Abstract

Background

Syntaxin-binding protein 1, encoded by STXBP1, is highly expressed in the brain and involved in fusing synaptic vesicles with the plasma membrane. Studies have shown that pathogenic loss-of-function variants in this gene result in various types of epilepsies, mostly beginning early in life. We were interested to model pathogenic missense variants on the protein structure to investigate the mechanism of pathogenicity and genotype–phenotype correlations.

Methods

We report 11 patients with pathogenic de novo mutations in STXBP1 identified in the first 4293 trios of the Deciphering Developmental Disorder (DDD) study, including six missense variants. We analyzed the structural locations of the pathogenic missense variants from this study and the literature, as well as population missense variants extracted from Exome Aggregation Consortium (ExAC).

Results

Pathogenic variants are significantly more likely to occur at highly conserved locations than population variants, and be buried inside the protein domain. Pathogenic mutations are also more likely to destabilize the domain structure compared with population variants, increasing the proportion of (partially) unfolded domains that are prone to aggregation or degradation. We were
unable to detect any genotype–phenotype correlation, but unlike previously reported cases, most of the DDD patients with STXBP1 pathogenic variants did not present with very early-onset or severe epilepsy and encephalopathy, though all have developmental delay with intellectual disability and most display behavioral problems and suffered seizures in later childhood.

Conclusion

Variants across STXBP1 that cause loss of function can result in severe intellectual disability with or without seizures, consistent with a haploinsufficiency mechanism. Pathogenic missense mutations act through destabilization of the protein domain, making it prone to aggregation or degradation. The presence or absence of early seizures may reflect ascertainment bias in the literature as well as the broad recruitment strategy of the DDD study.

Introduction

Brain function relies on the release of neurotransmitters from chemical synapses, which in turn relies on the rapid and regulated fusion of synaptic vesicles with the plasma membrane. The central machinery involved in the fusion process is composed of two conserved protein families: the SNARE (soluble N-ethylmaleimide-sensitive factor attachment receptor) and the SM (Sec1/Munc18) proteins (Südhof and Rothman 2009), now known as syntaxin-binding proteins. Heterozygous pathogenic variants in syntaxin-binding protein 1 (STXBP1, previously called Munc18-1), encoded by the STXBP1 gene, have been reported in nearly 200 patients with various types of developmental disorders, primarily epilepsy/epileptic encephalopathy, developmental delay, and intellectual disability (Saitsu et al. 2008, 2010; Otsuka et al. 2010; Milh et al. 2011; Neale et al. 2012; Epi 4K Consortium and Epilepsy Phenome/Genome Project 2013; Romaniello et al. 2014, 2015; Weckhuysen et al. 2013; Barcia et al. 2014; Carvill et al. 2014; Michaud et al. 2014; Tso et al. 2014; Keogh et al. 2015; Khaikin and Mercimek-Mahmutoglu 2016; Stamberger et al. 2016). Although the mechanism of disease is consistent with autosomal dominant haploinsufficiency, the mode by which pathogenic missense variants cause disease still remains unclear.

Many details about the molecular basis of membrane fusion remain to be established (Rizo and Xu 2015), but it is believed that STXBP1 plays an essential role in synaptic vesicle docking, priming, and fusion (Toonen and Verhage 2007; Südhof and Rothman 2009; Carr and Rizo 2010), and is highly expressed in the brain (Uhlén et al. 2015). To mediate these different functions, STXBP1 is involved in multiple types of interaction with SNAREs, of which the interaction with syntaxin-1 is the most important. Syntaxin-1 can be folded in an open and closed conformation. In the closed conformation, a short sequence at the N-terminus (the N-peptide) and an N-terminal three helix bundle (the Habc domain) are held in a compact autoinhibitory conformation that hinders interactions with other SNAREs to form the SNARE complex. STXBP1 initially binds syntaxin-1 in the closed conformation, accounting for the regulating function of the protein. Studies have suggested it is responsible for transporting syntaxin-1 to the plasma membrane in the closed conformation, where it facilitates vesicle fusion (Fig. 1) (Arunachalam et al. 2008; Han et al. 2009; Ma et al. 2011; Martin et al. 2013).

The crystal structure of STXBP1 in complex with syntaxin-1 shows that it binds in the closed conformation to the syntaxin-1 N-peptide and the Habc domain, PDB accession 3c98 (Misura et al. 2000; Burkhardt et al. 2008). STXBP1 adopts an arch-shaped structure consisting of three domains: domain 1, domain 2, and domain 3, where domain 3 is subdivided into domain 3a and 3b, assigned by Misura et al. (2000) (Fig. 2). Domain 1 and domain 3a form the cavity that binds the Habc domain and the SNARE motif of syntaxin-1. Furthermore, the opposite side of domain 1 binds to the N-peptide. STXBP1 binds to the four helix bundle of the SNARE complex (Dulubova et al. 2007; Shen et al. 2007) and in a compact autoinhibitory conformation that hinders interactions with other SNAREs to form the SNARE complex. STXBP1 initially binds syntaxin-1 in the closed conformation, accounting for the regulating function of the protein. Studies have suggested it is responsible for transporting syntaxin-1 to the plasma membrane in the closed conformation, where it facilitates vesicle fusion (Fig. 1) (Arunachalam et al. 2008; Han et al. 2009; Ma et al. 2011; Martin et al. 2013).

The crystal structure of STXBP1 in complex with syntaxin-1 shows that it binds in the closed conformation to the syntaxin-1 N-peptide and the Habc domain, PDB accession 3c98 (Misura et al. 2000; Burkhardt et al. 2008). STXBP1 adopts an arch-shaped structure consisting of three domains: domain 1, domain 2, and domain 3, where domain 3 is subdivided into domain 3a and 3b, assigned by Misura et al. (2000) (Fig. 2). Domain 1 and domain 3a form the cavity that binds the Habc domain and the SNARE motif of syntaxin-1. Furthermore, the opposite side of domain 1 binds to the N-peptide. STXBP1 binds to the four helix bundle of the SNARE complex (Dulubova et al. 2007; Shen et al. 2007) and

Figure 1. Cartoon representation of the role of STXBP1 in priming vesicle fusion, showing binding to syntaxin-1 (closed conformation) and other members of the SNARE complex.
there is evidence that this binding is mediated by the same cavity that binds syntaxin-1 (Shen et al. 2010; Xu et al. 2010) as well as domain 3a (Hu et al. 2011; Pari-sotto et al. 2014). Experiments with the STXBP1 homolog in yeast (Sec1p) suggested that the binding takes place via the groove between domains 1 and 2 (Hashizume et al. 2009; Weber-Boyvat et al. 2016). Every domain of STXBP1 therefore plays a putative role in its function.

