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Increased Survival and Partly Preserved Cognition in a Patient with ACO2-Related Disease Secondary to a Novel Variant

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Abstract

ACO2 encodes aconitase 2, catalyzing the second step of the tricarboxylic acid. To date, there are only six reported families with five unique *ACO2* mutations. Affected individuals can develop intellectual disability (ID), epilepsy, brain atrophy, hypotonia, ataxia, optic atrophy, and retinal degeneration. Here, we report an 18-year-old boy with a novel *ACO2* variant discovered on whole-exome sequencing. He presented with childhood onset ataxia, impaired self-help skills comparable to severe-profound ID, intractable epilepsy, cerebellar atrophy, peripheral neuropathy, optic atrophy, and pigmentary retinopathy. His variant is the sixth unique *ACO2* mutation. In addition, compared to mild cases (isolated optic atrophy) and severe cases (infantile death), our patient may be moderately affected, evident by increased survival and some preserved cognition (ability to speak full sentences and follow commands), which is a novel presentation. This case expands the disease spectrum to include increased survival with partly spared cognition.

Keywords

ACO2; ataxia; intellectual disability; epilepsy; pigmentary retinopathy

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AUTHOR CONTRIBUTIONS

Dr. Srivastava contributed to study conception and drafted the manuscript. Dr. Gubbels, Ms. Dies, Dr. Fulton, and Dr. Yu contributed to acquisition and interpretation of data and critically revised the manuscript for important intellectual content. Dr. Sahin contributed to study conception and critically revised the manuscript for important intellectual content.

CONFLICTS OF INTEREST

Dr. Yu serves as part-time consultant to Claritas Genomics, a provider of genetic testing services (non-equity professional services agreement).

INTRODUCTION

The tricarboxylic acid (TCA) cycle is an essential component of cellular metabolism in all living organisms. The cycle consists of a series of enzymatic steps that harvests energy from the catabolism of carbohydrates, proteins, and lipids. In eukaryotes, enzymes of the TCA cycle are located in the mitochondrial matrix of virtually every cell type.¹ Mutations affecting the biochemical steps of the cycle, though rare, result in mitochondrial disease with a spectrum of systemic and neurological manifestations.²

One such example of a primary TCA cycle defect is aconitase deficiency secondary to alterations in the gene *ACO2* encoding aconitase 2 (ACO2). Aconitase catalyzes the second step of the TCA cycle. Humans have two isoforms of aconitase: aconitase 1 (ACO1), which is a cytoplasmic protein, and ACO2, which is a mitochondrial protein.³ To date, there are only six reported families with pathogenic variants in *ACO2* (hereafter, we will denote *ACO2* variants with respect to transcript NM_001098) (Table 1). Pathogenic variants in *ACO2* have been implicated in a spectrum of presentations, ranging from isolated optic nerve atrophy⁴ to infantile cerebellar-retinal degeneration (MIM# 614559), a neurodegenerative phenotype characterized by profound intellectual disability (ID), focal/generalized epilepsy, progressive cerebral and cerebellar atrophy, truncal hypotonia, ataxia, optic atrophy, and retinal degeneration.⁵

Here, we report an 18-year-old boy with a mitochondrial encephalopathy due to a novel *ACO2* variant. His features include impaired self-help skills comparable to severe-profound ID, intractable epilepsy, cerebellar atrophy, dysarthria, truncal hypotonia, peripheral neuropathy, ataxia, optic atrophy, and pigmentary retinopathy. After extensive prior evaluation, he underwent whole-exome sequencing (WES), which revealed a likely pathogenic, compound heterozygous missense/frameshift variant in *ACO2*: c.2328_2331delGGAA (p.Lys776Asnfs*49); c.1091T>C (p. Val364Ala). We characterize the genotype and phenotype of his disease, expanding the genetic landscape and phenotypic spectrum associated with *ACO2*-related diseases. We also describe how diagnosis with WES changed his clinical management, affirming the role of this technology as an indispensable tool for the child neurologist caring for patients with unrecognized neurological or metabolic conditions.

