THERAPEUTIC ADVANCES in
Rare Disease

Patient Perspective

STXBP1: fast-forward to a brighter future – a patient organization perspective

James R. Goss, Benjamin Prosser, Ingo Helbig and Charlene Son Rigby

Ther Adv Rare Dis

2024, Vol. 5: 1–10

DOI: 10.1177/26330040241257221

***Abstract*:** Syntaxin-binding protein 1 related disorder (STXBP1-RD) is a rare neurologic disorder associated with global neurodevelopmental delay, intellectual disability, early-onset epilepsy, motor abnormalities, and autism. The underlying pathophysiology stems from a *de novo* mutation in the *STXBP1* gene, which codes for the STXBP1 protein. The STXBP1 protein is involved in synaptic vesicle fusion and neurotransmitter release. Pathogenic variants in the *STXBP1* gene generally result in haploinsufficiency, an impairment in neurotransmitter release, and subsequent dysfunction in neuronal communication. The STXBP1 Foundation was founded in 2017 to support families of children with STXBP1-RD and accelerate the development of effective therapies and, ultimately, a cure for the disorder. The Foundation initially supported research aimed at better understanding the complex phenotypic presentation of the disease as well as the development of animal and cellular models usable by the research community to more fully characterize STXBP1 function and disease pathogenicity. In 2023, the Foundation embarked on its STXBP1 Fast Forward Strategic Plan, which includes a prospective natural history study and substantive biomarker work to drive forward the development of new precision therapies for STXBP1-RD.

Plain language summary

STXBP1: fast-forward to a brighter future

STXBP1-related disorder (STXBP1-RD) is a rare and severe brain condition. It causes delays in development, learning problems, seizures starting at an early age, movement challenges, and sometimes autism. The main problem comes from a new mutation in the STXBP1 gene, which makes a protein needed for brain cells to communicate properly. When this gene doesn't work right, there's not enough of the protein, leading to trouble with brain cell communication. To help families dealing with this disorder and speed up the search for effective therapies, the STXBP1 Foundation started in 2017. At first, they funded studies to understand the disease better and create models for testing treatments. Then, in 2023, they launched the STXBP1 Fast Forward Strategic Plan. This plan includes studying how the disorder progresses naturally and researching markers that could help develop precise treatments for STXBP1-RD.

Keywords: biomarkers, neurodevelopmental disorder, patient advocacy group, precision medicine, synaptopathy

Received: 15 January 2024; revised manuscript accepted: 8 May 2024.

Correspondence to:
James R. Goss
STXBP1 Foundation, PO Box 1148, Holly Springs, NC 27540, USA
james.goss@stxbp1disorders.org

Benjamin Prosser
Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Center for Epilepsy and Neurodevelopmental Disorders (ENDD), Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Ingo Helbig
Division of Neurology, Department of Biomedical and Health Informatics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA

Center for Epilepsy and Neurodevelopmental Disorders (ENDD), Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Charlene Son Rigby
STXBP1 Foundation, Holly Springs, NC, USA

An overview of syntaxin-binding protein 1 related disorder

In 2008, five patients with Ohtahara syndrome, a severe, early-onset epilepsy characterized by a suppression-burst pattern on electroencephalogram (EEG) and severe psychomotor retardation, were described with a variety of pathogenic variants in the syntaxin-binding protein 1 gene (*STXBP1*), including missense, frameshift, splice site, and nonsense variants.[1] Soon after, patients presenting with other early-onset epileptic encephalopathies, including West syndrome, Lennox-Gastaut syndrome, and Dravet syndrome, were identified with *STXBP1* variants.[2–4] Since then, a variety of *de novo* variants in the *STXBP1* gene have been found in individuals, resulting in severe encephalopathies with a diverse set of phenotypes.[5–8] A recent estimate of the incidence of syntaxin-binding protein 1 related disorder STXBP1-RD is approximately 1:30,000 births[9] and *STXBP1* is the fifth most implicated gene associated with epileptic developmental disorders,[10] suggesting that the disease is not as rare as once believed.

The *STXBP1* gene (also known as *MUNC18-1*), located on chromosome 9q34.1, codes for the protein STXBP1. The STXBP1 protein is involved in synaptic vesicle fusion with the neuronal cell membrane *via* its multiple interactions with the soluble N-ethylmaleimide sensitive factor adaptor protein receptors (SNAREs) complex, and thus vital for neurotransmitter release.[11] The evolutionary importance of STXBP1 function is illustrated in several animal models. Deletion of Stxbp1 in mice or its homolog gene, ROP, in Drosophila results in loss of neurotransmitter release, neuronal degeneration, and early death.[12,13] Interestingly, knockout mice die shortly after birth but have normal brain architecture development during embryogenesis. Loss of function variants in either of the zebrafish homologs, Stxbp1a or Stxbp1b, lead to lack of movement, reduced brain activity, and early fatality or reduced locomotor activity and spontaneous electrographic seizures respectively[14] and inactivation of the homolog gene in *Caenorhabditis elegans* (Unc-18) causes paralysis.[15] The primary mechanism of disease formation is haploinsufficiency, and in animal models, haploinsufficiency has been demonstrated to recapitulate several patient phenotypes.[16,17]

