

Clinical and Genetic Spectrum of *STXBP1* Encephalopathy in the Korean Pediatric Population

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Purpose: Syntaxin-binding protein 1 (*STXBP1*) mutations are known to result in various phenotypes including Ohtahara syndrome, West syndrome, and autism, collectively referred as *STXBP1* encephalopathy. This study aimed to expand our understanding of the genotype-phenotype spectrum of *STXBP1* encephalopathy in the Korean pediatric population.

Methods: Ten patients with *STXBP1* mutations were enrolled for a retrospective chart review. The patients were investigated for developmental delay of unknown cause and epileptic encephalopathy at a single center.

Results: Ten different *STXBP1* mutations were identified. Three mutations had not previously been reported (c.1212A>C, c.1497C>G, c.1030-2A>G). Eight patients showed early-onset epileptic encephalopathy as the main feature, while the main feature was developmental delay and non-epileptic movements in two patients. The most commonly seen electroencephalographic change was focal/ multifocal epileptiform discharges, which were observed in nine patients (90%). The classical burst-suppression pattern was observed in four patients, two of which evolved to show hypsarrhythmia. All patients with seizures had drug-resistant epilepsy. The patients suffered from severe developmental delay regardless of seizure frequency. Six patients showed an associated movement disorder or behavioral disorder.

Conclusion: This study describes the *STXBP1* encephalopathy patients in Korean pediatric population, further expanding knowledge of its phenotype spectrum.

Keywords: *STXBP1* protein, human; Pediatrics; Epilepsy; Developmental disabilities

Introduction

Developmental and epileptic encephalopathy is a concept to ac-

knowledge that many genetic disorders show developmental impairment as a direct consequence of the genetic mutation, in addition to the detrimental effect of the frequent epileptic activity on

brain development [1]. In the era of next generation sequencing, increasing monogenic causes for developmental and epileptic encephalopathy are being discovered, providing us with new insight to its underlying patho-genetic mechanisms.

Syntaxin-binding protein 1 (STXBP1) (also known as MUNC18-1) is a member of the membrane trafficking proteins predominantly expressed in the brain, which is important for docking and fusion of the synaptic vesicles [2]. There is approximately 200 cases reported in the literature until date, mostly being *de novo* heterozygous mutations [3]. *STXBP1* mutation was first described in 2008, in patients with early infantile epileptic encephalopathy with suppression-burst (EIEE), or Ohtahara syndrome [4]. Subsequent studies showed that *STXBP1* mutation is responsible for approximately 22% of cases with Ohtahara syndrome, 6% of non-syndromic early onset epileptic encephalopathy and 2% of West syndrome [4-7]. Due to the expanding phenotype *STXBP1* encephalopathy was suggested to be more appropriate [8].

Recognition and classification of diverse phenotypes arising in *STXBP1* mutation is crucial to guide future management options. Here, we report 10 patients with *STXBP1* mutation from a single center and summarize the detailed clinical features, with the aim to expand its phenotypic spectrum in Korean pediatric population.

Materials and Methods

Patients with *STXBP1* mutation were identified from a cohort of 198 pediatric patients with developmental and epileptic encephalopathy of unknown etiology. Patient cohort was selected from the Division of Pediatric Neurology of the Seoul National University Children's Hospital from September 2012 to May 2019. All the patients had no obvious etiology based on clinical features, neuroimaging and metabolic screening. The first-tier test included chromosomal microarray or targeted multi-gene panel. Whole exome sequencing was conducted as a first-tier test as well as a second-tier test, in selected cases based on the decision of the child neurology expert consortium. Variants were evaluated and classified according to the guideline proposed by American College of Medical Genetics (ACMG) [9]. ClinVar database was searched for past variant reports [10]. Population frequency of variants were determined using 1000genome, ExaC, and gNomad database.

STXBP1 sequencing was performed on DNA from probands and family members using the Sanger method to identify parental origin whenever possible. Information obtained from medical records include age at seizure onset, symptoms at onset, duration from onset to diagnosis, previous diagnosis and treatment, associated psychiatric and behavior symptoms, response to antiepileptic drug (AED) treatment and developmental outcome. Each patient's

electroencephalography (EEG) was obtained and analyzed. Seizure types and epileptic syndromes were classified according to the 2017 International League Against Epilepsy guidelines [1].

