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Title

Cerebellar ataxia with normal intellect associated with a homozygous truncating variant in $CA8$

Running Title

Truncating mechanism for $CA8$ cerebellar ataxia

Bylines

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Conflict of Interest

The authors declare no competing financial interests.

Data Availability Statement

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The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.
Abstract

Biallelic pathogenic variants in \textit{CA8} cause cerebellar ataxia, mental retardation and dysequilibrium syndrome 3 (CAMRQ3), a rare form of hereditary ataxia characterised by cerebellar hypoplasia/atrophy, variable intellectual disability and often quadrupedal gait. The few cases reported in the medical literature are all caused by pathogenic homozygous or compound heterozygous missense variants in \textit{CA8}. We report a 9 year-old boy with marked gross motor delay, ataxia and progressive cerebellar atrophy with limited bipedal gait, but without intellectual disability. Singleton whole exome sequencing was performed. A novel homozygous truncating variant in \textit{CA8} (c.232C>T) with a predicted premature termination codon at position 78 (p.Arg78*) was identified. Both parents and the proband’s healthy sister are heterozygous for the variant. This variant is likely pathogenic and the cause of the condition in this child. Functional evidence in the form of a spontaneous mouse model involving homozygous intragenic deletion of the mouse analogue of \textit{CA8} with nonsense-mediated decay and similar clinical features to the proband support pathogenicity. Identification of this truncating variant broadens the genotypic and phenotypic spectrum of \textit{CA8}-related cerebellar ataxia.

Keywords
Cerebellar ataxia, hereditary ataxia, cerebellar hypoplasia, neurogenetics, medical genomics
Introduction

The hereditary ataxias, a heterogeneous group of conditions characterised by impaired balance and coordination, are collectively common and confer considerable morbidity across the lifespan. Increased availability of next generation sequencing (NGS) has permitted molecular characterisation of many of these conditions and improved understanding of their neuropathophysiological underpinnings. Considerable locus and clinical heterogeneity is now recognised. The existence of an autosomal recessive form of nonprogressive cerebellar ataxia with variable intellectual disability, disturbed equilibrium, cerebro-cerebellar hypoplasia and loss of bipedal locomotion was described as early as 1940 and further analysed in a large consanguineous Hutterite family in 1981. Diagnostic labels of ‘dysequilibrium’ syndrome, and Unertan syndrome have been historically applied, with ‘cerebellar ataxia, mental retardation and dysequilibrium syndrome’ (CAMRQ) now in use. Biallelic mutations in four genes have been implicated in CAMRQ, giving rise to four subtypes (CAMRQ1-4) caused by pathogenic variants in \textit{VLDLR}, \textit{WDR81}, \textit{CA8} and \textit{ATP8A2} respectively.

CAMRQ1 (MIM 224050), or \textit{VLDLR}-associated cerebellar hypoplasia (\textit{VLDLR}-CH), is the most well-described subtype, first described in the Hutterite population due to a founder mutation. In addition to the core features, \textit{VLDLR}-CH causes moderate-to-profound intellectual disability, dysarthria, inferior cerebellar and pontine hypoplasia and gyral simplification, frequently with quadrupedal gait. Confirmed mutations in \textit{WDR81} and \textit{ATP8A2} (causing CAMRQ2 and CAMRQ4) have only been described in a small number of families. There are no established genotype-phenotype correlations.
CAMRQ3 (MIM 613227) is caused by biallelic mutations in CA8, a gene that encodes carbonic anhydrase-related peptide 8 (CARP8). There are only three published cases, all caused by biallelic missense mutations (Table 1). We describe a 9 year-old boy with ataxia and normal intellect who has a homozygous novel truncating variant in CA8, to our knowledge the first published report of this mutational mechanism causing the condition. Our findings expand the phenotypic and genotypic spectrum of CA8-related cerebellar ataxia.

**Materials and Methods**

**Case Report**

The 9 year-old male proband is the first child born to healthy non-consanguineous Caucasian parents. Labour was induced at 35 weeks for spontaneous rupture of membranes after an otherwise uncomplicated pregnancy. He required brief support in the special care nursery for temperature regulation and feeding difficulties. Moderate generalised hypotonia was apparent in infancy, particularly involving the trunk and lower limbs, with severe head-lag that continued until age 5 years. He sat at 14 months and crawled at 18 months. First independent steps were achieved at 6 years with significant cerebellar ataxia. At 9 years, gait is wide-based, slow and limited to 10-20 steps, requiring assistance for longer distances. The proband has poor fine motor skills and incoordination with dysmetria. Speech was delayed with his first word at 12 months but no additional language until 24 months. At 9 years speech content and vocabulary is age-appropriate but with reduced velocity and significant dysarthria. He has poor oromotor coordination without bulbar or respiratory compromise. Visual acuity is normal although some tracking and accommodative difficulties are apparent.
He attends a mainstream school and does not have an intellectual disability. At no stage was there developmental plateau or regression.

