RESEARCH ARTICLE

Natural History and Developmental Trajectories of Individuals With Disease-Causing Variants in *STXBP1*

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Abstract

Background and Objectives

Pathogenic variants in *STXBP1* are among the major genetic causes of neurodevelopmental disorders. Despite the increasing number of individuals diagnosed without a history of epilepsy, little is known about the natural history and developmental trajectories in this subgroup and endpoints for future therapeutic studies are limited to seizure control.

Methods

We performed a cross-sectional retrospective study using standardized questionnaires for clinicians and caregivers of individuals with *STXBP1*-related disorders capturing medical histories, genetic findings, and developmental outcomes. Motor and language function were assessed using Gross Motor Function Classification System (GMFCS) scores and a speech impairment score and were compared within and across clinically defined subgroups.

Results

We collected data of 71 individuals with *STXBP1*-related disorders, including 44 previously unreported individuals. Median age at inclusion was 5.3 years (interquartile range 3.5–9.3) with the oldest individual aged 43.8 years. Epilepsy was absent in 18/71 (25%) of individuals. The range of developmental outcomes was broad, including 2 individuals presenting with close to

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Glossary

GMFCS = Gross Motor Function Classification System; **HPO** = Human Phenotype Ontology; **IQR** = interquartile range; **OR** = odds ratio; **STXBP1** = syntaxin-binding protein 1.

age-appropriate motor development. Twenty-nine of 61 individuals (48%) were able to walk unassisted, and 24/69 (35%) were able to speak single words. Individuals without epilepsy presented with a similar onset and spectrum of phenotypic features but had lower GMFCS scores (median 3 vs 4, $p < 0.01$) than individuals with epilepsy. Individuals with epileptic spasms were less likely to walk unassisted than individuals with other seizure types (6% vs 58%, $p < 0.01$). Individuals with early epilepsy onset had higher speech impairment scores ($p = 0.02$) than individuals with later epilepsy onset.

Discussion

We expand the spectrum of *STXBP1*-related disorders and provide clinical features and developmental trajectories in individuals with and without a history of epilepsy. Individuals with epilepsy, in particular epileptic spasms, and neonatal or early-onset presented with less favorable motor and language functional outcomes compared with individuals without epilepsy. These findings identify children at risk for severe disease and can serve as comparator for future interventional studies in *STXBP1*-related disorders.

Introduction

STXBP1-related disorders lead to an increasingly recognized group of neurodevelopmental disorders with a broad phenotypic spectrum, including early-onset epileptic encephalopathies, movement disorders, and behavioral issues.[1-7] *STXBP1* encodes the syntaxin-binding protein 1 (STXBP1 or MUNC18-1), which is essential in the modulation of the soluble N-ethylmaleimide–sensitive factor attachment protein receptor complex during synaptic vesicle release through exocytosis.[8-10]

The first description of *STXBP1*-related disorders in 2008 was in individuals with early infantile developmental and epileptic encephalopathy,[11] previously classified as Ohtahara syndrome.[4] Since then, the phenotypic spectrum has largely expanded, and individuals with various epilepsy types and syndromes have been reported.[1-3,5-7,12] A recent study reviewed data from a large cohort and reported epilepsy in 89% of individuals.[12] While previous studies mainly included individuals with early-onset epilepsy syndromes,[7,13,14] little is known about the natural history and developmental trajectories of individuals without epilepsy compared with the broader *STXBP1* population. In a Danish cohort, the incidence of *STXBP1*-related encephalopathy was reported in 1.1/100,000 births.[7] However, the estimated incidence predicted by a gene-specific variant model was estimated higher (3.3–3.8/100,000 births), indicating that a large subcohort of *STXBP1*-related disorders may currently remain unidentified.[15] Considering the prevalence of 1.2% intellectual disability in developed countries and the increasing availability of exome-wide and genome-wide molecular testing, the number of individuals diagnosed with *STXBP1*-related neurodevelopmental disorders is likely to increase.[16,17] As the development of novel therapeutics rapidly progresses,[18,19] it is essential to define clinical endpoints beyond seizure control.

Here, we present systematic data on natural history and developmental trajectories of 71 individuals with disease-causing variants in *STXBP1*, including 18 individuals without a history of epilepsy. Hypothesizing that the presence of epilepsy may indicate a more severe disease burden, we aim to investigate the influence of epilepsy, epileptic spasms, and the age at epilepsy onset on developmental outcomes of individuals with *STXBP1*-related disorders.

Methods

Study Design, Setting, and Participants

Participants were referred from the German-speaking family association STXBP1 e.V. and from collaborating child neurologists in Germany, the Netherlands, Switzerland, Austria, and the United States. The German-speaking family association was chosen as patient advocacy group to assure recruitment of a representative number of individuals. The association has an interest in participating in clinical research to improve the knowledge of the condition. Physicians who were asked to participate were involved in previous collaborative research and known for their status as regional centers for *STXBP1*-related disorders. We sent questionnaires, focusing on current and retrospective clinical and diagnostic data, genetic findings, and developmental features, out to both caregivers and physicians caring for individuals with *STXBP1*-related disorders. All data were included in this study. The solicitation period was between February 2021 and September 2022. The only eligibility criterion was the diagnosis of a *STXBP1*-related disorder, and all recruited participants were included in the analysis. The study size was determined at the end of recruitment, with no predefined value. Selected genotypical and phenotypical data of 27 individuals from our cohort have been included in previous publications.[12,20,21] Reporting of this study complied with