This study aims to further explain why pathogenic variants in STXBP1 lead to disease and hopefully thereby gain a better understanding of the protein. Here we describe 11 new patients from the Deciphering Developmental Disorder (DDD) study with pathogenic de novo mutations in STXBP1 (Deciphering Developmental Disorders Study 2017). We provide detailed clinical data and analyze the structural consequences of the de novo missense mutations. We will also compare pathogenic missense variants from the literature with the population missense variants from the Exome Aggregation Consortium (ExAC) (Lek et al. 2016), which is depleted of severe pediatric conditions and therefore assumed not to have individuals with severe intellectual disability (ID) or significant developmental delay, to investigate the differences between tolerated and nontolerated missense variants from a protein structure perspective.

Materials and Methods

Ethical compliance

The DDD study has UK Research Ethics Committee approval (10/H0305/83, granted by the Cambridge South REC, and GEN/284/12 granted by the Republic of Ireland REC). Postdiagnostic phenotyping was carried out within the Phenotypes in Intellectual Disability study, with independent Ethics Committee approval (11/EE/0330, granted by Cambridge Central REC).

Clinical and genomic data

All patients were recruited as family trios into the DDD study from Regional Genetic Services across the United Kingdom because of an unexplained developmental disorder, including severe neurodevelopmental disorders, and/or congenital anomalies, abnormal growth parameters, dysmorphic features, and unusual behavioral phenotypes. Genome-wide microarray and whole exome sequencing were performed on all patients as detailed previously (Deciphering Developmental Disorders Study 2015). De novo mutations in STXBP1 (NM_003165.3; ENSG00000136854) were identified in 11 of the 4293 probands (0.26%) using trio exome sequencing, and deposited into the DECIPHER database (Bragin et al. 2014). The results were subsequently validated in diagnostic molecular genetic laboratories by Sanger sequencing, and given to the families via their clinical teams.

Clinical characteristics at the time of recruitment to the DDD project were provided by referring clinical geneticists via the DECIPHER website (Bragin et al. 2014) using HPO terminology (Koehler et al. 2014). Diagnosed patients were subsequently invited to participate in detailed research phenotyping, and 10 patients participated. The assessment protocol included a structured medical history interview, neurological examination, assessment of global and specific functional abilities (Vineland Adaptive Behavior Scales), and standardized behavior questionnaire appropriate for young people with ID (Developmental Behavior Checklist) (Einfeld and Tonge 2002).

Structural analysis

The structural analysis was performed on PDB accession 3c98 (Misura et al. 2000; Burkhardt et al. 2008), which contains the rat structure of STXBP1 (100% sequence identity to the human protein) in complex with the N-terminal part of syntaxin-1. The structures were analyzed and figures made using CCP4mg (McNicholas et al. 2011). The structural analysis of STXBP2 (64% sequence identity to STXBP1) was performed on PDB accession 4cca. The structures were superposed using the superpose function from the CCP4 suite to compare residue coordinates. The superpose function is based on secondary structure and iterative three-dimensional alignment of protein backbone C-alpha atoms (Krissinel and Henrick 2004). Residue conservation was retrieved from ConSurf (Dodge et al. 2006).
et al. 1998; Ashkenazy et al. 2010), and used 150 sequences predicted to be structurally similar to PDB accession 3c98. Residue accessibility, a measure of how buried individual amino acids are within the domain structure, was calculated using Naccess (Hubbard and Thornton 1993). The change in thermodynamic stability upon mutation and the number of amino acid interactions were calculated using FoldX PositionScan and PrintNetworks commands (Schymkowitz et al. 2005).

Population missense variants, 71 in total, were retrieved from the ExAC, MA (http://exac.broadinstitute.org). Read data were manually checked for all variants to exclude mosaic variants. The ExAC lists pathogenic variants in the second isoform of STXBP1 (Uniprot identifier: P61764-2 instead of P61764-1), where the last 18 amino acids are replaced by 27 different ones. Since the structure is based on the first isoform, the six variants in the last part of the protein were discarded. This leaves 65 population variants to be used for analysis.

Results

Patient data

Table 1 lists detailed phenotypes of all 11 patients with de novo STXBP1 mutations identified by the DDD study, with DECIPHER IDs (Bragnin et al. 2014). Seven patients were female and four were male; their age range was 1.6–15.5 years. Only two were born prematurely (<37 weeks completed gestational age). None of the patients had intrauterine growth retardation and only one patient had microcephaly (head circumference <2nd centile). Although six patients had some facial dysmorphism, there were no consistent dysmorphic findings in this group. Other low-frequency findings in these patients included gastroesophageal reflux, tapered fingers, eye problems, and joint laxity.

All patients had developmental delay, with age range for starting to walk independently from 2.1 to 8 years. All patients had ID with speech and language delay, and four patients have not yet developed speech. Only one patient developed speech at an appropriate age, though it is still very limited. Six patients were assessed using the Vineland Adaptive Behavior Scales (parental interview) and all had low scores with four in the severe ID range (26, 33, 30, and 31) and two in the moderate ID range (47 and 45). On average, communication skills and daily living skills were as expected for global ability, whereas motor skills and social abilities were slightly stronger than expected. Eight patients had behavioral problems, three having some form of anxiety and three having stereotypies or repetitive behaviors such as hand flapping. Two patients had been diagnosed with an autism spectrum disorder and three patients showed some aggressive behaviors. Where available and with one exception, standardized questionnaire ratings indicated much higher risk of behavior problems than expected for age or ID severity, with highest scores in the self-absorbed, disruptive, and communication disturbance domains.

