

UPDATE

Janus-faced spatacsin (SPG11): involvement in neurodevelopment and multisystem neurodegeneration

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Hereditary spastic paraplegia (HSP) is a heterogeneous group of rare motor neuron disorders characterized by progressive weakness and spasticity of the lower limbs. HSP type 11 (SPG11-HSP) is linked to pathogenic variants in the *SPG11* gene and it represents the most frequent form of complex autosomal recessive HSP. The majority of SPG11-HSP patients exhibit additional neurological symptoms such as cognitive decline, thin corpus callosum, and peripheral neuropathy. Yet, the mechanisms of *SPG11*-linked spectrum diseases are largely unknown. Recent findings indicate that spatacsin, the 280 kDa protein encoded by *SPG11*, may impact the autophagy-lysosomal machinery. In this update, we summarize the current knowledge of SPG11-HSP. In addition to clinical symptoms and differential diagnosis, our work aims to link the different clinical manifestations with the respective structural abnormalities and cellular *in vitro* phenotypes. Moreover, we describe the impact of localization and function of spatacsin in different neuronal systems. Ultimately, we propose a model in which spatacsin bridges between neurodevelopmental and neurodegenerative phenotypes of *SPG11*-linked disorders.

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Introduction

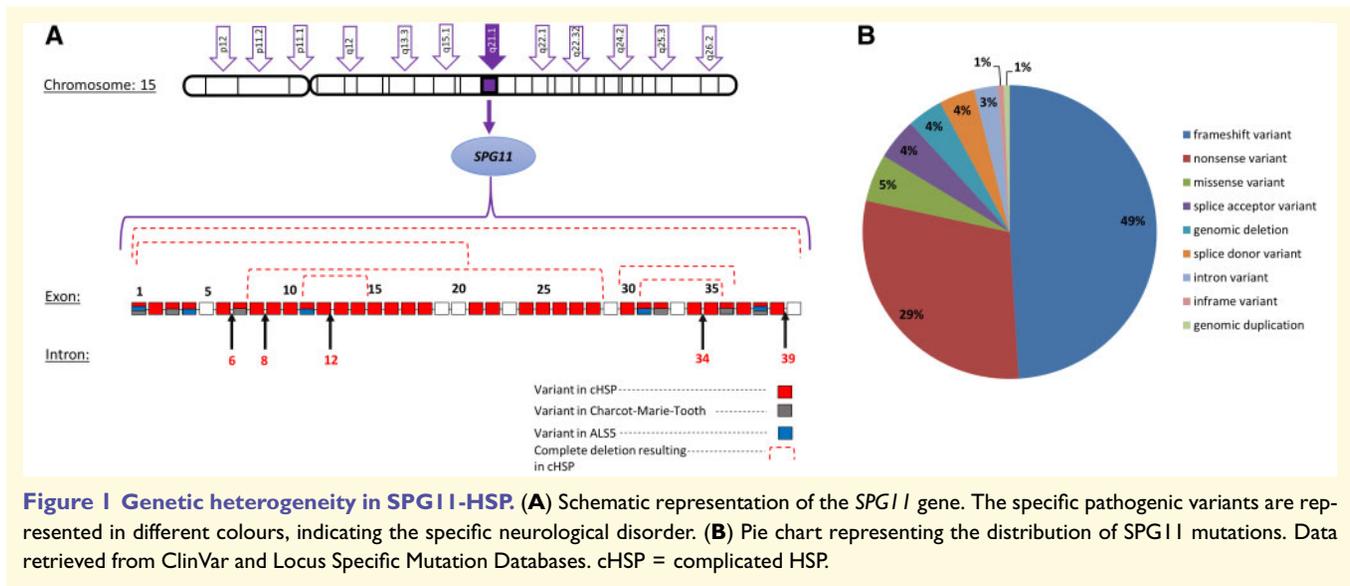
The human *SPG11* gene is located on chromosome 15q21.1 and spans an 8-kb region that encompasses 40 exons encoding spatacsin (Fig. 1A). More than 100 pathogenic variants in *SPG11* have been identified

throughout the gene without any evidence for clustering in mutational ‘hot spots’ (Denora *et al.*, 2016). The vast majority of *SPG11* mutations are frameshift or nonsense mutations, suggesting truncation of the protein encoded by *SPG11* and in all likelihood, loss of function (Fig. 1B).

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The molecular aspects of spatacsin have predominantly been obtained by studying its interaction partners in non-neuronal cells. However, because of the prominent involvement of the central and peripheral nervous systems in hereditary spastic paraplegia type 11 (SPG11-HSP), this update focuses on data from neuronal models in correlation with clinical phenotypes of neurodevelopment and degeneration (Fig. 2).