On last examination at age nine years there were no significant dysmorphic features. His weight and height were on the 5th percentile and head circumference on the 30th percentile. Although nystagmus was not present, he had difficulty with visual tracking and smooth pursuit. He had marked cerebellar dysarthria. His gait was wide based and unsteady, and he showed truncal instability. There was normal tone in his limbs and normal strength. Reflexes in the upper limbs were normal but reflexes in the lower limbs were increased with subtle crossing and an extensor right plantar response. The Romberg test was normal. He had an intention tremor and difficulties with rapid alternating movements.

Magnetic resonance imaging (MRI) of the brain was normal at 2 years, but repeat MRI at age 7 years showed cerebellar vermian and hemispheric cerebellar atrophy superiorly with thinning of the folia (Figure 1). Normal investigations included chromosomal microarray, urine and plasma metabolic screens, transferrin isoforms, lysosomal enzymes, mitochondrial and POLG gene panel, mitochondrial deletion/duplication panel and testing for the FXN intron 1 GAA expansion that underlies most Friedreich ataxia.

**Whole exome sequencing**

Informed consent was obtained for genomic testing and case report publication, in accordance with institutional requirements. Singleton whole exome sequencing with deletion/duplication analysis was undertaken on the proband. DNA was extracted from peripheral blood using QIAamp DNA blood mini kits (Qiagen, Düsseldorf, Germany) and sample quality confirmed using the Agilent Tape Station Genomic DNA ScreenTape (Agilent...
Technologies, Santa Clara, CA, USA). Sequencing was performed at PerkinElmer Genomics Laboratory (Branford, CT, USA) by massively-parallel sequencing using a targeted sequence capture method (Agilent v6CREv2) followed by NGS of the amplified captured regions (Illumina, San Diego, CA, USA). Alignment to reference genome GRCh37 was performed. The DRAGEN (Dynamic Read Analysis for GENomics) Bio-IT Platform (Illumina) generated annotated variant calls within the target region and copy number variation (CNV) analysis was performed using the DRAGEN CNV platform (Illumina). Variants were classified according to the American College of Medical Genetics and Genomics (ACMG) variant classification criteria.
Results

A novel homozygous nonsense variant in exon 2 of CA8 (NM_004056.4[CA8]: c.232C>T) was identified, with predicted substitution of arginine for a premature termination codon (PTC) at position of 78 of the protein (p.Arg78*). This variant has not previously been reported in clinical cases and is absent in population databases (gnomAD, 1000 Genomes). Both healthy parents were heterozygous for the variant by polymerase chain reaction (PCR) amplification and Sanger sequencing, as was the proband’s healthy younger sister. There were no additional variants of interest considered compatible with the phenotype of the proband.

Discussion

CAMRQ3 is a rare hereditary ataxia reported in only three published families to date. Core features of (usually superior) cerebellar atrophy, ataxia and dysarthria appear to be fully penetrant, while intellectual disability, tremor and loss of bipedal gait are variably reported. This is similar to VLDLR-associated cerebellar hypoplasia. Possible progression of the phenotype has been reported in one individual with CAMRQ3 (Table 1). The proband reported here has not experienced clinical regression but the differing MRI findings between age 2 and age 7 may suggest some progression the phenotype. The small number of individuals reported precludes meaningful conclusions around genotype-phenotype correlation. It would appear, however, that intellectual disability and quadrupedal gait are not necessary in making the diagnosis, and a diagnostic label of ‘CA8-related cerebellar ataxia’ is proposed in place of CAMRQ3 in this child.

There has been some debate regarding the biological mechanisms driving the quadrupedal gait tendency which has historically defined the condition. Quadrupedal...
locomotion may represent an adaptive compensation for impaired balance and coordination rather than a devolution of bipedality\textsuperscript{16, 17} although it is generally not seen in other early-onset ataxias. The proband has achieved limited bipedal gait with intensive support and physical therapy, possibly representing a modified phenotype and extending the phenotypic spectrum of the condition.