Eight patients had a history of epilepsy. Seizure types, severity, and age of onset varied widely within the group. One patient had severe infantile encephalopathy, one patient had absence seizures with a single generalized tonic–clonic seizure, one patient had absence seizures with several generalized seizures, three patients had complex partial seizures, and two patients had seizures with no further details available. An additional patient also had unilateral twitching and clenched hands during infancy only, though it is unclear if these were seizures. The remaining two patients had not had any seizures at the last clinical assessment. Only one of the patients had early infantile epileptic encephalopathy. Nine patients had abnormal neurological findings, particularly tremor, which was seen in six patients. Hypotonia and ataxia were each seen in three patients. Ten patients had neuroimaging (MRI brain in nine and CT brain in one), but only one MRI brain scan was reported to be abnormal (bilateral symmetrical abnormalities in anterior temporal lobe).

Variant data and comparative protein modeling

The 11 de novo STXBP1 mutations identified in DDD include three frameshift, two nonsense, and six missense changes (Table 1; note that the same missense pathogenic variant was present in two unrelated patients). Table 2 lists the results of standard in silico analyses of the pathogenicity of the five missense variants identified in our patients. Four of these variants have been reported previously, all in patients with early-onset epileptic encephalopathy. The c.533C>T; p.(Thr178Ile) variant has not been reported previously.

The literature contains over 50 reported missense variants in STXBP1 resulting in developmental disorders (summarized in Stamberger et al. 2016), and there are a further 65 population missense variants retrieved from the ExAC database (Lek et al. 2016), which are presumed to be benign with respect to severe childhood disorders. The location of unique missense variants in these two datasets – disease-causing and population – is shown in the two-dimensional (2D) protein domain structure in Figure 2 and the three-dimensional (3D) protein structure in Figure 3. Both types of missense variants are scattered throughout the different structural domains of the protein, with no significant enrichment of disease-causing variants in any specific area of the protein, reflecting the
| DECIPHER ID | 258815 | 261841 | 260459 | 261220 | 269500 | 270001 | 261234 | 273873 | 258242 | 263903 | 272650 |
|-------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Age at recruitment (age at last clinical assessment) | 6.41 (13) | 8.21 (12.75) | 11.97 | 7.56 | 10.78 | 1.93 | 12.01 (15.5) | 1.61 | 11.32 | 10.27 (15.1) | 6.5 (12.92) |
| Sex | F | F | F | M | F | F | M | F | M | M | F |
| Gestation (weeks) | 40 | 39 | 38 | 41 | 39 | 40 | 33 | 40 | 35 | 42 | 42 |
| Birth weight (centile), kg | 3.544 (62) | 3.373 (65) | 4.053 (99) | 4.45 (96) | 2.8 (17) | 3.46 (55) | 1.9 (34) | 3.2 (33) | 2.5 (54) | 3.86 (73) | 3.2 (33) |
| Last recorded OFC (centile), cm | 51 (13) | 54 (78) | 56 (90) | 54 (57) | 48 (1) | 46.5 (2) | 55 (48) | 47 (16) | 56.2 (79) | 54 (36) | 52.5 (62) |
| Developmental delay | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Age at independent walking | 2.1 years | 8 years | Unknown | 2.5–3 years | Unknown | 3 years | Unknown | Unknown | 2.5–3 years | Unknown | Unknown |
| S&L development | First words 2–2.5 years. Can speak in short sentences | No speech | No speech | No speech | No speech | Single words from 2.5 years, limited vocabulary of single words only | First words 20 months | First words 8 months | First words 5 years | First words 3–5, can use words and short sentences |
| ID (VABC score) | Yes (47) | Yes (33) | Yes | Yes | Yes | Unknown | Unknown | Yes (30) | Yes | Yes | Yes (31) |
| Behavioral problems | Anxiety and phobia | Stereotypic behavior, sociable, mild anxiety | Unknown | Autism | Stereotypic behavior | Aggressive and compulsive behavior, bruxism | Unknown | Aggressive and difficult behavior | Unknown | Echolalia | Yes (45) |
| Developmental Behavioral Checklist total problem behavior centile (stratified by age and ID severity) | 90 | 90 | 94 | 90 | 90 | 94 | 90 | 90 | 94 | 90 | 90 |
| Seizures | Absence seizures, single tonic–clonic generalized seizure at age 10 years | No (unilateral "twitching" and clenched hands during infancy only) | Yes (complex partial) | No | Complex partial, frequent and brief from age 15, worsened on risperidone | Infantile encephalopathy (severe seizure disorder in the first year of life) | Focal seizures at 6 weeks, stopped after ~2 months | Infrequent generalized seizures (3 in total!), absence seizures | Possible febrile seizures at 8 months, complex partial seizures (clusters) |

(Continued)
Table 1. Continued.

| DECIPHER ID | 258815 | 261841 | 260459 | 261220 | 265950 | 270001 | 261234 | 273873 | 258242 | 263903 | 272650 |
|-------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Facial dysmorphism | Down-slanted palpebral fissures, anteverted nares, malar flattening | Round face, up-slanted palpebral fissures, depressed nasal bridge, malar flattening, narrow mouth | Protruding ear | Frontal bossing, broad face, short nose, depressed nasal bridge, narrow mouth | Flat occiput | Deep philtrum, malar flattening, exaggerated cupid's bow |
| Neurological findings | Fine tremor | Infantile axial hypotonia, tremor, broad-based gait, poor coordination | Possible tremor | Truncal ataxia | Infantile hypotonia, pain insensitivity | None | Developmental regression, resting tremor, head titubation | Ataxia with broad-based gait, unilateral hand tremor |
| MRI brain | Normal | Normal | Normal | No | Abnormal bilateral symmetrical abnormal gray-white matter differentiation in anterior temporal lobes | Normal | Normal CT | Normal | Normal | Normal |
| STXBP1 mutation | c.704G>A; p.Arg235Gln | c.1631G>T; p.Gly544Val | c.437_438delCCinsC; p.Leu147fs*18 | c.713+1delACinsA; p.Ser240fs*8 | c.568C>T; p.Arg190Trp | c.568C>T; p.Arg190Trp | c.778G>T; p.Glu260* | c.1651C>T; p.Glu551Cys | c.148dupA; p.Leu50fs*14 | c.1099C>T; p.Arg367* | c.533C>T; p.Phe1178Le |
| Other features | Gastroesophageal reflux and sleep disturbances in infancy | Astigmatism, tapered fingers, inverted nipples, increased body weight | Talipes | Joint laxity | Bilateral moderate enophthalmos, hearing impairment, divergent squint, menarche at 9 years | Gastroesophageal reflux, constipation, broad palm, tapered fingers, broad nasal bridge, squint | Constipation | Well, placid baby, high-pitched voice | Joint laxity |

