

# Further Delineation of the Clinical Phenotype of Cerebellar Ataxia, Mental Retardation, and Disequilibrium Syndrome Type 4

Saud Alsahli<sup>1</sup>, Muhammad Talal Alrifai<sup>1,2</sup>, Saeed Al Tala<sup>3</sup>, Fuad Al Mutairi<sup>1,4</sup> and Majid Alfadhel<sup>1,4</sup>

<sup>1</sup>King Abdullah International Medical Research Center (KAIMRC), King Saud bin Abdulaziz University for Health Sciences, King Abdulaziz Medical City, Ministry of National Guard-Health Affairs (MNGHA), Riyadh, Saudi Arabia. <sup>2</sup>King Abdullah International Medical Research Center (KAIMRC), Division of Neurology, Department of Pediatrics, King Abdulaziz Medical City, Ministry of National Guard-Health Affairs (MNGHA), Riyadh, Saudi Arabia. <sup>3</sup>Division of Genetics, Department of Pediatrics, Armed Forces Hospital, Khamis Mushayt, Saudi Arabia. <sup>4</sup>King Abdullah International Medical Research Center (KAIMRC), Division of Genetics, Department of Pediatrics, King Abdulaziz Medical City, Ministry of National Guard-Health Affairs (MNGHA), Riyadh, Saudi Arabia.

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## ABSTRACT

**BACKGROUND:** Cerebellar ataxia, mental retardation, and disequilibrium syndrome (CAMRQ) is a heterogeneous group of genetic disorders that have been grouped by shared clinical features; all of these features are transmitted via an autosomal recessive mechanism. Four variants of this syndrome have been identified so far, and each one differs in terms of both clinical and genotypical features. CAMRQ4 is a rare genetic disorder characterized by mental retardation, ataxia or an inability to walk, dysarthria and, in some patients, quadrupedal gait.

**METHODS:** We investigated three Saudi families with CAMRQ4. Blood samples were collected from the affected patients, their parents, and healthy siblings. DNA was extracted from whole blood, and whole-exome sequencing was performed. Findings were confirmed by segregation analysis, which was performed on other family members.

**RESULTS:** Thus far, 17 patients have been affected by CAMRQ4. Genetic analysis of all patients, including our current patients, showed a mutation in the aminophospholipid transporter, class I, type 8A, member 2 gene (*ATP8A2*). A series of common phenotypical features have been reported in these patients, with few exceptions. Ataxia, mental retardation, and hypotonia were present in all patients, consanguinity in 90% and abnormal movements in 50%. Moreover, 40% achieved ambulation at least once in their lifetime, 40% had microcephaly, whereas 30% were mute. Magnetic resonance imaging (MRI) of the brain was normal in 60% of patients.

**CONCLUSIONS:** We described the largest cohort of patients with CAMRQ4 syndrome and identified three novel mutations. CAMRQ4 syndrome should be suspected in patients presenting with ataxia, intellectual disability, hypotonia, microcephaly, choreoathetoid movements, ophthalmoplegia, and global developmental delay, even if brain MRI appears normal.

**KEYWORDS:** CAMRQ4, ataxia, mental retardation, intellectual disability, disequilibrium syndrome, *ATP8A2*, WES, encephalopathy, hypotonia, chorea, choreoathetoid movements, optic atrophy