Virtually all STXBP1-RD patients demonstrate developmental delay and intellectual disability, the majority of whom can be classified as severe to profound. Approximately 85–90% of patients have some form of epilepsy, which usually manifests within the first year. Several types of seizures have been reported in individuals, with the most common being focal-onset seizures, generalized-onset seizures, and epileptic spasms.[8] About 90% of patients have other neurological features such as dystonia, spasticity, ataxia, hypotonia, and tremors, and behavioral issues, including hyperactivity, anxiety, aggressiveness, and autism which are common occurrences in subsets of patients. Significant impacts of the disease include loss of autonomy, difficulties in socialization and schooling for the patients, and emotional and daily life activities for the caregivers.[18]

The STXBP1 Foundation

The STXBP1 Foundation was founded in 2017 by six families with the mission of accelerating effective treatments for STXBP1-RD while supporting patients and families today. With a priority focus on advancing research, the Foundation formed a Scientific Advisory Board in January 2018 to guide research plans. At the first-ever global STXBP1 researcher meeting, convened June 2019, we held a set of breakout sessions to build our first set of research priorities, which included: (1) the need for a regulatory-compliant, prospective natural history study; (2) open access to standardized animal models and other technologies; (3) a better understanding of the biology and phenotypic expression of *STXBP1* within humans and model species; and (4) a better understanding of the effects of STXBP1 protein overexpression.

These four priorities saw significant advancements over the next 3 years. Early on, the research and clinical communities realized that significant groundwork was needed before a robust longitudinal prospective natural history study could be launched. There were questions concerning the breadth of symptoms experienced by STXBP1-RD patients and a concomitant uncertainty of what behavioral domains are most affected and how best these domains should be assessed. Multiple studies to characterize STXBP1-RD were conducted, including retrospective natural history

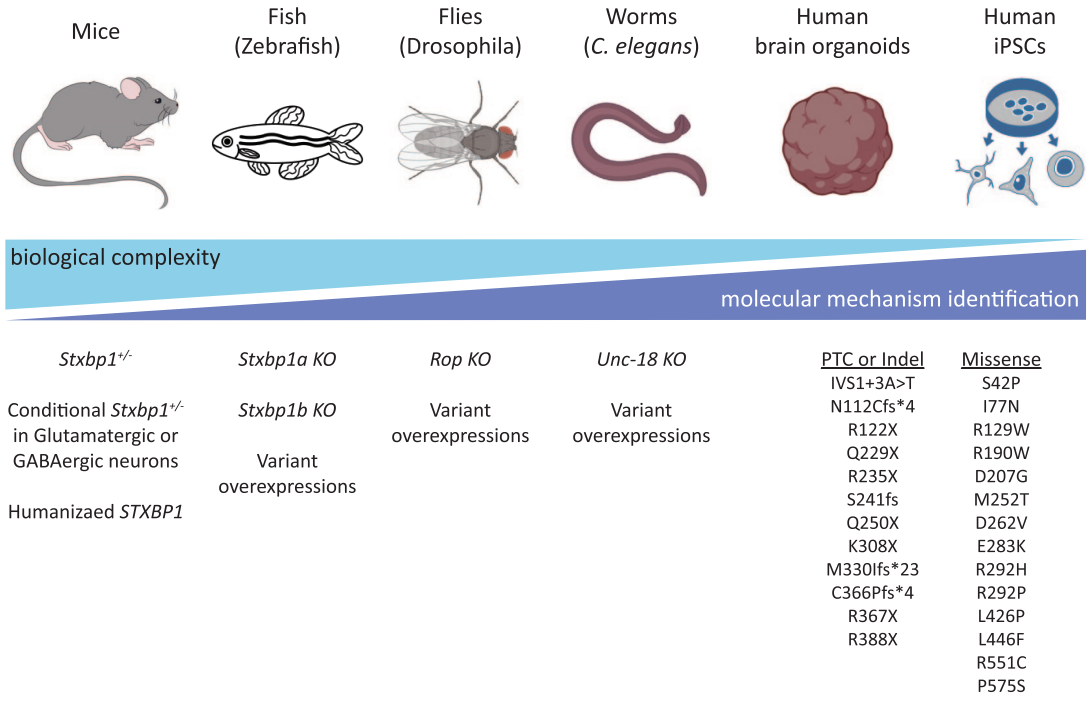

Figure 1. Model systems to study STXBP1-RD biology and pathology. A range of *in vitro* and *in vivo* model systems are necessary to fully study the biology and function of the *STXBP1* gene and protein and understand the effects of various genomic variants on basic function and subsequent pathologies. Examples of variants available, as of December 2023, for different model systems are shown.
STXBP1-RD, syntaxin-binding protein 1 related disorder.

