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De novo and dominantly inherited *SPTAN1* mutations cause Spastic Paraplegia and Cerebellar Ataxia

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Abstract

Background—Pathogenic variants in *SPTAN1* have been linked to a remarkably broad phenotypical spectrum. Clinical presentations include epileptic syndromes, intellectual disability, and hereditary motor neuropathy.

Objectives—We investigated the role of *SPTAN1* variants in rare neurological disorders such as ataxia and spastic paraplegia.

Methods—We screened 10,000 NGS datasets across two international consortia and one local database, indicative of the level of international collaboration currently required to identify genes causative for rare disease. We performed *in silico* modelling of the identified *SPTAN1* variants.

Results—We describe 22 patients from 14 families with five novel *SPTAN1* variants. Of six patients with cerebellar ataxia four carry a *de novo SPTAN1* variant, two showed a sporadic inheritance. In this group one variant (p.Lys2083del) is recurrent in four patients. Two patients have novel *de novo* missense mutations (p.Arg1098Cys, p.Arg1624Cys) associated with cerebellar ataxia, in one patient accompanied by intellectual disability and epilepsy. We furthermore report a recurrent missense mutation (p.Arg19Trp) in 15 patients with spastic paraplegia from seven families with a dominant inheritance pattern in four and a *de novo* origin in one case. One more patient carrying a *de novo* missense mutation (p.Gln2205Pro) has a complex spastic ataxic phenotype. Through protein modelling we show that mutated amino acids are located at crucial interlinking positions, interconnecting the three-helix bundle of a spectrin repeat.

Conclusions—We show that *SPTAN1* is a relevant candidate gene for ataxia and spastic paraplegia. We suggest that for the mutations identified in this study, disruption of the interlinking of spectrin helices could be a key feature of the pathomechanism.

Introduction

The paradigm of "one gene - one phenotype" is increasingly challenged by the discovery of considerable pleiotropy for many genes, leading to overlapping disease spectra across many neurological diseases such as motor neuron and frontotemporal diseases, motor neuropathies, spastic paraplegia, and Charcot-Marie-Tooth disease. The widespread implementation and increasing accessibility of next generation sequencing (NGS) platforms accelerates the identification of new gene-disease-relations in rare disease (RD) patients and constantly broadens our understanding of such overlapping clinical entities.[1–3]

Of recent and particular interest is the wide range of Mendelian gene-disease links established for neurological diseases and mutations in the *SPTAN1* gene. Initially, *SPTAN1* mutations were reported to be linked with severe epileptic syndromes and intellectual disability.[4] In these patients, brainstem and cerebellar atrophy, cerebral hypomyelination and microcephaly are frequently observed. We recently identified three families with nonsense mutations causing a hereditary motor neuropathy (HMN) phenotype.[5] An independent report of a *de novo* nonsense mutation in a HMN patient confirmed the pathogenicity of *SPTAN1* nonsense mutations.[6] Later *SPTAN1* was also implicated in a sensory-motor peripheral neuropathy phenotype accompanied by a developmental disorder.[7] There are also two reports on putatively recessively inherited *SPTAN1* variants in hereditary spastic paraplegia (HSP) patients.[8,9] One patient with severe intellectual disability was reported with cerebellar ataxia, but pathogenicity of the *SPTAN1* intronic variant could not be conclusively established due to a pathological expansion at the *ATXN8* locus. Other reported symptoms include autism and migraine. Aggregation of spectrin complexes was found in cortical neurons differentiated from induced pluripotent stem cells (iPSCs) of a patient with epileptic encephalopathy, however, this aggregate formation cannot be considered as a pathogenic hallmark for all other epilepsy associated *SPTAN1* mutations.[4,10] In contrast, nonsense-mediated mRNA decay haploinsufficiency was proposed in the HMN cohort.[5]

The *SPTAN1* gene encodes for α-II-spectrin, a major component of the cytoskeleton expressed in all cells, except erythrocytes, which express α-I-spectrin.[11] In neurons, α-II-

spectrin forms a complex with one of four β-spectrins (*SPTBN1*, *SPTBN2*, *SPTBN4*, *SPTBN5*) through heterotetramerization of two α- and two β-subunits (Figure 1). While the α-subunit is always α-II-spectrin in neuronal cells, the composition of the complex in terms of its β-spectrin can differ depending on the cell type and even its subcellular localization.[12,13] The canonical function of the spectrin complex is to interlink actin rings, giving rise to the membrane-associated periodic skeleton (MPS). The α-II-spectrin protein counts 20 spectrin repeats, each composed of a three-helix bundle.[14] Additionally the spectrin complex has a scaffolding function ensuring the correct localization of several ankyrins, known to be of critical importance for neuronal development and homeostasis (Figure 1).[14]

The exact genotype-phenotype correlations remain enigmatic in *SPTAN1*-related diseases. At least 35 different mutations in *SPTAN1* scattered across the entire gene have been identified, but why these mutations affect diverse neuronal subtypes at different developmental timepoints remains unexplained (Figure 2). Several β-spectrins have also been implicated in neuronal disease. *SPTBN1* (β-II-spectrin) mutations have been associated with intellectual disability and autism, while *SPTBN2* (β-III-spectrin) mutations cause both dominant and recessive cerebellar ataxia.[15–17] Furthermore, *SPTBN4* (β-IV-spectrin) is associated with a neurodevelopmental disorder with hypotonia, neuropathy and deafness.[18,19] Apart from Mendelian gene-disease links, defects in spectrins are also implicated in degenerative and psychiatric conditions.[20] These 'spectrinopathies' illustrate the vital nature of the spectrin complex in neuronal development and homeostasis.