Given the paucity of truncating \textit{CA8} variants in the medical literature and disease databases, additional functional evidence was considered in assigning pathogenicity to the p.(Arg\textsuperscript{78*}) variant. This variant is located in exon 2, predicting nonsense-mediated decay (NMD). Should the transcript escape NMD, there would be loss of the majority of the carbonic anhydrase domain, including a pfam-predicted active site at position 87. There is a spontaneous mouse model in which a biallelic 19 bp deletion in the murine homolog of \textit{CA8} that results in ataxia and truncal dystonia, providing persuasive evidence for a loss of function mechanism\textsuperscript{18}. Homozygous \textit{wdl} mutant mice exhibit reduced \textit{CA8} mRNA transcript and protein levels compared to wildtype, indicating NMD and supporting a loss of function mechanism\textsuperscript{18}.

The ataxic phenotype in \textit{wdl} mice is mediated through disordered granule cell proliferation and circuit patterning of cerebellar Purkinje cells, providing insight into the pathogenesis of the condition\textsuperscript{19}. Development of paraneoplastic cerebellar degeneration due to anti-CARP8 antibodies targeting Purkinje cells in an individual with malignant melanoma provides additional \textit{in vivo} evidence that loss of function of this protein results in cerebellar dysfunction\textsuperscript{20}.

We conclude that the p.(Arg\textsuperscript{78*}) variant is likely pathogenic and is the cause of this child’s condition. A likely pathogenic classification was applied, based on ACMG criteria\textsuperscript{15}, in
consideration of the variant’s absence in population databases (PM2), compelling functional
evidence for a loss of function mechanism (PS3) and close concordance of clinical features
with those reported in in an animal model. In this family, molecular characterisation has
permitted diagnostic confidence and informed reproductive planning. Identification of this
truncating variant broadens the mutational spectrum of this rare condition and highlights the
value of whole exome sequencing in diagnosis of rare forms of hereditary ataxia.

References

3. Schurig V, Orman AV, Bowen P. Nonprogressive cerebellar disorder with mental
   Homozygosity mapping and targeted genomic sequencing reveal the gene responsible for
   cerebellar hypoplasia and quadrupedal locomotion in a consanguineous kindred. Genome
   low-density lipoprotein receptor VLDLR cause cerebellar hypoplasia and quadrupedal


Figure Legends

Table 1. Previously reported cases of CAMRQ3 with confirmed pathogenic or likely pathogenic variants in \textit{CA8}

Figure 1. Brain MRI showing progressive cerebellar atrophy. Midline sagittal and coronal T1-weighted images at age two years (left column) and seven years (right column) showing progressive cerebellar atrophy affecting both the vermis and superior cerebellar hemispheres.
Clinical and Research Publication
Consent Form

Genetic File Number: 55776

DETAILS OF PATIENT

First Name: ___________________________ Surname: ___________________________

Date of Birth: ___________________________

I, ___________________________, being the patient/legal capacity appointee, or being the parent/legal guardian/next of kin of ___________________________, being the patient/legal capacity appointee, or being the parent/legal guardian/next of kin of ___________________________, give my consent for medical research or scientific publication of clinical information.

VCGS Clinician to request person giving consent to please circle agree or do not agree option

I agree do not agree (please select) that clinical details, including information about medical conditions, the family history, and results of investigations (including images such as X-rays and pictures taken through a microscope) may be included.

I agree do not agree (please select) that a drawn family tree (pedigree) may be included (if applicable).

I agree do not agree (please select) that clinical photographs may be included (if applicable).

I understand that the clinical information will be published without names attached, however I understand that complete anonymity cannot be guaranteed. It is possible that someone may recognise the information provided as related to me or my family.

I understand that the clinical information may include details of medical conditions and their prognosis, and treatment that has been provided or may be required in the future.

I understand that the clinical information may be published in a journal that is distributed worldwide, which go mainly to doctors and other health professionals, but may also be seen by others including academics, students and journalists. It may be placed on the journal’s website and made available on other medical/scientific websites or linked to in social media.

I understand that in some cases, the journal may require an additional consent form to be completed at a later date.

I understand that I may revoke my consent up until the information is committed to publication in a medical journal (‘gone to press”).

I understand that after the article has “gone to press”, it is not possible to revoke my consent.

I understand that VCGS staff will not publish images on non medical/scientific websites such as Google, but others may take images published in medical/scientific publications and place those images on non medical/scientific websites. VCGS has no control over such actions.

