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Biallelic loss-of-function *UBA5* mutations in a patient with intractable West syndrome and profound failure to thrive

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ABSTRACT – Mutation of the gene encoding ubiquitin-like modifier-activating enzyme 5 (*UBA5*) causes autosomal recessive early-onset epileptic encephalopathy. UBA5 acts as an E1-activating enzyme in the ubiquitin-fold modifier 1 pathway, which is important for unfolded protein elimination and regulation of apoptosis, and has been linked to human diseases. We identified biallelic mutations in *UBA5* in a Japanese boy with intractable West syndrome, profound failure to thrive, and severe cerebral and cerebellar atrophy. The boy presented with epileptic spasms and hypsarrhythmia at the age of three months. He was diagnosed with West syndrome, however, treatments with adrenocorticotropic hormone and several antiepileptic drugs were ineffective. MRI findings were initially normal, but subsequently showed a progression of cerebellar and cerebral atrophy. By the age of seven years, he had not achieved any developmental milestones; he had daily epileptic spasms and tonic seizures and profound failure to thrive. Gene analysis revealed novel compound heterozygous mutations in *UBA5*; a microdeletion encompassing the entire *UBA5* gene and a putative disease-causing missense mutation in the catalytic domain. These biallelic variants may have caused loss of function, accounting for the observed clinical symptoms. Intractable infantile epileptic spasms, failure to thrive, and severe neurological impairment may be characteristic of patients with *UBA5* mutations.

Key words: cerebellar atrophy, epileptic spasm, gene analysis, infantile spasm, ufmylation

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Ufmylation is a type of post-translational protein modification that resembles ubiquination and involves the addition of ubiquitine-fold modifier 1 (UFM1) to target proteins. The E1-activating enzyme, ubiquitin-like modifier-activating enzyme 5 (UBA5), together with E2-conjugating enzyme and E3 ligase, mediate this transfer. These enzymes, which are part of the ubiquitine proteasome system, operate in the endoplasmic reticulum to regulate unfolded protein elimination and apoptosis (Colin *et al*., 2016; Muona *et al*., 2016).

West syndrome is an epileptic encephalopathy characterized by clustered epileptic spasms, a hypsarrhythmia pattern on EEG, and developmental delay. The causes of the disease vary and include brain malformations, metabolic defects, infections, and genetic abnormalities. Recent whole-exome sequencing (WES) studies have revealed that West syndrome is associated with heterogenous genetic backgrounds that are enriched with *de novo* variants of candidate genes including *TBL1XR1, WDR45,* and *GABRA1* (Saitsu *et al*., 2014; Kodera *et al*., 2016; Nakashima *et al*., 2016). Meanwhile, biallelic variants of other candidate genes also cause West syndrome. Based on recent studies, biallelic variants of *UBA5* were found in patients with early-onset epileptic encephalopathies including West syndrome (Colin *et al*., 2016; Duan *et al*., 2016; Muona *et al*., 2016); patients presented with intractable seizures, severe intellectual disability, movement disorders, and acquired microcephaly. Here, we present a patient with West syndrome with severe clinical symptoms and biallelic *UBA5* mutations.

