Natural History Study of STXBP1-Developmental and Epileptic Encephalopathy Into Adulthood

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ABSTRACT

Background and objectives: Pathogenic STXBP1 variants cause a severe early-onset developmental and epileptic encephalopathy (STXBP1-DEE). We aimed to investigate the natural history of STXBP1-DEE
in adults focusing on seizure evolution, presence of movement disorders and the level of functional (in)dependence.

**Methods:** In this observational study, patients with a minimum age of 18 years carrying a (likely) pathogenic *STXBP1* variant were recruited through medical genetics departments and epilepsy centers. Treating clinicians completed clinical questionnaires and performed semi-structured video examinations while performing tasks from the (modified) Unified Parkinson Disease Rating Scale when possible.

**Results:** 30 adult patients were included for summary statistics, with video recordings available for 19/30. Median age at last follow-up was 24 years (range 18-58 years). All patients had epilepsy, with median onset age of 3.5 months. At last follow-up, 80% of adults had treatment resistant seizures despite long periods of seizure freedom in 37%. Tonic-clonic, focal and tonic seizures were most frequent in adults. Epileptic spasms, an unusual feature beyond infancy, were present in 3 adults. All individuals had developmental impairment. Periods of regression were present in 59% and did not always correlate with flare-ups in seizure activity. 87% had severe or profound intellectual disability, 42% had autistic features and 65% significant behavioral problems. Video examinations showed gait disorders in all 12 patients able to walk including postural abnormalities with external rotation of the feet, broad-based gait, and asymmetric posture/dystonia. Tremor, present in 57%, was predominantly of the intention/action type. Stereotypies were seen in 63%. Functional outcome concerning mobility was variable ranging from independent walking (50%) to wheelchair dependence (39%). 71% of adults were non-verbal and all were dependent on caregivers for most activities of daily living.

**Discussion:** STXBP1-DEE warrants continuous monitoring for seizures in adult life. Periods of regression are more frequent than previously established and can occur into adulthood. Movement disorders are often present and involve multiple systems. While functional mobility is variable in adulthood, STXBP1-DEE frequently leads to severe cognitive impairments and a high level of functional dependence. Understanding the natural history of STXBP1-DEE is important for prognostication and will inform future therapeutic trials.
INTRODUCTION

Syntaxin binding protein 1 (STXBP1), Sec1/Munc18, encoded by the chromosome 9 located gene STXBP1, is a key regulator of synaptic vesicle docking and fusion through its interaction with the Soluble NSF Attachment Protein Receptors or SNARE-proteins. Normal functioning of STXBP1 is critical for neurotransmitter release at all synapses and essential for neuronal survival. Pathogenic genetic variants in STXBP1 were first identified in 2008 in patients with Ohtahara syndrome, a severe neonatal-onset developmental and epileptic encephalopathy (DEE). Subsequently, the phenotypic spectrum of STXBP1-developmental and epileptic encephalopathy (STXBP1-DEE) has significantly broadened including other DEEs such as West syndrome, Lennox-Gastaut syndrome, unclassified early infantile DEEs and, less frequently, neurodevelopmental disorders with infrequent or no seizures. STXBP1-DEE is usually caused by heterozygous de novo variants affecting STXBP1. Parental mosaicism has been reported and recently a homozygous missense variant has been described and functionally studied. At the protein level, haploinsufficiency/loss of function is the main proposed disease mechanism. This is supported by the fact that more than half of the reported variants are protein truncating variants (PTV) or splice site variants and smaller or larger indels or deletions. Furthermore, missense variants can lead to decreased protein expression at the synapse. However, recent studies suggest alternative molecular mechanisms such as a dominant negative or gain-of-function effect of specific missense variants. To date, no obvious genotype-phenotype correlations have been identified. STXBP1-DEE is now recognized as one of the more frequent monogenic DEEs with a predicted incidence of 3.30–3.81 per 100,000 births. In addition to intellectual disability and epilepsy, movements disorders such as tremor and ataxia are frequent. Extrapyramidal features have been described in a few adolescents and adults with STXBP1-DEE though information on the prevalence of this clinical symptomatology at adult age is missing.
To date, no studies have systematically investigated the clinical presentation of STXBP1-DEE in adulthood. Such studies are important to inform prognostication, genetic counseling, and to understand the natural history of the disease with the prospect of future therapeutic trials. Here, we analyzed the clinical features of 32 adult patients with disease causing STXBP1-variants with special focus on epileptology in different phases of life, movement disorders, and functional independence in adulthood.