F, female; M, male; OFC, orbitofrontal cortex circumference; ID, intellectual disability; VABC, Vineland Adaptive Behavior Scales.
STXBP1 Mutations and Developmental Delay

Table 2. Analysis of the five missense variants identified in STXBP1 in the DDD Study.

| DECIPHER ID  | 258815 | 261841 | 265950 and 270001 | 273873 | 272650 |
|--------------|--------|--------|-------------------|--------|--------|
| DECIPHER ID  | 258815 | 261841 | 265950 and 270001 | 273873 | 272650 |
| STXBP1 mutation | c.704G>A; p.Arg235Gln | c.1631G>T; p.Gly544Val | c.568C>T; p.Arg190Trp | c.1651C>T; p.Arg551Cys | c.533C>T; p.Thr178Ile |
| Phylop       | 5.69   | 5.61   | 2.38              | 5.61   | 5.61   |
| Grantham distance | 43       | 109    | 101              | 180    | 89     |
| Align GVGD   | C35    | C0     | C65              | C0     | C15    |
| SIFT         | 0      | 0      | 0                | 0      | 0      |
| PROVEAN      | Damaging | Damaging | Damaging     | Damaging | Damaging |
| Polyphen 2   | 0.994  | 1      | 0.999            | 0.897  | 0.535  |
| Mutation taster | Disease causing | Disease causing | Disease causing | Disease causing | Disease causing |
| SNPs&GO      | Disease related | Disease related | Disease related | Disease related | Neutral |
| ClinVar dbSNP | c.704G>A; p.Arg235Gln (rs794727970) | Not present | c.568C>T; p.Arg190Trp (rs796053355 - pathogenic) | Not present | Not present |
| ExAC Protein domain | Not present | Not present | Not present | Not present | Not present |
| Literature (Phenotype) | Stamberger et al. (2016) (EOEE) | Weckhuysen et al. (2013) (EOEE→West) | Carvill et al. (2014)(EOEE); Epi 4k Consortium 2014 (EOEE→LSG) | Neale et al. (2012) (autism study); Weckhuysen et al. (2013) (EOEE); Stamberger et al. (2016) (2 patients both with EOEE) | Not previously reported |

Phylop (Pollard et al. 2010), Grantham distance (Grantham 1974), Align GVGD (Tavtigian et al. 2006), SIFT (Kumar et al. 2009), PROVEAN (Choi et al. 2012), Polyphen-2 (Adzhubei et al. 2010), Mutation Taster (Schwarz et al. 2010), SNPs&Go (Calabrese et al. 2009), dbSNP (Sherry et al. 2001), ExAC (Lek et al. 2016). EOEE, early-onset epileptic encephalopathy; LGS, Lennox–Gastaut syndrome.

fact that STXBP1 is a highly constrained gene, with fewer loss-of-function variants (pLI = 1) and missense variants (z = 5.22) than expected throughout the gene (Lek et al. 2016).

Amino acids that are altered in the population versus those that cause disease nonetheless differ significantly in terms of their residue accessibility (RSA) (Fig. 4A), their sequence conservation (Fig. 4B), the number of interacting amino acids within the STXBP1 domain (Fig. 4C), though not with syntaxin-1 (Fig. 4D), and their predicted change in structural stability upon mutation (Fig. 4E). The RSA of the disease-associated pathogenic variants is significantly lower than that of the population variants and of all residues in the protein (Wilcoxon–Mann–Whitney test, P = 6e-8 and P = 2e-5, respectively), while the population variants are also significantly different from all residues in the protein (P = 2e-5). This indicates that the pathogenic changes are significantly more often located in the core of the protein, while the population variants are more likely to occur on the surface, as has been noted in the 1000 genomes project (de Beer et al. 2013). Additionally, the residue conservation of the disease-causing variants is significantly higher than that of the population variants and of all residues in the protein (P = 1e-9 and P = 8e-7, respectively), while the conservation of the population variants is significantly lower than all residues in the protein (P = 3e-6). The amino acids where pathogenic variants occur have significantly more interactions with other amino acids in STXBP1 than those where population variants occur (P = 5e-5), though there is no difference in interactions with syntaxin-1. Pathogenic variants are predicted to be significantly more destabilized, relative to the wild type, than population variants (P = 7e-10).

Interestingly, variants of Val84 and His445 occur in both the patient and population datasets, though different amino acids are substituted between the datasets: Val84Ile and His445Tyr (number of alleles present in ExAC was 54 and 1, respectively; Grantham distances of 29 and 83, respectively) occurred in developmentally normal individuals, whereas Val84Asp and His445Pro (Grantham distances of 121 and 77, respectively) were found to cause developmental problems in children. At position 84, isoleucine (the population variant) is present in 30% of the 150 aligned sequences used for conservation analysis (Ashkenazy et al. 2010), while aspartic acid (the pathogenic variant) is not present in any of the aligned sequences; in addition, isoleucine shares many amino acid properties with valine, while aspartic acid is very different being both larger and negatively charged. At position 445,
histidine is only present in 28% of aligned sequences and, although the location tolerates a wide variety of amino acids, tyrosine (the population variant) is present in 14% of alignments, while proline (the pathogenic variant) is never present. Moreover, since the residue is located in a helix, the restricted backbone $\phi$ and $\psi$ angles necessitated by a proline residue would likely break or substantially destabilize the helix. In both cases (positions 84 and 445), the pathogenic variant is predicted to cause a substantial decrease in protein stability relative to the wild type, while the population variant is predicted to be slightly stabilizing. These examples demonstrate the value of protein structural analysis in predicting whether a specific pathogenic variant will be deleterious or not.

The structural context of the five missense changes identified by the DDD study is shown in Figure 5, and their effect on the protein structure is considered in detail below. All the DDD variants are in domain 2 of the protein structure. Table 2 lists evidence for their pathogenicity.

