Case Report

Severe spinal cord hypoplasia due to a novel ATAD3A compound heterozygous deletion

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A B S T R A C T

Biallelic deletions extending into the ATPase family AAA-domain containing protein 3A (ATAD3A) gene lead to infantile lethality with severe pontocerebellar hypoplasia (PCH). However, only 12 such cases have been reported worldwide to date, and the genotype-phenotype correlations are not well understood. We describe cases associated with the same novel biallelic deletions of the ATAD3A and ATAD3B/3A regions in Japanese siblings with severe spinal cord hypoplasia and multiple malformations, including PCH, leading to neonatal death. The ATAD3A protein is essential for normal interaction between mitochondria and endoplasmic reticulum and is important for mitochondrial biosynthesis. The cases were evaluated using whole-genome sequencing for genetic diagnosis of mitochondrial disease. Spinal cord lesions associated with biallelic compound heterozygous deletion extending into the ATAD3A gene have not been reported. In addition, the ATAD3A deletion was 19 base pairs long, which is short compared with those reported previously. This deletion introduced a frameshift, resulting in a premature termination codon, and was expected to be a null allele. The pathological findings of the atrophic spinal cord showed gliosis and tissue destruction of the gray and white matter. We describe spinal cord lesions as a new central nervous system phenotype associated with a biallelic compound heterozygous deletion extending into the ATAD3A gene. Biallelic ATAD3A deletions should be considered in cases of mitochondrial disease with spinal cord hypoplasia and PCH.

1. Introduction

Disruption of ATAD3 (ATPase family AAA-domain containing protein 3) cluster, specifically ATAD3A, was recently shown to be an important cause of various neurological syndromes. The ATAD3 cluster, located on chromosome 1p36.33, is composed of three paralogs (ATAD3A [MIM:612316], ATAD3B [MIM:612317], and ATAD3C [MIM:617227]) formed through two tandem segmental duplications

Abbreviations: ATAD3A, ATPase family AAA-domain containing protein 3A; ATAD3B, ATPase family AAA-domain containing protein 3B; ATAD3C, ATPase family AAA-domain containing protein 3C; PCH, pontocerebellar hypoplasia; bp, base pairs; mtDNA, mitochondrial DNA; Apgar, an appearance, score, grimace, activity and respiration; SNVs, single nucleotide variants; IUGR, intrauterine growth restriction; PCR, polymerase chain reaction; RARS2, arginyl-tRNA synthetase 2, mitochondrial; SLC25A46, solute carrier family 25 member 46; MRI, magnetic resonance imaging.

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The ATAD3 locus is one of the most common causes of nuclear-encoded pediatric mitochondrial disease; however, the repetitive nature of the locus means ATAD3 diagnoses may be frequently missed [9]. To date, only 12 cases of biallelic deletions extending into the ATAD3A gene have been reported globally (Table 1), and the genotype-phenotype correlations are not well-understood. We describe new cases of novel biallelic deletions of the ATAD3 and ATAD3B/ATAD3A regions in three Japanese patients associated with severe spinal cord hypoplasia and multiple malformations, which led to their neonatal death. We describe spinal cord lesions with PCH as a new central nervous system phenotype associated with biallelic compound heterozygous deletions extending into the ATAD3A gene.

### Table 1
Clinical findings in cases with biallelic compound heterozygous deletion extending into the ATAD3A gene.

| ATAD3 deletion | This study | Harel et al. (2016) | Desai et al. (2017) | Peeters-Scholte et al. (2017) | Zheng Yie Yap et al. (2021) | Family1 | Family2 |
|----------------|------------|---------------------|---------------------|-----------------------------|-----------------------------|---------|---------|
| II-1           | II-2       | II-3                | F7, II-1             | S1a/S1b/S2/S3/S4             | S1a/S1b/S3/S4               | Family1 | Family2 |
| ATAD3 deletion | unknown    | 3B/3B/3A           | 3B/3B/3A            | 3A/3A                       | 3A/3A                       | 3A/3B/3A | 3A/3A |
| kbp            |            | 38                 | 3B/3B/3B/3B/3B      | 3A/3A                       | 3A/3A                       | 3A/3B/3A | 3A/3A |
|                |            | 68 kb              |                     | 3A/3A                       | 3A/3A                       | 3A/3B/3A | 3A/3A |
| Age at death   | 19 bp      | 19                 | 19                   | 19                          | 19                          | 19       | 19     |

| Fetal presentation | + | + | + | + | + | + | + |
| Early delivery     | + | + | + | + | + | + | + |

### 2. Patient reports

We describe three affected siblings born to healthy Japanese parents who are unrelated by blood. The pedigrees and ATAD3 genotypes of the family are shown in Fig. 1A. Their clinical features are summarized in Table 1.

Patient II-1 was born in 2005 by emergency cesarean section because of a non-reassuring fetal status at 36 weeks and 4 days of gestation, weighed 2234 g, and had an Apgar score of 2/2. Intrauterine growth restriction (IUGR) was detected at approximately 28 weeks of pregnancy. After birth, resuscitative endotracheal intubation was required for severe neonatal asphyxia. Corneal clouding, low-set ears, joint contractures, seizures, hyperlactacidemia, and metabolic acidosis were observed during intensive care. He died at 15 h after birth from respiratory and circulatory insufficiency. An autopsy was performed. The chromosome (G-banding) was a normal male type, and no further genetic analysis was conducted.