Given the association of *SPTBN2* with cerebellar ataxia and preliminary reports of *SPTAN1* mutations in two recessive HSP families, as well as the cerebellar atrophy frequently noted in epilepsy patients carrying *SPTAN1* mutations, we set out to delineate the role of *SPTAN1* variants in these rare neurological disorders. Through a screen of more than 10,000 NGS datasets, we describe 22 patients from 14 families with five novel *SPTAN1* variants.

Materials and methods

Patient Cohort

We used 10,000 NGS datasets of RD patients from two large international consortia and one local database. First, 3,500 HSP and ataxia NGS datasets, including 2,500 datasets from the PREPARE ataxia network and a further 1,000 HSP datasets were provided by the Genesis database.[21] Genesis is a NGS data-sharing and analysis platform (https://www.tgp-foundation.org), housing a number of rare disease consortia. Additionally, the Solve-RD EU-Horizon 2020 (http://solve-rd.eu/) network includes more than 12,000 NGS datasets from which we used 3,500 datasets from patients in the European Reference Network for Rare Neurological Disorders (ERN-RND). Diseases covered in the ERN-RND include Cerebellar Ataxias and Hereditary Spastic Paraplegias, Choreatic syndromes, Dystonias, Paroxysmal Movement Disorders, Frontotemporal Dementia and Leukodystrophies. They were analyzed through the RD-Connect Genome-Phenome Analysis Platform (GPAP). Finally, 3,600 datasets were included from patients with different neurological diseases, belonging to a series of 14,500 NGS datasets generated in a diagnostic context at the Institute of Medical Genetics and Applied Genomics (IMGAG, Tübingen). Part of these

datasets (>1,200) have been contributed to the Solve-RD consortium for secondary use in research setting and overlap with the NGS datasets stated above in ERN-RND. Finally, 30,000 more NGS datasets belonging to patients with other RD phenotypes are available for analysis through the abovementioned consortia. All genetic and patient data is handled and processed according to the General Data Protection Regulation (GDPR) rules set by the EU, as approved in a Data Management Plan submitted to the University of Antwerp. All patients and/or their legal representatives signed an informed consent locally, the study was approved by the Ethics Committee of the participating centers.

Genetic Studies

Conventional NGS methodology was used (see Supplementary Material). There were no relevant variants in genes known for ataxia, HSP or other Rare Neurological Disorders (RND) found for any of the patients described in this study. Filtering was performed first by searching for variants affecting conserved amino acids in the *SPTAN1* gene shared across multiple families but absent from gnomAD.[22] After the identification of a *de novo* deletion, an additional search was performed for conserved variants absent from gnomAD with a possible *de novo* inheritance. Where possible Sanger segregation analysis was performed (see carrier status in Figure 3 and Supplementary Figure 2). Primer sequences are available upon request. Paternity testing by DNA fingerprinting and STR analysis was performed where available (families A, B, M and N) to confirm the *de novo* status of variants. All *SPTAN1* variants are mapped to transcript NM_001130438.2.

In-depth phenotyping

Standardized in-depth phenotyping was used for all patients with a *SPTAN1* variant. Clinical information was collected per patient in a cross-center harmonized fashion using a questionnaire. Phenotypes evaluated were not only limited to the specific phenotypic traits associated with HSP and ataxia but also evaluated the presence of epilepsy, intellectual disability and peripheral neuropathy, all symptoms known to be associated with other *SPTAN1* variants. Representative MRI images are shown in Supplementary Figure 1. Data of performed Nerve Conduction Studies (NCS) is available in Supplementary Table 1.

Structural modelling of *SPTAN1* variants

We evaluated the consequence of each *SPTAN1* missense variant using three-dimensional (3D) models of the α-II-spectrin protein structure. For the p.Arg19Trp mutation, the crystal structure of the α-II-spectrin tetramerization domain was used (PDB entry 3F31).[23] No experimental structure is known for other parts of the α-II-spectrin protein. Given the extensive size (2472 amino acids) of α-II-spectrin, the Phyre2 webserver was used to model at least two neighboring spectrin repeats at the location of each missense variant,[24] resulting in five different 3D models (corresponding to amino acid positions 890–1170, 1339–1549, 1446–1762 and 1873–2472). Across all generated models, the top template hit used for homology modelling by Phyre2 was either PDB entry 3edv, 1u4q or 1u5p, all relevant structures of spectrin repeats.[25,26] For missense variants, the impact of the variant was predicted and modelled using the Missense3D server.[27] Protein structures were visualized using the UCSF ChimeraX molecular visualization program.[28]

Data availability

The raw NGS data cannot be made publicly available for confidentiality policies in the consortia involved. The NGS data in Solve-RD is available through the RD-Connect GPAP after an embargo period of 6 months. All other data generated during this study is available upon reasonable request.