Table 1 Demographic Data of the Cohort

Cases, n	71
Age at inclusion, y, median (IQR)	5.3 (3.5–9.3), n = 71
Sex, n (%)	
Female	29/71 (41)
Male	42/71 (59)
Age at symptom onset, mo, median (IQR)	2 (1–9), n = 61
Age at genetic diagnosis, mo, median (IQR)	29 (11.5–57.5), n = 71
Diagnostic delay, mo, median (IQR)	19 (6–51.5), n = 61
Epilepsy, n (%)	53/71 (75)
Epileptic spasms, n (%)	20/70 (29)
Generalized-onset epilepsy, n (%)	30/70 (43)
Focal-onset epilepsy, n (%)	39/70 (56)
NA, n (%)	1/70 (1)
Epilepsy onset	
Age at epilepsy onset, mo, median (IQR)	2 (1–7), n = 49
Neonatal onset (<1 mo), n (%)	12/49 (24)
Early onset (≥1 mo–2 y), n (%)	31/49 (63)
Late onset (≥2 y), n (%)	6/49 (12)
Seizure freedom at inclusion, n (%)	23/50 (46)
Age at seizure remission, mo, median (IQR)	16.5 (6.8–35.7), n = 20
Predicted protein function (n = 71), n (%)	
Protein loss	33 (46)
Residual protein function	38 (54)

Abbreviation: IQR = interquartile range.

Strengthening the Reporting of Observational studies in Epidemiology criteria.

Phenotypic Annotations and Clinical Assessment

We translated clinical information into Human Phenotype Ontology (HPO) terminology. HPO provides a standardized vocabulary to describe phenotypic abnormalities. The structure of the ontology is tree-like, and each phenotype has a more general phenotype superimposed on it so that phenotypes can be analyzed computationally and compared across the cohort. For example, the traits "Focal impaired awareness seizure" (HP:0002384) and "Focal clonic seizure" (HP:0002266) are both subordinate to the term "Focal-onset seizure" (HP:0007359), and this in turn is subordinate to the terms "Seizure" (HP:0001250) and "Abnormal nervous system physiology" (HP:0012638).[22] We combined the HPO terms "Abnormality of movement" and "Abnormal central motor function" to capture all motor and movement disorders. Children younger than 3 years were excluded from assessment of "Neurological speech impairment" (HP:0002167). A detailed list of all terms used along with an ontology tree capturing abnormalities of the nervous system and abnormalities of the musculature can be found in eTable 1 and eFigure 1 (links.lww.com/WNL/C957).

We assessed motor development based on (1) motor milestones such as head control, unassisted sitting, standing, and unassisted walking (for individuals aged 1.5 years and older) and (2) Gross Motor Function Classification System (GMFCS) scores (for children aged 3 years and older).

We analyzed expressive language assessing the ability to speak at least 1 word and to build 2-word combinations (for individuals aged 2 years and older). In addition, providers and caregivers were asked to assign a speech impairment score value to each individual aged 3 years and older based on a scoring system ranging from 0 (age-appropriate speech) to 4 (no consistent communication with caregivers).

We analyzed developmental outcomes within and across different subgroups comparing (1) individuals with and without a history of epilepsy and (2) individuals with epileptic spasms and individuals with other seizure types. In addition, we stratified developmental trajectories according to the age at epilepsy onset into (1) neonatal onset (first month of life), (2) early onset (second month of life until the end of second year of life), and (3) late onset (from the third year of life onward). One individual was excluded from the epilepsy subgroup analysis because of unknown seizure type. Epilepsy remission was defined as absence of seizures for at least 12 months.