The study protocol was approved by the Institutional Review Board of Seoul National University Hospital (IRB No.H-2001-134-1096), and the study was conducted in accordance with relevant guidelines and regulations. Written informed consent by the patients was waived due to the retrospective nature of our study.

Results

1. Genetic identification of *STXBP1* mutations

Total of 10 different variants were identified in 10 patients (Table 1 and Fig. 1). Four variants were missense mutations, another four variants were nonsense mutations, and two variants were splicing mutations. Three novel variants (c.1212A > C, c.1497C > G, c.1030-2 A > G) and seven variants previously reported as pathogenic [3,8,11-15] were identified. All novel variants were not found in either 1000Genomes or ExAC control database. Total of eight patients' parental DNA sample were available for segregation analysis. All eight patients tested harbored *de novo* mutation.

2. Clinical characteristic of *STXBP1* mutations

The clinical characteristics of 10 patients with *STXBP1* mutations are summarized in Table 1. Patient's median age was 7 years old (range, 1 to 11). Head circumference was normal in all patients. Brain magnetic resonance imaging from all patients did not show remarkable abnormalities. Among 10 patients, nine patients had confirmed electroclinical seizures. The median age of seizure onset was 6 months old (range, 3 days to 7 years). Three patients showed neonatal onset seizures, presenting within 1 month of age and other five patients presented as early onset epilepsy, presenting with seizures before the age of 3 years. In remaining two patients seizure was not a main clinical feature; patient 6 was being followed up for global developmental delay and ataxia before his first seizure at age of 7 years. Patient 7 never had clinical seizures until the age of 8 years at last follow-up, but suffered from head dyskinesia, bruxism, and hand stereotypy.

All patients showed significant global developmental delay regardless of seizure frequency. All domains of development were delayed, but the language domain was always more severely affected compared to the motor domain. Only one patient was able to achieve any word output, whereas four patients achieved the milestone of walking. Three patients (patient 4, 6, 7) were already being followed for a global developmental disorder before seizure occurrence. Two patients (patient 6, 7), who's seizure was not a dominant feature, showed clear developmental regression during fol-

