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Functional Cellular Analyses Reveal Energy Metabolism Defect and Mitochondrial DNA Depletion in a Case of Mitochondrial Aconitase Deficiency

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Abstract

Defects in the tricarboxylic acid cycle (TCA) are associated with a spectrum of neurological phenotypes that are often difficult to diagnose and manage. Whole-exome sequencing (WES) led to a rapid expansion of diagnostic capabilities in such disorders and facilitated a better understanding of disease pathogenesis, although functional characterization remains a bottleneck to the interpretation of potential pathological variants. We report a 2-year-old boy of Afro-Caribbean ancestry, who presented with neuromuscular symptoms without significant abnormalities on routine diagnostic evaluation. WES revealed compound heterozygous missense variants of uncertain significance in mitochondrial aconitase (*ACO2*), which encodes the TCA enzyme ACO2. Pathogenic variants in *ACO2* have been described in a handful of families as the cause of infantile cerebellar-retinal degeneration syndrome. Using biochemical and cellular assays in patient fibroblasts, we found that ACO2 expression was quantitatively normal, but ACO2 enzyme activity was less than 20% of that observed in control cells. We also observed a deficiency in cellular respiration and, for the first time, demonstrate evidence of mitochondrial DNA depletion and altered expression of some TCA components and electron transport chain subunits. The observed cellular defects were completely restored with *ACO2* gene rescue. Our findings demonstrate the pathogenicity of two VUS in *ACO2*, provide novel mechanistic insights to TCA disturbances in ACO2 deficiency, and implicate mitochondrial DNA depletion in the pathogenesis of this recently described disorder.

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CONFLICTS OF INTEREST

The authors declare that no conflict of interest exists.

Keywords

Whole Exome Sequencing; mtDNA Depletion; Neurodegenerative Disease; TCA Cycle; Mitochondrial Aconitase Deficiency

1. INTRODUCTION

Recessive defects in genes encoding critical enzymes and proteins in the tricarboxylic acid (TCA) cycle have been associated with a broad spectrum of neurological and neuromuscular phenotypes (1, 2). In more classical inborn errors of metabolism, pathway metabolites are utilized for biochemical screening, confirmation, and monitoring of disease. In contrast, the cellular effects of TCA cycle defects are diverse and complex, limiting the utility of similar diagnostic approaches. Next-generation whole-exome sequencing (WES) has made the molecular diagnoses of rare Mendelian diseases more tractable (3), including those affecting the TCA cycle (4). Despite the ever growing collection of publically available completed exomes, clinical WES frequently identifies numerous novel variants of uncertain significance (VUS) in potential disease-causing genes. Such variants, particularly in TCA cycle defects, may not directly contribute to clinical phenotypes, and, as these defects generally constitute newly reported disease-genes, the phenotypic spectra may not yet be fully elaborated. This uncertainty has implications for appropriate management and adequate counseling of affected families (5).

This diagnostic difficulty is compounded by the relative lack of knowledge concerning the underlying pathogenic mechanisms in TCA defects (6). Although wide-spread disruption of energy metabolism and oxidative phosphorylation (OXPHOS) is part of the presumed pathogenic mechanism, recent studies of defects in other key TCA cycle components have hinted at more diverse pathophysiological mechanisms, including defects in mitochondrial DNA (mtDNA) maintenance (1, 6). A better understanding of the pathophysiological consequences of deficiencies in TCA cycle components might pave the way for more specific diagnostic tests and new therapeutic approaches.

The TCA cycle enzyme aconitase (EC 4.2.1.3) is the second step in the TCA cycle, reversibly catalyzing the conversion of citrate into isocitrate. In humans, aconitase is expressed as two isoforms – cytosolic (soluble) aconitase (ACO1) and mitochondrial aconitase (ACO2). The mitochondrial isoenzyme ACO2 is encoded by the nuclear gene of the same name (*ACO2*) (7), and pathogenic variants in *ACO2* have recently been reported to cause infantile cerebellar-retinal degeneration syndrome (OMIM 614559) (8, 9). This syndrome is characterized by diverse neurological symptoms that include ophthalmological abnormalities, truncal hypotonia, muscle atrophy, seizures, and impaired motor and cognitive skills (8). To date, all reported cases have resulted from compound heterozygous or homozygous inheritance of missense variants in *ACO2*. The pathophysiologic defects resulting from deficiency of ACO2 in humans have not yet been fully described.

