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Spectrins: molecular organizers and targets of neurological disorders

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Abstract

Spectrins are cytoskeletal proteins that are expressed ubiquitously in the mammalian nervous system. Pathogenic variants in *SPTAN1*, *SPTBN1*, *SPTBN2* and *SPTBN4*, four of the six genes encoding neuronal spectrins, cause neurological disorders. Despite their structural similarity and shared role as molecular organizers at the cell membrane, spectrins vary in expression, subcellular localization and specialization in neurons, and this variation partly underlies non-overlapping disease presentations across spectrinopathies. Here, we summarize recent progress in discerning the local and long-range organization and diverse functions of neuronal spectrins. We provide an overview of functional studies using mouse models, which, together with growing human genetic and clinical data, are helping to illuminate the aetiology of neurological spectrinopathies. These approaches are all critical on the path to plausible therapeutic solutions.

Introduction

Neuronal spectrins coordinate the positioning and stabilization of multifunctional nanodomains and microdomains of ion channels, cell adhesion molecules, membrane transporters and scaffolding proteins[1–3]. Together with actin, spectrin forms a submembrane cytoskeleton thought to impart mechanical resilience to neuronal processes and mediate signalling events[4]. Additionally, spectrins promote vesicle and organelle transport[2]. Given the multifaceted roles of spectrins in neurons, it is not surprising that pathogenic variants in spectrin genes lead to neurodevelopmental disorders. Clinical variants in four (*SPTAN1*,

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SPTBN1, *SPTBN2* and *SPTBN4*) of the six spectrin genes expressed in the nervous system have been genetically and functionally linked to neurological disorders whose clinical presentations include intellectual disability (ID), developmental delay (DD), seizures, movement disorders and behavioural abnormalities (Table 1). The advent of accessible whole-exon sequencing for clinical diagnosis has enabled the identification of genes and variants underlying new neurological spectrinopathies and measurably expanded the list of individuals affected by these rare disorders.

Spectrinopathies of the nervous system diverge in their primary clinical diagnoses but overlap in their syndromic presentations, underscoring both the functional similarities and the distinct cellular and subcellular specializations of spectrins (Fig. 1). Spectrins are broadly expressed in the nervous system and form elongated, rod-like polypeptides directly coupled to the actin cytoskeleton to form remarkably regular networks that line the cell membrane spanning the neuron. These arrays form by assembling a basic motif that comprises α-spectrin and β-spectrin heterodimers, which then form head-to-head tetramers that crosslink F-actin (Fig. 2). This meshwork integrates into the cytosolic side of the plasma membrane by direct association with membrane lipids and ankyrins[1,2] (Fig. 2a). These structural hubs bring together other molecular partners, whose specific identities determine local function, and their disruption drives the underlying pathophysiology of the different spectrinopathies.

In this Review, we describe the function of spectrins in mammalian neurons and summarize recent advances in delineating their cell-type-specific and neuronal-domain-specific localization and functional specialization. We examine how impaired expression and pathogenic variants in spectrin genes lead to altered protein function, the physiological and behavioural consequences of these changes in mouse models, and their relationship to clinical presentations in humans. Lastly, we discuss potential mechanistic overlap across spectrinopathies of the nervous system and future directions that may inform the rational design of therapeutic approaches.

Spectrins in neuron architecture and function

The basic set of a single α-spectrin, one giant β-spectrin and one canonical β-spectrin with ankyrin-binding activity (Fig. 2) already present in bilaterians expanded markedly in vertebrates through whole-genome duplication events[3]. Non-mammalian vertebrates express four β-spectrin genes. *SPTB* encodes βI-spectrin, the red blood cell β-spectrin[5,6] also found in neurons; *SPTBN1* encodes βII-spectrin, first characterized in the brain[7,8] and expressed in all tissues; *SPTBN4* encodes βIV-spectrin, found in the nervous system, pancreatic islets[9] and cardiomyocytes[10]; and *SPTBN5* encodes the giant βV-spectrin, which lacks the ankyrin-binding sequence and is expressed at modest levels in the cerebellum and in auditory hair and photoreceptor cells[11,12] (Fig. 2d,f). Mammals also express βIII-spectrin, encoded by *SPTBN2*, which was first identified in the brain[13,14] (Fig. 2d) and detected at high levels in the pancreas, kidney, reproductive tissues and skin. In addition, vertebrates express αII-spectrin, which is encoded by *SPTAN1* and associates ubiquitously with β-spectrins[15] (Fig. 2c); and αI-spectrin, encoded by *SPTA1*, which is found exclusively in mammalian

erythrocytes[16]. Alternative splicing expands the spectrin complement[17]. For example, βIV-spectrin is also expressed as isoforms lacking portions of the N or C termini[9,18] (Fig. 2e).

Molecular architecture of spectrins

Spectrins are elongated molecules formed by in-tandem spectrin repeats (SRs), each containing 99–114 residues and extending approximately 100 nm in length. Crystal structures show that SRs adopt a left-handed, anti-parallel, three-helix coiled-coil topology, and are connected by short α-helical linkers[19]. Canonical βI–βIV-spectrins contain 16 full SRs and a partial 17th SR[20], two N-terminal tandem calponin homology (CH) domains, an ankyrin-binding site in SRs 14 and 15, and a C-terminal pleckstrin homology (PH) domain (Fig. 2d). βIV-spectrin has an additional sequence between the final SR and the PH domain[9] (Fig. 2d). Giant βV-spectrin contains 29 full SRs plus a partial 30th SR[11] (Fig. 2f). By contrast, αII-spectrin, the obligatory partner of neuronal β-spectrins, contains 20 SRs, an Src-homology 3 (SH3) domain in SR9, a calmodulin (CaM)-binding loop in SR10 and calcium-binding EF hand domains near the C terminus (Fig. 2c). Complementary motifs in SRs 1 and 2 of βI–βIV-spectrins and SRs 20 and 21 of αII-spectrin enable the antiparallel lateral assembly of α–β-spectrin heterodimers[21] (Fig. 2b). Spectrin dimers assemble into tetramers via head-to-head non-covalent association between partial repeats in each α-spectrin and β-spectrin subunit (Fig. 2b–d). Atomic force microscopy studies show that the tertiary structure of SRs imparts elasticity to the molecules[22]. This spring-like property facilitates elastic recovery of spectrin molecules when subjected to shear stress during circulation in erythrocytes[23] or mechanical tension in axons during growth and fasciculation[24,25], and offers a rationale for the formation of long-range ordered spectrin–actin assemblies in neurons[26].

Spectrins and the neuronal cytoskeleton

The ability of spectrins and actin to form long-range ordered networks was first observed via electron microscopy in erythrocytes, where they organize as a hexagonal lattice, in which six spectrin tetramers about 60–80 nm in length crosslink short actin protofilaments[27] capped by adducin[28,29] and tropomodulin[30,31]. Visualization using 3D-stochastic optical reconstruction microscopy (3D-STORM) confirmed that native erythrocytic spectrin tetramers adopt a relaxed conformation[32] and also revealed a similar 2D polygonal spectrin lattice in the somatodendritic compartment of cultured rodent neurons[33]

(Fig. 1). This 2D assembly progressively develops both in the soma and in dendrites in vitro and depends on actin polymerization and βII-spectrin for its formation[33]. Images obtained with 3D-STORM suggest that the somatodendritic spectrin lattice contains tetramers of αII/βIII-spectrin in their extended conformation. However, whether βIII-spectrin is an essential component of this structure and the functional role of the 2D lattice are not known. In accordance with spectrin's canonical role, it is possible that one function is to stabilize protein complexes in those neuronal regions or to modulate endocytosis; this remains to be clarified.

The membrane-associated periodic skeleton (MPS), consisting of actin, spectrins and binding partners, is conserved across organisms and neuron tyes[34–37]. The MPS is composed

of submembrane actin rings periodically spaced at ~190 nm throughout axons and mature dendrites, which corresponds to the extended conformation of spectrin tetramers[33–35] (Fig. 1). This remarkable periodicity of the actin lattice is established very early in neuronal development and is likely to be conserved across neuron types and species. In mouse neurons, the MPS is detected in the proximal axon of cultured neurons as early as day in vitro 2 (DIV2) and propagates towards distal axon regions as neurons mature[38]. However, a recent study suggests that the MPS nucleates from multiple periodic patches along the growing axon that expand and coalesce into a single scaffold[39]. That the youngest part of the axon with the lowest actin–spectrin periodicity has the greatest axonal diameter implicates the gradual MPS assembly in the progressive constriction of the growing axon[39,40]. The assembly and integrity of the MPS depend on both actin and spectrin. Perturbation of actin using destabilizing drugs and depletion of βII-spectrin, the most ubiquitous β-spectrin in neurons, by in vitro short hairpin RNA knockdown or in vivo genetic knockout prevents the assembly of the MPS or disrupts its stability. These disruptions correlate with deficits in the structural integrity of the axon initial segment (AIS), the growth of axons in vitro and in vivo, and the formation of long-range axonal tracts in mouse brains[33,34,38,41,42].

Early studies in *Caenorhabditis elegans* showed that worms lacking β-spectrin are more prone to axon breakage upon movement[43], which suggests that spectrins and the MPS promote the integrity and mechanical stability of axons under mechanical stress[26]. This protection could be due, in part, to the unfolding properties and intrinsic flexibility of the SRs[22,44], which probably confer tension-buffering properties on the MPS and allow axons to stretch reversibly without compromising their structural or functional properties[45]. Several studies also support the idea that spectrins and the MPS are critical regulators of axon diameter. Loss of either βII-spectrin or the actin-capping protein α-adducin, a component of the axonal MPS, results in axon enlargement and degeneration in mouse models[40,41]. The ability of the MPS to regulate axonal radial contractility is facilitated by the actin-binding protein non-muscle myosin II, which associates with periodic F-actin rings via its head domains[46,47].

Other functions attributed to the actin and spectrin-based MPS include serving as a signalling platform (discussed below) and acting as a diffusion barrier at the AIS that selectively filters proteins to contribute to neuronal polarity[48,49]. In addition, direct crosstalk between the MPS and microtubules, in which these two cytoskeletal networks depend on each other for their formation, stability and regulation, has been proposed[50].

Subcellular localization and functional specialization

Despite their remarkable structural and functional domain similarities, β-spectrins are differentially expressed across neuronal types and preferentially localized to specific functional compartments (for example, the AIS, nodes of Ranvier (NoR), dendrites and spines, and the postsynapse) (Fig. 1). We next discuss the preferential segregation of neuronal spectrins and what is known about how this spatial distribution is molecularly codified.