Table 1. Clinical and genetic features of *STXBP1* encephalopathy patients

Variable	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Sex/age	F/7 years	F/5 years	F/3 years	M/7 years	F/1 year	M/11 years	F/8 years	F/2 years	F/3 years	M/3 years
Seizure onset	3 days	13 months	3 months	24 months	10 days	7 years	Never	10 days	2 months	2 months
Seizure type	T	Ba, T	T, Es, My, At	T, Ba	T, Es	My	NA	T, Es	T, GTC	Es
Seizure frequency	Daily→weekly	Daily→weekly	Weekly→monthly	Weekly→Frequent daily	Daily→Daily	Monthly	Never	Frequent daily→weekly	Daily→Monthly	Daily→ Seizure-free
EEG	B-S with multifocal spikes→focal spikes	Focal and generalized spikes	B-S with multifocal spikes→H	Focal spikes	B-S with multifocal spikes→H	Focal spike	Diffuse background slowing	B-S with multifocal spikes	Focal spikes	Focal spikes
Brain MRI	Normal	Normal	Normal	Normal	Normal	Diffuse background slowing	Normal	Normal	Normal	Normal
DD before seizure	NA	No	No	Yes	NA	Yes	Yes	NA	No	No
Regression	No	No	No	No	No	Yes (after seizure onset)	Yes	No	No	No
Neurologic state at the last follow-up	Severe GDD	Few words, climbed stairs	Pointed to objects, walked while holding	No word output, walked alone	Severe GDD	No word output but walked alone→bedridden state	Rolled over, babbling→bedridden state, no word output	Bedridden state, no eye contact	Severe GDD, standing up by self	Severe GDD, bedridden state
Other symptoms	None	Hyperactivity, disruptive behavior	None	Hyperactivity, disruptive behavior	None	Hyperactivity, disruptive behavior	Head tremor, dyskinesia, bruxism, hand stereotypy	Truncal dystonia, hypotonia	Bruxism, hand stereotypy	None
AEDs	LEV, CNZ, VPA	VPA, LEV, OXC, TPM	VPA, VGB, LEV	VPA, LEV, LTG, OXC	VPA, VGB, LEV, LCS, LTG	ataxia, tremor TPM, LEV, CLB, VPA	None	LEV, VGB, CLB	VPA, CLB	None
Clinical diagnosis	EIEE → EE, unspecified	EE, unspecified	EIEE → WS	EE, unspecified	EIEE → WS	EE, unspecified	Rett syndrome	EIEE	EE, unspecified	EE, unspecified
Variant/previous report	c.703C>T, reported [11]	c.1212A>C, novel	c.1099C>T, reported [3]	c.1651C>T, reported [13]	c.88-2A>G, reported [14]	c.1497C>G, novel	c.1439C>T, reported [12]	c.1030-2 A>G	c.1162C>T, reported [15]	c.1631G>T, reported [16]
Segregation	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	NA	NA	<i>De novo</i>
Pathogenicity (ACMG criteria)	Pathogenic (PVS1, PS2, PM2, PP3, PP5)	Likely pathogenic (PS2, PM2)	Pathogenic (PVS1, PS2, PM2, PP3, PP5)	Pathogenic (PS2, PM1, PM2, PP3, PP5)	Pathogenic (PVS1, PS2, PM2, PP3, PP5)	Pathogenic (PVS1, PS2, PM2)	Pathogenic (PS2, PM1, PM2, PP5)	Pathogenic (PVS1, PM2, PP3)	Pathogenic (PVS1, PM2, PP5)	Pathogenic (PS2, PM1, PM2, PP5)

STXBP1, syntaxin-binding protein 1; T, tonic; Ba, behavior arrest; Es, epileptic spasms; My, myoclonic; At, atonic; NA, not available; GTC, generalized tonic-clonic; EEG, electroencephalography; B-S, burst-suppression; H, hypersarhythmia; MRI, magnetic resonance imaging; DD, developmental delay; GDD, global developmental delay; AED, antiepileptic drug; LEV, levetiracetam; CNZ, clonazepam; VPA, valproic acid; OXC, oxcarbazepine; TPM, topiramate; LTG, lamotrigine; VGB, vigabatrin; LCS, lacosamide; CLB, clobazam; EIEE, early infantile epileptic encephalopathy; EE, epileptic encephalopathy; WS, West syndrome; ACMG, American College of Medical Genetics; PVS, pathogenic very strong; PS, pathogenic strong; PM, pathogenic moderate; PP, pathogenic supporting.

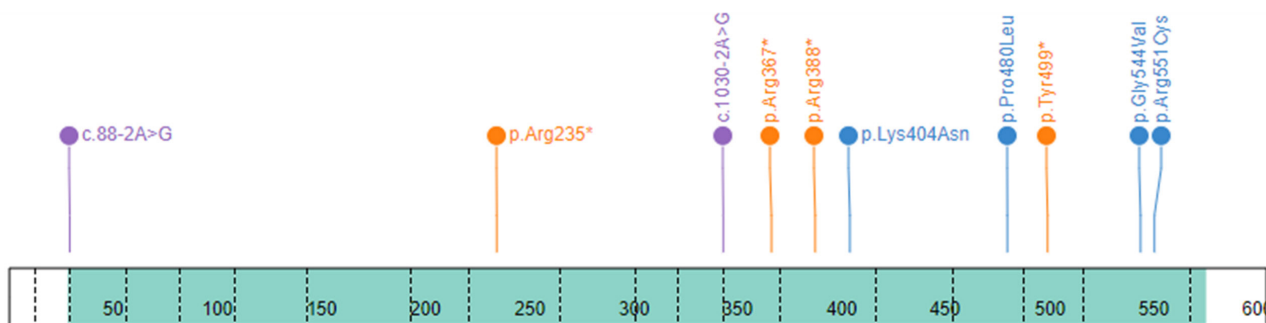


Fig. 1. Positions of syntaxin-binding protein 1 (*STXBP1*) variants.

low-up. In patient 6, motor function deterioration was observed after seizure onset, which progressed to worsening ataxia and dysphagia. Patient 7 showed developmental regression being evident at the age of 2 years without any clinical seizures, which lead to initial misdiagnosis as Rett syndrome.

