

Epilepsy Course and Developmental Trajectories in *STXBPI-DEE*

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Abstract

Background and Objectives

Clinical manifestations in *STXBPI* developmental and epileptic encephalopathy (DEE) vary in severity and outcome, and the genotypic spectrum is diverse. We aim to trace the neurodevelopmental trajectories in individuals with *STXBPI-DEE* and dissect the relationship between neurodevelopment and epilepsy.

Methods

Retrospective standardized clinical data were collected through international collaboration. A composite neurodevelopmental score system compared the developmental trajectories in *STXBPI-DEE*.

Results

Forty-eight patients with de novo *STXBPI* variants and a history of epilepsy were included (age range at the time of the study: 10 months to 35 years, mean 8.5 years). At the time of inclusion,

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Glossary

ASM = antiseizure medication; **DEE** = developmental and epileptic encephalopathy; **FCD** = focal cortical dysplasia; **ID** = intellectual disability.

65% of individuals (31/48) had active epilepsy, whereas 35% (17/48) were seizure free, and 76% of those (13/17) achieved remission within the first year of life. Twenty-two individuals (46%) showed signs of developmental impairment and/or neurologic abnormalities before epilepsy onset. Age at seizure onset correlated with severity of developmental outcome and the developmental milestones achieved, with a later seizure onset associated with better developmental outcome. In contrast, age at seizure remission and epilepsy duration did not affect neurodevelopmental outcomes. Overall, we did not observe a clear genotype-phenotype correlation, but monozygotic twins with de novo *STXBPI* variant showed similar phenotype and parallel disease course.

Discussion

The disease course in *STXBPI*-DEE presents with 2 main trajectories, with either early seizure remission or drug-resistant epilepsy, and a range of neurodevelopmental outcomes from mild to profound intellectual disability. Age at seizure onset is the only epilepsy-related feature associated with neurodevelopment outcome. These findings can inform future dedicated natural history studies and trial design.

Disease-causing variants in *STXBPI* are among the most common causes for neurodevelopmental disorders and epilepsy with a frequency of up to 1:26,000.¹ *STXBPI* is a crucial presynaptic protein involved in neurotransmitter release^{2,3} and the most frequent member of SNARE complex-related genes involved in neurodevelopmental disorders and epilepsy.⁴

The association between pathogenic variants in *STXBPI* and Ohtahara syndrome was first reported in 2008.⁵ Since then, the clinical features of patients with *STXBPI* encephalopathy have been extensively described, leading to the definition of *STXBPI* developmental and epileptic encephalopathy (*STXBPI*-DEE) as a neurodevelopmental disorder characterized by intellectual disability (ID), epilepsy (in 95% of patients), neurologic impairment, and behavioral abnormalities.⁶ Nevertheless, seizure history and developmental outcomes present a considerable degree of variability, with no prognostic factors identified to date.

Several genetic neurodevelopmental disorders currently represent prime targets for gene therapy or gene regulation approaches.^{7,8} However, given the considerable variability in *STXBPI* phenotypes, the best outcome measure and therapeutic window remain unknown.

Defining developmental trajectories and discrete subgroups in *STXBPI*-related disorders is a prerequisite for designing more precise natural history studies. Seizure history is considerably variable between individuals, developmental outcomes range in severity, and prominent age-dependent clinical features have been observed in subgroups of individuals. Accordingly, the heterogeneity and disease-specific features need to be considered through a natural history study to identify the domains and windows for possible therapeutic interventions and to plan for clinical trials. However, longitudinal data are

limited for *STXBPI*-related disorders, and there remains a need for targeted studies aiming to assess the developmental trajectories and natural history of individuals with *STXBPI*-DEE. We investigated 48 individuals with de novo *STXBPI* variants to define their clinical features, trace the neurodevelopmental trajectories, and dissect the relationship between neurodevelopment and epilepsy.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

Written informed consent was obtained for genetic analysis and any clinical and instrumental investigation performed. All clinical data used in this study were gathered during a routine diagnostic and clinical activity. Clinical data were provided to the principal investigator by each referring clinician in a de-identified format in the form of a structured questionnaire. The study complies with anonymized retrospective studies regulations and was reviewed by the local Ethics Committee.

Inclusion Criteria and Genetic Analysis

Patients were recruited from those followed up between 2010 and 2020, at 20 neuropediatric clinical centers in 4 different countries (eTable 1, links.lww.com/NXG/A522). The study included individuals with de novo *STXBPI* variants and a history of epilepsy, aged >10 months. Molecular testing was performed in the context of standard diagnostic protocols by certified Genetic Laboratories using gene panel or whole-exome sequencing through next-generation sequencing approaches. Sequencing of parental DNA was performed in all included cases. Individuals for whom parental DNA sequencing was not available were not included in the study. *STXBPI* variants were interpreted according to the American College of Medical Genetics and Genomics classification.

