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VIEWS & REVIEWS

STXBP1 encephalopathy

Connecting neurodevelopmental disorders with α-synucleinopathies?

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Abstract

De novo pathogenic variants in *STXBP1* encoding syntaxin1-binding protein (STXBP1, also known as Munc18-1) lead to a range of early-onset neurocognitive conditions, most commonly early infantile epileptic encephalopathy type 4 (EIEE4, also called STXBP1 encephalopathy), a severe form of epilepsy associated with developmental delay/intellectual disability. Other neurologic features include autism spectrum disorder and movement disorders. The progression of neurologic symptoms has been reported in a few older affected individuals, with the appearance of extrapyramidal features, reminiscent of early onset parkinsonism. Understanding the pathologic process is critical to improving therapies, as currently available antiepileptic drugs have shown limited success in controlling seizures in EIEE4 and there is no precision medication approach for the other neurologic features of the disorder. Basic research shows that genetic knockout of STXBP1 or other presynaptic proteins of the exocytic machinery leads to widespread perinatal neurodegeneration. The mechanism that regulates this effect is under scrutiny but shares intriguing hallmarks with classical neurodegenerative diseases, albeit appearing early during brain development. Most critically, recent evidence has revealed that STXBP1 controls the self-replicating aggregation of α-synuclein, a presynaptic protein involved in various neurodegenerative diseases that are collectively known as synucleinopathies, including Parkinson disease. In this review, we examine the tantalizing link among STXBP1 function, EIEE, and the neurodegenerative synucleinopathies, and suggest that neural development in EIEE could be further affected by concurrent synucleinopathic mechanisms.

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Glossary

CSPα = cysteine-string protein α; **EIEE4** = early infantile epileptic encephalopathy type 4; **FCD** = focal cortical dysplasia; **Hsp70** = heat shock protein 70; **PD** = Parkinson disease; **SNARE** = soluble N-ethylmaleimide-sensitive factor attachment protein receptor; **STXBP1** = syntaxin binding protein 1; **STXBP1-E** = *STXBP1* encephalopathy.

Syntaxin binding protein 1 (STXBP1), also known as Munc18-1, is a member of the Sec1/Munc18-1 family of proteins that are important regulators of the secretory and synaptic vesicle fusion machinery that underpins hormonal and neuronal transmission, respectively. STXBP1 promotes vesicular priming by opening syntaxin-1A, a critical step that allows it to engage in the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex.[1] Genetic ablation of *Stxbp1* in mice leads to perinatal paralysis-induced lethality,[2] demonstrating that STXBP1 is involved in synaptic transmission. STXBP1 also plays an important role in the early stages of neuritogenesis, which depends on vesicular fusion.[3]

Early infantile epileptic encephalopathy 4 (EIEE4) (MIM 612164) is linked to mutations in *STXBP1*,[2,4] and pathogenic de novo variants of *STXBP1* lead to a range of neurologic features, including autism and movement disorders.[5,6] EIEE4 is a severe early-onset form of developmental delay/intellectual disability that is often associated with intractable seizures. Individuals with EIEE4 exhibit a broad spectrum of seizure types, including epileptic spasms, focal seizures, and tonic seizures. Seizure onset is most common in the first year of life, with the most frequent EEG features being burst suppression and hypsarrhythmia. However, as epilepsy is not universally present in individuals with de novo *STXBP1* variants,[7] *STXBP1* encephalopathy (STXBP1-E) constitutes a more appropriate term. A range of neuroradiologic features have been reported in patients with STXBP1-E who have undergone MRI, including cerebral atrophy, abnormal myelination, and hypoplasia of the corpus callosum.[7–9] Focal cortical dysplasia has been confirmed after epilepsy surgery in 2 patients,[7,8] one of whom had a normal MRI scan. It is therefore possible that subtle abnormalities in cortical development are present in more individuals, but that these remain underdiagnosed due to limitations of current neuroradiologic techniques. Seizures, when present, are frequently resistant to polytherapy, and there is no targeted therapy for the other neurologic symptoms of the condition. Therefore, a more precise therapeutic approach is highly desirable.[5]

Over the last 2 decades, research has linked variants in a range of presynaptic proteins, which are ultimately involved in mediating neurotransmitter release, to both early-onset neurodevelopmental conditions such as EIEE and to later-onset neurodegenerative conditions such as synuclein (*SNCA*)–related Lewy body dementia (MIM 127750[10]) and Parkinson disease (PD) (MIM 168601 and 605543[11]; table 1). Interestingly, recent studies have reported a few older patients with STXBP1-E who displayed symptoms reminiscent of juvenile-onset parkinsonism, with tremor and prominent extrapyramidal features.[12,13] Such a neurodegenerative course is in line with animal knockdown studies of *STXBP1* and other major presynaptic vesicle proteins such as syntaxin-1B, cysteine-string protein α (CSPα), and SNAP25, which have reported the presence of widespread neurodegeneration (table 2).

Whereas the pathologic mechanism of EIEE4 is generally thought to stem from haploinsufficiency, leading to reduced levels of functional STXBP1 protein, other lines of evidence are supportive of a gain of toxic function mechanism leading to neurodegeneration. This review gathers the currently available evidence linking *STXBP1* variants to either a loss of function or a gain of toxic function. We suggest that in addition to the neurodevelopmental phenotype, STXBP1-E contains a neurodegenerative aspect stemming from a proaggregative effect of pathogenic variants on α-synuclein, a key protein in the pathogenesis of PD.