Six patients showed additional symptoms other than seizure. Behavior problem was seen in five patients showing autistic like features including hyperactivity/disruptive behavior ($n = 3$), bruxism ($n = 2$), hand stereotypy ($n = 2$). Neurologic symptoms were seen in three patients, including tremor ($n = 2$), ataxia ($n = 1$), dystonia ($n = 1$), hypotonia ($n = 1$), and dyskinesia ($n = 1$).

Four patients (patient 1, 3, 5, 8) were initially diagnosed as EIEE (or Ohtahara syndrome) with background burst-suppression pattern with multifocal spikes. Two patients (patient 3, 5) went on to develop hypsarrhythmia with epileptic spasms and diagnosis was changed to West syndrome. A total of six patients (patient 1, 2, 4, 6, 9, 10) eventually evolved to unspecified epileptic encephalopathy with focal and multifocal spike discharges (Fig. 2). Patient 7 showed irregular high amplitude delta activities in the background activity. All patients except patient 7 ($n = 9$) showed either focal or multifocal epileptiform discharges in EEG.

Tonic seizure was the most common type seen ($n = 8$), but multiple types of seizure semiology are observed including epileptic spasms ($n = 4$), myoclonic ($n = 2$), and behavior arrest ($n = 2$) seizure. Six patients overall showed more than one type of seizure semiology.

Regarding treatments, all patients with epilepsy were refractory to antiepileptic medications, requiring more than two types of AEDs, and none achieved seizure freedom through medication. Of note, patient 10 showed significance response to ketogenic diet, leading to cessation of all AEDs.

Discussion

Here we identified 10 Korean patients with *STXBP1* mutation, which included three novel mutations. We described detailed phe-

notypes and genotypes of the patients with *STXBP1* encephalopathy. Due to its rarity, the clinical spectrum of *STXBP1* encephalopathy is not yet well known, but recent large cohort study suggests that intellectual disability and epilepsy is the two main major components of *STXBP1* encephalopathy.

Epilepsy was observed in 95% of *STXBP1* encephalopathy patients. Among the patients with available information, about half was taking more than three AEDs and one-third was suffering from frequent seizure. On the other hand approximately one-third achieved seizure freedom [3]. Consistent with the above observation, nine patients (90%) in the current cohort showed epilepsy and all patients required more than two AEDs.

In the same study, moderate to severe intellectual disability was observed in 88.4% of patient. Notably, developmental delay was present before seizure onset in 64.3%, and 7% of patients were observed to have just developmental delay without any seizure. Other previous studies also observed that some degree of developmental delay was often observed prior to any seizure onset, thus it is considered an independent domain from epilepsy [3,12]. Indeed, in our cohort all patients showed profound developmental delay or severe intellectual disability and 30% of patients showed developmental delay before seizure onset.

However, it is often difficult to assess whether seizure activity or epileptiform discharges have any effect on developmental process or whether developmental delay is totally separate phenotype in *STXBP1* encephalopathy. It was suggested that developmental regression is rarely seen and does not seem to be related to seizure activity [3]. In the current cohort, patient 6 and 7 showed clear regression during follow-up. Interestingly, both patients were different from typical patients presenting with early onset epileptic encephalopathy, as seizure was not their main feature and both showed predominantly non-epileptic movement symptoms. Patient 7 followed Rett syndrome like features with regression starting at age of 3 with autistic features, and patient 6 showed many resemblances to the phenotype previously described as ataxia-tremor-retardation syndrome [16]. Patient 6 showed clear motor func-