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I have been given the opportunity to ask questions and I am satisfied with the explanation and the answers to my questions. I hereby give my consent:

Signature: ___________________________ Date: _________________

VCGS CLINICIAN: I have a good understanding of the service and I have explained the process to the above named and answered any questions. In my opinion, she/he/they has understood this explanation and has signed and consents without compulsion.

Clinician’s Name: ___________________________ Signature: ___________________________ Date: _________________

If interpreter service used:

Name of Interpreter: ___________________________ Signature: ___________________________ Date: _________________

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CA8-related cerebellar ataxia: novel truncating mechanism

**CAMRQ3**
- Cerebellar ataxia
- Mental retardation
- Dysequilibrium

*Cause*: biallelic pathogenic variants in *CA8*...

- p.Ser100Pro
- p.Gly162Arg
- p.Arg237Gln
- c.232C>T
  - p.(Arg78*)

9 year-old male
- Congenital ataxia
- Limited bipedal gait
- Cerebellar atrophy
- Normal intellect

Previously published variants all *missense*

![Whole exome sequencing](Image)
Table 1. Previously reported cases of CAMRQ3 with confirmed pathogenic or likely pathogenic variants in *CA8*

<table>
<thead>
<tr>
<th>Family</th>
<th>Nucleotide Change</th>
<th>Predicted Protein Change</th>
<th>Variant Type</th>
<th>Clinical Presentation</th>
<th>MRI Findings</th>
<th>Source, Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.298T&gt;C</td>
<td>p.Ser100Pro</td>
<td>Missense</td>
<td>CAMRQ3 Mild ID</td>
<td>n/a</td>
<td>Türkmen et al*: 4 affected siblings</td>
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<td></td>
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<td>Cerebellar ataxia, dysarthria, Tremor</td>
<td>Cerebellar ataxia, dysarthria, Tremor, Quadrupedal gait</td>
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<td></td>
<td>Cerebellar ataxia, dysarthria, Tremor</td>
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<tr>
<td>2</td>
<td>c.710G&gt;A</td>
<td>p.Arg237Gln</td>
<td>Missense</td>
<td>CAMRQ‡ Ataxia</td>
<td>Cerebellar hypoplasia</td>
<td>Najmabadi et al‡: single proband</td>
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<td>Cerebellar hypoplasia</td>
<td>Cerebellar hypoplasia</td>
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<tr>
<td>3</td>
<td>c.484G&gt;A</td>
<td>p.Gly162Arg</td>
<td>Missense</td>
<td>CAMRQ3 Mild ID</td>
<td>Cerebellar volume loss (possibly progressive)</td>
<td>Kaya et al‡: 7 affected individuals, 3 related families</td>
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<td>Cerebellar ataxia, dysarthria, tremor</td>
<td>Cerebellar hypopa genesis, peritrigonal white matter irregularity</td>
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<td></td>
<td>Delayed/absent walking</td>
<td>PET Hypometabolic cerebellar hemispheres, temporal lobes, mesial cortex</td>
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<td></td>
<td></td>
<td>No quadrupedal gait</td>
<td>PET Hypometabolic cerebellar hemispheres, temporal lobes, mesial cortex</td>
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<tr>
<td>4</td>
<td>c.232C&gt;T</td>
<td>p.Arg78*</td>
<td>Nonsense</td>
<td>CAMRQ3 Cerebellar ataxia</td>
<td>Cerebellar atrophy (vermis, superior cerebellar hemispheres), progressive</td>
<td>This report</td>
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<td></td>
<td>Significant gross motor delay</td>
<td>Cerebellar atrophy (vermis, superior cerebellar hemispheres), progressive</td>
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<td></td>
<td>Generalised hypotonia</td>
<td>Cerebellar atrophy (vermis, superior cerebellar hemispheres), progressive</td>
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<td>No quadrupedal gait, walking limited</td>
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<td>to 10-20 steps</td>
<td>Cerebellar atrophy (vermis, superior cerebellar hemispheres), progressive</td>
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<td>Normal intellect</td>
<td>Cerebellar atrophy (vermis, superior cerebellar hemispheres), progressive</td>
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</table>

†Transcript NM_004056.4
‡Limited clinical information available

ACMG denotes American College of Medical Genetics and Genomics; CAMRQ3, ‘cerebellar ataxia, mental retardation and dysequilibrium syndrome 3’; ID, intellectual disability; n/a, not available; PET, positron emission tomography.
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