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Heterozygous Variants in *SPTBN1* Cause Intellectual Disability and Autism

Jill A. Rosenfeld[1,a], **Rui Xiao**[1,2], **Mir Reza Bekheirnia**[1,3], **Farah Kanani**[4], **Michael J. Parker**[5], **Mary K. Koenig**[6], **Arie van Haeringen**[7], **Claudia Ruivenkamp**[7], **Joana Rosmaninho-Salgado**[8], **Pedro M. Almeida**[8], **Joaquim Sá**[8], **Jorge Pinto Basto**[9], **Emily Palen**[10], **Kathryn F. Oetjens**[10], **Lindsay C. Burrage**[1,11], **Fan Xia**[1,2], **Pengfei Liu**[1,2], **Christine M. Eng**[1,2], **Undiagnosed Diseases Network**, **Yaping Yang**[1,2,*], **Jennifer E. Posey**[1], **Brendan H. Lee**[1]

[1.]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, 77030, USA

[2.]Baylor Genetics Laboratories, Houston, Texas, 77030, USA

[3.]Renal Section, Department of Pediatrics, Baylor College of Medicine, Houston, Texas, 77030, USA

[4.]Sheffield Clinical Genetics Service, Sheffield Children's NHS Foundation Trust, Sheffield, UK

[5.]The Wellcome Centre for Ethics and Humanities/Ethox Centre, Nuffield Department of Population Health, University of Oxford, Oxford, UK

[6.]Department of Pediatrics, University of Texas Health Science Center, Houston, Texas, 77030, USA

[7.]Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands

[8.]Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

[9.]Molecular Diagnostics and Clinical Genomics, CGC Genetics, Porto, Portugal

[10.]Autism & Developmental Medicine Institute, Geisinger, Danville, Pennsylvania, 17822, USA

[11.]Texas Children's Hospital, Houston, Texas, 77030, USA

Abstract

Spectrins are common components of cytoskeletons, binding to cytoskeletal elements and the plasma membrane, allowing proper localization of essential membrane proteins, signal

[a]**Corresponding author:** Jill A. Rosenfeld, MS; Department of Molecular & Human Genetics, Baylor College of Medicine; One Baylor Plaza, Rm R804, MS BCM225; Houston, TX 77030; mokry@bcm.edu.

[*]Current affiliation: AiLife Diagnostics, Pearland, Texas, 77584, USA

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Conflict of Interest

The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing conducted at Baylor Genetics Laboratories.

transduction, and cellular scaffolding. Spectrins are assembled from α and β subunits, encoded by *SPTA1* and *SPTAN1* (α) and *SPTB*, *SPTBN1*, *SPTBN2*, *SPTBN4* and *SPTBN5* (β). Pathogenic variants in various spectrin genes are associated with erythroid cell disorders (*SPTA1*, *SPTB*) and neurologic disorders (*SPTAN1*, *SPTBN2*, *SPTBN4*), but no phenotypes have been definitively associated with variants in *SPTBN1* or *SPTBN5*. Through exome sequencing and case matching, we identified seven unrelated individuals with heterozygous *SPTBN1* variants: two with *de novo* missense variants and five with predicted loss-of-function variants (found to be *de novo* in two, while one was inherited from a mother with a history of learning disabilities). Common features include global developmental delays, intellectual disability, and behavioral disturbances. Autistic features (4/6) and epilepsy (2/7) or abnormal EEG without overt seizures (1/7) were present in a subset. Identification of loss-of-function variants suggests a haploinsufficiency mechanism, but additional functional studies are required to fully elucidate disease pathogenesis. Our findings support essential roles of *SPTBN1* in human neurodevelopment and expand the knowledge of human spectrinopathy disorders.

Keywords

SPTBN1 ; spectrin; neurodevelopmental disorder; spectrinopathy

INTRODUCTION

Spectrins are a family of cytoskeletal proteins that bind to cytoskeletal elements, such as actin filaments, microtubules and intermediate filaments, functioning as cytoskeletal integrators and linking the cytoskeleton to the plasma membrane (Liem, 2016). Spectrin was first discovered in erythrocyte “ghosts,” which result from osmotically lysing erythrocytes to release cytoplasmic components (V. T. Marchesi & Steers, 1968). Originally thought to be limited to erythrocytes, spectrins were later found in most cell types, including brain, kidney, skeletal muscles, cardiac muscles and intestines, as a common cytoskeletal component (Burridge, Kelly, & Mangeat, 1982; Glenney & Glenney, 1983). Spectrins are composed of α and β subunits, which interact with each other in an antiparallel manner to form rod-shaped heterodimers. Two spectrin heterodimers form an elongated, head-to-head tetramer through the interaction of the amino termini of the α subunits and the carboxyl termini of the β subunits (Liem, 2016). In mammals, two genes encode the spectrin α subunits. *SPTA1* produces the αI spectrin found in erythrocytes, whereas *SPTAN1* expresses at least 4 isoforms of αII spectrins present in most non-erythroid cells (Cianci, Zhang, Pradhan, & Morrow, 1999). Five types of spectrin β subunits (βI, βII, βIII, βIV and βV) in mammals are encoded by *SPTB*, *SPTBN1*, *SPTBN2*, *SPTBN4* and *SPTBN5* respectively. βI is the only β subunit expressed in erythrocytes, and the other β spectrins exist in nonerythrocytes (Derbala, Guo, Mohler, & Smith, 2018; Liem, 2016). Spectrin α and β subunits share significant sequence homology, and both contain structural features similar to actin-crosslinking protein α-actinin. One of the common structural features of spectrins is the presence of multiple copies of short units, knowns as spectrin repeats, each of which comprises about 106 amino acid residues and forms 3 α-helices (Liem, 2016). The spectrin repeats usually appear in tandem, and the number of copies ranges from 14 to 27 in human spectrin proteins. In addition to spectrin repeats, spectrin α subunits have 3 tandem copies

of EF-hand domains at the carboxyl terminus, whereas spectrin β subunits have 2 tandem copies of calponin homology domains at the amino terminus, which interact with actin and are also called actin-binding domains, and a pleckstrin homology (PH) domain at the carboxyl terminus of their long isoforms, serving as a ligand binding site for phospholipids involved in signal transduction. In spectrin α subunits, an SH3 (SRC homology 3) domain, which is also involved in signal transduction, is located in the 9th spectrin repeat from the amino terminus (Derbala et al., 2018; Machnicka et al., 2014). The spectrin repeats in the β subunits also bind phospholipids and hydrophobic compounds (Diakowski, Szopa, & Sikorski, 2003), and the 14th-15th spectrin repeats contain an ankyrin binding site (Davis et al., 2009). In turn, ankyrins mediate interactions with many spectrin binding partners including phospholipids, channels, transporters and receptors (Machnicka et al., 2014).