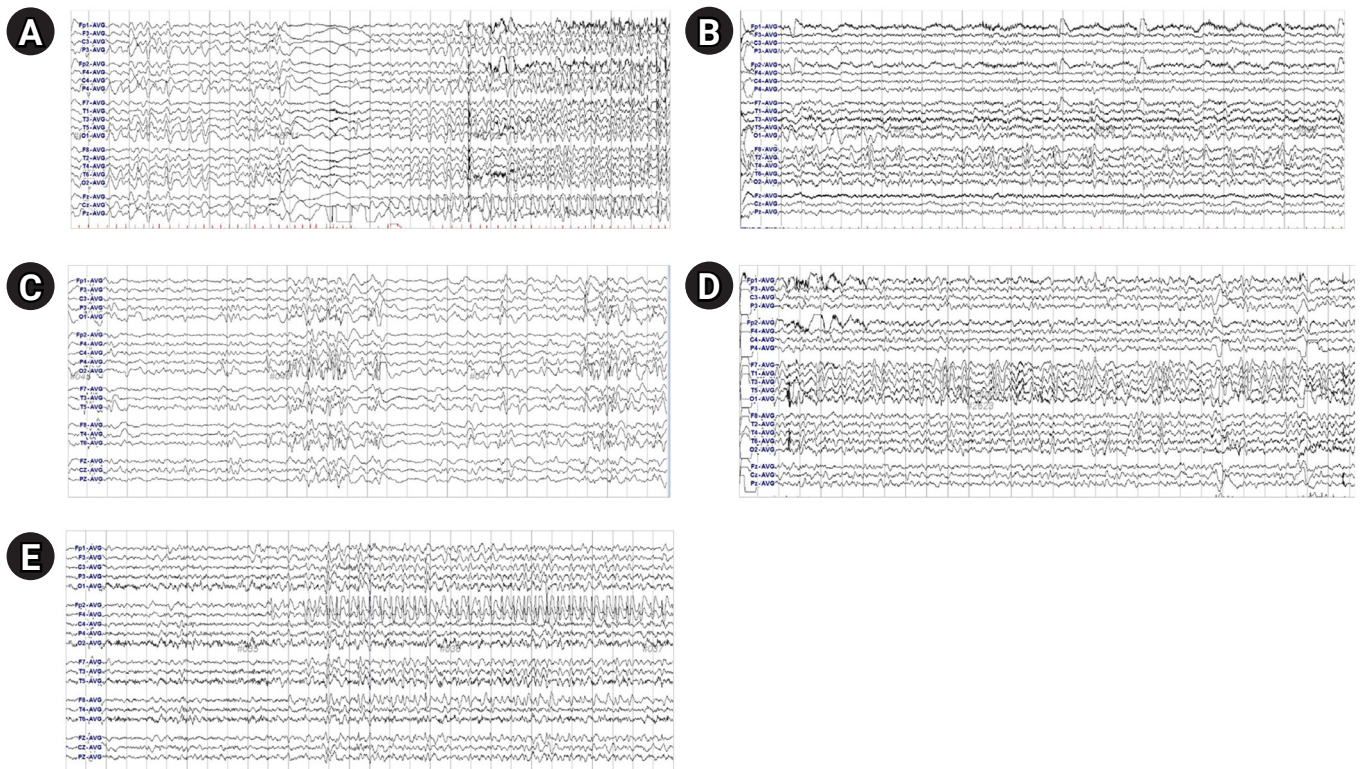


Fig. 2. Electroencephalograms of patients with syntaxin-binding protein 1 (*STXBP1*) mutations showing focal epileptiform discharges. (A) Patient 1, central area. (B) Patient 2, right fronto-temporal area. (C) Patient 3, right or left occipital area. (D) Patient 4, left temporo-occipital area. (E) Patient 6, right frontal area.

tion regression after seizure onset with worsened ataxia and newly developed dysphagia. Above two patients support that seizure activity and intellectual development are indeed independent domains.