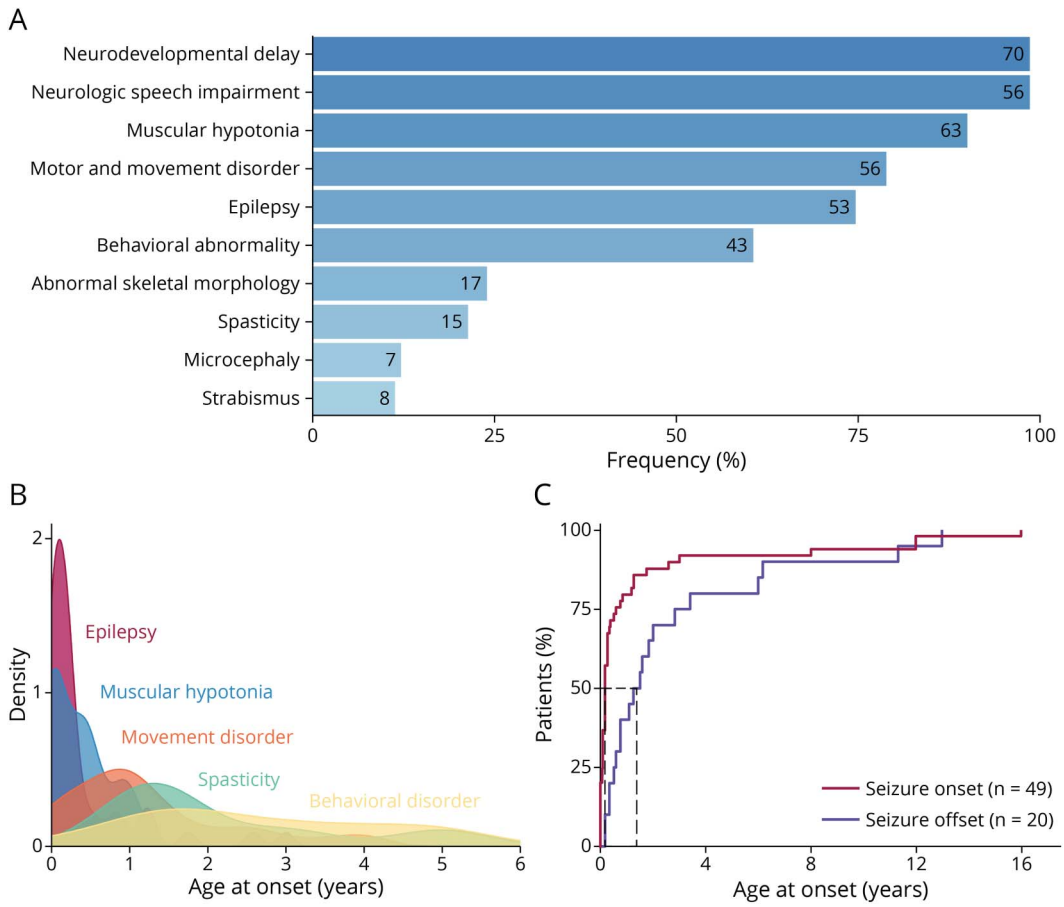
Statistical Analysis

Data analysis was performed using the R statistical framework.[23-26] Individuals with missing data were excluded from subgroup analyses, and sample sizes are indicated (n=) for each corresponding analysis. The Fisher exact test was used for comparison of the frequencies of clinical features. After testing for normal distribution of data by histogram visualization and the Shapiro-Wilk test, nonparametric tests were used to perform statistical analysis: The Exact Wilcoxon-Mann-Whitney test for comparison of subgroups related to seizure types and the Kruskal-Wallis test for comparison of epilepsy onset subgroups. Sensitivity analyses were not conducted. Reported p values were 2-sided, with $p \leq 0.05$ considered statistically significant. p values were adjusted for multiple hypothesis testing using the Bonferroni procedure holding a false discovery rate of 5%. The findings are presented as odds ratios (ORs) with 95% CIs.

Standard Protocol Approvals and Patient Consents

The study adheres to the principles set out in the Declaration of Helsinki. All individuals or guardians gave their informed consent before the questionnaires were sent out to caregivers and physicians. The study was approved by the ethics

Figure 1 Clinical Spectrum and Disease Course of *STXBP1*-Related Disorders



(A) Frequencies of reported Human Phenotype Ontology terms across the cohort. The absolute frequency of each term is indicated (n=). (B) Age at onset of typical symptoms including epilepsy (n = 49), muscular hypotonia (n = 47), motor and movement disorders (n = 39), spasticity (n = 6), and behavioral disorders (n = 25). (C) Age at onset of epilepsy (median 2 months, IQR 1–7 months, n = 49) and age at last seizure (epilepsy offset) (median 16.5 months, IQR 6.8–35.7 months, n = 20). IQR = interquartile range.

committee of the Medical Faculty of the University of Heidelberg (S-318/2018).

Data Availability

Anonymized data not published in this article can be made available on reasonable request from any qualified investigator.

Results

Demographic Data

We collected data of 73 individuals from 73 unrelated families. Two individuals with variants of unknown significance were excluded from the main analysis, and clinical information can be found in supplementary material (links.lww.com/WNL/C957). Thirty-eight of 61 (62%) of physicians and 46/73 (63%) of families who were asked to participate answered the questionnaires. We collected data from 27 questionnaires answered by physicians, 37 answered by caregivers and physicians, and 9 answered by caregivers with additional data from medical reports. All individuals were alive at time of study inclusion and had a median age of 5.3 years (interquartile range [IQR] 3.5–9.3 years) and a maximum age of 43.8 years. Twenty-nine of 71 individuals (41%) were female and 42/71 (59%) male. Median age of onset of first symptoms was 2 months (IQR 1–9 months, n = 61) followed by a median age of molecular confirmation of 29 months (IQR 11.5–57.5 months, n = 71), resulting in a median diagnostic delay of 19 months (IQR 6–51.5 months, n = 61) (Table 1).

No Apparent Genotype-Phenotypic Associations Regarding Clinical Spectrum and Developmental Trajectories

Genetic testing was performed using either single, panel or (trio-)exome sequencing. All individuals included in the analysis had a likely pathogenic or pathogenic variant in *STXBP1*. Comprehensive data on variant classifications can be found in eTable 2 (links.lww.com/WNL/C957). Individuals carried 58 unique variants in *STXBP1*, including 38 missense (54%), 13 splice-site (18%), 9 frameshift (13%), 7 nonsense variants (10%), and 4 larger deletions (6%). In 51 families, segregation analysis was performed, resulting in the