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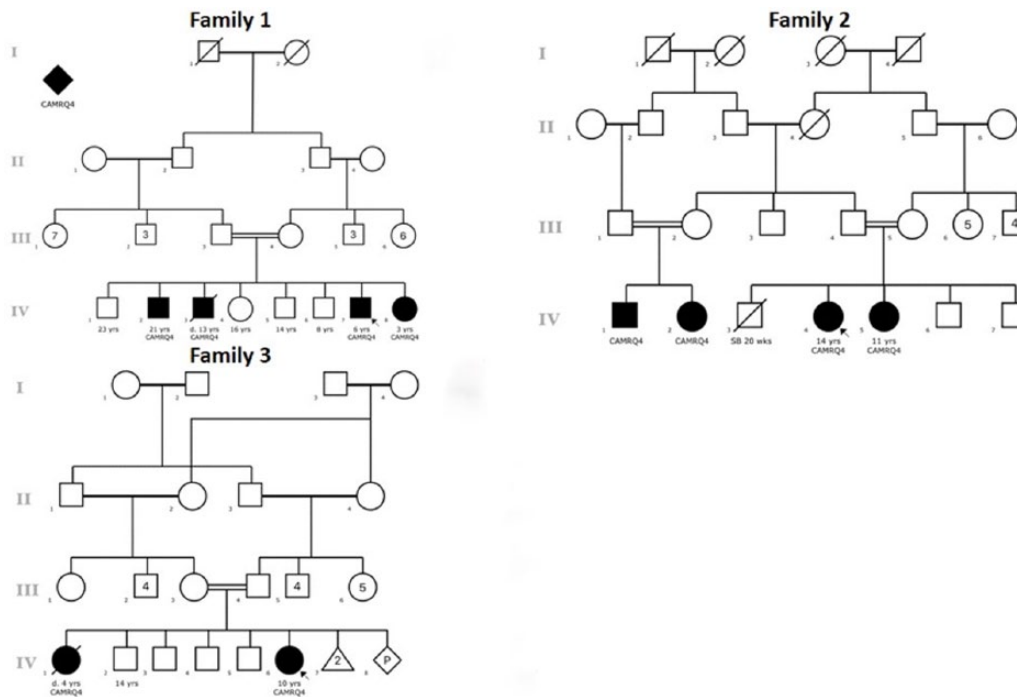
**CORRESPONDING AUTHOR:** Majid Alfadhel, King Abdullah International Medical Research Center (KAIMRC), Division of Genetics, Department of Pediatrics, King Abdulaziz Medical City, Ministry of National Guard-Health Affairs (MNGHA), PO Box: 22490, Riyadh 11426, Saudi Arabia. Email: dralfadhel@gmail.com

## Introduction

Cerebellar ataxia, mental retardation, and disequilibrium syndrome (CAMRQ) is a heterogeneous group of genetic disorders that have been grouped by shared clinical features; all of these features are transmitted by autosomal recessive mechanisms. Four variants of the syndrome (types 1–4) have been identified thus far, and each one differs in terms of clinical and genotypical features. Four genes have been reported to be responsible for this genetically heterogeneous disorder: *VLDLR* (type 1), *WDR81* (type 2), *CA8* (type 3), and *ATP8A2* (type 4).<sup>1–4</sup> Cerebellar ataxia, mental retardation, and disequilibrium syndrome type 4 (CAMRQ4, OMIM# 615268) is a rare, autosomal recessive genetic disorder characterized by

mental retardation, ataxia or inability to walk, dysarthria and, in some patients, quadrupedal gait. This syndrome is caused by mutations which disrupt the *ATP8A2* gene, which is present on chromosome 13q12. Genetic analysis of all patients, including our own patients, showed homozygous or compound heterozygous mutations in the aminophospholipid transporter, class I, type 8A, member 2 gene (*ATP8A2*) in Turkish and Spanish patients and a de novo–balanced translocation, t(10;13), which disrupted the *ATP8A2* gene, in a French patient.<sup>5</sup> The *ATP8A2* gene is highly expressed in the retina, brain, spinal cord, and testis; the highest levels have been reported in the cerebellum.<sup>3,4</sup> *ATP8A2* encodes for phospholipid-transporting ATPase IB, which belongs to the P4-ATPases subfamily and is





**Figure 1.** Family pedigrees of current patients.

only present in eukaryotic organisms.<sup>6</sup> The ATP8A2 complexes with cell cycle control protein 50A (CDC50A) to form a flippase in the Golgi apparatus and plasma cell membranes. The ATP8A2-CDC50A complex drives energy from ATP hydrolysis to transport aminophospholipids from the exoplasmic leaflet to the cytoplasmic leaflet, thus maintaining an asymmetric distribution of phospholipids across the bilayer membrane.<sup>7</sup> Studies have shown that the ATP8A2:CDC50A complex catalyzes the inwards movement of phosphatidylserine and, to a lesser extent, phosphatidylethanolamine.<sup>8</sup>

Herein, we investigated three Saudi families with 10 members affected by this condition, each family with a distinct mutation affecting the *ATP8A2* gene.