Table 1 *STXBP1* Composite Developmental Score (*STXBP1_DevScore*)

Domains	Score	Explanation
Development course		
Examination at birth	0 - 1	0 = delay/abnormality (for examination at birth and neonatal period: presence of neurologic abnormalities) 1 = typical development (for examination at birth and neonatal period: absence of neurologic abnormalities)
Neonatal period	0 - 1	
Infancy	0 - 1	
After 1 year/early childhood	0 - 1	
Developmental milestones at last examination (≥ 3 years old)		
Head control	0 - 0.5 - 1	0 = no skill (eye contact: absent)
Eye contact	0 - 0.5 - 1	0.5 = partially acquired (eye contact: intermittent)
Walking	0 - 0.5 - 1	1 = acquired
Speech	0 - 0.5 - 1	
Neurologic and behavioral features at last examination		
Neurologic examination	0 - 0.5 - 1	0 = abnormal
		0.5 = mildly abnormal
		1 = unremarkable
Behavior	0 - 1	0 = abnormal
		1 = no abnormalities
Total	0 (profound developmental disorder)	
	‡	
	10 (typical development)	

Only individuals carrying pathogenic or likely pathogenic variants were included in the study.⁹ All identified variants (missense, stop, indel, frameshift, and splice site) were validated by Sanger Sequencing and reported according to the RefSeq transcript NM_003165. Microrearrangements encompassing the *STXBP1* gene were also included.

Collection of Clinical Data and Developmental Score System Design

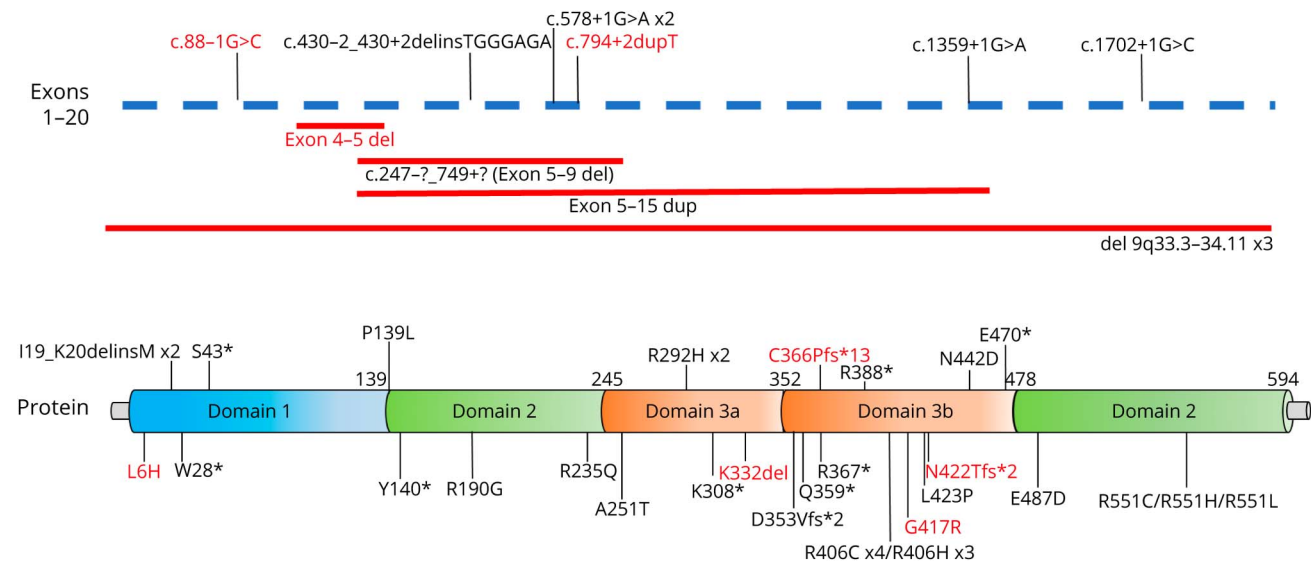
The following set of clinical data was required for eligibility: family history, seizure history (age at onset and seizure freedom, seizure types, EEG and antiseizure medications at the onset, follow-up, and last examination), neurologic examination, brain MRI, neurodevelopmental milestones and outcomes, and behavioral features. Epileptic seizures were defined according to the 2017 International League Against Epilepsy Classification criteria.¹⁰ Patients were identified as seizure free after they haven't had seizures for a period 3 times in duration compared with the longest preintervention interseizure interval.¹¹

Development was assessed through developmental milestones (eye contact, head control, walking, and speech) and neurologic examination by certified neurologists. Behavioral abnormalities were evaluated based on the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*¹² by each

referring clinician. The cognitive outcome was defined by each treating clinician as mild, moderate, severe, or profound ID (for individuals >6 years old) or cognitive delay (for individuals <6 years old), based on age-appropriate metrics; however, this metric was not included in the developmental score system.

Based on expert consensus, a composite developmental score system (referred to as *STXBP1_DevScore*) was created, to enable the quantitative comparison of developmental trajectories and outcomes in different individuals using a standardized framework. The score includes 10 domains comprising of development course, degree of development (assessed by developmental milestones), and neurologic and behavioral features. The score is based on the observed and reported clinical features and the rates of skills acquisition in the *STXBP1* population. A maximum of 10 points corresponds to typical development, and a minimum of 0 points corresponds to profound developmental impairment in each domain (see Table 1 for scoring details). The score was applied only to individuals of at least 3 years of age at the time of the study. The relationship between epilepsy course and development for each individual was evaluated by assessing the correlation between the total *STXBP1_DevScore* and its subscores with age at seizure onset, epilepsy outcome (seizure

Figure 1 *STXBP1* Variants Over Exons and Linear Protein Structure



free vs active epilepsy), age at seizure freedom (if any), and epilepsy duration.