Table 2 GMFCS and Speech Impairment Score

GMFCS score (n = 39)		
Score	**Criteria**	**n (%)**
0	Unrestricted and age-appropriate motor skills present.	1 (3)
1	Free walking in all everyday situations. Climbing stairs possible without holding on. Running and hopping is limited due to balance and coordination problems.	1 (3)
2	Free walking is possible in most environments. Difficulties exist on uneven ground or inclines. Stair climbing is only possible with holding on. Running and hopping is only possible with major problems or no longer possible.	9 (23)
3	Free walking is only possible with walking aids. Climbing stairs is possible only by handrail or with assistance. For longer distances an active wheelchair is used.	5 (13)
4	In everyday life, a wheelchair is used that is moved with external assistance or, if necessary, with an electric drive. Locomotion without assistive devices is not possible.	13 (33)
5	Head position and trunk position can be maintained against gravidity if necessary. Aids are required for sitting and standing. Locomotion only with external assistance in wheelchair.	10 (26)
Speech impairment score (n = 57)		
Score	**Criteria**	**n (%)**
0	Age appropriate	1 (2)
1	In simple language, not age-appropriate	2 (4)
2	Using few words	13 (23)
3	Using gestures and sounds for communication	28 (49)
4	No communication	13 (23)

Abbreviation: GMFCS = Gross Motor Function Classification System.

detection of 42 de novo variants (82%) and 2 parental mosaics (4%). In 7 families (14%), the inheritance of only 1 parent was tested and found to be negative.

We did not find statistically significant differences between the genotype and clinical features or developmental trajectories in our cohort. Detailed information is given in the Supplementary analysis of genotype-phenotype associations, eFigures 2 and 3 (links.lww.com/WNL/C957).

Phenotypic Spectrum and Disease Course

Almost all individuals in our cohort had neurodevelopmental abnormalities (99%, n = 70/71), neurologic speech impairment (98%, n = 56/57), and muscular hypotonia (90%, n = 63/70). Motor and movement disorders were present in 56/71 (79%) individuals, occurring at a median age of 1 year (IQR 0.5–1.5, n = 39). The most frequent symptoms in this category were involuntary movements, such as tremor and motor stereotypies (49%, n = 35/71) as well as ataxia (71%, n = 49/69). Behavioral abnormalities were present in 43/71 (61%) of individuals occurring at a median age of 3 years (IQR 1.5–4 years, n = 25) and including bruxism (26%, n = 17/65), a short attention span (24%, n = 17/71), and autistic behavior (27%, n = 19/71). Eight of 71 individuals (11%) showed aggressive behavior occurring at a median age of 13 years (IQR 10.1–13.5 years, n = 4).

Data regarding the presentation of *STXBP1*-related disorders during pregnancy and during birth and neonatal period are given in eTable 3 (links.lww.com/WNL/C957).

Fifty-three of 71 individuals (75%) had a history of epilepsy, including 20/70 individuals (29%) with epileptic spasms. Thirty-nine of 70 individuals (56%) had focal-onset seizures, and 30/70 individuals (43%) had generalized-onset seizures. The median age at epilepsy onset was 2 months (IQR 1–7 months, n = 49) with the latest epilepsy onset occurring at 16 years in 1 individual. Most individuals (70%, n = 33/47) had a maximum seizure frequency of more than 5 seizures per day, and 32/42 individuals (76%) received a rescue medication at least once in their disease course. At time of inclusion, 13/50 individuals (26%) had more than 1 seizure per week, while 23/50 individuals (46%) were seizure-free at a median age of 16.5 months (IQR 6.8–35.7, n = 20) (Figure 1).

Neurologic Development Varies in Children With *STXBP1*-Related Disorders

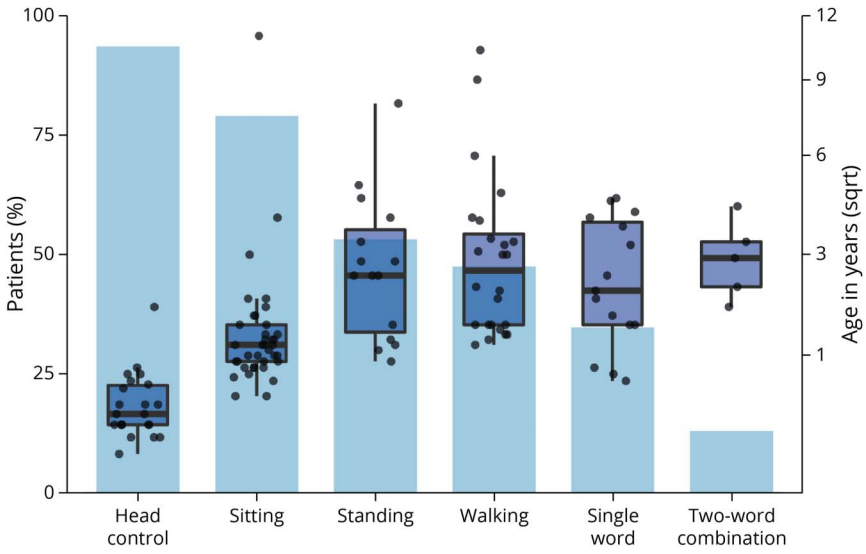
All but 1 individual (variant: p.[Lys425Arg]) had mild-to-severe neurodevelopmental delay (Table 2). This individual was diagnosed with attention deficits and had focal motor epilepsy at the age of 12 years. One other individual (variant: p.[Arg551Cys]) showed mild motor impairment (GMFCS 1) and was able to communicate with few words at the age of 7 years (speech impairment score 2). This individual had focal and generalized epilepsy between the third and seventh year of life. Additional symptoms included also motor and movement disorders, muscular hypotonia, and behavioral abnormalities.

