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A case of ATR-X syndrome with mitochondrial respiratory chain dysfunction

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ABSTRACT

Alpha-thalassemia X-linked intellectual disability (ATR-X) syndrome is caused by a mutation in *ATRX*, which is essential for proper chromatin remodeling. ATRX dysfunction leads to dysregulation of many genes due to abnormal chromatin remodeling, and causes a multisystem disorder in patients with ATR-X. Because mitochondrial disorders also show multisystem involvement, whether mitochondrial function is affected in patients with ATR-X is of interest. Here, we report a case of a 4-year-old male with a mutation (NM_000489.4: c.736C > T p.Arg246Cys) in *ATRX*, who showed mitochondrial dysfunction with complex I deficiency. The results from our study suggest that target genes of the ATRX protein may include those responsible for mitochondrial function, and mitochondrial dysfunction may contribute to some ATR-X phenotypes.

1. Introduction

Alpha-thalassemia X-linked intellectual disability (ATR-X) syndrome is caused by a mutation of the chromatin regulator gene, *ATRX*, and is characterized by severe intellectual disability, mild hemoglobin H disease, facial dysmorphism, skeletal and genital abnormalities, and characteristic posture and/or behavior (Gibbons et al., 2008). Although not clarified completely, the pathophysiological mechanism of ATR-X syndrome may involve epigenetic modifications, and the various clinical symptoms may be due to abnormal expression of target genes regulated by the ATRX protein. Because mitochondrial disorders also show multisystem involvement, determination of whether mitochondrial function is affected in patients with ATR-X is of interest. Nevertheless, investigation of mitochondrial function in patients with ATR-X syndrome has not been reported. In the present case report, we describe an ATR-X syndrome patient with mitochondrial dysfunction as determined with the results of enzyme activity assays and analysis with blue native polyacrylamide gel electrophoresis (BN-PAGE).

2. Clinical report

A male patient was born by normal spontaneous vaginal delivery at 40 weeks of gestation and weighed 3318 g (+0.7 SD). No family history of neuromuscular disease was reported. Beginning in the neonatal period, he showed retracted breathing above the clavicles and weak suckling. At 1 month of age, he was admitted to our hospital for severe respiratory insufficiency. He required transient mechanical ventilation and tube feeding. On physical examination, he was cyanotic with apnea and showed severe retracted breathing. His weight at admission was 3980 g (−0.7 SD), and poor weight gain was noted. He showed facial dysmorphisms, including hypertelorism, a small triangular nose, and a tented upper lip. His muscle tone was diminished, and his deep tendon reflex was normal. Cryptorchidism was noted. Due to repetitive respiratory failure, noninvasive positive pressure ventilation was introduced and continued until 13 months of age. Tonic seizures emerged at 9 months. Sodium valproate treatment was started, and seizure control was achieved. His global development was profoundly delayed; he was

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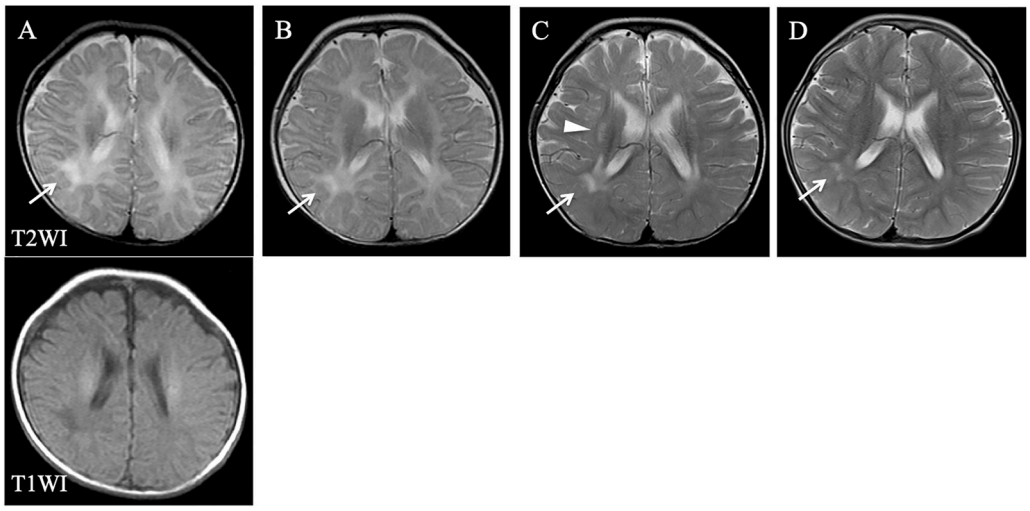

Fig. 1. Magnetic resonance images at 2 months (A), 5 months (B), 13 months (C), and 4 years of age (D). Increased signal intensity is observed on T2WI in the white matter, especially around the trigones (arrow) and around the body of the lateral ventricle (arrowhead). This finding was most prominent at 13 months, and was not noticeable at 4 years. T1WI, T1-weighted image; T2WI, T2-weighted image.