SPG11-HSP

Bi-allelic pathogenic variants in *SPG11* most frequently cause complicated HSP (Fig. 1B). They are also the most common cause of complicated autosomal recessive HSP, followed by pathogenic variants in *SPG15*, now known as *ZFYVE26*. Patients with SPG11-HSP show progressive spasticity and paraparesis as predominant symptoms. Additional symptoms can be classified according to CNS or peripheral nervous system (PNS) involvement. CNS-related symptoms comprise cognitive deterioration, parkinsonism, psychosis, and visual impairments, whereas PNS-linked symptoms include neuropathy and sphincter disturbance; other signs such as peripheral lymphoedema and obesity are also often present (Winner *et al.*, 2004; Hehr *et al.*, 2007; de Bot *et al.*, 2013; Schule *et al.*, 2016; Faber *et al.*, 2018a).

Within the clinical spectrum of *SPG11* mutations, no clear-cut genotype-phenotype correlation exists to explain the specific functional implications of single mutations depending on their localization within the gene.

Differential diagnosis of SPG11-HSP

As the observed symptoms and age at onset are highly variable in overall HSP, the differential diagnosis is broad and has been discussed in detail elsewhere (Fink, 2014). In turn,

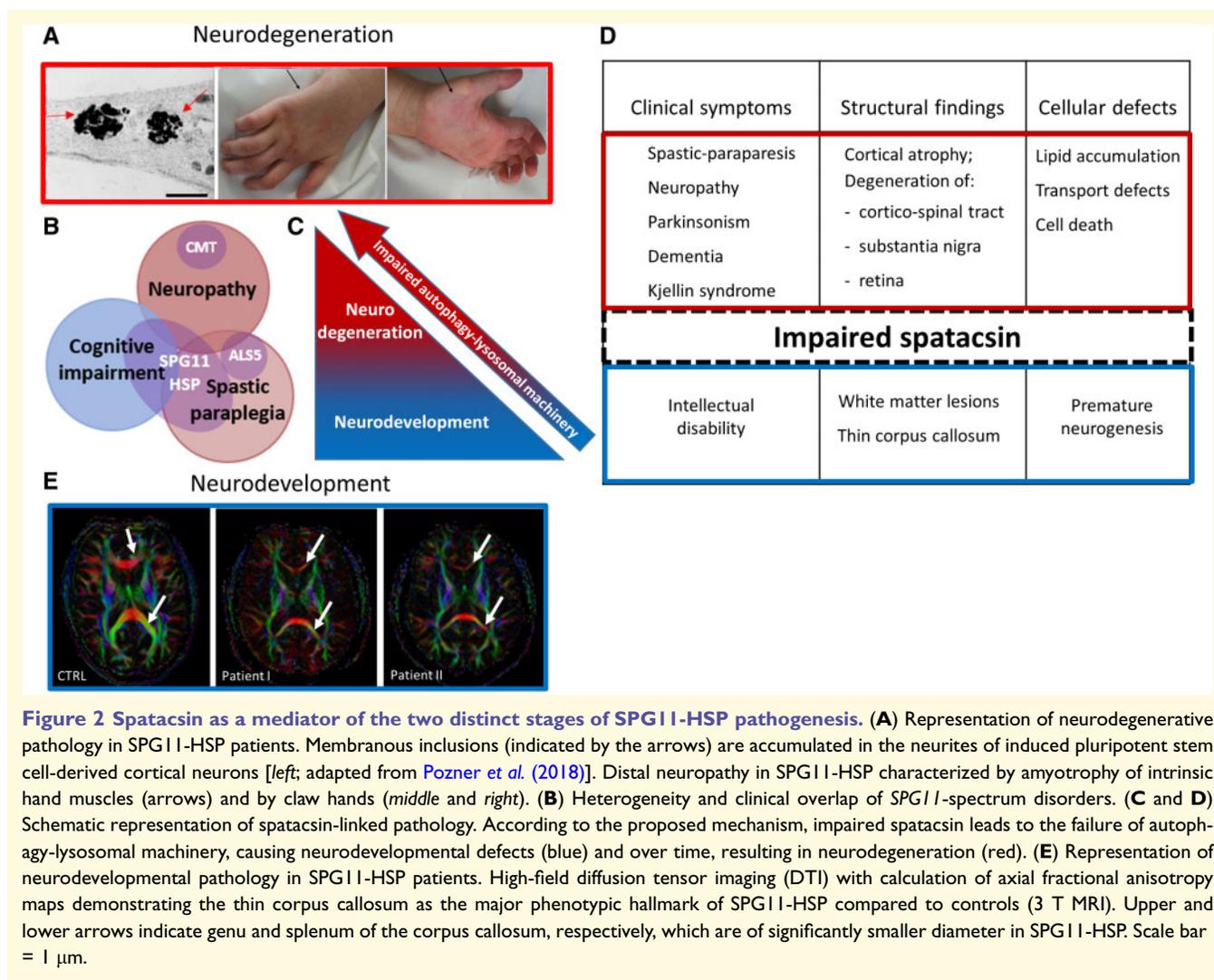
SPG11-HSP presents as an early-onset motor neuron disease with complicating symptoms that may steer the differential diagnosis.