CLINICAL REPORT

The patient first presented at 15 months of age with acute truncal ataxia in the setting of a suspected viral illness. After this initial event, he developed several more episodes of illness-associated ataxia, each with gradual resolution. Eventually, the ataxia became constant.

In addition to ataxia, he demonstrated delayed development. He pulled to stand at 12 months and started cruising at 15 months. He has never walked independently. He said his first word besides “mama” between ages 4–5 years. He started speaking in full sentences years later, though he has poor articulation. At age 17 years, he underwent neuropsychological assessment with adaptive skills measurements suggesting that his overall level of functioning was comparable to severe-profound ID.

Seizures started at age 2 years and evolved into intractable generalized epilepsy unresponsive to multiple medication trials including anticonvulsants, ketogenic diet, and cannabidiol oil. He also takes gabapentin to improve pain associated with suspected peripheral neuropathy (based on diminished ankle reflexes and atrophic feet). His anticonvulsant regimen previously included valproic acid, which his team stopped after the diagnosis of aconitase deficiency due to worsening of neuropathic leg pain.

He experienced vision decline in the setting of progressive pigmentary retinopathy. Electroretinography and examination under anesthesia around age 4 years revealed markedly attenuated responses, optic atrophy, and pigmentary retinopathy. The pigmentary retinopathy progressed over time (Figure 1), leading to legal blindness.

On his latest examination at age 18 years, his growth parameters were normal apart from short stature (z-score -4.75). His general examination was notable for bifid uvula, submucous cleft palate, and bilateral pes planovalgus. He could follow commands and talk in full sentences, though his speech was dysarthric, requiring parental interpretation. He had intermittent alternating esotropia, intermittent nystagmus, and low visual acuity. He had axial hypotonia and appendicular hypertonia. His reflexes were 3+ in the upper extremities, 2+ at the knees, and diminished at the ankles. He exhibited ataxic movements of his trunk and limbs. He could not ambulate and used a wheelchair for mobility.

Prior to WES, he underwent extensive evaluations which did not provide a diagnosis. Brain MRI revealed mild cerebellar atrophy (Figure 2). Plasma amino acids revealed hyperalaninemia. Urine organic acids demonstrated drug metabolites. Skeletal muscle rotenone-sensitive NADH-cytochrome c reductase activity – reflecting electron transport chain (ETC) complexes I and III – was 0.2 $\mu\text{mol/min/g}$ wet weight (lower limit of control range 0.2). Muscle biopsy at age 2.5 years was non-diagnostic. Normal biochemical tests included plasma/CSF lactate, plasma acylcarnitine profile, plasma very long chain fatty acids, leukocyte coenzyme Q10 concentration, CSF neurotransmitter metabolites, urine free sialic acid, and leukocyte lysosomal enzyme analysis. Chromosomal microarray revealed a likely benign, maternally inherited 278 kb deletion in 6q14.1. Sequencing studies for specific considerations as well as mtDNA genome sequencing were unremarkable. Other normal evaluations included audiometry at age 12.5 years and echocardiogram/electrocardiogram at age 18 years.

METHODS

Exome sequencing was performed by Claritas Genomics using an orthogonal next-generation sequencing platform approach⁶, combining Illumina Trusight One sequencing (targeting 11.9 Mb of human disease-relevant genes) on the MiSeq with Proton Ampliseq whole exome sequencing. On the MiSeq platform, 29.5 million total reads were generated (mean read length of 150 bp) for a mean coverage of 185x. 96.0% of reads were alignable to UCSC hg19, 77.6% of which were within the targeted region. 98.1% of the target was covered by least 20 reads. Variant calling using the GATK platform identified a total of 7990 variants: 7776 SNVs (96.2% found in dbSNP), 108 insertions (59.3% found in dbSNP) and 106 deletions (60.4% found in dbSNP). For the Proton platform, a sequencing library was

prepared using AmpliSeq Hi-Q protocol targeting a 57.7 Mb exome, followed by sequencing on Ion Proton, generating a total of 25.3 million reads and covering the target to a mean depth of 69x. Analysis using the Ion Torrent Variant Caller identified a total of 51823 variants. Variant interpretation and analysis was performed using the WuXi NextCODE analytic platform.