**METHODS**

**Patient recruitment and genotyping**

32 patients were recruited through an international network of clinicians, mostly consisting of medical geneticists and epileptologists, following patients with STXBP1-DEE. For inclusion in the study, patients had to be at least 18 years old and have a pathogenic or likely pathogenic STXBP1 variant according to the American College of Medical Genetics and Genomics (ACMG) guidelines which was (re-)assessed by use of the program VarSome (eTable 1). Thirteen patients were previously published (patients 7, 8, 9, 10, 12, 14, 15, 16, 17, 18, 19, 20, 24, and 31) and updated information with focus on clinical features at adult age was included in this study. Variants of previously unpublished patients were identified by collaborating research and diagnostic laboratories, and parental and family segregation studies were performed where possible.

**Phenotyping**

Clinical data was collected using 3 clinical questionnaires completed by referring clinicians. One questionnaire focused on epileptology and development, one on movement disorders and extrapyramidal features and one on mobility, communication and functional independence in adulthood. This last questionnaire integrated two standardized scales: the Functional Mobility Scale to evaluate mobility and the Katz scale to evaluate self-reliance concerning activities of daily living. Seizure types were classified according to the International League Against Epilepsy Classification. Age at epilepsy
improvement was defined as the age at which a clinically relevant decrease in seizure frequency was seen, as noted in the medical records of the treating physicians. The reported effect of anti-seizure medications (ASM) was based on the clinical impression of the treating physician. Seizure freedom was defined as the period without seizures being at least three times longer than the longest inter-seizure interval in the preceding year. Cognitive outcome was defined based on the level of adaptive functioning as proposed by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5).

**Video-examination**

Referring clinicians were asked to video record their patients while performing tasks of the motor subscale of the modified Unified Parkinson’s Disease Rating Scale (MDS-UPDRS)35 adapted to a level feasible for their patient(s). Because of the variability in performance level of individual patients, the different items could not be scored numerically, but videos were systematically assessed for the presence of the following features: hypomimia, bradykinesia, postural stability, gait abnormalities, tremor, ataxic features and stereotypes. Video recordings were reviewed independently by the clinical neurologists coordinating the study (H.S., E.G., S.W) and an independent neurologist specialized in movement disorders (D.C.) after which a consensus was reached.

**Statistical analyses**

Clinical data on 30/32 patients was included for summary statistics; data of 2 patients was excluded (see results section). As we did not have complete information on all patients, denominators in the results section indicate the number on whom information on the clinical feature addressed was available. A Chi-Square test was used to compare variant type (missense vs other) and seizure outcome (seizure-free vs not seizure free), presence of regression (yes vs no), cognitive outcome (moderate-severe ID vs severe-profound ID), and functional outcome concerning mobility (ambulatory vs wheelchair dependent) and communication (some speech vs non-verbal); and to compare seizure outcome with cognitive outcome, and functional outcome concerning mobility and speech. A Fisher’s exact test was used in case any of the
cells had an expected count below 5. A nonparametric Mann-Whitney U test was used to look for a correlation between seizure onset age and cognitive outcome and functional outcome concerning mobility and speech. Statistical analyses were performed using SPSS statistics software (IBM SPSS Statistics 28). Reported tests were performed 2-tailed with an alpha-level for significance of $p < 0.05$.

**Standard Protocol Approvals, Registrations, and Patient Consents**

Written informed consent for participation/publication in this study was obtained from all participants or their parents or legal guardians. Separate consent forms for publication of pictures/videos were obtained when applicable.

**Data Availability Statement**

Deidentified data can be shared by request from any qualified investigator.