STXBP1 encephalopathy was known to be most commonly associated with burst suppression and hypsarrhythmia, as it was initially described mostly in Ohtahara syndrome [11,12,17]. However recent large cohort study suggest most commonly described changes are focal or multifocal activities, seen in 64% of the cases [3]. Our patients also showed that focal or multifocal epileptiform discharges were the most common finding (60%), and classical burst-suppression patterns were seen in only proportion of the patients (40%). However, patients who were followed up long term showed that patients with burst-suppression pattern often evolve to hypsarrhythmia or non-specific focal patterns. Thus it is possible some patients did have burst-suppression pattern at one point but was never recorded. The range of abnormal EEG findings was wide in the current cohort, and they showed little correlation with their developmental status or seizure activity, in accordance to previous reports [3,18].

The reason why the clinical presentation of patients with *STXBP1* mutation varies so much is yet unknown. Previous evi-

dence supported the hypothesis that haploinsufficiency is the main pathogenic mechanism underlying *STXBP1* encephalopathy, which may explain for such phenotypic heterogeneity [19]. However, there is growing evidence that *STXBP1* mutation have more than one mechanism of pathophysiology. Recently, a homozygous *STXBP1* mutation was found to cause Lennox-Gastaut syndrome, showing that *STXBP1* also have a dominant-negative effect [20]. Thus mechanism of *STXBP1* encephalopathy still needs much further research in the future.

Another important aspect of *STXBP1* mutation to consider is mosaicism. Both somatic and germline mosaicism of *STXBP1* mutation has been reported. It is interesting that focal epileptiform discharges are commonly seen in *STXBP1* encephalopathy, especially since there were two reports of significant improvement of seizure with epilepsy surgery [8,21]. According to these reports, presence of focal cortical dysplasia have been confirmed by tissue pathology in both cases. One patient was confirmed to also harbor somatic mosaicism for homozygosity of *STXBP1* mutations in the dysplastic tissue.

Pathogenic role of somatic mosaicism on *STXBP1* encephalopathy is still unknown, but this finding suggest they may play an important role in cortical development.

On the other hand, germline mosaicism can cause major problem in genetic diagnosis of *STXBP1* mutation and in family genetic counseling. Germline mosaicism of *STXBP1* has been reported previously from an unaffected parent of a patient [22,23]. Assumption of *de novo* mutation based on parental Sanger sequencing maybe incorrect, as it is unlikely to detect low-rate mosaicism [24]. Therefore it is possible that unrecognized parental *STXBP1* mosaicism are present among the current cohort as well. Recently, the high fold coverage of next-generation sequencing allow for the detection of even very low levels of mosaicism in the blood cells [25]. This has opened a new era of genetic testing, which will hopefully broaden our knowledge of mosaicism in *STXBP1* encephalopathy.

Current study has several limiting factors. Selection bias is present due to primary identification of cases from pediatric clinics with significant epilepsy or developmental delay. Given the wide variety of phenotypes, it is plausible that there are cases of *STXBP1* encephalopathy with milder symptoms or different phenotype were overlooked. There are also possibility that patients with focal cortical dysplasia or other cortical malformation harbor *STXBP1* mutation and were never considered for genetic testing, in the absence of the classical features.

In conclusion, *STXBP1* encephalopathy showed a wide clinical spectrum of phenotypes from severe epileptic encephalopathy such as Ohtahara syndrome or West syndrome to unknown neurodevelopmental retardation without epilepsy. Their seizure types and EEG findings are also diverse. Therefore it is important to consider *STXBP1* mutation even in patients without seizure, or predominantly focal EEG changes without history of burst suppression. Further research is needed to discover the full range of phenotypes of *STXBP1* encephalopathy in order to elucidate underlying disease mechanism.

Conflicts of interest

Jieun Choi is an associate editor, Ki Joong Kim and Jong-Hee Chae are the editorial board members of the journal, but They was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Author contribution

Conceptualization: WJK and JHC. Data curation: WJK, YKS, YJK, and SYK. Formal analysis: WJK and YKS. Funding acquisition: KJK and JHC. Methodology: WJK and YKS. Project administration: HK, BCL HH, JC, and JHC. Visualization: WJK and YJK. Writing-original draft: WJK. Writing-review & editing: BCL, KJK, and JHC.

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