Clinical spectrum of early-onset epileptic encephalopathies associated with *STXBP1* mutations

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ABSTRACT

Objectives: Heterozygous mutations in *STXBP1*, encoding the syntaxin binding protein 1, have recently been identified in Ohtahara syndrome, an epileptic encephalopathy with very early onset. In order to explore the phenotypic spectrum associated with *STXBP1* mutations, we analyzed a cohort of patients with unexplained early-onset epileptic encephalopathies.

Methods: We collected and clinically characterized 106 patients with early-onset epileptic encephalopathies. Mutation analysis of the *STXBP1* gene was done using sequence analysis of the exon and intron–exon boundaries and multiplex amplification quantification to detect copy number variations.

Results: We identified 4 truncating mutations and 2 microdeletions partially affecting *STXBP1* in 6 of the 106 patients. All mutations are predicted to abolish *STXBP1* function and 5 mutations were proven to occur de novo. None of the mutation-carrying patients had Ohtahara syndrome. One patient was diagnosed with West syndrome at disease onset, while the initial phenotype of 5 further patients did not fit into a specific recognized epilepsy syndrome. Three of these patients later evolved to West syndrome. All patients had severe to profound mental retardation, and ataxia or dyskinetic movements were present in 5 patients.

Conclusion: This study shows that mutations in *STXBP1* are not limited to patients with Ohtahara syndrome, but are also present in 10% (5/49) of patients with an early-onset epileptic encephalopathy that does not fit into either Ohtahara or West syndrome and rarely in typical West syndrome. *STXBP1* mutational analysis should be considered in the diagnostic evaluation of this challenging group of patients. ***Neurology*® 2010;75:1159–1165**

GLOSSARY

AED = antiepileptic drugs; **EME** = early myoclonic encephalopathy; **EOEE** = early-onset epileptic encephalopathy; **ILAE** = International League Against Epilepsy; **MAQ** = multiplex amplicon quantification; **STR** = short tandem repeat.

Epileptic encephalopathies are devastating conditions in which frequent or severe epileptic seizures but also abundant interictal paroxysmal activity contributes to the progressive deterioration of cerebral functions. Within the group of epileptic encephalopathies, several syndromes are distinguished based on their onset age, seizure type, and interictal EEG pattern.[1,2] The etiologies of epileptic encephalopathies are heterogeneous and often symptomatic including structural brain defects and metabolic disorders. However, cases caused by genetic defects have been described. The known causal genes encode proteins with diverse functions including a subunit of voltage-gated ion channels (i.e., *SCN1A*), a serine/threonine

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kinase (i.e., *CDKL5*), and a homeobox protein (i.e., *ARX*).[3] De novo heterozygous mutations in *STXBP1* have been identified in a subset of patients with Ohtahara syndrome.[4] Ohtahara syndrome, or early infantile epileptic encephalopathy with suppression-burst pattern, is one of the earliest developing forms of epileptic encephalopathy. This disorder is characterized by intractable seizures, mainly tonic spasms, with onset within the first 3 months of life, and a characteristic interictal suppression-burst pattern on EEG. Prognosis is poor, with severe psychomotor retardation and a high mortality rate during infancy. Surviving patients often show a remarkable age-dependent evolution; 75% evolve into West syndrome after the age of 3 to 4 months.[5] This age-dependent transition suggests a common pathologic mechanism at least in some patients.

In this study, we analyzed *STXBP1* in a cohort of patients with different types of early-onset epileptic encephalopathies in order to further delineate the clinical phenotypes associated with *STXBP1* mutations.

METHODS **Patients.** We retrospectively included 106 patients with unexplained early-onset epileptic encephalopathies. All patients were referred to us previously by collaborating child neurologists of Western European or Australian university hospitals for genetic testing in a research setting. All patients had onset of seizures within the first 9 months of life followed by slowing of psychomotor development with or without additional neurologic deficits such as spasticity or ataxia. Routine diagnostic screening including at least MRI of the brain, metabolic screening (amino acids in blood and urine, organic acids in urine, and lactate in blood), and karyotyping was negative for all patients. Mutations in *SCN1A*, *ARX*, and *CDKL5* were tested for and excluded in several patients in whom the phenotype was compatible with the respective associated syndromes. However, mutations in these genes were not systematically excluded in the complete cohort, since this was not the aim of the study.

