***ADCK3* mutations with epilepsy, stroke-like episodes and ataxia: a POLG mimic?**

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The authors declare no financial or other conflicts of interest.

ABSTRACT:

Background

Defects of CoQ10 metabolism cause a variety of disorders ranging from isolated myopathy to multisystem involvement. *ADCK3* is one of several genes associated with CoQ10 deficiency presenting with progressive cerebellar ataxia, epilepsy, migraine and psychiatric disorders. Diagnosis is challenging due to the wide clinical spectrum and overlap with other mitochondrial disorders.

Methods

We provide a detailed description of 3 new and one previously reported patients from three families with novel and known mutations in the *ADCK3* gene focussing on the epileptic semiology and response to treatment. In 3 patients, we used exome sequencing to identify the *ADCK3* mutation and in 2 measurement of skeletal muscle CoQ10 was performed.

Results

All four patients presented with childhood onset epilepsy and progressive cerebellar ataxia. Three patients had epilepsia partialis continua and stroke-like episodes affecting the posterior brain. EEG showed focal epileptic activity in the occipital and temporal lobes. Genetic investigation revealed *ADCK3* mutations in all patients including a novel change in exon 15: c.T1732G, p.F578V. There was no apparent genotype-phenotype correlation.

Conclusion

ADCK3 mutations cause a combination of progressive ataxia and acute epileptic encephalopathy with stroke-like episodes. The clinical, radiological and electrophysiological features of this disorder mimic the phenotype of polymerase gamma (POLG) related encephalopathy. We suggest that *ADCK3* mutations should be considered in the differential diagnosis of mitochondrial encephalopathy with POLG-like features.

INTRODUCTION:

Coenzyme Q10 (CoQ10), the major form of endogenous ubiquinone in humans, is a fat-soluble substance present in most eukaryotic cells. It is synthesized in the mitochondrial inner membrane and comprises a benzoquinone ring, derived from tyrosine or phenylalanine and decaprenyl tail, which is synthesized in the cytosol via the mevalonate pathway. The process of CoQ10 synthesis is complex and involves several enzymatic steps (1). The major function of CoQ10 is to transport electrons from complexes I and II to complex III in the respiratory chain, but it is also required for pyrimidine nucleoside biosynthesis and is involved in modulating apoptosis (2). It is still not clear whether the pathophysiological mechanism of CoQ10 deficiency reflects the bioenergetic defect or to impairment of its other functions (3). Thirteen genes are known to be involved in the biosynthesis of human CoQ10 (*PDSS, PDSS2, COQ2, COQ3, COQ4, COQ5, COQ6, COQ7, ADCK3, ADCK4, COQ9, COQ10a, COQ10B*). Mutations in eight of them (*PDSS1, PDSS2, COQ2, COQ4, COQ6, ADCK3, ADCK4, COQ9*) are associated with human diseases (4). CoQ10 deficiency comprises a continuum of overlapping phenotypes classified either as primary, i.e. caused by mutations in genes involved in CoQ10 synthesis, or secondary, those caused by defects in genes unrelated to CoQ10 biosynthesis, but which result in CoQ10 deficiency.

Five major phenotypes have been described (3) with primary CoQ10 deficiency including myopathic and encephalopathic forms, but overlap is common. Diagnosis of CoQ10 deficiency disorders is challenging especially in the paediatric population where patients often present with non-specific phenotypes such as neonatal seizures or encephalopathy.

We report a series of 4 patients with *ADCK3* mutations (MIM606980) and CoQ10 deficiency presenting with a phenotype resembling that seen in polymerase gamma (POLG) related disease (5). The clinical features of these cases are compared with previously reported cases with particular focus in the seizures frequency, semiology and response to treatment.

PATIENTS AND METHODS:

Our study was approved by the Regional Committee for Medical and Health Research Ethics, Western Norway (IRB00001872). All study participants provided written informed consent. The clinical and genetic features of the patients are summarized in table 1.

Case reports

Patient 1 is a 35 year old woman, the second child of non-consanguineous parents. After a normal birth and early psychomotor development, she developed focal and secondarily generalized seizures aged 7 years. From her early teens, she became increasingly ataxic and developed a fine tremor involving the head and upper extremities. Examination at the age of 34 years showed cerebellar dysarthria and mild gait and appendicular ataxia. Muscle tone and power, deep tendon reflexes and Romberg test were all normal. At 34 she remains ambulatory and works full-time. Her inter-ictal EEG shows bilateral occipital and right temporal epileptic activity. MRI at the age 31 years showed mild cerebellar atrophy affecting mainly the superior vermis and high T2-signal symmetrically in the nuclei dentatii ("white teeth" sign). Treatment with Ubiquinone 300 mg 3 times daily for 6 months has not yet given any apparent improvement.