Here, we present a 2-year-old boy with mild neurodegenerative symptoms, in whom clinical WES revealed two missense variants of uncertain significance in *ACO2* (OMIM-100850). WES was undertaken after extensive clinical and laboratory evaluations failed to reveal a

diagnosis. We undertook *in vitro* biochemical characterization of patient-derived fibroblasts as a means of confirming the pathogenicity of the suspected variants and gaining mechanistic insights to the disorder through qualitative and/or quantitative assessments of the TCA cycle, oxidative phosphorylation, and mtDNA maintenance.

2. MATERIALS AND METHODS

2.1. Participants

The proband, mother, and father provided informed consent to be enrolled in the study. The study was approved by Institutional Review Board (IRB) of BCM and the Ethics Committee of The University of the West Indies.

2.2. Whole Exome Sequencing (WES)

Clinical whole-exome sequencing was undertaken by Baylor-Miraca Medical Genetics Laboratory and the details of their clinical sequencing platform have been reported previously (10). In brief, however, after capturing targeted exome, variants are called from reads with a minimum of 20× depth coverage, resulting in a minimum variant call rate of 94.6% for single-nucleotide variants (SNVs) and 88.2% for insertions or deletions (indels) (11). Variants of suspected clinical significance are confirmed by di-deoxy (Sanger) sequencing.

2.3. qPCR

Relative copy number of mtDNA in fibroblasts was analyzed using real-time qPCR method as described before (12). The beta 2 microglobulin gene (*B2M*) was used as the nuclear gene (nDNA) normalizer for the calculation of mtDNA/nDNA ratio. The ND1 region of human mtDNA was amplified using forward primer 5' GTCAACCTCGCTTCCCCACCCT 3' and reverse primer 5' TCCTGCGAATAGGCTTCCGGCT', giving a fragment of 108bp; A fragment of human beta 2 microglobulin gene was amplified using forward primer 5' CGACGGGAGGGTCGGGACAA 3' and reverse primer 5' GCCCGCGAAAGAGCGGAAG 3' giving a fragment of 118bp. Real-time qPCR analysis was performed (using Bio-Rad iTaq™ SYBR Green Supermix with ROX) and fluorescent signal intensity was recorded and analyzed on The Bio-Rad iCycler Sequence Detection System. Dissociation curves for the amplicons were generated after each run to confirm that the fluorescent signals were not attributable to nonspecific signals (primer-dimers). The mtDNA content (mtDNA/B2M ratio) was calculated using the formula: mtDNA content = $1/2^{Ct}$, where $Ct = Ct^{mtDNA} - Ct^{B2M}$.

2.4. Fibroblast Cell Culture

Primary skin fibroblasts from the proband and controls were grown in DMEM (HyClone) with 25 mM Glucose and 5mM Pyruvate supplemented with 10% FBS, 1% Antibiotic-Antimycotic, and 50 ug/mL uridine. Cells were grown in 37°C to 70-80% confluence and experiments performed on cells pooled from lines at similar passage number.

2.5. Gene Rescue

Fibroblasts from the affected individual were stably infected with pLenti6.3/V5-DEST lentiviral mammalian expression vector system (ThermoFisher) expressing *ACO2* cDNA (NM_001098.2). HEK293T cells were plated in DMEM (HyClone) with 10% FBS (Atlanta Biologicals) and transfected with pLenti – ACO2 in Opti_DMED medium (HyClone) packaging mix (5 µg pMDLg/pRRE, 2.5 µg RSV-rev, and 3 µg MD2.VSVG) with Lipofectamine 2000 (Life Technologies). Infectious lentiviral supernatant was collected at 48 hours and 60 hours, filtered, and used to infect growing fibroblasts for 72 hours before selection by blasticidin (10 µg /mL).