Standard protocol approvals and patient consents. Parents or the legal representative of each patient signed an informed consent form for participation. The study was approved by the Commission for Medical Ethics of the University of Antwerp and by the Human Research Ethics Committee of Austin Health.

Mutation analysis. Genomic DNA was extracted from peripheral blood of all patients, and their unaffected parents when possible. Sequence analysis of the 20 exons of *STXBP1* was performed using flanking intronic primers. The presence of identified mutations was also tested for in 250 control individuals.

The in-house developed technique multiplex amplicon quantification (MAQ) was used to screen the genomic region containing *STXBP1* for copy number variations (www.multiplicon.com). The multiplex PCR reaction consists of 14 test amplicons located in the genomic region of *STXBP1* and 6 reference amplicons randomly located on different chromosomes. The size of the identified microdeletions was determined by a SYBR® Green real-time PCR assay and haplotype analysis of short tandem repeat (STR) markers localized in the region surrounding the *STXBP1* gene.

Effect of the intronic mutation c.1029 + 1G>T on exon splicing was investigated by extracting total RNA of the patient. We were not able to obtain an RNA sample of the patient carrying the intronic mutation c.429 + 1G>A. Online available splice sites prediction programs were therefore used to investigate the possible effect of this mutation.

Mutations were numbered according to the published cDNA sequence (NM 003165) with nucleotide + 1 corresponding to the A of the ATG translation initiation codon and the MDI/HGVS Mutation Nomenclature Recommendations were followed (http://www.hgvs.org/mutnomen).[6]

Detailed information about the different methods used for the mutation identification is available in appendix e-1 on the *Neurology*® Web site at www.neurology.org.

RESULTS **Clinical features of the cohort and of patients with *STXBP1* mutations.** Of the 106 included patients, 9 patients were diagnosed with Ohtahara syndrome, 32 patients had West syndrome, 14 had migrating partial seizures of infancy, and 2 patients had early myoclonic encephalopathy (EME). The general term early-onset epileptic encephalopathy (EOEE) was used for the remaining 49 patients for whom the clinical or EEG features did not fit into a specific epilepsy syndrome. Patients had different degrees of mental retardation, ranging from moderate to profound. All patients were of Caucasian descent; most patients had a Western European or Australian background, and a minority had origins in Eastern Europe, North Africa, or Turkey.

Disease-causing mutations were identified in 6 patients (5.7%). The frequency of mutations in syndromes recognized by the International League Against Epilepsy (ILAE) was West syndrome 1/32 (3%), migrating partial seizures of infancy 0/14, EME 0/2, and Ohtahara 0/9. In contrast, of the 49 patients with EOEE, 5 (10.2%) had *STXBP1* mutations.

The table gives an overview of the clinical data of patients carrying a mutation. Seizure onset age ranged between 3 days and 4.5 months of life. Patient 4 presented at the age of 4.5 months with epileptic spasms and hypsarrhythmia on EEG, consistent with West syndrome. The 5 other patients had an earlier onset, between 3 days and 10 weeks of life. The presenting seizure type was tonic, clonic, myoclonic, or partial seizures or epileptic spasms. EEG performed at seizure onset showed focal or bilateral synchronous discharges. Patient 1 was initially diagnosed with benign neonatal (myoclonic) seizures, but later psychomotor retardation suggested a more severe epilepsy syndrome. Patients 2, 3, 5, and