**RESULTS**

**Study cohort and STXBP1 variants**

32 adult patients, 17 females and 15 males, from 31 families were referred for our study. Detailed clinical information is presented in eTable 2. In this cohort, 30 different STXBP1 variants were identified (eTable 1): 15 missense variants (the p.(Glu283Lys) variant was present in 2 unrelated patients), 6 splice site variants, 4 stop gains, 2 frameshift variants, 2 indels and 1 deletion. 7/30 were known recurrent variants. In 26/31 (84%) families STXBP1 variants occurred *de novo*. Two sisters carried the same p.(Ile439Phe) variant that was not detected in blood-derived DNA of their parents and 2 healthy siblings, so germline mosaicism in one of the parents was suspected. For 3 other patients variant inheritance was unknown/incomplete though pathogenicity was considered certain as it concerned known missense variants (patient 6, p.(Arg190Gln), patient 16, p.(Glu283Lys)), or a frameshift variant (patient 4, p.(Ser121Ilefs*21)). 1 variant was present in a mosaic state (patient 17, p.(Arg367*), see below).
After careful review, 2/32 patients were excluded from summary statistics. Patient 6 had a history of Rubella encephalitis as a child which may significantly influence the phenotype. Patient 17 had a very mild phenotype with normal development, borderline intellect and a few tonic-clonic (TC) seizures at adult age, not consistent with a diagnosis of STXBP1-DEE. She carried a somatic mosaic pathogenic STXBP1-variant in blood which was present in a heterozygous state in her daughter diagnosed with STXBP1-DEE. The following sections describe the features in the remaining cohort of 30 patients. Median age at last follow-up was 24 years (range 18-58 y).

**Epilepsy**

All 30 patients had a history of epilepsy. Median age at seizure onset was 3.5 months (range: 1 d (with suspected seizures in utero)- 19 y) and 24/30 (80%) had seizure onset within the first year of life. At last follow up, 5/30 (17%) patients had been seizure-free for 10 to 25 years, having become seizure free at age 8 months to 11 years. 24/30 (80%) patients had active epilepsy, with (several) seizures per day in 9/24 (38%) patients, weekly seizures in 10/24 (42%) patients, and monthly seizures in 4/24 (17%). Patient 5 had a history of few TC seizures only. In patient 31 possible very rare focal seizures were reported at last follow-up (not counted as having active epilepsy). Notably, 11/30 (37%) patients had prolonged seizure-free periods (range 1-16 y, median 3 y) in early childhood or adolescence with later seizure recurrence, and in 7 of them ASMs could (temporarily) be withdrawn.

*Evolution of seizure types*

Patients had 1-3 different seizure types at onset. Focal seizures (n=14), epileptic spasms (ES, n=8), TC seizures (n=4) and tonic seizures (n=4) were most frequent. From infancy through adolescence patients had 1-7 seizure types, most frequently including focal seizures (n=21), tonic seizures (n=19), TC (n=16), and ES (n=11). Four patients had periods of status epilepticus (specified convulsive in 2). Adult patients still had 1-5 different seizure types with TC seizures (n=18), focal seizures (n=16, often with impaired awareness and/or motor components), and tonic seizures (n=11) as most frequent seizure types. Less
frequent were atonic seizures \( (n=4) \), myoclonic seizures \( (n=3) \) and ES \( (n=3, \text{ example in Video 1}) \). Periods of status epilepticus were observed in 3 adult patients (specified convulsive in 1 and non-convulsive in 1).

Evolution of EEG pattern

The interictal EEG at epilepsy onset was normal in 4/21 (19%) patients. Focal or multifocal interictal epileptiform abnormalities (IED) were present in 10/21 (48%) and focal slowing in 5/21 (24%). Five children had hypsarrhythmia and 2 with a burst suppression pattern. EEG in adults did not differ significantly from EEG during childhood through adolescence and showed background slowing (8/18 adults, 44%) with focal/multifocal IED (12/18, 67%) and/or focal slowing (5/18, 28%). Two patients always had a normal EEG, and in 2 additional patients, the EEG was normal at their most recent recording (at 17 and 54 years of age).

Response to anti-seizure medication (ASM)

The ASMs most frequently reported to be effective before adulthood were valproic acid (VPA, 13/26, 50%), benzodiazepines (clobazam (CLB) /clonazepam (CLN) / nitrazepam, 11/31, 35%), vigabatrin (10/16, 62%), lamotrigine (LTG, \( n=7/16, 44\% \)) as well as levetiracetam (LEV, 5/16, 33%), and phenobarbital (5/15, 33%). Ketogenic diet therapies (KDT) reduced seizure frequency in 2/5 (40%) and adrenocorticotropic hormone (ACTH) in 4/6 (67%). Adult patients with active epilepsy were (still) treated with 1-5 ASMs (mean: 3 ASMs) at last follow up. The ASMs most frequently reported as effective in adulthood were LTG (6/14, 43%), VPA (6/14, 43%) and benzodiazepines (CLB/CLN, 5/14, 36%), followed by LEV (4/12, 33%) and topiramate \( (n=3/9, 33\%) \). Vagal nerve stimulation was (partially) effective in 5/6 (83%) patients with VNS, and KDT in 1. (Denominator indicating number of patients in whom anti-seizure treatment was trialed.)
Developmental trajectory, cognitive outcome and behavioral features