2.6. Cellular respiration assay

XF24 extracellular flux analyzer from Seahorse Biosciences was used to measure the rates of oxygen consumption in fibroblasts. Cells were plated the previous day of experiment on the XF24 cell culture microplates at a density of 60,000 cells per well. XF24 cartridge was equilibrated with the calibration solution overnight at 37° C. XF assay media (5mM glucose, 2mM Pyruvate, in DMEM (Seahorse Biosciences)) was prepared and pH adjusted to 7.0 on the day of the experiment. XF assay media was used to prepare cellular stress reagents to provide the following final concentrations: 500 nM Oligomycin, 500 nM FCCP, 100 nM Antimycin A and 100 nM Rotenone. All the reagents were loaded in the ports as suggested by Seahorse Biosciences. Oxygen consumption rates (OCRs) were measured for 3 min with 3 min of mixing and 2 min of waiting period. After the assay was completed, cells in each well were trypsinized and counted using Vi-Cell XR (Beckman Coulter) cell counter and the counts were used to normalize the OCR rates. OCR was expressed as nmoles oxygen/min/ 1000 cells.

2.7. Western Blot

Whole cell lysates were prepared in RIPA buffer (50 mM TrisHCl pH7.4, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.1% SDS). After centrifugation of the lysate (10000g, 4 min), soluble proteins were isolated in the supernatant, and protein concentration was determined according to the Bradford-Lowry method. Samples were mixed with equal volume of 2× SDS-PAGE sample loading buffer (0.5M Tris-Cl, pH6.8, 10%SDS, 50% glycerol, 2% β-mercaptoethanol, and 5% bromophenol blue). Protein samples were separated on a 10% SDS-polyacrylamide mini-gel. Proteins were transferred electrophoretically to polyvinylidene difluoride membranes for 1h and 15min at 100V. Membranes were blocked for 3h in 5% milk-PBS and incubated overnight with primary antibodies from Abcam (CS, mito-cocktail, ACO2) and GeneTex (SUCLG1 SUCLG2). GAPDH was used as a loading control. After three washes with PBS-0.05% Tween 20, the membranes were incubated for 2 h with horseradish peroxidase-conjugated secondary antibody (Bio-Rad) diluted in 5% milk-PBS. The secondary antibody was detected using the chemilluminant ECL Plus reagent (Millipore, USA), and the membrane was viewed with the ChemiDocMP Imaging System (Bio-Rad).

2.8. Aconitase enzyme activity

The activity of mitochondrial aconitase was measured on the basis of conversion of citrate into α -ketoglutarate coupled with NADP reduction (Sigma) at 340 nm and was normalized for total protein as previously described (13).

2.9. Citrate Synthase enzyme activity

Citrate synthase (CS) activity was determined by measuring the reduction of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) at 412 nm, which is coupled to the reduction of acetyl-CoA by citrate synthase in the presence of oxaloacetate. The reaction mixture consists of 10 mM potassium phosphate (pH 7.5), 100 mM DTNB, 50 mM acetyl-CoA, and 250 mM oxaloacetate. All activities were calculated as nmoles/min/mg protein, and expressed as a percentage of control activity, according to Kennedy et al. (14).

2.10. Statistical Analysis

All data are expressed as means $\pm$ standard derivations. Group means were compared by ordinary one-way analysis of variance (ANOVA) performed using GraphPad Prism 6.0 (GraphPad Software Inc., La Jolla, CA, USA). A $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Clinical report

A now 3-year-old boy of mixed Afro-Caribbean and East Indian ancestry first presented at age 6 months of age with truncal ataxia, which progressed to more distal hypotonia, ultimately resulting in ataxia requiring support for ambulation. When seen at age 18 months, he exhibited global developmental delay together with dysmorphic facial features that included down slanting palpebral fissures, prominent forehead, and droopy eyelids. His growth parameters and trajectory were otherwise normal. He was noted to have occasional myoclonic jerks during times of illness. Other clinical features included moderate to severe sensorineural hearing loss bilaterally, and cog-wheel eye saccades. Optic nerves appeared normal. Neurological examination revealed prominent cerebellar involvement with oculomotor dyspraxia, truncal unsteadiness and disequilibrium, gait ataxia, mild limb dysmetria and reduced muscle tone.

He had an extensive diagnostic work-up that included array comparative genomic hybridization (aCGH or chromosome microarray (v9.1, BCM)), screening for metabolic abnormalities (acylcarnitine profile, urine organic acids, plasma amino acids), serum lactate, serum alpha-fetoprotein (AFP), basic chemistry (CHEM-7) and liver function tests (Alkaline phosphatase, Gamma-glutamyl transferase), all of which were normal. Magnetic resonance imaging (MRI) of the brain revealed a small (2×3 cm), stable, arachnoid cyst in the left temporal lobe region with an otherwise unremarkable cerebellum. MRI of the spine was also within normal limits.

