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Short communication

Alpha-thalassemia X-linked intellectual disability syndrome identified by whole exome sequencing in two boys with white matter changes and developmental retardation

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ABSTRACT

Alpha-thalassemia X-linked intellectual disability (ATRX) syndrome is a genetic syndrome caused by mutation of the *ATRX* gene associated with chromatin remodeling. Recently, a wide spectrum of brain MRI abnormalities and clinical manifestations has been recognized. We describe two male patients with genetically confirmed ATRX syndrome, both presented with developmental delay and white matter changes without typical clinical characteristics of ATRX. Whole-exome sequencing revealed the presence of *ATRX* mutations: a novel c.6472A>G mutation in Case 1 and a previously reported c.6532C>T mutation in Case 2. These two cases expanded the genetic and clinical spectrum of ATRX syndrome, including brain MRI abnormalities. Our results suggest that male patients with developmental delay and widespread white matter changes, even without distinctive facial dysmorphism and hematologic abnormalities, should be suspected as ATRX syndrome. We support the clinical utility of whole-exome sequencing, particularly in ultra-rare neurological diseases with nonspecific developmental disabilities and atypical presentation.

1. Introduction

White matter disease is a clinically and genetically heterogeneous disorder mainly recognized by brain magnetic resonance imaging (MRI) (Kohlschutter and Eichler, 2011). The precise diagnosis and the identification of genetic cause remain as a challenge in many cases with white matter changes. Even comprehensive MRI-based approaches and laboratory findings may not always lead to a precise diagnosis (Schiffmann and van der Knaap, 2009).

Alpha-thalassemia X-linked intellectual disability (ATRX) syndrome is a genetic syndrome caused by mutations in *ATRX*. This condition is characterized by developmental delay, facial dysmorphism, alpha-thalassemia, and genital anomalies. Since the typical case was first described in 1990 (Wilkie et al., 1990), a broad spectrum of atypical clinical manifestations was noted (Pavone et al., 2010; Basehore et al., 2014). Moreover, Wada et al. recently reported various neuroimaging findings of ATRX syndrome, which included white matter abnormalities, as well as nonspecific brain atrophy (Wada et al., 2013).

In this study, we present two cases of genetically confirmed ATRX syndrome that were identified by whole-exome sequencing (WES) of patients presented with nonspecific severe developmental retardation and unexplained white matter changes without typical thalassemia traits.

2. Materials and methods

2.1. Patients

2.1.1. Case 1

A 17-month-old boy was referred to our hospital for global developmental delay. He was born at 40 weeks of gestation by cesarean section because of failure to progress (birth weight = 2.8 kg). He was the second baby of nonconsanguineous Korean parents whose first baby was a normal girl. During the neonatal period, intensive care was provided due to poor oral intake and tachypnea. Thereafter, he received a high-calorie diet via a feeding tube. He showed delayed developmental

Abbreviations: ATRX, alpha-thalassemia X-linked intellectual disability; MRI, magnetic resonance imaging; WES, whole-exome sequencing; FLAIR, fluid-attenuated inversion recovery; CBC, complete blood count; RBC, red blood cell; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction.

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milestones. He could not sit up with assistance and his height, weight, and head circumference were 72 cm (<3 percentile), 7.8 kg (<3 percentile), and 44 cm (<3 percentile), respectively, at 17 months of age. He displayed a high-arched palate, clubfoot deformity, and undescended testes. The muscle tone of the upper extremities was normal, whereas it was increased in the lower extremities. Chromosomal analysis revealed a normal male karyotype (46, XY). Blood tests, including hemoglobin, serum creatine kinase, metabolic screening with blood ammonia, lactate, amino acids, urine organic acids, thyroid function tests, tandem mass screening, arylsulfatase A and β-galactosylcerebrosidase activities, and very long chain fatty acid profiles, were all normal. Abdominal ultrasonography showed bilateral small echogenic kidneys, although he had normal renal function. Echocardiography was unremarkable. Brain MRI showed multiple symmetric deep and subcortical lesions with high signal intensities on T2 and fluid-attenuated inversion recovery (FLAIR) images (Fig. 1A). After the age of 4 years, facial dysmorphisms, such as low-set ears, flat nasal bridge, microophthalmia, hypertelorism, and epicanthic fold, were recognized (Fig. 2). He started to walk a few steps without assistance at the age of 6 years.