analyses to better understand the broad phenotypic spectrum observed in patients[8,19–22] and the establishment of a disease concept model to assess the lived experience of individuals with STXBP1-RD and their families.[18] These studies have provided a much clearer picture of the STXBP1-RD landscape, including a better appreciation of the significant phenotypic heterogenicity observed in the patient population, the first impressions of potential genotype/phenotype relationships in regard to seizure types experienced in the patient population, and an initial assessment of clinical outcome measures that could be utilized in the STXBP1-RD population in a prospective natural history study and future drug trials. The additional clinical knowledge learned during these 3 years also led the Foundation to request and engage in an FDA Patient Listening Session in April of 2022 as an initial step to help the Agency begin to better understand the complex nature of STXBP1-RD.

In addition to characterization of patient populations, an expansion in the number of STXBP1 animal and *in vitro* models grew significantly, and characterization of models has been undertaken. Today, animal models exist for *C. elegans*, zebrafish, *drosophila*, mice, and macaques,[14,17,23–25] and numerous patient-derived and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-derived induced pluripotent stem cell (iPSC) lines became available (Figure 1). The open-access approach outlined at the 2019 meeting has been borne out in models being deposited at Jackson Labs and shared *via* material transfer agreements. Today, a list of available animal models and iPSC lines is found on our website (www.stxbp1foundation.org). The commitment of the research community to open access has accelerated research in this ultrarare disease.

The mechanism of STXBP1's role in neurotransmitter release as part of the SNARE complex has been well established for over a decade.[11,26,27] As the protein continues to be studied, additional roles for STXBP1 have been suggested, including roles in cellular protein trafficking[22,28,29] and in regulation of the neuronal transcriptome.[30,31] An increase in STXBP1

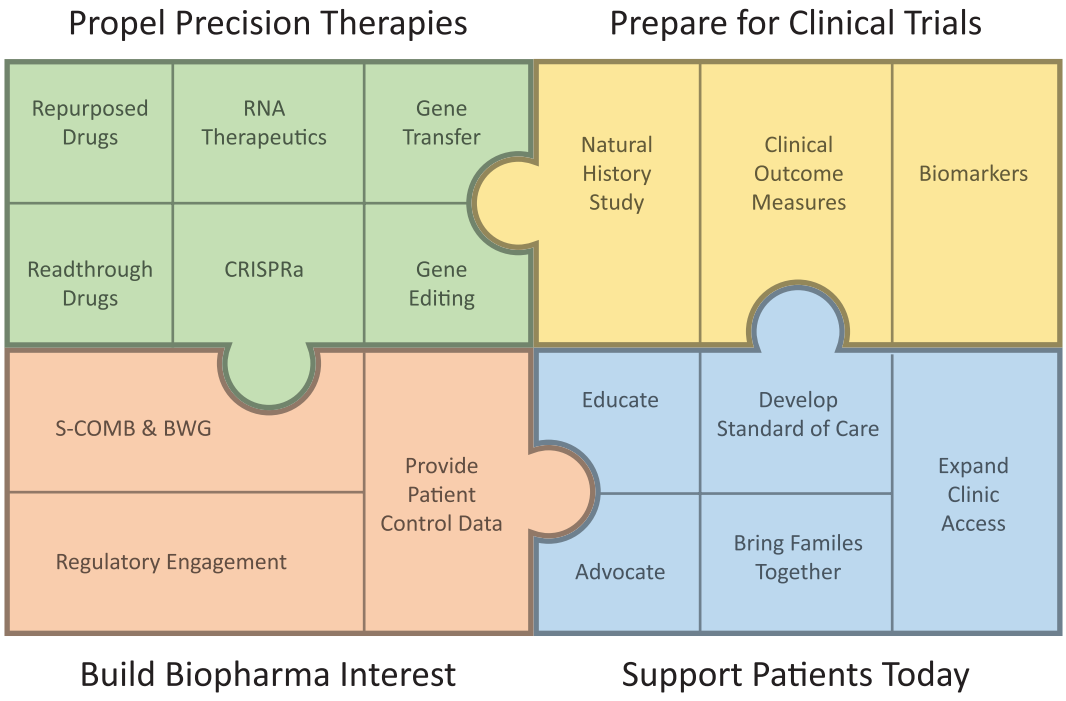

Figure 2. STXBP1 fast-forward strategic plan. The Fast Forward Strategic Plan adopted by the STXBP1 Foundation in 2023 is comprised four principal components, all functioning together to accelerate the Foundation's goals of finding transformative therapies for STXBP1-RD while improving the lives of our patients and families. Each principal component is comprised of various endeavors, being advanced by the Foundation and our partners, to ensure these goals are met.
STXBP1-RD, syntaxin-binding protein 1 related disorder.

