and language development delay. He crawled till the age of 2 years, stood up at 18 months old and walked only at 32 months old. He has a very slow writing and gets tired quickly. He talks slowly and stretches the words. Brain MRI showed enlargement of the vermis sulci especially the upper part of the vermis, the cerebellar hemispheres are normal. He was also found to be homozygous to ADCK3 stop codon mutation. He was also treated with Q10 without any improvement.

At the age of 7.5 years of age, he has scanning speech and seems to have learning difficulties. His walk is very clumsy and has an ataxia. He walks on a wide base. He has dysmetria and intention tremor. Romberg test is positive. His cranial nerves were normal apart from bilateral esotropia, saccadic pursuit, with horizontal nystagmus at the end and often vertical nystagmus while looking lateral and upwards. His tendon reflexes are normal, Babinski is flexor. He is studying in a special education center with his both sisters, and he is diagnosed with severe motor and learning developmental delay.

Family-2

A family with three siblings of whom two are affected with SCA. The parents are healthy first degree cousins.

Case-1 (F2-P1)

A 20 years age old male patient diagnosed with SCA. He was born after normal pregnancy and delivery. He is healthy in general. He had a motor, language and learning developmental delay. He crawled till the age of 18 months, stood up at 2 years and walked only at 2.5 years old. He has muscle weakness and hypotonia. He talks slowly and stretches the words. He is studying in a special education center and he is diagnosed with severe intellectual disability.

Brain MRI showed enlargement of the vermis sulci especially the upper part of the vermis, the cerebellar hemispheres are hypoplastic and enlargement of the 4th ventricle. At the age of 20 years of age, he has scanning speech and mild mental retardation. He has dysmetria and intention tremor. He has clumsy walk, ataxia and Romberg test is positive. His cranial nerves were normal apart. His tendon reflexes are normal. Metabolic evaluation (urine organic acid, blood amino acids and lactate) was within normal range.

Case-2 (F2-P2)

A 16 years old female patient diagnosed with SCA. She was born after normal pregnancy and delivery. She is healthy in general. She had a motor, language and learning development delay. She crawled till the age of 20 months, stood up at 2 years and walked only at 3 years old. She has muscle weakness and hypotonia. At the age of 16 years, she has scanning speech clumsy walk, she has dysmetria, intention tremor, ataxia and mild cognitive disability. Romberg test is positive. She is studying in a special education center with her brother and she is diagnosed with moderate intellectual disability.

Brain MRI showed enlargement of the vermis sulci accompanied with mild vermis and cerebellar hemispheres hypoplasia. Metabolic evaluation (urine organic acid, blood amino acids and lactate) was unremarkable.

Family-3

This is a family with eight siblings (two males and six females) of whom one is affected with SCA. The parents are healthy first degree cousins.

Case-1 (F3-P1)

A 29 years age old female patient diagnosed with SCA. She was born after normal pregnancy and delivery. At the age of 1 year she was reported to have motor delay. She crawled till the age of 22 months, stood up at 2.5 years old and she walked at the age of 3.5 years old. She has muscle weakness and hypotonia. She talks slowly and stretches the words. Her cognitive development was normal but she has bradyphrenic thinking and she graduated from a college. Brain MRI showed enlargement of the fourth ventricle accompanied with hypoplasia of both vermis and cerebellar hemispheres. At the age of 29 years of age, although she speaks coherently, her speech is extremely slow with stretching words. She has clumsy walk and she walks on a wide base, ataxia and her Romberg and Hoffman tests are positive. Her cranial nerves were normal apart and her tendon reflexes were normal. According to the parents, her siblings are unaffected.

The above data are also summarized in Tables 1 and 2.

WES Revealed Mutations in ADCK3 Validated by Sanger Sequence

WES in all cases reveal causal variants in the *ADCK3* gene (hg19), responsible for primary Coenzyme Q10 deficiency (OMIM #612016). The first variant, chr1:227170682C > T; NM_020247.4; c.1027C > T; p.Gln343Ter, causing a truncated protein at position 343; and the second variant, chr1:227174317C > T;; NM_020247.4; c.1823C > T, p.Ser608Phe. The missense variant was predicted to be damaging (Polyphen2 HDIV, P Polyphen2 HVAR, SIFT dbNSFP MutationTaster).