Patient II-2 was born in 2007 by repeat cesarean section at 38 weeks and 1 day of gestation, weighed 2390 g, and had an Apgar score of 2/2. IUGR was detected at 36 weeks of pregnancy. Similar to Patient II-1, endotracheal intubation was required just after birth; the patient died on day 20 from respiratory and circulatory insufficiency. Corneal clouding, joint contractures, seizures, hyperlactacidemia, metabolic acidosis, and hyperalanemia were observed. A head CT was performed immediately after birth, and postmortem magnetic resonance imaging (MRI) of the head and spinal cord was performed. An autopsy was not approved, but skin and liver biopsies were performed. Mitochondrial respiratory chain enzyme activity in the liver was within normal range. The chromosome (G-banding) was a normal female type. The comparative genomic hybridization array test showed no obvious abnormal findings, and no further genetic analysis was performed.

Patient II-3 was born in 2011 by repeat cesarean section at 38 weeks and 1 day of gestation, weighed 2294 g, and had an Apgar score of 3/2.

| Symptoms | This study | Harel et al. (2016) | Desai et al. (2017) | Peeters-Scholte et al. (2017) | Zheng Yie Yap et al. (2021) | Family1 | Family2 |
|----------|------------|---------------------|---------------------|-----------------------------|-----------------------------|---------|---------|
| Respiratory insufficiency | + | + | + | + | + | + | + |
| Hypertrophic cardiomyopathy | + | + | + | + | + | + | + |
| Seizures | + | + | + | + | + | + | + |
| Contractures | + | + | + | + | + | + | + |
| Congenital cataract/ corneal clouding | + | + | + | + | + | + | + |

* Died intrapartum during a lengthy labor, ND = no data, NR = not reported.
IUGR was detected during pregnancy. Similar to that in patients II-1 and II-2, endotracheal intubation just after birth was required; the patient died on day 35 from renal and circulatory insufficiency. Seizures, hyperlactacidemia, and metabolic acidosis were observed. An autopsy was not performed, but samples of the liver and myocardium were collected by needle biopsy, and enzyme activity was measured. Only myocardium samples showed decreased activity, leading to the diagnosis of complex I deficiency (% citrate synthase ratio = 4.3%).

2.1. Whole-genome sequencing and sanger sequencing analysis

As shown in Fig. 1A, patients II-2 and II-3 had compound heterozygosity with a paternally derived 38 kbp ATAD3B/3A deletion and maternally derived 19 bp deletion in ATAD3A exon 6. The paternal-derived deletion was an ATAD3B/ATAD3A (NC_000001.10) deletion (g.1416465_1417219\(\_\)1454520\(\_\)1455274\_del), and that from the mother was an ATAD3A (NM_001170535) deletion (c.646_664del:p.Ala216Serfs^*235) (Fig. 1B, C). There are no reports of a 19 bp ATAD3A deletion in large human genome datasets (gnomAD, GenomeAsia 100 K, jMorp14KJPN [Japanese multiomics reference panel], Biobank Japan) [15–18]. No other genes with homozygous or compound heterozygous with potentially pathogenic variants were found in the WGS of patient II-3. In patient II-1, the only specimens available were formalin-fixed paraffin-embedded sections and the dried umbilical cord. DNA extracted from the fixed section was not amplified by polymerase chain reaction (PCR). DNA extracted from the umbilical cord showed no PCR amplification of 5 kbp and no verification of the 38 kbp-del of paternal origin, but a short sequence of 163 bp was amplified by PCR amplification, revealing a deletion of maternal origin. No amplification was detected even in short PCRs of approximately 400 and 600 bp, unrelated to ATAD3, which was attributed to the low DNA quality.

2.2. Head and spinal imaging findings

Only patient II-2 underwent head imaging. Head computed tomography (immediately after birth) showed cerebellar and brainstem hypoplasia and diffuse hypoabsorption areas in the bilateral cerebral white matter. Head MRI (postmortem) revealed a high signal on T2-weighted images, suggesting a history of encephalomalacia in the cerebellum, brainstem, and most of the cerebral cortex. MRI of the spinal cord showed prominent thinning of the spinal cord (Fig. 2 A–E).

2.3. Autopsy findings

An autopsy was performed on patient II-1. Macroscopically, the spinal cord was thin and < 2 mm in diameter, indicating hypoplasia. In addition, myelin sheaths were obscured, and extensive gliosis due to destruction of the gray and white matter was observed (Fig. 2F–I).