Results

Genetic findings

Shared across Solve-RD, PREPARE and Genesis consortia as well as the IMGAG database, we analyzed in total more than 10,000 whole-exome or whole-genome sequencing datasets. We identified the c.6247_6249delAAG, p.Lys2083del variant in four sporadic probands with ataxia (patients A:II:3, B:II:1, C:II:1 and D:II:2). We also identified the c.55C>T, p.Arg19Trp *SPTAN1* variant in seven probands with HSP (patients E:II:1, F:II:1, G:II:1, H:III:1, I:II:1, J:II:1 and K:III:5). Through segregation analysis, we found that the p.Arg19Trp variant follows a dominant inheritance pattern in four different families (families G, H, I and K) and arose *de novo* in one additional index patient (patient F:II:1). In the last two families, families E and J, a segregation pattern could not be determined, although patient J:I:2 is reported as affected and thus a dominant inheritance pattern is probable. The p.Lys2083del variant was shown to be *de novo* in two index patients (patients B:II:1 and D:II:2). A *de novo* variant was suspected but not conclusively proven in two more index patients (patients A:II:3 and C:II:1) (Figure 3). We therefore re-evaluated the patient cohort for conserved *de novo* variants in the *SPTAN1* gene, leading to the identification of three additional *SPTAN1* variants (c.3292C>T, p.Arg1098Cys; c.4870C>T, p.Arg1624Cys; c.6614A>C, p.Gln2205Pro) linked to ataxia with or without epilepsy and intellectual disability; all were demonstrated to be *de novo* (Figure 3, L–M). Identified variants are furthermore absent from more than 30,000 NGS datasets from patients with other RD phenotypes that do not include HSP or ataxia symptoms (see Patient Cohort).

Clinical findings

We identify 22 patients belonging to 14 families. Two recurrent mutations were found; p.Lys2083del and p.Arg19Trp. The first associated with predominant cerebellar ataxia (4/22), the latter with spastic paraplegia (15/22). Furthermore, we describe three *de novo* missense mutations: p.Arg1098Cys, p.Arg1624Cys and p.Gln2205Pro.

Cerebellar ataxia sub-group (p.Lys2083del mutation)

Four unrelated patients (A:II:3, B:II:1, C:II:1 and D:II:2) with a p.Lys2083del mutation were diagnosed with cerebellar ataxia. Gait instability was the initial symptom in all four patients. The overall phenotype is described as slowly progressive cerebellar ataxia and mild intellectual disability with early to juvenile disease onset (range 3–25 years). Patient B:II:1 showed a more complex phenotype with co-occurrence of hearing impairment, paroxysmal dyskinesia and dystonic movement in the upper limbs and toes as well as cervical dystonia. Also (sub-)cortical myoclonus was present responding to carbamazepine, lamotrigine and regressing fully under zonisamide. Patient D:II:2 showed signs of pyramidal

tract involvement. MRI showed marked cerebellar atrophy in all patients and a thin corpus callosum in one (D:II:2).

Hereditary Spastic Paraplegia sub-group (p.Arg19Trp mutation)

This group consists of 15 patients. The onset age ranges from congenital to adolescence (0 – 13y). One multigenerational family was included (Figure 3, family K) with in total 12 affected members across three generations; detailed clinical data was available for 7 of them. The index patient (K:III:5) showed a congenital onset pure spastic paraplegia with marked leg spasticity (Ashworth 3–4). Most of the other family members showed a similar phenotype albeit with a variable grade of severity. Three patients, a mother and two of her three sons (K:III:4; K:IV:3 and K:IV:4), developed a more complex phenotype, with evidence of cerebellar and peripheral neuropathic involvement. NCS/EMG confirmed the presence of a sensory-motor mainly axonal neuropathy with possibly superimposed mild focal demyelinating features. No MRI abnormalities were present in all (7) examined patients.

In addition, four other families (G, H, I and J) were included. All showed early onset (range 6–8 years) pure spastic paraplegia and slowly progressed to moderate reduction in ambulation distance, except patient H:III:1 who is wheelchair dependent at age 40. Only the father of family J (I:I:1) had evidence of a mixed sensorimotor axonal neuropathy. Of note, patient I:II:1 had Rolandic epilepsy of maternal inheritance based on medical history. In patient G:II:1, walking difficulties similarly started at age 6, with the patient recently being diagnosed with bilateral optic atrophy.

Finally, two sporadic patients were included. Patient E:II:1 showed an early (3 years) onset spastic paraplegia with high arched feet. Patient F:II:1 showed an early onset form of spastic paraplegia complicated with distal weakness, optic neuropathy, unilateral vestibular areflexia and hypermobility.

Additional complex spastic and ataxia phenotypes

Three more patients were found to harbour a *de novo SPTAN1* mutation, while displaying the different extremes of the spastic ataxia spectrum. The phenotype of patient M:II:1, bearing a *de novo* p.Arg1624Cys mutation, is dominated by early onset cerebellar ataxia, dystonic head movement disorder and distal muscular atrophy. Patient L:II:1, bearing a p.Arg1098Cys mutation, displays a cerebellar ataxia with severe intellectual disability and seizures. Interestingly, serial brain MRI at age 4 and 9 showed a hypoplastic cerebellum, progressive cerebellar cortical atrophy and a FLAIR-hyperintense superior cerebellar cortex. Lastly, clinical examination of patient N:II:1, bearing a *de novo* p.Gln2205Pro mutation, is in line with spastic cerebellar ataxic gait, epilepsy, mild intellectual disability and scoliosis. Brain MRI revealed hypoplasia of the vermis and cerebellar hemispheres together with parietal polymicrogyria.