- Thr178 (mutated to Ile) is positioned in the center of an $\alpha$-helix, where its side chain hydroxyl forms an hydrogen bond with the backbone oxygen of Gln175. This bond would be lost upon substitution to isoleucine, but it is uncertain if this causes the variant to be deleterious. Perhaps the slightly larger size of isoleucine destabilizes the protein fold. Thr178 is highly structurally conserved (99%) and the only other amino acid observed at this position is the even smaller amino acid alanine.
- Arg190 (mutated, in two unrelated DDD children, to Trp) is buried and forms three hydrogen bonds, two with the backbone oxygen of Tyr499 and one with the backbone oxygen of Leu494. This residue is 100% conserved in the alignment. Replacement by tryptophan will not only result in the loss of these hydrogen bonds, it will also disrupt the protein fold. Tryptophan is less flexible (two side chain torsion angles compared to four in arginine) and substantially larger, causing steric clashes with other residues in the core of the structure.
- Arg235 (mutated to Gln) is located in a loop and partakes in a network of electrostatic interactions. One of
those interactions is a hydrogen bond between its own backbone oxygen and Ne. This is the only interaction that would remain upon changing arginine to glutamine; all others would be lost. Furthermore, arginine is the only residue that is able to occupy this very specific space, hence its 100% residue conservation.

"Gly544 (mutated to Val) resides in a loop and is preceded by Gly543. Both the residues are 100% structurally conserved and have \( \varphi \) and \( \psi \) angles (103° and 23° for Gly543 and 99° and 150° for Gly544) that other residues are unable to have. These torsion angles allow the loop to perfectly bridge the preceding \( \beta \)-strand with the following \( \alpha \)-helix in this limited space. Moreover, substitution of Gly544 to valine would also result in a steric interference with the overlying loop. Substitution of this residue to aspartic acid and serine (Gly441Ser in STXBP2) has already been found to be deleterious for the protein (Cetica et al. 2010) indicating that any pathogenic variant of Gly544 is likely to result in loss of function.

"Arg551 (mutated to Cys) is sandwiched between two negatively charged residues, forming a salt bridge with one and a hydrogen bond with the other. Replacement to cysteine would result in a loss of these interactions and thereby compromise the stability of the protein. Arg551 is 99% conserved, indicating the importance of this residue at this position.

Discussion

It is well established that heterozygous loss-of-function variants in STXBP1 – including whole gene deletion, intragenic deletion, stop gain, frameshift, and splice donor/acceptor variants – result in a severe childhood developmental phenotype associated with seizures and ID. The gene is highly evolutionarily constrained, with just four apparent loss-of-function variants in the ExAC database of population variation (Lek et al. 2016). These four variants (three frameshift and one splice donor variant) are all located at very C-terminal end of the protein, after the end of the structured domain, and absent from the first isoform, where they are unlikely to cause loss of function.

In order to try and elucidate the mechanism of pathogenicity of missense variants in STXBP1, we compared population missense variants (from ExAC) with pathogenic missense changes (from DDD and the literature). Although in principle the existence of a single population variant does not rule out pathogenicity, it is unlikely that the observed population variants in STXBP1 are pathogenic, since severe early-onset childhood disorders have specifically been excluded from ExAC. We evaluated missense changes in the 3D domain structure, but were unable to find any positional correlation with pathogenicity. Nonetheless, pathogenic missense variants are significantly more likely to be buried within the domain, at highly conserved residues that are involved in a network of intramolecular interactions within STXBP1 (Fig. 4). There is no evidence that pathogenic variants directly affect the binding interaction with syntaxin-1. We also assessed the effect of both pathogenic and population variants on the predicted thermodynamic stability of the protein domain, relative to the wild type (Schymkowitz et al. 2005), and found that the pathogenic changes were significantly more likely to destabilize the domain (Fig. 4). This provides good evidence that the mechanism of pathogenicity for missense variants in STXBP1 is haploinsufficiency, through destabilization of the native folded state of the protein domain, making it prone to misfolding, aggregation, and degradation (Nielsen et al. 2017). This mechanism has previously been suggested by Saitis et al. (2012), where direct evidence for destabilization and aggregation was observed for several pathogenic missense variants in STXBP1.

Heterozygous pathogenic variants in the STXBP1 gene can be associated with ID phenotypes, with or without epilepsy. The ID–epilepsy phenotypes include early...
infantile epileptic encephalopathy (IEE) or Ohtahara syndrome (Saito et al. 2008), West syndrome (Deprez et al. 2010; Otsuka et al. 2010), Dravet syndrome (Carvill et al. 2014), infantile spasms (Mignot et al. 2011; Carvill et al. 2014; Michaud et al. 2014; Boulry-Kryza et al. 2015), neonatal-onset or early-onset focal epilepsy (Vatta et al. 2012; Romaniello et al. 2014), partial complex epilepsy (Hamdan et al. 2011), and nonsyndromic epilepsy (Hamdan et al. 2009; Deprez et al. 2010). These phenotypes are invariably associated with ID and often with ataxia, tremor, and sometimes with a movement disorder (Kanazawa et al. 2010). Respiratory complex I and IV deficiency and lactic acidemia without respiratory complex deficiency have also been reported in single patients with STXBP1-related epilepsy phenotypes (Barcia et al. 2014; Keogh et al. 2015; Li et al. 2016), as has atypical Rett syndrome variant (Olson et al. 2015; Romaniello et al. 2015). However, intellectual disability phenotypes without seizures have only been reported previously in a small number of patients with heterozygous STXBP1 pathogenic variants (Hamdan et al. 2011; Campbell et al. 2012; Rauch et al. 2012; Gburek-Augustat et al. 2016; Stambberger et al. 2016). These patients usually have severe ID, often in combination with ataxia and tremor, with additional findings of autism, attention-deficit disorder, and movement disorder in one or more patients. Stambberger et al. recently reviewed the phenotypic spectrum of 147 patients with STXBP1 encephalopathy, including 45 previously unreported patients. A majority of these patients presented with early-onset epilepsy and encephalopathy (EOEE) or Ohtahara syndrome. Only 4 of the 45 (<10%) previously unreported patients with STXBP1 pathogenic variants had ID without seizures. Table S1 summarizes the clinical findings and STXBP1 pathogenic variants in 12 previously reported patients with STXBP1 pathogenic variants and ID without any seizures, as well as the two patients from our cohort.