Case study

The patient was a Japanese boy, born at 40 weeks by normal delivery. His weight at birth was 2.802 kg (-1.2 standard deviation [SD]), his height 48.0 cm (-0.8 SD), and his head circumference 33.0 cm (-0.3 SD). The patient had no family history of epilepsy or any neurological disorders. Until the age of three months, he was bottle-fed and showed no eye pursuit or social smiling. At that time, he started having epileptic spasms, which appeared as clusters, five to six times daily. Interictal EEG demonstrated hypsarrhythmia, however, initial MRI was normal and the boy had no signs of metabolic defects. Because of his developmental delay prior to seizure onset, he was diagnosed with symptomatic West syndrome. At the age of four months, he was treated with adrenocorticotropic hormone (ACTH), which decreased the frequency of his spasms to one per day. Nevertheless, after the ACTH therapy, he presented with rigidity and evident gastro-oesophageal reflux. His oral intake decreased and he thus started to receive nasoenteric feeding. Thereafter, the frequency of spasms, which were resistant to valproate, topiramate, and clobazam, gradually increased. He had focal motor seizures at four years of age. By seven years of age, he had not gained any developmental milestones and showed spastic quadriplegia. At that age, his height was 90 cm (-6.3 SD), his body weight 6.4 kg (-4.0 SD), and his head circumference 43 cm (<3rd percentile). He showed a profound failure to thrive, no facial expressions, micrognathia, and tapering fingers (*figure 1A*), and experienced epileptic spasms and focal motor seizures on a daily basis. His interictal EEG exhibited frequent 2-3-Hz spikes and waves in the occipital region, as well as some sharp waves in the frontal and fronto-polar region. His MRI showed severe atrophy in the cerebrum and cerebellum *(figure 1B*).

Genetic analyses

The study was approved by the respective Institutional Review Boards of Yokohama City University School of Medicine and Showa University School of Medicine. We performed WES analysis for the patient, as previously described (Mizuguchi *et al*., 2017), and searched for possible pathogenic single nucleotide variants (SNVs) and small indels. We also examined copy number variants (CNVs) using two algorithms: the eXome-Hidden Markov Model (XHMM) (Fromer and Purcell, 2014) and the program based on relative depth of coverage ratios developed by Nord *et al*. (2011). We detected a microdeletion of about 3.2 Mb within the 3q22.1 region encompassing *UBA5* (Chr3:129762317-132948291) (*figure 1C; left*). We confirmed that this deletion was inherited from the father using quantitative-polymerase chain reaction methods (*figure 1C; right*). In addition, we identified a hemizygous missense variant, c.214C>T: (p.Arg72Cys), in *UBA5* (NM_024818.3). Sanger sequencing confirmed that this variant was inherited from the mother (*figure 1D*). Allele frequency of this missense variant was 0.0003472 in the east Asian population and 0.0000413 in the total population, according to the ExAC database (http://exac.broadinstitute.org). The missense variant, c.214C>T (p.Arg72Cys), was predicted to be disease-causing based on SIFT, PolyPhen2, and Mutation Taster (*supplementary table 1*). These results indicated that this patient had compound heterozygous variants of *UBA5*.

Discussion

In this study, we detected biallelic variants of *UBA5* in a Japanese boy with intractable West syndrome, profound failure to thrive, and severe neurological impairments, presumably due to severe cerebral and cerebellar atrophy. In recent studies, 18 patients with early-onset encephalopathy and biallelic variants of