RESULTS

To search for a genetic diagnosis for our patient, we undertook an analysis of variants generated by orthogonal exome sequencing guided by key phenotypes reported in this case. Using the WuXi NextCODE software platform, we searched for variants in genes associated with the following Human Phenotype Ontology (HPO) terms⁷: ID (HPO code 0001249), seizures (HPO code 0001250), ataxia (HPO code 0001251), pigmentary retinopathy (HPO code 0000580), and pes planus (HPO code 0001763). These search terms interrogated a set of candidate gene set in which we found 68 rare (AF <1%), potentially molecularly impactful (missense/nonsense/splice acceptor and donor site/coding indel) variants. Eighteen of these variants were consistent with an autosomal recessive inheritance model. Of these, only in the case of *ACO2* the gene was linked to the majority of the preselected HPO-terms.

The patient is compound heterozygous for a missense mutation in *trans* with a frameshift deletion in *ACO2*: c.2328_2331delGGAA (p.Lys776Asnfs*49; also called c.2325delTAAG); c.1091T>C (p.Val364Ala) based on transcript NM_001098. The father was found to be heterozygous for the deletion, and the mother was found to be heterozygous for the point mutation. Neither variant is present in the Exome Aggregation Consortium (ExAC; <http://exac.broadinstitute.org/>) or 1000 Genomes (<http://www.1000genomes.org>) databases. The missense variant is benign according to PolyPhen-2 (score 0.052; <http://genetics.bwh.harvard.edu/pph2/>), but damaging according to SIFT (score 0; <http://sift.jcvi.org/>) and disease causing according to Mutation Taster (<http://www.mutationtaster.org/>). The variant occurs at a position that is evolutionarily conserved across vertebrate species (Figure 3).

DISCUSSION

We have presented an 18-year-old young man with severely impaired self-help skills, intractable epilepsy, dysarthria, truncal hypotonia, peripheral neuropathy, ataxia, and pigmentary retinopathy secondary to a novel, likely pathogenic variant in *ACO2* discovered on WES. This variant is the sixth unique *ACO2* alteration associated with disease and the second reported *ACO2* compound heterozygous missense/frameshift alteration. It is likely pathogenic for a number of reasons. Not only is the missense change absent from control databases, it is also deleterious according to multiple *in silico* tools. Simultaneously, the frameshift variant truncates the last 5 residues of the ACO2 protein prior to the stop codon while adding 48 extra residues in their place. The combined effect of the two variants, in *trans*, is likely loss of function, consistent with the autosomal recessive mode of inheritance of *ACO2*-related diseases.

Our case helps delineate the full clinical spectrum associated with *ACO2*-related diseases (Table 1). We suggest that the mild end of the spectrum is characterized by isolated optic atrophy, without retinal dystrophy or cognitive involvement, and survival well into adulthood, exemplified by two individuals (Patient 1 and 2)⁴ who presented between ages 3–5 years with decreased visual acuity and optic disk pallor but who are still alive in their 4th–5th decade of life without other neurological symptoms. In contrast, the severe end of the spectrum may be associated with optic atrophy without retinal dystrophy combined with cerebellar atrophy, cardiorespiratory compromise (central apnea, bradycardia), and infantile death, features shared by two siblings (Patient 3 and 4).⁴ Our patient may be moderately affected, similar to the presentation of the individuals reported by Spiegel et al.⁵ Overlapping characteristics between our patient and this cohort include ID, epilepsy, significantly impaired motor abilities, cerebellar atrophy (but not cerebral atrophy), truncal hypotonia, peripheral neuropathy, ataxia, optic atrophy, and retinal degeneration. The oldest individual in this cohort (18 years) had profound ID and remained in a vegetative state. Relative to this degree of impairment, our patient's cognition is somewhat spared, allowing him to communicate in full (albeit dysarthric) sentences and follow commands.

