Complex hereditary spastic paraplegia associated with episodic visual loss caused by ACO2 variants

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Abstract
Most patients with homozygous or compound heterozygous pathogenic ACO2 variants present with muscular hypotonia features, namely, infantile cerebellar-retinal degeneration. Recently, two studies reported rare familial cases of ACO2 variants presenting as complex hereditary spastic paraplegia (HSP) with broad clinical spectra. Here, we report the case of a 20-year-old Japanese woman with complex HSP caused by compound heterozygous ACO2 variants, revealing a new phenotype of episodic visual loss during febrile illness.

The ACO2 gene on chromosome 22 encodes the aconitase 2 (ACO2) protein in the mitochondrial matrix; ACO2 catalyzes the stereospecific isomerization of citrate to isocitrate in the tricarboxylic acid (TCA) cycle. Pathogenic ACO2 variants were first reported in eight individuals from two Arab families, and they had infantile cerebellar-retinal degeneration (ICRD, OMIM#614559). Subsequently, ~20 cases of pathogenic homozygous or compound heterozygous ACO2 variants have been reported, including mild cases such as isolated optic atrophy (optic atrophy 9, OMIM#616289)–⁶. Most patients initially present with muscular hypotonia, ataxia, seizures, progressive optic atrophy, retinal degeneration, and intellectual disabilities. Decreased aconitase activity in fibroblastic or lymphoblastic cells suggests that impaired energy metabolism in the TCA cycle is a major cause of symptoms in patients with pathogenic ACO2 variants. Recently, cases from two families with pathogenic ACO2 variants represented by early- or late-onset spastic paraplegia with intellectual disability and broad clinical spectra were reported⁴,⁵. Here, we describe pathogenic variants in the ACO2 gene presenting as complex hereditary spastic paraplegia (HSP) with a new phenotype of episodic visual loss after every febrile infection and progressive optic atrophy. This is the third familial report and the first Asian patient with complex HSP caused by pathogenic ACO2 variants.

The proband was born to nonconsanguineous healthy parents at 38 weeks gestational age after unremarkable delivery. She did not have a family history of neuromuscular disorders or motor development delay. Her birth weight was 2482 g, and her head circumference was 32 cm. Her motor development was delayed, and she could not walk independently at 1 year and 10 months because of progressive lower limb spasticity. Physiotherapy was subsequently provided, and she started walking independently at 2 years and 6 months. Her cognitive level was moderate disability (estimated development quotient: 50) at this time.

From 3 years of age, she experienced recurrent encephalopathy-like episodes, episodic visual loss, ataxia, and altered consciousness after every febrile illness episode. During febrile illness, she often accidentally hit her head on the wall because of her poor vision. Her visual loss recovered after defervescence, although the other symptoms remained for several weeks. In the acute phase, magnetic resonance imaging (MRI) of the cerebrum and
variants were con-
disease-causing by MutationTaster (prob 0.999). Both predicted to be deleterious by SIFT (score 0.04) and
Gly259Asp variant inherited maternally (Fig.1C). We
maternally and paternally inherited, respectively. The
Gly259Asp and p.Asn716Lys variants were found to be
located in exon 17 (NM_001098.2: c.2148 C > G, p.
Aconitase enzymopathy is more difficult to diagnose
than other TCA enzymopathies. One reason is the poor
abnormalities in metabolic screening samples. An eleva-
tion of lactate levels in the blood and CSF and of specific
organic acids in the urine, which is typically detected in
other TCA enzymopathies, is not observed in aconitase
enzymopathy. Another reason is the broad clinical spec-
trum of ACO2-related disorders, ranging from isolated
optic atrophy to syndromic optic atrophy, such as ICRD
that involves hypotonia, retinal degeneration, severe
encéphalopathy, epilepsy, and cerebellar ataxia.6 Moreover,
new phenotypes associated with spastic paraplegia
were recently reported, including in this study. Table 1
shows the clinical manifestations of ACO2-related dis-
orders (optic atrophy 9, ICRD, and complex HSP).4–6,12 Most patients with ACO2-related disorders have optic
dwine involvement, which might be a hallmark feature of
aconitase enzymopathy, but not all patients have optic
atrophy.4–12 Thus, biochemical testing and clinical phe-
notypes are insufficient for diagnosing aconitase enzy-
mopathy, thereby indicating the importance of WES.

Figure 1D shows the structure of the ACO2 protein and
reported variants in ACO2-related disorders, including in
this study. There is no hot-spot region in any ACO2-
related disorder, and there seems to be poor

The electroencephalogram showed diffuse
slow waves and focal spikes compatible with nonspecific
encephalopathy; subsequently, antiepileptic drug therapy
was initiated. However, despite treatment with the med-
ications, episodic attacks repeatedly occurred after every
disease episode of fever. Her lower limb spasticity and reflexes
progressed with sustained clonus and extensor plantar responses.