Genetics of STXBP1-E

A broad range of pathogenic germline heterozygous *STXBP1* variants have been reported in nearly 200 affected individuals, including frameshift, missense, splice site, and nonsense sequence variants, together with whole gene and intragenic deletions (figure 1). Most variants occur de novo in simplex families, although germline mosaicism with recurrence in siblings has occasionally been reported. A combination of germline and somatic mosaicism for a multi-exonic *STXBP1* deletion has also been found in an individual with STXBP1-E and focal cortical dysplasia, with evidence of a mosaic homozygous deletion in resected dysplastic brain tissue.[6] The distribution of pathogenic variants is surprisingly widespread in the exonic sequence, although structural analysis of pathogenic missense variants has demonstrated that they are significantly more likely to occur at highly conserved locations and to be buried inside the protein core region. A number of recurrent variants have been reported, particularly in domains 2 and 3. Large clinical cohort studies have not been able to provide clear phenotypic–genotypic correlations. Affected individuals with deletion, frameshift, splice site, or nonsense variants are not clearly different in terms of the severity or range of neurologic or developmental presentations, at least in childhood, compared to those individuals with missense variants.[7] This suggests a common pathologic mechanism that remains to be established.

Table 1 Variants in presynaptic proteins implicated in human neurodevelopmental disorders, epilepsy, and neurodegenerative diseases

Variants in affected individuals	Observed phenotypes (MIM numbers if available)
Heterozygous and homozygous variants in *UNC13A* encoding Munc13-1	• De novo heterozygous p.Pro814Leu variant in *UNC13A* (Munc13-1) associated with delayed neurologic development, dyskinetic movement disorder, and autism[47] • Homozygous truncating variant in *UNC13A* associated with syndrome of microcephaly, cortical hyperexcitability, and fatal myasthenia
De novo variants in *STXBP1* encoding Munc18-1/2	• De novo heterozygous missense or loss of function variants associated with early infantile epileptic encephalopathy (MIM 612164), intellectual disability, autism movement disorder,[4,48,49] and parkinsonism
De novo and inherited variants in *STX1B* encoding syntaxin-1B	• *STX1B* deletions, truncating and missense variants associated with fever-associated epilepsy syndromes ranging from simple febrile seizures to severe epileptiform encephalopathies (MIM 616172)[50] • Polymorphism in *STX1B* associated with susceptibility to Parkinson disease[51]
De novo variant in *SYT1* encoding synaptotagmin-1	• De novo heterozygous p.Ile368Thr variant associated with early-onset dyskinetic movement disorder, severe motor delay, and profound cognitive impairment[52]
Inherited missense variants in *LRRK2* encoding leucine-rich repeat serine/threonine-protein kinase 2	• Inherited heterozygous missense variants associated with Lewy body Parkinson disease, nigrostriatal cell loss without α-synuclein-positive Lewy bodies or Lewy neurites, progressive supranuclear palsy, and multiple system atrophy (MIM 168600; 607,060)[8,53,54]
Variants of genes encoding Ras-associated (Rab) proteins	• Homozygous or compound heterozygous loss of function and missense variants in *RAB3GAP1*, *RAB3GAP2*, and *RAB1B* associated with syndromic neurodevelopmental disorders characterized by eye, nervous system, and endocrine abnormalities, Warburg Micro syndrome (MIM 600118; 614,225 and 614,222), and Martsolf syndrome (MIM 212720); neurologic involvement includes intellectual disability, microcephaly, and progressive spastic paraplegia[55,56] • Inherited hemizygous missense and truncating variants in *GDI1* associated with nonsyndromic X-linked intellectual disability (MIM 300849)
Variants in *SNCA* encoding α-synuclein	• Inherited heterozygous missense, truncating and copy number variants in *SNCA* associated with Lewy body dementia (MIM 127750), Parkinson disease (MIM 168601 and 605543), paralysis, and motor deficits[11,57]
Variants of genes encoding synapsins	• Inherited hemizygous truncation variants in *SYN1* associated with intellectual disability, autism, and epilepsy (MIM 300491)[58] • Truncating and missense variants in *SYN2* associated with autism[59,60] and polymorphism associated with predisposition to idiopathic generalized epilepsy • Polymorphisms in SYN3 associated with psychiatric disease[33]
De novo variants in *SNAP25* encoding synaptosomal-associated protein	• De novo missense and truncating variants in both isoforms of *SNAP25* associated with intellectual disability, epilepsy, and cerebellar ataxia (MIM 616330)[61]
Variants in *SYNJ1* encoding synaptojanin1	• Autosomal recessive missense and truncating *SYNJ1* variants associated with neonatal refractory epilepsy and neurodegenerative disease (MIM 617389)[32] • A recurrent homozygous missense variant p.Arg258Gln associated with early-onset parkinsonism (MIM 615530)[62,63]
Variants in *PPP3CA* encoding protein phosphatase 3, catalytic subunit, alpha isoform	• De novo missense and nonsense variants in PPP3CA associated with early-onset epileptic encephalopathy (MIM 617711)[64]
Variants in *DNM1* encoding dynamin 1	• De novo missense variants associated with early-onset epileptic encephalopathy (MIM 616346)[65,66]