protein has been observed in neurological disorders, including schizophrenia and multiple sclerosis,[32] and overexpression of munc18-1 in transgenic mice has been associated with schizophrenia-like behavioral abnormalities.[33] While haploinsufficiency appears to be the primary pathological mechanism in STXBP1-RD, there is an observation that some missense variants might cause protein instability, misfolding, and aggregation, resulting in wild-type protein depletion.[34,35] This finding directly led to the idea that protein chaperones could be a potential treatment option for STXBP1-RD and the first clinical trial investigating the use of 4-phenylbutyrate in patients with STXBP1-RD.[35,36]

STXBP1 fast-forward strategic plan

By 2022, research efforts had begun to expand to therapeutic development, which was welcomed by the patient community. Indeed, by spring of 2022, there were nine therapies in early development pipelines known to the STXBP1 Foundation across gene therapies, RNA therapies, and small molecules. The STXBP1 Foundation realized additional efforts were required to support and accelerate the development of these early therapeutic pipelines. With the guidance of its Scientific Advisory Board, the STXBP1 Foundation developed a new 3-year plan, STXBP1 Fast Forward, which was launched in January 2023. STXBP1 Fast Forward conceptualization included studying strategies of other patient advocacy groups, including Foundation for Angelman Syndrome Therapeutics (https://cureangelman.org) and the Rett Syndrome Research Trust (https://reverserett.org/research/).

STXBP1 Fast Forward has four pillars (Figure 2):

1. Propel Precision Therapies for STXBP1-RD. We are energetically pursuing several therapeutic strategies for STXBP1-RD. Pursuing multiple strategies at the same time gives us more 'shots-on-goal' for effective therapies and reduces risk.

2. Prepare for Clinical Trials. With multiple therapies potentially entering clinical trials in the next couple of years, it is imperative that we maximize the likelihood that clinical trials are successful – and that we see improvements in the symptoms that are important to our patients and families. We are leading and orchestrating a number of clinical trial readiness projects, including natural history studies, identifying clinical outcome measures (endpoints), biomarker development, and engaging with regulatory agencies to educate them on STXBP1-RD and what is important to patients.
3. Build Biopharma Company Interest. We need to build awareness and interest from biotech and pharmaceutical companies, who will ultimately lead the development and commercialization of STXBP1-RD therapies. Our goal is to de-risk STXBP1-RD through our research and clinical trial readiness initiatives, thus making STXBP1-RD an attractive condition for companies to invest in.
4. Support Patients Today. We are focused on enabling the precision therapies of tomorrow, but we also realize it's critical to support our patients and families now. We are broadening access to clinical expertise for STXBP1-RD, both through clinical centers for STXBP1 and by developing a standard of care for STXBP1-RD patients.

Propel precision therapies for STXBP1

Treatment options for STXBP1-RD are limited and focused on seizure control. Antiseizure medications are the primary intervention for seizures. Drug resistance is very common, resulting in frequent changes in medication over the lifespan; alternatives include dietary therapy and vagal nerve stimulation.[7] Notably, there are no drugs available to address the cognitive differences and developmental delays associated with STXBP1-RD; physical, occupational, and speech therapy are used to help the patient meet some specific needs but are limited in their ability to help the majority of patients adequately compensate for the severe nature of their developmental delay. The lack of adequate therapies to address the multiple disease manifestations of STXBP1-RD is a major unmet medical need.

Repurposed drugs and precision therapies, like gene therapy, are actively being pursued and supported by the Foundation. Aside from the aforementioned 4-phenylbutyrate clinical trial, the Foundation is pursuing large-scale drug screens in model systems to identify currently available drugs that could significantly impact disease symptoms and quality of life for STXBP1-RD individuals. Several research groups are investigating a variety of precision therapy approaches. Gene replacement is intended to add a fully working copy of the *STXBP1* gene in cells to counteract haploinsufficiency and is being investigated by several groups. In one example, rescue of haploinsufficiency by an AAV-Stxbp1 construct in a mouse model of STXBP1 encephalopathy reversed abnormal hindlimb clasping, reduced the number of spike-wave discharges and myoclonic seizures, reversed anxiety-like behaviors, and rescued cognitive and nest-building behaviors.[37] Importantly, these results were achieved in adult animals. Viral-mediated delivery of *STXBP1* transcription factor regulators is an approach being investigated to increase the expression of the *STXBP1* gene to relieve haploinsufficiency, as is CRISPR activation (CRISPRa), which utilizes a guide RNA to direct a CRISPR effector coupled to a transcriptional activator. Antisense oligonucleotide (ASO) gene therapy is an active area of research. ASOs can be used to induce exon splicing to remove specific variants in the *STXBP1* mRNA or used to block RNA-degrading micro RNAs, thus increasing the amount of functional STXBP1 protein. Like ASOs, antisense long non-coding RNAs, known as SINEUPs, can be used to increase translation from mRNA and increase endogenous STXBP1 protein levels. Table 1 summarizes the state of STXBP1 therapeutic development as of early 2024. These therapies are at various stages of development, some will continue to be pursued, eventually entering clinical trials, others will undoubtedly be abandoned, while new therapies and approaches will be added in the coming years. Regardless, any of these precision therapies, if proven successful, could address most, or maybe all, of the disease manifestations associated with STXBP1-RD.