Axonal spectrins: axon initial segment.

αII-Spectrin and βIV-spectrin are the most abundant spectrin tetramers at the AIS, where they incorporate into the MPS with a ~190 nm periodicity[51–53] (Fig. 1). αII-Spectrin associates with both βIV-spectrin-ΣI and βIV-spectrin-ΣVI isoforms, which depend on ankyrin-G for their recruitment to this axonal domain[54,55]. Right after axonal specification and before AIS formation, periodic αII/βII-spectrin tetramers can already be detected in the proximal axon[38]. However, as neurons mature, both the βII-spectrin signal and its periodicity at the AIS diminish, whereas ankyrin-G and βIV-spectrin levels increase to become highly periodic by DIV12 (ref. [38]). Early βII-spectrin expression promotes the formation of a periodic βIV-spectrin assembly at the AIS and proper AIS development[38]. Consistent with these roles, neurons cultured in vitro and mouse brains lacking βII-spectrin exhibit fragmented AIS[41,42,48] and expanded localization of βIV-spectrin beyond the normal AIS boundaries[48]. Similarly, loss of αII-spectrin, which lowers βII-spectrin levels by more than 70%, results in a reduction in the number of AISs in the mouse cortex[52]. The remaining AISs are fragmented, with lower levels and less periodic distribution of βIV-spectrin[52]. Impaired AIS formation in either αII-spectrin- or βII-spectrin-deficient neurons occurs despite a significant increase in levels of the βIV-spectrin-ΣVI isoform[41,52]. These results are surprising given that βIV-spectrin-ΣVI, which lacks actin binding but interacts with ankyrin-G, restores AIS morphology and the clustering of ankyrin-G and other critical AIS components of neurons lacking βIV-spectrin[56]. Unexpectedly, βIV-spectrin periodicity at the AIS is not affected by chemical perturbation of the submembrane actin and microtubule lattices[52]. Thus, the organization and function of βIV-spectrin in the AIS do not require the submembrane cytoskeleton but, rather, depend on ankyrin-G binding[53,57]. Targeting of βIV-spectrin to the AIS can be further modulated by phosphorylation-dependent conformational changes in 480 kDa ankyrin-G, the giant isoform that localizes at the AIS and promotes its development[56,57]. βI-Spectrin, the major β-spectrin in erythrocytes, is not normally found at the AIS but re-localizes to this domain in parvalbumin-positive interneurons lacking βIV-spectrin[58]. However, βI-spectrin cannot compensate for loss of AIS βIV-spectrin. Unlike βII-spectrin and βIV-spectrin, βI-spectrin preferentially binds ankyrin-R[59], which cannot be recruited to the AIS; therefore, βI-spectrin is unable to stabilize ion channels and other AIS components[58]. Together, spectrins regulate the morphology, structural integrity and macromolecular composition of the AIS, which is required for efficient action potential initiation and propagation in the nervous system.

Axonal spectrins: nodes of Ranvier.

Spectrins collaborate with ankyrins to position macromolecular complexes that are essential for the ultrastructural organization and function of NoR[60]. Ankyrin-G and βIV-spectrin are confined to the nodal gap and organized with a ~190 nm periodicity[61,62] (Fig. 1). Early in development, βIV-spectrin-ΣI is the predominant isoform at NoR in mouse neurons; however, βIV-spectrin-ΣVI levels increase robustly after birth, quickly exceeding βIV-spectrin-ΣI in abundance[63]. Loss of ankyrin-G or βIV-spectrin does not disrupt the clustering of voltage-gated sodium channels (Na_V) at NoR of sensory neurons owing to a compensatory mechanism whereby ankyrin-R and βI-spectrin concentrate at the nodes to stabilize these channels[59]. Studies in conditional knockout mouse models that selectively lack either βI-spectrin or βIV-spectrin, or both, only in peripheral sensory neurons (PSNs)

demonstrated a hierarchy of nodal spectrins. βIV-Spectrin is the main nodal spectrin; however, βI-spectrin can fully compensate for its loss at NoR[64]. Although nodal β-spectrins are not required for Na_V clustering during development, they are essential for maintaining Na_V assemblies at NoR and the structural integrity of sensory axons, and their loss leads to axonal degeneration[64]. βII-Spectrin localizes to the paranodal region of NoR, where it is periodically organized[62,65] (Fig. 1) and required for paranode-dependent clustering of nodal Na_V[66]. Loss of βII-spectrin disrupts the paranode–juxtaparanode membrane barrier and leads to diffusion of voltage-activated $K_V1.2$ potassium channels into paranodes and nodal gaps[67]. αII-Spectrin also forms a periodic cytoskeleton at nodes and paranodes[68] (Fig. 1). Loss of αII-spectrin disrupts the periodicity of βIV-spectrin at the nodal gap and of βII-spectrin at paranodes, impairs NoR assembly and maintenance, disrupts the restricted juxtaparanode localization of $K_V1.2$ and causes axon degeneration[68]. These studies underscore the critical roles of nodal spectrins in organizing key ion channels and cytoskeletal components at NoR, which are essential for fast action potential propagation in myelinated axons and for maintaining axon integrity.

Somatodendritic spectrins.

As in axons, αII/βII-spectrin tetramers organize the MPS in dendritic shafts and in a subset of spine necks of mature (DIV16-21) cultured mouse neurons, and probably in vivo[33,38,41,69,70] (Fig. 1). The formation of the dendritic MPS depends on the expression and local concentration of βII-spectrin[33,38]. Ankyrin-B, a βII-spectrin binding partner, is a critical regulator of βII-spectrin dendritic levels and dendritic MPS formation[38]. Loss of ankyrin-B results in a twofold increase in dendritic βII-spectrin in DIV10 neurons without overall changes in its total brain expression or in the organization of the axonal MPS[38,71]. Although the MPS is very irregular in DIV10 control dendrites, at this timepoint βII-spectrin and α-adducin exhibit a highly periodic distribution in all dendrites of neurons lacking ankyrin-B, quantitatively similar to their axonal periodicity[38]. The periodic distribution of βII-spectrin at spine necks is less penetrant and detected in 25–50% of spines, depending on culture conditions and super-resolution imaging modalities[33,69]. By contrast, F-actin rings are periodically organized at the neck of every spine[69], suggesting that other β-spectrins may selectively help to assemble the dendritic MPS in a subset of spines.

Tetramers containing βIII-spectrin are likely to contribute to the periodic organization of actin and other MPS components in spines. βIII-Spectrin is highly enriched in dendrites and at the neck of spines, as shown by confocal and platinum replica electron microscopy[72,73] (Fig. 1). A periodic βIII-spectrin signal has also been detected by STORM in dendritic shafts of mature neurons[33]. Consistent with a functional role for βIII-spectrin in spines, its knockdown decreases the density of dendritic spines and alters the formation of the constricted necks of spines in hippocampal neuronal cultures[73]. Deficits in βIII-spectrin also reduce the number of synapses and impair the localization of critical postsynaptic density (PSD) components, including metabotropic glutamate receptor 1α (mGluR1α) and delta-2 glutamate receptor (GluRδ2) within spines of cerebellar Purkinje neurons[72,74,75]. These deficits, which are likely to arise from a failure of βIII-spectrin's essential role in localizing and scaffolding membrane proteins at dendritic domains, alter cerebellar excitability and cause motor impairments in mouse models[72,74].

Multiple spectrins localize at the spine head, including βII-spectrin and βIII-spectrin (Fig. 1), whose signals do not appear to co-localize with PSD markers[70]. This localization pattern contrasts with previous reports that βII-spectrin selectively interacts with NMDA receptor subunits through binding sites distinct from those of members of the PSD95/SAP90 family[76]. βI-Spectrin is localized throughout dendrites[77,78] and enriched at the PSD[79–81]. Interestingly, the βI-spectrin signal at the PSD does not co-localize with αII-spectrin, which is found mostly at the spine neck[81]. Whether βI-spectrin functions in its monomeric form in this subsynaptic domain, is integrated into spectrin tetramers or is periodically organized remains to be determined. βI-Spectrin may have both structural and functional roles in dendritic spines through its interaction with F-actin and the small GTPase RAC3 (ref. [82]). Expression of the actin-binding domain of βI-spectrin stabilizes actin filaments in dendritic spines by reducing its depolymerization rate[82]. Functionally, these changes disrupt the dynamic regulation of actin in spines and the morphological and electrophysiological plasticity of spines, as evidenced by increases in the sizes of spine heads, in the trafficking of AMPA receptors into spines and in AMPA receptor-mediated synaptic responses[82]. The inability of a mutant ankyrin-G that lacks βIV-spectrin binding to rescue deficits in spine development associated with ankyrin-G loss prompted the suggestion that βIV-spectrin may localize to spines and participate in the modulation of spine morphology[83]. However, the binding site for βIV-spectrin in ankyrin-G is shared with other β-spectrins. Thus, the specific β-spectrin(s) that modulate the roles of ankyrin-G in spines, and whether βIV-spectrin localizes to spines, remain to be determined through super-resolution microscopy, biochemical and functional studies.

Spectrins in intracellular transport.

The capacity of spectrins to act as transport facilitators has critical physiological consequences[41,42,84]. The implication of spectrin in organelle transport was first suggested by the co-migration of βII-spectrin with axonal organelles in the optic nerve[85], and by the formation of complexes between βII-spectrin and kinesin motors KIF3A, KIF5B and KIF1A and the $p150^{Glued}$ dynactin subunit[41]. Other spectrins also associate with motor proteins or their adaptors. For instance, αII-spectrin interacts and travels with similar velocity to KIF3 in rat optic nerves[86]. βIII-Spectrin binds the dynactin actin-related protein 1 (Arp1) subunit[87]. Lastly, βV-spectrin binds KIF3A and myosin VIIa motors and the dynactin subunit dynamitin (p50) in photoreceptor cells[12]. Spectrins also interact with various organelles[12,41,85,88] and probably operate as adaptors or accessory proteins that promote the recruitment of microtubule-based motors to cargoes. For example, βII-spectrin associates with synaptic vesicles and lysosomes in mouse brain lysates, and its loss impairs their bidirectional axonal transport in hippocampal and cortical neurons[41,42]. Similarly, βV-spectrin forms complexes with opsin-containing vesicles in vivo and promotes their recruitment to myosin VIIa and transport to the outer segments of photoreceptor cells[12].