Evidence for a loss of function mechanism

Although the genetic link between *STXBP1* and encephalopathy has been well-documented, it remains unclear whether the pathology stems from a loss of STXBP1 function or from a gain of pathologic function associated with the aggregation of *STXBP1*-mutated proteins. However, the fact that many variants lead to similar pathologic outcomes points to a loss of function/haploinsufficiency mechanism. Many pathogenic variants in *STXBP1*, such as the recurrent variant NM_003165.3: c.539G>A; Cys180Tyr,[4] are located in the hydrophobic core of the protein and have been hypothesized to destabilize the secondary structure of STXBP1, thereby decreasing its thermostability and thus vesicular exocytosis.[4,14] Consistent with these previous findings, Suri and collaborators[15] used in silico structural modeling to demonstrate that the protein stability of *STXBP1* pathogenic missense variants was typically reduced compared to that of wild-type *STXBP1*. This provides some evidence that the mechanism of pathogenicity for missense variants in *STXBP1* is haploinsufficiency through destabilization of the native folded state of the protein, making it prone to misfolding, aggregation, and degradation. This viewpoint aligns with the findings of other groups who have provided direct evidence of the destabilization and aggregation of several pathogenic missense variants in *STXBP1*.[4,16–18]

Table 2 Knockout of various exocytic proteins leads to paralysis and neurodegeneration

Knockout mice of exocytic proteins	Observed phenotypes
STXBP1/Munc18-1$^{-/-}$	• Severe paralysis and neuronal apoptosis[2] • Neurodegeneration, tau phosphorylation, neurofibrillary tangles, and accumulation of insoluble proteins[27] • Neuronal cell death and early Golgi abnormalities[31]
Syntaxin-1B$^{-/-}$	• Paralysis and motor function impairments[28] • Disrupted brain morphology and neuronal cell death[28] • Reduced spontaneous and evoked neurotransmitter release[45] • Cell death and neurodegeneration[31]
SNAP25$^{-/-}$	• Cell death and neurodegeneration[29,31]
Cysteine-string protein α	• Decrease in SNAP25 and impaired SNARE assembly[67] • Neurodegeneration

Abbreviation: SNARE = soluble N-ethylmaleimide-sensitive factor attachment protein receptor.

STXBP1-E: A disorder of vesicular fusion, cortical development, neuritogenesis, or neurodegeneration?

Understanding the pathology of STXBP1-E is critical to finding new therapeutic approaches. In the following sections, we gather the evidence pointing to either a gain or a loss of function of STXBP1 in vesicular fusion, early brain development and neuritogenesis, and neurodegeneration.

Pathogenic *STXBP1* variants affect vesicular fusion

Given the critical role of STXBP1 in exocytosis, loss of function has been suggested as a potential pathologic mechanism that leads to defective synaptic transmission. Indeed, several *STXBP1* variants are unable to regulate normal neuronal exocytosis, resulting in defective synaptic transmission.[14,19]

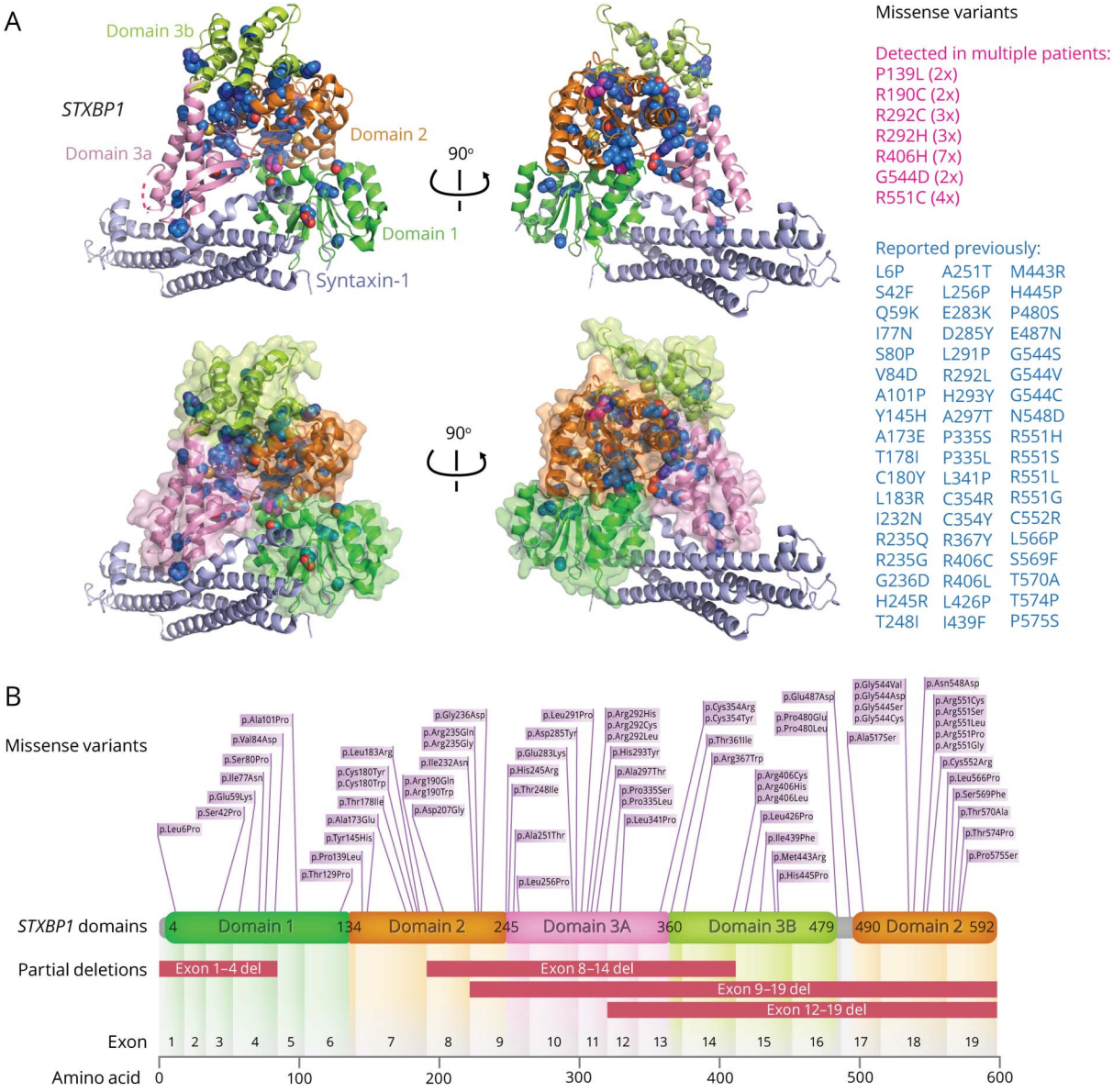
Although the common pathogenic variants STXBP1^{Cys180Tyr} and STXBP1^{Cys552Arg} are able to form a normal binary complex with syntaxin-1A, 2 studies have reported a defect in exocytosis.[14,19] First, STXBP1^{Cys180Tyr} was unable to rescue stimulated exocytosis at 37°C.[14] Second, STXBP1^{Cys552Arg} was unable to accelerate SNARE-mediated lipid content mixing, suggesting an impairment in membrane fusion. Although this mutant bound normally to the *cis*-SNARE complex, it did not promote the *trans*-SNARE zippering required for the fusion reaction.[19] Finally, other loss of function variants have been described, such as Pro335Leu[18] or Pro335Ala.[20] These variants are located within domain 3A, which is critical for vesicular priming,[14,21] and are unable to undergo the conformational change that triggers the opening of syntaxin-1A. Taken together, these lines of evidence point to an imbalance in vesicular fusion due to reduced STXBP1 activity.