Patient 2 is a 34 year old man, the third child of healthy parents. His similarly affected older sister died aged 22 years (Patient 3). He was born at term after a normal pregnancy and delivery and walked aged 12 months, with an unsteady gait. He developed focal and generalized seizures aged 7 years. At age 8 had one episode of epilepsia partialis continua affecting the right arm and leg and a stroke-like episode with vomiting, headache and right hemiparesis. He also had migraine-like episodes at age of 18 with two episodes of severe headache with nausea leaving him bedridden for one day. He has been seizure free from the age of 29 years on lamotrigine and sodium valproate. He developed progressive ataxia from late teenage and examination aged 33 years showed marked scanning dysarthria, gait ataxia,

dysmetria, intension tremor and dysdiadochokinesia. He scored 11 points on the SARA scale (scale for assessment and rating of ataxia). Inter-ictal EEG shows focal, bilateral occipito-temporal epileptic spikes. MRI of the brain showed cerebellar atrophy with “white teeth” sign and a focal, cortical stroke-like lesion in the right occipital lobe (figure 1A-C). Cerebral FDG-PET showed cerebellar hypometabolism and focal, asymmetrical hypometabolism in the right occipital lesion (Figure 1D-E). Treatment with Ubiquinone 300 mg 3 times daily was started aged 33 years and at six month follow-his score had fallen to 6 points on the SARA scale. Both patient and family reported improved balance, coordination and daily function. MRI of the brain remained unchanged.

Patient 3, the older sister of patient 2, was born at term after normal pregnancy and delivery. She developed normally until age 3 years when she developed motor difficulties, dysarthria, and progressive ataxia. She developed epilepsy aged 7 years, which was classified as focal with secondary generalization. EEG showed bilateral multifocal temporo-occipital epileptic spikes. Her ataxia worsened in her late teens and she developed tremor and cognitive impairment and was admitted repeatedly with status epilepticus and epilepsia partialis continua. MRI showed cerebellar atrophy with dentate hyperintensity and multiple bilateral cortical stroke-like lesions throughout the cortex. She died at the age of 22 years in intractable status epilepticus.

Patient 4, a 22 years old women, has been published previously (6), but is included here with a more detailed clinical description. She is the first child of healthy non consanguineous parents. She developed motor difficulties and ataxia at 2 years and epilepsy at 3 that was classified as focal with secondary generalization. She became wheel chair dependant at age 12, developed feeding difficulties requiring a gastrostomy and lost speech aged 14. She was admitted with status epilepticus and epilepsia partialis continua at age 14 and repeatedly thereafter. EEG showed left temporo-occipital activity. MRI of the brain showed progressive

cerebellar atrophy with multiple bilateral cortical stroke-lesions in the occipital lobes. She started deoxyubiquinon 1000 mg per day aged 18 years without any significant improvement.

Pathology

Open muscle biopsy from vastus lateralis muscle was performed in patients 1, 2 and 4. Sequential histochemical assay of cytochrome oxidase (COX) and succinate dehydrogenase (SDH) activity was performed as previously described (7). Post-mortem examination of the brain was performed in patient-3 with standard histological methods including hematoxylin and eosin and immunohistochemistry for the astrocytic marker GFAP and microglial marker HLA-DR.

Genetics

DNA was purified from blood using the Qiasymphony instrument (Qiagen, Hilden Germany) and muscle using a QIAamp DNA Mini Kit, Qiagen. MtDNA quantification and deletion assessment were performed in muscle DNA from patient 2 as previously described (8). Sanger sequencing of all coding exons of *POLG* and *C10*or*f2* in patient 2 were normal. Whole exome sequencing was performed at HudsonAlpha Institute for Biotechnology (Huntsville, AL) using Roche-NimbleGen Sequence Capture EZ Exome v3 kit and paired-end 100nt sequencing on the Illumina HiSeq. Data quality control and analysis was performed at our in-house pipeline as previously described (9).

CoQ measurement

CoQ10 was extracted from muscle homogenate as previously described (10) and its concentration was determined by reverse phase high-performance liquid chromatography with electrochemical detection.

RESULTS:

Histopathological studies:

Muscle biopsies from patients 1 (32 years) and 4 (4 years) were unremarkable. The biopsy of patient 2 aged 23 years was similarly normal except for the presence of a single COX negative fibre.