## Materials and Methods

### Human subjects

We investigated three Saudi families with this CAMRQ4. All patients underwent a comprehensive clinical evaluation by a clinical geneticist, a neurologist, and an ophthalmologist. We also reviewed their medical records and summarized their clinical presentations and laboratory findings. Our institution's clinical exome consent was obtained from the parents of all patients, and the study received ethical approval from King Abdullah International Medical Research Center (KAIMRC; reference number: IRB C/202/17).

### Genetic analysis

Blood samples were collected from all affected patients, their parents, and healthy siblings. DNA was extracted from whole

blood, and a whole-exome sequencing (WES) analysis was performed on the proband from each of the 3 families. For segregation analysis, Sanger sequencing was performed on DNA acquired from the parents, affected siblings, and other healthy siblings. The pathogenicity of the identified variants was predicted by taking into consideration the biochemical and biophysical differences between the wild type and the changed amino acid. In silico predictors (Polyphen-2, SIFT, and MutationTaster) were also used.

## Results

### Family 1

Four members of Family 1 (Figure 1) were affected by this syndrome. All 4 members were siblings, although only 2 were available for examination. The parents are first-degree cousins, and there was no history of similar illness in other relatives. The proband in family 1 was a 6-year-old man (Patient 1A, Figure 1) and his sister, a 3-year-old girl (Patient 1B, Figure 1). The other affected members were an older male sibling, aged 21 years, and another male sibling, who died at the age of 13 years. Patient 1A was the product of a full-term normal spontaneous vaginal delivery (NSVD) with an uneventful pregnancy. His birth weight was 3.1 kg (10th-25th percentile); length and head circumference were within normal limits. The first abnormality noted was hypotonia at the age of 2 months, and he subsequently showed a delay in all developmental domains. In addition, he began to lag in all growth parameters (weight, height, and head circumference; now all <3rd percentile). In terms of gross motor assessment, he is currently wheelchair bound and cannot walk, crawl, sit, raise his chest, or hold

his head. In terms of fine motor control, he cannot hold objects or feed himself. Language and social assessment shows that he cannot speak or recognize his parents, although the patient demonstrated good eye contact. His examination was significant for ataxia, microcephaly, hypotonia, and choreoathetotic movements. Patient 1B underwent a similar course. She was the product of a full-term NSVD with normal growth parameters, and her first abnormality was noted at the age of 1 month. She also suffers from ataxia, microcephaly, hypotonia, and choreoathetotic movements. In both Patient 1A and Patient 1B, hearing and fundoscopic examinations were reported to be normal. All molecular and biochemical investigations including comparative genome hybridization (CGH) microarray, liver function test,  $\gamma$ -glutamyltransferase, purines, pyrimidines, guanidinoacetate, cerebrospinal fluid (CSF) lactic acid, and creatine were unremarkable. Brain magnetic resonance imaging (MRI) was not available for either patient. According to the parents, the other 2 siblings shared a similar outlook.

The WES revealed a novel homozygous variant in exon 20 of the *ATP8A2* gene in Patient 1A, c.1741C>T (p.Arg581STOP). This substitution relates to a highly conserved nucleotide that is predicted to result in the conversion of arginine to a premature stop codon. In silico software prediction showed that this variant is damaging with a high probability. Sanger sequencing for the other 2 affected siblings, the other healthy siblings, and the parents confirmed the homozygous status for the affected siblings and the heterozygous status of the parents.