3. Discussion

We identified novel compound heterozygous deletions in the ATAD3A and ATAD3B/3A regions in siblings from a healthy non-consanguineous family associated with severe spinal cord hypoplasia, cerebellar hypoplasia, seizures, contractures, and corneal clouding. Twelve cases of biallelic deletions extending into the ATAD3A gene have been reported (Table 1). Two of these cases had a 38-kb ATAD3B/3A deletion along with a larger 68-kb ATAD3C/3A deletion [10,13]. The other ten had biallelic ATAD3B/3A deletions, including five cases with homozygous or compound heterozygous 38-kb deletions [5,11]. These
Clinical manifestations are quite similar, indicating that biallelic ATAD3A deletion is an important cause of severe PCH leading to early death. In our cases, the deletion of ATAD3A was for 19 bp, which is short compared with the lengths of deletions reported previously for this locus [5,10,11,13]; however, the short deletion was expected to be a null allele because of introduction of a premature termination codon through a frameshift. In addition to the biallelic large deletions in ATAD3B/3A, severe PCH and early postnatal death have been reported in cases of homozygous nonsense and missense mutations of ATAD3A [11,12]. Based on previously reported cases, genetic conditions that highly reduce ATAD3A expression rather than ATAD3B expression are mainly associated with neonatal lethality. Although ATAD3A and 3B proteins were not measured in these cases, deletion of ATAD3B was found only in one allele, emphasizing the effect of ATAD3A loss. Furthermore, imaging findings of the central nervous system revealed PCH, which is associated with biallelic ATAD3A deletions, and spinal cord hypoplasia, which has not been fully described. Spinal cord hypoplasia involves tissue destruction and gliosis of the gray and white matter, with few neurons remaining. The lesion may have developed during a chronic course that began in utero. Neurons depend primarily on mitochondrial oxidative phosphorylation for their energy and have a limited ability to upregulate glycolysis compared to astrocytes and oligodendrocytes [19]. Therefore, neurons are more susceptible to mitochondrial dysfunction, and gliosis likely forms after neuronal destruction.

Spinal cord involvement in patients with mitochondrial disorders is not uncommon and has been reported in patients with PCH [20]. PCH is currently classified into 11 types, although ATAD3A deletion is excluded [21]. Among these classifications, PCH type 1E [solute carrier family 25 member 46 (SLC25A46)] and PCH type 6 [arginyl-tRNA synthetase 2, mitochondrial (RARS2)] are derived from the etiologic genes of mitochondrial diseases, and PCH type 1E is characterized by respiratory failure requiring tracheal intubation immediately after birth, congenital contractures, and spinal cord involvement, which is consistent with the clinical findings of ATAD3A deletion. However, spinal cord lesions in PCH1 are limited to degeneration and reduction of the anterior horn cells, which differs from the present case, in which tissue destruction of the entire spinal cord was observed, albeit with anterior horn predominance. When PCH is associated with spinal cord hypoplasia, biallelic ATAD3A deletions should be considered as differential.

This study had some limitations. ATAD3A deletion could only be demonstrated in one allele in the case in which pathological autopsy was performed. Of the two remaining cases in which ATAD3A deletion was found in both alleles, spinal cord hypoplasia was confirmed in only one. Therefore, additional cases should be evaluated to clarify the relationship between biallelic ATAD3A deletion and spinal cord lesions.

4. Conclusions

We reported cases of siblings with severe spinal cord hypoplasia as a new central nervous system phenotype accompanied by neonatal death associated with compound heterozygous deletions in the ATAD3A and ATAD3B/ATAD3A regions. In cases of fetal cerebellar hypoplasia and spinal cord hypoplasia, compound heterozygous deletions in the ATAD3A gene must be identified to differentiate the disease and are important for predicting prenatal prognosis.

Ethics approval

This is an observational retrospective patient report that did not involve any research-based patient intervention. All interventions were intended to diagnose and treat the patient. No aspect of this report contradicts the Helsinki Declaration of 1975, as revised in 2000.

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Patient consent

Written informed consent for the publication of these data was obtained from the patient's mother.

Fig. 2. Imaging and autopsy findings. (A, B) Head computed tomography (CT) of patient II-2 was performed immediately after birth. (A) Cerebellar and brainstem hypoplasia (long arrow). (B) Diffused hypoabsorption areas in the bilateral cerebral white matter. (C-E) Head and spinal magnetic resonance imaging (MRI) of patient II-2 was performed postmortem. (C, D) Axial T2-weighted image shows strong signals in the cerebellum, brainstem, and most of the cortex above the bilateral tentorium. (E) Sagittal T1-weighted image shows prominent thinning of the spinal cord (long arrow). (F) Appearance of thin spine in patient II-1 (scale bar 500 mm). (G-I) Microscopic findings of spine of patient II-1. (G) The spinal cord is thin, and the white and gray matter is obscure (hematoxylin–eosin staining, scale bar 500 μm). (H) Myelin sheaths are indistinct (Kluver–Barrera stain). (I) Extensive gliosis is demonstrated (immunohistochemistry for glial fibrillary acidic protein).
Author contributions

TE, TT, and KM conceptualized this report, were involved in clinical practice, and drafted the initial manuscript; TN, YS, MS, MT, KI, YN, NA, LS, YY, KN, YK, TF, AO, AO, and YO coordinated and supervised data collection and critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Declaration of Competing Interest

Authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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