Unlike other complicated types of HSP, SPG11-HSP frequently presents with parkinsonism in addition to the upper motor neuron phenotype, i.e. as a ‘pallido-pyramidal syndrome’ (PPS). The PPS comprises different multisystem neurodegenerative disorders. It may be caused by several types of genetic Parkinson’s disease, by genetic neurometabolic disorders, by neurodegeneration with brain iron accumulation (NBIA), other rare neurogenetic diseases, and by atypical Parkinson’s disease (Tranchant *et al.*, 2017). Similar to SPG11-HSP, most of the mechanisms in PPS are linked to lysosomal pathology and may present during neurodevelopment and neurodegeneration (Ysselstein *et al.*, 2019). α -Synuclein pathology is observed in atypical Parkinson’s disease and in some genetic causes of PPS, including Kufor-Rakeb syndrome and Gaucher’s disease. Whether α -synucleinopathy is also present in SPG11-HSP remains to be determined.

Mild cases of cerebral palsy may also present as lower limb spasticity along with additional neurological symptoms (Ashwal *et al.*, 2004). Differential diagnosis relies on a history of pregnancy and delivery, cerebral imaging studies, asymmetry of symptoms and rate of progression.

Mutations in *SPG11* have also been found in cases of early-onset autosomal recessive amyotrophic lateral sclerosis (termed ALS5) (Orlacchio *et al.*, 2010). However, the clinical classification distinguishing ‘HSP with neuropathy’ from ALS constitutes a spectrum, highlighting the need for exact and standardized clinical characterizations of patients for the future genotype-phenotype correlations (Fig. 2B and E).

In the case series describing patients with autosomal recessive Charcot-Marie-Tooth (ARCMT) disease, presenting with pure lower motor neuron phenotype, *SPG11* mutations were found in 43% of the patients, appearing as a



significant cause for the disease (Montecchiani et al., 2016). None of these patients exhibited signs of spasticity, retinal disease or cerebellar dysfunction (Montecchiani et al., 2016). In other types of HSP, the shared vulnerability of both cortico-spinal motor neurons and of spinal motor neurons is rarely observed. Thus, SPG11-HSP should be routinely tested in ARCMT disease. Of note, biallelic pathogenic variants in *ZFYVE26/SPG15* (encoding spatacsin) and *SPG48* (now known as *AP5Z1*; encoding *ZNF267*) cause autosomal recessive complicated HSP. According to the published case reports, these are clinically undistinguishable from *SPG11* but are observed at lower frequencies (Erfanian Omidvar et al., 2019). As a result of the limited number of reports, it remains unclear whether the clinical spectra of *SPG11*, *ZFYVE26/SPG15* and *AP5Z1/SPG48* are truly identical or whether there are predominant symptoms in any of the three entities (Pensato et al., 2014). Because of the lack of genotype-specific symptoms, the genetic work-up of autosomal recessive HSP with complicating symptoms should include

the analysis of *ZFYVE26/SPG15* and *AP5Z1/SPG48* genes in *SPG11*-negative cases.

Clinical and morphological characteristics of impaired neurodevelopment in SPG11-HSP

In comparison to pure HSP, complex SPG11-HSP starts earlier in life, with a peak age of diagnosis in the second decade of life (Winner et al., 2004; Schule et al., 2016). The clinical manifestation encompasses mostly subtle childhood-onset neurodevelopmental deficits, culminating in a broad range of symptoms, from subtle learning difficulties to severe intellectual disability (Siri et al., 2010). Many families report poor school performance preceding gait abnormalities (Faber et al., 2016). Because of the wide range of phenotypes, many patients remain undiagnosed during early childhood when motor symptoms remain mild or are not yet present.

On a structural level, a thin corpus callosum is the major phenotypic hallmark of the disorder (Fig. 2E). Most patients

with a thin corpus callosum show a hyperintensity of the forceps minor of the corpus callosum, which has been termed the ‘ears of the lynx’ sign (Riverol *et al.*, 2009; Pascual *et al.*, 2019). Moreover, transcranial magnetic stimulation reveals impairment of transcallosal inhibition, reflecting functional impairments of cortico-cortical projections (Winner *et al.*, 2006).