All patients had developmental delay that became apparent in the first 2 years of life and in 22/29 (76%) before the age of 1 year. In at least 7 patients developmental delay preceded seizure onset. One or more episodes of regression of variable severity occurred in 16/27 (59%). In 7/16 patients, episodes of regression correlated with seizure onset or increased seizure activity. Notably, 7/27 (26%) had loss of skills beyond early childhood (range 4-23 y) mainly affecting loss of verbal communication and walking. At last follow-up, 10/30 (33%) patients had profound or severe-profound ID, 16/30 (53%) severe ID and 4/30 (13%) moderate or moderate-severe ID.

11/26 (42%) patients were diagnosed with ASD or showed autistic features. Stereotypies were noted in 14/30 (47%) patients mostly including hand stereotypies (n=6), body rocking (n=3) and previously described figure-of-eight head stereotypies (n=2). 17/26 (65%) had behavioral or psychiatric problems including aggressive behavior (n=7), self-mutilation (n=4), hyperactivity (n=3), compulsive symptoms (n=1), and episodes of psychosis or auditory hallucinations at adult age in 2 sisters. Seven adults received medication for behavioral/psychiatric problems at last follow-up.

Clinical neurological examination with specific focus on movement disorders and extrapyramidal features at adult age

Movement disorders including gait disturbance and extrapyramidal features reported by referring clinician

In 26/30 (87%) patients, movement disorders were reported by the referring clinicians (Table 1). The 4 patients in whom this was not described had severe spastic tetraparesis. Most frequently reported were tremor (17/30, 57%) and gait disturbances (present in 10/19 patients able to walk, 53%). Less frequent were hypomimia (8/30, 27%), bradykinesia in 3/30 (10%) and rigidity (2/30, described as cogwheel rigidity in 1. Ataxia was not further specified in 8 patients. Patient 13 was effectively treated with propranolol for his tremor, patient 1 was trialed on levodopa and rasagiline with uncertain effect.
Movement disorders including gait disturbance and extrapyramidal features as assessed by video examinations

For 19/30 (63%) patients video recordings were available (Table 1 and eTable 3) including clinical examination capturing (some) items of the motor MDS-UPDRS motor subscale in 11/19. The most frequently observed features were hypomimia (15/18, 83%), bradykinesia (5/8, 63%) and different types of tremor (9/17, 56%) most frequently including intention/action tremor of the hands (n=8). In 5 patients, the tremor was irregular or jerky, with clinically convincing myoclonic features in 2/5. In all 12 patients able to walk (walking with limited assistance included), (mild) gait abnormalities were noted most frequently including postural abnormalities of the feet with external rotation with/without eversion (n=8) and broad-based gait (n=7). Stereotypies were observed in 12/19 (63%) patients, with figure-of-eight head stereotypies in 5. Representative videos of gait abnormalities, tremor and stereotypies are available in supplementary Videos 2 to 4. The videos of 2 patients showed choreatic dyskinesia (Video 5).

Additional neurological findings reported by referring clinician

Abnormal muscle tone was a frequent neurological co-morbidity and consisted of spasticity/hypertonia in 13/29 (45%) patients and (axial) hypotonia in 8/29 (28%). Two patients had uni- and bilateral foot drop suggesting possible (primary or secondary) involvement of the peripheral nervous system although no nerve conduction studies were available. Furthermore, 2 patients had cortical visual impairment and 3 oral apraxia or dyspraxia.

Neuroimaging

MRI imaging reports were available for 29/30 (97%) patients. In 22/29 (76%) patients, brain MRI was normal including 7/9 patients in whom imaging was (also) performed at adult age. 4/29 (14%) patients had some degree of cerebral atrophy. In one (patient 19) mild atrophy was reported at adult age but was not described in imaging reports at 4 and 13 years old. 4/29 (14%) patients had other non-specific
imaging findings including right parietal gyral asymmetry and FLAIR hyperintensities in patient 14, a small infarction in patient 23, small hippocampi with incomplete rotation in patient 24 and a suspected temporal myelination defect in patient 32.