Patients who were not seizure free at the last visit were defined as having active epilepsy. In this group, epilepsy duration was defined as time from the first seizure to the last examination. Similarly, to analyze the correlation between *STXBP1*_DevScore and age at seizure offset, patients with active epilepsy were included, using their age at last examination as age at last seizure.

Statistical Analysis

Statistical analyses were performed with one-way ANOVA or 2-way Student *t* test, using Prism GraphPad software. Correlation analysis for *STXBP1*_DevScore was performed using the R Statistical Package. Statistical significance was reported with a *p* value cutoff of 0.05.

Data Availability

Anonymized individual clinical data that are not published within this article will be made available by request from any qualified investigator.

Results

We collected data from 48 individuals (18 females, 38%) with de novo *STXBP1* variants and a history of epilepsy (eTable 2, links.lww.com/NXG/A522), including 12 individuals previously reported in the literature (see references 6, 13–16). The mean age at inclusion was 8.5 years (range: 10 months–35 years). Three individuals deceased between age 21 months and 11 years because of intractable seizures and respiratory complications.

Genotypic and Phenotypic Spectrum in *STXBP1*-DEE

Genetic Findings

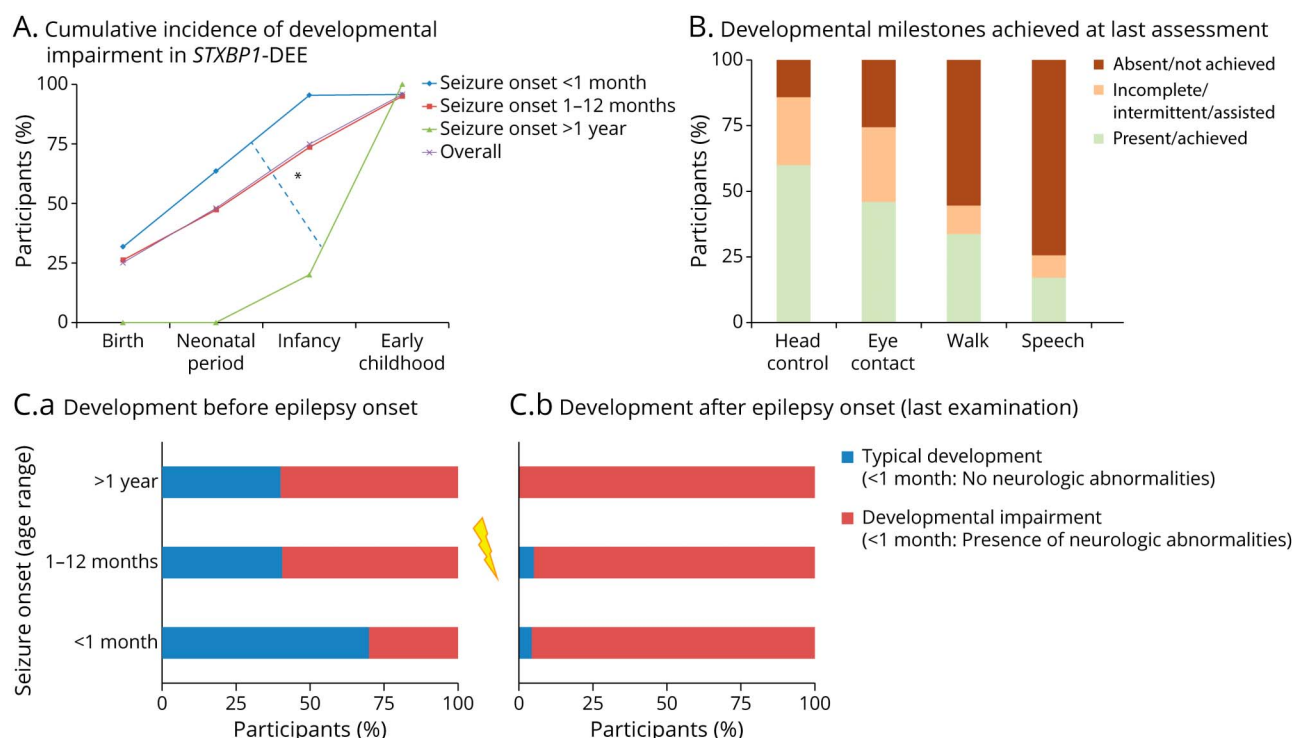
We identified 38 unique de novo *STXBP1* heterozygous variants (16 missense variants, 41%), with eight novel variants (Figure 1, in red). The variants were distributed across all the functional domains of *STXBP1* protein, with distinct recurrent variants: p.Arg406Cys (4 individuals; 8%), p.Arg406His (3 individuals; 6%), del 9q33.3–34.11 (3 individuals; 6%), p.Arg551Cys/His/Leu (3 individuals; 6%), p.Ile19_Lys20delinsMet (2 individuals; 4%), and c.578+1G>A (2 individuals; 4%).

Epilepsy Phenotypes and Histories

No family history for epilepsy or neurocognitive deficits was reported in 27 individuals (56%), whereas 6 (13%) reported a family history of seizures (two in first-degree relatives). The family history was unknown for the remaining 15 individuals.

