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Child Neurology: Progressive Cerebellar Atrophy and Retinal Dystrophy - Clues to an Ultra-Rare ACO2-Related Neurometabolic Diagnosis

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Abstract

Pathogenic bi-allelic variants in *ACO2*, which encodes the enzyme mitochondrial aconitase, are associated with the very rare diagnosis of *ACO2*-related Infantile Cerebellar Retinal Degeneration (OMIM 614559). We describe the diagnostic odyssey of a 4-year-old female patient with profound global developmental delays, microcephaly, severe hypotonia, retinal dystrophy, seizures, and progressive cerebellar atrophy. Whole exome sequencing (WES) revealed two variants in *ACO2*; c.2105_2106delAG (p.Gln702ArgfsX9), a likely pathogenic variant, and c.988C>T (p.Pro330Ser) which was classified as a variant of uncertain significance

(VUS). While the VUS was confirmed to be maternally inherited, the phase of the other variant could not be confirmed due to lack of a paternal sample. Functional biochemical studies were performed on a research basis to clarify the interpretation of the VUS, which enabled clinical confirmation of the diagnosis of ACO2-related Infantile Cerebellar Retinal Degeneration for our patient.

Case Presentation

A 4-year-old girl presented to the Neurogenetics clinic for an initial assessment of developmental delay and hypotonia. The family history was significant for an elder brother who passed away at 13 months in Somalia with a complex neurological history involving global developmental delays, abnormal eye movements, and an unconfirmed seizure episode. Parents were healthy, non-consanguineous and of Somali ancestry. The pregnancy, delivery and neonatal history for our patient were reportedly normal. She developed intermittent seizure-like episodes starting at 3 months, accompanied by worsening hypotonia. The patient's neurodevelopment plateaued at 4 months of age, with subsequent significant global developmental delays noted. By the time she was 7 months old, she was also observed to have abnormal eye movements. Magnetic resonance imaging (MRI) of the brain performed at 11 months revealed a typical-appearing brain, including cerebellum (Figure 1 A, B).

At 4 years of age, shortly after immigrating with her mother to Canada from Somalia, the patient developed generalized tonic-clonic seizures requiring the initiation of levetiracetam, with good control thereafter. An MRI brain with magnetic resonance spectroscopy (MRS) performed at 4 years revealed new diffuse, severe atrophy of the cerebellum and vermis compared with the MRI from 11 months of age (Figure 1C), as well as abnormal signal of the cerebellar cortex and dentate nuclei. Atrophy and abnormal signal of the hippocampi were also noted, in keeping with

bilateral mesial temporal sclerosis (Figure 1D). MRS was normal. Maculopathy and retinal dystrophy were observed on Ophthalmology assessment.

On examination at 4 years, the patient was generally non-dysmorphic in appearance, but microcephalic. Anthropometry revealed her weight at the 57th percentile for age (WHO Chart for Canada), and her head circumference of 47 cm, less than the 2nd percentile for age (Nellhaus Chart). General examination was unremarkable. Neurological examination revealed minimal interaction with her surroundings, lack of speech, inability to fix or follow, severe visual and hearing impairment, very significant axial and appendicular hypotonia, decreased muscle bulk and strength, diminished deep tendon reflexes, clumsy voluntary movements and inability to sit, stand or walk.

Initial investigations included chromosomal microarray and a metabolic screen consisting of lactate to assess for mitochondrial disorders, transferrin isoforms to assess for congenital disorders of glycosylation (CDGs), plasma amino acids and ammonium to assess for aminoacidopathies and urea cycle defects, urine organic acids to assess for organic acidaemias, plasma acylcarnitines to assess for fatty acid oxidation defects; urine mucopolysaccharides and oligosaccharides to assess for storage disorders, and very long chain fatty acids and phytanic acid to assess for peroxisomal disorders. After this testing returned with normal results, whole-exome sequencing (WES) was initiated. As only the mother was available to provide a parental sample at the time, “duo” testing was arranged with her sample and the proband’s.