Table Clinical characteristics of patients with an *STXBP1* mutation

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Sex/age at study, y	M/11	M/6	M/11	F/13	F/6	M/11
Age at onset	5 wk	6 wk	3 d	4.5 mo	10 wk (apneic attacks at birth, spontaneous remission at d 3; investigations all normal; etiology?)	4 wk
Initial symptoms	Asymmetric myoclonus	Myoclonus	Tonic spasms, asymmetric partial seizures	Epileptic spasms	Tonic seizures, clonic seizures	Epileptic spasms, secondarily generalized partial seizures
EEG at onset (age)	Lateralized epileptic activity (5 wk)	Lateralized epileptic activity (6 wk)	Lateralized epileptic activity (1 wk)	HS (4.5 mo)	Bilateral synchronous epileptic activity (10 wk)	Frequent multifocal epileptic activity, independently from occipital and temporal regions bilaterally (4 wk)
Response to initial treatment	Immediate seizure-free on VGB	No effect of PB	No effect of PB	VGB, NTZ, ACTH, VPA; seizure-free at 8 mo	Immediate seizure-free on PB and VGB	No effect of PB
Age at transition to West, mo	NA	4.5	2	NA	NA	4
EEG	NA	HS (5 mo)	HS (4 mo)	NA	NA	HS (4 mo)
Follow-up treatment and response	VGB stop at 3 mo; seizure-free	VGB, CNZ, prednisone, TPM, GBP, CLB, VPA, LTG, LEV, IVIg; therapy resistant	VPA, VGB, CNZ; seizure-free at 6 mo; AED stop at 3 y	AED stop at 2 y; seizure-free	AED stop at 1.5 y; seizure-free	PB, VGB, pyridoxine, prednisolone, VPA, LTG, CNZ, TPM, LEV; therapy resistant; worse on VGB at age 13 wk; improvement on VGB when reintroduced 2 y later
Current seizures type	None	Daily myoclonic and tonic seizures	None	None	None	Refractory epileptic spasms, rare tonic seizures
Current treatment	No AED	VPA, LTG, LEV	No AED	No AED	No AED	LTG, VGB, LEV
MRI (age)	Normal (4 y)	Normal (8 wk)	Widened pre-pontine CSF space: subtle brainstem atrophy? (2 y 5 mo)	Increased left frontal extracerebral space (4.5 mo), normal follow-up MRI (2 y 5 mo)	Slight dilatation of temporal horns (1 y 11 mo)	Initially normal (3 and 12 mo); nonspecific small focal lesion (right precentral and postcentral gyrus) and mildly delayed myelination in both anterior temporal lobes and frontal pole; sylvian fissures prominent (2 y)
Last EEG (age)	Slow background; no epileptic activity (10 y 8 mo)	Multifocal sharp waves and (poly) SW, predominant over left posterior hemisphere (2 y 1 mo)	Slow background; no epileptic activity (9 y 6 mo)	Slow background; no epileptic activity (5 y 6 mo)	Normal (1 y 11 mo)	Slow background; rare independent bilateral mid and posterior temporal epileptic discharges (7 y 9 mo)
Cognition[a]	Severe mental retardation	Profound mental retardation	Profound mental retardation	Severe mental retardation	Severe mental retardation	Profound mental retardation
Neurologic examination	Severe ataxia	Wheelchair-bound, dyskinetic	Wheelchair-bound	Subtle hypertonia and ataxia	Ataxia, hypotonia	Wheelchair-bound, truncal hypotonia, dyskinetic, figure of 8 head tremor
Additional features	Hyperactivity	Hyperactivity	Hyperactivity	Stereotypic behavior	None	Cortical visual impairment; precocious puberty
Clinical diagnosis	EOEE	EOEE with evolution to West	EOEE with evolution to West	West	EOEE	EOEE with evolution to West
Mutation	c.1434G>A	c.893_894delAG	c.1029 + 1G>T	c.963 + ?_(*1967+?) del	c.(?_-120)_37 + ?del	c. 429 + 1G>A
Predicted effect	p. Trp478X	p.Glu278GlyfsX15	p.[Lys343AsnfsX13; Tyr344_Glu603del Ins11]	p.Thr322_Glu603 del	No protein	Destruction of splice site and truncated protein

Abbreviations: AED = antiepileptic drug; CLB = clobazam; CNZ = clonazepam; EOEE = early-onset epileptic encephalopathy; GBP = gabapentin; HS = hypsarrhythmia; IVIg = IV immunoglobulin; LEV = levetiracetam; LTG = lamotrigine; NA = not applicable; NTZ = nitrazepam; PB = phenobarbital; SW = spike-wave; TPM = topiramate; VGB = vigabatrin; VPA = valproic acid.

