

A splice variant in *ATAD3A* expands the clinical and genetic spectrum of Harel-Yoon syndrome

Ilana Hanes, MD, MSc, Hugh J. McMillan, MD, MSc, Yoko Ito, PhD, Kristin D. Kernohan, PhD, Joanna Lazier, MD, Matthew A. Lines, MD, and David A. Dymont, DPhil, MD

Correspondence

Dr. Dymont
ddyment@cheo.on.ca

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ATAD3A is a mitochondrial AAA + ATPase protein localized between the inner and outer mitochondrial membrane¹; its role includes the stabilization of mitochondrial DNA, the regulation of mitochondrial fission/fusion, and the regulation of cholesterol homeostasis.^{1,2} Harel-Yoon syndrome (HYS) can result from biallelic deletions in the *ATAD3* gene cluster (containing *ATAD3A*, *ATAD3B*, and *ATAD3C*) and is associated with cerebellar and brainstem atrophy, hypotonia, encephalopathy, and death in the first days and weeks of life.^{3,4} A less severe presentation has been reported in those with biallelic missense variants.^{4,5} This attenuated form of HYS presents with developmental delay, cataracts, seizures, and optic and cerebellar atrophy with individuals living into adulthood.^{4,5} The findings are consistent with a genotype-phenotype correlation based on the variant type.^{6,7}

We report a child with a homozygous variant in the *ATAD3A* splice region, representing a loss of function variant. Progressive cerebellar atrophy occurred but was a less prominent feature.