In total, 49/62 individuals (79%) were able to sit unassisted at a median age of 1.2 years (IQR 0.9–1.5 years, n = 37), and 29/61 individuals (48%) were able to walk unassisted at a median age of 2.6 years (IQR 1.5–3.5 years, n = 24). Twenty-four of 69 (35%) individuals were able to speak 1 word at a median age of 2.2 years (IQR 1.5–3.9 years, n = 15) (Figure 2). Thirty-seven of 47 individuals (79%) living in Germany had an institutional certificate of disability, and 44/48 (92%) received long-term care allowance by the social system. Twenty-nine of 30 individuals (97%) attended a class for children with special needs.

Higher Functional Levels in Individuals Without Epilepsy

Comparing the age at study inclusion across the epilepsy subgroups, we did not find significant differences. The phenotypic spectrum and age at onset in individuals with and without a history of epilepsy did not differ significantly after

Figure 2 Developmental Milestones in Individuals With *STXBP1*-Related Disorders



The light blue bars indicate the percentage of patients who reached a given milestone including head control (94%, n = 58/62), sitting (79%, n = 49/62), standing (53%, n = 33/62), unassisted walking (48%, n = 29/61), speaking single words (35%, n = 24/69), and speaking 2-word combinations (13%, n = 8/61). The dark blue boxplots indicate the age when a given individual had reached a particular developmental milestone including head control (n = 22), sitting (n = 37), standing (n = 15), unassisted walking (n = 24), speaking single words (n = 15), and speaking 2-word combinations (n = 5). The age of achievement of motor milestones in our cohort was delayed compared with the ages given by the World Health Organization (sitting without support: mean [SD] = 6 months [1.1]; standing alone: mean [SD] = 11 months [1.9]; walking alone: mean [SD] = 12.1 months [1.8]).[36]

multiple testing using the Fisher exact test and the Exact Wilcoxon-Mann-Whitney test, respectively (eFigure 4, links.lww.com/WNL/C957). Individuals without a history of epilepsy had lower GMFCS scores (median: 3, n = 12) than individuals with epilepsy (median: 4, n = 27; Exact Wilcoxon-Mann-Whitney test: $p < 0.01$). The ability to walk unassisted was 4 times more likely to be achieved by individuals without a history of epilepsy (71%, n = 12/17) compared with individuals with epilepsy (39%, n = 17/44; OR 3.7, 95% CI 1–16; Fisher exact test: nominally significant, unadjusted $p = 0.04$). Individuals without epilepsy were 2 times more likely to speak a single word (44%, n = 8/18) than individuals with epilepsy (31%, n = 16/51; OR 1.7, 95% CI 0.5–6; Fisher exact test: unadjusted $p = 0.39$) (Figure 3). None of the individuals without epilepsy (n = 15) were able to communicate age-appropriate or with simple language (speech impairment score 0 or 1) in comparison with 3/42 individuals with epilepsy (7%; OR inf, 95% CI 0.1–inf; Fisher exact test: $p = 0.56$).

Epileptic Spasms Are Associated With More Severe Developmental Impairment

We stratified developmental trajectories in our cohort depending on the presence of epileptic spasms compared with other types of seizures. Individuals with epileptic spasms (n = 11) had higher median GMFCS scores (5 vs 4) than individuals with other seizure types (n = 20) and were less likely to achieve the ability to walk unassisted (6%, n = 1/16) compared with individuals with other seizure types (58%, n = 18/31; OR 0.05, 95% CI 0.4–0.001; Fisher exact test: significant after multiple testing, unadjusted $p < 0.01$).

The ability to use a single word was 3 times more likely to be achieved by individuals without a history of epileptic spasms (42%, n = 15/36) compared with individuals with epileptic spasms (17%, n = 3/18; OR 3.5, 95% CI 0.8–22.1; Fisher exact test: unadjusted $p = 0.08$).