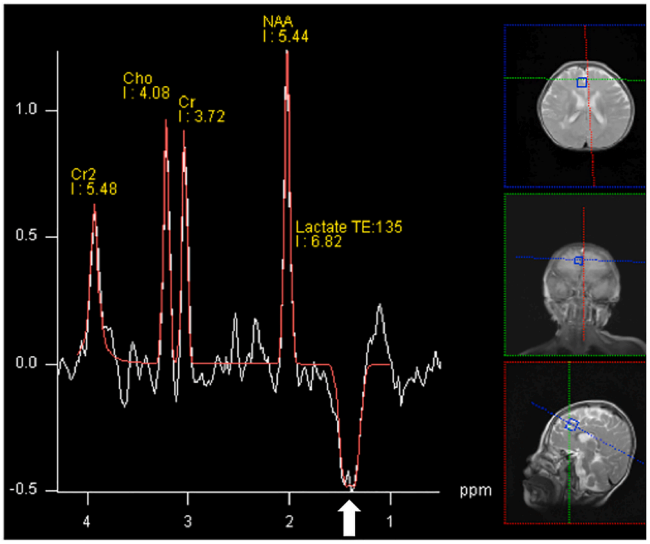

Fig. 2. Magnetic resonance spectroscopy of the white matter of the frontal lobe at 13 months of age shows an elevation of the peak corresponding to lactate (arrow).

able to hold his head up at 16 months, and was able to turn over at 17 months. At 4 years of age, the patient was able to sit up with support, but had not yet acquired the ability to stand. He was not able to speak meaningful words. He showed various and complicated gastrointestinal symptoms, especially gastroesophageal reflux, aerophagia, and constipation. He showed a stereotyped movement and a gesture that induced vomiting by putting his hand in his mouth. Brilliant cresyl blue staining of a peripheral blood smear did not identify hemoglobin H inclusion bodies.

The lactate-to-pyruvate ratio in his cerebrospinal fluid at 2 months was mildly elevated at 20.4 (11.0 mg/dL and 0.54 mg/dL, respectively). In addition, lactate-to-pyruvate ratio in his blood examined at the same time was elevated to 24.5 (15.2 mg/dL and 0.62 mg/dL, respectively). On the other hand, his blood concentration of alanine was not elevated (145 nmol/mL). Head T2-weighted magnetic resonance imaging showed hyperintensity of the white matter, especially in the terminal zone and around the body of the lateral ventricle. This finding was most prominent at 13 months of age and was relatively obscure at 4 years of

Table 1
The results of an enzyme assay for oxidative phosphorylation in fibroblast cells from the patient.

	CO I	CO II	CO II + III	CO III	CO IV	CS
Control						
Crude activity (%)	**47.0**	**56.0**	**35.1**	**66.3**	**58.3**	**67.0**
CS ratio (%)	**69.3**	**82.7**	**51.1**	**95.5**	**85.7**	
CO II ratio (%)	**82.9**		**60.0**	**113.9**	**100.9**	
Patient						
Crude activity (%)	**16.2**	**39.2**	**38.4**	**28.0**	**55.3**	**42.2**
CS ratio (%)	**38.0**	**91.8**	**88.6**	**64.0**	**129.0**	
CO II ratio (%)	**40.9**		**93.9**	**68.8**	**136.9**	

CO I, complex I; CO II, complex II; CO III, complex III; CO IV, complex IV; CS, citrate synthase.

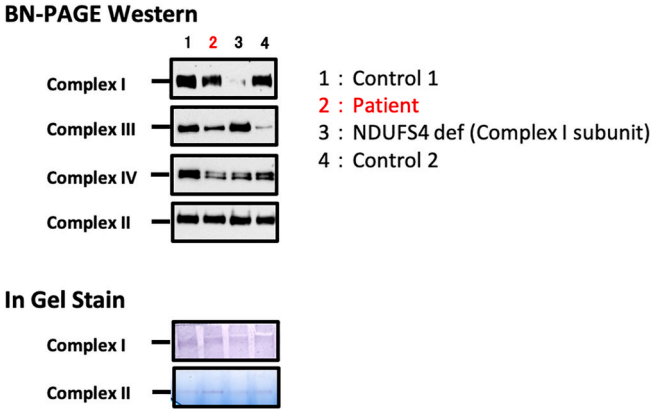

Fig. 3. Analysis with blue native polyacrylamide gel electrophoresis (BN-PAGE) using mitochondria isolated from fibroblasts established from the patient as well as from two healthy controls and a patient with NDUFS4 deficiency (positive control for complex I deficiency). The percentage amount of assembled complex I was estimated by densitometry of three independent BN-PAGE immunoblots using assembled complex II as a control. The mean relative value of two controls was defined as 100%. In gel enzyme staining of each complex was performed as described previously (Van Coster et al., 2001). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