It remains unclear whether the thin corpus callosum phenotype is a result of congenital thinning/hypoplasia or whether it is related to progressive atrophy (Nakamura *et al.*, 1995; Teive *et al.*, 2001). Several reports support the possibility of congenital processes, due to unchanged thickness of the thin corpus callosum during follow-up examinations (Nakamura *et al.*, 1995; Teive *et al.*, 2001; Sperfeld *et al.*, 2004). Others suggest that the thin corpus callosum is due to neurodegeneration, occurring as a secondary process to the gliosis in white matter (Casali *et al.*, 2004; Hourani *et al.*, 2009). The main challenge of addressing this question is the frequent delay of genetic diagnosis until motor symptoms occur. As the clinical observations may not be sufficient to determine the nature of the thin corpus callosum, experimental models will be needed. Experiments to date support the idea of a neurodevelopmental origin of a thin corpus callosum that worsens over time due to the degeneration of projection neurons. Thus, the premature neurogenesis observed in an SPG11-HSP patient-derived induced pluripotent stem cell (iPSC) 2D and 3D neuronal model, combined with reduced neuronal complexity, may account for the potential vulnerability of the projection neurons, resulting in a thin corpus callosum (Perez-Branguli *et al.*, 2014, 2019). Moreover, one *SPG11 in vivo* murine model exhibited thinning of the corpus callosum after only 4 months, prior to general brain atrophy (Branchu *et al.*, 2017).

In addition to a thin corpus callosum, mild to severe enlargement of the lateral ventricles has been observed in the majority of patients (Pensato *et al.*, 2014). White matter lesions in the frontal, occipital, temporal and periventricular regions were found in a subset of patients (Winner *et al.*, 2004; Stevanin *et al.*, 2007; Denora *et al.*, 2016; da Graca *et al.*, 2018; Faber *et al.*, 2018a). Additional longitudinal clinical evaluation revealed differences between grey and white matter involvement. Thus, while the cortex and the spinal cord progressively deteriorated, diffusion tensor imaging (DTI) revealed only static involvement of the white matter (Faber *et al.*, 2018a). This observation suggests a neurodevelopmental origin of white matter impairments, linking it to imaging results found in leukodystrophies. Yet, there is no evidence of defects in the myelin sheath-forming cells, oligodendrocytes and Schwann cells in SPG11-HSP, suggesting that the white matter deficits may result from a ‘dying back’ of the axons.

There is also indirect evidence for neurodevelopmental deficits in SPG15-HSP: age at onset occurs as early as in *SPG11* and thinning of the corpus callosum and periventricular white matter hyperintensity along with cognitive

impairment is present (Pensato *et al.*, 2014). However, these developmental abnormalities were not observed in a *Zfyve26/Spig15* knock-out mouse model and should therefore be further assessed in the future, in different models (Khundadze *et al.*, 2013).

Taken together, the currently available data suggest that progressive cognitive decline in SPG11-HSP patients is at least partially of neurodevelopmental origin.

Clinical and morphological characteristics of neurodegeneration in SPG11-HSP

The progressive nature of SPG11-HSP, combined with observations of severe motor neuron degeneration in patients, render neurodegeneration a prominent characteristic of the disease (Winner *et al.*, 2004). The ongoing neurodegeneration affects both the CNS and PNS.

The predominant SPG11-HSP symptoms of spasticity and paraplegia are attributed to axonal degeneration in the corticospinal tract and peripheral nerves (Winner *et al.*, 2004). Indeed, clinical reports affirm that progressive upper and lower motor neuron degeneration occurs in SPG11-HSP (Stevanin *et al.*, 2008; Orlacchio *et al.*, 2010; Iskender *et al.*, 2015; Denora *et al.*, 2016). The severity of the reduction of spinal cord volume in SPG11-HSP patients correlates with the duration of the disease.

The frequently observed motor neuropathy is remarkably similar to that observed in slowly progressive ALS (Faber *et al.*, 2018a). In addition to clinical similarities with ALS, neurons from the medulla oblongata and spinal cord of SPG11-HSP patients accumulate lysosome-like structures (Denora *et al.*, 2016). These clinical and neuropathological findings are in agreement with reports of juvenile ALS caused by *SPG11* mutations (Orlacchio *et al.*, 2010; Daoud *et al.*, 2012).

Aside from motor neuron degeneration, initial imaging studies identified increasing grey matter atrophy in the basal ganglia, the thalamus, and the precentral gyri (Hehr *et al.*, 2007; Franca *et al.*, 2012). Recent findings support a more widespread involvement of grey matter in the disease, including the amygdala, red nucleus and nucleus accumbens, as well as the superior temporal sulcus, cingulum, parahippocampal, parietal and paracentral regions (Faber *et al.*, 2018a). The extent of cortical atrophy renders it challenging to associate the damage to a distinct region. However, it may account for the progressive nature of the cognitive decline occurring in SPG11-HSP. Indeed, dementia has previously been reported in a number of SPG11-HSP patients (Fraidakis *et al.*, 2016; Faber *et al.*, 2018a).