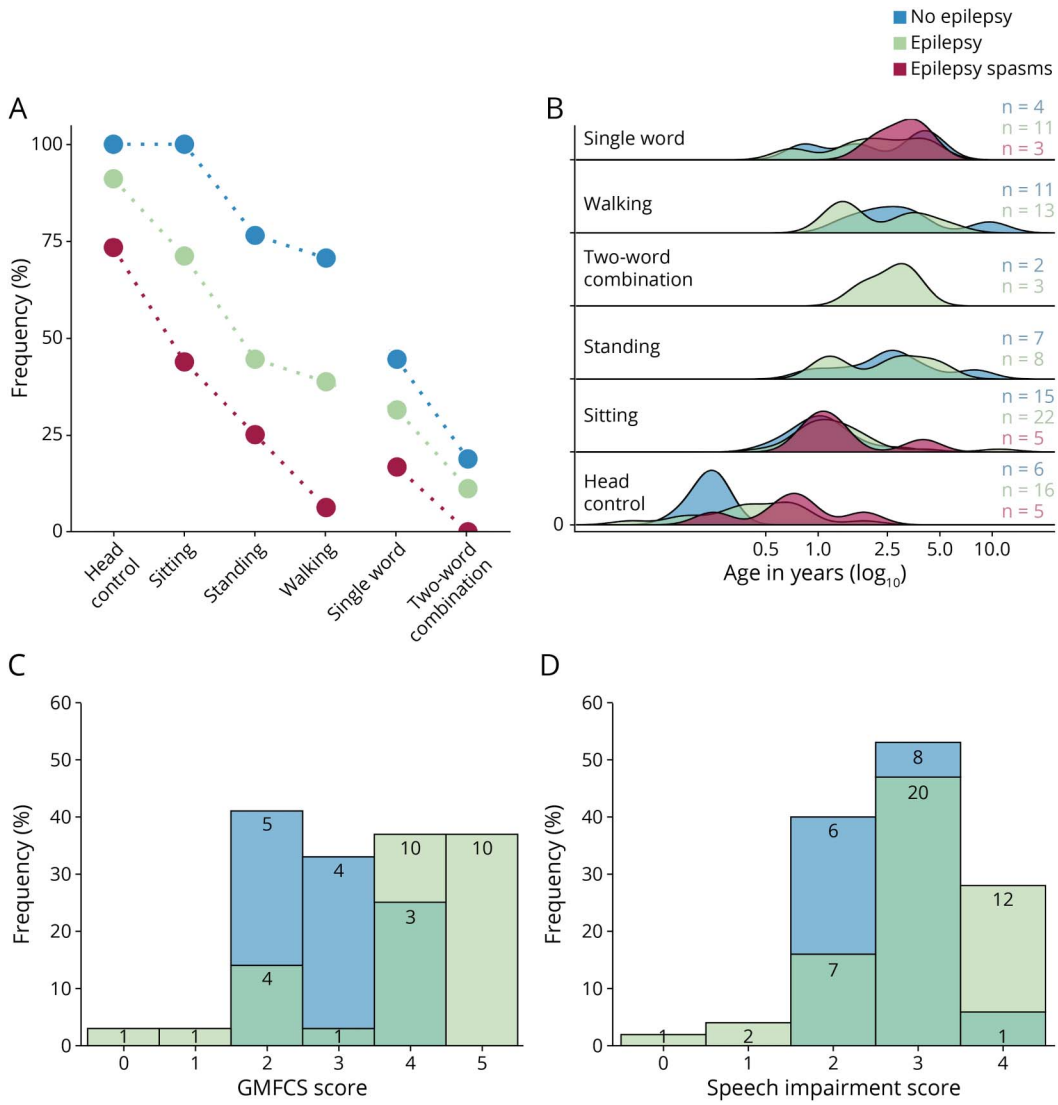
Individuals with (n = 15) and without epileptic spasms (n = 30) had each a median speech impairment score of 3. Three of 30 individuals (10%) without epileptic spasms were able to communicate age-appropriate or with simple language (speech impairment score 0 or 1) whereas none of the individuals with epileptic spasms reached this degree of communication (n = 0/15; OR 0, 95% CI 0–4.9; Fisher exact test: $p = 0.54$). Distribution of GMFCS scores and speech impairment scores are shown in eFigure 5 (links.lww.com/WNL/C957). The age at achievement of milestones did not differ significantly between epilepsy subgroups (Figure 3).

Impact of Age at Epilepsy Onset on Motor and Speech Development

We found that individuals able to walk unassisted had later epilepsy onset (median 5.8 months, n = 16) than individuals dependent on help for ambulation (median 1.5 months, n = 24; Exact Wilcoxon-Mann-Whitney test: $p = 0.01$). We found that individuals with neonatal epilepsy onset had higher GMFCS scores (median 4.5, n = 8) compared with individuals with epilepsy onset between the age of 2 months and 2 years (median 4) and individuals with epilepsy onset after the age of 2 years (median 2.5).

We found that individuals able to speak a single word had later epilepsy onset (median 5.8 months, n = 16) than nonverbal individuals (median 1 month, n = 31; Exact Wilcoxon-Mann-Whitney test: $p < 0.01$). Individuals who communicated age-appropriate or in simple language (speech impairment score 0 or 1) had a later epilepsy onset (median 10 months, n = 3) than individuals that used single words or nonverbal communication (median 2 months, n = 36). Individuals with

Figure 3 Developmental Outcome of Individuals Across Different Subgroups



Comparison of individuals with no history of epilepsy (in blue), individuals with epilepsy including focal-onset and generalized-onset seizures as well as epileptic spasms (in green), and individuals who had epileptic spasms (in red) regarding their developmental trajectories. (A) Frequency of individuals across subgroups achieving each milestone including head control ($n_{no\ epilepsy}$ = 17/17, $n_{epilepsy}$ = 41/45, n_{spasms} = 11/15), sitting ($n_{no\ epilepsy}$ = 17/17, $n_{epilepsy}$ = 32/45, n_{spasms} = 7/16), standing ($n_{no\ epilepsy}$ = 13/17, $n_{epilepsy}$ = 20/45, n_{spasms} = 4/16), walking ($n_{no\ epilepsy}$ = 12/17, $n_{epilepsy}$ = 17/44, n_{spasms} = 1/16), speaking a single word ($n_{no\ epilepsy}$ = 8/18, $n_{epilepsy}$ = 16/51, n_{spasms} = 3/18), and speaking 2-word combinations ($n_{no\ epilepsy}$ = 3/16, $n_{epilepsy}$ = 5/45, n_{spasms} = 0/18). (B) Age of achievement of milestones across epilepsy subgroups. (C) Distribution of GMFCS score rankings comparing individuals with and without epilepsy. Frequencies are relative within the subgroups; absolute number of individuals who had a certain score value are indicated on each bar. The distribution of GMFCS scores in individuals without epilepsy is shown in blue and the distribution of individuals with epilepsy in green bars. (D) Frequency of speech impairment score rankings comparing individuals with and without epilepsy. Absolute frequencies are indicated on each bar. The distribution of speech impairment scores in individuals without epilepsy is shown in blue and the distribution of individuals with epilepsy in green bars. GMFCS = Gross Motor Function Classification System.