[a] Severe mental retardation defined as IQ < 40, profound mental retardation defined as IQ < 25.

6 were initially diagnosed with atypical forms of West syndrome. We grouped these 5 patients under the general term EOEE based on their initial presentation. Three patients (patients 2, 3, and 6) evolved to West syndrome with the appearance of hypsarrhythmia by 5 months of age.

To exclude the possibility that a burst-suppression pattern was not described in the original EEG proto-

col, we reexamined all EEG recordings. All patients had an EEG at seizure onset and regular follow-up EEGs in the active epilepsy phase, so although we cannot exclude the presence of a burst-suppression pattern for a very short time outside the recording period, this seems unlikely.

The response to antiepileptic drugs (AED) varied; 2 patients became seizure-free 1 week after starting AED, 2 patients some months later, while 2 patients had intractable seizures despite multidrug treatment. All patients had severe to profound mental retardation. Ataxia was present in 3 patients; the remaining 3 were wheelchair-bound due to profound mental retardation, sometimes in combination with hypotonia or dyskinetic movements. Head growth was normal in all patients.

Mutation analysis. ***Sequence analysis.*** Sequence analysis of the exons and the exon–intron boundaries of *STXBP1* revealed mutations in 4 patients. A nonsense mutation c.1434G>A, predicted to create a stop codon at position 478 of the protein (p.Trp478X), was found in patient 1. Patient 2 carried a 2–base pair deletion c. 893_894delAG predicting a frameshift mutation (p.Glu278GlyfsX15). Two intronic mutations affecting different donor splice sites were identified: c.1029 + 1G>T in patient 3 and c.429 + 1G>A in patient 6. All 4 mutations were absent in 250 control individuals and 3 were proven to arise de novo. The potential de novo origin of p.Glu278GlyfsX15 mutation could not be tested since DNA samples of the parents of patient 2 were not available.

To assess the effect of the c.1029 + 1G>T mutation on mRNA splicing, we extracted RNA from lymphocytes of patient 3 and 1 control individual. Three aberrant transcripts were observed in the cDNA of the patient compared to the control. The mutation created a new splice site located 1 nucleotide before the normal splice site. Utilization of this new splice site resulted in a deletion of the G at position 1029 (r.1029delG) on transcript level and a shift of the reading frame (p.Lys343AsnfsX13) on protein level. The second transcript contained 107 additional bases (r.[1029 + 1_1029 + 107ins;1029 + 1g>u]) and resulted from the use of a cryptic splice site within intron 12. Intron retention of the entire intron 12 created the third transcript (r.[1029 + 1_1030–1ins;1029 + 1g>u]). The 2 last transcripts had the same predicted effect on protein level: replacement of the normal C-terminus by 11 different amino acids (p.Tyr344_Glu603delins11). Total RNA of patient 6 carrying the c.429 + 1G>A mutation could not be obtained. This mutation affected the G of the GT consensus sequence in the splice donor site of intron 6 and in silico analysis with prediction programs suggests complete abolition of this splice site.