Non-neurological comorbidities
In 22/30 (73%) patients, non-neurological comorbidities were reported. 14/30 (47%) patients reported gastrointestinal problems including constipation (n=6), problems with feeding/weight maintenance (n=4) and gastro-oesophageal reflux (GORD, n=2). 4/30 (13%) patients received food/liquids through a percutaneous endoscopic gastrostomy tube (PEG-tube). 6/30 (20%) patients were reported to have significant problems with sleep initiation or maintenance. Other recurrent non-neurological comorbidities were (severe) scoliosis (n=4), joint laxity (n=3), and (mild) dysmorphic features (n=3).

Communication, mobility and functional independence in adulthood
Communication problems were present in all patients, with 20/28 (71%) individuals being non-verbal (Figure 1A). Motor disability was variable and ranged from walking (short distances) independently to completely non-(Figure 1B). All patients were largely dependent for activities of daily living such as washing, dressing, toileting and feeding (Figure 2). This need for constant care translated to a significant number of adult patients (14/27 (52%)) living in residential care most of the time. Nine (33%) patients went to the daycare center and 4 (15%) were at home predominantly.

Death and cause of death
2/30 patients died after their last follow-up at the ages of 36 and 23 years. The reported cause of death was a post-surgical infection and sepsis respectively.
Genotypic and phenotypic correlations

No (statistically significant) correlation was found between variant type (missense vs other) and seizure outcome, cognitive outcome, presence of regression and functional outcome concerning mobility and communication. Likewise, correlation was found between seizure outcome (seizure free vs not seizure free) and cognitive outcome or functional outcome concerning mobility and communication. Interestingly, there was a significant correlation between the age of seizure onset and the level of functional mobility at last follow-up, with the median age at seizure onset being lower in the group of patients who were wheelchair dependent (median: 25 d) compared to the group of ambulatory patients (median: 10 mo; Mann-Whitney-U, \( p = 0.004 \)). This correlation was not seen with regards to cognitive outcome, presence of regression or the level of verbal communication. (Data available upon request.)

DISCUSSION

In this study, we analyzed the phenotypic features of STXBP1-DEE in adulthood to delineate the natural history of the disease. Our study cohort included 30 adult patients with disease causing STXBP1-variants including missense variants in about half of the patients, next to protein truncating variants, indels and splice site variants.

Epilepsy in adult STXBP1-DEE

Although epilepsy is not a mandatory feature of STXBP1-DEE, all patients included in our study had epilepsy which was active at adult age in 80%. This may be related to the relatively small number of patients included in our study, or due to referral bias as many patients were recruited through collaborating (child) neurologists with interest in epilepsy. Although seizures typically started in infancy or early childhood (before the age of 1 year in 80%), one patient had seizures starting at adult age only. Seizure onset age beyond early childhood has been reported rarely in previous studies as well.\(^\text{12}\) Focal seizures and ES were the most frequent seizure types at onset, whereas in adulthood, TC seizures, focal
and tonic seizures were most frequent. Notably, in 3 patients ES were described in adulthood which is uncommon as ES are epileptic events typical of infancy. Since these patients also had tonic seizures, we cannot exclude that in this context ES and tonic seizures may lie on a spectrum as the differentiation often relies on duration, with epileptic spasms lasting less than 2 seconds and tonic seizures more than 2 seconds. However, ES in adults had the typical pattern of series of spasms seen in younger patients. As such, we propose that adolescents or adults with unsolved DEE with ongoing apparent ES deserve genetic scrutiny for STXBP1.

Even though our cohort was limited due to size and potential recruitment bias, we stipulate that seizures in STXBP1-DEE may represent a life-long burden and may continue to be drug-resistant in adulthood. At last follow up, 80% of this study cohort had active epilepsy with seizure frequencies ranging from multiple seizures a day to monthly seizures. In keeping with this, most adults still received multiple ASMs. Notably, more than a third experienced prolonged periods of seizure freedom in childhood or adolescence, in some patients to an extent that ASMs could be temporarily withdrawn. The underlying mechanisms for this phenomenon are unknown so far and represent an area in need of further investigation. Altogether, our results underscore the importance of continuous monitoring for seizures in adolescents and adults with STXBP1-DEE, including in patients who have been seizure-free for a prolonged period of time. A summary of key findings and recommendations for clinicians taking care of (adult) STXBP1-DEE patients can be found in Table 2.