Structural modelling of *SPTAN1* variants

The *SPTAN1* gene encodes the sizeable α-II-spectrin protein, which forms the functional spectrin complex through heterotetramerisation with one of four different β-spectrins.

Although initially no structural damage was predicted by the Missense3D server, we pinpointed the p.Arg19Trp mutation to a crucial location in the tetramerization interface of the spectrin complex (Figure 4). The Arginine to Tryptophan amino acid substitution results in a loss of positive charge, which could in turn result in a partial loss of this electrostatic interaction at the tetramerization interface.

Recently and highly relevant to the current study, a mouse model with a spontaneous p.Arg1098Gln mutation in the *Sptan1* gene was shown to develop progressive ataxia with tremors and seizures, with weakened binding of calmodulin as the proposed pathomechanism.[29] Through protein homology modelling we similarly identified the Arg1098 residue to be located at a position where electrostatic interactions across the spectrin repeat are highly likely. We hypothesize that the loss of a positive charge due to the p.Arg1098Cys mutation results in the loss of stabilizing interhelical electrostatic interactions in the 10th spectrin repeat.

We identified the p.Arg1624Cys and p.Gln2205Pro substitutions to be located at similar locations in different spectrin repeats. We identify the p.Arg1624Cys mutation to be located in the 14th spectrin repeat with a possible electrostatic interaction with the Glu1549 residue. We analogously hypothesize a pathomechanism where the loss of positive charge results in destabilization of the interlinked α-helices. Although for the p.Gln2205Pro mutation there is no loss of charge resulting from the missense mutation, there is a predicted loss of hydrogen bond interlinking the Gln2205 and Cys2120 residues at the start of the 20th spectrin repeat. This bond is predicted to be lost due to the mutation (Figure 4). Furthermore, the introduction of a buried Proline is likely to destabilize the protein domain. To add to these observations, the recurrent p.Lys2083del mutation is predicted to be located across from two Aspartic acid residues (protein positions 2002 and 2003). Taken together, these observations form a common pattern, suggesting destabilization of spectrin repeats through disturbances in either electrostatic interactions or hydrogen bonds.

Discussion

The current study investigated the impact of *SPTAN1* mutations in cerebellar ataxia and HSP as well as other rare neurological disorders (see Methods). Given the association of ataxia with *SPTBN2*, earlier reports of *SPTAN1* related epilepsy syndromes showing cerebellar structural abnormalities on brain MRI and the known widespread and diverse functions of α-II-spectrin in neuronal cells, we postulated a role for *SPTAN1* mutations in rare neurological disorders, most notably HSP and cerebellar ataxia.

We report five novel *SPTAN1* mutations through a screen of 10,000 NGS datasets. In four HSP families, a recurrent p.Arg19Trp mutation segregates as a dominantly inherited trait, while in one additional patient this mutation occurs *de novo*. This finding is in contrast with earlier reported putatively recessive *SPTAN1* variants causing HSP. In the first paper, segregation data presented to support his recessive inheritance pattern was limited to one trio only; recently, a second report was made of a homozygous missense variant in one family. None of these variants are reported to be recurrent.[8,9] In our cohort of 10,000 NGS datasets, we were not able to identify any segregating *SPTAN1* variants that fit a

recessive inheritance pattern. We secondly identified a recurrent p.Lys2083del mutation in four cerebellar ataxia patients; two are *de novo* mutations, two occur in sporadic patients. Three other *de novo* missense mutations in *SPTAN1* are associated with cerebellar ataxia with possible co-occurrence of epilepsy, intellectual disability and/or pyramidal signs (Table 1). Although both the p.Arg19Trp and p.Lys2083del mutations are recurrent in the very large datasets used for this study, the identification of these patients also shows the relative rarity of *SPTAN1* mutations and the need to share NGS data for RD patients in large consortia.

The large number of patients and families presented in our study allows us to define the contours of a very broad phenotypical spectrum linked to *SPTAN1* mutations. There are two clearly delineated groups of patients with a respectively predominantly HSP phenotype linked with the p.Arg19Trp mutation and a cerebellar ataxia phenotype associated with the p.Lys2083del mutation. A third group consists of patients with more complex phenotypes, typically with cerebellar ataxia as the defining feature in the developmental delay. Two of these patients showed a more syndromic clinical phenotype with varying grades of intellectual disability and epilepsy. One of these patients (M:II:1) showed a superior cerebellar cortex hyperintense FLAIR signal, a relatively rare feature which was also reported in a patient with congenital non-progressive cerebellar ataxia due to a *SPTBN2* mutation,[17] hinting towards further phenotypical overlap between mutations in the different spectrin genes.

Strikingly, in a multigenerational family three out of 12 patients present with an HSP phenotype complicated by cerebellar ataxia and a sensory-motor axonal neuropathy with demyelinating features, while the other family members have a consistently pure HSP phenotype. This illustrates that the same *SPTAN1* mutation, segregating within the same kinship, can result in a marked variability of the clinical expression. Generally, however, most p.Arg19Trp patients do still display a pure HSP phenotype.