All 48 individuals had a history of seizures, with a median onset of 1 month (range 1 day–6 years) (Table 2). Seizure types at onset consisted mostly of focal motor seizures (31 individuals; types reported: tonic-clonic, myoclonic, focal to bilateral tonic-clonic) and spasms (14 individuals). Focal nonmotor seizures with impaired awareness and atypical absences were also present at onset in four individuals. Seizures presented mostly at daily frequency, with multiple seizures per day, and occurred in clusters in 12 individuals (25%). During the disease course, the patients developed spasms or different types of focal motor seizures and generalized onset motor seizures. Status epilepticus was reported in three individuals (6%), two at seizure onset and 1 after 2 years following onset.

Figure 2 Neurodevelopmental Features in Individuals With *STXBP1*-DEE Stratified per Age Range at Seizure Onset



(A) Cumulative incidence of neurodevelopmental impairment from birth to early childhood in the overall cohort ($n = 48$) and per age at seizure onset (<1 month $n = 23$, 1–12 months $n = 20$, >1 year $n = 5$). $*p = 0.033$, one-way ANOVA. (B) Developmental milestones achieved at last assessment in $n = 36$ individuals with *STXBP1*-DEE ≥ 3 years old (median age 8.35 years, range 3–35 years). (C.a) Development before epilepsy based on age range at seizure onset: neurologic abnormalities before epilepsy onset were evident in 7/23 individuals (30%) with seizure onset <1 month; signs of developmental impairment before epilepsy onset were evident in 12/20 (60%) of patients with seizure onset between 1 and 12 months and in 3/5 (60%) of patients with seizure onset >1 year. (C.b) Development after epilepsy onset based on age range at seizure onset: only 2/48 individuals (4%) did not present with impaired development after epilepsy onset at the last examination (at 10 and 13 months).

At the time of the study, 17 individuals (35%) achieved seizure freedom, and antiseizure medications (ASMs) were discontinued in 11 individuals (23%). The median duration of seizure-freedom was 48 months (range 3 months–11 years). Most of these individuals became seizure-free within the 1st year of life (14; 82%). The median epilepsy duration in this latter group was 6.5 months (range: 0.3–11.7 months). Individuals with later remission had a median duration of 13 years (range: 2–31 years).

Thirty-one individuals (65%) had active epilepsy at inclusion (median follow-up of 5.4 years, range: 10 months–28 years). Seizure frequency at last follow-up remained daily in 16 individuals (33%); however, it decreased to weekly or monthly in the remainder of the cohort with active epilepsy. Six (13%) individuals were seizure free for at least 1 year (median 22.5 months, range: 12–60 months) before seizure recurrence.

Antiseizure medications ranged from 1 to 8 different drug trial(s) per individual. At last follow-up, 22 patients were still on polytherapy treatment. The most used ASMs were phenobarbital (24 individuals, 50%), valproate (20; 42%), vigabatrin (15; 31%), adrenocorticotropic hormone (ACTH) (13; 27%), pyridoxine (14; 31%), levetiracetam (15; 31%), benzodiazepines (11; 23%),

topiramate (11; 23%), and carbamazepine (11; 23%). One individual (patient 8) underwent resective epilepsy surgery with a dramatic benefit on seizure frequency (further discussed in the following section).

EEG with burst suppression at seizure onset was reported in 16 individuals (33%) and hypsarrhythmia was reported in 3 (6%). Sixteen individuals had focal or multifocal epileptiform discharges. Last EEG was abnormal in 33 individuals (abnormal background activity, with or without focal or multifocal paroxysmal activity), whereas it was reported to be almost unremarkable in 5 individuals and was not available in 10.

Neurologic Status and Brain Imaging

We observed a range of common neurologic features in our cohort of 48 individuals with *STXBP1*-related disorders and epilepsy. At last follow-up (mean age 8.5 years), almost half of the patients (21 individuals, 46%) presented with hypotonia, both axial or generalized, or associated with distal hypertonia; 11 individuals (23%) presented with tetraplegia or tetraparesis, both spastic or flaccid (mean age 8.7 years). Ataxia was reported in 5 individuals. Other neurologic features were observed including tremors, erratic eye movements, nystagmus, severe dystonia,

Table 2 Epilepsy Course in Individuals With *STXBPI* Disorders Grouped by Age at Seizure Onset

	Seizure onset (age range groups)			Whole cohort
	<1 mo	1–12 mo	>12 mo	
Seizure onset (individuals, %)	23, 48%	20, 42%	5, 10%	48
Age at seizure onset: median (range)	7.5 d (1–25 d)	2 m (1–11 m)	2.8 y (1.3–6 y)	1 m (1 d–6 y)
Seizure remission (individuals, %)	8, 33%	9, 45%	0%	17, 35%
Age at seizure remission: median (range)	7 m (0.75–12 m)	12 m (1.96 m–31 y)	—	7.5 m (21 d–31 y)
Epilepsy duration: median (range)	7 m (0.75–11 m)	7 m (0.8 m–31 y)	—	7 m (10 d–31 y)

dyskinesia, dysarthria, myoclonus, and choreoathetosis. High pain threshold was reported by caregivers in one patient. Three individuals (6%) were reported with postnatal microcephaly. In 5 individuals, neurologic examination was unremarkable. Fifteen individuals (31%) presented with motor stereotypies, involving mainly the hands, and oral stereotypies and stereotypies, involving the head. Nine individuals (19%) had autistic traits, 3 (6%) had hyperactivity, and 2 (4%) presented with wake bruxism. Oppositional and self-aggressive behaviors were reported in 2 individuals. In 5 individuals (10%), no behavioral concerns were reported. Ten individuals (21%) presented sleep disturbances. ID of variable degree was observed in all individuals aged over 6 years (23, 48%): severe in 17 (74%), mild in three (13%) and profound in three (13%). Among the individuals <6 years old (25; 52%), only two (8%) showed no signs of cognitive delay, whereas three (12%) showed mild delay, three (12%) moderate, 17 (68%) severe, and 1 (4%) profound delay.