In Family 1 all of the three patients harbored a homozygous c.1027C > T; p.Gln343Ter allele variant inherited from both parents and was validated by Sanger sequencing. Both patients from Family 2 were compound heterozygous for both variants. The mother was heterozygous for c.1823C > T, p.Ser608Phe allele and the father was heterozygous for c.1027C > T; p.Gln343Ter allele while, the healthy sibling was carrier for the reference alleles. The segregation was validated by Sanger sequencing. The patient from family-3 arrived to the Bnai Zion genetic institute recently; we realized that she lives in the same village as our previous two families. We tested her for the mutated alleles directly and

Table 1 Clinical characteristics of patients with ADCK3 mutations

Patient	Gender	Family	Age of onset (years)	Muscle involvement	Cerebellar ataxia	Spasticity	Developmental retardation	Epilepsy	Ophthalmic involvement	Refs.
1	M		1.5	Muscle weakness and hypotonia	Yes	NA	Yes (mild intellectual regression)	Yes-epilepsia partialis continua	Strabismus	[6]
2	F		1.5	Muscle weakness and hypotonia	Yes	NA	Motor and speech delay	Yes-epilepsia partialis continua	Ptosis	
3	F		3	Muscle weakness and hypotonia	Yes	NA	Motor and speech delay	Yes-epilepsia partialis continua	Ptosis	
4	M		3	Early exercise intolerance and muscle weakness	Yes	NA	NA	Yes myoclonic		[20]
5	M	A	11	NA	Yes	Yes	Psychomotor retardation	NA	NA	[5]
6	M	A	4	Exercise intolerance	Yes	Yes	Psychomotor retardation	NA	NA	
7	M	A	7	Exercise intolerance	Yes	NA	Psychomotor retardation	NA	NA	
8	F	A	8	Exercise intolerance	Yes	NA	NA	NA	NA	
9	M		4	NA	Yes	NA	Mild	NA	NA	
10	M		5	NA	Yes	NA	NA	NA	NA	
11	F		3	NA	Yes	NA	Moderate mental retardation	NA	NA	
12	M	B	3	Exercise intolerance and myoclonus	Yes	Yes with dystonic posture	Depression and mild cognitive	Yes	Saccadic eye movement	[8]
13	M	B	9	Exercise intolerance and myoclonus	Yes	NA	Mild cognitive speech and coordination difficulties	NA	Saccadic eye movement	
14	M	B	NA	NA	Yes	NA	Motor delay	Yes	NA	
15	F	C	2	Exercise intolerance 3rd decade	Yes	Pyramidal symptoms right side	Normal	No	Normal	
16	M	C	1	Exercise intolerance 3rd decade	Yes	No	Normal with bradyphrenia	No	Normal	
17	F		15	Muscle weakness	Yes	Yes	NA	Yes (myoclonus)	Slow ocular pursuits	[9]
18	M		15	Muscle weakness	Yes	Yes (tremor)	NA	Yes (myoclonus)	NA	
19	F		27	Muscle weakness	Yes	Yes (tremor)	Normal	Yes (myoclonic)	Normal	
20	F		1	Muscle weakness	Yes	No	Yes	Yes	Normal	
21	F		2	Muscle weakness	Yes	Yes (tremor)	Yes (depression)	No	Nystagmus	

Table 1 (continued)

Patient	Gender	Family	Age of onset (years)	Muscle involvement	Cerebellar ataxia	Spasticity	Developmental retardation	Epilepsy	Ophthalmic involvement	Refs.
22	F		2	No	Yes	No	Motor delay, cognitive, psychiatric impairment	No	Nystagmus, rod and cone dysfunction	[10]
23	F		Childhood	No	No	No	Normal	No	Normal	
24	F	1	2	Weakness, exercise intolerance	Yes	No	Moderate developmental delay	No	Strabismus bilateral	Present report
25	F	1	1	Weakness, exercise intolerance	Yes	No	Moderate developmental delay	No	Bilateral esotropia, saccadic pursuit	
26	M	1	1	Weakness, exercise intolerance	Yes	No	Severe developmental delay	No	Bilateral esotropia, saccadic pursuit and nystagmus	
27	M	2	1	Weakness, exercise intolerance	Yes	No	Cognitive impairment severe	No	Nystagmus	
28	F	2	1	Weakness, exercise intolerance	Yes	No	Cognitive impairment severe	No	Nystagmus	
29	F	3	1	Weakness, hypotonia, exercise intolerance	Yes	No	Normal with bradyphrenia	No	No	