Cognitive and behavioral features
Developmental delay is a core clinical feature of STXBP1-DEE and usually already evident in the first 2 years of life. Our study revealed episodes of regression in more than half of the patients, including loss of communicative or motor skills beyond early childhood in 26%, which is significantly more frequent than previously described. A correlation with seizure activity was not always present. None of the adults in this cohort had mild-moderate ID although this was previously reported in a small proportion of patients. This may be partially explained by a selection bias towards patients with a more severe presentation that
might favor inclusion in research studies. An alternative explanation could be an increased diversion from developmental expectations with age as was recently suggested in a study addressing development and behavioral characteristics in STXBP1-DEE. Problems with social interaction, including autistic features, often with additional behavioral problems were present in more than half of our cohort and 23% received medical treatment for psychiatric/behavioral problems at last follow-up, indicating that behavioral problems still form a significant problem at adult age.

**Extrapyramidal features and other movement disorders**

Through the use of semi-structured video examinations including tasks from the motor part of the MDS-UPDRS, in addition to clinical questionnaires, we aimed to reduce inter-observer variability and increase the possibility of identification of discriminatory findings. Structured video review resulted in a higher yield of movement abnormalities compared to data collection through questionnaires alone (Table 1). Gait abnormalities, including broad based gait and postural abnormalities of the feet, were most frequent. Hypomimia was seen or reported in more than half of the patients but was often associated with a hypotonic facies, which would influence facial expression. Tremor, present in 56%, was mostly of the action/intention type. In the questionnaires, a resting tremor was more frequently reported than observed on video examinations, indicating that the momentary observations on video examination may not have captured all different tremor types. Nevertheless, central review of video material showed that tremors often had a jerky/irregular appearance with clear myoclonia in some. Interestingly, and strengthening our finding, a recent study characterized the tremor in some STXBP1-patients as a tremor-like subcortical myoclonus. Altogether, these findings are not compatible with a pure extrapyramidal movement disorder and suggest multi-system involvement. Functional imaging studies including dopamine transporter imaging, although not straightforward in this patient population, could shed a light on the relative contribution of different systems involved. We further note that certain medications taken by some of this adult cohort, such as valproic acid and neuroleptic drugs, may also induce or worsen movement disorders such as tremor or bradykinesia. Of interest, 1 patient (patient 1) received treatment
with levodopa (+- rasagiline) after her neurologist noticed extrapyramidal features. On subsequent investigation by a different neurologist, these features were no longer observed. It is uncertain whether this represents a true effect of levodopa, but a trial of levodopa would be of interest in STXBP1-DEE patients with disruptive extrapyramidal features.

(Neurological) comorbidities and functional independence

All adult patients with STXBP1-DEE in our study had significant limitations in verbal communication, with the majority of patients (71%) being non-verbal. Functional mobility was more variable ranging from independent walking on all or level surfaces (50%) to being mostly or completely wheelchair-bound (39%). We note that some patients were able to walk short distances independently but used a wheelchair for longer distances. Mobility in STXBP1-DEE is likely influenced by several clinical features such as gait ataxia, (axial) hypotonia, spasticity and dystonia and skeletal problems such as foot deformities and scoliosis. Of interest, we found a statistically significant correlation between age at seizure onset and level functional mobility. Further investigation is warranted to look whether this can be replicated in larger study samples and whether or not this represents a causal relationship. Gastro-intestinal problems were the most frequently reported non-neurological comorbidities (47%) with 13% of patients requiring a PEG tube. All adult patients in our study were partially or completely dependent for most activities of daily living.