Prepare for clinical trials

In April 2023, the STXBP1 Foundation established the STXBP1 Clinical Outcome Assessment

Table 1. STXBP1 therapies in development.

Type of therapy	Mechanistic approach	Developer(s)	Stage of development
Drug repurposing	4-Phenylbutyrate	Academic	Clinical trial
	Single repurposed drug candidate	Biopharma	Preclinical/New drug formulation
	In vitro screen of large FDA-approved drug library for increases in STXBP1 gene expression	Academic/Biopharma	Discovery/*In vitro* screening of potential drug candidates
	In silico testing of large drug library for interactions with specific STXBP1 variants	Academic/Biopharma	Discovery/*In vitro* and *in vivo* screening of potential drug candidates
Gene transfer	AAV-based STXBP1 gene replacement	Biopharma	Late preclinical
	AAV-based STXBP1 gene regulator	Biopharma	Early preclinical/*In vitro* testing of drug candidate
	AAV-based STXBP1 gene replacement	Biopharma	Discovery/drug candidate screening
CRISPR	CRISPRa-based STXBP1 gene activation	Academic	Discovery/*In vitro* drug candidate identification
RNA therapy	ASO-based, block micro-RNA to increase STXBP1 translation	Academic/Biopharma	Early preclinical/*In vitro* testing of drug candidate
	ASO-based, target regulatory site to increase STXBP1 translation	Biopharma	Early preclinical/*In vitro* testing of drug candidate
	ASO-based, target potential poison exons	Academic	Discovery/Target and drug candidate identification
	ASO-based, unknown mechanism	Pharma	Discovery/Drug candidate identification
	ASO-based, unknown mechanism	Pharma	Discovery/Drug candidate identification
	SINEUP-based, increased STXBP1 translation	Academic	Discovery/Drug candidate identification
Readthrough	Suppressor tRNA	Biopharma	Late preclinical
	Premature stop readthrough drug	Academic	Early preclinical/*In vitro* testing of drug candidate

AAV, adeno-associated virus; ASO, antisense oligonucleotide; CRISPRa, CRISPR activation; STXBP1, syntaxin-binding protein 1.

and Biomarker Consortium (S-COMB). This multi-stakeholder consortium, including academic researchers, clinicians, and industry, is focused on establishing a common set of tests and outcome measures in patients that can be used in clinical trials to establish whether a treatment successfully improves the symptoms of STXBP1-RD that matter to patients, and ensuring that data, knowledge, and research resources are shared.

As previously noted, multiple studies phenotyping patients with STXBP1-RD have been completed, and ongoing studies collecting patient-reported data through RARE-X and Simons Searchlight, as well as electronic medical

record data through Ciitizen, are being conducted. These efforts, along with lessons learned in the 4-phenylbutyrate trial, provided adequate foundational work to enable the design of a regulatory-compliant, prospective natural history study.

The STARR (STXBP1 Clinical Trial Readiness) Study was launched in the United States in July 2023. Importantly, the goal of this study is to prepare for clinical trials by gaining a better understanding of how STXBP1-RD progresses over time and to develop a standardized set of clinic assessments and biomarkers for STXBP1-RD patients. The study is being conducted at four clinical sites: Children's Hospital of Philadelphia, Children's Hospital Colorado, Baylor/Texas Children's Hospital, and Weill-Cornell. The STXBP1 Foundation and Children's Hospital of Philadelphia are leading the study, with Ingo Helbig, MD, from Children's Hospital of Philadelphia serving as the study's Principal Investigator. The STARR study is being conducted in two phases: a 12-month cross-sectional phase and a 2–5-year longitudinal phase. The cross-sectional phase is being conducted in five separate age cohorts; the primary purpose is to evaluate multiple clinical outcome measures and use the data collected to select a set of outcome measures that can be used for future clinical trials. The longitudinal phase will be a regulatory-compliant study to follow STXBP1-RD disease progression in all ages with the primary goal of obtaining control data that may be utilized in future interventional drug trials.