Haploinsufficiency has also been proposed in recent studies using cultured *Stxbp1* knockout neurons[18] and heterozygous *Stxbp1*$^{+/-}$ mice and cultured neurons.[22,23] Interestingly, in vitro, both heterozygous and homozygous deletion of *Stxbp1* led to an inhibition of synaptic transmission[18,23] whereas in vivo, in the heterozygous state, no effect on neurotransmitter release was detected, although STXBP1 levels were reduced.[22] When disease-causing *STXBP1* variants were expressed in *Stxbp1*$^{+/-}$ mouse neurons, they all resulted in severely decreased protein levels,[22] some more than expected for haploinsufficiency, suggesting an additional gain of function (or dominant negative) mechanism such as templating/co-aggregating effects of some of the *STXBP1* variants on the wild-type protein. These additional effects have been characterized in vitro in cultured cells,[17] and in vivo.[18] Indeed, studies have demonstrated that *STXBP1* variants act as a template to promote the aggregation of wild-type STXBP1.[17,18] The *Stxbp1*$^{+/-}$ mouse model[22] is likely to be an excellent resource for future studies of STXBP1-E as it recapitulates some of the key human phenotypic features.[22]

Pathogenic *STXBP1* variants affect cortical development

Hamada et al.[24] recently demonstrated that knockdown of *Stxbp1* expression by in utero electroporation of small hairpin RNA resulted in abnormal cortical neuron migration in mice. This effect was almost completely rescued by re-expression of *Stxbp1*, suggesting that *Stxbp1* could play a role in cortical development by affecting both cortical neuron migration and neuritogenesis. However, no change in the morphology of the cortex could be detected in the *Stxbp1* knockout.[2] In patients with STXBP1-E, MRI is often reported as normal, or demonstrating subtle changes such as cerebral atrophy, myelination abnormalities, and hypoplasia of the corpus callosum.[7,8] However, diagnostic MRI may miss subtle abnormalities in cortical development; for example, Stamberger et al.[5] reported a patient with a de novo germline *STXBP1* variant who had undergone epilepsy surgery prior to genetic diagnosis and presented with evidence of a focal cortical dysplasia (FCD) type 1a with abnormal radial cortical lamination in resected brain. More recently, Uddin et al.[6] reported 7 patients with heterozygous de novo variants in *STXBP1*, 3 of whom had suspected or confirmed FCD. One patient in this cohort

Figure 1 Mutations in *STXBP1* linked to early infantile epileptic encephalopathy type 4 (EIEE4)



(A) The structure of STXBP1 is shown in the ribbon diagram in complex with syntaxin-1A (blue; top panels). The bottom panels represent the STXBP1 surface shown with transparency to highlight that the mutations lie within the hydrophobic core of the protein and not at the surface. Residues that have been found to be mutated in EIEE4 patients are represented by magenta spheres for those identified in multiple patients, and blue for those that have been reported only once. (B) Schematic diagram of the *STXBP1* gene, illustrating the location of point mutations and partial deletions in each of its domains (updated from references 7 and 15).

underwent epilepsy surgery, confirming a FCD type 1b as well as mosaicism for homozygosity of *STXBP1* mutations in the dysplastic tissue. Further clinical studies are required to assess the effect of heterozygous *STXBP1* mutations on cortical development, and the possible role of somatic mosaicism as a second hit in the *STXBP1* gene in patients with focal onset to their seizures.