3.2. Whole-Exome Sequencing

Whole-exome sequencing (WES) using a whole blood sample from the proband revealed two heterozygous novel missense variants of uncertain significance in the *ACO2* gene. The first, in exon 17 (g.chr22:41923953; c.2135C>T; p.P712L, NM_001098) was predicted to be damaging by PolyPhen-2 (15) and possibly damaging by SIFT (16), and was observed once in the ESP5400 database (<http://evs.gs.washington.edu/EVS>; AA – 1/3737) and four times in the ExAC database (<http://exac.broadinstitute.org/>; 3/10,400 Africans, 1/66,724 Europeans) with no homozygotes reported. The second variant, in exon 15 (g.chr22:41922323; c.1819C>T; p.R607C, NM_001098) was considered damaging by Poly-Phen2 and probably damaging by SIFT, and was not observed in any of the aforementioned public databases. Both variants were confirmed by Dideoxy-sequencing and observed to be in *trans* configuration based on parental studies, with the p.P712L variant being paternally inherited and the p.R607C being maternally inherited. Both missense variant affect amino acid residues that are highly conserved from human to yeast (supplemental figure 1). Other missense variants identified as being potentially related to the clinical phenotype (supplemental table 1) were considered to be unlikely given the full clinical phenotype, inheritance pattern, and predicted protein effect. We thus focused our attention on the variants in *ACO2*.

3.3. Biochemical Testing

Primary fibroblasts from the patient exhibited only 20% of the total aconitase enzyme activity (ACO1 and ACO2) compared to control primary fibroblasts (Figure 1A). Additionally, mitochondria isolated from patient fibroblasts were tested for ACO2 activity, and were also observed to be approximately 20% of control cell activity (supplemental figure 2). To confirm that this deficiency was the result of the observed mutations in *ACO2*, we undertook gene rescue experiments by virally introducing wild-type *ACO2* into the patients cells. Importantly, whole cell aconitase activity was completely restored upon transduction of patient cells with ectopically-expressed wild-type *ACO2* (Figure 1A). We did not detect a significant reduction in ACO2 protein levels in patient cells compared to controls (Figure 1B) on Western Blot analysis, suggesting that the observed enzymatic defect is not caused by reduced stability of the protein. Next, we measured the activity of citrate synthase (CS; E.C. 2.3.3.1) in patient fibroblasts as a biochemical marker of mitochondrial mass. CS activity was observed to be comparable in patient and control cells, while the rescued fibroblasts exhibited a 50% increase in CS activity (Figure 1C). Interestingly, immunoblotting suggested that there is an overall similar increase in CS protein expression for both patient and patient-rescued fibroblasts compared to controls (Figure 1D).

Given an expected defect in intermediate oxidative metabolism resulting from ACO2 deficiency, cellular respiration studies were performed. Measuring oxygen consumption in the presence of glucose and pyruvate, we observed a significant defect in cellular respiration in the patient's fibroblasts (Figure 2). Both patient and control cells had similar basal respiration rates and responded similarly to the ATP inhibitor oligomycin, indicating the patient's cells have unaltered coupling efficiency (17). With the addition of FCCP, however, we observed that patient fibroblasts had significantly lower total respiratory capacity and

minimal reserve respiratory capacity, compared to control cells, such that when control cells were operating at maximal respiration rate patient cells had 40% less capacity. Importantly, introduction of wild type *ACO2* into the patient fibroblasts completely rescued the cellular respiration defects, indicating that these defects are specific for the observed *ACO2* deficiency.

Because the patient fibroblasts exhibited a severe defect in cellular respiration (Figure 2) and aconitase deficiency has been associated with mtDNA depletion in yeast (18), we used qPCR to assess mtDNA copy number relative to nDNA copy number. We observed that the patient fibroblasts exhibited 50% reduction in mtDNA copy compared to control cells and that mtDNA levels were restored to control levels with introduction of wild type *ACO2* into the patient cells (Figure 3).

Finally, to determine whether there was any evidence for effects of *ACO2* deficiency on expression of other TCA cycle enzymes and OXPHOS complexes, we performed Western Blot analysis for a subset of representative proteins (Figure 4). We observed increases in the steady-state level of subunits of respiratory chain complex II (SDHA) and complex III (UQRC2), as well as subunits of TCA enzyme Succinyl-CoA Synthetase (SUCLG1 and SUCLG2) (Figure 4). The increased levels of all of these proteins (except SUCLG1) were reduced to normal levels in the rescued cell line, suggesting that these changes were a consequence of *ACO2* deficiency.