NA data not available

Table 2 Biochemical molecular and imaging data of patients with ADCK3 mutations

Patient	Lactate	MRC	Cerebellar atrophy	Histology	Genotype	Oral Coq10 therapy	Refs.
1	Elevated CSF and plasma	Muscle-low, fibro-normal	Severe vermis and cerebellar atrophy and stroke-like anomalies	NA	Homozygous 1655G > A, p. Glu551Lys (E551K)	No benefit	[6]
2	Elevated CSF	Muscle- low	Severe vermis and cerebellar atrophy and stroke-like anomalies	NA	Compound heterozygous: paternal allele: 636C > T, p. Arg213Trp (R213W) and maternal allele: 815G > T, p. Glu272Val (G272V)	No benefit	
3	Elevated CSF	Muscle low	Severe vermis and cerebellar atrophy and stroke-like anomalies	NA	Compound heterozygous: paternal allele: 636C > T, p. Arg213Trp (R213W) and maternal allele: 815G > T, p. Glu272Val (G272V)	No benefit	
4	Elevated plasma	NA	Yes	NA	Compound heterozygous: paternal allele: 815G > A; p. Gly272Asp. (G272D) and maternal allele: c.[1812_1813insG]	No benefit	[20]
5	Elevated plasma	Fibro low	Yes	NA	Homozygous: donor splice site mutation, 1398 + 2T-C, in intron 11, p. Asp420Trp fsX40,Ile467 AlafsX22)	NA	[5]
6	Elevated plasma	Fibro low	Yes	NA	Homozygous: donor splice site mutation, 1398 + 2T-C, in intron 11, p. Asp420Trp fsX40,Ile467 AlafsX22)	NA	
7	Elevated plasma	Fibro low	Yes	NA	Homozygous: donor splice site mutation, 1398 + 2T-C, in intron 11, p. Asp420Trp fsX40,Ile467 AlafsX22)	NA	
8	Elevated plasma	Fibro low	Yes	NA	Homozygous: donor splice site mutation, 1398 + 2T-C, in intron 11, p. Asp420Trp fsX40,Ile467 AlafsX22)	NA	
9	NA	Fibro low	Yes	NA	Homozygous: exon3 500_521delinsTTG, p. Gln167LeufsTer36	NA	
10	NA	NA	Yes	NA	Compound heterozygous: c.(1541A > G), p.Tyr514Cys and c. (1750_1752 delACC), p. Thr584 del	NA	

Table 2 (continued)

Patient	Lactate	MRC	Cerebellar atrophy	Histology	Genotype	Oral Coq10 therapy	Refs.
11	NA	NA	NA	NA	Compound heterozygous: c. 993C > T, p. Lys314_Gln360 del and c. 1645G > A, Gly549Ser	NA	
12	NA	NA	Yes	NA	Homozygous; p.R348X	NA	[8]
13	NA	NA	Yes	NA	Homozygous; p.R348X	NA	
14	NA	NA	Yes		Homozygous; p.R348X	NA	
15	Normal lactate peaks by MRS	NA	Yes	Normal	Compound heterozygous: p.R348X/p.L379X	NA	
16	Normal lactate peaks by MRS	Muscle 23% reduction in complex II + III	Yes	Normal	Compound heterozygous: p.R348X/p.L379X	NA	
17	NA	NA	Yes	NA	c.811C > T, p.R271C/ c.911G > A, p.A304V	No significant improvement	[9]
18	NA	NA	Yes	NA	NA	NA	
19	NA	Muscle-low	Yes	COX-, lipid	Homozygous: c.911C > T, p.A304V	No significant improvement	
20	NA	NA	Yes	Normal	Homozygous: c.895C > T, p.R299W	No significant improvement	
21	NA	Muscle-low	Yes	cox-, lipid	Heterozygous: c.1286A > G‹ p.y429c/?	No significant improvement	
22	Normal	Decrease in complexes I + III, IV	Yes (prominent)	Normal EM	Compound heterozygous: c.1977C > G, p.Pro602Arg and c. (1750_1752 delACC), p. Thr584 del	Mild improvement	[10]
23	NA	NA	Yes (prominent)	NA	Compound heterozygous: c.1977C > G, p.Pro602Arg and c. (1750_1752 delACC), p. Thr584 del	NA	
24	NA	Fibro 30% reduction in complex II + III	Yes (prominent)	NA	Homozygous: c.1027C > T; p.Gln343Ter	No significant improvement	Present report
25	NA	Fibro 32% reduction in complex II + III	Yes (prominent)	NA	Homozygous: c.1027C > T; p.Gln343Ter	No significant improvement	
26	NA	NA	Vermis sulci's enlargement normal cerebellar hemispheres	NA	Homozygous: c.1027C > T; p.Gln343Ter	NA	
27	NA	NA	Yes (prominent)	NA	Compound heterozygous: c.1027C > T; p.Gln343Ter and c.1823C > T, p.Ser608Phe	NA	