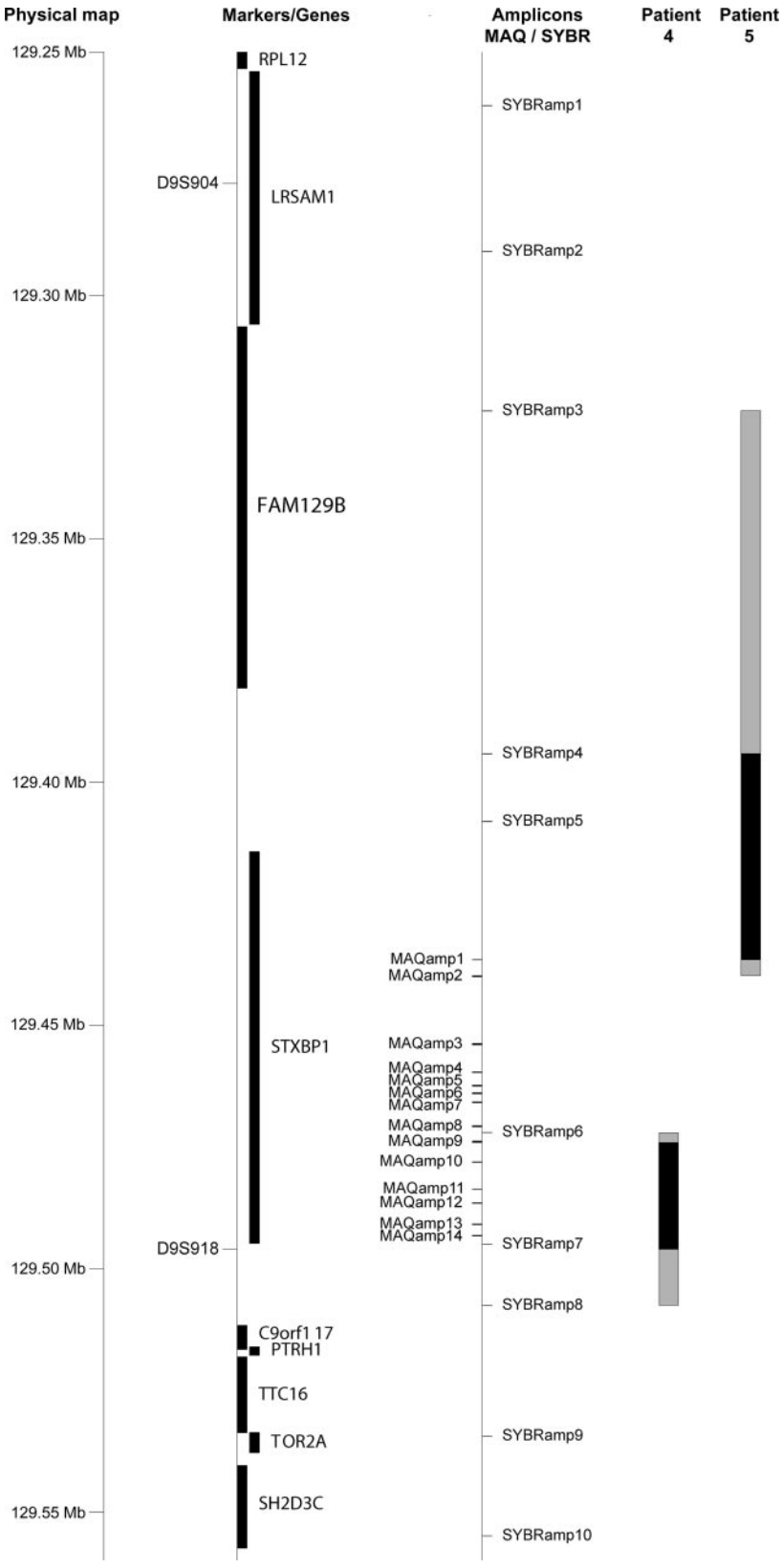
Copy number analysis. Copy number analysis of the genomic region of *STXBP1* using the MAQ technique revealed a heterozygous microdeletion in 2 patients (figure 1). The chromatogram of patient 4 indicated a deletion of the last 6 amplicons at the 3′ side of the gene. Confirmation of the deletion was obtained by a SYBR® Green real-time PCR assay with 5 amplicons (figure e-1, A and C). This deletion was not observed in the DNA of the parents. Haplotype analysis of the STR marker D9S918 showed that patient 4 did not inherit an allele from his mother. The total length of this deletion ranged between 23 and 35.4 Kbp with a centromeric breakpoint between *STXBP1* exons 11 and 12 and the telomeric breakpoint after the last exon. The deletion affected the *STXBP1* exons 12 to 20 and was predicted to result in a transcript, if any, missing the last 281 bases. The open reading frames of the adjacent genes were not affected (figure 1).