Post-mortem examination of the brain in patient 3 showed severe cerebellar atrophy and multifocal fibrotic infarct-like changes in the occipital and frontal cortex. Microscopic examination showed widespread, multifocal infarct-like changes throughout the cerebral cortex with neuronal loss and eosinophilic neuronal necrosis accompanied by astrocytic and microglial proliferation. Similar changes were also seen in the striatum and amygdala. In the cortex, neuronal loss affected predominantly the superficial (II-III) and deep (IV-V) cortical layers suggestive of laminar cortical necrosis. In the cerebellum and brainstem there was severe neuronal loss, eosinophilic neuronal necrosis and astrogliosis in the cerebellar cortex, affecting the Purkinje cells and neurons of the granule cell layer, dentate nucleus and inferior olivary nucleus of the medulla oblongata, whereas the pons and mesencephalic structures, including the substantia nigra, were preserved.

CoQ10 levels:

CoQ10 levels in frozen muscle samples from patients 1 and 2 (table 2) were low, both when related to protein content and the mitochondrial marker, citrate synthase (CS). In patient 1 the CoQ10 concentration was 10% (normalized to protein) and 24% (normalized to CS) of control mean, while in patient 2 the concentration was 34% (normalized to protein) and 60% (normalized to CS).

Genetic findings:

In families 1 and 2, *ADCK3* mutations were identified by whole exome sequencing and confirmed by Sanger sequencing. In family 1, we found a homozygous mutation in exon 7: c.895C>T, which is predicted to give the amino acid change p.R299W. In patient 2, we found compound heterozygous mutations in exon 7: c.895C>T, p.R299W and exon 15:c.T1732G, p.F578V. We confirmed these were *in trans* by parental testing.

The findings in family 3 have been reported previously: this patient was homozygous for c.895C>T, p.R299W. Currently, no connection between families 1 and 3 is known. The p.R299W mutation has been reported (6). The novel mutation p.F578V was absent in 400 in house controls and public databases including the 1000 genomes (11) and Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: http://exac.broadinstitute.org) [June 2015 accessed]. Both the 299 and 578 residues are highly conserved and the mutations are predicted to affect the function of the protein by all protein predictions tools in the AlaMut software (Interactive Biosoftware, Rouen, France).

No mtDNA deletions or depletion were found in the muscle of patient 2.

DISCUSSION

We report 4 cases of CoQ10 deficiency due to mutations in the *ADCK3* gene. The phenotype of our patients is compatible with earlier reports with all having a slowly progressive cerebellar ataxia with cerebellar atrophy. Additionally, our patients had epilepsy complicated by episodes of status epilepticus and clinical and imaging evidence of stroke-like episodes (4). While ataxia and encephalopathy are well-recognised features of CoQ10 deficiency, the epileptic semiology and stroke like episodes have not been systematically studied.

Including our cases, a total of 78 patients from 45 families with primary CoQ10 deficiency have been reported (6, 12, 13). Epilepsy was present in 16 (20%) the majority of whom (11) had mutations in the *ADCK3* gene suggesting that among patients with CoQ10 deficiency, those with *ADCK3* mutations are particularly prone to epileptogenesis. Of the 11 patients with *ADCK3* mutations and epilepsy, six had epilepsia partialis continua and 5 developed stroke-like episodes with cortical lesions (Table 1&3) (14). EEG in our 4 patients showed that epileptic activity was focused in the occipital and temporal lobes. The combination of occipital predilection and an acute on chronic course with stroke-like lesions on MRI associated with periods of intense epileptic activity are similar to those seen in patients with POLG related disorders (5, 15, 16).

While CoQ10 deficiency is a potentially treatable cause of disease, the steroid resistant nephrotic syndrome is reported to have a better treatment response than the neurological features. Seizures appear to respond poorly to oral CoQ10 supplementation (6, 12, 13, 17-25) and this may be due to reduced oral bioavailability. CoQ10 is poorly absorbed and as a lipid-soluble factor must be ingested together with significant quantities of fat to facilitate uptake. Bioavailability will depend both on dosage and how much crosses the blood-brain barrier (14). In the absence of formal trials these data are currently unavailable. Three of our patients have received oral deoxyubiquinone: one has not been on treatment long (patient 1) while

another (patient 4) started late and was already immobilised meaning that motor benefits would be hard to detect. Patient 2 showed a measurable improvement of ataxia. While improvement of the ataxia has been described previously (14), no definite antiepileptic effect has been shown. A potential preventative effect with regard to status epilepticus and associated stroke-like episodes cannot be excluded, but showing this will require systematic longitudinal studies, which are currently lacking. Moreover, as in our patient4, lack of clinical response in some patients may be due to delayed treatment start (18, 26). Thus, further studies are needed to characterise therapeutic responses to different CoQ10 derivatives, doses and organ-specific bioavailability.