Table 2 (continued)

Patient	Lactate	MRC	Cerebellar atrophy	Histology	Genotype	Oral Coq10 therapy	Refs.
28	NA	Fibro 29% reduction in complex II+III	Yes (prominent)	NA	Compound heterozygous: c.1027C > T; p.Gln343Ter and c.1823C > T, p.Ser608Phe	NA	
29	NA	NA	Yes (prominent)	NA	Compound heterozygous: c.1027C > T; p.Gln343Ter and c.1823C > T, p.Ser608Phe	Significant improvement	

NA not available, *Fibro* fibroblasts, *MRC* mitochondrial respiratory chain activities

found that she is compound heterozygous, resembling the case in family2. The three families descended from different seemingly unrelated clans.

Biochemical Investigations

Fibroblast from the patients was studied for two purposes; to confirm the pathogenicity of the variants and to examine the effect of Coenzyme Q supplementation. For enzymatic studies the activity of the CoQ dependent succinate cytochrome *c* reductase i.e. mitochondrial respiratory chain complexes II + III was measured in the presence and absence of CoQ1 (a soluble CoQ analogue) in the reaction while the CoQ independent succinate dehydrogenase was measured for comparison. The activities of succinate cytochrome *c* reductase were significantly lower in the patient's fibroblasts albeit only with a partial 29–32% reduction (Fig. 1a) this reduction was also evident when compared to succinate dehydrogenase (Fig. 1b). The addition of coenzyme Q_1 in the reaction markedly elevated succinate cytochrome *c* reductase in all cells (Fig. 1a) the relative elevation (II + III + Q1/III + Q) was somewhat higher in the patients cells (Fig. 1b) however the difference between controls and patients was not statistically significant. Enzymatic activities were also measured in cells grown for 72 h in the presence of CoQ10 (Fig. 1a, b). Here, the decreases observed in unsupplemented medium were not detected. The relative elevation was evident (II + III + Q10/II + III) (Fig. 1b). Normalization to citrate synthase disclosed the same trends as normalization to succinate dehydrogenase (results not shown).

Mitochondrial ATP production (Fig. 2a) and intracellular ROS production (Fig. 2b) in the patients fibroblasts were not significantly different in the controls. Nevertheless, Coenzyme Q_{10} supplementation did show a positive effect in patients from family 1 by increasing (15–30%) ATP production, while having no significant effect on control cells. Coenzyme Q_{10} supplementation significantly decreased ROS production in all cells but to a greater extent in the patients cells (28–34% decrease) than in controls (22% decrease).

Discussion

We describe our findings in six SCA patients from three apparently, unrelated families. We describe two novel mutations in *ADCK3* gene. In the first family, three patients have homozygous alleles for termination mutation (Gln343Ter) while in the other two families, the patients have compound mutated alleles (Gln343Ter and Ser608Phe). Previous reports show the most common primary CoQ deficiency is due to ADCK3 mutations and is mainly associated with autosomal recessive spinocerebellar ataxia (ARCA2, SCAR9) [5–10]. The mutations, Gln343Ter and Ser608Phe,