WES revealed two variants in the *ACO2* gene, c.2105_2106delAG (p.Gln702ArgfsX9), a pathogenic variant, and c.988C>T (p.Pro330Ser), a variant of unknown significance (VUS), with the latter variant confirmed to be maternally-inherited. As the father was not available for testing, we could not definitively confirm whether the two variants were *in trans* (i.e., bi-allelic). Based

on the clinical phenotype of the proband, these variants were highly suggestive of causing the very rare disease ACO2-related Infantile Cerebellar Retinal Degeneration. This is a neurometabolic condition caused by defective function of mitochondrial aconitase, an enzyme involved in the tricarboxylic acid (TCA) cycle, which catalyzes the conversion of citrate to isocitrate.¹

Given that it was impossible to confirm the bi-allelic status of our patient's *ACO2* variants without a paternal sample, and that the maternally-inherited variant was of uncertain clinical significance, we performed functional biochemical studies necessary to confirm the diagnosis by proving dysfunction of the *ACO2* gene product. The protein level of mitochondrial aconitase (Aco2) in lymphoblastoid cell line (LCL) extracts from the proband were 52% that of her mother, as determined by immunoblotting with antibodies specifically recognizing Aco2, with actin as a loading control (Figure 2A). Aco2 activity in LCL extracts from the mother and proband were compared using an in-gel activity assay as previously described,² which revealed Aco2 activity in the proband's cells was 45-50% of that observed in her mother (Figure 2B). The proband showed 40% reduced mtDNA content compared to her mother (Figure 2C), by quantitative PCR. However, the oxygen consumption rate (OCR) of proband and mother LCLs were virtually identical using glucose as an energetic substrate (Figure 2D) by Seahorse analysis. Further studies are required to determine if OCR is impaired when substrates other than glucose are utilized. These results enabled clinical confirmation of the diagnosis of ACO2-related Infantile Cerebellar Retinal Degeneration for our patient.

Discussion

Childhood-onset cerebellar atrophy is associated with considerable clinical and genetic heterogeneity, with over 340 genetic conditions associated with cerebellar atrophy listed in the

OMIM (Online Mendelian Inheritance in Man) database. These include many ultra-rare genetic disorders and inborn errors of metabolism (IEM). The inheritance pattern of these disorders is commonly autosomal recessive, however, X-linked, mitochondrial, and *de novo* dominant inheritance are also prominent. Determining a rational diagnostic approach is critical given such a broad differential diagnosis. Neuroimaging is an essential initial step, as brain MRI and MRS can reveal important clues which help focus the diagnostic pathway by identifying additional structural abnormalities or characteristic MRS patterns.³ Additional clues can be gleaned from careful history-taking, including detailed family history, and elucidation of key clinical features, including the age of onset of symptoms, progression of symptoms, and the presence of multi-systemic involvement. A broad-based approach to genetic testing which incorporates clinical and imaging information is often required to confirm the diagnosis. Next-generation sequencing approaches such as whole exome sequencing (WES) are widely available, however interpreting the results of broad-based DNA sequencing is challenging for clinicians, given the high incidence of variants of uncertain significance (VUS). These are genetic variants that have insufficient or inconclusive evidence for their pathogenicity⁴, which usually require follow-up molecular and functional analyses to aid interpretation.

Although autosomal recessive ACO2-related Infantile Cerebellar Retinal Degeneration is an ultra-rare neurologic disorder, with less than 50 patients reported in the literature to date, it is an important diagnostic consideration in a pediatric patient who presents with progressive cerebellar atrophy, visual impairment, hypotonia, global delays, and seizures. This pan-ethnic disorder was initially described in 2012¹, in eight patients with bi-allelic pathogenic variants in *ACO2* associated with early-infantile onset of severe axial hypotonia, truncal ataxia, seizures, progressive microcephaly, esotropia, optic atrophy, and later retinal dystrophy.⁵ There has been a

broad range of phenotypes associated with pathogenic bi-allelic variants in *ACO2* since the original report, which renders recognition of this clinical entity difficult. These include complicated hereditary spastic paraplegia^{6,7,8}, episodic ataxia with mild delays and predominantly expressive language impairment⁹, neonatal hypotonia with severe central apnea⁹, isolated or syndromic optic neuropathy^{10,11} and severity of the clinical course ranges from death in early childhood to survival into adulthood with minimal functional impairment. Heterozygous pathogenic variants in *ACO2* are also known to cause autosomal dominant optic atrophy.^{12,13} This clinical and genetic heterogeneity further highlights the need for careful clinical evaluation of patients with suspected rare neurometabolic disorders, as appropriate functional testing to investigate the consequences of individual VUS can provide key evidence of pathogenicity.

In many cases, functional interrogation of a variant may be the only option to obtain conclusive evidence of its pathogenicity and is essential to confirm or reject a potential diagnosis with certainty. Previous work has shown that abnormalities of *ACO2* protein levels and/or enzyme activity can variably affect mitochondrial DNA (mtDNA) content and mitochondrial respiratory function.^{6,12,14} Reduction in *ACO2* activity most likely determines the severity of the clinical phenotype; abnormal transcript and protein levels do not always correlate with impaired *ACO2* function, as enzymatic activity may be regulated independently of protein levels.