In conclusion, we report that patients with *ADCK3* mutations can mimic the phenotype seen in POLG-related encephalopathy with progressive cerebellar ataxia complicated by epilepsy with occipital predilection, status epilepticus and associated stroke-like episodes. We recommend, therefore, mutations in *ADCK3* gene be considered in the differential diagnosis of patients with POLG-like phenotypes.

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Table 1 Summary of cases reported in this paper

Family	Patient	Onset	Age		Cerebellar atrophy	Stroke like lesions	Epilepsy		Mutations
			Current	Death			Type	Focus	
1	1	7	35		+	-	F,GTC	Occ/Temp	c.895C>T, p.R299W homozygous
2	2	7	34		+	+	F,GTC,EPC	Occ/Temp	c.895C>T,p.R229W, c.1732T>G, p.F578V heterozygote
2	3	3		22	+	+	F,GTC,EPC	Occ/Temp	c.895C>T,p.R229W, c.1732T>G, p.F578V heterozygote
3	4	2	22		+	+	F,GTC,EPC	Occ/Temp	c.895C>T, p.R299W homozygous

Abbreviations: F – focal seizure; GTC - generalised tonic clonic seizure; EPC- epilepsia partialis continua;

Occ – occipital; Temp – temporal.

Table 2. CoQ10 levels in patients 1 and 2

		µg CoQ/g protein	µg CoQ/CS	Citrate synthase (CS)
Patient 1		12.5	0.55	22.5
Patient 2		40.9	1.36	30
Control	Mean	122	2.25	53
	Range	87-158	1.51-2.99	34-72

Table 3. Summary of previously reported patients with ADCK3 mutations.

Families (N=Patients)	Onset (years)	Age (years)	Cerebellar ataxia	Cerebellar atrophy	Epilepsy	Epilepsy classification	Mutations
1 (4)	7-11	29-42	+	+	NK	NK	c.1398+2T>C homozygous
2(1)	4	18	+	+	NK	NK	c.500_521 delinsTTG homozygous
3(1)	5	17	+	+	NK	NK	p.Y514C/ p.T584del
4(1)	3	30	+	+	NK	NK	p.L314_Q 360del/ p.G549S
5(1)	1,5	16	+	+ SLL	+	GTC/ EPC	p.E551K homozygous
6(2)	1,5	13,14	+	+ SLL	+	GTC/ EPC	p.R213W/ p.G272V
7(1)	3	17	+	+	+	Myoclonus	p.G272D/ c.1812_13insG
8(3)	3-9	26-31	+	+	+	Myoclonus	p.R348X homozygous
9(2)	1,2	20,30	+	+	NK	NK	p.R348X/ p.L379X
10(2)	15,18	46/NK	+	+	+	Myoclonus	c.811C>T, p.R271C/ c.911G>A, pA304V
11(1)	27	50	+	+	+	Myoclonus	c.911C>T, p.A304V homozygous
12(1)	1	18	+	+	+	NK	c.895C>T, p.R299W homozygous
13(2)	1,5 -2	20,26	+	+	-		c.1286A>G, p.Y429C heterozygous
14(2)	2-NK	20,32	+	+	NK		p.T584delACC(c.1750_1752delACC) and p.P502R
15(2)	10,14	35,32	+	+	NK		C.1844_1845insG, p.S616Lfs*114

Abbreviation: NK-Not known; + Present; - Absent; EPC- epilepsia partialis continua; GTC- generalised tonic clonic seizure, SLL-Stroke like lesions.

Figure legends

Figure 1

MRI (A-C) and FDG-PET (D-F) of the brain of patient 2 at the age of 34 years. A: sagittal T1-weighted image showing midline cerebellar atrophy, particularly of the superior vermis. B: axial T2-weighted image showing symmetrical hyperintense signal in the nucleus dentatus consistent with “white teeth” sign which often accompanies efferent cerebellar degeneration (i.e. loss of cerebellar output). C: axial T2-weighted image showing a stroke-like lesion in the right lateral occipital lobe. D-F: FDG-PET scan of the brain showing decreased glucose metabolism in the cerebellum (D and E) and right occipital lesion (F).

Figure 1