In patient 5, the MAQ assay and the SYBR® Green real-time PCR assay revealed a heterozygous deletion at the 5′ side of *STXBP1* (figure e-1, B and D). This deletion was not observed in the DNA samples from the parents. Patient 5 was heterozygous for marker D9S904, proving that this marker was not deleted. The total length of this deletion ranged between 42.3 and 116 Kbp with a centromeric breakpoint in front of the first *STXBP1* exon and the telomeric breakpoint between exon 1 and 2. Both the first exon including the transcription start site and the proximal promoter region of *STXBP1* were affected. This deletion is predicted to prevent gene transcription. This deletion may also affect the neighboring gene *FAM129B,* encoding the hypothetical protein LOC64855 (figure 1).

DISCUSSION Haploinsufficiency of *STXBP1* has recently been described to cause Ohtahara syndrome in 5 patients.[4] One of the patients had a 2.0-Mb deletion encompassing the complete *STXBP1* gene and functional analysis of the 4 missense mutations present in the other patients suggested loss of function. Seizure onset in these patients was between 10 days and 3 months of life. All had a burst-suppression pattern on the initial EEG. One patient rapidly became seizure-free, while 4 evolved to West syndrome. Three of them continued to have daily intractable seizures. All were profoundly mentally retarded and had diplegia or quadriplegia.

The first indication that *STXBP1* may be associated with other phenotypes was presented in a recent study investigating patients with mental retardation and autism.[7] Truncating *STXBP1* mutations were identified

Figure 1 Map of deleted regions in patients 4 and 5



Map of the region surrounding *STXBP1* (chromosome 9q33.3-q34.11) showing the location of the genes, short tandem repeat markers, and amplicons analyzed in the multiplex amplicon quantification assay and the SYBR® Green real-time PCR assay. The physical distance was based on the UCSC Genome Browser: http://genome.ucsc.edu. For each patient, the minimum size of the deletion is indicated in black. The gray regions represent the region in which the breakpoints are located.

in 2 patients with severe mental retardation and epilepsy. Seizures onset age was 6 weeks in one patient and 2 years and 9 months in the other. They could not be diagnosed with a specific epilepsy syndrome.

To delineate the phenotypic spectrum associated with *STXBP1* mutations, we screened 106 patients with early-onset epileptic seizures and mental retardation of unknown etiology. Disease-causing mutations were identified in 6 patients (5.7%). Seizure onset age ranged between 3 days and 4.5 months of life. One patient had West syndrome starting at 4.5 months. The epilepsy phenotypes of the 5 other patients did not meet criteria for any syndrome recognized by the ILAE and patients were therefore grouped under the more general term EOEE.

In striking contrast to the initial study on *STXBP1* mutations, none of our mutation-positive patients had Ohtahara syndrome as none had a burst-suppression pattern on EEG. Even more remarkable is the observation that none of the Ohtahara patients in our study carried a *STXBP1* mutation. We had detailed clinical information on 7 out of 9 patients with Ohtahara syndrome, and the clinical characteristics did not seem different from the ones described in the article on *STXBP1* mutations in Ohtahara syndrome. Two patients were referred to us by experienced neuropediatricians with a diagnosis of Ohtahara without further clinical data, so we cannot exclude that they had atypical forms. The discrepancy between our findings and the high mutation rate (5/13) found in the aforementioned study can be explained by sample size bias or different ethnic/racial background of the patients.

Treatment response varied widely. Remarkably, 2 patients became immediate seizure-free on vigabatrin (one patient on phenobarbital as well). Two further patients eventually became seizure-free using combination therapy including vigabatrin. Although it is too early to draw any conclusions in this small patient group, this is a better response than generally seen in these patients with early-onset epilepsy and mental retardation and a trial of vigabatrin seems justified in patients with a *STXBP1* mutation.