The availability of a crystal structure of the tetramerization domain of *SPTAN1* in addition to the well-established composition of the spectrin repeat allowed us to approach the range of mutations using *in silico* protein modelling. A common mechanistic theme arises when realizing that the amino acids identified in this study are located at crucial linking positions within the spectrin repeats. The p.Arg19Trp substitution creates a loss of positive charge and results in a destabilization of electrostatic interaction at the heterotetramerization site.[23] Previous studies have postulated that the first helix of the α-II-spectrin protein binds with the two C-terminal helices of the β-spectrins, forming a functional spectrin repeat. This three-helix bundle was also dependent on a salt bridge formed by the positively charged Arg19 residue with an Aspartic acid residue of the β-spectrin helix.

Recently, a mouse model with a spontaneous p.Arg1098Gln mutation in the *Sptan1* gene was shown to develop progressive ataxia with tremors and seizures, mimicking the phenotype seen in the patient with the p.Arg1098Cys variant in *SPTAN1* and reminiscent of mice with βIII spectrin deficiency.[29,30] In general, the *SPTAN1* gene is moderately conserved for missense variants, with a 90% confidence interval for the observed/expected ratio of missense variants being 0.55–0.62. The Arg1098 residue has a PhyloP score of 7.1794; indicative of a conserved amino acid so both the glutamine and the cysteine

substitutions of this same residue might result in similar effects. It was shown that the p.Arg1098Gln variant in the mouse *Sptan1* caused a weakened binding of calmodulin, therefore enhancing the *in vivo* proteolysis by calpain. Through protein modelling we are analogously able to hypothesize that the loss of a positive charge induced by the p.Arg1098Cys substitution results in a loss of stabilizing electrostatic interactions.

The same pattern also applies to the p.Arg1624Cys, p.Lys2083del and p.Gln2205Pro substitutions, all targeting strategically positioned (charged) amino acids interlinking different helices in the spectrin repeats. Interestingly, interhelical interactions have been shown to be of importance in the stabilization of spectrin repeats before.[26] Even more so, a mutation in dystrophin has been shown to destabilize its spectrin-like domain due to a change in electrostatic interactions, leading to Duchenne Muscular Dystrophy.[31]

Numerous *SPTAN1* mutations have been reported previously, obscuring a thorough literature search.[4,6,39–48] In order to provide at least a partial overview, we have recapitulated those with conclusive Mendelian inheritance patterns based on segregation and recurrency of mutations in Figure 2. The clinical spectrum ranges from early-onset severe epileptic syndromes, intellectual disability, HSP, spinocerebellar ataxia to late-onset mild HMN, with some patients displaying a combination of phenotypical traits. In addition, the first patient presenting with a sensory involvement was recently described.[7] Although age of onset, disease severity and some phenotypical traits can differ, correlations can be made between *SPTAN1* variants and the predominantly associated phenotype, such as the p.Lys2083del mutation in the cerebellar atactic phenotype and the p.Arg19Trp mutation in the HSP phenotype.

Although it remains challenging to draw clear-cut genotype-phenotype correlations for all *SPTAN1* variants, several interesting trends can be delineated: C-terminal in-frame insertions, deletions and duplications are highly linked to severe, early-onset epileptic encephalopathies and West syndrome. These variants are located within the spectrin dimerization domain, and one has been shown to cause spectrin aggregates in patient-derived iPSCs.[10] In contrast, nonsense mutations are seen more frequently in patients with mild HMN, where a haploinsufficiency mechanism is proposed due to nonsense-mediated decay.[5] The mutations presented in the current study typically affect positively charged Arginine or Lysine residues, producing the hypothesis that the disease mechanism is driven by the disruption of stabilizing electrostatic interactions.

How *SPTAN1* mutations affect different neuronal subtypes at different stages of life remains unanswered. In comparison to *SPTAN1*, mutations in the β-spectrins display a more limited phenotypical spectrum per gene to date. Since it is known that the composition of the spectrin complex in terms of the β-spectrin differs depending on the subcellular localization and specific neuron type, future work could uncover additional molecular evidence that results into divergent pathomechanisms between *SPTAN1* variants.

In conclusion, we report recurrent *de novo* and dominantly inherited *SPTAN1* mutations as a cause of both pure and complex HSP and cerebellar ataxia phenotypes in 22 patients from 14 families. The nature and position of the variants within the α-II-spectrin protein

