Case Report: Infantile Cerebellar-Retinal Degeneration With Compound Heterozygous Variants in ACO2 Gene—Long-Term Follow-Up of a Sibling

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Infantile cerebellar-retinal degeneration (ICRD) is an extremely rare, infantile-onset neurodegenerative disease, characterized by autosomal recessive inherited, global developmental delay (GDD), progressive cerebellar and cortical atrophy, and retinal degeneration. In 2012, a biallelic pathogenic variant in ACO2 gene (NM_001098.3) was found to be causative of this disease. To date, approximately 44 variants displaying various clinical features have been reported. Here, we report a case of two siblings with compound heterozygous variants in the ACO2 gene. Two siblings without perinatal problems were born to healthy non-consanguineous Korean parents. They showed GDD and seizures since infancy. Their first brain magnetic resonance imaging (MRI), electroencephalography, and metabolic workup revealed no abnormal findings. As they grew, they developed symptoms including ataxia, dysmetria, poor sitting balance, and myopia. Follow-up brain MRI findings revealed atrophy of the cerebellum and optic nerve. Through exome sequencing of both siblings and their parents, we identified the following compound heterozygous variants in the ACO2: c.85C > T (p.Arg29Trp) and c.2303C > A (p.Ala768Asp). These two variants were categorized as likely pathogenic based on ACMG/AMP guidelines. In conclusion, this case help to broaden the genetic and clinical spectrum of the ACO2 variants associated with ICRD. We have also documented the long-term clinical course and serial brain MRI findings for two patients with this extremely rare disease.

Keywords: infantile cerebellar-retinal degeneration, ACO2 gene, aconitase hydratase, optic atrophy, global developmental delay

INTRODUCTION

Infantile cerebellar-retinal degeneration (ICRD, MIM #614559) is a rare, autosomal recessive, infantile-onset neurodegenerative disease. It is characterized by truncal hypotonia, epilepsy, developmental delay, progressive cerebellar and cortical atrophy, optic nerve atrophy, and retinal degeneration (Spiegel et al., 2012; Sharkia et al., 2019). In 2012, Spiegel et al. analyzed eight patients from two families and reported that homozygous variants of the 1-aminocyclopropane-1-carboxylic
acid oxidase two gene (ACO2, NM_001098.3) were the cause of ICRD (Spiegel et al., 2012). To date, approximately 44 variants with varying clinical features have been reported. Here, we report a case of ICRD in two siblings cause by compound heterozygous variants in ACO2. This report can help expand the genomic and clinical spectrum of ACO2-related ICRD.

CASE DESCRIPTION

Two siblings without antenatal and perinatal problems were born to healthy non-consanguineous Korean parents. There was no family history of developmental delay, ataxia, or vision impairment.

Patient 1 (Older Sister)

She was a girl referred at the age of 30 months for occupational and physical therapies for delayed development from early infancy. The Bayley Scales of Infant Development II (BSID-II) test showed global developmental delay (GDD). She had been admitted to the hospital several times for febrile seizures. Her first brain magnetic resonance imaging (MRI) showed no significant abnormalities, and electroencephalography (EEG) suggested partial seizures. She did not take anticonvulsants because she had no partial seizures other than generalized tonic-clonic type convulsion accompanied by high fever. With age, she developed symptoms such as ataxia, dysmetria, poor sitting balance, strabismus, and myopia. Ophthalmic examination revealed atrophy of the bilateral optic nerves at age of 9 years. Follow-up brain MRI showed mild atrophy of the bilateral cerebellum (Figures 1A,B). At 9 years of age, her total intelligence quotient (TIQ), which was evaluating using the Korean Wechsler Intelligence Scale for Children-III was 31, indicating severe intellectual disability. A metabolic work-up, including blood lactic acid, pyruvic acid, amino acids, and urine organic acid tests, showed no abnormal findings. At 19 years of age, she speaks only a few words and can walk or lean against a wall. Further, she has severe vision impairment that allows her to only discern light. Follow-up brain MRI showed no significant changes (Figures 1C,D).