Life expectancy

There is almost no data on life expectancy in STXBP1-DEE. Here, we report the oldest patient to date, a woman aged 58 years. Taking into account a previous report of a patient 56 years old\textsuperscript{12}, we can conclude patients with STXBP1-DEE can live well up to their sixties at least. 2/30 (7%) adult patients included in our study died in their 3\textsuperscript{rd} and 4\textsuperscript{th} decade. The cause of death in both patients was not related to seizures. However, our study recruited adults with STXBP1-DEE, and consequently cannot assess early mortality in STXBP1-DEE. More systematic studies looking at the frequency and cause of death are warranted.
Neurodevelopment versus neurodegeneration

Although the early disease course of STXBP1-DEE suggests a primary problem in neurodevelopment, STXBP1 had also been implicated in neurodegeneration. First, both in vitro and in vivo studies have shown that STXBP1 is critical for neuronal survival and maintenance. Furthermore, a recent study revealed that STXBP1 controls the self-replicating aggregation of α-synuclein, a protein involved in various neurodegenerative diseases including Parkinson disease. By definition, neurodegenerative disorders deteriorate with age, hence, a study of older patients with STXBP1-DEE provides a unique opportunity to look for clinical signs of neurodegeneration. In this regard, the presence of developmental regression beyond early childhood (present in 26% in our cohort) was remarkable, especially as this was not always clearly related to flair-ups in seizure activity. However, these periods of regression occurred at certain points in life only, with plateauing rather than showing a protracted course. The mechanisms underlying this unusual phenomenon remain to be identified. Another argument favoring a role of neurodegeneration are the movement abnormalities we described, particularly the extrapyramidal features that seem to be more prevalent in older patients. We did not find convincing clinical evidence for neurodegeneration when comparing MRI imaging reports from adult and childhood age which showed possible mild progressive cerebral atrophy in only 1 out of 7 patients with information available. Further studies using serial MRI-imaging are necessary to draw any further conclusions.

Strengths and limitations of this study, future directions

This is the first study to systematically investigate the clinical presentation of STXBP1-DEE in adulthood. We propose this study could serve as a pilot study for future (prospective) natural history studies on STXBP1-DEE extending into adulthood.

Our study however has some limitations: the study design was cross-sectional with the inclusion of retrospective data and as such not ideal to evaluate disease evolution over time. Although the video...
examinations were of added value to the clinical questionnaires, we acknowledge they reflect momentary observations and some clinical features of interest could not be reliably assessed on video.

Future natural history studies should preferably include longitudinal prospective data and use standardized scales and questionnaires adapted to the population studied to harmonize data collection. Repeated semi-structured video examinations and serial brain MRI over time would be particularly interesting to further investigate the clinical correlates of a possible neurodegenerative component. The presence of ES in adulthood should be confirmed with simultaneous video-EEG and polygraphic recordings.

There is an unmet need for disease-modifying therapies for STXBP1-DEE as current therapeutic options are largely symptomatic and mainly directed at seizure control. Proceeding insights into the disease mechanisms underlying STXBP1-DEE are now paving the way for more targeted therapies that should tackle the whole gamut of comorbidities including neurodevelopmental, movement disorders, behavioral and psychiatric and gastrointestinal problems, as well as seizures. Further studies confirming our findings suggestive of a slowly progressive disease course in at least some of the STXBP1-DEE patients, would provide rationale to also study the effect of targeted therapy initiation in adolescence/adulthood. Understanding the natural history of STXBP1-DEE will be essential for the selection of relevant clinical outcome measures in future therapeutic trials.
TABLES AND FIGURES

Table 1: Movement disorders including extrapyramidal features in adults with STXBP1-developmental and epileptic encephalopathy.

<table>
<thead>
<tr>
<th>Questionnaires</th>
<th>Video examinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feature present**</td>
</tr>
<tr>
<td>Hypomimia</td>
<td>8/30 (27%)</td>
</tr>
<tr>
<td>Bradykinesia</td>
<td>3/30 (10%)</td>
</tr>
<tr>
<td>Rigidity</td>
<td>2/30 (7%)</td>
</tr>
<tr>
<td>Tremor (hands/arms)</td>
<td>17/30 (57%)</td>
</tr>
<tr>
<td>postural</td>
<td>3</td>
</tr>
<tr>
<td>action/intention</td>
<td>7</td>
</tr>
<tr>
<td>resting</td>
<td>7</td>
</tr>
<tr>
<td>Postural instability</td>
<td>-</td>
</tr>
<tr>
<td>Gait disturbances</td>
<td>10/19 (53%)</td>
</tr>
<tr>
<td>Broad-based/ataxic</td>
<td>8</td>
</tr>
<tr>
<td>External rotation + foot eversion</td>
<td>2</td>
</tr>
<tr>
<td>Reduced arm swing</td>
<td>1</td>
</tr>
<tr>
<td>Asymmetric posture/dystonic features</td>
<td>3</td>
</tr>
<tr>
<td>Ataxia (not further specified)</td>
<td>8/30 (27%)</td>
</tr>
<tr>
<td>Stereotypies</td>
<td>14/30 (47%)</td>
</tr>
</tbody>
</table>