Consistent with their pivotal roles in maintaining cytoskeleton in erythrocytes, defective erythroid spectrins lead to weak and fragile red blood cells. Pathogenic variants in *SPTA1* and *SPTB*, which encode erythroid spectrin α and β subunits respectively, have been associated with autosomal dominant and autosomal recessive hemolytic disorders, including elliptocytosis, spherocytosis, pyropoikilocytosis and neonatal hemolytic anemia (Gallagher & Forget, 1996; Gallagher et al., 1997; Gallagher et al., 1995; S. L. Marchesi et al., 1987). Additionally, pathogenic variants in non-erythroid spectrin genes have been associated with neurological and developmental disorders. *De novo* heterozygous variants in *SPTAN1* were identified in several unrelated patients with epileptic encephalopathy, brain atrophy, progressive microcephaly, and developmental delay (Hamdan et al., 2012; Saitsu et al., 2010). In an 11-generation pedigree descended from Abraham Lincoln's grandparents and two additional families, in-frame deletions and a missense variant in *SPTBN2* were identified to cause autosomal dominant spinocerebellar ataxia 5 (SCA5), a slowly progressive disorder with variable age of onset (Ikeda et al., 2006). In addition, homozygous stopgain and frameshift variants in *SPTBN2* were found in two consanguineous families with spinocerebellar ataxia, consistent with autosomal recessive inheritance (Elsayed et al., 2014; Lise et al., 2012). Biallelic variants in *SPTNB4* have been identified in multiple unrelated families with neurodevelopmental disorders (Anazi et al., 2017; Knierim et al., 2017; Pehlivan et al., 2019; Wang et al., 2018). Common features of these patients include congenital hypotonia, areflexia, feeding difficulties, muscle fiber atrophy, and profound developmental delay. The remaining human spectrin genes, *SPTBN1* and *SPTBN5*, have not yet been definitively associated with human diseases, although *de novo SPTBN1* variants (one missense, one nonsense) have been identified in two males as part of a large screen of individuals with autism spectrum disorder (Iossifov et al., 2014). In this report, we describe heterozygous loss-of-function or *de novo* variants in *SPTBN1* in seven unrelated patients with overlapping neurodevelopmental disorders. Our findings suggest the essential roles of spectrin βII subunits in human development and add a new member to the spectrinopathy disorders.

METHODS

Editorial policies and ethical considerations

Informed consent for publication of clinical information and photographs was obtained for all individuals, using Institutional Review Board-approved research protocols.

Patient ascertainment

De novo variants in *SPTBN1* were identified via clinical trio exome sequencing in individuals 1 and 2. As *SPTBN1* was not a known human disease gene, individual 1 subsequently enrolled in the Undiagnosed Diseases Network (UDN) for further phenotypic and genetic evaluations. Subsequent review of Baylor Genetics Laboratories' clinical exome sequencing database identified individual 3 with a heterozygous loss-of-function *SPTBN1* variant. Additional individuals 4 and 5 with *SPTBN1* loss-of-function variants were identified in the DECIPHER database (Firth et al., 2009). Family and/or clinician contact was established with individuals 6 and 7 with *SPTBN1* heterozygous loss-of-function variants through a "Participant Page" on the UDN website for individual 1.

SPTBN1 variant identification

Individuals 1 and 2 underwent clinical trio exome sequencing, and individual 3 underwent proband exome sequencing, all at Baylor Genetics Laboratories, according to previously described protocols (Yang et al., 2014). In individual 3, Sanger sequencing was used to confirm the *SPTBN1* variant in the proband and to test the parents. Individual 4 underwent exome sequencing through the Deciphering Developmental Disorders Study (Deciphering Developmental Disorders Study, 2015) and genome sequencing through the 100,000 Genomes Project (Caulfield et al., 2019). Individual 5 underwent trio exome sequencing using the Agilent SureSelectXT Human All Exon v5 kit on the Hiseq4000 platform, according to previously described protocols (van der Sluijs et al., 2019). Individuals 6 and 7 underwent clinical proband exome sequencing, at CGC Genetics and GeneDx Laboratories, respectively, with Sanger sequencing of the identified *SPTBN1* variants in the probands and mothers.

RESULTS

Genomic analyses

Trio exome sequencing identified *de novo SPTBN1* missense variants in individuals 1 and 2, located in the second calponin homology domain and in the 15th spectrin repeat, respectively (Table 1, Figure 1). Predicted *SPTBN1* loss-of-function variants were identified in the remaining individuals, located in the calponin homology domains in individuals 3-6 and in one of the spectrin repeats in individual 7 (Table 1, Figure 1). Parental studies showed variants in individuals 1-3 and 5 to be *de novo,* while the variant in individual 6 was maternally inherited. The mothers of individuals 4 and 7 were negative for the variants, while their fathers were not available for testing. All *SPTBN1* variants are absent from the gnomAD general population database (Karczewski et al., 2020), and missense variants are located at evolutionarily conserved positions (Figure 1). No coding-region variants were predicted to alter splicing (Supplemental table 1).