A clinical study on a large patient cohort consisting of 22 *SPG11* mutation carriers, reported degeneration of the substantia nigra and reduced dopamine transporter density as common findings in SPG11-HSP (Faber *et al.*, 2018b). The latter was indicative of a disruption of presynaptic dopaminergic pathways even in patients without extrapyramidal motor symptoms (Faber *et al.*, 2018b). These observations are in line with reports of parkinsonism in SPG11-HSP

patients, partly as a presenting symptom and partly with a positive L-DOPA response (Anheim *et al.*, 2009; Paisan-Ruiz *et al.*, 2010; Guidubaldi *et al.*, 2011; Vanderver *et al.*, 2012; Wijemanne *et al.*, 2015).

Interestingly, central retinal degeneration has also been reported in SPG11-HSP, adding a neuro-ophthalmological aspect termed Kjellin syndrome to the complex phenotype of the disease (Orlen *et al.*, 2009). In this case, retinal impairment becomes evident after the onset of spastic paraplegia (Puech *et al.*, 2011).

All of these clinical symptoms of progressive neurodegeneration have also been described in *ZFYVE26/SPG15* and partly also in *AP5Z1/SPG48* patients, further emphasizing the large clinical overlap of these related entities (Hanein *et al.*, 2008; Kara *et al.*, 2016). The fact that, unlike in SPG11-HSP, pathogenic variants of *ZFYVE26/SPG15* were not detected in a case series on patients with autosomal recessive pure neuropathy without pyramidal symptoms, may be at least partially explained by the low frequency of *ZFYVE26/SPG15* mutations (Montecchiani *et al.*, 2016).

Spatacsin: spatio-temporal expression and function

To understand the cellular dysfunction involved in the pathogenesis of the neurodevelopmental and neurodegenerative features of SPG11-HSP, we focus on spatacsin, the protein encoded by *SPG11*.

Spatacsin is a large protein (2443 amino acids; ~280 kDa), which is highly conserved across vertebrates. Human spatacsin shares 85%, 76% and 73% sequence identity with its homologues in dogs, mice and rats, respectively (Stevanin *et al.*, 2008). According to *in silico* analyses, spatacsin is predicted to contain a short-coiled coil domain, a glycosyl hydroxylase F1 signature, a leucine zipper, a Myb domain and four transmembrane domains (Paisan-Ruiz *et al.*, 2008). Spatacsin is associated with other proteins linked to HSP, i.e. spastizin (encoded by *ZFYVE26/SPG15*) and the fifth adaptor protein complex (AP-5), which is localized to the late endosomal compartment (Slabicki *et al.*, 2010; Hirst *et al.*, 2011, 2013; Murmu *et al.*, 2011; Chang *et al.*, 2014).

Expression of spatacsin in the developing and adult brain

Existing data on the localization of spatacsin are scarce and inconsistent. The major reason for reported discrepancies in the protein expression is a lack of specific antibodies. The development of alternative techniques to reliably detect the protein expression of spatacsin is crucial to enable the study of its temporal and spatial expression, function and interpretation of disease phenotypes.

The available data from different experimental species indicate a widespread expression of spatacsin in the CNS during development. *In vivo* rodent studies demonstrate strong and ubiquitous expression of spatacsin during embryonic development in the brain, spinal cord and in

regions outside the CNS of rat and mouse models (Stevanin *et al.*, 2007; Murmu *et al.*, 2011; Perez-Branguli *et al.*, 2014). Similarly, *SPG11* mRNA is highly expressed in zebrafish throughout embryogenesis, from the one-cell stage to at least 3 days of development (Southgate *et al.*, 2010; Martin *et al.*, 2012).

In adults, studies regarding spatacsin expression have mostly been confined to the CNS because of its prominent involvement in SPG11-HSP. The first study to examine spatacsin expression in the brain indicated strong mRNA expression in the human cerebral cortex and cerebellum (Stevanin *et al.*, 2007). In the mouse cortex, spatacsin expression was restricted to neurons and was not present in astrocytes (Perez-Branguli *et al.*, 2014). Overall, in the CNS, regions with a high density of neurons tend to exhibit the strongest expression of spatacsin as it is expressed mainly in neuronal cells.

On a subcellular level, spatacsin was detected in axons and dendrites of both mouse cortical neurons and human embryonic stem cell (HUES6)-derived cortical neurons (Perez-Branguli *et al.*, 2014). In both neuronal models, spatacsin was found in the cytosol and it co-localized with microtubules, actin, and synaptic markers (Perez-Branguli *et al.*, 2014). In non-neuronal models (e.g. fibroblasts or HeLa cells; Hirst *et al.*, 2011, 2013, 2015) spatacsin co-localized to late endosomes/lysosomes suggesting a role in vesicular trafficking.