strongly suggests a common mechanistic theme, of spectrin repeats destabilized due to loss of electrostatic interaction or loss of hydrogen bond.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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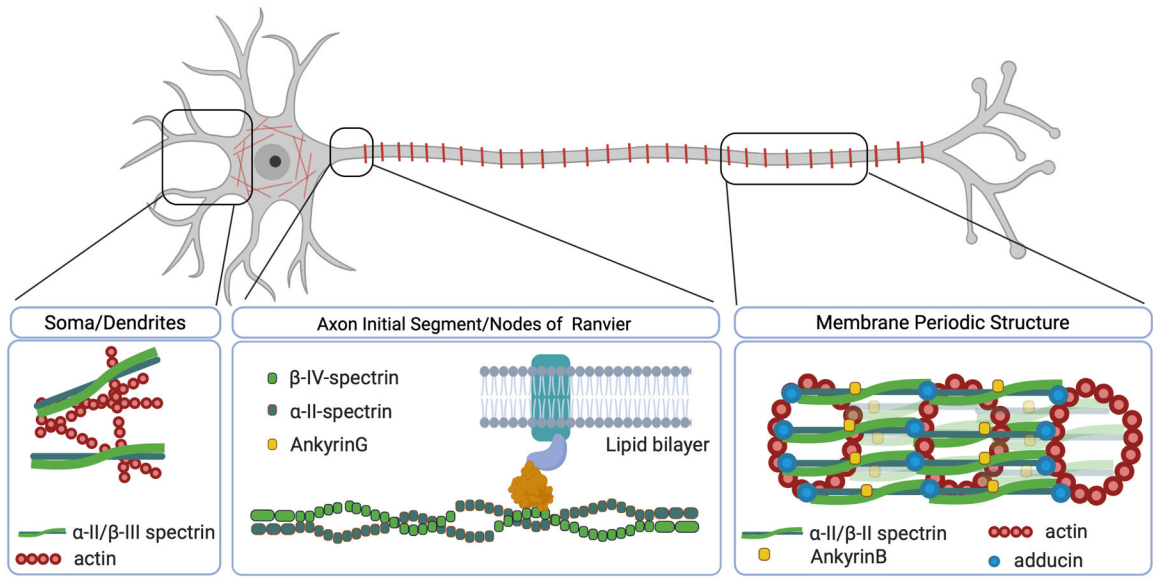
Figure 1: Function, structure, and subcellular compositions of spectrin.
Left, the mesh-like structure formed in the somatodendritic area. Middle, the scaffolding
role of the α-II/beta-IV spectrin complex. Right, Membrane Periodic Structure (MPS) with
actin rings along the distal axon.

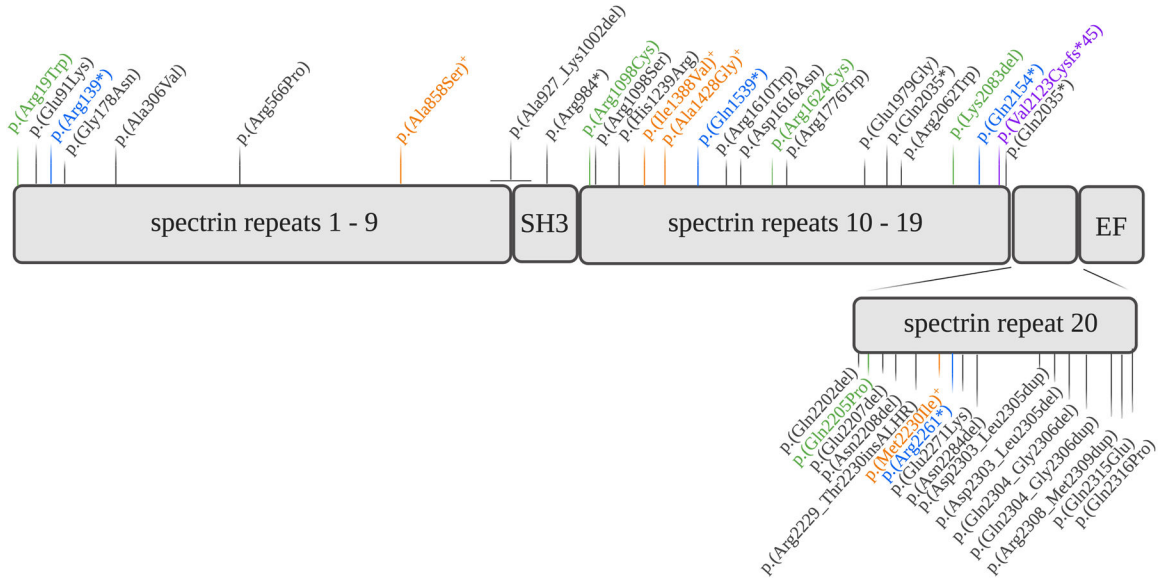**Figure 2: Graphical representation of SPTAN1 mutations reported with conclusive Mendelian inheritance.**