β-Spectrins are recruited to intracellular membranes via coupling of their PH domains to membrane phospholipids. Expression of the K2207Q βII-spectrin PH domain mutant, which is incapable of binding phosphoinositide[89], cannot restore axonal transport in neurons lacking βII-spectrin[41,90]. Similarly, reconstituted motility assays using cytoplasmic material and liposomes from squid axons showed that expression of a dominant-negative

construct containing the PH domain of βII-spectrin or βIII-spectrin impaired organelle and liposome retrograde motility[88]. These studies indicate that the association of β-spectrins with membrane lipids is required for axonal transport driven by dynein or dynactin[88]. β-Spectrins also bind integral membrane proteins on vesicles, as suggested by the direct interaction of βII-spectrin with synapsin I[91]. The association of β-spectrins with vesicles could be mediated by ankyrin-B, which binds surface phosphoinositol trisphosphate lipids in organelles and regulates axonal transport[71]. However, the Y1874A mutant form of βII-spectrin, which does not bind ankyrin-B, rescues synaptic vesicle dynamics in neurons lacking βII-spectrin, which suggests that ankyrin-B and βII-spectrin promote axonal transport through independent pathways[41].

How can spectrins simultaneously integrate into the axonal MPS and associate with motors and cargoes? Are the axonal transport deficits in neurons lacking β-spectrins secondary to MPS disruption? It is possible that high F-actin concentrations combined with the low-affinity association between F-actin and the CH domains of β-spectrins partition spectrin molecules between motor protein-bound and MPS-associated pools. Interestingly, βII-spectrin regulates axonal cargo transport from early axonal development in the absence of the MPS, which forms a few days later[41]. However, the secondary effects of acute MPS disassembly on axonal organelle trafficking warrant further investigation.

Spectrins in signal transduction.

The ability of spectrins to periodically organize functionally related membrane proteins suggests that the MPS may serve as a structural platform that regulates signalling events spatially and temporally. Multiple signalling molecules, including G-protein-coupled receptors (GPCRs), cell adhesion molecules and receptor tyrosine kinases (RTKs), are periodically distributed in axons and co-localize with the MPS near the ankyrin-binding region of the spectrin tetramer[4]. The incorporation of a subset of signalling molecules into the MPS is potentiated (or only detected) following extracellular stimulation[4]. Thus, the initial association of these molecules with the MPS probably increases their local concentration, amplifies their recruitment through multivalent interactions with ankyrins and spectrins, and promotes the formation of signalling hubs[4]. In support of this view, periodically localized cannabinoid type 1 receptor (CB1) and neural cell adhesion molecule 1 increased their association with the MPS upon treatment with a CB1 agonist, which resulted in RTK transactivation and activation of the ERK cascade in neurons[4]. Interestingly, ERK signalling causes degradation of spectrin and the MPS by calpain, which provides a feedback loop mechanism for neuronal signal attenuation[4].

Insights from mouse models

The high evolutionary conservation of spectrin genes across mammals has made knock-in, global and conditional knockout mouse models of spectrins valuable tools for discerning their roles in the nervous system and investigating the pathogenic mechanisms of nervous system spectrinopathies (Fig. 3; see Supplementary Table S1).

Sptan1 mouse models

Although global loss of αII-spectrin causes early lethality around mouse embryonic day 12.5–16.5 and craniofacial, neural tube and cardiac anomalies, haploinsufficiency does not affect the lifespan or lead to obvious abnormalities[92]. Mice with conditional loss of αII-spectrin in all neural progenitors (Nestin-Cre; *Sptan1*$^{f/f}$) survive for the first month of life, providing a powerful tool to define the in vivo roles of αII-spectrin during early CNS development[52]. Juvenile Nestin-Cre; *Sptan1*$^{f/f}$ mice exhibit generalized seizures with nearly continuous limb movements and abnormal EEG discharges. These epileptic presentations are in line with cellular and anatomical brain abnormalities that can alter neuronal excitability, including fewer and fragmented AISs in the cerebral cortex, disrupted cortical lamination (probably owing to arrested neuronal migration) and reduced complexity of dendritic arborization in the mouse cortex and in hippocampal neuron cultures[52]. Loss of αII-spectrin also resulted in fewer Purkinje neurons, which show fragmented and shorter AISs, disrupted formation of Pinceaux terminals from basket cells, thinning of their dendrites and altered organization of axonal projections. Brains from Nestin-Cre; *Sptan1*$^{f/f}$ mice exhibited increased immunostaining for β-amyloid precursor protein in Purkinje neurons, the thalamus and the cortex, suggesting widespread neuronal degeneration[52]. CRISPR-mediated deletion of *Sptan1* in embryonic rat forebrain via in utero electroporation disrupted neuronal polarity, AIS organization and dendritic development[93]. Loss of αII-spectrin also impaired callosal axon growth and guidance, and resulted in corpus callosum dysgenesis, defective GABAergic innervation and reduced frequency of miniature inhibitory postsynaptic currents in cortical pyramidal neurons, all consistent with hyperexcitability and epileptic presentations.

αII-Spectrin also promotes the organization of excitable axonal domains in PSNs. Young adult Advillin-Cre; *Sptan1*$^{f/f}$ mice with selective αII-spectrin loss in PSNs exhibit severe ataxia, motor impairments and reduced nerve conduction velocity associated with preferential degeneration of myelinated proprioceptor and mechanoreceptor axons[68]. Affected sensory axons show a reduced number of NoR, disrupted paranodal junctions and mislocalized $K_V1.2$ channels, suggesting mechanical weakening of the axons at NoR[68]. Degeneration of peripheral axons is consistent with motor neuropathy phenotypes reported for some affected individuals carrying disease-linked *SPTAN1* variants (described below)[94–96].

Models of αII-spectrin deficits in selective neurons have provided insights into its roles in nervous system function. However, the in vivo characterization of morphological and behavioural phenotypes associated with pathogenic *SPTAN1* variants predicted to operate via dominant or dominant-negative mechanisms has lagged. The identification of a mouse line carrying the spontaneous p.R1098Q mutation[97] allows us to study how heterozygous expression of the p.R1098C or p.R1098S variants (Fig. 4a), in the same residue, causes combined ataxia, spasticity, ID and seizures in humans[98]. Initial evaluations show that heterozygous p.R1098Q mice develop progressive ataxia with tremors, cerebellar degeneration and extensive neuronal loss in the neocortex and hippocampus[97]. Substitutions at p.R1098 are predicted to intrinsically enhance proteolysis of αII-spectrin by calpain,

which may destabilize the submembrane spectrin cytoskeleton, disrupt axon and dendritic development and integrity, and cause global neurodegeneration[97].

Sptbn mouse models

Conditional loss of βI-spectrin in all neural progenitors (Nestin-Cre; *Sptbn*$^{f/f}$) does not lead to any discernible alterations in the structural organization of the AIS of cortical neurons or in motor performance[58]. Similarly, mice selectively lacking βI-spectrin in PSNs (Advillin-Cre; *Sptbn*$^{f/f}$) show normal proprioceptive responses, nerve conduction velocity and nodal Na_V channel clustering[64]. Thus, although βI-spectrin is highly expressed in multiple neuronal types, it does not seem to be essential for central or PSN function, probably owing to compensation by other neuronal β-spectrins.

Sptbn1 mouse models

Whole-body loss of the embryonic liver fodrin (ELF) isoform of βII-spectrin, which lacks the PH domain, results in mid-gestational embryonic lethality with severe growth delay and aberrant development of the heart, gut, liver and brain[99]. Mice with complete loss of βII-spectrin in all neural progenitors (Nestin-Cre; *Sptbn1*$^{f/f}$) exhibit early lethality at around 5 weeks, accompanied by global DD, overt hyperactivity and motor deficits, tremors and seizures[41,42]. Brain βII-spectrin haploinsufficiency also leads to global DD and social interaction deficits in young adult mice[42]. DD was also observed upon selective loss of βII-spectrin in cortical projection neurons (Nex-Cre; *Sptbn1*$^{f/f}$), suggesting neuronal-autonomous mechanisms[42]. Selective loss of βII-spectrin in auditory hair cells (Atoh1-Cre; *Sptbn1*$^{f/f}$) causes deafness, probably owing to its critical roles in organizing the actin cytoskeleton around the base of hair cell stereocilia rootlets[100]. Behavioural effects of brain βII-spectrin deficiency in mice are consistent with autism spectrum disorder (ASD), attention deficit and hyperactivity disorder (ADHD), and learning, motor and hearing deficits reported in carriers of *SPTBN1* variants (discussed below)[42,101].

βII-Spectrin is required for the establishment of long-range cerebellar axons and of axonal tracts connecting the cerebral hemispheres in the mouse brain, and for the proper formation of neocortical layers that give rise to callosal-projecting neurons[41,42]. That βII-spectrin regulates brain cytoarchitecture is demonstrated by the presence of corpus callosum hypoplasia in humans with *SPTBN1* variants and in mice with complete loss or haploinsufficiency of βII-spectrin in the entire brain or only in cortical projection neurons[41,42]. βII-Spectrin also has critical roles in myelinated PNS axons. At paranodes, βII-spectrin provides a barrier that promotes the polarized assembly of macromolecular complexes[67]. Mice lacking βII-spectrin in PSNs (Advillin-Cre; *Sptbn1*$^{f/f}$) exhibit impairments in motor coordination and proprioceptive responses[67].

Sptbn2 mouse models

Studies in whole body βIII-spectrin hypomorph mice have demonstrated its role in stabilizing surface excitatory amino acid transporter 4 (EAAT4) in dendrites of Purkinje neurons[102–104]. Loss of βIII-spectrin also reduces dendritic levels of the glutamate transporter GLAST and ankyrin-R, and sodium currents in Purkinje neurons[103,104]. In addition, expression of the p.E532_M544del βIII-spectrin variant associated with

spinocerebellar ataxia type 5 (SCA5) impairs localization of mGluR1α in Purkinje neuron dendritic spines and long-term potentiation in knock-in mice[74] (Fig. 3). It is likely that combined deficits in cerebellar glutamatergic transmission and in the firing of Purkinje neurons in βIII-spectrin-deficient mice may intrinsically diminish Purkinje neuron output and cause their degeneration. Beyond progressive deficits in motor function, βIII-spectrin-deficient mice exhibit myoclonic seizures, underperform in a subset of cognitive and memory tasks, and show thinning of dendrites of prefrontal cortex neurons[72,104,105]. These behavioural phenotypes correlate with cognitive deficits of probands homozygous for the recessive human C627* variant, which is predicted to eliminate most βIII-spectrin expression[105]. Interestingly, neither βIII-spectrin haploinsufficient mice nor individuals hemizygous for the C627* variant show obvious cerebellar or cortical dysfunction[72,105,106], which suggests a total loss-of-function mechanism. By contrast, multiple individuals heterozygous for de novo missense *SPTBN2* variants develop severe childhood-onset cerebellar ataxia and ID[107–113]. These findings point to strong gain-of-function or dominant-negative effects, which may not be recapitulated by loss-of-function models and warrants further investigation.