Role of spatacsin during development

The first evidence for the functional involvement of spatacsin in neurodevelopment originated from experiments with embryonic zebrafish. Morpholino knock-down of *SPG11* led to developmental defects, which included locomotor abnormalities, aberrant branching of spinal cord motor neurons at the neuromuscular junction (Martin *et al.*, 2012) and impaired neuronal differentiation (Southgate *et al.*, 2010). Similarly, a recent *Spg11* knockout mouse model exhibited early-onset cognitive and motor defects which were linked to a failure of lysosomal lipid clearance, and subsequent lysosomal accumulation of lipids in neurons (Branchu *et al.*, 2017).

In humans, a major step towards understanding human neurodevelopment resulted from an investigation of iPSC-derived neural progenitor cells (NPCs) from SPG11-HSP patients. SPG11-NPCs exhibited cell cycle alterations of reduced S and G2/M phases, pointing towards decreased neural proliferation (Mishra *et al.*, 2016). The underlying mechanism was attributed to impairments of the Wnt/ β -catenin signalling pathway, which has a prominent role during neural development (Clevers, 2006). In SPG11-NPCs, over-activation of GSK3 β resulted in a reduction of β -catenin levels, which in turn compromised cellular proliferation (Mishra *et al.*, 2016). A follow-up study revealed an increased asymmetric division rate of patients' NPCs as the underlying cause of the observed proliferation deficits in cerebral organoids (Perez-Branguli *et al.*, 2019). SPG11-

NPCs undergo premature neurogenesis resulting in a smaller size of SPG11 cerebral organoids (Perez-Branguli *et al.*, 2019). In both experimental models, these neurodevelopmental defects were rescued upon application of tideglusib, a specific GSK3 inhibitor (Mishra *et al.*, 2016; Perez-Branguli *et al.*, 2019).

Role of spatacsin during neurodegeneration

To date, two different *Spg11* knock-out mouse models have been published. While in the first model (Varga *et al.*, 2015) spatacsin was disrupted by inserting a gene-trap cassette in the first intron of *Spg11*, the recent model (Branchu *et al.*, 2017) was generated by inserting a stop codon in exon 32 of the gene. Both models exhibit neurodegenerative features, including a neuronal loss in the motor cortex and the cerebellum (Varga *et al.*, 2015; Branchu *et al.*, 2017). However, the latter (Branchu *et al.*, 2017) exhibits early symptoms and more extensive signs of neurodegeneration, e.g. lower motor neurons, muscular atrophy, and memory deficits. The differences are consistent with the vast heterogeneity reported in human phenotypes. However, the different strategies of gene knock-out may at least partially account for this difference.

A follow-up study using the recent mouse model (Branchu *et al.*, 2017) identified gangliosides as the lipid type accumulating in the lysosomes, resulting in neurodegeneration that was rescued by inhibition of ganglioside synthesis (Boutry *et al.*, 2018). Similarly, the inhibition of ganglioside synthesis by miglustat improved the motor phenotype of the *SPG11* zebrafish model (Boutry *et al.*, 2018).

Additional evaluation of cellular models derived from the *Spg11* knock-out mouse (Branchu *et al.*, 2017) linked the loss of spatacsin to lysosomal cholesterol accumulation, eventually leading to reduced level of cholesterol in the plasma membrane, resulting in impaired calcium homeostasis (Boutry *et al.*, 2019). Both cellular phenotypes—altered cholesterol distribution and calcium dysregulation—are known to be tightly linked to neurodegeneration (Mattson, 2007; Zundorf and Reiser, 2011; Arenas *et al.*, 2017).

Further insights into spatacsin-linked neurodegeneration were obtained from *in vitro* measurements of axonal transport. Anterograde axonal trafficking of synaptophysin-positive vesicles was significantly reduced in *SPG11*-patient-derived neurons, as well as in *SPG11*-silenced mouse cortical neurons (Perez-Branguli *et al.*, 2014). The observation of axonopathy was supported by the downregulation of axonal genes, decreased neuritic complexity and membranous inclusions within the axons (Perez-Branguli *et al.*, 2014). Defects in neuritic complexity were also present in a model of patient iPSC-derived cortical neurons and in CRISPR-Cas9-mediated *SPG11* knock-out lines (Pozner *et al.*, 2018). *SPG11*-HSP neuronal cells also exhibited increased cell death and accumulation of membranous inclusions, all of which were rescued by the application of the previously mentioned GSK3 inhibitor, tideglusib (Pozner *et al.*, 2018). Thus, GSK3 inhibition may constitute a first therapeutic target in *SPG11* related disorders.