Green, mutations identified in this study. Orange, mutations identified in HSP patients. Blue, mutations identified in HMN patients. In purple, mutation causative of sensorimotor neuropathy and developmental disorder. In black, mutations associated with epilepsy, autism or intellectual disability.
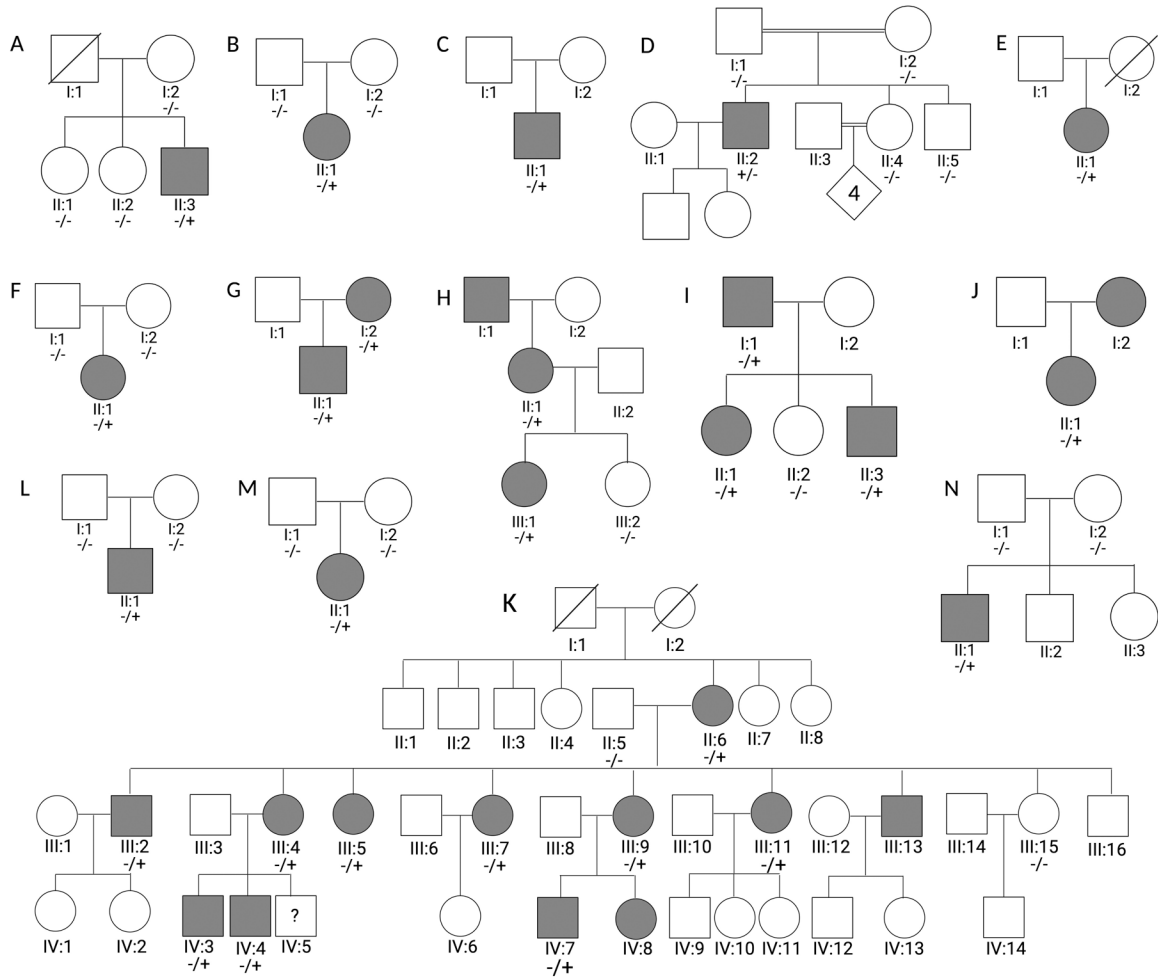
Figure 3: Pedigrees of families with SPTAN1 mutations.
Pedigrees of families showing the segregation of SPTAN1 variants
(NM_001130438.2:c.6247_6249delAAG, p.Lys2083del in families A-D),
(NM_001130438.2:c.55C>T, p.Arg19Trp in families E-K), (NM_001130438.2:c.3292C>T,
p.Arg1098Cys in family L), (NM_001130438.2:c.4870C>T, p.Arg1624Cys in family M),
(NM_001130438.2:c.6614A>C, p.Gln2205Pro in family N); Affected (black symbols) and
unaffected individuals (white symbols). Mutation results are indicated as carrier (−/+) or
non-carrier (−/−).
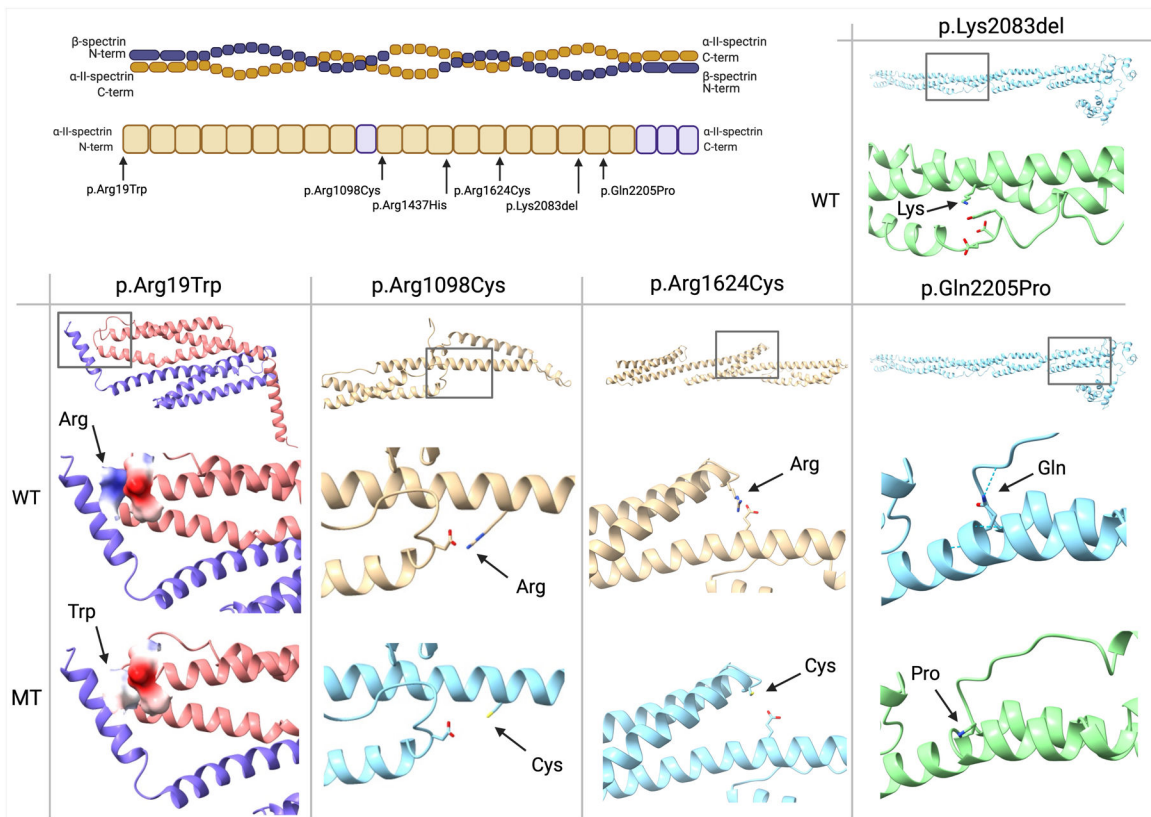
Figure 4: Protein modelling of respective SPTAN1 variants.
Top left panel: overview of the tetramer structure of the spectrin complex. Below, a graphical depiction of the α-II-spectrin protein structure with indication of the mutation locations. Top right panel: indication of the wild type Lys2083 residue, located across from three negatively charged residues. Bottom panel: comparison of the predicted wild type (WT) and mutant (MT) structures for every respective variant, with an overview of the location of the variant above. Positively charged residues are colored dark blue, negatively charged residues red. A hydrogen bond is indicated with a dotted line.