Perhaps mediating the patient's phenotype is the varied impact of each allele on enzyme structure and function. Based on structural determination of porcine mitochondrial aconitase (which has 97% sequence identity with human *ACO2*), *ACO2* likely contains 4 different domains. The first three domains (beginning with the N-terminal domain) are connected to the fourth (C-terminal swivel domain) by a hinge-linker region. In the center of the folded enzyme is the citrate-binding active site, composed of residues from each of the four domains in addition to a catalytic iron-sulfur cluster. The first three domains are tightknit and enclose the iron-sulfur cluster, while the fourth domain hinges with the other three domains to create a cleft that funnels toward the iron-sulfur cluster.^{8–11} In our patient, the frameshift variant truncates the very end of the C-terminal domain while inserting 48 additional residues. The resulting domain, though extended, may still be able to carry out some of its structural role. In contrast, the missense variant may be more detrimental by affecting specific interactions with the iron-sulfur cluster. Further analysis is necessary to test this hypothesis.

The neurological and ophthalmological findings seen in our patient and others with *ACO2*-related diseases may be related to mitochondrial dysfunction in energy dependent structures. Neuronal loss in the cerebellum is evident with mitochondrial diseases¹² and may explain the cerebellar atrophy, dysarthria, nystagmus, and ataxia seen in our patient. Likewise, the optic nerve and retina are highly energy dependent structures, leaving them susceptible to damage in states of impaired energy production.^{13,14}

Interestingly, despite the importance of the TCA cycle in mitochondrial functioning, biochemical evidence of mitochondrial dysfunction in aconitase deficiency may be absent. Our patient had subtle examples of such biochemical abnormalities, including elevated plasma alanine and minimally reduced activity of ETC complexes I and III. However, his plasma and CSF lactate levels were normal, and there was no detectable lactate peak on MR spectroscopy. In other reports of *ACO2*-related diseases, there have been normal values reported for plasma lactate^{4,5,15}, CSF lactate⁵, plasma amino acids^{5,15}, and urine organic

acids^{5,15} including aconitic, citric, and isocitric acids.⁵ Mitochondrial respiratory chain enzyme activities have also been normal⁴ or slightly abnormal with evidence of mildly reduced glutamate oxidation.⁵ Currently, there is only one lab in the United States that offers clinical aconitase enzymatic activity testing. In essence, biochemical testing alone (including muscle ETC activity) may be insufficient to diagnose *ACO2*-related diseases. With the advent of next-generation sequencing (NGS), more diagnoses of aconitase deficiency may become apparent.

Importantly, under certain circumstances, WES offers a number of advantages over traditional diagnostic approaches. For our patient, diagnosis through WES changed clinical management. Specifically, he stopped taking valproic acid for epilepsy, and he started consistent usage of mitochondrial multivitamin supplementation which he had trialed in the past. One of the downsides of repeated targeted biochemical and molecular testing such as in our patient's case is passage of time, which can be deleterious in suspected neurometabolic or neurodegenerative conditions, especially if established or possible treatments are available. Our patient eluded a diagnosis for 17 years. Moreover, depending on the institution or laboratory, biochemical testing or DNA sequencing may not be readily available on a clinical basis. In situations like this, a non-biased NGS based approach may be advantageous and cost-effective, a conclusion reached by other studies examining the utility of WES for different populations, including neurometabolic diseases.¹⁶