Patient 2 (Younger Sister)

She also showed GDD with BSID-II at 22 months of age. At 3 years of age, she was admitted to the intensive care unit with a diagnosis of status epilepticus. Her first brain magnetic resonance imaging (MRI) showed no significant abnormalities, and electroencephalography showed epileptic discharges in the frontal and occipital lobes. She took anticonvulsants, including valproate and topiramate, until 7 years of age. At 4 years of age, she had a decreased response to visual stimuli, and a visual evoked potential study showed optic neuropathy, which led to complete blindness. She showed no abnormalities in the metabolic workup. However, her symptoms developed earlier and were more severe than her sister’s symptoms. At
9 years of age, her TIQ was 43, indicating severe intellectual disability. Follow-up brain MRI at 7 years of age revealed hydrocephalus and atrophies of the cerebellum, brain stem, and optic nerve (Figures 1E,F). At 17 years of age, she is unable to walk and cannot speak meaningful words. Follow-up brain MRI showed no significant changes (Figures 1G,H).

**Exome Sequencing**

We performed exome sequencing (ES) of DNA from the siblings. Genomic DNA was extracted from proband blood. All exon regions of all human genes (~22,000) were captured by Twist Human Core Exome Kit (Twist Bioscience, South San Francisco, CA, United States). The captured regions of the genome were sequenced using the sequencing machine Novaseq 6,000 (Illumina, San Diego, CA, United States). The raw genome sequencing data analysis, including alignment to the GRCh37/hg19 human reference genome, variant calling, and annotation, was conducted using open-source bioinformatics tools and in-house software. We extracted evidence data on the pathogenicity of variants from previous studies and disease databases, including ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and UniProt (https://www.uniprot.org/). The results of ES revealed the following compound heterozygous variants in ACO2: c.85C>T (p.Arg29Trp) and c.2303C>A (p.Ala768Asp). These variants were validated by paired-end Sanger sequencing. The variant segregation analysis of the unaffected parents demonstrated that they were heterozygous carriers of each variant (Figure 2). No other homozygous or compound-heterozygous pathogenic or likely pathogenic variants were identified in the known Mendelian disease genes in the exome sequencing data of the siblings.

