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

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Abstract
Pathogenic biallelic 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 revealed 2 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 neurologic history involving global developmental delays, abnormal eye movements, and an unconfirmed seizure episode. Parents were healthy, nonconsanguineous, 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 of age, she was also observed to have abnormal eye movements. MRI of the brain performed at 11 months revealed a typical-appearing brain, including the cerebellum (Figure 1, A and 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. A brain MRI 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) and 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 (World Health Organization Chart for Canada) and her head circumference of 47 cm, less than the second percentile for age (Nellhaus Chart). General examination was unremarkable. Neurologic 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 acidemias, 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. Because the father was not available for testing, we could not definitively confirm whether the 2 variants were in trans (i.e., biallelic). 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 cycle, which catalyzes the conversion of citrate to isocitrate.

WES revealed 2 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. Because only the mother was available to provide a parental sample at the time, “duo” testing was arranged with her sample and the proband’s.

Given that it was impossible to confirm the biallelic 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 was 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, 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 mitochondrial DNA (mtDNA) content compared with
that of her mother’s (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 whether OCR is impaired when substrates other than glucose are used. 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 more than 340 genetic conditions associated with cerebellar atrophy listed in the Online Mendelian Inheritance in Man database. These include many ultrarare genetic disorders and inborn errors of metabolism. 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 because brain MRI and MRS can reveal important clues that help focus the diagnostic pathway by identifying additional structural abnormalities or characteristic MRS patterns. Additional clues can be gleaned from careful history-taking, including detailed family history, and elucidation of key clinical features, including the age at onset of symptoms, progression of symptoms, and the presence of multisystemic involvement. A broad-based approach to genetic testing that incorporates clinical and imaging information is often required to confirm the diagnosis. Next-generation sequencing approaches such as 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 pathogenicity, which usually require follow-up molecular and functional analyses to aid interpretation.

Although autosomal recessive ACO2-related infantile cerebellar retinal degeneration is an ultrarare 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 2012, in 8 patients with biallelic 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. There has been a broad range of phenotypes associated with pathogenic biallelic variants in ACO2 since the original report, which renders recognition of this clinical entity difficult. These include complicated hereditary spastic paraplegia, episodic ataxia with mild delays and predominantly expressive language impairment, neonatal hypotonia with severe central apnea, and isolated or syndromic optic neuropathy, 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. Previous work has shown that abnormalities in ACO2 activity most likely determine the severity of the clinical phenotype; abnormal transcript and protein levels do not always correlate with impaired Aco2 function because enzymatic activity may be regulated independently of protein levels.

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 mtDNA content and mitochondrial respiratory function. 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 because enzymatic activity may be regulated independently of protein levels.

Functional testing can be performed through 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. Clinicians will need to become familiar with these types of investigations because 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 neurologic presentations.

**Conclusion**

Complex neurologic 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 through functional studies, which can provide definitive evidence of their pathogenicity.

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**Disclosure**

The authors report no relevant disclosures. Go to Neurology.org/N for full disclosures.

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**Appendix Authors**

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References


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