Clinical exome sequencing reports did not contain any other strong candidates to explain the individuals' phenotypes, with the exception of a *de novo* missense variant of uncertain significance in *IL17RD* (NM_017563.3:c.682C>T, p.Arg228Cys) in individual 2 and a missense variant of uncertain significance in *SMARCA4* (NM_001128849.1:c.3986G>A, p. Arg1329His; not present in mother, father not available) in individual 4. Pathogenic variants in *IL17RD* cause hypogonadotropic hypogonadism-18 with or without anosmia (HH18; OMIM 615267), although this condition is typically inherited in either a recessive or digenic manner (Miraoui et al., 2013). Heterozygous pathogenic variants in *SMARCA4* cause Coffin-Siris syndrome (CSS4; OMIM 614609), characterized by fifth digit clinodactyly/hypoplasia (which was not seen in individual 4), neurodevelopmental delay, and characteristic facial features and hirsutism. Seizures have been reported in some individuals with CSS, and *SMARCA4*-associated CSS more commonly causes growth impairment and behavioral abnormalities (Schrier Vergano et al., 2013 Apr 4 [Updated 2018 Feb 8]). Individual 3, who had mitochondrial dysfunction, had a single heterozygous variant of uncertain significance in *NDUFS2* (NM_004550.4:c.227T>C, p.Ile76Thr), associated with autosomal recessive mitochondrial complex I deficiency (OMIM 618228). His phenotype was not thought to fit this condition, which is characterized by failure to thrive, optic atrophy, cardiomyopathy, developmental regression, and brain atrophy, so the possibility of a second, unidentified *NDUFS2* variant was thought unlikely. Mitochondrial DNA sequencing in individual 3 identified two apparently homoplasmic variants of uncertain significance (m.1282G>A (12S rRNA) and m.14256T>C (p.I140V, *ND6*)). These variants were present in control databases mtDB and MitoMap and were bioinformatically predicted to be benign, so familial testing was not pursued. Individual 2 was also identified to carry a *de novo* ~410-kb 17q22 duplication by chromosomal microarray (arr[hg19] 17q22(54214083-54624225)x3dn), which encompasses all of a single gene, *ANKFN1*, although variants in this gene have not been described to cause disease in humans. All other individuals had normal clinical chromosomal microarray testing.

Clinical features

Clinical features of probands are summarized in table 1. All individuals demonstrate developmental delays, and the older individuals have intellectual disability, mostly mild to moderate, although individual 3 is nonverbal. Of note, individual 6's mother, who also carried the *SPTBN1* variant, did not have intellectual disability but had learning disabilities, mainly in math, and completed schooling through the ninth grade. Additionally 6/7 individuals demonstrate abnormal behaviors, with ADHD specifically noted in four and autism spectrum disorder or autistic features in four. The oldest individual (#1, age 25 years) underwent multiple formal neurodevelopmental evaluations, receiving a diagnosis of pervasive developmental disorder, not otherwise specified, at age 3 years; by age 8 years her relative strengths in language suggested that other possible diagnoses included ADHD-combined form, oppositional defiant disorder, and a severe nonverbal learning disability. Formal evaluations were also completed in individuals 5 and 7, providing diagnoses of ADHD, with or without autistic disorder, respectively. Individual 3 did not have formal testing for autism but clearly met diagnostic criteria due to language delay (not speaking until age 5, and without meaningful language skills at age 11), perseverative behaviors, inappropriate social behaviors, and stereotypies. The youngest individual (#2, age 4 years)

has abnormal movements thought to be stereotypies; a normal EEG ruled out seizure activity in him.

Epilepsy is present in 2/7 individuals, a third had a single febrile seizure, and a fourth had abnormalities on EEG without overt seizures. Two of the three remaining individuals had EEGs that were normal. Epilepsy in individual 7 was characterized by two seizure types including absence and generalized tonic-clonic seizures that started by age 3, with EEG capturing focal left central parietal discharges. His seizures were controlled with oxcarbazepine. The generalized epilepsy (with a clustering pattern) in individual 4 was refractory to treatment (with current medications being levetiracetam & sodium valproate).

While most individuals have some mild dysmorphic features, no consistent, recognizable pattern is present (Figure 2). Growth parameters are within normal range, with the exception of microcephaly in individual 4 (measurement not available) and at least borderline macrocephaly in individuals 2 and 7 (Z scores of 2.63 and 2.1, respectively). Both individuals with missense variants have high and narrow palates, with one having a submucosal cleft. Three individuals have short and/or tapered fingers (Figure 2). Additional features are present in only single individuals, including mild ascending aorta dilation (Z score 2.7) in the oldest individual, hemihypertrophy (mild – one leg longer than the other), and mitochondrial dysfunction (decreased complex I activity measured in fibroblasts).

DISCUSSION

We identified seven individuals with neurodevelopmental disorders and heterozygous variants in *SPTBN1*: either *de novo* missense variants (individuals 1, 2) or predicted loss-of-function variants (individuals 3-7, occurring *de novo* in two and inherited from a mother with learning disabilities in one). Common phenotypes include global developmental delays leading to intellectual disability, as well as various behavioral abnormalities. Consistent with the previous finding of *de novo SPTBN1* variants in two individuals with autism spectrum disorder (Iossifov et al., 2014), three individuals had a diagnosis of autism spectrum disorder, and autistic features were present in a fourth. Epilepsy was present in a minority. All predicted loss-of-function variants occur before the penultimate exon of this 36-exon gene, so they are predicted to lead to nonsense-mediated decay, which would therefore suggest a haploinsufficiency mechanism. In support of this, the pLI (probability of loss-of-function intolerance) score for *SPTBN1* is 1, and the observed/expected ratio of loss-of-function variants in gnomAD is 0.04 (Karczewski et al., 2020). Additionally, homozygous knockout of *Sptbn1* in mice is lethal (Tang et al., 2003). Other autosomal dominant non-erythrocyte spectrinopathies (SCA5 due to *SPTBN2* variants and epileptic encephalopathy due to *SPTAN1* variants) are thought to have dominant negative etiologies (Clarkson, Gillespie, Perkins, Lyndon, & Jackson, 2010; Hamdan et al., 2012; Ikeda et al., 2006; Saitsu et al., 2010). In contrast, for other non-erythrocyte β spectrins, biallelic loss of function is required to lead to disease (Elsayed et al., 2014; Knierim et al., 2017; Lise et al., 2012; Wang et al., 2018). Therefore, further studies to determine if truncated βII spectrin is produced in individuals with *SPTBN1* loss-of-function variants would be of interest to further elucidate disease etiology.