**Molecular Dynamic Stimulation**

Both variants have been reported at an extremely low frequency in large population cohorts (https://gnomad.broadinstitute.org/). The heterozygous variant c.2303C>A on ACO2 changes the amino acid Ala to Asp at codon 768 in exon 18. However, this has not been reported in large population cohorts (GenomAD). The programs MODELLER (https://salilab.org/modeller/) and GROMACS (https://www.gromacs.org/) were used to visualize and analyze the ACO2 protein structure. The p. Ala768Asp variant showed probable damage to the protein structure (Figure 3A). Structural modeling was performed based on the protein data bank structure (1C96.pdb) of the taurus ACO2 gene, which has a highly similar sequence (identity = 97%) to the human ACO2 gene. The distance between two helices is predicted to be increased, and nearby residues (Asp 773, Arg 994) were dragged by charge changes due to the variant, resulting in the relocation of Asp773 and subsequently destabilizing loop structures. Molecular dynamic stimulation was performed to evaluate the effect of this variant on structural stability. The structure of the variant had a larger...
variation in the root-mean-square deviation compared to the WT (Figure 3B). A missense variant is commonly associated with disease incidence, and the rate of benign missense variants is relatively low. The siblings’ phenotypes were highly specific for this disease. This variant was categorized as likely pathogenic according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines (PM2, PP1, PP2, PP3, and PP4) [3]. The heterozygous variant c.85C>T on ACO2 changes the amino acid Arg to Trp at codon 29 in exon 2. It has been reported at an extremely low frequency in large population cohorts. The allele frequency in GenomAD is 0.00003. In silico prediction of this variant showed contradictory results. It is predicted to be disease-causing by Mutation Taster (http://www.mutationtaster.org/) and Combined Annotation Dependent Depletion (score: 28.6, https://cadd.gs.washington.edu). Another in silico tool, REVEL (https://labworm.com/tool/revel) and MetaSVM (https://sites.google.com/site/jpopgen/dbNSFP) predicted this variant to be tolerated or benign. We could not predict the pathogenicity of this variation using protein structural modeling. However, the heterozygous variant c.85C>T on ACO2 was co-segregated in affected family members and confirmed as trans with other likely pathogenic variants after the segregation analysis. Further, the phenotypes of the patients were consistent with ICRD. Thus, this variant was categorized as likely pathogenic according to the ACMG/AMP guidelines (PM2, PM3, PP1, PP2, and PP4) (Richards et al., 2015).
| Reference | Our patients | Spiegel et al. | Metodev et al. | —— | Sadat et al. (2016) | —— | Shrivastava et al. (2017) | Kalman et al. | Bouwkamp et al. (2018) | —— | Marrilli et al. | —— | Sherkia et al. | —— | Fukada et al. (2019) | Ji soo park et al. | Patrick R. et al. |
|-----------|--------------|---------------|---------------|-----|--------------------|-----|--------------------------|--------------|-------------------------|-----|------------------|-----|----------------|-----|------------------|-------|----------------|
| No. of family | 1 | 2 | 3 | —— | 1 | 2 | —— | 1 | 1 | 1 | 5 | —— | —— | 1 | 1 | 1 | 1 | 1 |
| No. of patients | 2 | 5 | 3 | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 1 |
| Onset age | 30/22 mon | 2–6 months | 5/3 years | at birth | 5 months | 5 months | 3 months | 5 months | 2/8 years | 5/3/2 months | NA | 7 months | 1 day | 5 weeks | 2/6 months | 2 months | 12/20 months | 20 months |
| Current age | 19 | 17 years | 0.5–18 years | 36 years | 61 days | 10 years | 3 years | 17/14 years | 14 years | 46 months | 9 years | 28/14 years | 3 months | 5 years | 2/6 months | 7 months | 12/15 years | 15 years |
| Motor skills | Walk with assist | None | Wheelchair bound | Ataxic gait | NA | Wheelchair bound | Normal | Walk with assist | Walk with assist | Walk with assist | None | None | Walk alone | None | —— | Wheelchair bound | Normal | Walk with assist |
| Cognitive skills | A few words | None | Smiles, recognize family | NA | NA | Full sentence | Normal | A few words | Mild cognitive impairment | A few words | None | None | Some speech | None | None | None | Dysarthria |
| Hypotonia (%) | ++ | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Cerebellar ataxia (%) | ++ | − | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Seizures (%) | ++ | − | + | − | − | + | + | − | NA | − | + | − | + | + | − | + | + |
| Cortical atrophy (%) | ++ | − | + | − | + | − | + | + | + | + | + | + | + | − | + | + | + |
| Cerebellar atrophy (%) | ++ | − | + | − | + | − | + | + | − | NA | + | + | + | − | + | + | + |
| Optic atrophy (%) | ++ | − | + | − | − | + | − | − | − | − | − | − | − | − | − | − | − |
| Hearing loss (%) | ++ | − | + | − | − | + | − | − | − | − | − | − | − | − | − | − | − |
| Ethnicity | Northeast Asian | Arab | French | Algerian | NA | African – Caribbean | Arab | Caucasian | Mixed European | NA | Arab | Caucasian | Hispanic | Caucasian | African | Caucasian | Northeast Asian | Arab |
| Genotypes | c.2328Cc.2328G | c.2328Cc.2328G | c.2328Cc.2328G | c.2328Cc.2328G | c.2328Cc.2328G | c.2328Cc.2328G | c.2328Cc.2328G | c.2328Cc.2328G | c.2328Cc.2328G | c.2328Cc.2328G | c.2328Cc.2328G | c.2328Cc.2328G | c.2328Cc.2328G | c.2328Cc.2328G | c.2328Cc.2328G | c.2328Cc.2328G | c.2328Cc.2328G |
| Enzyme activity | NA | 12% | 59/86% | 5% | NA | 20% | NA | NA | NA | NA | NA | NA | NA | 50% | 30/20% | NA | 66% | 12% |