A patient registry and longitudinal natural history study are also being launched in Europe by the European STXBP1 Consortium (ESCO), a research consortium that currently includes nine countries (http://stxbp1eu.org); the STXBP1 Foundation has provided initial funding to ESCO. Importantly, the STARR and ESCO natural history studies have collaborated to align the protocols of the studies, including the age cohorts, visit cadence, and core assessments. This alignment is critical to enable maximal insights from these studies to inform clinical trials.

In addition to natural history studies, the Foundation and our research community are very engaged in the development of biomarkers for STXBP1-RD. A major focus in this area is identifying potential STXBP1-specific EEG signatures as a potential biomarker to monitor the response to treatment. One comparison of EEGs from children with STXBP1-RD to EEGs from neurotypical children revealed a decrease in the excitatory/inhibitory ratio.[38] Other EEG data comparisons are ongoing with promising initial results. Metabolomic, proteomic, and transcriptomic studies on body fluids (blood, cerebral spinal fluid, urine) from STXBP1-RD individuals and age- and family-matched controls are being carried out by several investigators with the goal of identifying biomarkers for disease progression and/or drug response and target engagement. To help accelerate this biomarker work, the Foundation started a Biomarker Working Group (BWG) as part of the S-COMB initiative. The BWG allows various STXBP1 stakeholders to share results and collaborate in the design and implementation of new biomarker studies. In addition, the Foundation maintains a biospecimen repository *via* a collaboration with COMBINEDBrain (https://combinedbrain.org/biorepository). As of December 2023 there were over 1200 biosamples available from approximately 120 individuals (STXBP1-RD patients and controls) for research use.

Build biopharma company interest

In 2019, when the Foundation mapped out its first set of priorities, there was only one new therapy starting to be developed, the repurposing of 4-phenylbutyrate, and there were no identified biopharma companies that acknowledged they were developing therapies for STXBP1-RD. By the end of 2022 more than nine potential therapies were in development at various biopharma companies. The Foundation, through its support of the STARR and ESCO studies, the S-COMB, and the BWG, is working to make STXBP1-RD an attractive candidate disorder for therapy development. Through the STARR and ESCO studies we aim to provide patient control data to our biopharma partners as well as set up multiple clinical sites capable of running clinical drug trials for STXBP1-RD. We are working closely with our biopharma partners in the development of EEG and body fluid biomarkers.

Another crucial avenue through which the Foundation contributes to advancing future drug development involves active collaboration with

regulatory agencies. In addition to the FDA Patient Listening Session held in April 2022, in October 2023, the Foundation hosted a public Patient-Focused Drug Development (PFDD) meeting for the FDA. The PFDD dealt with the health implications and daily challenges associated with STXBP1-RD, along with an examination of the existing landscape of treatment approaches. These meetings serve the purpose of enlightening the FDA about the varied clinical manifestations of STXBP1-RD, highlighting the complexities in assessing patient capabilities. The overarching goal is to assist regulatory authorities in comprehending how forthcoming therapies can optimally enhance the well-being of our patients. As we move forward with the STARR study, we will continue to engage with the Agency to confirm the data we are collecting meets their expectations. *Via* our collaboration with ESCO and alignment of protocols, we will also work to make sure our data is in accordance with European regulatory requirements.

Support patients today

As we engage with Biopharma and work towards supporting future precision medicines for STXBP1-RD we are also working to improve our patients' lives, and those of their caregivers, today. We have worked with Children's Hospital of Philadelphia and Children's Hospital of Colorado to help set up and/or expand multidisciplinary clinics where our kids can be more fully assessed by a healthcare team during a single visit, and we are working to open additional multidisciplinary clinics in other geographical areas of the country. We are currently working, in collaboration with our physician partners, on developing a 'Standard of Care for STXBP1-RD' that can be shared with the patient/caregiver community and provided to primary care physicians who may have little to no experience in seeing or treating STXBP1-RD patients.

Through our yearly family meetings, our website, and *via* social media, the Foundation works to educate our caregivers and keep them updated on the latest in drug development and biomarker work, and we continue to advocate at the local, state, and federal levels on behalf of our caregivers and patients. In October 2021, we launched STXBP1 Global Connect (https://www.stxbp1globalconnect.org), an affiliation of associations and parent representatives from countries around the world with the goal of connecting our communities of parents, scientists, and medical professionals across the globe with the common purpose of developing transformative therapies for this rare genetic disease.