The predominantly neurologic phenotypes observed in our cohort are consistent with βII spectrin's important roles in the nervous system. Experimental evidence from human brains shows expression of the gene in all examined brain regions from development into adulthood (values for $\log_2$ of reads per kilobase per million ranging from 2.83-6.76 prenatally and from 4.33-7.14 postnatally; data obtained from brainspan.org) (Miller et al., 2014). βII spectrin has been shown to bind to neurotransmitter-containing synaptic vesicles via synapsin-I (Sikorski, Sangerman, Goodman, & Critz, 2000), to help maintain structural integrity of neurons (Galiano et al., 2012), to partner with α-synuclein to regulate neurite growth during synaptogenesis (Lee, Lee, & Im, 2012), and to allow proper myelination by Schwann cells (Susuki et al., 2018). Furthermore, dysfunction of βII spectrin has been implicated in *SPTAN1*-associated epileptic encephalopathy, where mutant αII spectrin forms abnormal aggregates with both βII and βIII spectrin, leading to improper localization of voltage-gated sodium channels and epilepsy (Saitsu et al., 2010). However, only 2/7 in our cohort had epilepsy; *SPTBN1*-associated epilepsy may be less severe and/or less penetrant than that associated with *SPTAN1* due to lack of disruption of βIII spectrin and/or differences in how βII spectrin function is being disrupted.

Some additional features of note are seen in the individuals with *de novo* missense variants. Individual 1 has a mildly dilated ascending aorta, and individual 2 has mild hemihypertrophy. It is possible that these are rare manifestations of the *SPTBN1*-related neurodevelopmental disorder, that they result from a specific property conferred by the missense variants, or that they are unrelated to the *SPTBN1* variant. The aortic dilation may be a feature with a later age of onset, although age of onset in individual 1 is unknown, as she did not have an echocardiogram until adulthood as part of a comprehensive clinical research evaluation. Roles of βII spectrin support the possibility of a connection between these phenotypes and the *SPTBN1* variants. βII spectrin is important in cardiac development and function, although most studies implicate it in maintenance of cardiocytes' electrical function (Derbala et al., 2018). Disruption of βII spectrin's interaction with ankyrin-B results in arrhythmia, due to improper trafficking of membrane proteins, including ion channels (Smith et al., 2015). Additionally, βII spectrin serves as an adaptor for TGF-β signaling through interaction with Smad3/4 (Tang et al., 2003), and heterozygous variants disrupting SMAD3 function cause Loeys-Dietz syndrome-3, a key feature of which is aortic aneurysms (Chesneau et al., 2020). The hemihypertrophy present in individual 2 is also interesting, given that epigenetic silencing of *SPTBN1* is a feature of Beckwith-Wiedemann syndrome (BWS), and heterozygous *Sptbn1* knockout mice are considered a phenocopy of BWS (Chen et al., 2016; Yao et al., 2010). Although individual 2 does not show signs of BWS other than macrocephaly and mild hemihypertrophy, he is being followed to monitor for tumor development, and no tumors have been identified at this time. He has not had 11p15 methylation studies.

Missense variants in individuals 1 and 2 both affect evolutionarily conserved residues (Figure 1), and the gene is overall intolerant to missense variation (missense Z-score of 4.54, with observed/expected ratio of missense variants in gnomAD of 0.66 (Karczewski et al., 2020); Supplemental figure 1). The variant in individual 1 affects the second calponin homology domain; these domains are important for proper interaction with actin (Machnicka et al., 2014). The variant in individual 2 affects the 15th spectrin repeat, near the site of

ankyrin binding (Davis et al., 2009). Therefore, these variants could alter or abrogate the ability of βII spectrin to interact with these partners. Furthermore, variations in phenotypes could be attributed to effects in different domains of βII spectrin, as has been proposed for *SPTBN4* (Pehlivan et al., 2019). However, previous experiments changing Asp1880 and Asp1881 (the residue changed to asparagine in individual 2) to alanines did not alter βII spectrin's ability to bind ankyrin (Davis et al., 2009), and bioinformatic predictions of variant pathogenicity are mixed for the Asp1881Asn variant, with a CADD phred score of 24 (Supplemental table 1). Therefore, while some evidence exists that the missense variants in individuals 1 and 2 may be causing dysfunction of βII spectrin, both of these variants are still considered to be of uncertain significance.

Individuals reported here are subject to an ascertainment bias and may represent the severe end of a phenotypic spectrum caused by *SPTBN1* variants. As illustrated by the mother of individual 6, who only presented with learning disabilities, by the previously reported nonsense variant in an individual with autism spectrum disorder without intellectual disability (Iossifov et al., 2014), and by the presence of predicted loss-of-function variants in the gnomAD database (although at a lower than expected frequency), haploinsufficiency of this gene may demonstrate variable expressivity. Furthermore, genetic variants outside of *SPTBN1* may influence the phenotypes in individuals reported here. Individual 4 carries a *SMARCA4* variant of uncertain significance, and pathogenic variants in *SMARCA4* can cause neurodevelopmental features including behavioral problems and epilepsy (Schrier Vergano et al., 2013 Apr 4 [Updated 2018 Feb 8]). Individual 2, whose missense *SPTBN1* variant carries weaker predictions for pathogenicity, has two additional *de novo* variants found by exome sequencing and chromosomal microarray, although neither of these could be directly implicated as causing disease. Of note, while phenotype information is limited due to younger age, individual 2's neurodevelopmental phenotype seems milder than others in our cohort, as his delays were mainly restricted to motor skills, and the only behavioral concerns have been possible stereotypic movements. It remains possible that the p.D1881N variant in individual 2 has a milder, hypomorphic impact on protein function, or this variant may not be contributing to his phenotype.