Only 1 of the 11 probands reported here presented with an infantile epileptic encephalopathy phenotype, though another seven had some form of seizures and all had severe ID. Nonetheless, there is a clear underrepresentation of severe, early-onset epilepsy phenotypes in our patient cohort relative to those previously published with mutations in STXBP1. This is unlikely to be related to the type of pathogenic variant or its location, as patients in our cohort had a range of stop gain, frameshift, and missense pathogenic variants spread across the protein. In addition, four of the five missense pathogenic variants that were identified in our cohort have been previously reported to cause early-onset epileptic encephalopathy (Table 2). This may reflect variability of the phenotype associated with STXBP1 pathogenic variants, or the modifying effects of variants in other genes. Interestingly, STXBP1 knockout mice display a complete loss of neurotransmitter secretion from synaptic vesicles throughout development leading to neurodegeneration, though seizures have never been described (Verhage et al. 2000). Another possible explanation for the lack of a severe early-onset epilepsy phenotype in our cohort could be ascertainment bias in the literature, which is absent from the DDD Study due to its wide eligibility criteria (Wright et al. 2015). We were unable to detect any genotype–phenotype correlation to explain the different presentation of these cases, and therefore suggest that STXBP1 variants resulting in loss of function can cause a broad developmental phenotype associated with severe ID with or without a variety of other behavioral and neurological problems, particularly seizures/epilepsy, ataxia, and tremor.

In conclusion, analysis of de novo pathogenic variants in STXBP1 in a cohort of children with developmental disorders confirms that loss-of-function variants in this gene cause severe developmental delay with or without seizures. Pathogenic variants in all domains of the protein encoded by STXBP1 can result in the phenotype of ID with or without seizures, indicating that this phenotype does not correlate with the type or location of the pathogenic variant, but is consistent with a haploinsufficiency mechanism. Structural analysis of the STXBP1 confirms that the pathogenic missense variants are mostly buried inside the protein domain at highly conserved residues where a variant is likely to destabilize the domain and potentially lead to protein aggregation or degradation.

Acknowledgments

The DDD study presents independent research commissioned by the Health Innovation Challenge Fund (grant number HICF-1009-003), a parallel funding partnership between the Wellcome Trust and the Department of Health, and the Wellcome Trust Sanger Institute (grant number WT098051). The views expressed in this publication are those of the author(s) and not necessarily those of the Wellcome Trust or the Department of Health. The research team acknowledges the support of the National Institute for Health Research, through the Comprehensive Clinical Research Network. Postdiagnostic phenotyping was carried out within the Phenotypes in Intellectual Disability study funded by the Newlife Foundation for Disabled Children. The authors have no conflicts of interest to declare.

Conflict of Interest

None declared.
References

Adzhubei, I. A., S. Schmidt, L. Peshkin, V. E. Ramensky, A. Gerasimova, P. Bork, et al. 2010. A method and server for predicting damaging missense mutations. Nat. Methods 7:248–249.

Arunachalam, L., L. Han, N. G. Tassew, Y. He, L. Wang, L. Xie, et al. 2008. Munc18-1 is critical for plasma membrane localization of syntaxin1 but not of SNAP-25 in PC12 cells. Mol. Biol. Cell 19:722–734.

Ashkenazy, H., E. Erez, E. Martz, T. Pupko, and N. Ben-Tal. 2010. ConSurf 2010: calculating evolutionary conservation in sequence and structure of proteins and nucleic acids. Nucleic Acids Res. 38(Suppl 2):W529–W533.

Barcia, G., N. Chemaly, S. Gobin, M. Milh, P. Van Bogaert, C. Barnerias, et al. 2014. Early epileptic encephalopathies associated with STXBP1 mutations: could we better delineate the phenotype? Eur. J. Med. Genet. 57:15–20.

de Beer, T. A. P., R. A. Laskowski, S. L. Parks, B. Sipos, N. Goldman, and J. M. Thornton. 2013. Amino acid changes in disease-associated variants differ radically from variants observed in the 1000 genomes project dataset. PLoS Comput. Biol. 9:e1003382.

Boutry-Kryza, N., A. Labalme, D. Ville, J. de Bellescize, R. Touraine, F. Prieur, et al. 2015. Molecular characterization of a cohort of 73 patients with infantile spasms syndrome. Eur. J. Med. Genet. 58:51–58.

Bragin, E., E. A. Chatzimichali, C. F. Wright, M. E. Hurles, H. V. Firth, A. P. Bevan, et al. 2014. DECIPHER: database for the interpretation of phenotype-linked plausibly pathogenic sequence and copy-number variation. Nucleic Acids Res. 42: D993–D1000.

Burkhardt, P., D. A. Hattendorf, W. I. Weis, and D. Fasshauer. 2008. Munc18a controls SNARE assembly through its interaction with the syntaxin N-peptide. EMBO J. 27:923–933.

Calabrese, R., A. Capriotti, P. Fariselli, P. L. Martelli, and R. Casadio. 2009. Functional annotations improve the predictive score of human disease-related mutations in proteins. Hum. Mutat. 30:1237–1244.

Campbell, I. M., S. A. Yatsenko, P. Hixon, T. Reimschisel, M. Thomas, W. Wilson, et al. 2012. Novel 9q34.11 gene deletions encompassing combinations of four Mendelian disease genes: STXBP1, SPTAN1, ENG, and TOR1A. Genetics in medicine: official journal of the American College of Medical. Genetics 14:868–876.

Carr, C. M., and J. Rizo. 2010. At the junction of SNARE and SM protein function. Curr. Opin. Cell Biol. 22:488–495.

Carvill, G. L., S. Weckhuysen, J. M. McMahon, C. Hartmann, R. S. Moller, H. Hjalgrim, et al. 2014. GABRA1 and STXBP1: novel genetic causes of Dravet syndrome. Neurology 82:1245–1253.

Cetica, V., A. Santoro, K. C. Gilmour, E. Sieni, K. Beutel, D. Pende, et al. 2010. STXBP2 mutations in children with familial haemophagocytic lymphohistiocytosis type 5. J. Med. Genet. 47:595–600.

Choi, Y., G. E. Sims, S. Murphy, J. R. Miller, and A. P. Chan. 2012. Predicting the functional effect of amino acid substitutions and indels. PLoS ONE 7:e46688.

Deciphering Developmental Disorders Study. 2015. Large-scale discovery of novel genetic causes of developmental disorders. Nature 519:223–228.

Deciphering Developmental Disorders Study. 2017. Prevalence and architecture of de novo mutations in developmental disorders. Nature 542:433–438.