Brain MRI was unremarkable in 25 (52%) individuals and revealed mild cortical atrophy in seven individuals (15%), thin corpus callosum in seven (15%), and hypo-/delayed myelination in four (8%) individuals. Additional findings included focal hyperintensities in temporal subcortical white matter, reduced volume of cerebellar hemisphere, basal ganglia hyperintensity, arachnoid cyst, temporal focal cortical dysplasia (FCD) IB and mesial temporal sclerosis (FCD IIIA), and thickening of the fusiform gyrus.

Genotype-Phenotype Correlation

We compared the electroclinical phenotypes of individuals carrying the same *STXBPI* genotype. Four individuals were found to carry the recurrent variant p.Arg406Cys and three the variant p.Arg406His. All but one individual with these variants had severe phenotypes with early-onset seizures. The only exception was a single individual with childhood-onset seizures and severe ID. Three individuals were identified with variants affecting the p.Arg551 hotspot, including p.Arg551-Cys, p.Arg551His, and p.Arg551Leu. All individuals had infantile seizure onset (range 10–16 months). Two individuals with p.Ile19_Lys20delinsMet had late seizure onset (11 and 17 months), no seizure remission, and mild to moderate ID; however, both acquired the ability to walk and had simplified language. Furthermore, neuroimaging performed during

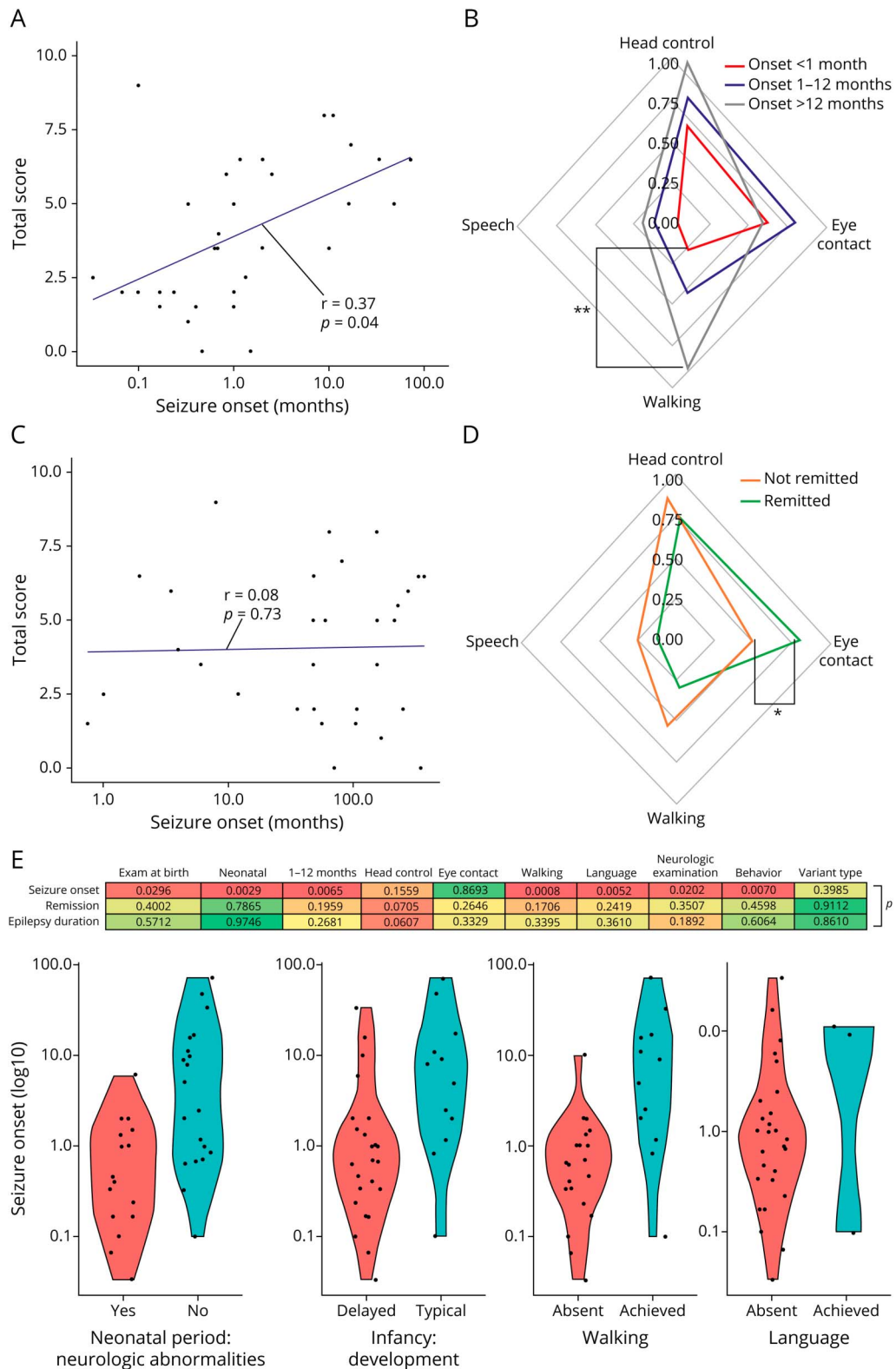
childhood was abnormal in both individuals, indicating left temporal pole FCD IB and left mesial temporal sclerosis (FCD IIIa) in one, and T2-weighted focal hyperintensities in the subcortical white matter in temporal poles and smaller size of the left cerebellar hemisphere in the other. The individual with FCD IIIa underwent a lobectomy of the left temporal lobe at 3 years of age and had a dramatic reduction of seizure frequency (from daily seizures to monthly) and improvement of development; the *mTOR* pathway genes panel performed on the resected tissue was negative. Recurrent c.875G>A (p.Arg292His) was present in two individuals: both had infantile spasms starting in the first month of life, severe developmental delay, and sleep disturbances.