* denominator indicating patients in whom the features could be reliably assessed on video examination.
# number of patients in whom the feature was also noticed by the referring clinician in the questionnaire

Table 1 summarizes the presence of movements disorders including extrapyramidal features in our adult cohort. In the video examination column, denominators indicate the number of patients in whom this specific feature could be addressed.
Table 2: Key findings and recommendations for the clinician taking care of patients with STXBP1-developmental and epileptic encephalopathy.

1. STXBP1-DEE warrants continuous monitoring for seizures in adolescence and adulthood.
   - In 80% of patients seizures started in the first year of life although onset in adulthood was present in 1.
   - 38% of patients had prolonged periods of seizure-freedom in childhood or adolescence with seizure recurrence at later age.
   - 80% of adults had active epilepsy *
   - Tonic-clonic, focal and tonic seizures were most frequent in adulthood. In 3 patients, epileptic spasms continued into adulthood.

2. Movement disorders of multisystem involvement are a frequent comorbidity requiring special attention.
   - > 50% of adults had tremor, predominantly of the action/intention type, a resting tremor was less frequent.
   - Gait abnormalities were present in all adults, most often including ataxic gait and postural abnormalities of the feet.
   - Extrapyramidal features such as hypomimia and bradykinesia may be more frequent in adolescence/adulthood. (cogwheel) rigidity represents a rare feature.
   - Stereotypic movements were present in > 60%.

3. A multidisciplinary approach including occupational therapy, physiotherapy and speech therapy should continue into adulthood to optimize functional outcome.
   - Developmental delay was evident in the first 2 years of life in all patients.
   - Periods of developmental regression were present in 59% including regression beyond early childhood in 27% mostly including loss of verbal communication and/or functional mobility.
   - > 70% of adults were non-verbal. Functional mobility was variable ranging from independent walking (50%) to complete wheelchair dependency (39%) and is likely influenced by several clinical features such as gait ataxia, abnormal muscle tone and skeletal problems.
   - 87% of adults had severe to profound ID.*

4. Ongoing attention for psychiatric comorbidities is warranted.
   - > 20% of patients required medical treatment for psychiatric/behavioral problems in adulthood.

# a selection bias toward patients with (active) epilepsy is possible due to recruitment through epilepsy center
* a selection bias towards more severely affected patients included in research is possible
**Figure 1:** Functional mobility and level of verbal communication at adult age.

**Legend figure 1:** Figure with charts showing the functional outcome at last follow-up concerning verbal communication (panel A) and mobility assessed with the Functional Mobility Scale (panel B).
**Figure 2:** Level of functional independence at adult age.

*Legend figure 2:* Figure with charts showing the functional outcome at last follow-up concerning activities of daily living assessed with the Katz scale.
Online supplementary videos

Video 1: Video showing a cluster of violent epileptic spasms in a 19-year-old man with STXB1P1-DEE.

Video 2: Video showing 4 adult patients with STXB1P1-DEE with mild to pronounced gait abnormalities (1): 21-year-old man with mildly broad-based gait with external rotation of the feet and pes planus; (2): 19-year-old woman with more pronounced broad based gait with external rotation and eversion of feet while walking and pes planus; (3): 20-year-old man with pronounced ataxic gait with external rotation of the feet; (4): 22-year-old woman with broad-based gait with asymmetric posturing and dystonia of the left arm and impression of hypertonia in the legs

Video 3: Different tremor types in 3 adult patients with STXB1P1-DEE, a jerky/irregular component is often present.

Video 4: Stereotypies including figure-of-eight head stereotypies in 3 adult patients with STXB1P1-DEE.

Video 5: Dyskinesia including choreiform movements in 2 patients with STXB1P1-DEE.
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Natural History Study of STXBP1-Developmental and Epileptic Encephalopathy Into Adulthood
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