The seven individuals reported here with *SPTBN1* variants support an expansion of knowledge of human spectrinopathies, showing that heterozygous disruption of βII spectrin leads to a neurodevelopmental disorder characterized by developmental delays and intellectual disability, in addition to a spectrum of abnormal behaviors including autism spectrum disorder. Combined with the occurrence of epilepsy in some individuals, these phenotypes support a critical role for *SPTBN1* in development and function of the nervous system. Expression of *SPTBN1* is broad, not limited to the nervous system (Derbala et al., 2018; Hayes et al., 2000). Therefore, effects of these *SPTBN1* variants may extend beyond the nervous system, with musculoskeletal abnormalities seen in some individuals and mild aortic dilation in one individual. Identification of additional individuals with *SPTBN1*-related neurodevelopmental disorder will help to further refine the associated phenotypic spectrum. Additional studies may also help clarify if this disorder is due to *SPTBN1* haploinsufficiency or a gain-of-function/dominant negative mechanism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability Statement

The variant data presented in this paper are deposited in ClinVar at:

https://www.ncbi.nlm.nih.gov/clinvar/variation/636237/

https://www.ncbi.nlm.nih.gov/clinvar/variation/988010/

https://www.ncbi.nlm.nih.gov/clinvar/variation/988771/

https://www.ncbi.nlm.nih.gov/clinvar/variation/1047862/

or DECIPHER at:

https://www.deciphergenomics.org/patient/417903

https://www.deciphergenomics.org/patient/359450

References

Anazi S, Maddirevula S, Salpietro V, Asi YT, Alsahli S, Alhashem A, … Alkuraya FS (2017). Expanding the genetic heterogeneity of intellectual disability. Hum Genet, 136, 1419–1429. doi: 10.1007/s00439-017-1843-2 [PubMed: 28940097]

Burridge K, Kelly T, & Mangeat P (1982). Nonerythrocyte spectrins: actin-membrane attachment proteins occurring in many cell types. J Cell Biol, 95, 478–486. doi: 10.1083/jcb.95.2.478 [PubMed: 6183274]

Caulfield M, Fowler T, Billins T, Boardman-Pretty F, Clark T, Counter P, … Hill S (2019). The National Genomics Research and Healthcare Knowledgebase v5, from doi:10.6084/m9.figshare.4530893.v5

Chen J, Yao ZX, Chen JS, Gi YJ, Munoz NM, Kundra S, … Mishra L (2016). TGF-beta/beta2-spectrin/CTCF-regulated tumor suppression in human stem cell disorder Beckwith-Wiedemann syndrome. J Clin Invest, 126, 527–542. doi: 10.1172/JCI80937 [PubMed: 26784546]

Chesneau B, Edouard T, Dulac Y, Colineaux H, Langeois M, Hanna N, … Plaisancie J (2020). Clinical and genetic data of 22 new patients with SMAD3 pathogenic variants and review of the literature. Mol Genet Genomic Med, 8, e1132. doi: 10.1002/mgg3.1132 [PubMed: 32154675]

Cianci CD, Zhang Z, Pradhan D, & Morrow JS (1999). Brain and muscle express a unique alternative transcript of alphaII spectrin. Biochemistry, 38, 15721–15730. doi: 10.1021/bi991458k [PubMed: 10625438]

Clarkson YL, Gillespie T, Perkins EM, Lyndon AR, & Jackson M (2010). Beta-III spectrin mutation L253P associated with spinocerebellar ataxia type 5 interferes with binding to Arp1 and protein trafficking from the Golgi. Hum Mol Genet, 19, 3634–3641. doi: 10.1093/hmg/ddq279 [PubMed: 20603325]

Davis L, Abdi K, Machius M, Brautigam C, Tomchick DR, Bennett V, & Michaely P (2009). Localization and structure of the ankyrin-binding site on beta2-spectrin. J Biol Chem, 284, 6982–6987. doi: 10.1074/jbc.M809245200 [PubMed: 19098307]

Deciphering Developmental Disorders Study. (2015). Large-scale discovery of novel genetic causes of developmental disorders. Nature, 519, 223–228. doi: 10.1038/nature14135 [PubMed: 25533962]

Derbala MH, Guo AS, Mohler PJ, & Smith SA (2018). The role of betaII spectrin in cardiac health and disease. Life Sci, 192, 278–285. doi: 10.1016/j.lfs.2017.11.009 [PubMed: 29128512]

Diakowski W, Szopa J, & Sikorski AF (2003). Occurrence of lipid receptors inferred from brain and erythrocyte spectrins binding NaOH-extracted and protease-treated neuronal and erythrocyte membranes. Biochim Biophys Acta, 1611, 115–122. doi: 10.1016/s0005-2736(03)00032-4 [PubMed: 12659952]

Elsayed SM, Heller R, Thoenes M, Zaki MS, Swan D, Elsobky E, … Bolz HJ (2014). Autosomal dominant SCA5 and autosomal recessive infantile SCA are allelic conditions resulting from SPTBN2 mutations. Eur J Hum Genet, 22, 286–288. doi: 10.1038/ejhg.2013.150 [PubMed: 23838597]

Firth HV, Richards SM, Bevan AP, Clayton S, Corpas M, Rajan D, … Carter NP (2009). DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. Am J Hum Genet, 84, 524–533. doi: 10.1016/j.ajhg.2009.03.010 [PubMed: 19344873]

Galiano MR, Jha S, Ho TS, Zhang C, Ogawa Y, Chang KJ, … Rasband MN (2012). A distal axonal cytoskeleton forms an intra-axonal boundary that controls axon initial segment assembly. Cell, 149, 1125–1139. doi: 10.1016/j.cell.2012.03.039 [PubMed: 22632975]

Gallagher PG, & Forget BG (1996). Hematologically important mutations: spectrin variants in hereditary elliptocytosis and hereditary pyropoikilocytosis. Blood Cells Mol Dis, 22, 254–258. doi: 10.1006/bcmd.1996.0105 [PubMed: 9075575]

Gallagher PG, Petruzzi MJ, Weed SA, Zhang Z, Marchesi SL, Mohandas N, … Forget BG (1997). Mutation of a highly conserved residue of betaI spectrin associated with fatal and near-fatal neonatal hemolytic anemia. J Clin Invest, 99, 267–277. doi: 10.1172/JCI119155 [PubMed: 9005995]

Gallagher PG, Weed SA, Tse WT, Benoit L, Morrow JS, Marchesi SL, … Forget BG (1995). Recurrent fatal hydrops fetalis associated with a nucleotide substitution in the erythrocyte beta-spectrin gene. J Clin Invest, 95, 1174–1182. doi: 10.1172/JCI117766 [PubMed: 7883966]