Finally, we report monozygotic twins with a de novo *STXBPI* pathogenic variant c.578+1G>A (splice site variant in exon 8 GT donor site) and parallel phenotypes and disease course. At 19–20 days after birth, both siblings had neonatal focal motor tonic and myoclonic seizures, with daily frequency, and bilateral tonic-clonic seizures during follow-up. Both achieved seizure remission at 3 months and remained seizure free until 2.5 and 4 years, when seizures relapsed. Their examination at birth was unremarkable, but development did not progress during infancy. At the last follow-up (4 years of age), both twins had severe developmental delay: they achieved head control but were nonambulatory and nonverbal, and eye contact was intermittent. Both also presented with hypotonia and stereotypies.

Developmental Trajectories in *STXBPI*-DEE

In our cohort, 46 individuals (96%) with *STXBPI*-DEE displayed a clinically evident developmental impairment by early childhood (Figure 2A). Twelve individuals (25%) showed an abnormal examination at birth, with hypotonia or jerky movements, and feeding difficulties. In individuals ≥ 3 years old (36, 75%), we assessed the developmental milestones at the last examination (median age 8.35 years, range 3–35 years) (Figure 2B). Head control was complete in 22 individuals (61%), incomplete in eight (22%), and not achieved in five (14%). Eye contact was present in 16 individuals (44%); it was intermittent in 10 (27%) and absent in nine individuals (25%). Twelve individuals (33%) could walk autonomously, four (11%) with assistance, and 20

Figure 3 Impact of Epilepsy on Development in Individuals With *STXBP1*-DEE



(A) *STXBP1*_DevScore distribution in $n = 36$ individuals with *STXBP1*-DEE ≥ 3 years old (median age 8.35 years, range 3–35 years) based on age at seizure onset (log₁₀ scale). Seizure onset <1 month ($n = 15$) 1–12 months ($n = 16$), >12 months ($n = 5$). (Pearson correlation coefficient). (B) Developmental milestones subscores (mean) stratified per age range at seizure onset. $**p = 0.0049$, one-way ANOVA. (C) *STXBP1*_DevScore distribution based on age at seizure offset or age at last examination in the case of active epilepsy (log₁₀ scale). Active epilepsy ($n = 23$), seizure free ($n = 13$) (Pearson correlation coefficient). (D) Developmental milestones subscores (means) stratified per epilepsy outcomes. Active epilepsy ($n = 23$), seizure free ($n = 13$). $*p = 0.0348$. (E) *STXBP1*_DevScore domain correlations with seizure onset, offset, and epilepsy duration. Violin plots show only significant correlations (Wilcoxon rank-sum test).

(56%) were not able to walk at the time of the last assessment. Similarly, six individuals (17%) acquired the ability to say a few meaningful words, and three individuals (8%) could say short sentences.

Because of the observed variability of developmental and epileptic outcomes, we asked whether epilepsy had an impact on development in individuals with *STXBPI*-DEE. When the individuals were grouped based on their age at seizure onset (<1 month, 1–12 months, and >12 months), the cumulative incidence of signs of neurodevelopmental impairment over time was different between seizure onset groups, especially between seizure onset <1 month and >12 months ($p = 0.033$, one-way ANOVA) (Figure 2A).

Next, we analyzed development before and after epilepsy onset. Development before epilepsy onset was referred to be typical (or with no neurologic abnormalities in the neonatal period) in 26 individuals (54%), whereas 22 (46%) showed developmental abnormality (or neurologic abnormalities in the neonatal period), independently from the age at seizure onset (Figure 2C, left).

After epilepsy onset, most individuals presented impaired development at last examination (Figure 2C, right). Two individuals with seizure onset at 0.3 and 10 months did not show signs of delay at the last follow-up (10 and 13 months).

We used the *STXBPI*_DevScore, a disease-specific scoring system, to assess the differences in development across individuals. We assessed development across various domains (see Table 1). Individuals with typical development or no abnormalities in each of the domains were assigned a score of 1, whereas individuals with observed delay or abnormalities were assigned a score of 0 or 0.5. We applied the *STXBPI*_DevScore only to the 36 individuals aged a minimum of 3 years old at the time of the study. The median overall *STXBPI*_DevScore in these individuals was 3.5 (range: 0–9).