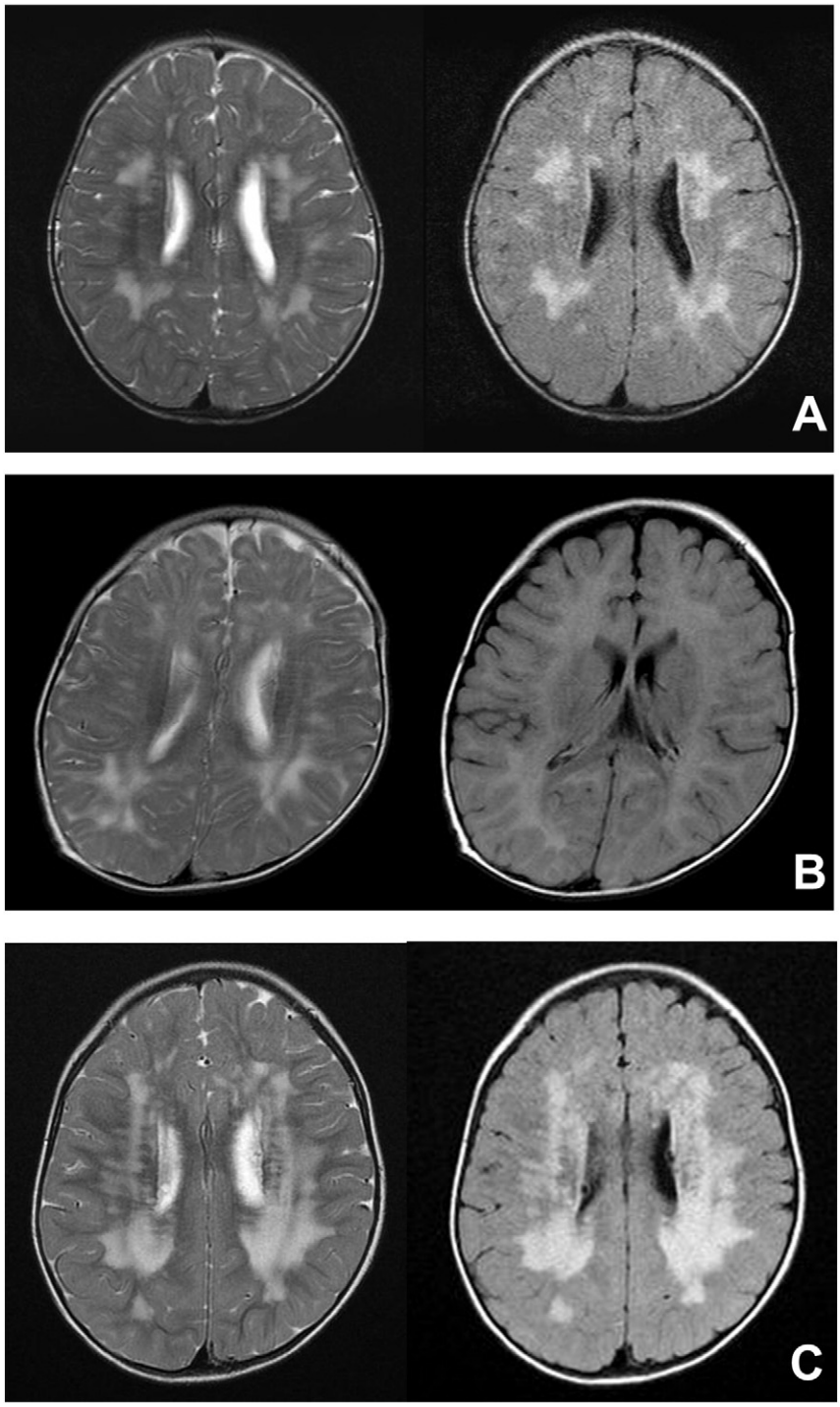

Fig. 1. White matter abnormalities on brain MRI in two patients with ATRX syndrome. [A] T2 and FLAIR axial images of Case 1 showing multiple symmetric deep and subcortical white matter lesions with high signal intensity. [B] T2 and FLAIR axial images of Case 2 at the age of 9 months showing widespread high signal intensity lesions in bilateral deep white matter. [C] Follow-up MRI of Case 2 performed at the age of 29 months showing increased extent of high signal lesions in white matter.