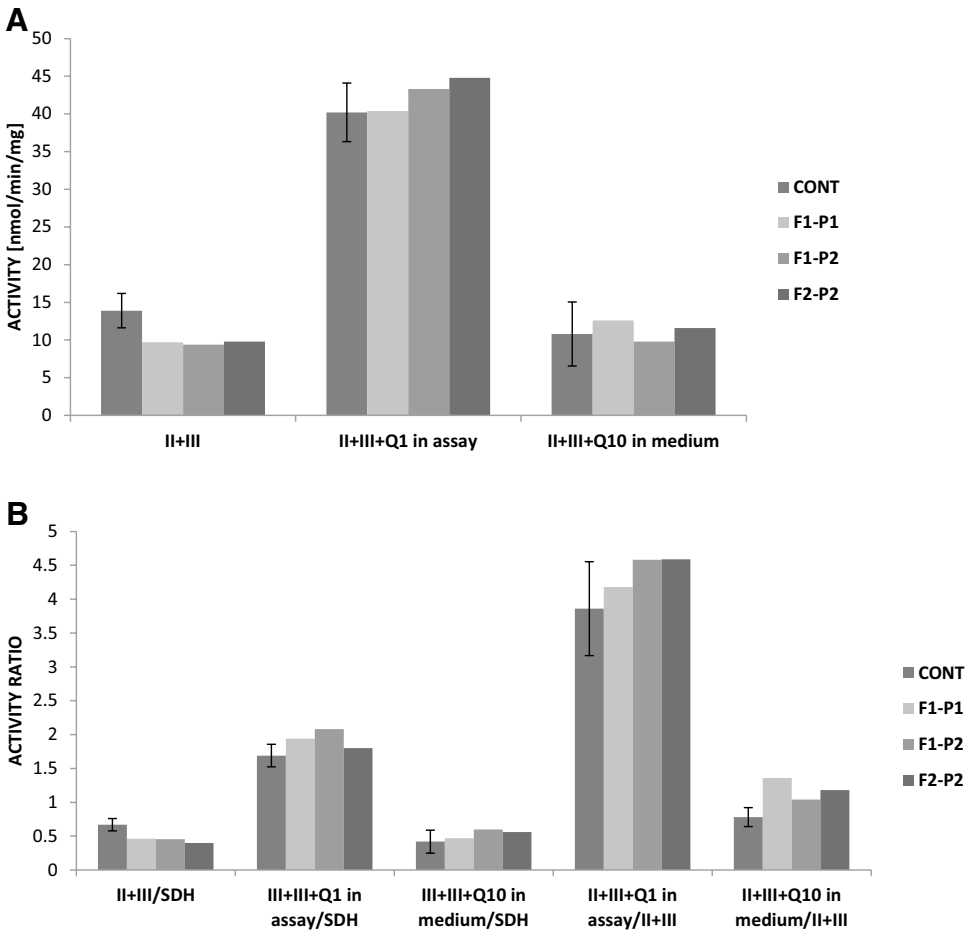

Fig. 1 Enzymatic activities. The enzymatic activities of succinate dehydrogenase (SDH) and succinate cytochrome *c* reductase (II + III) in the absence and presence of Coenzyme Q1 in the reaction or Coenzyme Q10 supplemented in the growth medium was determined in fibroblast homogenates by spectrophotometric methods. **a** Activities in normal controls (CONT n = 5) and two patients from family 1 (F1-P1, F1-P2) and one from family 2 (F2-P2) (mean of duplicate determinations). **b** Relative activity ratios

are located in conserved domains, the kinase conserved motif AxASx(A/G)QV (337–344) and ADCK3 C-terminal domain, respectively (Fig. 3).

As described in results section (summarized in Tables 1, 2), the phenotype is severe in two families (early onset, marked weakness, progressive ataxia and cognitive impairment) and mild to moderate in the third family. Three patients (two from family 1 and the patient from family 3) received Coenzyme Q_{10} supplementation. No significant improvement for the time being, was reported in family 1 similarly to most patients with *ADCK3* mutations reported (Table 2). Nevertheless, the patient from family 3 experienced a significant improvement after 3 weeks of oral CoQ10 supplementation (500 mg twice a day).