Pathogenic *STXBP1* variants affect neuritogenesis

The early-onset forms of STXBP1-E, such as EIEE4, are characterized by severe developmental delay/intellectual disability. This aspect has been linked to neuronal development, with important variation in dendritic tree complexity and length. Dendrite growth relies on the supply of membranous material,[3] notably through exocytosis. STXBP1 has been shown to play an important role in dendrite outgrowth, as neurons from *Stxbp1* knockout mouse embryos present a decreased total length of their dendritic tree,[25] a finding that has been confirmed in *Drosophila*.[26] The involvement of STXBP1 in the development and maintenance of neurites has also been highlighted by Yamashita et al.,[16] who observed impaired neurite outgrowth in an induced pluripotent stem cell–generated neuronal culture derived

from a patient carrying an *STXBP1* nonsense variant (Arg367*). Therefore, *Stxbp1* haploinsufficiency or knockout seems to influence neurite morphology. Whether these effects stem from a direct effect of loss of STXBP1 function on dendritic tree morphogenesis or result from neurodegeneration due to a secondary/concomitant gain of toxic function will need to be assessed.

Pathogenic *STXBP1* variants induce neurodegeneration

The first evidence that neurodegeneration could contribute to STXBP1-E came from an early homozygous *Stxbp1* knockout mouse study.[2] As expected from a loss of function, these mice exhibited perinatal paralysis-induced lethality. However, widespread neuronal degeneration was detected in utero,[2] suggesting that *STXBP1* deletion triggers a neuronal cell death program that leads to neurodegeneration. Remarkably, no disruption in the developing cortex has been described in either homozygous[2] or heterozygous[22] *Stxbp1* knockdown mice. This was confirmed in a recent study, which showed that *Stxbp1* knockdown mice present widespread neurodegeneration despite normal early stages of spinal motor circuit formation.[24] The latter result suggests that neurotransmitter release is dispensable for the formation of neural circuits.[24] The study also demonstrated defects in the trafficking of syntaxin-1A,[24] postsynaptic density 95, the tyrosine receptor kinase B, and deleted in colorectal cancer receptors,[27] and pathologic similarities to Alzheimer disease, such as defects in tau phosphorylation, the development of neurofibrillary tangles, and the accumulation of insoluble proteins, suggesting a link with other neurodegenerative conditions.

Evidence for a role in neurodegeneration also comes from in vitro studies. The expression of STXBP1-E variants in hippocampal neurons leads to an increased proportion of pyknotic nuclei and extensive cell death.[17] Furthermore, neurites from these STXBP1-E variant-expressing neurons also exhibit classical signs of neurodegeneration, including the presence of neuritic spheroids that are inherent to neurodegenerative conditions such as Alzheimer disease.

Interestingly, *STXBP1* is not the only synaptic gene whose disruption leads to neuronal degeneration, as genetic ablation of other major presynaptic vesicle proteins produces similar neurodegenerative phenotypes.[28–30] For example, *syntaxin-1B* knockout mice display paralysis and motor function impairments as well as neuronal cell death,[28,31] while *SNAP25* and *CSPa* knockout mice exhibit neuronal cell death and neurodegeneration (table 2). Finally, different variants in the same genes encoding presynaptic proteins have been linked to both early and later onset neurodegeneration in humans; for example, autosomal recessive missense and truncating variants in the synaptojanin 1 (*SYNJ1*) gene are associated with both severe neonatal-onset refractory epilepsy with a neurodegenerative disease course (MIM 617389[32]) and early-onset parkinsonism (MIM 615530[33]). Together, these findings suggest a key role of presynaptic vesicle proteins in supporting neuronal survival.[29]

Toxic protein aggregation is a recurrent feature of neurodegenerative disorders, and the fact that many *STXBP1* variants exhibit strong aggregation when expressed in cells in vitro suggests the possibility of a gain of pathogenic function via neurodegeneration.[4,14] Neurodegenerative diseases are often associated with toxic protein aggregation based on their ability to partially or fully unfold and to act as templates for co-aggregation of endogenous wild-type protein.[34,35] STXBP1 joins the pool of such proteins, as STXBP1-E variants can co-aggregate wild-type STXBP1 in vitro, indicating that these variants have the ability to recruit new wild-type monomers to generate large aggregates.[17] This has been confirmed in neurosecretory cells and rat hippocampal neurons, where *STXBP1* variants dominantly sequester wild-type STXBP1 into aggregates.[17] It is worth noting that a recent study carried out in vitro and in vivo also found co-aggregation of wild-type and mutant STXBP1 but did not find toxicity associated with these aggregates.[18]

Evidence for the chaperoning activity of *STXBP1* for α-synuclein, controlling its self-replicating aggregation