We observed a correlation between age at epilepsy onset and *STXBPI*_DevScore ($p = 0.03$, Pearson correlation coefficient, Figure 3A), suggesting that the developmental trajectories and outcomes are more favorable when epilepsy onset is after 12 months. This correlation was evident also in the developmental milestones achieved by the different ages at seizure onset groups (Figure 3B), with a prominent difference in the ability to walk between individuals with seizures onset <1 and >12 months ($p = 0.0049$, one-way ANOVA).

We then analyzed the impact of epilepsy outcomes in neurodevelopment. No correlation was observed between *STXBPI*_DevScore and age at seizure offset ($p = 0.64$, Pearson correlation coefficient) (Figure 3C) or the epilepsy duration ($p = 0.86$, Pearson correlation coefficient, data not shown). However, when assessing the duration in individuals with active epilepsy, the age at last evaluation was used, and we acknowledge that the absence of a correlation

between development and epilepsy duration can be limited. Similarly, no prominent differences were found between individuals with seizure remission and individuals with active epilepsy regarding head control, walking, and speech milestones. However, we noticed a difference ($p = 0.0348$, t test) in eye contact, being more present in individuals who achieved seizure remission (Figure 3D).

Finally, we analyzed the correlation between all domains of the *STXBPI*_DevScore and age at seizure onset, seizure remission, and epilepsy duration (Figure 3E). Seizure onset was significantly correlated with an abnormal examination at birth, presence of neurologic abnormalities in the neonatal period and of signs of delay in infantile period, walking and speech ability, abnormal neurologic examination, and behavioral abnormalities (Wilcoxon rank-sum test). Age at seizure remission and epilepsy duration did not show any significant correlation with any of the *STXBPI*_DevScore domains. No correlation was observed between the variant type (missense vs others) and seizure onset, offset, or epilepsy duration.

Discussion

We report detailed phenotypic data and developmental trajectories of a cohort of 48 individuals with *STXBPI*-related epilepsy. The epileptic phenotype in our *STXBPI* cohort shows considerable variability in seizure types and onset. One-third of individuals became seizure free and most of them (76%) within the first year of life. We did not identify any prognostic factors regarding epilepsy offset. About half of the individuals showed developmental impairment before epilepsy onset.

The most common first-line ASMs, including phenobarbital, reflected the predominant neonatal-infantile seizure onset in individuals with *STXBPI* variants with no superiority of one specific ASM or ASMs combination. Dramatic efficacy of levetiracetam has been reported,^{17,18} given the specific mechanism of action of this drug, which binds SV2A and modulates the neurotransmitter release system. However, we were not able to confirm this finding in our cohort.

We assessed the impact of epilepsy on developmental outcomes in our cohort using a composite developmental score, *STXBPI*_DevScore. The developmental milestones that could be achieved were very limited for most individuals, with speech being the domain with the greatest observed delay and impairment. When we stratified development based on age at seizure onset, we observed an almost direct proportionality: patients with later seizure onset have more favorable developmental outcomes, especially when assessing the ability to walk.

When we analyzed the impact of epilepsy remission on developmental outcomes, we found little difference between individuals with remitted epilepsy and with active epilepsy. The individuals with later epilepsy onset still had seizures at

the time of the study, which affected their ability to make eye contact, which in this case is regarded as a trait associated with autistic behavior, rather than a visual engagement defect. However, our observation may suggest a greater frequency of features associated with autism in individuals with *STXBPI*-DEE and active epilepsy. Thus, seizure control could have a beneficial impact on behavioral and interactive skills despite not having an impact on global development. We did not analyze the social interaction in our cohort, but a recent study¹⁹ showed that social motivation is present in greater frequency in the *STXBPI* cohort than in mixed ID cohorts; therefore, the precise genetic etiology may be a discriminating factor in behavioral features.

These observations provide evidence that age at epilepsy onset but not epilepsy outcome correlates with neurodevelopmental outcome in *STXBPI*-DEE. We were not able to conclude whether the relationship between age at epilepsy onset and development stands as a causal relationship or a contributing factor or whether there is a genetic basis for the difference in baseline development. These conclusions are limited by the number of individuals in this cohort and by the absence of a control group of individuals with *STXBPI*-related disorders without epilepsy (estimated 5% of the reported cases in the literature⁶).

The *STXBPI*_DevScore was elaborated for this study to compare the development of individuals with *STXBPI* using a standardized framework that integrates developmental trajectories with developmental outcomes. Disease-specific scoring systems have been elaborated for other rare disorders based on the need for an internal control (e.g., Aicardi-Goutieres syndrome,²⁰ Batten disease,²¹ SMA,²² and Niemann-Pick type C²³). As different genetic disorders and DEEs can have unique natural disease courses, a distinct scale system that assesses development across various domains within *STXBPI* disorders is especially critical to ensure that meaningful differences such as the acquisition of certain skills are captured between individuals with *STXBPI* variants. The *STXBPI*_DevScore is not intended for clinical and diagnostic use. However, we aim to further develop and validate this framework in prospective studies, adding more granularity and specificity to each domain, including metrics to measure cognition.

Nevertheless, the results of this study suggest the existence of distinct subgroups in the *STXBPI* population that vary with regard to their epilepsy course, developmental trajectories, and outcomes; these phenotypic groups should be further investigated in the context of natural history studies.