Mutations in *ADCK3* lead to variety of disorders ranging from isolated myopathy to multisystem involvement. In some patients the clinical spectrum overlap with other mitochondrial disorders including epilepsia partialis continua, stroke-like episodes and a disorder that mimic the phenotype of polymerase gamma (POLG) related encephalopathy [5–10, 20, 21]. The pleotropic effect of *ADCK3* gene, raised the question whether there is any evidence for genotype–phenotype correlation in *ADCK3* gene. To address this point we focused on *ADCK3* mutations and ARCA2 patients. We screened the published literature and investigated four leading genetic databases including ClinVar, HGMD, ExAc and LOVD. Since there are an overlapping data we decided to summarize and present (Fig. 3) some of the variants assigned in ExAc and HGMD website. In brief, in the ExAc database, there are nine hundreds and nineteen ADCK3 gene registered variants. More than one-third (339 variants) of these variants are located within the coding region and of those, thirty-three variants are defined as loss of function (LoF) mutations (19 frameshift, 9 stop-codon and 5 Splicing). Then in order to analyze the biallelic ADCK3 variants genotype–phenotype correlation, we summarized the available data of forty-three ADCK3 pathogenic variants from HGMD-professional (http://www.hgmd.cf.ac.uk/ac/all.php). These mutations (41 damaging and 2 likely damaging variants) were reported in thirteen publications (Fig. 3). Adding our novel mutations to the 41 damaging ones, the ADCK3 mutations can be stratified to different subgroups as the following: twenty-three (53%) missense and four nonsense (9.3%) mutations, two splicing (4.6%), seven small deletions (16.2%), four small insertions and duplications (9.3%), one gross deletion and one complex rearrangement mutations.

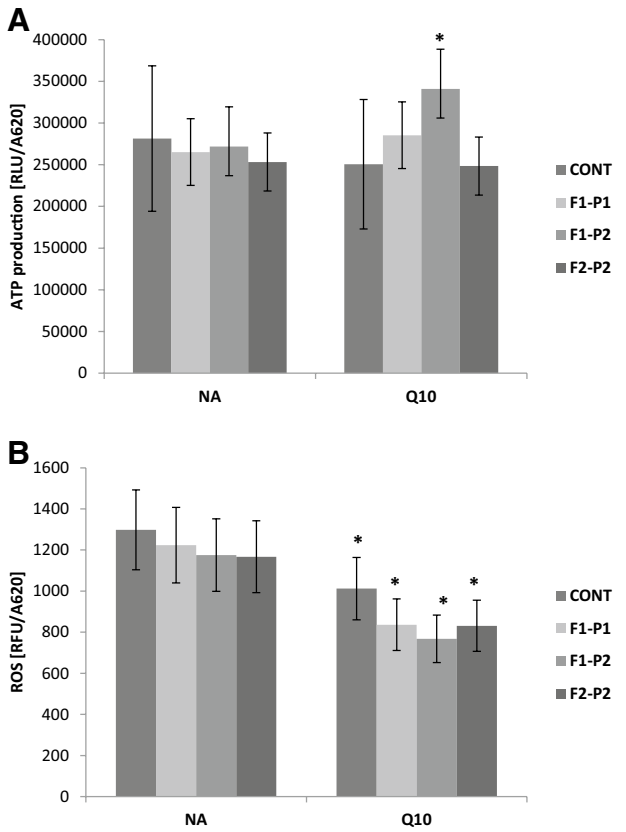

Fig. 2 ATP and ROS production. ATP and ROS production was determined in fibroblasts from normal controls (CONT n = 4) and two patients from family 1 (F1-P1, F1-P2) and one from family 2 (F2-P2) without added (NA) or in the presence of Coenzyme Q10 in the growth medium for 72 prior to assay. **a** Mitochondrial ATP production (RLU) relative to cell content (A620) determined in digitonin permeabilized cells. **b** Intracellular ROS production determined by H_2DCFDA (RFU) relative to cell content (A620). Measurements were performed in triplicates on at least two separate occasions. *$p < 0.05$ students *t* test compared to untreated cells

In this context, we found that a direct consequence of twenty-one variants (47%) of the listed mutations cause to null ADCK3 synthesis (including early truncation which in general lead to the protein degeneration). Interestingly, four mutations (9.3%) are located within the conserved ADCK Kinase amino acid motifs while; fourteen mutations (32%) are located within the C-terminal domain (Fig. 3).