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References

1. Berg, JM., Tymoczko, JL., Stryer, L. [cited 2016 Sep 29] The Citric Acid Cycle. 2002. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK21163/>
2. Rustin P, Bourgeron T, Parfait B, Chretien D, et al. Inborn errors of the Krebs cycle: a group of unusual mitochondrial diseases in human. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 1997 Aug 22; 1361(2):185–97. [PubMed: 9300800]
3. Beinert H, Kennedy MC. Aconitase, a two-faced protein: enzyme and iron regulatory factor. *FASEB J*. 1993 Dec; 7(15):1442–9. [PubMed: 8262329]
4. Metodiev MD, Gerber S, Hubert L, Delahodde A, et al. Mutations in the tricarboxylic acid cycle enzyme, aconitase 2, cause either isolated or syndromic optic neuropathy with encephalopathy and cerebellar atrophy. *J Med Genet*. 2014 Dec; 51(12):834–8. [PubMed: 25351951]
5. Spiegel R, Pines O, Ta-Shma A, Burak E, et al. Infantile cerebellar-retinal degeneration associated with a mutation in mitochondrial aconitase, *ACO2*. *Am J Hum Genet*. 2012 Mar 9; 90(3):518–23. [PubMed: 22405087]
6. Chennagiri N, White EJ, Frieden A, Lopez E, et al. Orthogonal NGS for High Throughput Clinical Diagnostics. *Sci Rep*. 2016 Apr 19; 6:24650. [PubMed: 27090146]
7. Köhler S, Vasilevsky NA, Engelstad M, Foster E, et al. The Human Phenotype Ontology in 2017. *Nucleic Acids Res*. 2017 Jan 4; 45(D1):D865–76. [PubMed: 27899602]
8. Lauble H, Kennedy MC, Beinert H, Stout CD. Crystal structures of aconitase with isocitrate and nitroisocitrate bound. *Biochemistry*. 1992 Mar 17; 31(10):2735–48. [PubMed: 1547214]

9. Robbins AH, Stout CD. Structure of activated aconitase: formation of the [4Fe-4S] cluster in the crystal. *Proc Natl Acad Sci USA*. 1989 May; 86(10):3639–43. [PubMed: 2726740]
10. Zheng L, Kennedy MC, Beinert H, Zalkin H. Mutational analysis of active site residues in pig heart aconitase. *J Biol Chem*. 1992 Apr 15; 267(11):7895–903. [PubMed: 1313811]
11. Robbins AH, Stout CD. The structure of aconitase. *Proteins*. 1989; 5(4):289–312. [PubMed: 2798408]
12. Lax NZ, Hepplewhite PD, Reeve AK, Nesbitt V, et al. Cerebellar ataxia in patients with mitochondrial DNA disease: a molecular clinicopathological study. *J Neuropathol Exp Neurol*. 2012 Feb; 71(2):148–61. [PubMed: 22249460]
13. Barot M, Gokulgandhi MR, Mitra AK. Mitochondrial dysfunction in retinal diseases. *Curr Eye Res*. 2011 Dec; 36(12):1069–77. [PubMed: 21978133]
14. Carelli V, Ross-Cisneros FN, Sadun AA. Mitochondrial dysfunction as a cause of optic neuropathies. *Prog Retin Eye Res*. 2004 Jan; 23(1):53–89. [PubMed: 14766317]
15. Sadat R, Barca E, Masand R, Donti TR, et al. Functional cellular analyses reveal energy metabolism defect and mitochondrial DNA depletion in a case of mitochondrial aconitase deficiency. *Mol Genet Metab*. 2016 May; 118(1):28–34. [PubMed: 26992325]
16. Tarailo-Graovac M, Shyr C, Ross CJ, Horvath GA, et al. Exome Sequencing and the Management of Neurometabolic Disorders. *New England Journal of Medicine*. 2016 Jun 9; 374(23):2246–55. [PubMed: 27276562]

Figure 1.