Deprez, L., S. Weckhuysen, P. Holmgren, A. Suls, T. Van Dyck, D. Goossens, et al. 2010. Clinical spectrum of early-onset epileptic encephalopathies associated with STXBP1 mutations. Neurology 75:1159–1165.

Dodge, C., R. Schneider, and C. Sander. 1998. The HSSP database of protein structure—sequence alignments and family profiles. Nucleic Acids Res. 26:313–315.

Dulubova, I., M. Khvotchev, S. Liu, I. Huryeva, T. C. Südhof, and J. Rizo. 2007. Munc18-1 binds directly to the neuronal SNARE complex. Proc. Natl Acad. Sci. 104:2697–2702.

Einfeld, S., and B. Tonge. 2002. Developmental behaviour checklist. 2nd ed. University of New South Wales and Monash University, Clayton, Melbourne.

Epi 4K Consortium, Epilepsy Phenome/Genome Project. 2013. De novo mutations in epileptic encephalopathies. Nature 501:217–221.

Ghreki-Augustat, J., S. Beck-Woedl, A. Tzscha, P. Bauer, M. Schoening, and A. Riess. 2016. Epilepsy is not a mandatory feature of STXBP1 associated ataxia-tremor-retardation syndrome. Eur. J. Paediatr. Neurol. 20:661–665.

Granthon, R. 1974. Amino acid difference formula to help explain protein evolution. Science 185:862–864.

Hamdan, F. F., A. Piton, J. Gauthier, A. Lortie, F. Dubeau, S. Dobrzeniecka, et al. 2009. De novo STXBP1 mutations in mental retardation and nonsyndromic epilepsy. Ann. Neurol. 65:748–753.

Hamdan, F. F., J. Gauthier, Y. Araki, D.-T. Lin, Y. Yoshizawa, K. Higashi, et al. 2011. Excess of de novo deleterious mutations in genes associated with glutamatergic systems in nonsyndromic intellectual disability. Am. J. Hum. Genet. 88:306–316.

Han, L., T. Jiang, G. A. Han, N. T. Malintan, L. Xie, L. Wang, et al. 2009. Rescue of Munc18-1 and-2 double knockdown reveals the essential functions of interaction between Munc18 and closed syntaxin in PC12 cells. Mol. Biol. Cell 20:4962–4975.

Hashizume, K., Y.-S. Cheng, J. L. Hutton, C. C-h, and C. M. Carr. 2009. Yeast Sec1p functions before and after vesicle docking. Mol. Biol. Cell 20:4673–4683.

Hu, S.-H., M. P. Christie, N. J. Saez, C. F. Latham, R. Jarrott, L. H. Lua, et al. 2011. Possible roles for Munc18-1 domain 3a and Syntaxin1 N-peptide and C-terminal anchor in SNARE complex formation. Proc. Natl Acad. Sci. 108:1040–1045.
Kanazawa, K., S. Kumada, M. Kato, H. Saitsu, E. Kurihara, and N. Matsumoto. 2010. Choreo–ballistic movements in a case carrying a missense mutation in syntaxin binding protein 1 gene. Mov. Disord. 25:2265–2267.

Keogh, M. J., D. Daud, A. Pyle, J. Duff, H. Griffin, L. He, et al. 2015. A novel de novo STXBP1 mutation is associated with mitochondrial complex I deficiency and late-onset juvenile-onset Parkinsonism. Neurogenetics 16:65–67.

Khaikin, Y., and S. Mercimek-Mahmutoglu. 2016. STXBP1 mutations and developmental delay. Mov. Disord. 25:2265

Kumar, P., S. Henikoff, and P. C. Ng. 2009. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat. Protoc. 4:1073–1081.

Lek, M., K. Karczewski, E. Minikel, K. Samocha, E. Banks, T. Fennell, et al. 2016. Analysis of protein-coding genetic variation in 60,706 humans. Nature 536:285–291.

Li, D., E. Bhoj, E. McCormick, F. Wang, J. Snyder, T. Wang, et al. 2016. Early infantile epileptic encephalopathy in an STXB1 patient with lactic acidemia and normal mitochondrial respiratory chain function. Case Rep. Genet. 2016:5.

Ma, C., W. Li, Y. Xu, and J. Rizo. 2011. Munc13 mediates the transition from the closed syntaxin–Munc18 complex to the SNARE complex. Nat. Struct. Mol. Biol. 18:542–549.

Martin, S., V. M. Tomatis, A. Papadopulos, M. P. Christie, N. T. Malintan, R. S. Gormal, et al. 2013. The Munc18-1 domain 3a loop is essential for neuroexocytosis but not for syntaxin-1A transport to the plasma membrane. J. Cell Sci. 126:2353–2360.

McNicholas, S., E. Potterton, K. Wilson, and M. Noble. 2011. Presenting your structures: the CCP4 mg molecular-graphics software. Acta Crystallogr. Sect. D: Biol. Crystallogr. 67:386–394.

Michaud, J. L., M. Lachance, F. F. Hamdan, L. Carmant, A. Lortie, P. Diadori, et al. 2014. The genetic landscape of infantile spasms. Hum. Mol. Genet. 23:4846–4858.

Mignot, C., M.-L. Moutard, O. Trouillard, I. Gourfinkel-An, A. Jacquette, B. Arveiler, et al. 2011. STXBP1-related encephalopathy presenting as infantile spasms and generalized tremor in three patients. Epilepsia 52:1820–1827.

Milh, M., N. Villeneuve, M. Chouchane, A. Kaminska, C. Laroche, M. A. Barthez, et al. 2011. Epileptic and nonepileptic features in patients with early onset epileptic encephalopathy and STXBP1 mutations. Epilepsia 52:1828–1834.

Misura, K. M., R. H. Scheller, and W. I. Weis. 2000. Three-dimensional structure of the neuronal-Sec1–syntaxin 1a complex. Nature 404:355–362.

Neale, B. M., Y. Kou, L. Liu, A. Ma’Ayan, K. E. Samocha, A. Sabo, et al. 2012. Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature 485:242–245.

Nielsen, S. V., A. Stein, A. B. Dinitzen, E. Papaleo, M. H. Tatham, E. G. Poulsen, et al. 2017. Predicting the impact of Lynch syndrome-causing missense mutations from structural calculations. PLoS Genet. 13:e1006739.