At 18 years of age, she was admitted to our hospital with
acute psychomotor agitation after infection. Cerebral and
spinal MRI, CSF analysis, metabolic screening, and oph-
thalmological evaluations were performed during admis-
sion. Laboratory results showed no abnormalities, whereas cerebral MRI showed mild cerebellar vermis
atrophy, and ophthalmoscopy showed bilaterally pale
optic discs and suspected optic atrophy (Fig. 1A, B). These
findings suggested a genetic cause for the complex HSP.
Written informed consent was obtained from her parents
in accordance with the Review Board and Ethics Com-
mittee of Kyoto University, and whole-exome sequencing
(WES) was performed when she was 19 years old. Trio-
based WES was performed using the SuperSelect XT
Human All Exon v6 (Agilent Technologies, Santa Clara,
CA). Captured libraries were sequenced using NovaSeq
6000 (Illumina, San Diego, CA). WES identified com-
pound heterozygous missense variants in ACO2. The first
variant was in exon 6 (NM_001098.2: c.776 G > A, p.
Gly259Asp) and was predicted to be deleterious by SIFT
(score 0; http://sift.jcvi.org/) and disease-causing by
MutationTaster (prob 1; http://www.mutationtaster.org/).
This variant is known to be a disease-causing mutation:
rs786204828 (pathogenic)6. The second variant was
located in exon 17 (NM_001098.2: c.2148 C > G, p.
Asn716Lys) and has not been reported as a pathogenic
variant; it was found to have an extremely low allele fre-
quence (1.59 × 10−6) in the Genome Aggregation Data-
base (http://gnomad.broadinstitute.org). This variant was
predicted to be deleterious by SIFT (score 0.04) and
disease-causing by MutationTaster (prob 0.999). Both
variants were confirmed by Sanger sequencing; the p.
Gly259Asp and p.Asn716Lys variants were found to be
maternally and paternally inherited, respectively. The
unaffected younger sister had a heterozygous p.
Gly259Asp variant inherited maternally (Fig. 1C). We
evaluated the pathogenicity of these two variants in
accordance with the 2015 guidelines of the American
College of Medical Genetics and Genomics. The c.776 G
> A, p.Gly259Asp and c.2148 C > G, p.Asn716Lys variants
were classified as pathogenic and likely pathogenic,
respectively. At the age of 20 years, the proband had
severe cognitive function (estimated intelligence quotient:
~30) and moderate visual impairment. She walked on her
toes with spastic scissor gait and required a walking aid.

Mitochondrial ACO2 is a critical enzyme in the TCA
cycle, which is the primary source of cellular metabolic
energy. Other TCA enzymopathies, such as deficiencies of
alpha-ketoglutarate dehydrogenase, fumarase, succi-
nate dehydrogenase, and succinyl-CoA synthase, have
been previously reported to cause severe encephalopathy
with muscle hypotonia, developmental delay, and retinitis
pigmentosa.7–11 The underlying pathophysiological
mechanism may involve a disruption of energy metabo-
lism and oxidative phosphorylation.7 Therefore, aconitase
deficiency is also thought to disrupt cellular energy
metabolism; consistent with this, a study reported mito-
ochondrial dysfunction in the fibroblasts of an aconitase-
deficient patient12.

ACO2-related disorders have optic
damage, retinal degeneration, severe
encéphalopathy, epilepsy, and cerebellar ataxia.6 Moreover,
new phenotypes associated with spastic paraplegia
were recently reported, including in this study. Table 1
shows the clinical manifestations of ACO2-related dis-
orders (optic atrophy 9, ICRD, and complex HSP)4–6,12 Most patients with ACO2-related disorders have optic
ATP7B-related disorders, the novel characteristic
phenotype of lactate levels in the blood and CSF and of specific
organic acids in the urine, which is typically detected in
other TCA enzymopathies, is not observed in aconitase
enzymopathy. Another reason is the broad clinical spec-
trum of ACO2-related disorders, ranging from isolated
optic atrophy to syndromic optic atrophy, such as ICRD
that involves hypotonia, retinal degeneration, severe
encéphalopathy, epilepsy, and cerebellar ataxia.6 Moreover,
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orders (optic atrophy 9, ICRD, and complex HSP)4–6,12 Most patients with ACO2-related disorders have optic
Atypical optic atrophy, which might be a hallmark feature of
aconitase enzymopathy, but not all patients have optic
atrophy.4,12 Thus, biochemical testing and clinical phe-
notypes are insufficient for diagnosing aconitase enzy-
mopathy, thereby indicating the importance of WES.