Glenney JR Jr., & Glenney P (1983). Fodrin is the general spectrin-like protein found in most cells whereas spectrin and the TW protein have a restricted distribution. Cell, 34, 503–512. doi: 10.1016/0092-8674(83)90383-5 [PubMed: 6352052]

Hamdan FF, Saitsu H, Nishiyama K, Gauthier J, Dobrzeniecka S, Spiegelman D, … Michaud JL (2012). Identification of a novel in-frame de novo mutation in SPTAN1 in intellectual disability and pontocerebellar atrophy. Eur J Hum Genet, 20, 796–800. doi: 10.1038/ejhg.2011.271 [PubMed: 22258530]

Hayes NV, Scott C, Heerkens E, Ohanian V, Maggs AM, Pinder JC, … Baines AJ (2000). Identification of a novel C-terminal variant of beta II spectrin: two isoforms of beta II spectrin have distinct intracellular locations and activities. J Cell Sci, 113 (Pt 11), 2023–2034. [PubMed: 10806113]

Ikeda Y, Dick KA, Weatherspoon MR, Gincel D, Armbrust KR, Dalton JC, … Ranum LP (2006). Spectrin mutations cause spinocerebellar ataxia type 5. Nat Genet, 38, 184–190. doi: 10.1038/ng1728 [PubMed: 16429157]

Iossifov I, O'Roak BJ, Sanders SJ, Ronemus M, Krumm N, Levy D, … Wigler M (2014). The contribution of de novo coding mutations to autism spectrum disorder. Nature, 515, 216–221. doi: 10.1038/nature13908 [PubMed: 25363768]

Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alfoldi J, Wang Q, … MacArthur DG (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. Nature, 581, 434–443. doi: 10.1038/s41586-020-2308-7 [PubMed: 32461654]

Knierim E, Gill E, Seifert F, Morales-Gonzalez S, Unudurthi SD, Hund TJ, … Schuelke M (2017). A recessive mutation in beta-IV-spectrin (SPTBN4) associates with congenital myopathy, neuropathy, and central deafness. Hum Genet, 136, 903–910. doi: 10.1007/s00439-017-1814-7 [PubMed: 28540413]

Lee HJ, Lee K, & Im H (2012). alpha-Synuclein modulates neurite outgrowth by interacting with SPTBN1. Biochem Biophys Res Commun, 424, 497–502. doi: 10.1016/j.bbrc.2012.06.143 [PubMed: 22771809]

Liem RK (2016). Cytoskeletal Integrators: The Spectrin Superfamily. Cold Spring Harb Perspect Biol, 8. doi: 10.1101/cshperspect.a018259

Lise S, Clarkson Y, Perkins E, Kwasniewska A, Sadighi Akha E, Schnekenberg RP, … Nemeth AH (2012). Recessive mutations in SPTBN2 implicate beta-III spectrin in both cognitive and motor development. PLoS Genet, 8, e1003074. doi: 10.1371/journal.pgen.1003074 [PubMed: 23236289]

Machnicka B, Czogalla A, Hryniewicz-Jankowska A, Boguslawska DM, Grochowalska R, Heger E, & Sikorski AF (2014). Spectrins: a structural platform for stabilization and activation of membrane channels, receptors and transporters. Biochim Biophys Acta, 1838, 620–634. doi: 10.1016/j.bbamem.2013.05.002 [PubMed: 23673272]

Marchesi SL, Letsinger JT, Speicher DW, Marchesi VT, Agre P, Hyun B, & Gulati G (1987). Mutant forms of spectrin alpha-subunits in hereditary elliptocytosis. J Clin Invest, 80, 191–198. doi: 10.1172/JCI113047 [PubMed: 3597773]

Marchesi VT, & Steers E Jr. (1968). Selective solubilization of a protein component of the red cell membrane. Science, 159, 203–204. doi: 10.1126/science.159.3811.203 [PubMed: 5634911]

Miller JA, Ding SL, Sunkin SM, Smith KA, Ng L, Szafer A, … Lein ES (2014). Transcriptional landscape of the prenatal human brain. Nature, 508, 199–206. doi: 10.1038/nature13185 [PubMed: 24695229]

Miraoui H, Dwyer AA, Sykiotis GP, Plummer L, Chung W, Feng B, … Pitteloud N (2013). Mutations in FGF17, IL17RD, DUSP6, SPRY4, and FLRT3 are identified in individuals with congenital hypogonadotropic hypogonadism. Am J Hum Genet, 92, 725–743. doi: 10.1016/j.ajhg.2013.04.008 [PubMed: 23643382]

Pehlivan D, Bayram Y, Gunes N, Coban Akdemir Z, Shukla A, Bierhals T, … Lupski JR (2019). The Genomics of Arthrogryposis, a Complex Trait: Candidate Genes and Further Evidence for Oligogenic Inheritance. Am J Hum Genet, 105, 132–150. doi: 10.1016/j.ajhg.2019.05.015 [PubMed: 31230720]

Rodrigues CH, Pires DE, & Ascher DB (2018). DynaMut: predicting the impact of mutations on protein conformation, flexibility and stability. Nucleic Acids Res, 46, W350–W355. doi: 10.1093/nar/gky300 [PubMed: 29718330]

Saitsu H, Tohyama J, Kumada T, Egawa K, Hamada K, Okada I, … Matsumoto N (2010). Dominant-negative mutations in alpha-II spectrin cause West syndrome with severe cerebral hypomyelination, spastic quadriplegia, and developmental delay. Am J Hum Genet, 86, 881–891. doi: 10.1016/j.ajhg.2010.04.013 [PubMed: 20493457]

Schrier Vergano S, Santen G, Wieczorek D, Wollnik B, Matsumoto N, & Deardorff MA (2013 Apr 4 [Updated 2018 Feb 8]). Coffin-Siris Syndrome. In Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mirzaa G & Amemiya A (Eds.), GeneReviews((R)). Seattle (WA): University of Washington. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK131811/.