4. DISCUSSION

Using WES, we identified two novel missense variants in trans in *ACO2* in a child who presented with truncal ataxia, hypotonia, developmental delay and hearing loss. We confirmed the pathogenicity of these variants through the use of functional biochemical assays performed in both patient- and gene-rescued-skin fibroblasts. We demonstrated defects in cellular respiration and mtDNA depletion in patient cells that is dependent on *ACO2* deficiency.

Pathogenic variants in mitochondrial aconitase were originally described in two families of Middle Eastern descent. The resulting defects in *ACO2* activity were associated with a relatively severe clinical course that included optic and cerebellar atrophy associated with hearing loss and severe to profound developmental delay (8). Subsequent to that original report (preceding the results on our patient), Metodiev *et al.* described an additional five subjects in three families (two consanguineous) from France and Algeria with other *ACO2* pathogenic variants (9). In that cohort, although the core symptoms of ataxia, optic nerve involvement and developmental delay were again noted, the severity of the associated symptoms varied widely. Our report therefore serves to further expand the phenotype associated with *ACO2* deficiency in a similar manner – our patient falls outside of the ethnic groups so far described, and did not have evidence of cerebellar atrophy on MRI or of significant optic nerve involvement at the time of evaluation. However, the aconitase activity is within the range of previously reported patient's with infantile cerebellar-retinal degeneration syndrome, and the *ACO2* protein activity is similar to at least one other patient (Figure 5).

Classic mtDNA depletion syndromes (MDS) have been described in association with neurodegenerative phenotypes, hearing impairments, and optic abnormalities. The majority of these syndromes have been linked to mutations in nuclear encoded genes known to function in mtDNA replication and/or maintenance, including *POLG1* (19), *TWINKLE* (20), and *DGUOK* (21). More recently, other genes with no previous known role in mtDNA maintenance have been associated with MDS, including *ABAT* (1) and *FXBL4* (2). Defects in subunits (*SUCLA2*, *SUCLG1*) of the TCA cycle enzyme succinyl-CoA synthetase have also been shown to be causes of MDS (22, 23). Depletion of mtDNA has previously been noted in yeast with deletion of mitochondrial aconitase (*ACO1*), and the mtDNA depletion in those mutant yeast was rescued by stable expression of a mutant form of Aco1 that is catalytically inactive (18). This finding suggests that in yeast, the mitochondrial aconitase catalytic activity and mtDNA maintenance functions of Aco1p are independent. The patient in this report has missense mutations that reduce enzymatic activity by 80% but the mutant ACO2 protein is stably expressed in the context of mtDNA depletion (Figures 1B, 3). This observed difference between yeast and human cells may indicate that mitochondrial aconitase (ACO2) activity is required for mtDNA maintenance in human cells or, alternatively, that these particular missense variants in *ACO2* result in conformational changes that disrupt some higher order protein complex required for mtDNA maintenance but do not reduce stability of the ACO2 monomer. Further studies are required to explore these possibilities. Importantly, however, this report represents the first description of mtDNA depletion in human cells from a patient with *ACO2* deficiency.

The mtDNA contains 13 protein-coding genes which comprise a subset of the protein subunits of complex I, III, IV, and V of the ETC. Depletion of mtDNA can result in defects of ETC in cells if the depletion reduces steady state level expression of mtDNA-encoded subunits (9, 24). Mouse embryonic fibroblasts deficient for *Sucla2* exhibit 50% depletion of mtDNA and similar cellular respiration defects (6). It is therefore not surprising that we observed OXPHOS defects in proliferating cells with 50% mtDNA depletion that is rescued with wild type ACO2 cDNA expression in patient cells. Interestingly, the ACO2-deficient patient cells showed *increased* representative subunits for complexes II and III and normal protein levels of representative subunits for other OXPHOS complexes. This observed up-regulation may be representative of a response to conditions of metabolic stress in order to promote cell survival (25). Therefore, our findings, although speculative, suggest the possibility that ACO2 (or closely related upstream or downstream metabolites) may have important roles in the regulation of some ETC subunits. It is interesting that CS expression is similarly increased in patient and patient-rescued cells (Figure 1D). It remains to be determined if the patient harbors any variants in *CS* that may affect expression levels. Furthermore, additional studies are required to determine if the 50% increase of CS enzyme activity observed in the rescued cells is a post-translational effect secondary to overexpression of ACO2 in the rescued cells or some other artifact. Finally, future studies incorporating a global approach to assessing the relative abundance of associated metabolic intermediates may help to provide a better context in which to interpret the observed abnormalities.