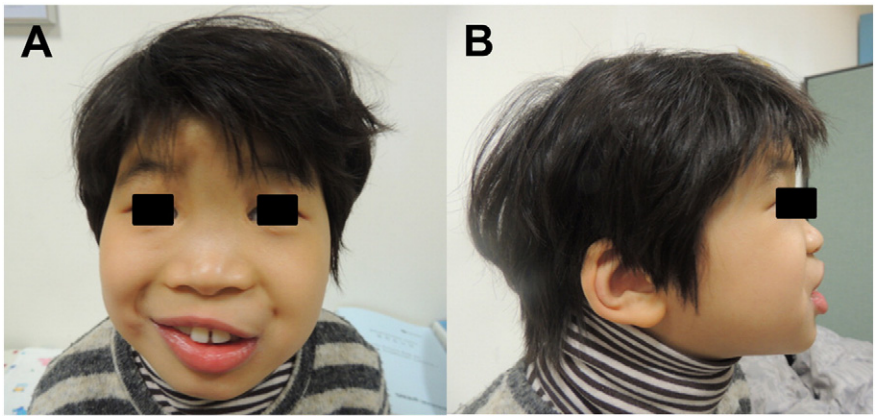

Fig. 2. (A, B) Facial features of Case 1 at the age of 9 years. Low-set ears, flat nasal bridge, micro-ophthalmia, hypertelorism, and epicanthic folds were noted. Consent of the parents for publishing the photographs was obtained.

He is currently 10 years old. His height, weight, and head circumference are 113.5 cm (<3 percentile), 16.8 kg (<3 percentile), and 48 cm (<3 percentile), respectively. He can walk, but is unstable and has severe intellectual disability, being able to say only a few single words. Hemoglobin H inclusion bodies were observed in 3 per 1000 RBCs after brilliant cresyl blue staining, and complete blood count (CBC) revealed a white blood cell count of 8350/μL, a red blood cell count of 4.44×10^6/μL, a hemoglobin level of 10.8 g/dL, hematocrit of 33.0%, a mean corpuscular volume of 74.3 fL, a mean corpuscular hemoglobin level of 24.3 pg, a mean corpuscular hemoglobin concentration of 32.7 g/dL, a red cell distribution width of 17.6%, and a platelet count of 423×10^3/μL.

2.1.2. Case 2

A 9-month-old boy was referred to our hospital for global developmental delay. He was born at 40 weeks of gestation by cesarean section (birth weight = 2.5 kg). There were no perinatal problems, although oligohydramnios was observed on prenatal ultrasonography. He was the first baby of nonconsanguineous Korean parents without a family history of intellectual disability. At 5 months of age, he could not control his head completely. At the age of 9 months, he could sit with assistance and reach out to get objects; however, he was still unable to roll over. He suffered from aspiration during feeding. At the age of 9 months, his head circumference was 40.5 cm (<3 percentile). He exhibited a high-arched palate and undescended testes. His muscle tone was hypotonic and his deep tendon reflexes were decreased. There were no abnormalities on CBC tests and serum creatine kinase level. He had a normal male karyotype (46, XY) and no abnormalities in a metabolic screening test. Abdominal ultrasonography showed no abnormalities. His brain MRI revealed increased T2 signal intensities in bilateral deep white matter (Fig. 1B). Follow-up MRI performed at the age of 29 months showed deterioration of white matter abnormalities (Fig. 1C), although at that time, he started to walk without assistance and speak a few meaningful words.

2.2. Genetic analysis

Blood samples were obtained from the two patients, whose parents provided informed consent. WES was performed on the two unrelated patients. Genetic variations were validated using Sanger sequencing.