We report the presence of brain MRI abnormalities in individuals with *STXBPI* variants and epilepsy. One patient with FCD IB underwent a successful lobectomy, and, notably, the *mTOR* gene panel on resected tissue resulted negative. A similar individual was reported with FCD IB and a germline *STXBPI* variant and lesional mosaicism of heterozygous and homozygous *STXBPI* variants; however, *mTOR* analysis was not performed.²⁴ Another case report described a patient with

FCD IA,²⁵ who benefited from surgery, but genetics was not performed on the resected tissue. A casual co-occurrence of the two conditions cannot be excluded. However, *STXBPI* may have a role in neuronal maturation and migration, especially radial migration.^{26,27} Therefore, a genetic diagnosis should not exclude epilepsy surgery evaluation in individuals with predominant focal electroclinical features. Fundamental research should address the hypothesis of the role of *STXBPI* in corticogenesis as a mechanism of neurodevelopmental disorder.

We also report two monozygotic twins with the c.578+1G>A variant. One other individual with c.578+1G>A variant and infantile-onset epileptic encephalopathy is reported in the literature.²⁸ The similarity of the phenotypes and the course of the disease between the two siblings, but not in the other reported case, points to shared modifier factors in the underlying genetic architecture that play a role in the phenotypic variability of *STXBPI* phenotypes.

Two probands of our cohort have a positive family history for seizure in one first-degree relative, but the segregation analysis confirmed a de novo variant in both cases. Although nearly all disease-causing *STXBPI* variants are de novo, mosaicism was reported in one family.²⁹ An autosomal recessive mechanism was described in one family with unaffected heterozygote members and affected siblings with homozygous variant in *STXBPI*, with an apparent gain-of-function effect on release probability and synaptic transmission.³⁰ Thus, these very rare cases should be taken into account during genetic counseling when discussing potential transmission risk.

Genotype-phenotype correlations seem to be limited or absent in our *STXBPI* cohort, as previously reported in the literature.⁶ The identified variants span all domains of *STXBPI*, with no preference of specific variant types for distinct domains. No significant differences were found in individuals with missense variants compared with all the other variants (stop, indel, frameshift, and splice site) regarding epilepsy onset, remission, and duration, suggesting that missense variants are equally disruptive for *STXBPI* protein function. A recent study used a computational framework to analyze the phenotypic landscape of >500 individuals with *STXBPI*-related disorders, being the most extensive analysis to date.³¹ The study shows that protein-truncating variants and deletions in *STXBPI* were more phenotypically similar compared with missense variants; furthermore, no significant phenotypic similarity was identified in the major recurrent variants in *STXBPI*. These findings confirm the complexity of *STXBPI*-related disorders.

The involvement of genetic modifiers or epigenetic factors might determine the expressivity of the disease, as suggested in other genetically determined epilepsies.^{32,33} One possible way to dissect the underlying causes of heterogeneity would be to look for common variants in other genes and/or regulatory regions in *STXBPI* individuals. Another important point is the possible emergence of age-dependent differences in individuals with different variants^{31,34}; therefore, prospective evaluation and adult studies are crucial as they

might highlight the presence of distinct natural histories in this condition.

STXBPI is one of the most common genes implicated in DEEs.³⁵ In adults with epilepsy and ID, *STXBPI* is the 3rd genetic diagnosis.³⁶ The frequency of *STXBPI* variants and the life-long clinical impact in individuals with *STXBPI*-related disorders call for a targeted therapy approach. Insights into possible targeted interventions have been recently given, with different approaches ranging from chemical chaperones^{37,38} to micro-RNAs modulation³⁹ that will likely be available for human trials in the upcoming few years. However, as the paradigm of clinical trials is changing for rare disorders and novel therapies, there is a need for studies leveraging longitudinal data for therapeutic end points that include cognitive and behavioral features, beyond epilepsy, and that are tailored to the individuals with *STXBPI*-related disorders.

The major limitations of our study include selection bias toward individuals with epilepsy, the limited number of individuals recruited, and the retrospective nature of data, which restricted some analyses to the evaluation of neurodevelopment and of epilepsy duration based on ages at last assessment. Nevertheless, the standardized data collection and the use of *STXBPI*_DevScore allowed us to address the heterogeneity in the retrospective data concerning neurodevelopment and, finally, to compare epilepsy and developmental trajectories of different individuals and to identify meaningful correlations.

Disease-causing variants in *STXBPI* lead to a severe neurodevelopmental syndrome with epilepsy. However, the epilepsy history and developmental trajectories in individuals with *STXBPI*-DEE show diverse patterns of progression. A disease-specific composite score is, therefore, necessary to quantify the developmental trajectories among different individuals and to unravel the relationship between epilepsy and development. Age at seizure onset was the only epilepsy-related feature associated with the neurodevelopment outcome in *STXBPI*-DEE. These observations point toward a deep developmental impact of *STXBPI* variants that goes beyond the impact of concomitant drug-resistant epilepsy. Our findings can inform future dedicated natural history studies and trial design. Given future clinical trials, an extensive prospective evaluation of individuals with *STXBPI*-DEE should be set, including detailed neurocognitive and psychosocial evaluations at different stages, that (1) delineate the detailed natural histories of the disease, taking into account the variability of epilepsy and developmental outcomes in subgroups; (2) identify appropriate and beneficial end-points and windows for therapeutic interventions; and (3) specifically address the genetic causes of developmental variability in the *STXBPI* population.

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Disclosure

The authors report no disclosures relevant to this manuscript. Go to Neurology.org/NG for full disclosures.

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Appendix (continued)

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