Our report further demonstrates the utility of WES for the diagnosis of TCA cycle deficits, and implicates quantitative deficits in oxidative capacity and mtDNA depletion, as well as

altered expression of at least some ETC and TCA proteins in the pathogenesis of ACO2 deficiency. Further investigation of the pathophysiology resulting from deficiency of TCA and ETC components is needed to explore potential novel diagnostic and therapeutic approaches in patients with these disorders. The combination of biochemical functional assays and WES can be a powerful tool in this quest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Two missense variants in *ACO2* identified by WES in a patient are reported.
- Patient fibroblasts are demonstrated to have mitochondrial aconitase deficiency
- Patient cells also exhibit cell respiration defect and mitochondrial DNA depletion.
- All cellular phenotypes are rescued by ectopic wild type *ACO2* expression.
- This is the first report showing *ACO2*-dependent mtDNA depletion in human cells.

Figure. 1. Aconitase deficiency in patient cells is rescued by ectopic wild type *ACO2* expression
1A. Aconitase enzyme activity measured in patient cells was significantly reduced compared to the mean activity of three controls and the patient cells rescued with wild type cDNA (**** $P < 0.0001$). **1B.** Western blot analysis of total protein extracts from cultured skin fibroblasts demonstrate similar expression of ACO2 in patient and control cells, with increased ectopic expression of wild type ACO2 in patient rescued cells. **1C.** CS enzyme activity is increased in the rescue cells (**** $P < 0.0001$), compared to the patient and control cells ($n=3$). **1D.** Expression of CS protein level in patient and patient-rescued fibroblasts is increased relative to controls. C1, C2 = control cell lines; Pt = patient cell line; Rs = patient-rescued cell line. Error bars indicate standard deviation for all data.

Figure 2. Cellular respiration defect in *ACO2* patient fibroblasts is dependent on *ACO2* deficiency
Cellular respiration analysis demonstrates reduced respiratory capacity in patient fibroblasts compared to controls (n=5) that is completely restored with ectopic expression of wild type *ACO2* in patient cells ("Patient + Rescue"). Experiment was done in triplicate. Error bars indicate standard deviation. "OCR" = oxygen consumption rate. Oligomycin = complex V (ATP synthase) inhibitor; FCCP = proton ionophore uncoupler; Antimycin & Rotenone = inhibitors of ETC complex III and complex I, respectively.

Figure 3. *ACO2* patient cells exhibit mtDNA depletion dependent on *ACO2* deficiency
Patient cells demonstrate an approximately 50% depletion of mtDNA content compared to controls (n=3) that is completely restored with ectopic expression of wild type *ACO2* in patient cells (“Rescue”). (*P<0.05). Error bars indicate standard deviation for all data.

Figure 4. Increased expression of some TCA cycle and ETC subunits in ACO2 deficiency

4A. Western blot analysis of total protein extracts from cultured skin fibroblasts of the selected subunits of TCA cycle enzyme succinyl-coA synthase, SUCLG1 and SUCLG2, demonstrate increased expression in patient cells that is at least partially reduced in patient cells ectopically expressing wild type *ACO2*. **4B.** Western blot analysis of selected subunits of ETC complex II (SDHA) and complex III (UQRC2) demonstrate increased expression in patient cells that is restored to control levels in patient cells ectopically expressing wild type *ACO2*. GAPDH was used as loading control. C1, C2 = control cell lines; Pt = patient cell line; Rs = patient-rescued cell line.

Figure 5. Clinical features of reported families with pathogenic variants in ACO2

ACO2 gene map depicts the amino acid residues with pathogenic variants identified in patient from this report and previously reported patients. Table compares the clinical features of aconitase activity and protein expression of the patient in this study with previously reported cases of infantile cerebellar-retinal degeneration.* May reflect either total aconitase activity or ACO2.