2.2.1. Whole-exome sequencing

Capturing genomic DNA, next-generation sequencing for WES and data processing was performed as previously reported (Seo et al., 2015). Among the called variants, common variants that are listed in public databases (dbSNP build 137; 1000 Genomes Project release 10.31.2012; NHLBI Exome Sequencing Project) were excluded, and only rare variants were considered as potential causative variants. The identified variants were annotated based on novelty, impact on the encoded protein, evolutionary conservation, and expression using an automated pipeline. PhyloP score and amino acid conservation were extracted from the UCSC genome annotation database (Pollard et al., 2010).

2.2.2. Orthologs

The proteins encoded by orthologs of *ATRX* in vertebrate species were identified by a BLAST search (Choi et al., 2011). GenBank accession numbers for these proteins included NP_000480 (*Homo sapiens*), NP_033556 (*Mus musculus*), XP_002720269.1 (*Oryctolagus cuniculus*), XP_005200807.1 (*Bos taurus*), XP_004940854.1 (*Gallus gallus*), NP_956947.2 (*Danio rerio*), and XP_002119349.1 (*Ciona intestinalis*). Multiple sequences were aligned using ClustalW2 software (Larkin et al., 2007).

2.2.3. Sanger sequencing

PCR amplification was performed with 10 pmol of each specific primer (Supplementary Table 1 online). The PCR conditions used are: 95 °C for 3 min, followed by 35 cycles (95 °C for 30 s, 59 °C for 30 s and 72 °C for 40 s) and a final extension at 72 °C for 5 min. Sanger sequencing reactions were run on an ABI-3730XL DNA Analyzer (Applied Biosystems).

3. Results

The exome run quality is shown in Supplementary Table 2 online. Five and two potentially disease-associated variants were identified as potentially causative genes by exome sequencing of Cases 1 and 2, respectively (Table 1). All the variants were confirmed by Sanger sequencing using specific primer pairs (see Supplementary Table 1 online). Among these six genes, *ATRX* variants were found from both of the patients and most likely to be pathogenic based on the known disease-association of the gene and functional assessment of the variants. Other five genes are less relevant to the clinical features of our patients, and their variants were located in residues with weaker evolutionary conservation and received less damaging scores compared to the *ATRX* variants (Table 1). Among the *ATRX* variants, the missense variant c.6472A>G (p.K2158E) was novel (Fig. 3A), whereas the missense variant c.6532C>T (p.R2178W) (Fig. 3B) was reported previously as a causative mutation of ATRX syndrome from Chinese patients, suggesting East Asian-specific presence of the variant (Badens et al., 2006). Both of the variants were not listed in the Exome Aggregation Consortium containing about 65,000 healthy individuals (ExAC, release 0.3, http://exac.broadinstitute.org). The novel variant in *ATRX* was inherited from the unaffected mother. Parental blood samples were

Table 1
Notable genetic variants in recessive model.

Case	Gene	Chromosome: position (hg19)	Nucleotide substitution	Zygosity	Coverage (ref.cov./ total cov.)	Impact on protein sequence	Amino acid change	Amino acid location/protein length	Number of species different from human/number of species with ortholog	PhyloP	SIFT	PolyPhen-2
1	*ALDH3B1*	11:67789295	G>C	Homozygous	0/25	Missense	p.R302P	302/468	25/88	1.074	0.06	0.893
1	*ATRX*	X:76814172	T>C	Hemizygous	0/74	Missense	p.K2158E	2158/2492	1/98	7.698	0.00	0.997
1	*GPRASP1*	X:101909416	T>C	Hemizygous	0/14	Missense	p.F192S	192/1395	6/52	1.099	0.51	0.999
1	*MED14*	X:40556377	T>C	Hemizygous	0/70	Missense	p.S517G	517/1454	28/98	4.689	0.33	0.167
1	*SMS*	X:21996141	C>T	Hemizygous	0/79	Missense	p.T190I	190/366	27/98	3.019	0.04	0.788
2	*ARHGAP6*	X:11196337	C>G	Hemizygous	0/21	Missense	p.L504F	504/974	12/95	−0.020	0.00	0.999
2	*ATRX*	X:76813089	G>A	Hemizygous	0/65	Missense	p.R2178W	2178/2492	0/97	4.160	0.00	1.000

Cov, coverage; ref, reference.

not available in Case 2. The two missense variants are located in the helicase C-terminal domain of ATRX and the altered amino acid residues are highly conserved across vertebrate species (Fig. 3C). In addition, the amino acid alterations were predicted to be damaging by the PolyPhen-2 and Sorting Intolerant From Tolerant (SIFT) programs (Table 1).