Functional testing can be done via omics strategies and biomarker studies, targeted biochemical, molecular and cell morphology assays, rescue experiments, transgenic expression in model systems, and studies in pluripotent stem cells.¹⁵ Clinicians will need to become familiar with these types of investigations as broad-based genetic testing, and subsequently the need to clarify results of unclear clinical significance, are now routine practice in the pediatric neurology clinic, particularly for the diagnosis of patients with complex neurological presentations.

Conclusion

Complex neurological presentations in young children often have a very broad differential diagnosis. Good-quality neuroimaging, thorough clinical evaluation, and initial general, genetic, and biochemical investigations can reveal important clues to help narrow a differential diagnosis. Next-generation sequencing is often necessary as a next step, however the interpretation of the results of this testing comes with unique challenges, with interpretation of VUS being of particular importance for clinicians. For patients with suspected neurometabolic disorders, VUS may be interrogated via functional studies, which can provide definitive evidence of their pathogenicity.

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Figure Legends:

Figure 1. Evolution of Neuroimaging With Age. Initial imaging with sagittal (A) and axial (B) T2 at the age of 11 months demonstrates a normal appearance to the cerebellum. Imaging at the age of 4 years demonstrates significant cerebellar and vermian volume loss (C). There is also new bilateral mesial temporal sclerosis (D) with small FLAIR hyperintense signal of the hippocampi (white arrow).

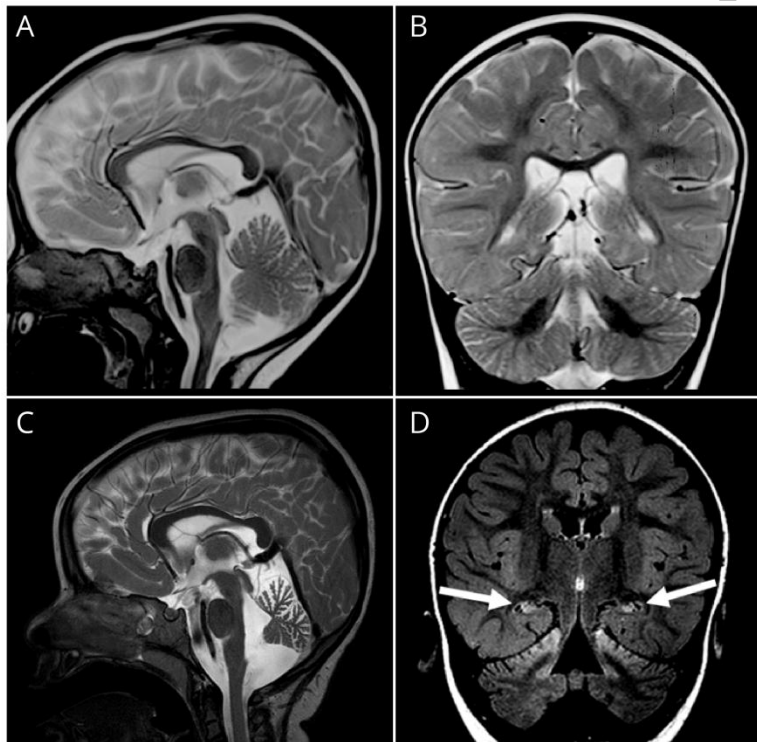
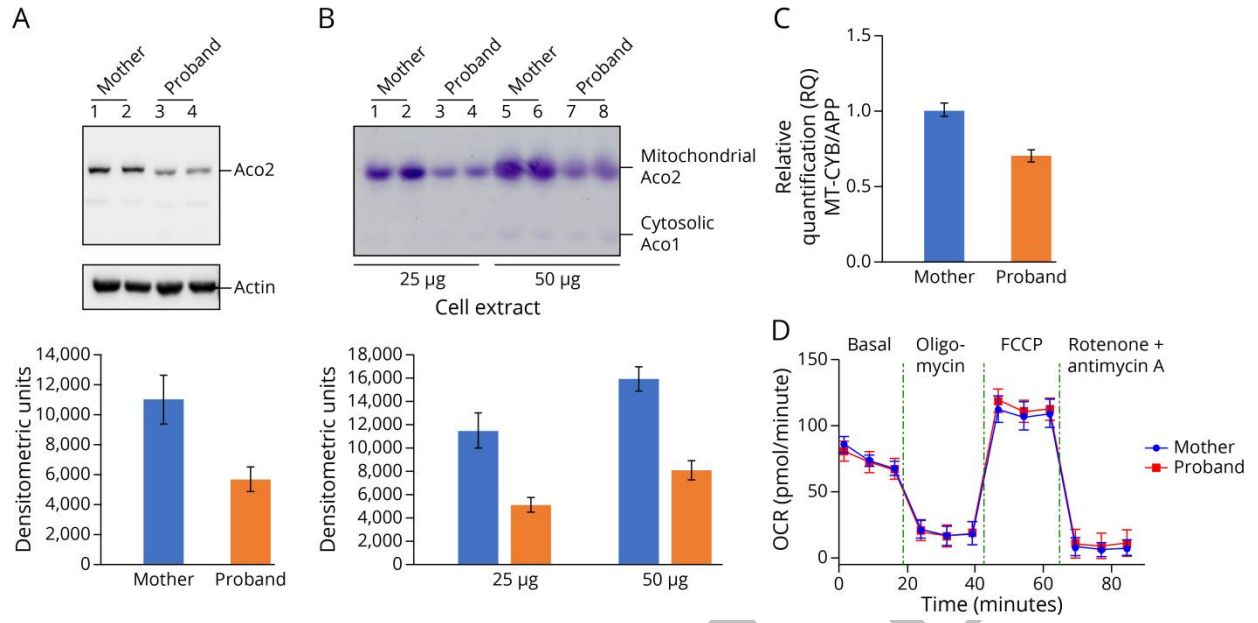