Additionally we summarized the clinical presentation and mutation details of 16 mutations described in 29 familial and sporadic ADCK3 related SCA patients (Tables 1, 2). The first important observation showed that there are more intra-familial phenotype correlation than inter-familial correlation Where, the clinical picture of patients from the same family do share similar phenotypes [5, 8, 9]. To emphasize this, we present here, two examples of families that have biallelic stop codons mutations, empirically; one may assume that these patients supposed to be severely affected. However, the first one is a nuclear family (family-1). Where, three affected siblings are homozygous for Gln343Ter mutation. All do share the same phenotype which includes severe ataxia, weakness, and developmental delay (moderate to severe). In the contrary, in the other family described by Gerards et al. [8] where two affected siblings, harbored compound termination mutations (p.R348X and p.L379X) their clinical phenotype, relatively was mild. Nevertheless, intra-familial correlation is entirely missing, in the family described by Blumkin et al., where, two affected sisters sharing the same compound mutations (c.1977C > G, p.Pro602Arg and c. (1750_1752 delACC), p. Thr584 del), their clinical phenotype extremely distinct, as the youngest patient presented a very severe neuropsychiatric phenotype while her oldest affected sister presented relatively, very mild phenotype [10]. On the other hand, we did not find strong genotype–phenotype correlation between our 3 apparently unrelated families (inter-familial correlation). Hence, clear phenotype diversity between families was observed, in terms disease severity, age of onset, neurological and ophthalmic symptoms and signs, intellectual disability, developmental delay and psychiatric features (Table 1) [5, 8, 9].

With respect to our biochemical assessment of CoQ10 deficiency, it was not feasible to obtain muscle biopsies (no consent) to analyze Coenzyme Q content and plasma Coenzyme Q content Plasma CoQ is influenced by dietary intake [22], we opted for an indirect approach to analyze Coenzyme Q status in fibroblasts by measuring a Coenzyme Q dependent enzymatic activity (succinate cytochrome c reductase, mitochondrial respiratory chain complexes II + III). This activity was also compared to a similar mitochondrial respiratory chain CoQ independent activity, which is an integral part of complex II (succinate dehydrogenase, SDH) in the presence and absence of CoQ1 in the enzymatic assay. We have previously successfully used this approach [15] and the decreased II + III activities observed are in accord with our molecular findings. However, the decrease in II + III activity was partial, and the response to CoQ1 in the assay or CoQ10 in the medium, was evident but limited relative to control cells, thus this type of assay would not be suitable as a sole diagnostic mean. Nevertheless, the II + III assay and especially the activity enhancement ratio II + III + Q/II + III, is useful to estimate CoQ status, as a complement to mutation analysis. Decreased mitochondrial respiratory chain activities were observed also in other *ADCK3* patients and these assays are therefore contributing to confirm the pathogenicity of many variants (Table 2) [5, 6, 8, 10] We did not observe any significant decrease in mitochondrial ATP production and increase in intracellular ROS production as we initially expected, but this is in line with the relatively subtle phenotype observed relative to other mitochondrial diseases [17, 18]. Interestingly Coenzyme Q_{10} supplementation increased ATP production above controls in cells