4. Discussion

ATRX syndrome, a kind of X-linked intellectual disability syndromes, is caused by *ATRX* mutations. ATRX is associated with chromatin remodeling and contains two important functional domains: the ATRX–DNMT3–DNMT3L (ADD) domain and the helicase domain; most *ATRX* mutations are clustered in the two domains (Badens et al., 2006; Gibbons et al., 2008). Affected male patients typically display developmental delay, intellectual disability, characteristic facial dysmorphism during infancy, alpha-thalassemia, and genital abnormalities. Other findings include minor skeletal abnormalities, microcephaly, short stature, gastroesophageal reflux, seizures, cardiac defects, and renal/urinary abnormalities. These clinical features might result from the abnormally regulated expression of many genes, as downregulation of alpha-globin expression by the mutated ATRX protein might result in alpha-thalassemia (Clynes and Gibbons, 2013; Ratnakumar and Bernstein, 2013).

ATRX plays a role in the normal development and organization of the brain. It interacts with MECP2 and cohesion, thereby co-regulating the expression of a subset of imprinted genes in the mouse brain (Kernohan et al., 2010). Reduced expression of the gene might cause hippocampal dysfunction in mice (Nogami et al., 2011). It has been known that the brain MRI in ATRX syndrome does not generally reveal remarkable findings, except for mild atrophy. However, a wide spectrum of brain MRI abnormalities has been recently recognized in this condition, including white matter abnormalities and delayed