Table 1:

Clinical characteristics of patients with *SPTAN1* mutations

SPTAN1 mutation	Patient	Inheritance	Etnicity	AAO (y)	Initial symptoms	ALE (y)	Cerebellar ataxia	Pyramidal signs	Muscle weakness	Sensory abnormalities	Intellectual disability	Epilepsy	Other features
p.Lys2083del	A:II:3	Sporadic	Caucasian	17	Atactic gait	42	Yes: LL *	-	-	-	+	-	-
	B:II:1	De novo	Caucasian	3	Atactic gait	28	Yes: LL *	+++	-	+	+	-	Cervical dystonia, paroxysmal dyskinesia, myoclonus, hearing impairment
	C:II:1	Sporadic	Caucasian	6	Atactic gait	20	Yes: LL *	-	-	-	+	-	-
	D:II:2	De novo	Zaza	25	Atactic gait	36	Yes: LL * Dysarthria	+	-	-	-	-	-
p.Arg19Trp	E:II:1	Unknown	Caucasian	3	Gait difficulty	15	-	+++ *	-	-	-	-	Pes cavus
	F:II:1	De novo	Caucasian	7	Gait difficulty	23	-	+++ *	LL: Distal	-	-	-	Optic neuropathy, myopia Left vestibular areflexia; Hypermobility
	G:II:1	AD	Caucasian	6	Gait difficulty	28	-	+++*	LL: Proximal	-	-	-	Pes cavus; optic neuropathy
	H:III:1	AD	Caucasian	7	Gait difficulty	40	-	+++*	LL: Proximal	-	-	-	-
	I:I:1			7	Gait difficulty	57	-	+++ *	LL: Distal	+	-	-	Sensory-motor axonal neuropathy
	I:II:1	AD	Caucasian	8	Gait difficulty	15	-	++ *	LL: Distal	-	-	+	-
	I:II:3			6	Gait difficulty	10	-	+++*	-	-	-	-	-
	J:II:1	Probable AD	Caucasian	8	Gait difficulty	22	-	++ *	LL: Distal	-	-	-	-
	K:III:4			13	Gait difficulty	18	Yes: LL	+++ *	LL: Distal	+	-	-	Sensory-motor demyelinating neuropathy; Pes cavus
	K:III:5			0	Gait difficulty	40	-	++ *	LL: Distal	-	-	-	Pes cavus

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SPTAN1 mutation	Patient	Inheritance	Etnicity	AAO (y)	Initial symptoms	ALE (y)	Cerebellar ataxia	Pyramidal signs	Muscle weakness	Sensory abnormalities	Intellectual disability	Epilepsy	Other features
	K:III:9			0	Gait difficulty	?	-	++*	-	-	-	-	-
	K:IV:3	AD	Kurdish	13	Gait difficulty	18	Yes: LL	+++*	LL: distal	+	-	-	Sensory-motor demyelinating neuropathy; Pes cavus
	K:IV:4			4	Gait difficulty	15	-	+++*	LL: proximal / distal	-	-	-	Sensory-motor axonal neuropathy; Pes cavus; febrile seizures; cardiac arrhythmia; patent foramen ovale
	K:IV:7			6	Gait difficulty	?	-	++*	-	-	-	-	-
	K:IV:8			6	Gait difficulty	?	-	++*	-	-	-	-	-
p.Arg1098Cys	L:II:1	De novo	Caucasian	0	Developmental delay	15	Yes: LL *	+	-	-	+++	+	-
p.Arg1624Cys	M:II:1	De novo	Caucasian	0	Clumsiness	34	Yes: LL *	-	LL: Distal	-	-	-	Dystonic head tremor
p.Gln2205Pro	N:II:1	De novo	Serbian	1	Developmental delay	26	Yes: LL*	++	-	-	++	+++	Scoliosis

Abbreviations: AD = Autosomal Dominant; AAO = age at onset; ALE = age at last examination; LL = lower limbs; UL = upper limbs; −/+/++/+++ = absent / mild / moderate / severe. Predominance of clinical phenotype indicated by *