What does the future hold for STXBP1

Over the past few years, the number of STXBP1-RD therapies in the developmental pipeline has increased dramatically. Over the next few years we anticipate several of these therapies to be in phase 1 or 2 clinical trials and additional therapies entering preclinical development. At this time, through our STARR study and biomarker work, we anticipate a standardized set of clinical outcome measures will have been chosen and evaluated and one or two biomarkers will be available. Both will further accelerate drug development as well as our understanding of STXBP1-RD disease progression. This acceleration in drug development and multiple 'shots-on-goal' will be needed as we anticipate some current therapies will not move forward due to pharmacological or other reasons.

We are hopeful that over the next several years, repurposed drugs may help reduce the severity of some STXBP1-RD symptoms for our patients while the promise of precision therapies, which will allow our kids autonomy and greater quality of life, begins to be realized. What we know now is that the knowledge base for STXBP1 is growing faster than it ever has before and we stand at the precipice of major advances.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Author contributions

James R. Goss: Conceptualization; Writing – original draft.

Benjamin Prosser: Conceptualization; Writing – review & editing.

Ingo Helbig: Conceptualization; Writing – review & editing.

Charlene Son Rigby: Conceptualization; Writing – original draft.

Acknowledgements
None.

Funding
The authors received no financial support for the research, authorship, and/or publication of this article.

Competing interests
The authors declare that there is no conflict of interest.

Availability of data and materials
Not applicable.

ORCID iD
James R. Goss https://orcid.org/0000-0002-7376-8059

References

1. Saitsu H, Kato M, Mizuguchi T, *et al.* De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat Genet* 2008; 40: 782–788.
2. Otsuka M, Oguni H, Liang J-S, *et al.* STXBP1 mutations cause not only Ohtahara syndrome but also West syndrome – result of Japanese cohort study. *Epilepsia* 2010; 51: 2449–2452.
3. Carvill GL, Weckhuysen S, McMahon JM, *et al.* GABRA1 and STXBP1: novel genetic causes of Dravet syndrome. *Neurology* 2014; 82: 1245–1253.
4. Allen AS, Berkovic SF, Cossette P, *et al.* De novo mutations in epileptic encephalopathies. *Nature* 2013; 501: 217–221.
5. Deprez L, Weckhuysen S, Holmgren P, *et al.* Clinical spectrum of early-onset epileptic encephalopathies associated with STXBP1 mutations. *Neurology* 2010; 75: 1159–1165.
6. Hamdan FF, Gauthier J, Dobrzeniecka S, *et al.* Intellectual disability without epilepsy associated with STXBP1 disruption. *Eur J Hum Genet* 2011; 19: 607–609.
7. Stamberger H, Nikanorova M, Willemsen MH, *et al.* STXBP1 encephalopathy: a neurodevelopmental disorder including epilepsy. *Neurology* 2016; 86: 954–962.
8. Xian J, Parthasarathy S, Ruggiero SM, *et al.* Assessing the landscape of STXBP1-related disorders in 534 individuals. *Brain J Neurol* 2022; 145: 1668–1683.
9. López-Rivera JA, Pérez-Palma E, Symonds J, *et al.* A catalogue of new incidence estimates of monogenic neurodevelopmental disorders caused by de novo variants. *Brain J Neurol* 2020; 143: 1099–1105.
10. Symonds JD and McTague A. Epilepsy and developmental disorders: next generation sequencing in the clinic. *Eur J Paediatr Neurol* 2020; 24: 15–23.
11. Rizo J and Xu J. The synaptic vesicle release machinery. *Annu Rev Biophys* 2015; 44: 339–367.
12. Harrison SD, Broadie K, van de Goor J, *et al.* Mutations in the Drosophila Rop gene suggest a function in general secretion and synaptic transmission. *Neuron* 1994; 13: 555–566.
13. Verhage M, Maia AS, Plomp JJ, *et al.* Synaptic assembly of the brain in the absence of neurotransmitter secretion. *Science* 2000; 287: 864–869.
14. Grone BP, Marchese M, Hamling KR, *et al.* Epilepsy, behavioral abnormalities, and physiological comorbidities in syntaxin-binding protein 1 (STXBP1) mutant zebrafish. *PLoS One* 2016; 11: e0151148.
15. Weimer RM, Richmond JE, Davis WS, *et al.* Defects in synaptic vesicle docking in unc-18 mutants. *Nat Neurosci* 2003; 6: 1023–1030.
16. Kovacevic J, Maroteaux G, Schut D, *et al.* Protein instability, haploinsufficiency, and cortical hyper-excitability underlie STXBP1 encephalopathy. *Brain J Neurol* 2018; 141: 1350–1374.
17. Chen W, Cai Z-L, Chao ES, *et al.* Stxbp1/Munc18-1 haploinsufficiency impairs inhibition and mediates key neurological features of STXBP1 encephalopathy. *eLife* 2020; 9: e48705.
18. Sullivan KR, Ruggiero SM, Xian J, *et al.* A disease concept model for STXBP1srelated disorders. *Epilepsia Open* 2023; 8: 320–333.
19. Balagura G, Xian J, Riva A, *et al.* Epilepsy course and developmental trajectories in *STXBP1*-DEE. *Neurol Genet* 2022; 8: e676.
20. Stamberger H, Crosiers D, Balagura G, *et al.* Natural history study of STXBP1-developmental and epileptic encephalopathy into adulthood. *Neurology* 2022; 99: e221–e233.