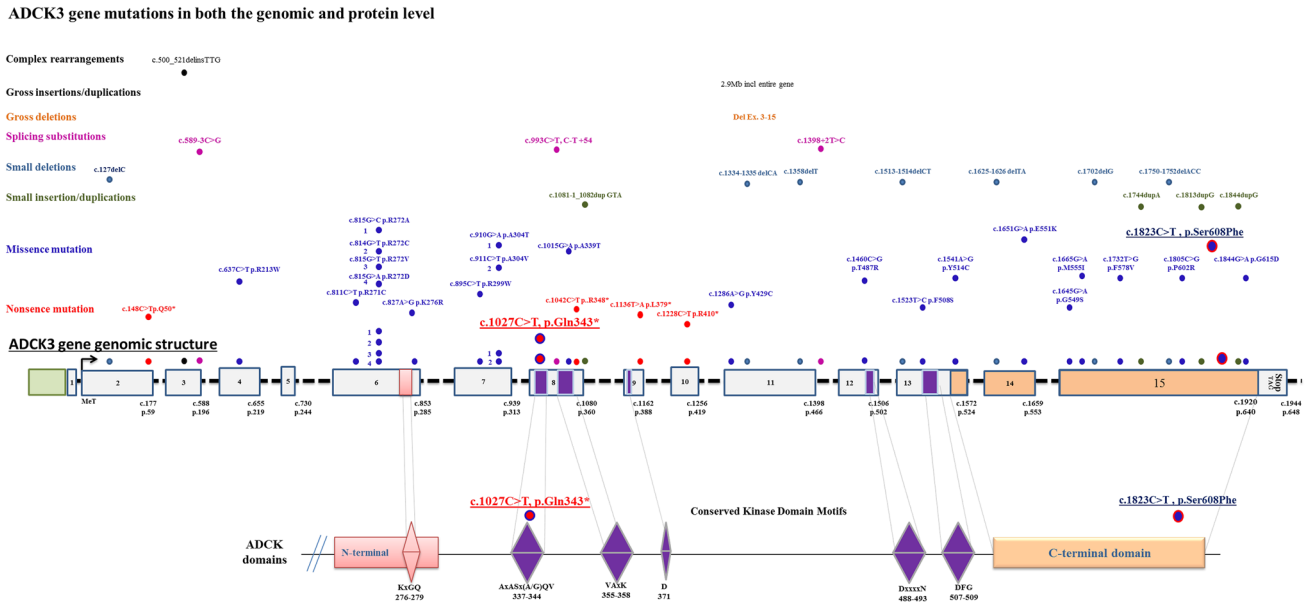

Fig. 3 ADCK3 gene mutations. Illustration of the ADCK3 genomic structure including exons 1–15 (boxes) and introns (scattered lines) (not depicted to the scale). For mutation identification (sequence, codon and amino acid numbering), we used the CDS of NM_020247.4 transcript as a reference. In the coding exons, we depicted the last nucleotide of the exon and the amino acid residue of the relevant codon (lower right corner of each exon). The schematic illustration of the genomic location of 41 known damaging mutations as registered at the HGMD, combined with our two novel mutations (c.1027C > T, p.Gln343Trer and c.1823C > T, p.Ser608Phe, bold and underlined). Each mutation was located vertically above its location in the specific exon, also represented by two blots. The mutations were grouped and colored according to their classification, as mentioned in the left upper part. The lower part, adapted from Clotilde Lagier-Tourenne et al. [5] ADCK3 domains are depicted on the diagram as follows: pink diamond, N-terminal domain conserved among all members of the ADCK family and containing the KxGQ motif; purple diamonds, the position of the conserved kinase motifs; orange rectangle, C-terminal domain specific for each ADCK subgroups. We used the same color of the conserved domain motifs to demonstrate their appropriate exonic coding sequence. Both mutations, c.1027C > T, p.Gln343* and c.1823C > T, p.Ser608Phe, that we identified in our patients, are located in the kinase conserved motif AxASx(A/G)QV (337-344) and in the ADCK3 C-terminal conserved domain, respectively

from family 1, we do not know the reason but speculate that the normal initial ATP production could be a result of some form of compensatory mechanism, although this is not evident from the succinate dehydrogenase activity. CoQ10 supplementation in the culture medium, did as expected decrease intracellular ROS in all cells albeit more in the patients. Still, ROS was initially not elevated in the patients fibroblasts and thus implication of this finding is presently unclear.

To summarize, we describe two novel *ADCK*3 mutations (one truncated and missense mutations) in three unrelated nuclear families with autosomal recessive spinocerebellar ataxia. Biochemical studies were in accord. We find more intra-familial genotype–phenotype correlation compared to the inter-familial correlation. CoQ10 supplementation improved the clinical phenotype in one out of three patients. Our findings expand the clinical, molecular and biochemical spectrum of *ADCK3* related CoQ10 deficiencies.

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Web Resources

The Human Genome Mutation Database, http://www.hgmd.cf.ac.uk/ac/all.php
The Clinical Variants database, https://www.ncbi.nlm.nih.gov/clinvar https://databases.lovd.nl
The gene cards database, https://www.genecards.org
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim
UCSC Genome Browser, http://www.genome.ucsc.edu

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