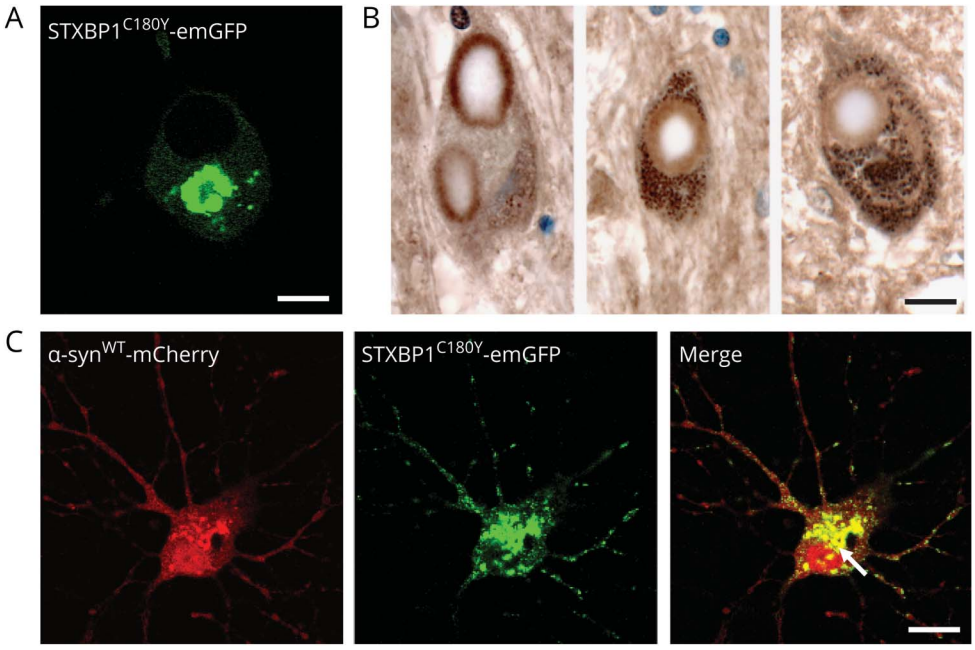
Some STXBP1-E variants were shown to form aggregates that display ring-like structures reminiscent of Lewy bodies upon overexpression in heterologous cells or primary rat hippocampal neurons (figure 2, A and B). These structures are positive for the aggregate-prone protein α-synuclein, a hallmark of the Lewy bodies present in patients with PD and related disorders.[10] Importantly, the neurons overexpressing STXBP1-E variants also contain STXBP1 aggregates that are positive for endogenous α-synuclein, suggesting the possibility of a gain of pathologic function associated with co-aggregation of STXBP1-E variants and α-synuclein.

Molecular chaperones help fold newly synthesized proteins, transport proteins into organelles, and reverse misfolding and aggregation of proteins.[36] A range of emerging evidence suggests that STXBP1 has a chaperoning function for α-synuclein.[17]

One major function of molecular chaperones is to prevent proteins and assembled protein complexes from aggregating[37] (figure 3). Knocking out *STXBP1* in neurosecretory cells (MKO-PC12 cells) is sufficient to significantly increase the number of endogenous and expressed α-synuclein aggregates, effects that can be fully rescued by re-expression of wild-type STXBP1 in these cells.[17] This suggests that STXBP1 plays a key role in chaperoning α-synuclein, and that *STXBP1* deletion is sufficient to promote α-synuclein aggregation.

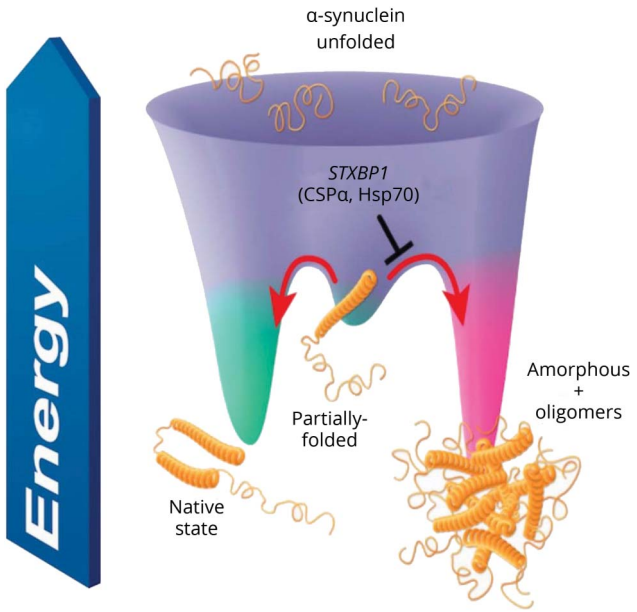
STXBP1-linked encephalopathy variants co-aggregate α-synuclein when expressed in rat hippocampal neurons and gene-edited neurosecretory cells in vitro. Expression of these mutants can effectively recruit endogenous α-synuclein into aggregates.[17] Conversely, overexpression of PD-linked

Figure 2 STXBP1^{C180Y}-GFP variant expressed in neurosecretory cells forms large aggregates, some displaying ring-like structures reminiscent of Lewy bodies



(A) Representative image shows Lewy body–like structure in MKO-PC12 cells expressing STXBP1^{C180Y}-emGFP (from Chai et al.,[17] 2016, with permission). Scale bar: 10 μm. (B) Immunohistochemistry using isodipeptide primary antibody (81D1C2) and rabbit polyclonal antibody for α-synuclein (Chemicon) in the substantia nigra of a patient with Parkinson disease with Lewy body dementia[46] (copyright 2003, National Academy of Sciences). Scale bar: 10 μm. (C) Representative confocal images of a hippocampal neuron at 8 days in vitro show aggregates positive for both α-synuclein^{WT}-mCherry and STXBP1^{C180Y}-emGFP (from Chai et al.,[17] 2016, with permission). Arrow indicates the co-localized aggregates. Scale bar: 20 μm.