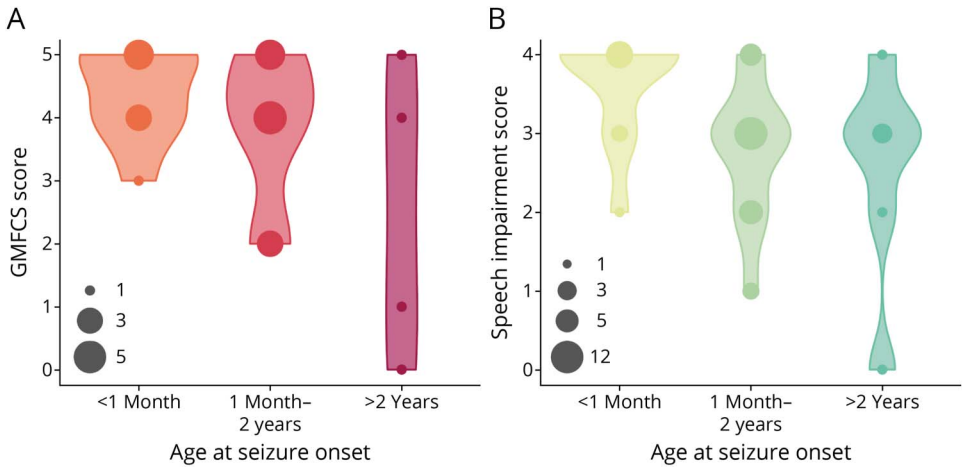
neonatal epilepsy had higher speech impairment scores (median 4, n = 10) than individuals with epilepsy onset between the age of 2 months to 2 years or after 2 years (median 3, $n_{2\ months–2\ years}$ = 23, $n_{after\ 2\ years}$ = 6; Kruskal-Wallis rank-sum test: p = 0.02). Distribution of GMFCS scores and speech impairment scores depending on age at epilepsy onset are shown in Figure 4.

No statistically significant correlations were found between neurodevelopmental outcomes and seizure freedom, age at epilepsy remission, and epilepsy duration.

Discussion

Since the first description of *STXBP1*-related disorders in individuals with Ohtahara syndrome, a broad phenotypic spectrum has been described in this increasingly recognized neurodevelopmental disorder. In this study, we aimed to delineate clinical and developmental outcomes given the known heterogeneity of developmental trajectories, stratifying patient cohorts by more precise information with regard to epilepsy histories, including type of epilepsy, onset, and duration. Owing to possible recruitment bias and a lack of

Figure 4 Distribution of Developmental Trajectories Across Different Epilepsy Onset Ranges



Individuals with a history of epilepsy were stratified with regard to seizure onset subgroups: neonatal onset (first months of life), early onset (second month of life until the end of second year of life), and late onset (from beginning of the third year of life). Point size indicates number of observations. (A) Distribution of GMFCS score across subgroups ($n_{<1\ m} = 8$, $n_{1\ m–2\ y} = 12$, $n_{>2\ y} = 4$). (B) Distribution of speech impairment score across subgroups ($n_{<1\ m} = 10$, $n_{1\ m–2\ y} = 23$, $n_{>2\ y} = 5$). GMFCS = Gross Motor Function Classification System.

population-wide studies, the estimated frequency of individuals without a history of epilepsy is likely higher than previously described[12] and will increase with the wider availability of genetic testing. As the development of therapeutic agents rapidly progresses, endpoints to measure outcomes beyond seizure freedom need to be defined. Here, we present the phenotypic spectrum, disease course, and developmental trajectories in a representative cohort of 71 individuals with *STXBP1*-related disorders, including 18 individuals without a history of epilepsy. Inclusion in our study was mainly performed through direct contact with affected families through parental groups in contrast with previous studies considering only individuals with epilepsy presenting in epilepsy clinics. This discrepancy may explain the higher rate of individuals without epilepsy, the higher rate of seizure freedom, and the different symptom correlations in our cohort.