Sptbn4 mouse models

Autosomal recessive variants in *Sptbn4* are associated with auditory and motor neuropathies, progressive ataxia with hindlimb paralysis and tremors in six lines of homozygous ‘quivering’ (*Sptbn4*$^{qv/qv}$) mice[114] (Fig. 3). These *Sptbn4* variants result in different truncations of βIV-spectrin and in its reduced expression, which correlates with the severity of the neurological phenotypes. For example, *Sptbn4*$^{qv3J/qv3J}$ mice, which carry a frameshift mutation at G2210 that is predicted to eliminate the PH domain and add a novel 49 amino acid extension in the C-terminal region, have disrupted NoR in the CNS but mostly normal PNS nodes[114–116]. By contrast, the nonsense Q1359* mutation in SR10 found in *Sptbn4*$^{qv4J/qv4J}$ mice[114] impairs the localization of nodal, paranodal and juxtaparanodal proteins at NoR in the PNS[115,116]. The more penetrant effect of the *Sptbn4*$^{qv4J/qv4J}$ mutation may be explained by the loss of the ankyrin-binding region of βIV-spectrin-ΣI and an extensively truncated βIV-spectrin-ΣVI isoform, combined with their reduced expressions[114,116]. Global loss of the βIV-spectrin-ΣI isoform recapitulates the quivering mice phenotypes[18,51], which underscores its roles in the organization and function of the AIS and NoR in vivo. Interestingly, a gene trap insertion mouse model that lacks full-length βIV-spectrin-ΣVI and βIV-spectrin-ΣI but might express N-terminal truncation fragments of βIV-spectrin-ΣI shows the hallmarks of the quivering mice phenotype, including a more severe progression than the isoform-specific knockout mice[54].

Functional evidence from mouse models suggests that pathogenic *SPTBN4* variants in the CNS operate principally through destabilization of the AIS followed by progressive neuronal dysfunction and degeneration. Conditional knockout of βIV-spectrin in the brain (Nestin-Cre; *Sptbn4*$^{f/f}$) causes tremors and poor motor performance, collateral to reduced expression of ankyrin-G and Na_V channels in the AIS of cortical neurons[58]. By contrast, Na_V clustering at CNS NoR is not affected because of compensation by βI-spectrin[64]. βIV-spectrin is also dispensable for normal clustering of Na_V channels at NoR in the PNS (Advillin-Cre; *Sptbn4*$^{f/f}$) through the functional compensation of the partners βI-spectrin and

ankyrin-R[59,64]. Interestingly, a nerve biopsy from a proband with compound heterozygous p.R504Q/p.R2435C βIV-spectrin variants showed normal NoR morphology and Na_V channel clustering, but weaker KCNQ2 immunoreactivity, an observation replicated in myelinated axons of *Sptbn4*$^{qv3J/qv3J}$ and ankyrin-G conditional knockout mice[115,117,118]. It is possible that the redundant ankyrin-R–βI-spectrin complex, which is important for the localization and maintenance of K_V3.1b at NoR[118], does not rescue KCNQ2. KCNQ2 deficiency may contribute to sensory nerve dysfunction and peripheral neuropathy.

Sptbn5 mouse models

The contribution of βV-spectrin to cochlear outer hair cell (OHC) function and auditory responses was recently investigated using a global knockout mouse model of *Sptbn5* (*Sptbn5*$^{-/-}$) generated by a targeted exon deletion[119]. These mice exhibit normal OHC electromechanical activity and auditory thresholds, probably owing to the undetectable expression of *Sptbn5* transcripts in OHCs. By contrast, *Sptbn5* transcripts are present in spiral ganglion neurons and βV-spectrin loss is associated with decreases in the number of afferent and efferent nerve fibres and in auditory brainstem response wave 1 amplitudes[119]. These data suggest that βV-spectrin promotes the maintenance and function of nerves in the peripheral auditory system but has no critical role in OHCs.

Spectrinopathies of the nervous system

Although pathogenic variants in spectrins are categorized as rare, their identification in individuals with neurological disorders is rapidly growing. Here we describe the spectrinopathies of the nervous system (Table 1) and summarize current knowledge regarding their clinical presentation (Fig. 5) and pathogenic mechanisms.

SPTAN1 spectrinopathy: epilepsy, ID and motor deficits

More than 40 pathogenic de novo and inherited *SPTAN1* variants have been linked to a wide range of neurological presentations[94–98,120–137] (Table 1 and Figs. 4a and 5; see Supplementary Table S2). The first association of αII-spectrin with human neurological diseases was the identification of de novo heterozygous in-frame deletion (p.E2207del) and in-frame duplication (p.R2308_M2309dup) variants in SR20 in two patients with West syndrome[120]. In addition to early-infantile epileptic encephalopathy (EIEE) with frequent severe seizures, these individuals presented with abnormal cortical and white matter development, corpus callosum thinning, hypomyelination, cerebellar atrophy, severe DD, ID and spastic quadriplegia[120]. Other studies have implicated de novo in-frame duplications and deletions in *SPTAN1* in EIEE and epilepsy[120–123,125,127,130,131,136]. More recently, heterozygous nonsense *SPTAN1* variants have been linked to hereditary motor neuropathy[94,95], a de novo heterozygous nonsense variant to sensorimotor peripheral neuropathy with DD[134], homozygous and biallelic missense variants to hereditary spastic paraplegia[133,134], and both de novo and dominantly inherited missense variants to cerebellar ataxia with ID, often accompanied by spasticity and seizures[98,136].

The corresponding amino acid position affected by pathogenic *SPTAN1* variants does not predict the presence or severity of specific neurological presentations. However, the

emerging picture points to a segregation of EIEE and West syndrome diagnosis with a cluster of variants in the spectrin dimerization domain in SR20 (Fig. 4a). The variability in variant type, disease onset and phenotypic spectrum suggests that *SPTAN1* variants operate through different mechanisms. For instance, aggregates of αII-spectrin have been detected in cortical mouse neurons expressing p.E2207del and p.R2308_M2309dup mutants[120], and in patient-derived induced pluripotent stem cell-derived glutamatergic neurons expressing a p.D2303_L2305dup variant[93], all of which are EIEE-linked variants, which suggests gain-of-function or dominant-negative effects. By contrast, αII-spectrin haploinsufficiency probably contributes to hereditary motor neuropathy presentations, given that hereditary motor neuropathy-linked nonsense *SPTAN1* variants cause degradation of the mutant transcript by nonsense-mediated decay[94]. *SPTAN1* variants associated with cerebellar ataxia and mild ID (p.K2083del), hereditary spastic paraplegia (p.R19W) and combined ataxia, spasticity, ID and seizure phenotypes (p.R1624C, p.R1098C and p.Q2205P) possibly destabilize SR folding through the loss of electrostatic interactions and hydrogen bonds[98]. Interestingly, the spontaneous p.R1098Q mutation in mouse αII-spectrin leads to progressive ataxia with tremors and seizures in mice[97]. Substitutions at p.R1098, near the start of SR10, which contains the calpain cleavage and CaM-binding sites, are predicted to alter αII-spectrin's CaM affinity and sensitivity to calpain proteolysis[97]. Overall, disease-linked *SPTAN1* variants impair the organization and maintenance of the neuronal actin–spectrin cytoskeleton, which has functional consequences in axonal development and connectivity, the formation and function of excitable axonal and synaptic domains, and neuronal excitability.

SPTBN1 spectrinopathy: DD, ID, epilepsy, ADHD and ASD

SPTBN1 variants cause an autosomal-dominant syndrome of early onset characterized by global developmental language and motor delays[42,101] (Table 1 and Figs. 4b and 5; see Supplementary Table S2). Affected individuals also co-present with mild to severe ID, seizures, movement abnormalities, hypertonia and hypotonia, and hearing impairments. Behavioural diagnoses include ASD, ADHD, sleep disturbances, anxiety, emotional liability and aggressive or self-injurious behaviours. Notably, ASD and ADHD are concurrent in a subset of probands, which supports the identification of *SPTBN1* as a top risk gene among genes with rare truncating variants that co-occur in these disorders[138]. Thirty-four unique heterozygous variants in *SPTBN1* have been identified in 33 affected individuals from 32 families, including 1 pair of siblings and 1 proband with 2 variants in *cis*[42,101] (Fig. 4b). Of those, 24 variants are missense, 4 are nonsense and 3 are canonical splice site variants, with 2 variants predicted to lead to in-frame deletions and 1 to a frameshift that introduces a premature stop codon. Three additional variants are predicted to cause protein frameshifts and loss of function[42,101]. Parental studies suggest that most individuals in these studies carry de novo variants. However, two maternal half-siblings inherited the p.R1003W variant from their unaffected mother, who shows low levels of mosaicism[42]. In addition, the carrier of the c.763+1G>A splice variant inherited it from his mother, who exhibits learning disabilities but not ID[101].

Approximately half of the variants cluster in the CH domains, which show a higher degree of missense variant constraint in the population than the rest of the protein (ExAC v.10)[139],

indicating its critical function. CH domain variants show deleterious effects on βII-spectrin's interactions with F-actin and αII-spectrin, and on cytoskeleton dynamics and neuronal morphology. Interestingly, variants within the proximal region of the second CH domain induce destabilizing structural effects on βII-spectrin that reduce its solubility and cause its aberrant accumulation within cytosolic aggregates together with F-actin and αII-spectrin[42]. Other variants affect βII-spectrin's interactions with ankyrins or membrane lipids because they result in truncated polypeptides that lack the ankyrin-binding motif and/or the PH domain.

The pathogenicity of clinically relevant *SPTBN1* variants is also supported by structure–function studies in which they failed to rescue deficits in axonal length, axonal organelle transport, AIS organization and dendritic development of cortical neurons lacking mouse βII-spectrin[42]. These results demonstrate the functional conservation between human and mouse βII-spectrin and highlight their multifaceted roles. Expression and functional studies in mouse neurons lacking βII-spectrin and patient-derived induced pluripotent stem cells suggest that loss-of-function mechanisms contribute to neuronal dysfunction. However, changes in the binding affinity for F-actin, in neuronal morphology and in the formation of cytosolic aggregates, which are not observed upon reduction in βII-spectrin levels, indicate that CH domain variants are likely to contribute to neurological deficits through dominant or dominant-negative effects that affect the submembrane cytoskeleton.

