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Original article

Loss-of-function mutations of *STXBP1* in patients with epileptic encephalopathy

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Abstract

Epileptic encephalopathy, which commences during early infancy, is a severe epileptic syndrome that manifests as age-dependent seizures and severe developmental delay. The syntaxin-binding protein 1 gene (*STXBP1*) is one of the genes responsible for epileptic encephalopathy. We conducted a cohort study to analyze *STXBP1* in 42 patients with epileptic encephalopathy. We identified four novel mutations: two splicing mutations, a frameshift mutation, and a nonsense mutation. All of these mutations were predicted to cause loss-of-function. This result suggests loss-of-function is a common mechanism underlying *STXBP1*-related epileptic encephalopathy. The four patients showed epileptic features consistent with *STXBP1*-related epileptic encephalopathy, but showed variable radiological findings, including brain volume loss and myelination delay.

Keywords: *STXBP1*-related epileptic encephalopathy; Ohtahara syndrome; West syndrome; Loss-of-function

1. Introduction

Epileptic encephalopathy, which commences during early infancy, is a severe epileptic syndrome that manifests as age-dependent seizures and severe developmental delay [1]. Epileptic encephalopathy occurring in the first few months of life is recognized as Ohtahara syndrome, otherwise known as early infantile epileptic encephalopathy with suppression-burst (EIEE) [2]. Seizure patterns of Ohtahara syndrome often change into those of West syndrome in the late infantile period. Those are representative of infantile-onset catastrophic epilepsies, causing marked deterioration of psychomotor development [3]. Recently, with the advance of molecular technologies, many genes responsible for this clinical syndrome have been identified [4]. The syntaxin-binding protein 1 gene (*STXBP1*) located on chromosome 9q34.11 is one of these genes.

In 2008, Saitsu et al. identified *de novo* *STXBP1* mutations in five patients with Ohtahara syndrome [5]. Subsequently, we identified novel *STXBP1* mutations associated with Ohtahara syndrome and West syndrome in two unrelated patients [6]. Thereafter, many *STXBP1* mutations have been reported [7–19]. Here, we report on the new results of our ongoing study of *STXBP1* in patients with epileptic encephalopathy.

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2. Materials and methods

This study was performed in accordance with an approval from the ethics committee of Tokyo Women's Medical University. Following our first *STXBP1* analysis [6], 42 patients with epileptic encephalopathy were recruited for this study. After obtaining written informed consent from the patients' families, blood samples were obtained from the patients and their parents. DNA was extracted from the blood samples using a QIAamp DNA extraction kit (Qiagen, Hilden, Germany). In cases with *STXBP1* mutations, parents' samples were analyzed to determine whether these were *de novo* mutations. Whole genome copy numbers were analyzed using SurePrint G3 Human CGH Microarray Kit 60K (Agilent Technologies, Santa Clara, CA), and all coding exons of *CDKL5* were analyzed as described previously [20]. Then, the patients who showed no genomic copy number aberrations and no *CDKL5* mutations were enrolled in this study. Nucleotide sequences of all 20 coding exons and intronic sequences adjacent to the splice junctions (60 intronic bases on average) were analyzed by Sanger sequencing as described previously [6]. The corresponding reference sequence numbers in this study are NG_016623.1 for the genome, NM_003165.3 for the mRNA, and NP_003156.1 for the protein (http://www.ncbi.nlm.nih.gov/).

3. Results

3.1. STXBP1 nucleotide alterations

We identified novel *STXBP1* nucleotide alterations in four unrelated patients (Table 1).

Patient 1 showed a 16-bp deletion in the splicing acceptor site of intron 3, c.170-17_170-2delGTTGTTTTGTTGTCTA (Fig. 1A), which is predicted to cause a splicing error. Patient 2 showed a G-to-A substitution in the splice donor site of intron 8, c.663+1G>A (Fig. 1B), which is also predicted to cause a splicing error. Patient 3 showed a nonsense mutation in exon 15, c.1303G>T (p.Glu435*) (Fig. 1C). Patient 4 showed a frame-shift mutation in exon 15, c.1347delC (p.Ile450Serfs*96) (Fig. 1D). These were determined to be *de novo* origins due to their absence in both parents.

3.2. Patients' reports

Patient 1 is an 8-year-old boy born at 28 weeks of gestation with asphyxia. His Apgar scores were 2 and 9 at 1 and 5 min, respectively. His birth weight was 1040 g (10th–50th centile). At the age of 3 months, he started to show partial seizures. His seizure patterns then changed into epileptic spasms, indicating West syndrome. Electroencephalography (EEG) patterns showed a continuous hypsarrhythmic pattern. Brain magnetic resonance imaging (MRI) examined at the age of 2 years and 9 months showed brain volume loss (Fig. 2A and B). At present, his height is 125 cm (50th centile), his weight is 18.7 kg (<3rd centile), and his occipitofrontal circumference (OFC) is 50.9 cm (3rd–10th centile). He still cannot control his head and cannot pursuit objects. He is aphasic and bedridden. Oral intake is impossible and tube feeding is required. Several series of spasms are observed every day. Neurological evaluation does not show any pyramidal signs and brain MRI does not show evidence of periventricular leukomalacia. Thus, we considered his

Table 1
Summary of the patients' information.

	Patient 1	Patient 2	Patient 3	Patient 4
General information				
Present age	8 years	4 years	3 years	2 years and 6 months
Gender	Male	Male	Female	Female
Epilepsy				
Age of initial seizure	3 months	2 days s/o	1 month	10 days
Finding of EEG	Hypsarrhythmia	Suppression-burst	Hypsarrhythmia	Hypsarrhythmia
Development				
Communication ability	None	None	None	None
Motor ability	Bedridden	Incomplete head control	Turning over and sitting	Bedridden
Others	Tube feeding			
Radiological features				
MRI finding	Volume loss	Volume loss	Hypomyelination	Volume loss
Molecular diagnosis				
Mutation stype	Splicing error	Splicing error	Nonsense	Frameshift
Nucleotide change	c.170-17_170-2delGTTGTTTTGTTGTCTA	c.663+1G>A	c.1303G>T	c.1347del
Amino-acid change	NA	NA	p.E435*	p.I450Sfs*96
Inheritance	*De novo*	*De novo*	*De novo*	*De novo*

s/o, suspect of; EEG, electroencephalography; MRI, magnetic resonance imaging; NA, not available.