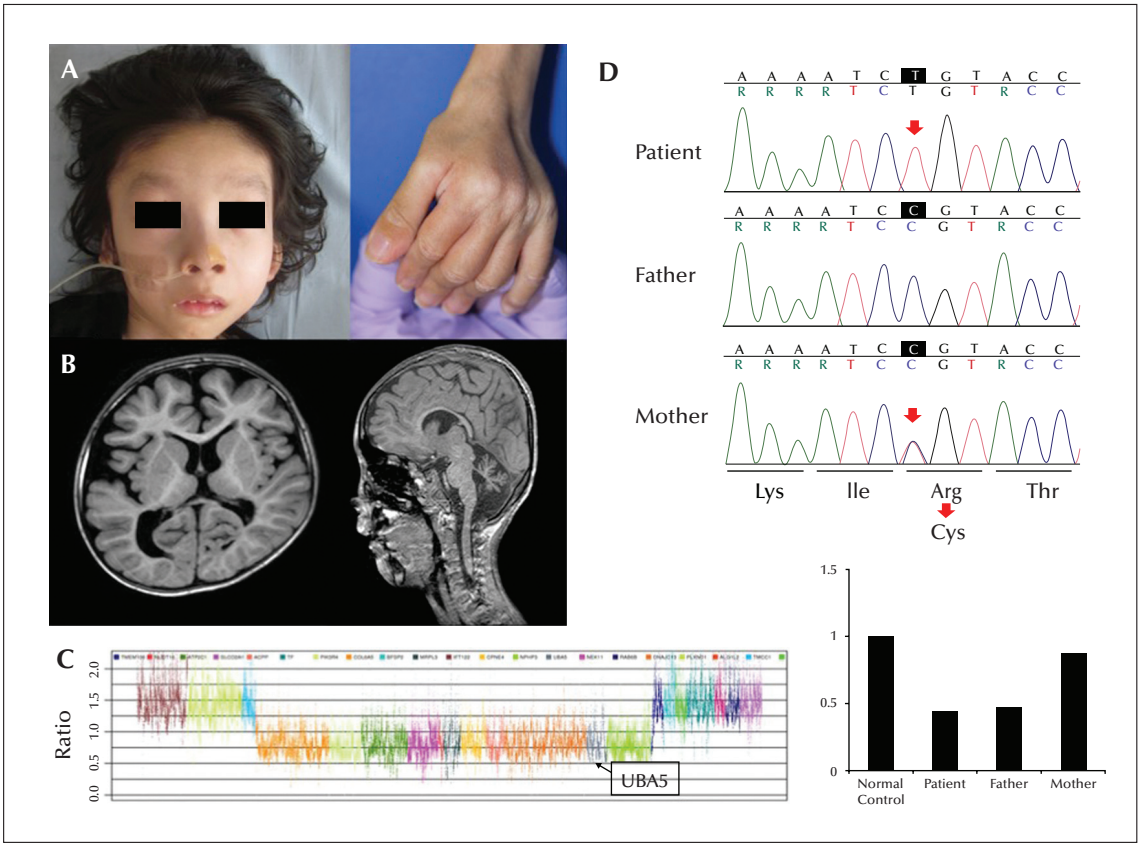

Figure 1. Dysmorphic features, MRI, and genetic analyses. (A) Images of the patient at seven years of age showing his expressionless face, micrognathia, and hands with tapering fingers. (B) T1-weighted MRI showing corpus callosum thinning, cerebellar atrophy, ventricular dilation, and white matter hyperintensities in periventricular regions. (C) Left: identification of a microdeletion in chromosomal band 3q22.1, encompassing the entire *UBA5* gene (3q22.1; Chr3:129762317-132948291; 3.2-Mb deletion); right: quantitative polymerase chain reaction confirmed that this deletion was inherited from the father. (D) Sequencing results showing the missense mutation, c.214C>T (p.Arg72Cys), present in the patient and his mother, but not his father.

UBA5 have been described (Colin *et al*., 2016; Duan *et al*., 2016; Muona *et al*., 2016; Arnadottir *et al*., 2017). These patients showed diverse clinical presentations, including hypotonia, spasticity, movement disorders, dysmorphic features, epilepsy, intellectual disabilities, vision defects, microcephaly, and failure to thrive. Their MRI displayed cerebral atrophy and cerebellar atrophy, as well as delayed myelination. Collin *et al*. reported five patients with *UBA5* variants and early-onset encephalopathy; in their report, intellectual disability, severe motor skill disability, microcephaly, and failure to thrive were observed in all cases (*table 1*). These features are consistent with the findings of our patient. The height of our patient was -6.3 SD and his weight was -4.0 SD by the age of seven years, and his failure to thrive was more severe than previously reported for patients with *UBA5* mutations. In addition, the patient had dysmorphic features such as expressionless face, micrognathia, and tapering fingers which have been previously reported for two patients (Muona *et al*., 2016). Consistent with our findings, 14 of the 18 patients reported with early-onset encephalopathy and *UBA5* biallelic variants also presented with mild to severe cerebral atrophy and cerebellar atrophy (cerebrum-limited atrophy in four patients and cerebellum-limited atrophy in six patients) (Arnadottir *et al*., 2017; Colin *et al*., 2016; Duan *et al*., 2016; Muona *et al*., 2016). West syndrome or infantile spasms were reported in eight patients; five were described in detail and showed poor response to treatment (Arnadottir *et al*., 2017; Colin *et al*., 2016; Muona *et al*., 2016). None of the treatments, including therapy with ACTH and several antiepileptic drugs, were effective for our patient.