SPTBN2 spectrinopathy: spinocerebellar ataxia with and without ID

Inherited autosomal dominant variants in *SPTBN2* cause late-onset SCA5 (refs. [75,140–142]) (Table 1 and Figs. 4c and 5; see Supplementary Table S2). In addition, de novo and autosomal recessive *SPTBN2* variants have been associated with early childhood ataxia, which often co-segregates with ID and DD[104,106,108–113,143–152] (Fig. 4c; see Supplementary Table S2). SCA5, the first brain spectrinopathy reported, was described in three unrelated families in the United States, France and Germany[153–155]. Symptoms appear anytime between early childhood and the seventh decade of life, but most frequently during adulthood. MRI and autopsy evaluations from these families show cerebellar cortical atrophy without other brain alterations, consistent with a pure form of cerebellar degeneration. Affected members of the American and French families are hemizygous for a 13 amino acid (p.E532_M544del) and a 5 amino acid (L629_R634delinsW) in-frame-insertion deletion in SR3, respectively. The German family carries the p.L253P variant in the second CH domain[75]. In addition, the SR2 p.T472M variant co-segregates with late-onset pure cerebellar ataxia in members of a family of Norwegian descent[140]. Interestingly, de novo variants that affect the same residues have been identified in unrelated patients with early childhood ataxia, DD and cognitive deficits, strongly supporting their pathogenic effects[107–111,143–145]. The growing number of cases also indicates that the association of *SPTBN2* variants with ataxia and various neurological presentations is more common than previously appreciated[156].

Evaluation of post-mortem samples from patients with SCA5 points to significant degeneration of the cerebellum with Purkinje neuron loss, thinning of the molecular layer and changes in the distribution of the EAAT4 and the glutamate receptor GluRδ2, probably

owing to their reduced stability at the surfaces of Purkinje neuron dendrites[75]. Changes in glutamate signalling secondary to deficits in βIII-spectrin levels and/or function could contribute to Purkinje neuron death and to motor and cognitive deficits. The p.L253P SCA5 variant significantly increases binding affinity for F-actin, suggesting that altered modulation of F-actin dynamics could also contribute to disease pathology[157].

SPTBN4 spectrinopathy: congenital hypotonia, neuropathy and deafness, with and without ID

Sixteen *SPTBN4* variants have been linked to a neurodevelopmental disorder with congenital hypotonia, neuropathy and deafness (NEDHND)[115,158–163] (Table 1 and Figs. 4d and 5; see Supplementary Table S2). These include 11 nonsense, 4 missense and 1 canonical splice site variants as well as 1 multi-exon deletion (Fig. 4d). Most affected individuals carry recessive homozygous *SPTBN4* variants; three have compound heterozygous variants. This suggests that variants in both *SPTBN4* alleles are necessary to induce pathogenicity. Common presentations among probands include lack of head control and ability to sit, stand and walk, congenital muscular hypotonia and axonal neuropathy, often accompanied by severe DD and ID. More than 50% of probands also experience seizures or have abnormal EEG recordings[115,158–163].

The first association of *SPTBN4* variants with NEDHND was the identification of a homozygous p.Q533* variant in a 10-year-old boy born to healthy heterozygous carrier parents[158]. A muscle biopsy revealed an absence of sarcolemma βIV-spectrin and the presence of demyelinating axonal motor neuropathy, probably owing to loss of sodium channels at neuromuscular junction sites. Six additional individuals with homozygous or compound heterozygous *SPTBN4* variants were reported to present with NEDHND, central vision impairment and ID[115]. Functional characterization of this subset of *SPTBN4* variants in rat hippocampal neurons indicated changes in the organization of the AIS, which are likely to alter neuronal excitability. In addition, a homozygous canonical splice site variant predicted to generate an in-frame βIV-spectrin polypeptide missing the protein sequence encoded by exon 19 was found in a sibling pair also born to heterozygous carrier parents[159]. Although these siblings shared severe hypotonia and axonal neuropathy, they did not exhibit ID or deafness, suggesting that the resulting βIV-spectrin, which is predicted to lack 49 amino acids at the beginning of SR7, is partially functional. The clinical spectrum associated with *SPTBN4* variants was recently expanded with the identification of five individuals carrying biallelic variants, who in addition to the common neurological presentations also developed horizontal nystagmus, abnormal EEG without seizures and choreoathetosis[160]. This broad nervous system phenotype underscores the critical functions of βIV-spectrin in the organization and maintenance of key neuronal domains in both the CNS and the PNS. Interestingly, the absence of symptoms in heterozygous carriers of pathogenic *SPTBN4* variants is consistent with the apparent lack of neurological phenotypes in mice with partial loss of βIV-spectrin[54]. It is possible that these partial deficiencies in βIV-spectrin are rescued by βI-spectrin[58,64].

Overlapping and diverging pathogenic mechanisms in spectrinopathies

Spectrinopathies of the nervous system are largely syndromic and display a wide range of clinical presentations within and across the affected spectrin genes (Table 1 and Fig. 5; see Supplementary Table S2). Underlying this variety is the intrinsic multifunctionality of spectrins, together with their cell-specific expression in the nervous system and their domain-specific localization in neurons (Fig. 1). Consequently, the penetrance and degree of pathogenicity of a spectrin variant is likely to be determined by the extent to which it affects some, or all, spectrin functions and its resulting effects at the cellular and circuit levels. Within this complexity, certain overlapping clinical features of brain spectrinopathies indicate similarities in the underlying molecular mechanisms and/or converging pathways (Fig. 5). For example, a large group of individuals affected by any of the four nervous system spectrinopathies present with DD, ID, seizures or movement abnormalities.

A convergent pathogenic mechanism may operate through αII-spectrin deficiency, either directly caused by αII-spectrin variants or through effects of β-spectrin variants on αII-spectrin function, or in the stability of the corresponding spectrin tetramers. For example, *SPTBN1* variants that cause βII-spectrin aggregation in cortical neurons sequester endogenous αII-spectrin within the aggregates[42]. Co-aggregation of αII-spectrin and βII-spectrin is also observed in patient-derived glutamatergic neurons expressing clinically relevant *SPTAN1* variants[93]. αII/βII-Spectrin aggregates interfere with normal protein function and may be inherently toxic to neurons. These aggregates also sequester F-actin and further disrupt cytoskeleton organization and dynamics, which underlie observed aberrant neuronal morphologies[42].

Several disease-linked βII-spectrin variants cluster in the CH domains and dysregulate binding to F-actin[42]. Molecular modelling suggests that their range of effects on F-actin binding affinity is probably due to both local and CH domain-wide conformational changes caused by modified intramolecular interactions that affect contacts at the βII-spectrin–F-actin interface. Modelling also predicts structural instability due to substantial charge changes and steric hindrance introduced by amino acid substitution in the CH domain, which probably underlies protein aggregation in cells[42]. Interestingly, the site of the pathogenic p.L250R βII-spectrin variant is conserved in βIII-spectrin (p.L253), and the p.L253P βIII-spectrin change causes SCA5 (refs. [42,75]). The L250/L253 site modulates βII-spectrin/βIII-spectrin affinity for F-actin[42,164], suggesting that the deficits in this function as a shared pathogenic mechanism. However, given that very few human variants have been reported in the CH domains of βIV-spectrin and that their effects on F-actin binding are unknown, it is not clear whether disruption of β-spectrin/F-actin is a universal pathogenic mechanism shared by all β-spectrinopathies.

Macro-scale disruption of the spectrin–actin cytoskeleton resulting from spectrin deficits disturbs the organization of functional neuronal macrodomains. Deficits in αII-spectrin, βII-spectrin and βIV-spectrin are associated with AIS loss, fragmentation or reduction in key AIS components such as ion channels[42,48,52,93,115] (Fig. 3a). These structural and macromolecular changes alter neuronal polarity, AP initiation and kinetics, and ion channel function, which may contribute to seizures and epileptic phenotypes in multiple patients

across these syndromes. Similarly, the roles of αII-spectrin, βII-spectrin and βIV-spectrin in organizing macromolecular complexes at NoR of PSN axons (Fig. 3a) likely underlie hypotonia and hypertonia, and neuropathy presentations associated with their selective disruptions[42,94–96,98,120,133,134,158–160].

Although failure to properly traffic or stabilize neuronal membrane proteins appears to be a shared mechanism of spectrinopathies, the individual spectrin partners, cell type expression and neuronal domain localization likely confer specificity (Fig. 1). For example, βIII-spectrin interacts with EAAT4 in the soma and dendrites of Purkinje neurons and with mGluR1α in dendritic spines[74,75,102]. Loss of βIII-spectrin or SCA5-linked variants cause deficient mGluR1-mediated long-term potentiation, Purkinje cell degeneration and cerebellar dysfunction through combined and cumulative effects of glutamate excitotoxicity and disrupted synaptic function[74] (Fig. 3a). Loss of βII-spectrin, or its pathogenic variants, affects axonal organelle transport, which probably contributes to altered protein distribution, diminished axonal growth in vitro and in vivo, and impairments in brain-wide axonal connectivity observed in probands and mouse models[41,42] (Fig. 3).

Deficits associated with spectrin dysfunction might also result from alterations in their binding partners, ankyrins, which are regulators of neuronal transport, membrane organization and long-range axonal connectivity[1–3,71,165]. For example, pathogenic *SPTBN4* variants impair βIV-spectrin binding to ankyrin-G and its clustering at the AIS[159]. Likewise, multiple βII-spectrin variants associated with the *SPTBN1* syndrome truncate the polypeptide prior to the ankyrin-binding domain and impair its binding to ankyrin-B[42]. In addition, SCA5-linked *SPTBN2* variants reduce ankyrin-R localization in Purkinje neuron dendrites, which is required for regulating Na_V levels and intrinsic excitability[103]. Dendritic underdevelopment has also been commonly observed across mouse and cellular models of either neuronal spectrin deficiency or *SPTAN1*, *SPTBN1* and *SPTBN2* spectrinopathies[42,72,74,97,104] (Fig. 3a). These effects suggest an additional pathogenic mechanism that can affect synaptic function; however, the functional roles of spectrin in dendrites and postsynaptic domains have not been extensively studied.

Concluding remarks and future directions

Pathogenic variants in four of the six spectrin genes expressed in the nervous system have been genetically and functionally associated with complex neurological syndromes. Although conditional knockout models of βI-spectrin (*SPTB*) did not show apparent neuronal or behavioural phenotypes[58,64], whether variants in this gene result in neurological deficits remains to be determined. A recent report linked de novo βV-spectrin (*SPTBN5*) variants to ID, aggressive behaviours and variable presentations including facial dysmorphisms and autistic behaviours in four unrelated individuals[166]. However, this study only evaluated the functional impact of putative pathogenic variants using in silico prediction tools. Thus, whether these variants are indeed pathogenic and the extent to which they affect βV-spectrin expression, localization or specialized functions in neurons remain to be established. Future studies that focus on discerning the neuronal types and domains in which βV-spectrin is expressed in the nervous systems, its functional roles and the impact

of putative disease variants using human samples and mouse models will shed light on the likelihood of pathogenicity of these and other *SPTBN5* variants.