Figure 3 Energy landscape of α-synuclein folding and misfolding



The folding and aggregation of α-synuclein are competing reactions. During the course of folding, chaperone molecules facilitate energetically favorable intramolecular interactions and prevent progression toward the aggregative unfolded state. α-Synuclein is prone to adopting misfolded states, ultimately leading to protein aggregation. Chaperones such as those indicated here regulate this aggregative process, thereby promoting folding to the native state conformation.

α-synuclein variants (Ala30Pro and Ala53Thr) causes endogenous and overexpressed wild-type STXBP1 to co-aggregate with α-synuclein.

STXBP1 may directly control the aggregation of α-synuclein, as its binding to both endogenous and overexpressed α-synuclein has been shown by pull-down assay.[17] This binding is potentiated by *STXBP1*-linked encephalopathy variants.

Taken together, these findings point to a critical role for STXBP1 in controlling α-synuclein aggregation, and suggest that STXBP1 acts as a molecular chaperone for α-synuclein. STXBP1 is already known to chaperone syntaxin-1A. Indeed, syntaxin-1A is ectopically expressed in rat kidney fibroblast cells and other non-neuronal cells lacking STXBP1. In these cells, syntaxin-1A localizes to the Golgi network or endoplasmic reticulum. Upon coexpression of STXBP1, syntaxin-1A primarily localizes correctly to the plasma membrane.[38,39]

Role of other α-synuclein chaperones in synaptic transmission and neurodegeneration

The significance of STXBP1 in the potential chaperoning of α-synuclein could have implications in synucleinopathies, including PD and Lewy body dementia. The primary component of the Lewy bodies that characterize these diseases are aggregates of α-synuclein, an abundant neuronal protein that

localizes in the presynaptic nerve terminals. Missense mutations (Ala30Pro, Glu46Lys, and Ala53Thr) of α-synuclein and duplications and triplications of the gene that encodes it are linked to early-onset PD.[40] It has previously been reported that α-synuclein aggregation is controlled by classical chaperones such as heat shock protein 70 (Hsp70).[41] Several studies have shown that Hsp70 and the 2 co-chaperones, CSPα and small glutamine-rich TRP protein (SGT), are chaperones that are involved in maintaining efficient neurotransmitter release and regulating synaptic vesicle exocytosis.[42] Knocking out CSPα in mice causes defects in synaptic transmission, cell death, and neurodegeneration.[42] Deletion of the *CSP* gene in flies also causes defects in synaptic transmission, leading to paralysis and premature death.[43]

Similar to CSPα, α-, β-, and γ-synuclein are abundant presynaptic proteins that are peripherally associated with synaptic vesicles. Strikingly, transgenic overexpression of human wild-type α-synuclein eliminates the lethal neurodegeneration in CSPα knockout mice, suggesting that upregulation of α-synuclein can compensate for loss of CSPα activity, restoring SNARE complexes to their correct levels.[30]

Considering that CSPα and Hsp70 have also been shown to chaperone α-synuclein and control its aggregative propensity, further experimental work is needed to assess whether STXBP1 has an overlapping chaperoning function for α-synuclein. It will be interesting to establish whether overexpression of *STXBP1* can compensate for loss of CSPα, or vice versa, to reduce α-synuclein aggregation and restore the deficits in synaptic exocytosis that lead to neurodegeneration.

Discussion

In this review, we provide an overview of the effects of pathogenic variants in STXBP1-E and discuss the current evidence related to the underlying pathophysiologic mechanisms of this disorder. Some studies point to a mechanism involving heterozygous loss of STXBP1 function, with subsequent reduced expression, leading to a defect in synaptic transmission. Although no obvious domain selectivity in missense variants can be seen, most tested STXBP1 variants have been shown to destabilize the native folded state of the protein, making it prone to misfolding, aggregation, and degradation. Neurons expressing *STXBP1* variants also exhibit classical signs of neurodegeneration, including neurite loss, pyknotic nuclei, and spheroid formation (figure 4A), phenotypes that suggest a possible gain of toxic function. STXBP1 was recently found to control the self-replicating aggregation of α-synuclein, a presynaptic protein that is involved in various neurodegenerative diseases, and missense variants have been shown to co-aggregate with both wild-type STXBP1 and α-synuclein. This suggests that disorders caused by *STXBP1* mutations may be involved in α-synuclein pathology.

There are still a limited number of older individuals with a confirmed molecular diagnosis of STXBP1-E.[2] This reflects current referral patterns for genetic testing, whereby primarily pediatric patients access diagnostic genetic testing.[44,45] Longitudinal clinical studies of cohorts of affected individuals with STXBP1-E, or studies of a larger cohort of older individuals, are required in order to clearly delineate the frequency of progressive neurologic symptoms, and to clarify if there is any genotypic–phenotypic correlation in the occurrence of later neurologic complications.

We propose that synaptic dysfunction caused by haploinsufficiency of *STXBP1* together with STXBP1 and α-synuclein aggregation as well as the loss of function of STXBP1 could lead to a unique combination of abnormal neurodevelopment, cell death, and neurodegeneration (figure 4B). In the longer term, targeting STXBP1 as a major regulator of α-synuclein aggregation may lead to improved clinical outcomes in the treatment of both synucleinopathies and STXBP1-E.