Impaired autophagy-lysosomal machinery: linking neurodevelopmental and neurodegenerative phenotypes

The most prominent defects observed in different models of *SPG11*-HSP are connected to autophagy (Ebrahimi-Fakhari *et al.*, 2016). Neuropathological studies revealed ubiquitin and p62 positive granules in medulla oblongata and spinal cord neurons of *SPG11*-HSP patients (Denora *et al.*, 2016) and an increase in p62 protein is usually correlated with a reduction in autophagy (Zatloukal *et al.*, 2002). Correspondingly, both *SPG11* mouse models exhibited autophagic defects (Varga *et al.*, 2015; Branchu *et al.*, 2017).

The mechanism underlying this phenomenon can be attributed to defects in the autophagic lysosomal reformation (ALR) process, which is crucial for lysosomal homeostasis during autophagy (Yu *et al.*, 2010). Spatacsin, together with its interaction partner spastizin, has been implicated in ALR initiation (Chang *et al.*, 2014). ALR impairment might also explain the observations of increased autophagosome accumulation and lysosomal enlargement frequently reported in *SPG11* models (Chang *et al.*, 2014; Renvoise *et al.*, 2014; Varga *et al.*, 2015). Interestingly, zebrafish oocyte model with mutated homologue of *ZFYVE26/SPG15*, named soufflé (*suf*) accumulated immature secretory precursors due to the lack of separated clathrin-coated buds (Kanagaraj *et al.*, 2014). Thus, a general failure of secretory vesicle formation mechanism may be the underlying cause of the observed ALR impairment.

The observed lysosomal aberration and subsequent lipid accumulation may be caused by spatacsin's role in intracellular cargo trafficking, mediated through its interaction with spastizin and AP-5. AP-5, in turn, has been implicated in the endosome to trans-Golgi network recycling of the cation independent mannose-6-phosphate receptor (CIMPR), a protein crucial for targeting lysosomal enzymes to the lysosomes (Hirst *et al.*, 2018). Indeed, blocking of the endosome to trans-Golgi network transport resulted in lipid accumulation reminiscent of *SPG11*-associated neurodegenerative processes (Lin *et al.*, 2018; Eising *et al.*, 2019).

Dysfunction of the autophagy-lysosomal machinery may mediate the pathogenic effects of *SPG11* mutations in both neurodevelopment and neurodegeneration (Fig. 2C). As neurons are post-mitotic cells, they are highly dependent on the process of autophagy for proper function and are therefore extremely vulnerable to an aberration of this process. Interestingly, an altered lysosomal function has also been reported in pure types of hereditary spastic paraplegia, including *SPAST*, *ATL1* and *REEP1* (Allison *et al.*, 2017). Lysosomal pathology has also been implicated in most of the corresponding mechanisms of PPS (Ysselstein *et al.*, 2019). Overall, the damage is cumulative and the detrimental consequences of the impairment become evident with the progression of the disease.

Table 1 Genetic models of SPG11-related pathology

Species	Genetic modification/mutation	Cellular phenotype	Affected cells	Affected regions	Thin corpus callosum	Cognitive impairment	Motor impairment	Neuro-developmental defects	Neuro-degenerative defects	References
Mouse	Cre-loxP system: exon 32	Lysosomal lipid accumulation, dystrophic axons, impaired calcium homeostasis	Cortical neurons, spinal motor neurons	Motor cortex, cerebellum, hippocampus, cortico-spinal tract	Yes	Yes	Yes (early onset)	Yes	Yes	Branchu <i>et al.</i> , 2017; Boutry <i>et al.</i> , 2018, 2019
Zebrafish	Gene trap cassette: intron 1	Autophagy-lysosomal impairments	Cortical neurons, Purkinje cells	Motor cortex, cerebellum	No	ND	Yes (late onset)	No	Yes	Varga <i>et al.</i> , 2015
	Morpholino knock-down: exon 26–intron 26 junction	Axonal growth defect, lipid accumulation	Spinal motor neurons	Hindbrain	–	ND	Yes (early onset)	Yes	Yes	Martin <i>et al.</i> , 2012; Boutry <i>et al.</i> , 2018
	Morpholino knock-down: exon 2–intron 2 and exon 4–intron 4 junctions	Impaired neuronal differentiation	Spinal motor neurons	Brain ventricles, hindbrain, eyes	–	ND	Yes (early onset)	Yes	No	Southgate <i>et al.</i> , 2010
Human nerve biopsies	Frameshift, nonsense and splice mutations	Hypomyelination of large nerve fibres, axonal accumulation of membranous material	Sensory neurons	Peripheral nervous system	–	–	Yes	–	Yes	Hehr <i>et al.</i> , 2007
Human post-mortem tissue	Nonsense mutations	Intracytoplasmic granular lysosome-like structures	Spinal motor neurons	Cortex, white matter, motor tracts in medulla oblongata and spinal cord	Yes	Yes	Yes	Yes	Yes	Denora <i>et al.</i> , 2016

ND = no data.