Based on combined evidence from our study (6 patients), the study in patients with Ohtahara syndrome (5 patients), and the study in patients with mental retardation (2 patients), several conclusions can be drawn when summarizing the clinical phenotypes of patients carrying a *STXBP1* mutation. First, the presenting epileptic phenotype can be Ohtahara syndrome (5/13) but is certainly not limited to this syndrome. In fact, most patients (6/13) presented with an atypical epilepsy syndrome that can be described as EOEE. One patient presented with West syndrome, and one patient had early global develop-

mental delay with seizures only starting in the third year of life. Interestingly, 8 patients out of the 13 evolved to West syndrome: 1 from the start, 4 from an initial Ohtahara syndrome, and 3 from EOEE. The variety of epilepsy phenotypes resulting from *STXBP1* mutations probably reflects maturational patterns of cerebral function: tonic and myoclonic seizures in the first 3 months of life and typical epileptic spasms after that period. In the EEG, this is reflected by lateralized epileptic activity in the first few weeks after birth, followed by bilateral synchronous epileptic activity in the somewhat more mature brain or a hypsarrhythmia pattern in case of diffuse epileptic brain dysfunction. Consistent with this theory, all patients who did not have immediate control of seizures at onset developed West syndrome later. The burst-suppression pattern seen in Ohtahara syndrome might be a sign of very severe epileptic dysfunction of the immature brain. Although 3 out of 5 patients with Ohtahara syndrome compared to 3 out of 8 patients without Ohtahara syndrome developed intractable epilepsy, the numbers are too small to conclude whether the presence of this pattern has a worse prognosis in the context of a *STXBP1* mutation.

Second, the phenotypes associated with *STXBP1* mutations seem to manifest in early infancy, ranging from 3 days to 4.5 months in 12 out of 13 patients. Still, we should keep in mind that onset before the age of 9 months was a selection criterion in our study, introducing a bias toward earlier onset ages.

Third, although *STXBP1* has so far been analyzed mainly in the context of epilepsy, it is noteworthy that the severity of the epilepsy varies widely from a few seizures that are immediately controlled to intractable epilepsy. Despite this, severe to profound mental retardation occurs in all mutation-positive patients. Interestingly, the severity of the epilepsy syndrome and the response to AED does not seem a discriminatory factor, suggesting that the neurodegeneration is an intrinsic property of *STXBP1* mutations and thus to some extent independent of frequent seizure activity. Additional behavioral disturbances such as hyperactivity and stereotypic behavior are frequent. Several patients never reach independent ambulation due to profound mental retardation, ataxia, hypertonia or hypotonia, dyskinetic movements, or a combination of these features. The pattern of symptoms observed in the majority of patients with an *STXBP1* mutation can be summarized as follows: early-onset epilepsy within the first 5 months of life, starting as either Ohtahara syndrome or as EOEE; frequent evolution to West syndrome; invariable development of severe mental retardation and behavioral disturbances; and severely compromised ambulation sometimes due to fixed neurologic deficits. In our series, 10.2% of patients with EOEE carried a *STXBP1* mutation. This number is likely to increase using more strict selection criteria based on the core phenotype as delineated earlier. The relative contribution of *STXBP1* mutations might also have been slightly different if the cohort had been systematically screened for mutations in all other relevant genes such as *SCN1A*, *ARX*, and *CDKL5*.

The pathologic mechanisms through which *STXBP1* mutations lead to epileptic encephalopathies and the associated neurodegeneration have to be further elucidated. *STXBP1* encodes syntaxin binding protein 1, more commonly called MUNC18-1, and is a neuron-specific protein of the SEC1 family of membrane-trafficking proteins. MUNC18-1 is expressed throughout the brain and a key component for calcium-dependent neurotransmitter release. Studies on homozygous and heterozygous *Stxbp1* knockout mice have shown the essential role of MUNC18-1 in synaptic vesicle release at both glutamatergic and GABAergic synapses.[8,9] In addition, MUNC18-1 seems to have a cell-intrinsic and essential function distinct from synaptic vesicle release.[10] A possible role in cytoskeletal dynamics is suggested by its copurification and subcellular colocalization with neuronal cytoskeletal proteins.[11] The functional profile of *STXBP1* differs clearly from the function of the other known genes for epileptic encephalopathies such as *SCN1A, ARX,* and *CDKL5* and suggests the involvement of many different pathologic mechanisms underlying these devastating entities.

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DISCLOSURE

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