### Family 2

Four members of Family 2 (2 sets of siblings) were also affected, as shown in Figure 1. The proband was a 14-year-old girl (Patient 2A), who presented to our clinic with her sister, Patient 2B, who was 11 years of age. Patient 2A was born at a full-term NSVD to first-degree cousins with normal growth parameters, ie, weight of 2.8 kg (5th–10th percentile), length of 53 cm (75th–90th percentile), and head circumference of 33 cm (10th–25th percentile). Her growth parameters remained normal until the age of 15 months when her head circumference fell below the 3rd percentile, followed later by her weight at the age of 2 years, and finally, her height at the age of 5 years. The mother observed her first abnormality, hypotonia, at the age of 3 months. She failed to meet the normal developmental milestones, cannot walk, sit with and without support, crawl, hold her head or raise her chest, and feed herself or speak. However, she can say “mama” and “dada” and roll over, and she can recognize both of her parents. On examination, her dysmorphic features included microcephaly, severe scoliosis, ataxia, severe hypotonia, and absent reflexes. Her ophthalmologic assessment revealed bilateral optic disc atrophy, fixed gaze, myopia, and ophthalmoplegia but her hearing assessment was normal. Brain MRI, nerve conduction studies, muscle biopsy, and an

electromyogram were within normal limits. All molecular and biochemical investigations, including CGH microarray, uric acid, carbohydrate-deficient transferrin, lactic acid, liver function test,  $\gamma$ -glutamyltransferase, purines, pyrimidines, guanidinoacetate, plasma and urine amino acids, very-long-chain fatty acids, biotinidase, CSF neurotransmitters, organic acids,  $\beta$ -galactosidase, tripeptidyl peptidase, palmitoyl protein thi-oesterase, mucopolysaccharide, urine oligosaccharides, and ammonia were unremarkable. Patient 2B followed a similar course to her older sister and was also the product of a full-term NSVD. Her growth parameters were normal at birth but started to lag at an earlier age compared with her sister. Her weight fell below the 3rd percentile when she was 10 months of age, her head circumference at 14 months of age, and her height at 25 months of age. Her mother noted her hypotonia at 1 month of age. As with her sister, she did not meet any developmental milestones, and cannot say a word; she can only “coo.” She is in a wheelchair, cannot walk, crawl, sit, feed herself, or recognize her parents or others. On examination, she had severe hypotonia, microcephaly, ataxia, choreoathetoid movement, and absent reflexes. On ophthalmologic assessment, there was a normal flat retina and macula, along with bilateral total optic disc atrophy and pallor. She cannot follow or fixate, and ophthalmoplegia was particularly notable. Her hearing assessment showed a severe bilateral hearing loss. Her nerve conduction study, muscle biopsy and electromyogram were normal, but her brain MRI showed ventriculomegaly. The results of the molecular and biochemical investigations were similar to her sister. Their affected cousins are reported to have a similar illness as Patients 2A and 2B, and they are also the product of a first-degree consanguineous marriage. Whole genome sequencing of Patient 2A revealed a homozygous intronic mutation (G>C) on chromosome 13 at position 26273310 in the 2 sisters; both parents are carriers of this variant.

### Family 3

Two siblings from Family 3 were affected, and their parents are double first cousins. Only Patient 3A, a 10-year-old girl, presented to our clinic; her sister died at 4 years of age. She was born as a full-term NSVD with normal growth parameters, ie, birth weight of 3.2 kg (50th–75th percentile), length of 54 cm (90th–95th percentile), and head circumference of 35 cm (on 50th percentile). Her first abnormality was hypotonia, which was noted at the age of 4 months. Her growth parameters were delaying gradually until the age of 12 months when her weight fell below the 3rd percentile, followed later, at 22 months of age, by her head circumference. Her height was normal until the age of 5 years when it also fell below the 3rd percentile. She did not meet any milestones during her development except for the fact that rolling over began at 10 years of age, and saying “mama” and recognizing her parents and others occurred at an