Author contributions

V. Lanoue: drafting/revising the manuscript, study concept or design, accepts responsibility for conduct of research and final approval, study supervision. Y.J. Chai: drafting/revising the manuscript, data acquisition, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, contribution of vital reagents/tools/patients, acquisition of data, statistical analysis, study supervision, obtaining funding. J.Z. Brouillet: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval. S. Weckhuysen: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval. E.E. Palmer: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval. B.M. Collins: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval, study supervision, obtaining funding. F.A. Meunier: drafting/revising the manuscript, study concept or design, accepts responsibility for conduct of research and final approval, study supervision, obtaining funding.

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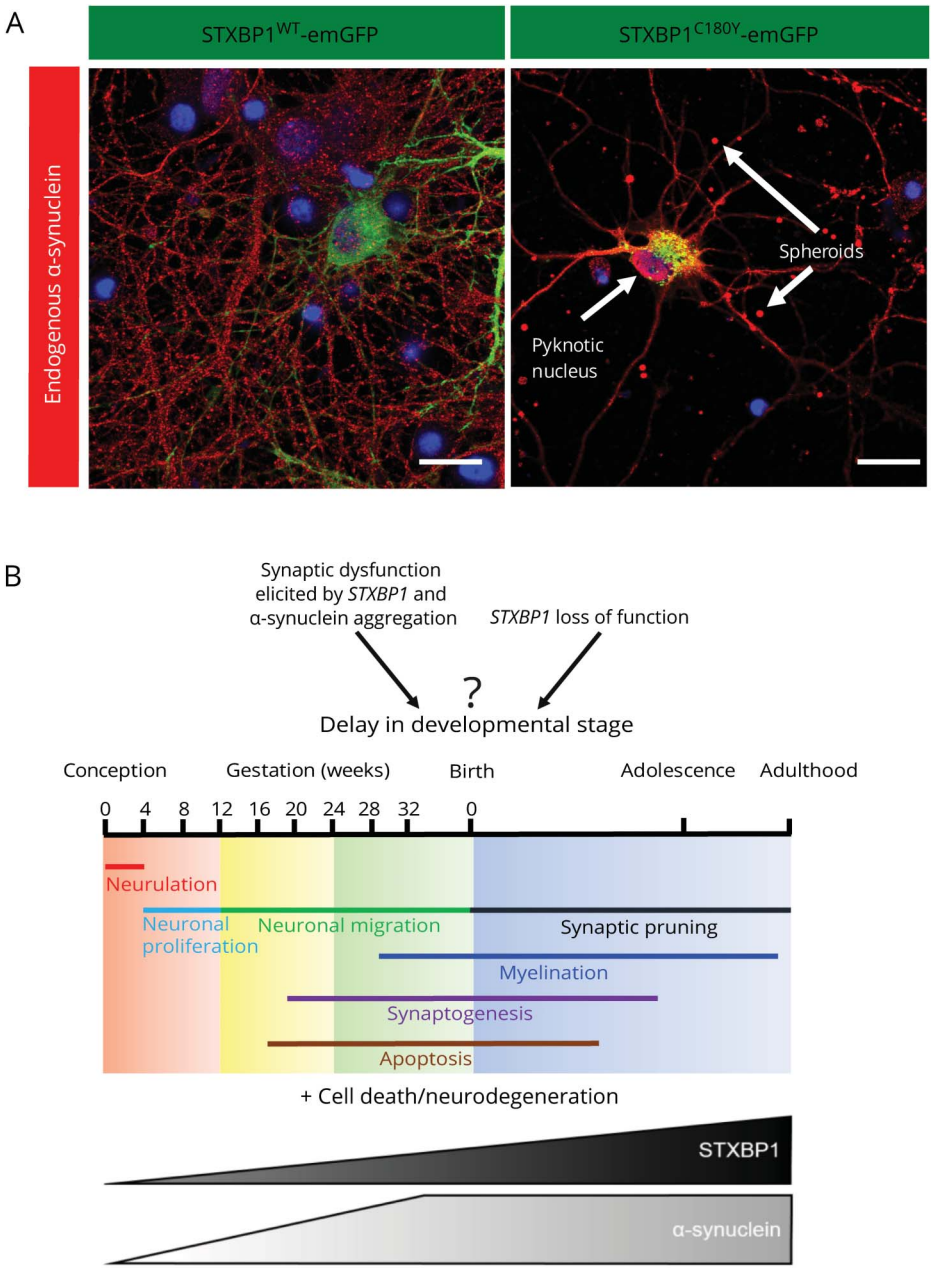
Figure 4 *STXBP1* mutations and haploinsufficiency may trigger aggregation and lead to aggregation-induced neurodegeneration



(A) Representative images of hippocampal neurons transfected with either STXBP1^{WT}-emGFP or STXBP1^{C180Y}-emGFP and labeled for endogenous α-synuclein (red). Nuclei were stained with DAPI (adapted from Chai et al.,[17] 2016, with permission). Scale bar: 20 μm. Spheroids and a pyknotic nucleus are highlighted. (B) *STXBP1* mutations and haploinsufficiency may trigger a pathogenic gain of function through both the corruption of native STXBP1 function and the perturbed chaperone activity for α-synuclein, leading to a delay in brain development and aggregation-induced neurodegeneration.

Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

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