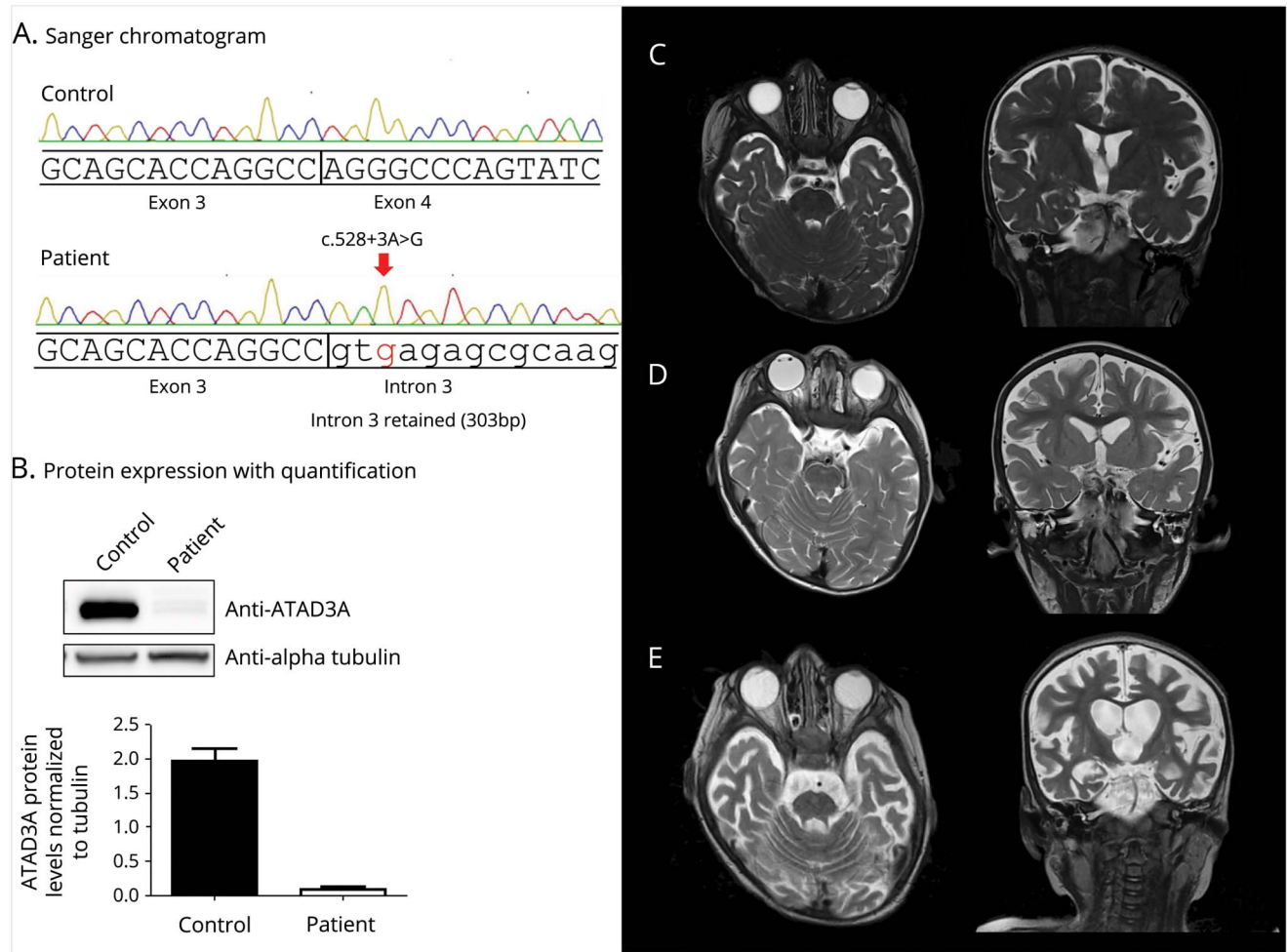
A term girl was born to healthy, consanguineous parents after an uncomplicated pregnancy. At 3 months of age, she had bilateral cataracts that were surgically removed. At 5 months old, she was admitted with pneumonia and noted to have axial hypotonia, hyporeflexia, and weakness. Her head circumference was 43.2 cm (35th percentile), and cranial nerve examination was normal. EMG revealed absent sensory responses and low motor nerve amplitudes consistent with a sensorimotor polyneuropathy with axonal features. Nerve and muscle biopsies confirmed an axonal polyneuropathy with no evidence of a storage disorder or mitochondrial disorder. Mitochondrial DNA sequencing and deletion analysis of muscle was unrevealing. By 8 months of age, developmental regression became apparent. She was no longer able to sit unsupported, and she had stopped rolling. MRI of the brain at 11 months old was unrevealing with a normal MR spectroscopy and EEG. Serum lactate was repeatedly normal. Biochemical testing for lysosomal and peroxisomal disorders was negative. Severe, bilateral sensorineural hearing loss was confirmed, and she developed failure-to-thrive requiring gastrostomy tube insertion. At 18 months old, she presented with bilateral ophthalmoplegia, partial left-sided ptosis, and visual impairment. She had upper and lower limb dystonic posturing that did not respond to levodopa-carbidopa supplementation. At 21 months of age, she experienced epilepsy partialis continua with intermittent generalized tonic-clonic seizures managed with levetiracetam, phenobarbital, piracetam, topiramate, and lacosamide. The ketogenic diet was initiated with no measurable clinical improvement seen. At 24 months of age, a tracheostomy was placed because of intermittent central and obstructive apneas and an inability to be weaned off mechanical ventilation. Repeat MRI showed progressive cerebral and cerebellar atrophy between studies performed at 20 and 24 months old (figure). At 32 months, she remains ventilated via tracheostomy with little interaction with her surroundings and ongoing seizures and dystonia.

From the Division of Neurology (I.H., H.J.M.), Children's Hospital of Eastern Ontario, Children's Hospital of Eastern Ontario Research Institute (H.J.M., Y.I., D.A.D.), University of Ottawa; Newborn Screen Ontario (K.D.K.), Ottawa; Department of Clinical Genetics (J.L., D.A.D.), and Division of Metabolics (M.A.L.), Children's Hospital of Eastern Ontario, University of Ottawa.