Sikorski AF, Sangerman J, Goodman SR, & Critz SD (2000). Spectrin (betaSpIIsigma1) is an essential component of synaptic transmission. Brain Res, 852, 161–166. doi: 10.1016/s0006-8993(99)02253-2 [PubMed: 10661507]

Smith SA, Sturm AC, Curran J, Kline CF, Little SC, Bonilla IM, … Mohler PJ (2015). Dysfunction in the betaII spectrin-dependent cytoskeleton underlies human arrhythmia. Circulation, 131, 695–708. doi: 10.1161/CIRCULATIONAHA.114.013708 [PubMed: 25632041]

Susuki K, Zollinger DR, Chang KJ, Zhang C, Huang CY, Tsai CR, … Rasband MN (2018). Glial betaII Spectrin Contributes to Paranode Formation and Maintenance. J Neurosci, 38, 6063–6075. doi: 10.1523/JNEUROSCI.3647-17.2018 [PubMed: 29853631]

Tang Y, Katuri V, Dillner A, Mishra B, Deng CX, & Mishra L (2003). Disruption of transforming growth factor-beta signaling in ELF beta-spectrin-deficient mice. Science, 299, 574–577. doi: 10.1126/science.1075994 [PubMed: 12543979]

van der Sluijs PJ, Aten E, Barge-Schaapveld D, Bijlsma EK, Bokenkamp-Gramann R, Donker Kaat L, … Santen GWE (2019). Putting genome-wide sequencing in neonates into perspective. Genet Med, 21, 1074–1082. doi: 10.1038/s41436-018-0293-0 [PubMed: 30287924]

Wang CC, Ortiz-Gonzalez XR, Yum SW, Gill SM, White A, Kelter E, … Rasband MN (2018). betaIV Spectrinopathies Cause Profound Intellectual Disability, Congenital Hypotonia, and Motor Axonal Neuropathy. Am J Hum Genet, 102, 1158–1168. doi: 10.1016/j.ajhg.2018.04.012 [PubMed: 29861105]

Yang Y, Muzny DM, Xia F, Niu Z, Person R, Ding Y, … Eng CM (2014). Molecular findings among patients referred for clinical whole-exome sequencing. JAMA, 312, 1870–1879. doi: 10.1001/jama.2014.14601 [PubMed: 25326635]

Yao ZX, Jogunoori W, Choufani S, Rashid A, Blake T, Yao W, … Mishra L (2010). Epigenetic silencing of beta-spectrin, a TGF-beta signaling/scaffolding protein in a human cancer stem cell disorder: Beckwith-Wiedemann syndrome. J Biol Chem, 285, 36112–36120. doi: 10.1074/jbc.M110.162347 [PubMed: 20739274]

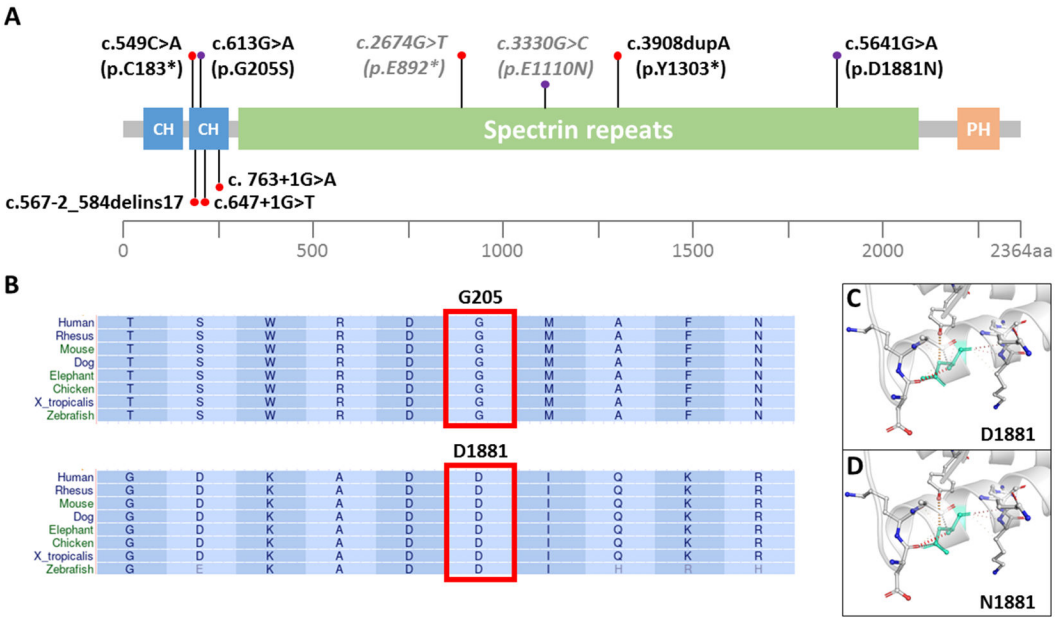

Figure 1. Location of identified variants within SPTBN1 protein domains.

(A) Schematic of βII spectrin illustrating location of protein domains and variants, with coding variants above the schematic and predicted splicing variants shown below. Red circles indicate predicted loss-of-function variants, while purple circles indicate missense variants. Two previously reported *de novo* variants indentified in males with autism spectrum disorder (Iossifov et al., 2014) are represented by gray, italicized text. CH = calponin homology domain; PH = plekstrin homology domain; aa = amino acids. **(B)** Residues affected by missense variants in individuals 1 and 2 are conserved across vertebrate species (obtained from UCSC Genome Browser, genome.ucsc.edu). **(C-D)** Visualization of wild-type aspartic acid at amino acid 1881 (light green, C) and its replacement by asparagine (light green, D). This residue is within the 15th spectrin repeat, near the site of ankyrin binding. Visualization is from DynaMut (Rodrigues, Pires, & Ascher, 2018), based on the crystal structure of the 14th-16th spectrin repeats (3EDV from Protein Data Bank, rcsb.org). Crystal structure for the second calponin homology domain, where individual 1's missense variant is located, was not available in Protein Data Bank.