Figure 1D shows the structure of the ACO2 protein and
reported variants in ACO2-related disorders, including in
this study. There is no hot-spot region in any ACO2-
related disorder, and there seems to be poor
genotype–phenotype correlation. Compared to previously
reported ACO2-related disorders, the novel characteristic
phenotype in the present patient was episodic visual loss
during febrile infection (Table 1). The phenotypes in the
present patient indicated that ACO2 plays a crucial role in
energy production in the optic nerve and retina, which are
highly energy-dependent structures14. Previous findings
suggested that phenotype variation and severity depend on
residual aconitase enzymatic activity12. Metodiev et al.
Fig. 1 Cerebral MRI, fundus photograph and genetic information of the patient. A Sagittal T1-weighted MRI showed mild atrophy of the cerebellar vermis at the age of 18 years. B Right and left fundus photographs showed a pale optic nerve head (white arrowhead) without retinal involvement at the age of 18 years. C Family pedigree. Shaded symbol indicates the proband. D Schematic structure of the ACO2 protein. Variants in patients with ACO2-related disorders (OPA9, ICRD, and complex HSP) are shown in the diagram. OPA9: optic atrophy 9 (red box), ICRD: infantile cerebellar-retinal degeneration (blue box), HSP: hereditary spastic paraplegia (purple box), magenta-highlighted mutations: present report.
| References          | Metodiev et al. | Srivastava et al. | Marelli et al. | Bouwkamp et al. | This study |
|---------------------|-----------------|-------------------|----------------|-----------------|------------|
| Clinical            | OPA9            | ICRD              | Complex HSP    |                 |            |
| presentation        |                 |                   |                |                 |            |
| Sex                 | Male            | Male              | Female         | Male            | Female     |
| Age at report (years) | 36             | 41                | Died 57 days   | Died 61 days    | 18         |
| Ethnicity           | French          | French            | Algerian       | Algerian        | N/R        |
| Infantile cerebellar-retinal degeneration | –              | –                 | +              | +               | +          |
| Optic atrophy       | +               | +                 | Edema of optic disks | +       | +        |
| Spastic paraplegia  | –               | –                 | –              | –               | –          |
| Intellectual disability | –             | –                 | N/A            | N/A             | Severe     |
| Seizure             | –               | –                 | N/R            | N/R             | +          |
| Episodic visual loss| –               | –                 | N/A            | N/A             | –          |
| Episodic ataxia     | –               | –                 | N/A            | N/A             | +          |
| Episodic behavior change | –             | –                 | N/A            | N/A             | –          |
| MRI finding         | No abnormality  | Moderate atrophy of the cerebellum | Moderate atrophy of the cerebellum | Moderate atrophy of the cerebellum | Mild atrophy of the cerebellar vermis |
| Mutation            | p.Leu74Val/p.Gly661Arg | p.Gly259Asp/p.Gly259Asp | p.Lys736Asn/p.Lys776Asn*49 | p.3364G->A/c.2328_2331delGGAAfs | p.Pro712Leu/p.Phe414Val |
| Enzyme activity (%) | 60              | 66                | <5             | N/P             | 30         |

+ present, − absent, MRI magnetic resonance imaging, N/R not reported, N/A not available, N/P not performed, OPA9 optic atrophy 9, ICRD infantile cerebellar-retinal degeneration, HSP hereditary spastic paraplegia.
reported a very severe case involving homozygous Gly259Asp variants. The patient presented with syndromic optic neuropathy along with cerebellar atrophy and died at 57 days because of central apnea. The aconitase enzymatic activity in the patient’s fibroblasts was extremely low (~5%). However, a case report of a mild phenotype despite a marked reduction in aconitase enzyme activity suggested poor genotype-phenotype correlations in \( ACO2 \) variants and the coexistence of genomic modifiers.

HSP is not a single disease; rather, it is a mixture of genetically heterogeneous conditions resulting in broadly overlapping clinical phenotypes. Using single-gene direct sequencing and next-generation sequencing technologies, various HSP-related gene variants have been identified. These genes encode proteins with diverse molecular functions, axonal transport, specific lipid metabolism, synaptic formation, axon development, and mitochondrial function. In addition to \( ACO2 \) variants, several other HSP-associated gene variants, such as those in \( PGN, HSPD1, DDHD1, REEP1, \) and \( MT-ATP6 \), have been found to impair mitochondrial function. Further reports on the causative genes of HSP would improve the understanding of the crucial role of mitochondrial dysfunction in HSP pathogenesis.

In conclusion, this case represents the third report of HSP caused by pathogenic \( ACO2 \) variants. Although most patients with \( ACO2 \)-related disorders present with muscular hypotonia features, it should be recognized that pathogenic \( ACO2 \) variants comprise one of the causes of complex HSP. Patients with \( ACO2 \)-related disorders should be evaluated for signs such as early-onset spastic paraplegia, especially those with episodic visual loss after febrile infection and progressive optic atrophy. The identification of pathogenic \( ACO2 \) variants in patients with HSP could contribute to the development of specific therapies against HSP caused by mitochondrial dysfunction.

HGV database

The relevant data from this Data Report are hosted at the Human Genome Variation Database at https://doi.org/10.6084/m9.figshare.hgv.2951 and https://doi.org/10.6084/m9.figshare.hgv.2954.

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Conflict of interest

The authors declare that they have no conflict of interest.

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