older age. She is in a wheelchair, and she cannot walk, crawl, sit with and without support, raise her chest or head. She can only say “mama,” and she does not interact with others. On examination, there was noticeable ataxia with choreoathetoid movements, lordoscoliosis, pectus excavatum, gum hypertrophy with abnormal bite, upturned nose, triangular face, and general atrophy of the muscles due to disuse. There were reduced reflexes and severe central hypotonia. Her hearing assessment was normal. On ophthalmologic assessment, we noted an abnormal gaze with lateral fixation bilaterally, bilateral ptosis, good eye control, mild myopia, mild optic disc atrophy bilaterally, and ophthalmoplegia. Her molecular and biochemical investigations, including chromosomal analysis, CGH microarray, uric acid, carbohydrate-deficient transferrin, lactic acid, liver function test,  $\gamma$ -glutamyltransferase, purines, pyrimidines, guanidinoacetate, plasma and urine amino acids, very-long-chain fatty acids, and creatine were unremarkable. Brain MRI, nerve conduction studies, and an electromyogram were all normal. According to the parents, her deceased sister had a similar course. The WES of Family 3 detected a previously unreported homozygous missense variant in exon 28 of the *ATP8A2* gene c.2749A>G (p.Asn917Asp). This was located in a highly conserved position, and in silico software prediction with PolyPhen-2, SIFT and MutationTaster showed that this variant is probably damaging. Sanger sequencing showed that the father and mother were heterozygous carriers and demonstrated good segregation in other family members.

## Discussion

In this study, we describe the largest number of patients diagnosed with CAMRQ4 syndrome. We also presented 3 novel genetic variants of CAMRQ4, which have not been described elsewhere. In addition, we compared our patients with previously reported cases from France, Turkey, and Spain (Table 1). Previous literature describes a total of 7 CAMRQ4 patients, although the clinical description of these known patients is insufficient. The first patient was reported in France, 4 were reported in Turkey and 2 were reported in Spain.<sup>3-5</sup>

In this study, we identified 10 further patients affected by this syndrome, meaning that a total of 17 patients have been identified as being having CAMRQ4 thus far. Phospholipid-transporting ATPase IB is made of 1148 amino acid residues and 46 exons and is highly expressed in the brain, retina, and spinal cord.<sup>3,4</sup> Four alleles and one haploinsufficiency have been reported in earlier literature as being causative factors for the disease. Our present cohort has expanded the molecular spectrum of this disorder by identifying three novel variants that are most likely to be pathogenic: a homozygous nonsense pathogenic variant [C.1741 C>T, p.Arg581X], a homozygous intronic pathogenic variant [G>C at position 26273310], and a homozygous missense pathogenic variant [C.2749A>G, p.Asn917Asp].

The phenotypical features of pediatric patients affected by these variants are similar, therefore providing further support

for the characterization of these clinical problems as a new syndrome. Cacciagli et al reported a patient in France with a de novo-balanced translocation, t(10;13), located between exons 27 and 28 of the *ATP8A2* gene; this translocation leads to a chimeric transcript between *ATP8A2* exons 1 to 27 and the *MPP7* exons. The chimeric transcript may have resulted in a dominant negative effect and may have affected the expression levels of this gene in the brain. There was no mutation in the other allele, and the translocation did not disturb any another gene. The reported phenotype of this patient was similar to our current cohort, with global developmental delay, hypotonia, and abnormal movements.<sup>3</sup> Uner Tan et al and Onat et al described a family in Turkey with CAMRQ4 syndrome and referred to this as Family C. Four members of the family were affected, 3 adult men and 1 adult woman, and all carried a homozygous missense mutation in the *ATP8A2* gene at c.1128C>G (p.Ile376Met). Their phenotypical features included intellectual disability, hypotonia, and hyporeflexia; they also had cerebellar signs such as truncal ataxia, dysarthria, and dysmetria.<sup>4,9</sup> Martin-Hernandez et al reported 2 unrelated patients in the pediatric age group, one with a homozygous mutation, c.1287G>T (p.Lys429Asn), and the other with a compound heterozygous mutation, c.1630G>C (p.Ala544Pro) and c.1873C>T, (p.Arg625Trp). The phenotypical features of these patients were similar to our present cohort in that they presented with intellectual disability, central hypotonia, chorea, and optic atrophy. The phenotypical features of those patients are similar. Ataxia, intellectual disability, and hypotonia were present in all patients, whereas consanguinity was present in 90% of patients. Of note, abnormal movements, described as chorea or choreoathetoid movements, were present in 50% of patients; 40% achieved ambulation at least once in their lifetime, 40% had microcephaly, and 30% were mute. Brain MRI in our current cohort, and the French patient, was normal with the exception of one patient who presented with ventriculomegaly. The brain MRIs from Family C showed mild atrophy of the corpus callosum, cerebral cortex, and inferior cerebellum. However, in Spanish patients, brain MRI showed mild cerebral atrophy and a thin corpus callosum. Delayed subcortical white matter myelination was noted in the temporal and frontal lobe of one patient, whereas poor myelination in the insula and anterior temporal lobes were noted in the other patient.