*D, days; mo, months; wk, weeks; NA, not available; +, present; −, absent.*
**DISCUSSION AND CONCLUSION**

In this study, we describe the clinical features of two siblings with two novel ACO2 variants from infancy to adolescence through a long-term follow-up. ACO2 (MIM #100850) encodes mitochondrial aconitase hydratase, which converts citrate to isocitrate in the tri-carboxylic acid (TCA) cycle (Mirel et al., 1998; Spiegel et al., 2012). The TCA cycle plays an important role in energy metabolism, and genetic defects in the TCA cycle are associated with various neurodegenerative disorders, including early-onset encephalopathies (Mirel et al., 1998; Briere et al., 2006). The biallelic variant of ACO2 has rarely been reported and exhibits various clinical manifestations from severe neurodegenerative disorders, such as ICRD, to mild ones, such as isolated optic atrophy 9 (MIM #616289) (Kelman et al., 2018; Sharkia et al., 2019; Blackburn et al., 2020; Gibson et al., 2020). Both of the presented siblings had clinical features commonly seen in ICRD, such as intellectual disability, cerebellar atrophy, and optic nerve atrophy. Although their numbers are limited, patients with ICRD have a wide range of age of onset and severity of phenotype (Sharkia et al., 2019). Table 1 shows the details of our and previously reported ICRD cases. Some reports state that residual aconitase activity in patient tissue or variant-specific assay in vitro is associated with clinical severity (Metodiev et al., 2014; Marelli et al., 2018; Blackburn et al., 2020). However, the methods of measuring aconitase activity differ across studies, hindering clinical application of aconitase activity measurement to the diagnosis of ACO2-related disorders. There are no useful metabolic biomarkers for the diagnosis of ICRD. Furthermore, sibling 2 demonstrated clinical symptoms, but characteristic radiologic findings, such as cerebellar atrophy on brain MRI, appeared several years later. Therefore, it is not easy for clinicians to suspect this extremely rare disease, ICRD.

To date, most of the known ACO2 variations have been diagnosed based on ES, and most of them are missense variants that require attention for interpretation (Marelli et al., 2018; Sharkia et al., 2019; Blackburn et al., 2020; Park et al., 2020). To interpret a missense variant as pathogenic or likely pathogenic, it is helpful to prove through various web-based in silico prediction software applications or protein structure modeling that the variant can induce physicochemical differences or evolutionarily conserved amino acid modification. In this study, both variants in the patients were missense variants, and the heterozygous variant c.2303C > A on ACO2 was predicted to modify the protein through homology modeling. However, for the other heterozygous variant c.85C > T on ACO2, we could not predict pathogenicity using in silico prediction tools or protein modeling. For the reasons mentioned above, we could not perform this variant-specific aconitase activity assay. Recently, it was reported that a 12-month-old infant with GDD and epilepsy had compound heterozygous missense variants in ACO2 through ES, and the c.85C > T on ACO2 variant was identical to ours (Bruel et al., 2019; Mau-

**DATA AVAILABILITY STATEMENT**

The datasets for this article are not publicly available due to concerns regarding participant/patient anonymity. Requests to access the datasets should be directed to the corresponding author.

**ETHICS STATEMENT**

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of Inha University Hospital (IRB No. 2020-05-032). All subjects provided written informed consent for clinical and molecular analyses. Written informed consent was obtained from the patient’s parent for publication of this case report and any accompanying images.

**AUTHOR CONTRIBUTIONS**

SJK and JEL designed the experiments. DJH, YSK, and JP helped recruit the patients and their family members. GHS and KL performed the experiments and helped with the Molecular dynamic stimulation. DJH and SJK wrote the manuscript. All authors contributed to the article and approved the submitted version.
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Conflict of Interest: GS and KL was employed by company 3 billion, Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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