21. O'Brien S, Ng-Cordell E, Astle DE, *et al*. STXBP1-associated neurodevelopmental disorder: a comparative study of behavioural characteristics. *J Neurodev Disord* 2019; 11: 17.

22. Abramov D, Guiberson NGL and Burré J. STXBP1 encephalopathies: clinical spectrum, disease mechanisms, and therapeutic strategies. *J Neurochem* 2021; 157: 165–178.

23. Hopkins CE, McCormick K, Brock T, *et al*. Clinical variants in *Caenorhabditis elegans* expressing human STXBP1 reveal a novel class of pathogenic variants and classify variants of uncertain significance. *Genet Med Open* 2023; 1: 100823.

24. Zhu B, Mak JCH, Morris AP, *et al*. Functional analysis of epilepsy-associated variants in STXBP1/Munc18-1 using humanized *Caenorhabditis elegans*. *Epilepsia* 2020; 61: 810–821.

25. Lu Z, He S, Jiang J, *et al*. Base-edited cynomolgus monkeys mimic core symptoms of STXBP1 encephalopathy. *Mol Ther* 2022; 30: 2163–2175.

26. Dulubova I, Khvotchev M, Liu S, *et al*. Munc18-1 binds directly to the neuronal SNARE complex. *Proc Natl Acad Sci U S A* 2007; 104: 2697–2702.

27. Sudhof TC and Rizo J. Synaptic vesicle exocytosis. *Cold Spring Harb Perspect Biol* 2011; 3: a005637.

28. Berkel AA, Santos TC, Shaweis H, *et al*. Loss of MUNC18-1 leads to retrograde transport defects in neurons. *J Neurochem* 2021; 157: 450–466.

29. Chai YJ, Sierecki E, Tomatis VM, *et al*. Munc18-1 is a molecular chaperone for α-synuclein, controlling its self-replicating aggregation. *J Cell Biol* 2016; 214: 705–718.

30. Van Berkel AA, Koopmans F, Gonzalez-Lozano MA, *et al*. Dysregulation of synaptic and developmental transcriptomic/proteomic profiles upon depletion of MUNC18-1. *eNeuro* 2022; 9: ENEURO.0186-22.2022.

31. Lammertse HCA, Moro A, Saarloos I, *et al*. Reduced dynamin-1 levels in neurons lacking MUNC18-1. *J Cell Sci* 2022; 135: jcs260132.

32. Tang F, Xiao D, Chen L, *et al*. Role of Munc18-1 in the biological functions and pathogenesis of neurological disorders. *Mol Med Rep* 2021; 23: 198.

33. Urigüen L, Gil-Pisa I, Munarriz-Cuezva E, *et al*. Behavioral, neurochemical and morphological changes induced by the overexpression of munc18-1a in brain of mice: relevance to schizophrenia. *Transl Psychiatry* 2013; 3: e221.

34. Martin S, Papadopulos A, Tomatis VM, *et al*. Increased polyubiquitination and proteasomal degradation of a Munc18-1 disease-linked mutant causes temperature-sensitive defect in exocytosis. *Cell Rep* 2014; 9: 206–218.

35. Guiberson NGL, Pineda A, Abramov D, *et al*. Mechanism-based rescue of Munc18-1 dysfunction in varied encephalopathies by chemical chaperones. *Nat Commun* 2018; 9: 3986.

36. Grinspan Z, Abila M, Basma N, *et al*. 4-Phenylbutyrate for children with STXBP1 and SLC6A1 mutations – a pilot clinical trial, https://aesnet.org/abstractslisting/4-phenylbutyrate-for-children-with-stxbp1-and-slc6a1-mutations—a-pilot-clinical-trial (2022, accessed 28 December 2023).

37. Chen W. Genetic rescue of mouse models of STXBP1 related developmental and epileptic encephalopathy in adulthood, https://aesnet.org/abstractslisting/genetic-rescue-of-mouse-models-of-stxbp1-related-developmental-and-epileptic-encephalopathy-in-adulthood (2021, accessed 28 December 2023).

38. Houtman SJ, Lammertse HCA, Van Berkel AA, *et al*. STXBP1 syndrome is characterized by inhibition-dominated dynamics of resting-state EEG. *Front Physiol* 2021; 12: 775172.