Finally, the prognosis of this syndrome is variable. In all, 2 of 10 patients reported in this study died due to recurrent respiratory infection. The remaining 8 patients are alive but experience severe hypotonia, intellectual disability, truncal ataxia, and dysarthria.

## Conclusions

CAMRQ4 should be suspected in patients presenting with ataxia, mental retardation, hypotonia, microcephaly, choreoathetoid movements or chorea, ophthalmoplegia, and global developmental delay, even if brain MRI appears to be normal.



**Table 1.** Clinical features of previously reported patients with CAMRQ4 syndrome compared with the current cohort of patients.

CLINICAL FEATURE	CURRENT COHORT	PREVIOUSLY REPORTED CASES		TOTAL
Age group	Pediatrics	Adults	Pediatrics	
Number of cases	10	4	3	17
Male/female ratio	4:6	3:1	0:3	7/10
Consanguinity	100%	100%	33.3%	88.2% (15/17)
Age of onset	1-4 mo	NA	1 mo	NA
Ataxia	100%	100%	100%	100%
Intellectual disability	100%	100%	100%	100%
Global developmental delay	100%	NA	100%	
Hypotonia	100%	100%	100%	100%
Microcephaly	100%	NA	NA	NA
Normal brain magnetic resonance imaging (MRI)	100% (3/3)	75%	33.3% (1/3)	14/17
Abnormal movements	87% (7/8), choreoathetoid	NA	100% (3/3), chorea	90% (10/11)
Walking or crawling	0% (0/10)	20%	0%	
Ophthalmoplegia	60% (3/5)	30%	66.6% (2/3)	62.5% (5/8)
Bilateral optic disc atrophy	60% (3/5)	30%	66.6% (2/3)	62.5% (5/8)
Hearing impairment	10% (1/5)	0%	33.3% (1/3)	16.6% (2/12)
Dysarthria or no speech	100%	100%		
Molecular findings in the ATP8A2 gene	[C.1741 C>T, p.Arg581X], intronic pathogenic variant [G>C at position 26273310], [C.2749A>G, p.Asn917Asp]	[C.1128C>G, p.Ile376Met]	De novo t(10;13) balanced translocation, [c.1287G>T, p.Lys429Asn], [c.1630G>C, p.Ala544Pro], [c.1873C>T, p.Arg625Trp]	
Prognosis	20% died. The rest are alive with ataxia, hypotonia, and mental retardation	14% died (1/7). The rest are alive with ataxia, hypotonia and intellectual disability		17.6% (3/17)

Abbreviation: NA, not applicable.

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### Author Contribution

SA and MA performed the majority of work associated with preparing, writing, and submitting the manuscript, and contributed to the clinical diagnosis and management of the patients. MTA summarized the clinical neurological data and contributed to the clinical diagnosis and management of the patients. SAT and FAM edited the manuscript and contributed to the clinical diagnosis and management of the patients. All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

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