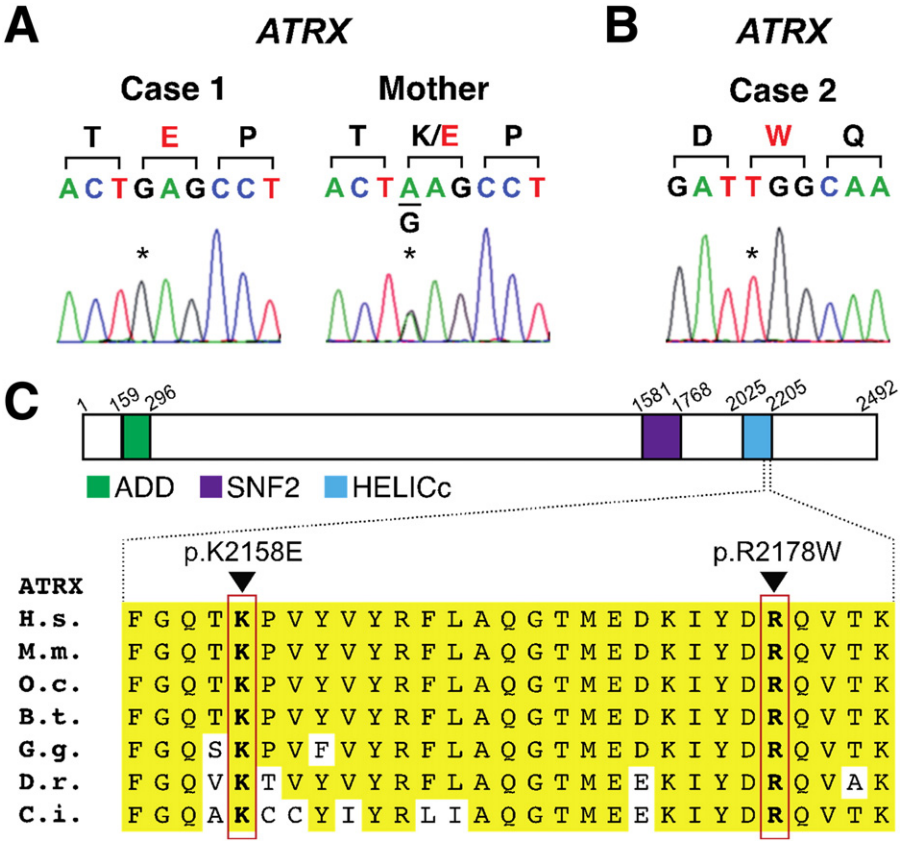

Fig. 3. *ATRX* variants. [A] Direct sequencing of *ATRX* in Case 1 confirmed a novel mutation, c.6472A>G (p.K2158E), which was inherited from the unaffected mother. [B] A reported mutation, c.6532C>T (p.R2178W), was detected in Case 2. [C] Multiple amino acid sequence alignments across vertebrate and invertebrate species display strong conservation around the variants. ADD, ATRX–DNMT3–DNMT3L domain; SNF2, SNF2-related domain; HELICc, Helicase C-terminal domain. H.s., *Homo sapiens*; M.m., *Mus musculus*; O.c., *Oryctolagus cuniculus*; B.t., *Bos taurus*; G.g., *Gallus gallus*; D.r., *Danio rerio*; C.i., *Ciona intestinalis*.

myelination, as well as nonspecific brain atrophy (Wada et al., 2013). Additionally white matter lesions usually seem to be localized around the trigones, rather than widespread and scattered (Wada et al., 2013). Our index cases showed remarkable widespread white matter changes on brain MRI. These findings supported the previous suggestion that ATRX is involved in normal myelination, or the mutated protein affects the expression of other proteins during myelination (Wada et al., 2013). Therefore, we emphasize the importance of brain MRI findings of unexplained white matter changes to suspect and establish a diagnosis of ATRX syndrome, as Wada et al. commented that they are very important and should be included in the diagnostic criteria of ATRX syndrome (Wada et al., 2013).

Upon identification of the presumably causative mutations, the patients were examined further to correlate with known features of ATRX syndrome. Alpha-thalassemia is considered as one of its essential features, albeit 10%–20% of patients have no hematologic abnormalities (Stevenson, 1993–2014). Hematologic screening of Case 1 revealed rare hemoglobin H inclusions (0.3%) and mild anemia. The characteristic facial features were not recognized until 4 years of age for Case 1. Case 2 had no distinct facial features, with the exception of a high-arched palate. Neither of our patients suffered clinical seizures. They were able to walk without assistance later in the course of the disease, although they displayed delayed motor development and cognitive impairment. Therefore, it may not be straightforward to suspect ATRX syndrome in cases with such atypical presentations, even after serial work-up to establish a diagnosis and identify disease etiology based on careful history taking, physical/neurological examination, and radiological findings.

The two missense mutations in *ATRX* are located in the helicase C-terminal domain and expected to alter protein function. The white matter abnormalities detected on brain MRI, as well as the milder clinical features of these patients, might be associated with the location of the mutations (Badens et al., 2006; Wada et al., 2013). Considering the milder phenotypes or various radiological findings of the cases, there might remain undiagnosed cases of ATRX syndrome, although the disease is rare and only single case with thalassemia has been reported in Korea to date (Yun et al., 2011).

In conclusion, these two cases expanded the genetic and clinical spectrum of *ATRX* mutation, including the spectrum of brain MRI abnormalities in ATRX syndrome. We propose that in cases of male patients with intellectual disabilities and abnormal signals in the white matter on brain MRI, ATRX syndrome should be considered even without distinctive facial dysmorphism and/or hematologic abnormalities. This study also supports the clinical utility of WES in the diagnosis of atypically presented cases of ultra-rare diseases under careful interpretation.

Author contributions

JSL, SL, MC and JHC wrote the manuscript. JSL and SL performed experiments and collected clinical data. BCL, KJK and YSH collected and interpreted clinical data and revised the manuscript. SL and MC performed genetic analysis. JHC and MC mentored JSL and SL through the process as a correspondence.

Declaration of conflicting interests

The authors declare no conflicts of interest with respect to the research, authorship, or publication of this article.

Ethical approval

This study was approved by the local institution review board.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2015.04.075.

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