Figure 2. Biochemical Studies of Mother and Proband. (A) Aco2 protein levels in Mother and Proband LCL cell extracts (20 μ g, per lane loaded in duplicate) were immunoblotted with anti-ACO2 antibodies. Actin was immunoblotted as a loading control. (B) The activities of mitochondrial aconitase (mito. Aco2) and the cytosolic aconitase isoform (cytos. Aco1) were determined in LCL extracts from Mother and Proband (25 and 50 μ g, each loaded in duplicate), using an in-gel activity assay. The band intensities were quantified using ImageJ software and expressed as the average of the duplicate densitometric units. The error bars represent the standard deviation. (C) The mtDNA copy number in Mother and Proband LCLs was determined by qPCR using isolated genomic DNA as a template and Taqman primers and probes targeting the mitochondrial MT-CYB gene encoding cytochrome b and the nuclear APP gene encoding the amyloid precursor protein to normalize the reactions. Relative quantification (RQ) of MT-CYB/APP is shown. The error bars represent the standard deviation. (D) Oxygen consumption rates (OCR) of LCLs (1×10^5 cells per well) from the Proband and Mother using glucose as an energetic substrate were measured using a Seahorse XFe24 Analyzer. Baseline oxygen consumption rate (OCR) was measured, after which the following reagents were sequentially injected into the wells - oligomycin (1 μ M), FCCP (1 μ M) followed by antimycin A + rotenone (1 μ M each). The data are representative of two independent experiments with 10 replicates for each cell line.



Appendix 1 Authors

Name	Location	Contribution
Noor Lail, MD	University of Toronto, Toronto, ON	Designed and conceptualized the study; analyzed the data; drafted the manuscript for intellectual content
Ashutosh K. Pandey, PhD	Rutgers-New Jersey Medical School	Major role in the acquisition of the data; interpreted the data; revised the manuscript for intellectual content
Venkatesh Sundararajan, PhD	Rutgers-New Jersey Medical School	Interpreted the data; revised the manuscript for intellectual content
Roberto D. Noland, B.S.	Rutgers-New Jersey Medical School	Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content
Gabriel Swanson, B.S.	Rutgers-New Jersey Medical School	Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content
Debkumar Pain, PhD	Rutgers-New Jersey Medical School	Interpreted the data; revised the manuscript for intellectual content

Helen Branson, MD	University of Toronto, Toronto, ON	Major role in the acquisition of the data; interpreted the data; revised the manuscript for intellectual content
Carolyn K. Suzuki, PhD	Rutgers-New Jersey Medical School	Major role in the design of experiments, acquisition and analysis of the data; interpreted the data; revised the manuscript for intellectual content
Grace Yoon, MD	University of Toronto, Toronto, ON	Designed and conceptualized the study; analyzed the data; revised the manuscript for intellectual content, overall study supervision

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