Olson, H. E., D. Tambunan, C. LaCoursiere, M. Goldenberg, R. Pinsky, E. Martin, et al. 2015. Mutations in epilepsy and intellectual disability genes in patients with features of Rett syndrome. Am. J. Med. Genet. A 167:2017–2025.

Otsuka, M., H. Oguni, J. S. Liang, H. Ikeda, K. Imai, K. Hirasawa, et al. 2010. STXBP1 mutations cause not only Ohtahara syndrome but also West syndrome—result of Japanese cohort study. Epilepsia 51:2449–2452.

Parisotto, D., M. Pfau, A. Scheutzow, K. Wild, M. P. Mayer, J. Malsam, et al. 2014. An extended helical conformation in domain 3a of Munc18-1 provides a template for SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complex assembly. J. Biol. Chem. 289:9639–9650.

Pollard, K. S., M. J. Hubisz, K. R. Rosenbloom, and A. Siepel. 2010. Detection of nonneutral substitution rates on mammalian phylogenies. Genome Res. 20:110–121.

Rauch, A., D. Wieczorek, E. Graf, T. Wieland, S. Endele, T. Schwarzmayr, et al. 2012. Range of genetic mutations with mitochondrial complex I deficiency and late-onset respiratory chain dysfunction. Case Rep. Genet. 2016:5.

Sabo, et al. 2012. Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature 485:242–245.

Saitis, H., M. Kato, T. Mizuguchi, K. Hamada, H. Osaka, J. Tohyama, et al. 2008. De novo mutations in the gene encoding STXB1 (MUNC18-1) cause early infantile epileptic encephalopathy. Nat. Genet. 40:782–788.

Saitis, H., M. Kato, I. Okada, K. E. Orii, T. Higuchi, H. Hoshino, et al. 2010. STXBP1 mutations in early infantile epileptic encephalopathy with suppression-burst pattern. Epilepsia 51:2397–2405.
Saitsu, H., M. Kato, and N. Matsumoto. 2012. Haploinsufficiency of STXBP1 and Ohtahara syndrome. Jasper’s Basic Mechanisms of the Epilepsies (Internet) Available at https://www.ncbi.nlm.nih.gov/books/NBK98196/: Bethesda (MD): National Center for Biotechnology Information (US).

Schwarz, J. M., C. Rodelsperger, M. Schuelke, and D. Seelow. 2010. MutationTaster evaluates disease-causing potential of sequence alterations. Nat. Methods 7:575–576.

Schymkowitz, J., J. Borg, F. Stricher, R. Nys, F. Rousseau, and L. Serrano. 2005. The FoldX web server: an online force field. Nucleic Acids Res. 33(Suppl_2):W382–W388.

Shen, J., D. C. Tarete, F. Paumet, J. E. Rothman, and T. J. Melia. 2007. Selective activation of cognate SNAREpins by Sec1/Munc18 proteins. Cell 128:183–195.

Shen, J., S. S. Rathore, L. Khandan, and J. E. Rothman. 2010. SNARE bundle and syntaxin N-peptide constitute a minimal complement for Munc18-1 activation of membrane fusion. J. Cell. Biol. 190:55–63.

Sherry, S., M. Ward, M. Kholodov, J. Baker, L. Phan, E. Smigieliski, et al. 2001. dbSNP: the NCBI database of genetic variation. Nucleic Acids Res. 29:308–311.

Stamberger, H., M. Nikanorova, M. H. Willemansen, P. Accorsi, M. Angriman, H. Baier, et al. 2016. STXBP1 encephalopathy: a neurodevelopmental disorder including epilepsy. Neurology 86:954–962.

Südhof, T. C., and J. E. Rothman. 2009. Membrane fusion: grappling with SNARE and SM proteins. Science 323:474–477.

Tavtigian, S. V., A. M. Deffenbaugh, L. Yin, T. Judkins, T. Scholl, P. B. Samollow, et al. 2006. Comprehensive statistical study of 452 BRCA1 missense substitutions with classification of eight recurrent substitutions as neutral. J. Med. Genet. 43:295–305.

Toonen, R. F., and M. Verhage. 2007. Munc18-1 in secretion: lonely Munc joins SNARE team and takes control. Trends Neurosci. 30:564–572.

Tso, W. W. Y., A. K. Y. Kwong, C. W. Fung, and V. C. N. Wong. 2014. Folinic acid responsive epilepsy in Ohtahara syndrome caused by STXBP1 mutation. Pediatr. Neurol. 50:177–180.

Uhlen, M., L. Fagerberg, B. M. Hallström, C. Lindskog, P. Oksvold, A. Mardinoglu, et al. 2015. Tissue-based map of the human proteome. Science 347.

Vatta, M., M. B. Tennison, A. S. Aylsworth, C. M. Turcott, M. P. Guerra, C. M. Eng, et al. 2012. A novel STXBP1 mutation causes focal seizures with neonatal onset. J. Child Neurol. 27:811–814.

Verhage, M., A. S. Maia, J. J. Plomp, A. B. Brussaard, J. H. Heeroma, H. Vermeer, et al. 2000. Synaptic assembly of the brain in the absence of neurotransmitter secretion. Science 287:864–869.

Weber-Boyvat, M., K. G. Chernov, N. Aro, G. Wohlfahrt, V. M. Olkkonen, and J. Jännti. 2016. The Sec1/Munc18 protein groove plays a conserved role in interaction with Sec9p/SNAP-25. Traffic 17:131–153.

Wexhuysen, S., P. Holmgren, R. Hendrickx, A. C. Jansen, D. Hasaerts, C. Dielman, et al. 2013. Reduction of seizure frequency after epilepsy surgery in a patient with STXBP1 encephalopathy and clinical description of six novel mutation carriers. Epilepsia 54:e74–e80.

Wright, C. F., T. W. Fitzgerald, W. D. Jones, S. Clayton, J. F. McRae, M. van Kogelenberg, et al. 2015. Genetic diagnosis of developmental disorders in the DDD study: a scalable analysis of genome-wide research data. Lancet 385:1305–1314.

Xu, Y., L. Su, and J. Rizo. 2010. Binding of Munc18-1 to synaptobrevin and to the SNARE four-helix bundle. Biochemistry 49:1568–1576.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Summary of the clinical findings and STXB1 pathogenic variants in 12 previously reported patients with STXB1 pathogenic variants and ID without any seizures, as well as the two patients from the DDD cohort.