Previous biochemical and biological analyses have shown that heterozygosity of hypomorphic and loss-of-function (LOF) mutations in *UBA5* result in a severe form of early-onset encephalopathy (Colin *et al*., 2016; Muona *et al*., 2016; Arnadottir *et al*., 2017). Moreover, two LOF mutations were shown to be probably

Table 1. Clinical features of the current and previously reported patients with mutations in *UBA5*.

	Arnadottir *et al.*, 2017		**Colin *et al.*, 2016**					**Present case**
Case	1	2	1	2	3	4	5	
Sex	F	F	M	M	F	F	F	M
Mutation	Missense c.1111G>A		Missense c.1111G>A	Missense c.1111G>A	Missense c.1111G>A	Missense c.778G>A	Missense c.169A>G	Missense c.214C>T
	Splice site c.684G>A		Nonsense c.904C>T	Nonsense c.904C>T	Frameshift c.971_972insC	Missense c.1165G>T	Missense c.503G>A	Deletion 3.2 Mb
Seizure type (onset)	ES (6mo)	ES (6mo)	ES (3mo)	ES (5mo)	ES (3mo)	-	-	ES (3mo)
Hypotonia	+	+	+	+	+	+	+	-
Spasticity	+	+	+	+	-	-	+	+
Movement disorder	NA	NA	+	-	-	+	+	-
Intellectual disability	+	+	+	+	+	+	+	+
Motor skill disability	+	+	+	+	+	+	+	+
Microcephaly	+	+	+	+	+	+	+	+
Failure to thrive	+	+	+	+	+	+	+	+

ES: epileptic spasms; F: female; M: male; mo: months; NA: Not available.

embryonic lethal in a mouse model (Tatsumi *et al*., 2011). Central nervous system specific knock-out mice for Ufm1 exhibit microcephaly and die within the first day of birth (Muona *et al*., 2016). In our patient, the 3q22.1 microdeletion and missense variant were observed as *trans* alleles. Microdeletion encompassing the whole *UBA5* gene lead to a deficit of *UBA5* expression. On the other hand, the pathogenicity of the c.214C>T:(p.Arg72Cys) variant is still uncertain. A previous study indicated that the amino acid changes located within the catalytic domain (aa.57-329) would cause drastic reduction in catalytic activity (Colin *et al*., 2016). The Arg72Cys variant is likely to be a LOF variant, however, further functional investigation is needed to clarify the pathogenicity of this missense variant. As a consequence of biallelic LOF mutations, the patient would be expected to present with severe clinical presentation.

In conclusion, we present a patient with a severe form of *UBA5* mutation-associated encephalopathy, manifesting with intractable West syndrome, profound failure to thrive, severe cerebral and cerebellar atrophy, and severe neurological impairment. A complex of intractable West syndrome, failure to thrive, postnatal microcephaly with cerebral and cerebellar atrophy, and dysmorphic features may be the characteristics of patients with *UBA5* variants. Our patient was shown to have an SNV and CNV as *trans* alleles and both variants were detected using WES data, indicating that WES is a powerful tool for comprehensive genetic analysis.

Supplementary data.

Supplementary table is available on the www.epilepticdisorders.com website.

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None of the authors have any conflict of interest to declare.

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TEST YOURSELF

(1) How is *UBA5* mutation-associated encephalopathy inherited?

(2) What does the *UBA5* gene encode?

(3) What are the characteristic features of patients with biallelic *UBA5* mutations?

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com, under the section "The EpiCentre".