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(A) Sanger sequencing of the patient's complementary DNA revealed that the entire 303 nucleotides of intron 3 are retained in *ATAD3A* gene (NM_018188.4). For this splicing assay, the patient's RNA was extracted from fibroblasts and reverse transcribed. The retention of intron 3 is predicted to result in a premature termination codon 69 amino acids downstream of the 3' end of exon 3. (B) Immunoblot analysis using an anti-*ATAD3A* antibody with an epitope located upstream of the c.528+3A>G variant (Abcam Inc., Toronto, ON, Canada; ab188386) shows a significant reduction of *ATAD3A* protein in the patient's fibroblasts ($*p < 0.001$, 2-tailed Student *t* test). Protein was extracted from the patient's fibroblasts and age-matched control fibroblasts. An anti-*ATAD3A* antibody with an epitope upstream of the c.528+3A>G variant (amino acids 24–28) was used. MRI axial (left) and coronal (right) T2-weighted sequences were performed at (C) 11 months old, (D) 20 months old, and (E) 24 months old. Progressive cerebral and cerebellar atrophy was seen. MR spectroscopy was normal at 11 months, with a lactate peak apparent at 20 months and 24 months old. Head circumference decreased from the 32nd percentile to 19th percentile between the 11-month and 24-month studies.

Exome sequencing was performed at a commercial laboratory with no pathogenic or likely pathogenic variants reported. The data files were reanalyzed under a research protocol (Care4Rare Consortium). A homozygous variant of “unknown clinical significance” was identified in the *ATAD3A* splice site (NM_018188.4 c.528+3A>G). This variant had never been observed in control databases (the Genome Aggregation Database and the Exome Aggregation Consortium) or within the Care4Rare internal control database. In silico programs predict that the variant has an impact on splicing (MaxEntScan, NNSPLICE, and GeneSplicer). Sequencing of complementary DNA showed that intron 3 was retained (figure, A). The insertion of the 303 nucleotides of intron 3 is predicted to result in a premature stop codon at 69 amino acids downstream of the 3' end of exon 3. Immunoblot analysis using an anti-*ATAD3A* antibody with an epitope located upstream of the c.528+3A>G variant (Abcam

Inc., Toronto, ON, Canada; ab188386) showed a significant reduction in *ATAD3A* (figure). The rarity of the variant, in addition to the observed introduction of a stop codon, and low protein expression consistent with the mechanism of disease, is consistent with the variant being likely pathogenic.

Biallelic loss-of-function variation in the *ATAD3* gene cluster has been associated with a severe phenotype that is lethal in the first days and week of life.^{4,5} Our patient experienced a period of apparently normal neurodevelopment before demonstrating regression at 5–8 months of age. An MRI of the brain was initially unrevealing, emphasizing that cerebellar or pontocerebellar atrophy is not always an early indicator of the disease. Clinically, she also showed epilepsy partialis continua that has not previously been described for those with HYS, although this is not uncommon in mitochondrial-related disease. The

ketogenic diet was initiated given a previous report,⁵ and no clinical benefits were seen over the course of several months.

The observation of a splice variant is also novel, and its identification required reanalysis of the commercially derived exome sequence data. The advantages of reanalysis of exome data are apparent, and the new annotation and diagnosis was because of the rapid pace of gene discovery. We hypothesize that a degree of functional ATAD3A is likely generated via a “leaky” splice variant that resulted in this less severe phenotype and slower progression. This report expands the clinical, radiologic, and genotypic information associated with HYS.

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Appendix Authors

Name	Location	Contribution
Ilana Hanes MD, MSc	Division of Neurology, Children’s Hospital of Eastern Ontario, University of Ottawa	Drafted the manuscript

Appendix (continued)

Name	Location	Contribution
Hugh J McMillan, MD, MSc	Division of Neurology, Children’s Hospital of Eastern Ontario, University of Ottawa	Edited the manuscript, major role in acquisition of sample material, analyzed and interpreted the data
Yoko Ito, PhD	Children’s Hospital of Eastern Ontario Research Institute, University of Ottawa	Performed experiments, analyzed and interpreted data, co-wrote manuscript
Kristin D. Kernohan, PhD	Newborn Screen Ontario, Ottawa	Study design, analyzed data, edited text
Joanna Lazier, MD	Department of Clinical Genetics, Children’s Hospital of Eastern Ontario, University of Ottawa	Acquisition of sample material, edited the draft document
Matthew A. Lines, MD	Division of Metabolics, Children’s Hospital of Eastern Ontario, University of Ottawa	Acquisition of sample material, analyzed and interpretation of data, edited the draft document
David A. Dymnt, DPhil, MD	Children’s Hospital of Eastern Ontario Research Institute, University of Ottawa	Acquisition of sample material, analyzed and interpreted results, edited the manuscript

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