Table 2 Human cellular models of *SPG11*-related pathology

Cell type	Patients, n	Observed phenotype	Neuro-developmental defects	Neuro-degenerative defects	References
Fibroblasts	ND	Lysosomal abnormality	–	–	Renoise <i>et al.</i>, 2014
	8	No significant alteration in autophagic/lysosomal markers	–	–	Kara <i>et al.</i>, 2016
Patient fibroblasts and spatacsin-depleted HeLa cells	ND	Autophagic lysosomal reformation (ALR) defects	No	Yes	Chang <i>et al.</i>, 2014
	3	Autophagy defects	–	–	Vantaggiato <i>et al.</i>, 2019
Patient iPSC-derived NPCs and neurospheres	3 ^a	Proliferation defect	Yes	No	Mishra <i>et al.</i>, 2016
Patient iPSC-derived neurospheres	3 ^a	Premature neurogenesis	Yes	No	Perez-Branguli <i>et al.</i>, 2019
Patient iPSC-derived cortical neurons	3 ^a	Axonal pathology and vesicle trafficking defects.	No	Yes	Perez-Branguli <i>et al.</i>, 2014
	3 ^a	Neurite impairment, increased cell death	No	Yes	Pozner <i>et al.</i>, 2018
Patient iPSC-derived organoids	3 ^a	Smaller organoid size	Yes	No	Perez-Branguli <i>et al.</i>, 2019
	2	Lipid metabolism	Yes	Yes	Boutry <i>et al.</i>, 2018

iPSC = induced pluripotent stem cells; ND = no data; NPC = neural progenitor cells.

^aIndicates the same patients.

Conclusions

In SPG11-HSP the neurodevelopmental origins of the disorder are evident in the juvenile stage of a patient's life and, over time, give rise to neurodegeneration.

Pathogenic variants in the *SPG11* gene encoding spatacsin are the most common cause of complicated autosomal recessive HSP, accounting for ~20% of cases with complicated HSP. Existing *in vivo* and *in vitro* models (Tables 1 and 2) link the protein function to development and neurodegeneration through its role in the lipid clearance process, which is mediated by a proper function of autophagic-lysosomal machinery (Fig. 2C and D). However, many questions regarding the exact localization and function of the protein remain unclear, mainly as a result of the difficulty in detecting the protein and limited knowledge of its structure. The development of alternative techniques to reliably detect the protein expression of spatacsin is crucial to enable an analysis of its temporal and spatial expression, determine its function and interpret disease phenotypes.

The majority of molecular experimental data stem from established knowledge of spatacsin's association with the AP-5 complex and spastizin ([Hirst *et al.*, 2013, 2015, 2018](#)). However, the fact that AP-5 knock-out did not affect the localization of spatacsin suggests that it might have other, AP-5 independent, functions ([Hirst *et al.*, 2013](#)). This notion is supported by interaction of *Drosophila SPG11* and *ZFYVE26/SPG15* orthologues exclusively with Rab7 and Rab26 ([Gillingham *et al.*, 2014](#)). As flies lack functional AP-5 complex, this interaction may shed light on alternative, vesicular trafficking associated pathways independent of the AP-5 complex. In addition, it has been shown that while mutations in *ZFYVE26/SPG15*, encoding spastizin, reduce the stability of spatacsin, mutations in *SPG11* have no effect on

spastizin level ([Vantaggiato *et al.*, 2019](#)). Thus, although both proteins are involved in ALR, only *ZFYVE26/SPG15* mutations lead to defects in the fusion between autophagosomes and endosomes ([Vantaggiato *et al.*, 2019](#)). Therefore, while dysfunction or lack of spatacsin may impair the autophagy-lysosomal machinery through disruption of either AP-5 complex or spastizin, it is likely that spatacsin acts through additional, distinct pathways.

It is important to add that the majority of data on the molecular function of spatacsin come from studies conducted on fibroblasts, HeLa cells, and animal models. Human neuronal models are essential in order to provide missing links regarding the function of the protein in patients. Recent advances in iPSC-derived 3D models may serve as a useful means of further studying the protein and its function in an environment that recapitulates human cellular organization. Advances in genome editing techniques can be of further benefit, by reconciling the issue of variability in the human patient-derived models. A combination of these methods provides an opportunity to determine the spatio-temporal localization of spatacsin and its mechanistic involvement in the pathogenesis of the disease.

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Competing interests

The authors report no competing interests

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