Various reports have also linked non-genetic spectrin deficits to neurological dysfunction. For instance, βIV-spectrin autoantibodies have been selectively detected in three unrelated individuals with paraneoplastic neuropathy, suggesting that they can either contribute to the development of neuropathic symptoms or serve as a biomarker[167,168]. In addition, βIV-spectrin levels are silenced through DNA methylation in the cerebral cortex of patients with Alzheimer disease, suggesting that impaired AIS and/or NoR function contributes to disease pathology[169]. The importance of maintaining proper βIV-spectrin levels during brain development is further demonstrated by significant associations between DNA methylation at the *SPTBN4* locus and severe delays in language and motor skills[170]. Spectrin levels have also been associated with neurodegeneration. As an example, increased levels of αII-spectrin and βII-spectrin breakdown products, generated by aberrant activation of calpain-dependent proteolysis, correlate with amyloid-β deposits and neurofibrillary tangles in the brains of individuals with Alzheimer disease[171,172]. αII-Spectrin and βII-spectrin are also enriched in Lewy bodies in the brains of individuals with Parkinson disease[173,174]. Interestingly, spectrin preferentially binds α-synuclein phosphorylated at Ser[129], which promotes aggregation and neurotoxicity. This enhanced interaction might reorganize the actin cytoskeleton and contribute to mitochondrial dysfunction in Parkinson disease[175].

Although our understanding of the neuronal roles of spectrins continues to advance, their biology in other cell types in the nervous system is largely understudied, and whether or how deficiency of spectrins in these cells may contribute to spectrinopathies is largely unknown. One study in mice lacking βII-spectrin in myelinating glial cells in both the CNS and the PNS showed that loss of the protein affected the formation and maintenance of NoR, and also altered action potential conduction velocities[176]. Given the marked importance of glial cells in neurodevelopmental disorders[177–179], this gap in knowledge deserves attention. We also lack a comprehensive understanding of the cell types and developmental stages that are vulnerable to disruptions in each spectrin. Therefore, cell-specific, spatiotemporal maps of spectrin expression in the brain and in the PNS in humans and in animal models used to study spectrinopathies will refine our knowledge of disease progression and define potential entry points for therapeutic interventions. For example, re-expression of βIV-spectrin in a mouse model that lacks full-length βIV-spectrin-ΣVI and βIV-spectrin-ΣI[54] partially restored PNS NoR organization independently of the intervention time[180]. CNS NoR restoration was slower and less efficient if the rescue started at a later timepoint, highlighting differences in the mechanisms and recovery window between CNS and PNS myelinated axons. Motor performance and axon functional parameters were only partially improved in these mice, probably because only 50% of PNS and 25% of CNS NoR were restored[180].

Lastly, additional knock-in animal models of variants linked to spectrinopathies will expand the toolbox to unambiguously discern pathophysiological mechanisms. However, because animal models often fail to recapitulate pathogenic effects observed in humans, harnessing the unique features of human induced pluripotent stem cells and CRISPR–Cas9 editing technology to establish cell-based 2D and 3D brain organoid models relevant to spectrinopathies is an attractive complementary approach[181]. Combined with ‘omics’ and

other unbiased high-throughput molecular technologies, this full arsenal will open avenues towards the identification of targets through traditional therapeutic discovery, an effort presently lacking for spectrinopathies. To this end, they will help to enable promising technologies that may offer alternative viable paths towards treatment, such as gene replacement and editing, and antisense oligonucleotide-based strategies[182].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Axon initial segment	(AIS). The domain 20-60μm long at the proximal axon–soma interface that has a high density of voltage-gated ion channels and other membrane proteins responsible for the initiation of the action potential.
De novo variants	Changes in the sequence of a gene that are seen for the first time in an individual but are not present in the parents.
Gain-of-function	A missense mutation (altered amino acid sequence) that results in enhanced or abnormal protein function.
Haploinsufficient	A gene for which 50% of normal protein expression is insufficient for normal function and may result in disease.
Juxtaparanode	A region adjacent to each side of a paranode in myelinated axons.
Knock-in mouse	A mouse in which an endogenous gene sequence of interest is altered by a one-for-one substitution with a transgene or by adding gene sequences that are not found within the locus.
Knockout mouse	A mouse in which expression of a gene of interest is inactivated.
Loss-of-function	A mutation that abolishes protein function, often by partial or complete loss of protein expression.
Nodes of Ranvier	(NoR). Ion channel-rich gaps along a myelinated axon that expose the neuronal membrane to the extracellular space and speed up the propagation of the action potential along the axon.

Paranode	A region adjacent to each edge of nodes of Ranvier (NoR) in myelinated axons.
Pinceaux terminals	The terminals of the Pinceau, a paintbrush-like network of cerebellar basket cell axon branchlets embracing the axon initial segment (AIS) of Purkinje neurons.
Postsynaptic density	(PSD). The protein-dense molecular network located beneath the membrane of dendritic spines of excitatory neurons.
Probands	The first individuals in a family who are suspected to be at risk of or affected by a genetic condition.
Somatodendritic	A neuronal region that includes the cell body and dendrites but excludes the axon.
Spectrinopathies	Diseases associated with loss or aberrant function of spectrins.

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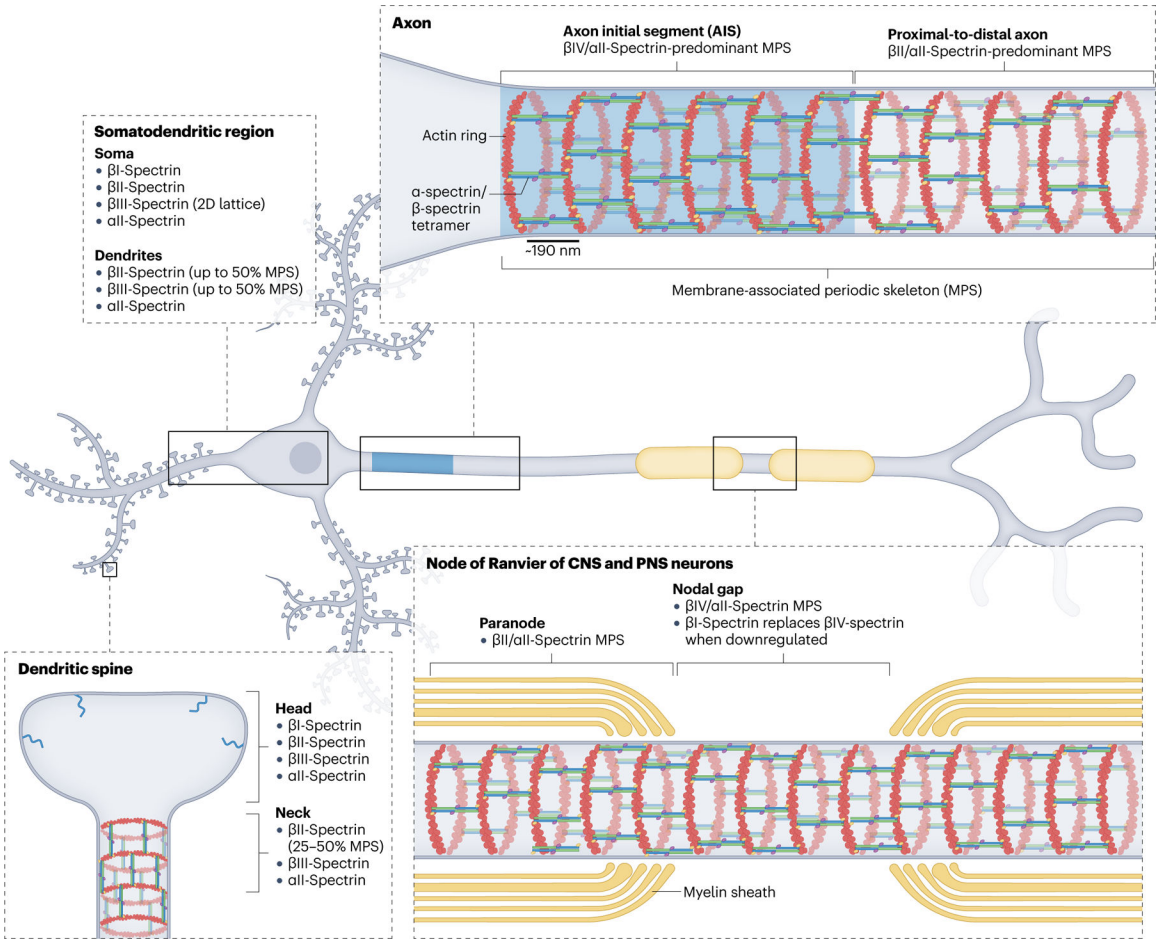

Fig. 1 |. Cellular localization and organization of neuronal spectrins.

Mammalian neurons express six of the seven spectrins, which follow a general pattern of domain localization and organization across different neuron types. βIV-spectrin is enriched at the axon initial segment (AIS), where, together with αII-spectrin, actin and other key molecules, it forms a membrane-associated periodic skeleton (MPS)[34]. The MPS is best characterized by actin rings enwrapping the circumference of neuronal processes with a ~190 nm periodicity, which is determined by their cross-linking by spectrin tetramers in their fully elongated conformation. The proximal-to-distal axon, including axonal branches, expresses βII-spectrin and αII-spectrin in high abundance together with relatively less abundant βIII-spectrin, all integrated into the MPS that spans the full axon. In myelinated axons of both the CNS and the PNS, βIV/αII-spectrins are localized and periodically organized in the nodal gap of nodes of Ranvier (NoR), flanked by βII/αII-spectrins in the paranode, also periodically distributed[60]. Upon loss of βIV-spectrin, βI-spectrin localizes to NoR and rescues βIV-spectrin function. This redundancy is not available at the AIS, probably because βI-spectrin localization depends on its molecular partner ankyrin-R, which is not recruited to the AIS[59]. Unlike in the axon, the probability of detecting the MPS in dendritic shafts of mature neurons, which includes βII-spectrin and βIII-spectrin, is about 50%[33]. In addition to the quasi-1D organization of the MPS, βIII-spectrin can form 2D

polygonal lattices in the soma and dendritic shaft. In dendritic spines, βII-spectrin and βIII-spectrin adopt MPS periodicity in the neck, but not in the head.