Our study has several main findings: First, beyond epilepsy, *STXBP1*-related disorders are associated with 3 major clinical features—(1) muscular hypotonia; (2) motor, movement, and coordination disorders; and (3) behavioral abnormalities. Identified in most individuals, muscular hypotonia was more common in our cohort than previously reported (90% vs 27%).[7] In addition, more individuals in our cohort had motor and movement disorders than previously reported (79% vs 50%–60%).[27,28] The third major symptom group were behavioral abnormalities, which were present in 61% of individuals in our cohort. With a frequency of 26%, bruxism was a common clinical symptom in line with a previous study.[29] Whereas autistic features were reported in 27% of individuals, the frequency of aggressive behavior was low (11%), in line with a previous study reporting that individuals with *STXBP1*-related disorders have a specific behavioral repertoire setting them apart from individuals with neurodevelopmental disorders of other etiologies.[30]

We observed substantial differences in developmental outcomes across individuals in our cohort, including 1 teenager with epilepsy and reported age-appropriate development. The developmental trajectories delineated in our cohort by quantification of developmental milestones can serve as a comparator for future interventional studies (Figure 2). An important developmental aspect of *STXBP1*-related disorders was the limited verbal communication, which is more prominent compared with individuals with other neurodevelopmental disorders.[30] Given that most individuals had high speech impairment scores, future studies may aim to capture communication skills with more refined measurements to further stratify the observed differences within this patient group.

The second finding of our study is that while individuals without epilepsy present with similar clinical features, developmental delay was less severe compared with individuals with epilepsy. Individuals without epilepsy were more likely to achieve gross motor skills and verbal communication. In our cohort, individuals without epilepsy were 4 times more likely to reach independent walking. In line, the 2 individuals in our cohort with close to age-appropriate motor development and 2 previously reported individuals with age-appropriate development had no history of epilepsy or late-onset epilepsy only.[31,32] Because other symptoms were only assessed in a binary way, more subtle differences in presentation were not captured for all symptoms, such as the severity of tremor. Future studies may aim at quantifying the severity of symptoms identified in our study.

Although developmental outcomes in individuals without epilepsy were more favorable than in individuals with epilepsy, outcomes varied widely on an individual level. Thus, most individuals were dependent on help for ambulation and nonverbal. So far, developmental outcomes in *STXBP1*-related disorders without a history of epilepsy have been reported in only 20 cases: 10 of 17 individuals with available information on motor skills were reported to walk and 4/13 to communicate

verbally. Variable intellectual disability was reported, ranging from "moderate" to "severe profound."[5-7,21,27,32-34]

Third, we found that individuals with epileptic spasms and early epilepsy onset were more likely to display neurodevelopmental delay. Individuals with epileptic spasms were less generally likely to reach developmental milestones, ambulation, and verbal communication. In addition, individuals with early epilepsy onset showed a more severe impairment of speech and ambulation, in line with a recent study.[13,14] Again, in the subgroup of individuals with epilepsy, neurodevelopmental outcomes had a wide range: 12 of 13 individuals (92%) without consistent communication (speech impairment score 4) had a history of epilepsy, but the only individual with age-appropriate language abilities had late-onset epilepsy. The frequency of unassisted walking and the use of single words in individuals with epilepsy were in the previously reported range of 33%–47% and 15%–57%, respectively.[7,13,14,28,30]

In our cohort, mainly selected through referral from the parental group, one-half of individuals were seizure-free at time of inclusion, in contrast to more severely affected cohorts with epilepsy in most individuals.[13,14] In line with previous studies, we found no significant impact of epilepsy remission or epilepsy duration on developmental outcomes. To further understand the influence of epileptic spasms on developmental outcomes, future studies should aim to include the duration of epileptic spasms into the analysis. However, our observation supports that cognitive development is mainly determined by the underlying etiology, rather than the epilepsy and seizure burden.[13,14] Nevertheless, a shorter lead time to treatment and early response to treatment in epileptic spasms in general have been associated with improved developmental and epilepsy outcomes, in addition to seizure control[35] arguing for early initiation of the appropriate treatment for epileptic spasms in *STXBP1* encephalopathy, a recurrent cause of epileptic spasms.

This study has several limitations because of its design: First, the age at study inclusion (median: 5.3 years) resulted in an underrepresentation of adult phenotypes. However, because symptom onset in *STXBP1*-related disorders usually occurs in infancy and childhood, our cohort is expected to be representative also for the adult population of individuals with *STXBP1*-related disorders.[13] Second, in general, it is very difficult to collect data of a representative cohort in rare genetic diseases because often only individuals with severe phenotypes receive genetic testing. We aimed to address this issue by recruiting study participants through collaborating physicians from 2 different continents and 5 countries as well in cooperation with the German family association, which may mitigate the bias of specialty care recruitment. However, we had a response rate of 62% (physicians) and 63% (families), what might risk selection bias. Third, although our data are mainly based on questionnaires filled-in by physicians (n = 64), in some individuals (n = 9), only data from caregivers were available. Owing to its retrospective design, this study only reports descriptive associations, and no causal relations can be inferred. Because there are no data from prospective natural history studies, retrospective modeling is necessary to provide baseline information for future study designs and therapeutic endpoint measurements for ongoing individual treatments.

In conclusion, we provide clinical features and developmental outcomes of individuals with *STXBP1*-related disorders, including a comparison of individuals with and without epilepsy. While individuals with and without epilepsy presented with similar spectrum of symptoms, individuals without an epilepsy history had higher functional levels of motor development and verbal communication. In addition, individuals with epileptic spasms and earlier epilepsy onset were less likely to ambulate independently and to communicate verbally, emphasizing epileptic spasms as a prognostic factor in this patient cohort. As expanded genetic testing is likely to identify an increasing number of individuals with *STXBP1*-related disorders without epilepsy, information on endpoints provided by our study will be critical to assess the therapeutic efficacy of current and future interventions in *STXBP1*-related disorders.

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Disclosure

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