age (Fig. 1). Magnetic resonance spectroscopy (MRS) at 13 months showed an elevation in the peak corresponding to lactate (Fig. 2). From these findings, the patient was suspected to have a mitochondrial respiratory chain disorder. Enzyme activity of mitochondrial respiratory chain complex I in fibroblasts was reduced to 38.0% of the normal control mean relative to citrate synthase (Table 1). Furthermore, blue native polyacrylamide gel electrophoresis (BN-PAGE) showed decreased complex I/II ratio of 46% of two controls, compatible to the enzyme activity. In addition, in gel stain also showed a decrease in complex I while quantification was not performed because of faint staining (Fig. 3). Accordingly, the patient was diagnosed with probable mitochondrial disease based on the diagnostic criteria of respiratory chain disorders proposed by Bernier et al. (2002). The patient began coenzyme Q_{10} and Vitamin B_2 therapy at 24 months. At 2 years of age, whole mitochondrial DNA (mtDNA) sequencings using his blood, fibroblasts and urinary sediments were performed, but no causative mtDNA mutations and deletions were identified. Next, we performed whole exome sequencing, which revealed a missense variant (NM_000489.4: c.736C > T p.Arg246Cys) in *ATRX* in the proband (hemizygous) and in his mother (heterozygous). This missense variant is a known pathogenic variant (Gibbons et al., 2008) and was described as an allelic variant of *ATRX* in OMIM. No other pathogenic or likely pathogenic variants were identified in genes associated with mitochondrial disorders.

This study was approved by the Institutional Review Board in Saitama Medical University (number 482), and written informed consent was obtained from the patient's parents.

3. Discussion

In this report, we identified decreased activity of mitochondrial respiratory chain complex I in a patient with ATR-X syndrome. To our knowledge, this is the first report to indicate a potential link between ATR-X syndrome and mitochondrial respiratory chain dysfunction.

Mitochondrial diseases are characterized by mitochondrial dysfunction (Alston et al., 2017). Because a wide variety of clinical phenotypes and underlying genetic mechanisms are present, biochemical demonstration of mitochondrial dysfunction is required to provide definite diagnosis of mitochondrial disease (Bernier et al., 2002). In our patient, mitochondrial dysfunction was suggested by complex I deficiency in fibroblasts, increased lactate concentration in MRS, and a mild increase in the lactate-to-pyruvate ratio in the cerebrospinal fluid and in the blood. The fact that no causative mtDNA mutations were identified prompted us to consider nuclear DNA abnormalities that result in mitochondrial dysfunction.

Many neurodevelopmental disorders are caused by a mutation in a gene involved in chromatin regulation (Fahrner et al., 2014). *ATRX* is one such gene, and its dysfunction induces improper chromatin remodeling as well as epigenetic dysregulation, which in turn perturbs appropriate expression of many developmental genes (Huidobro et al., 2013). Nevertheless, the ATRX target genes important for specific phenotypes of ATR-X syndrome remain unidentified (Law et al., 2010). Recently, Schenkel et al. (2017) identified a specific epi-signature of differentially hypo- and hyper-methylated genes in patients diagnosed with ATR-X syndrome. Intriguingly, *TFB2M*, which regulates mtDNA transcription and maintenance, is included among those dysregulated genes. Therefore, mitochondrial function may be disrupted in patients with ATR-X syndrome, as in our case.

Whether complex I deficiency in our patient was sufficient to cause his neurological phenotype is not clear. Indeed, the clinical features in our patient were not beyond the spectrum shown in patients with ATR-X syndrome. And the clinical features of our patient did not differ significantly from the ones of the patient previously reported associated with the same mutation. Also, his non-progressive clinical course was atypical for mitochondrial disorders. Therefore, we propose that mild mitochondrial dysfunction may partly underlie clinical features of patients with ATR-X syndrome. Because the mitochondrial dysfunction detected in our patient was not severe and was not detected with regular peripheral blood tests, more studies are required to uncover the significance of mitochondrial involvement in patients with ATR-X syndrome. MRS, which is a non-invasive test, may be useful for this purpose.

Since this is a case report, the causal relationship between the ATRX gene and mitochondrial function cannot be completely proved. However, we believe that ATRX gene mutations affect mitochondrial function because the ATRX gene disrupts the proper expression of many target genes including those involved in the regulation of mtDNA.

In conclusion, we report a patient with ATR-X syndrome who showed mitochondrial dysfunction. These results suggest that mitochondrial dysfunction may partly underlie ATR-X phenotypes and could be a therapeutic target.

CRediT authorship contribution statement

Kaori Aiba: Conceptualization, Investigation, Data curation, Writing – original draft. **Yuji Nakamura:** Resources, Project administration, Writing – review & editing. **Mari Sugimoto:** Resources, Project administration. **Yukiko Yatsuka:** Investigation, Resources. **Yasushi Okazaki:** Investigation, Resources. **Kei Murayama:** Investigation, Resources. **Akira Ohtake:** Investigation, Resources. **Kenji Yokochi:** Project administration, Writing – review & editing, Supervision. **Shinji Saitoh:** Writing – review & editing, Supervision.

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