Fundus photographs of the patient over time demonstrating progressive pigmentary retinopathy. (A), (B), and (C) correspond to fundus photographs of the left eye at age 10.0 years, the right eye at age 13.3 years, and both eyes at 16.5 years, respectively. Findings include a pale optic nerve head (solid black arrow), attenuated retinal arterioles (“>”), deposits of black bone spicule pigment (unfilled black arrow), and prominent appearance of choroidal vessels (unfilled black triangle).

Figure 2. Brain MRI of the patient demonstrating mild cerebellar atrophy. (A) Midsagittal T1- and (B) axial T2-weighted images of the patient at age 15 months showing normal cerebellar structures. (C) Midsagittal T1- and (D) axial T2-weighted images of the patient at age 9 years showed prominence of the fissures of the cerebellar hemispheres consistent with mild degree of parenchymal volume loss. (E) Midsagittal T1- and (F) axial T2-weighted images of the patient at age 13 years 3 months showed stable findings compared to prior study at age 9 years.

Figure 3.

Multiple sequence alignment of ACO2 homologues. The outline denotes the position corresponding to the p.Val364Ala variant in our patient.

Table 1

Characteristics of families with previously reported *ACO2* mutations in addition to our patient.

		Previously Reported Patients					Our Patient
Reference	5	4			15		
Genotype	Homozygous c.336C>G (p.Ser112Arg)	Homozygous c.336C>G (p.Ser112Arg)	c.220C>G (p.Leu74Val); c.1981G>A (p.Gly661Arg)	homozygous c.776G>A (p.Gly259Asp)	c.2208G>C (p.Lys736Asp); c.2325_2328delGAAG (p.Lys776Asnfs*49)	c.2135C>T (p.Pro712Leu); c.1819C>T (p.Arg607Cys)	c.2328_2331delGGAA (p.Lys776Asnfs*49); c.1091T>C (p.Val364Ala)
Number of patients described in each family	5 (IV-3, IV-5, V-2, V-5, V-7)	3 (III-1, III-2, III-3)	2 (Patient 1, Patient 2)	2 (Patient 3, Patient 4)	1 (Patient 5)	1	1
Ethnicity	Arab Muslim	Arab Muslim	French	Algerian	Unspecified	Afro-Caribbean/East Indian	Mixed European
ID	Moderate (1/5) Severe (1/5) Profound (3/5)	Severe (1/3) Profound (2/3)	-	N/A	Profound	Developmental delay	Severe-profound
Seizures	+ (4/5)	+ (2/3)	-	Epileptiform activity (1/2)	-	-	+
Cortical atrophy	+ (3/4 who had brain MRI)	+ (1/1 who had brain MRI)	-	-	-	-	-
Cerebellar atrophy	+ (3/4 who had brain MRI)	+ (1/1 who had brain MRI)	-	+ (2/2)	+	-	+
Hypotonia	+ (5/5)	+ (2/3)	-	+ (1/2)	+	+	+
Ataxia	+ (5/5)	+ (3/3)	-	N/A	+	+	+
Optic atrophy	+ (4/5)	+ (3/3)	+ (2/2)	Optic nerve edema (1/2), optic disk pallor (1/2)	+	-	+
Retinal dystrophy	+ (3/3 who underwent relevant evaluation)	+ (3/3)	-	Altered ERG (1/2)	Altered ERG	-	+
Sensorineural hearing loss	+ (2/5)	-	-	N/A	-	+	-
Age of presentation [symptoms]	2–6 months [hypotonia (5/5), ataxia (3/5), eye abnormalities (2/5)]	3–5 months [hypotonia (2/3), ataxia (2/3), seizures (1/3), eye abnormalities (1/3)]	3–5 years [decreased visual acuity (2/2), optic pallor (2/2)]	Birth [cardiovascular compromise (2/2)]	5 months (delayed head control)	6 months (ataxia)	15 months (ataxia)
Current age	0.5–18 years	2–9 years	36–41 years	Died 57–61 days of life (coma, cardiorespiratory arrest)	10 years	3 years	18 years

ERG = electroretinogram. N/A = not applicable.