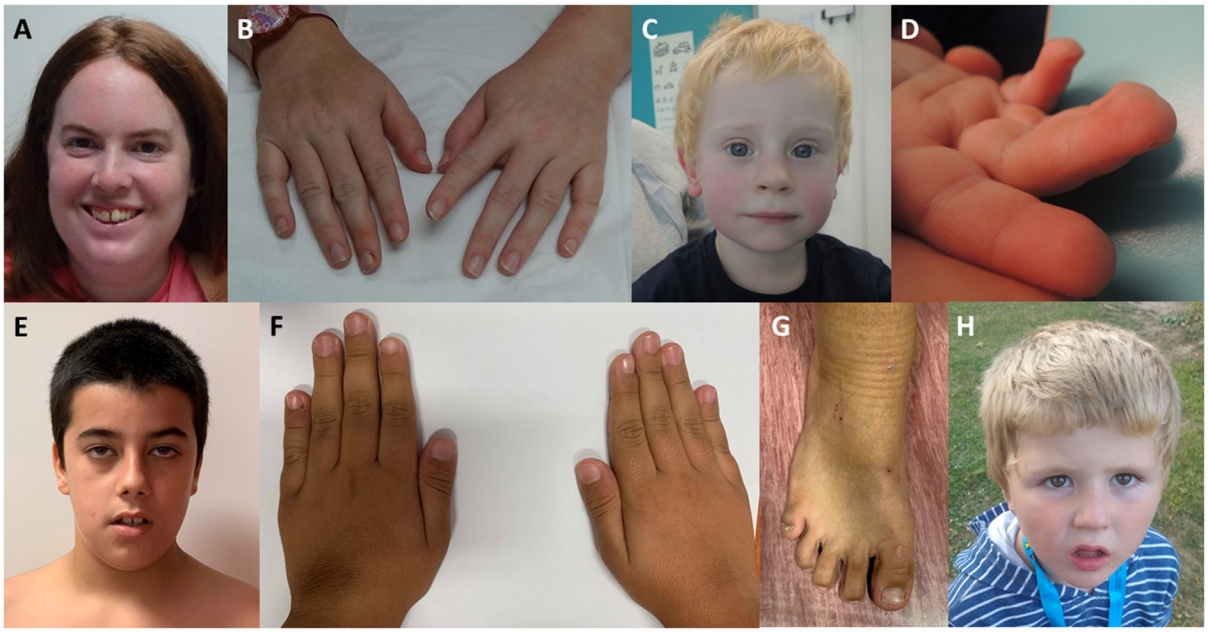

Figure 2. Physical features of individuals with *SPTBN1* variants.

(A-B) Individual 1 at 25 years of age. Note broad forehead and tapered fingers. **(C-D)** Individual 5 at 4 years of age. Note flat nasal bridge, uplifted earlobes, and fetal finger pads. **(E-G)** Individual 6 at 10 years of age. Note suspected shortened fourth and fifth metacarpals, hypoplastic nails, and hypoplastic fifth toe. **(H)** Individual 7 at 4 years of age.

Table 1.

Phenotypic features and *SPTBN1* variants in individuals 1-7

Individual	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Coordinate(s) [Chr2, hg19]	54844791	54880809	54844742-54844762	54844826	54843418	54845331	54870169
***SPTBN1* variant (NM_003128.2)**	c.613G>A (p.G205S)	c.5641G>A (p.D1881N)	c.567-2_584delins17	c.647+1G>T	c.549C>A (p.C183*)	c.763+1G>A	c.3908dupA (p.Y1303*)
Inheritance	*De novo*	*De novo*	*De novo*	Not maternal	*De novo*	Maternal	Not maternal
Sex, Age	F, 25y	M, 4y	M, 12y	F, 8y	M, 6y	M, 10y	M, 5y
Growth percentiles	Ht 26th; Wt 92nd; OFC 60th	Ht 35th; Wt 50th; OFC 99th (Z=+2.63 at 2y)	Ht 80th; Wt 75th; OFC 76th (at 10.5y)	Ht 8th; Wt 17th	Ht 90th; Wt 95th; OFC 54th (at 3y)	Ht 45th; Wt 97th; OFC 55th	Ht 85th; Wt 97th; OFC 98th (Z=+2.1)
DD/ID	Mild-moderate ID (IQ=53)	Motor delays	+ (nonverbal)	+ (global)	Mild ID	+ (ID)	+ (global, mild)
Behavioral	Formal diagnoses of ASD and ADHD, aggression, rigid behavior, OCD-like	Stereotypies	ASD, ADHD, OCD-like, motor tics	Autistic, aggression, Rett/Angelman-like	Formal diagnoses of ASD and ADHD	-	ADHD (diagnosed by BASC-3), disruptive behavior
Neuromuscular	-	Central hypotonia, spasticity	Diffuse hypotonia, mild weakness, Gower sign	-	-	-	-
Seizures/EEG	Right temporal focus of epileptiform activity; no seizures	Staring spells; EEG normal	EEG normal	Refractory generalized epilepsy, clustering pattern, onset age 1y	Single febrile seizure; EEG not performed	-	Absence and generalized tonic-clonic seizures, onset age 3y; focal left central parietal discharges on EEG
Craniofacial	Broad forehead, dolichocephaly, high/ narrow palate	Macrocephaly, frontal bossing, hypertelorism, flat nasal bridge; submucosal cleft	-	Microcephaly, flat occiput, long eyelashes, forehead hemangioma, mildly coarse features	Flat nasal bridge, uplifted earlobes	-	-
Musculoskeletal	Tapered fingers, mild joint laxity, kyphoscoliosis	Hemihypertrophy	Hyperextensible	Positional talipes at birth	Short fingers	Suspected short 4th & 5th metacarpals	-
Additional findings	Mildly dilated ascending aorta (Z=+2.7), irregular menstrual cycles	Small penis, vomiting & constipation, thin corpus callosum & tonsillar ectopia	Mitochondrial dysfunction (complex I activity in fibroblasts 17%; elevated lactate, pyruvate)	Cutis marmorata	Fetal finger pads	Hypoplastic nails; Mother with learning disabilities	-

+ = feature present; − = feature absent; ADHD = attention deficit hyperactivity disorder; ASD = autism spectrum disorder; DD = developmental delay; Ht = height; ID = intellectual disability; OCD = obsessive compulsive disorder; OFC = occipital frontal circumference; Wt = weight; y = years