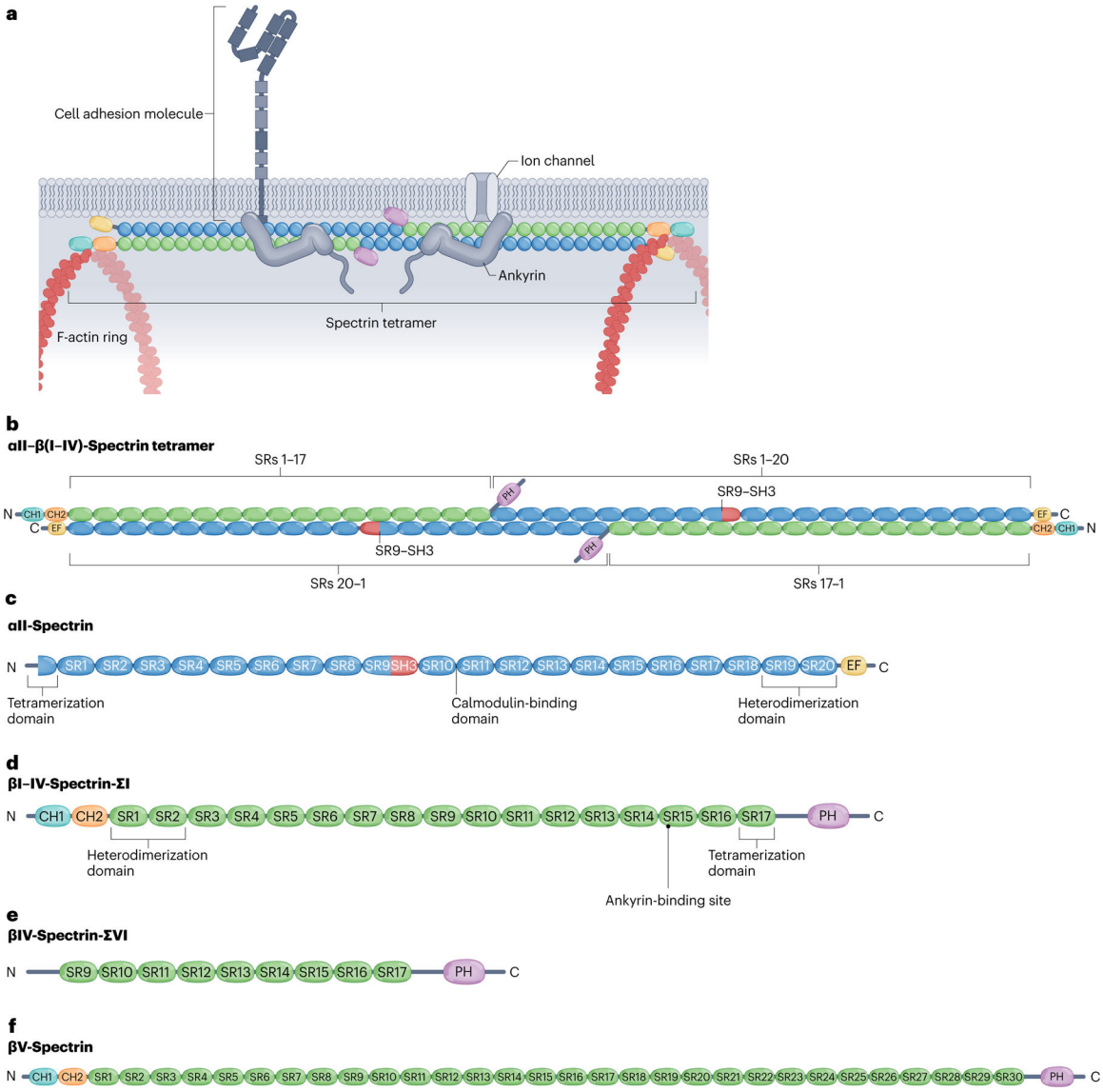

Fig. 2 |. Tetrameric assembly and structural domains of neuronal spectrins.

a, Canonical spectrins form heterotetramers of two α-units and two β-units that crosslink F-actin rings along the neuronal membrane. Spectrins bind ankyrins, which in turn stabilize membrane-spanning proteins such as cell adhesion molecules and ion channels. **b**, Spectrin tetramers assemble by linking heterodimers head-to-head via non-covalent association between the partial spectrin repeats (SRs) in the N terminus adjacent to SR1 in the α-spectrin subunits (blue) and partial SR17 at the N terminus of the β-spectrin subunits (green). Complementary motifs in SR1 and SR2 of βI–IV spectrins and SR19 and SR20 of αII-spectrin bind covalently to enable the antiparallel lateral assembly of α–β-spectrin heterodimers. **c**, αII-Spectrin spans 20 modular SRs (blue), a calcium-binding EF hand domain (yellow) close to the C terminus, an Src-homology 3 (SH3) domain (red) in SR9 and a calmodulin (CaM)-binding loop in SR10. **d**, Canonical βI–βIV-spectrins contain 16 full SRs and a partial 17th SR (green), two N-terminal tandem calponin homology (CH) domains (teal and orange), an ankyrin-binding site in SR15 and a C-terminal pleckstrin

homology (PH) domain (purple). The CH domains enable binding to actin and the PH domain binds membrane lipids. **e**, The alternatively spliced βIV-spectrin-ΣVI isoform, which is important for maintenance of the axon initial segment (AIS), lacks the CH domains and the first eight full SRs, but retains ankyrin-binding activity. **f**, Giant βV-spectrin contains 29 full SRs plus a partial 30th SR. Whether βV-spectrin associates with αII-spectrin is not clear.

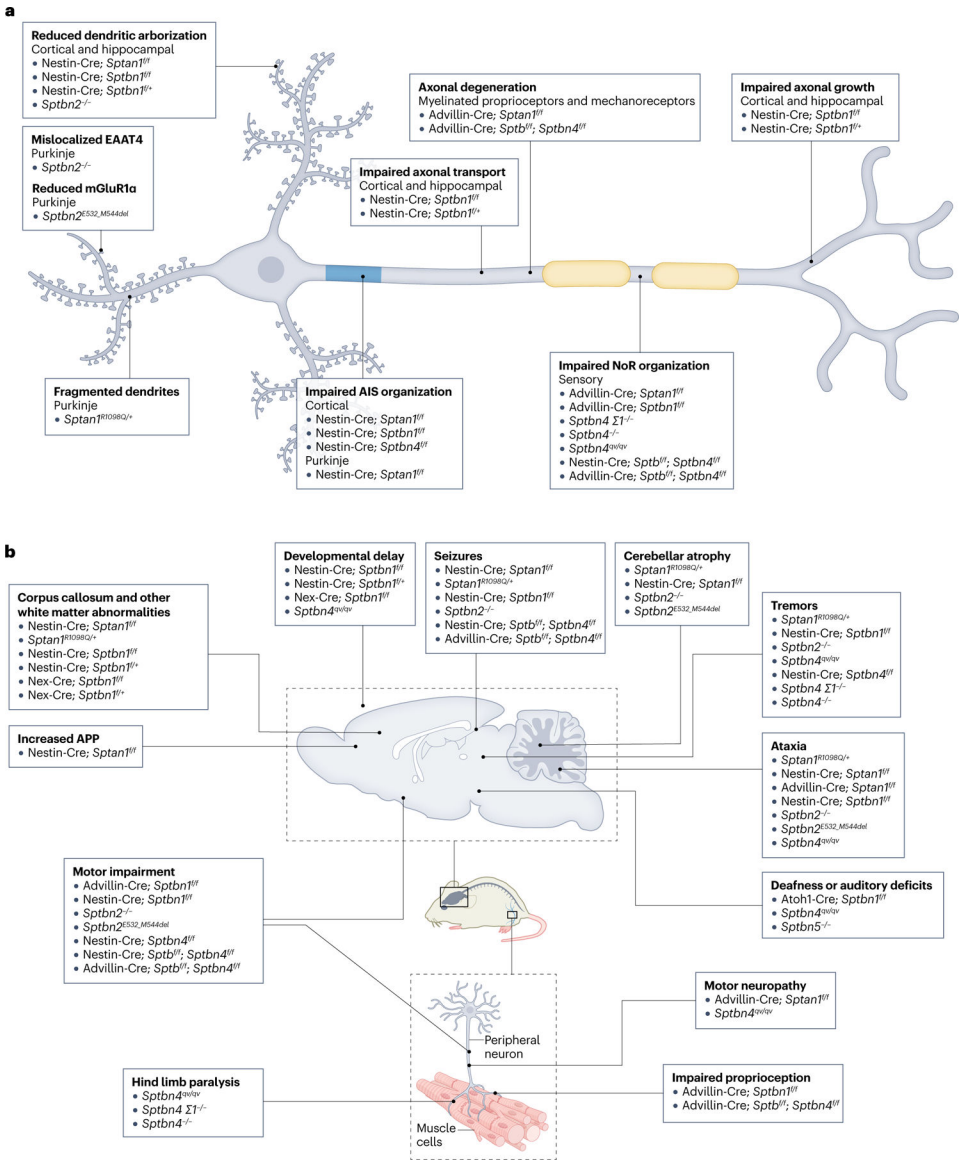

Fig. 3 |. Deficiencies in mouse models of neuronal spectrin dysfunction.

a, Loss, haploinsufficiency and mutations in spectrins in mice induce global, region and functional domain-specific neuronal defects in vivo and in vitro, including reduced dendritic arborization, axonal degeneration, protein mislocalization and reduced axonal transport. Neuron type and mouse model source (see Supplementary Table S1) indicated in parentheses. **b**, Major anatomical and functional phenotypes observed in mouse models of spectrin deficits in the CNS and PNS. Mouse model source (see Supplementary Table S1) indicated in parentheses. AIS, axon initial segment; APP, amyloid precursor protein; EAAT4, excitatory amino acid transporter 4; mGluR1α, metabotropic glutamate receptor type 1α; NoR, nodes of Ranvier.

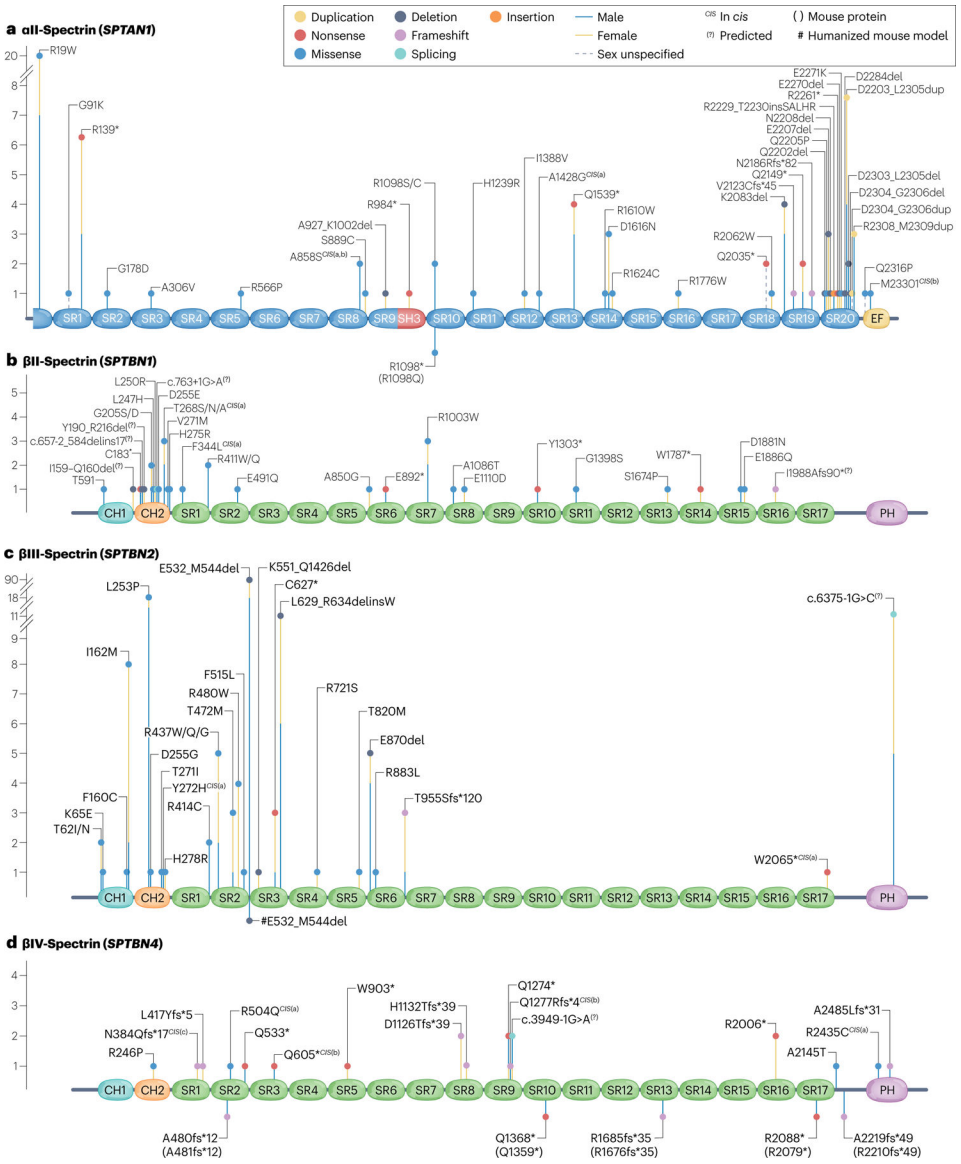

Fig. 4 |. Spectrin variants associated with neurological disorders.

a, Multiple reported αII-spectrin variants have been associated with neurological disorders. Variant types include missense (blue), nonsense (red), duplication (yellow), deletion (dark grey), splicing (teal), insertion (orange) and frameshift (violet). The *cis* superscript indicates compound heterozygous (in *cis*), with the letter in parentheses indicating the corresponding variant pair for a single individual. The sex of the reported individual is indicated by the lines below the dots (male, blue line; female, yellow line; unknown, discontinuous line). Number of individuals of each sex for each variant is indicated by the length of the corresponding line below the oval-shaped dot measured relative to the *y*-axis. Variants are distributed throughout the spectrin repeats (SRs; blue), with a cluster in the heterodimerization region (SRs 19–20). **b**, βII-Spectrin variants associated with a neurodevelopmental syndrome. These variants emerge largely de novo and are spread throughout the SRs (green), with a strong cluster in the second calponin homology (CH2;

orange) domain. **c**, βIII-Spectrin variants associated with ataxia, developmental delay (DD) and intellectual disability (ID). **d**, Reported human βIV-spectrin variants associated with disorders of the CNS and PNS. Only homozygous and compound heterozygous carriers manifest clinical presentations. The carrier of the N384Qfs*17$^{CIS(c)}$ variant also bears a maternally inherited deletion with a breakpoint spanning [chr19.g.(?_41,001,394)_(41,011,375_?)del (GRCh37)], which is predicted to delete exons 6–11 (ref. [160]). Knock-in mouse models are indicated in the lower part of the protein schematic, with the corresponding mutated site in the mouse spectrin homologue shown in parentheses. PH, pleckstrin homology; SH, Src-homology. Part **b** adapted from ref. [42], Springer Nature Limited.

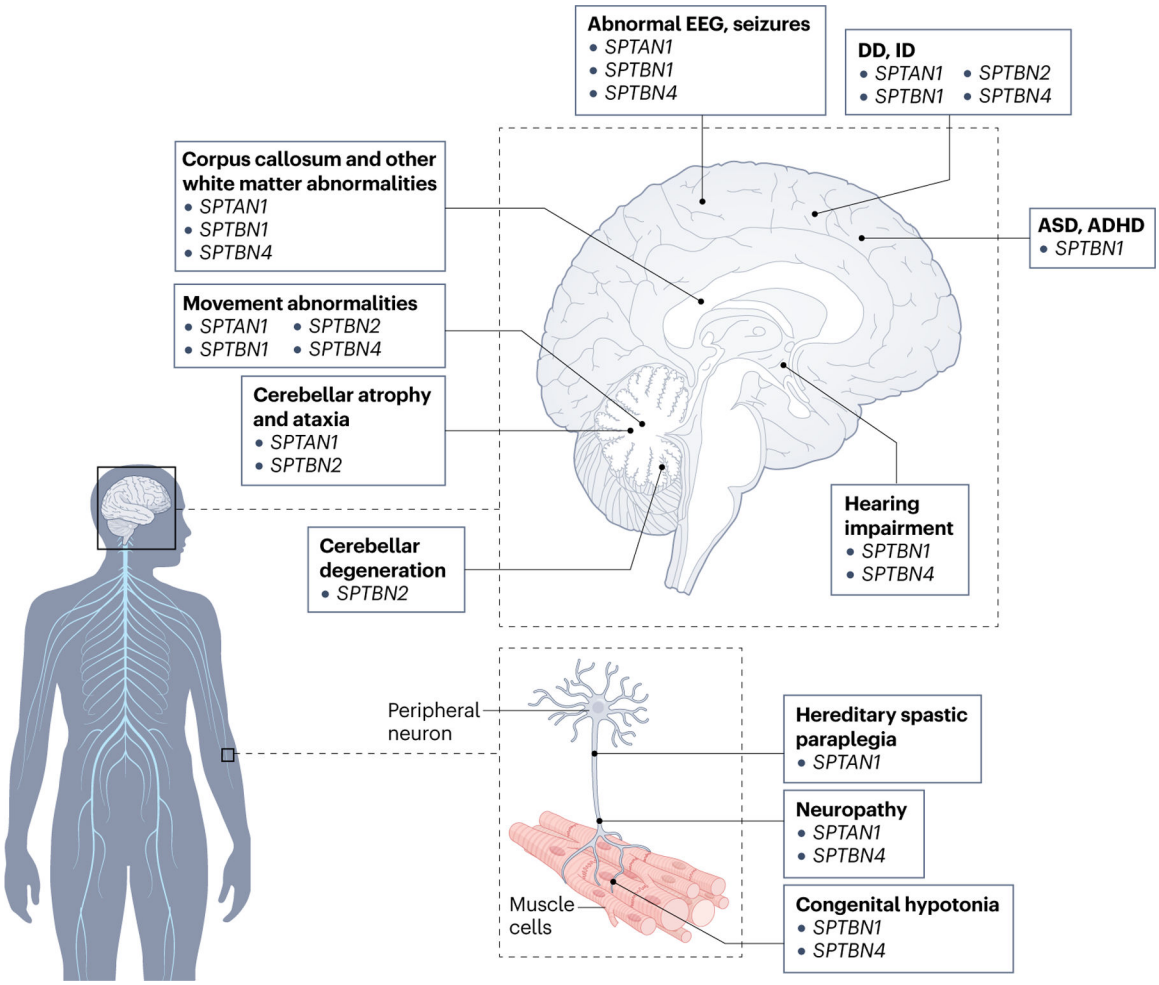

Fig. 5 |. Major phenotypes in humans with spectrinopathies of the nervous system.
Pathogenic variants in spectrins cause complex neurological syndromes in both the brain and periphery that have overlapping pathologies and clinical presentations across spectrin genes. Affected spectrin genes indicated in parentheses. ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; DD, developmental delay; ID, intellectual disability.

Table 1 |

Disorders associated with spectrin gene variants

Gene	Protein	Variant type	Inheritance	Common clinical diagnoses	OMIM entry
SPTAN1	αII-Spectrin	Missense, nonsense, in-frame insertions, in-frame deletions, duplications, frameshifts	De novo, inherited	Cerebellar ataxia, DD, DEE-5, ID, neuropathy, SP	182810
SPTBN1	βII-Spectrin	Missense, nonsense, in-frame deletions, frameshifts, splice site	De novo	ASD, ADHD, DDISBA, dysmorphisms, hypertonia or hypotonia, hearing impairments, ID, S	182790
SPTBN2	βIII-Spectrin	Missense, nonsense, in-frame deletion	De novo, inherited	Autosomal dominant spinocerebellar ataxia 5 (SCA5)	600224
				Spinocerebellar ataxia, autosomal recessive 14 (SCAR14)	615386
				Cerebellar ataxia, DD, ID	_[a]
SPTBN4	βIV-Spectrin	Missense, nonsense, in-frame deletion, frameshifts, canonical splice site	Inherited	Choreoathetosis, DD, ID, NEDHND, nystagmus, S	617519

ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; DD, developmental delay; DDISBA, developmental delay, impaired speech and behavioural abnormalities; DEE, developmental and epileptic encephalopathy; ID, intellectual disability; NEDHND, neurodevelopmental disorder with congenital hypotonia, neuropathy and deafness; S, infantile seizures and epilepsy; SP, spasticity, paraplegia or quadriplegia; OMIM, Online Mendelian Inheritance in Man (https://www.omim.org).

[a]No OMIM record.

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