

Hereditary spastic paraplegia: clinical features and pathogenetic mechanisms

Sara Salinas, Christos Proukakis, Andrew Crosby, Thomas T Warner

Hereditary spastic paraplegia (HSP) describes a heterogeneous group of genetic neurodegenerative disorders in which the most severely affected neurons are those of the spinal cord. These disorders are characterised clinically by progressive spasticity and weakness of the lower limbs, and pathologically by retrograde axonal degeneration of the corticospinal tracts and posterior columns. In recent years, genetic studies have identified key cellular functions that are vital for the maintenance of axonal homeostasis in HSP. Here, we describe the clinical and diagnostic features of the various forms of HSP. We also discuss the genes that have been identified and the emerging pathogenetic mechanisms.

Introduction

Hereditary spastic paraplegias (HSPs) are a clinically and genetically heterogeneous group of conditions that are characterised by the presence of lower limb spasticity and weakness.¹ The common pathological feature of these conditions is retrograde degeneration of the longest nerve fibres in the corticospinal tracts and posterior columns. The key diagnostic clinical findings are of lower limb spasticity and pyramidal weakness, with hyper-reflexia and extensor plantar responses.¹⁻³ The genetics of HSP are complex and all modes of inheritance (autosomal dominant, autosomal recessive, and X-linked recessive) have been described.^{1,3}

Few epidemiological studies of HSP have been done, but prevalence is estimated at 3–10 cases per 100 000 population in Europe.^{4,5} Onset is from early childhood through to 70 years of age and HSP is therefore a significant source of chronic neurodisability. Traditionally, it has been divided into pure (uncomplicated) HSP and complicated HSP, depending on the presence of other neurological features in addition to spastic paraparesis.^{2,3} This Review provides an overview of the clinical spectrum of HSP and the pathophysiological mechanisms that have been identified through the study of genes found to underlie HSP.

Clinical features and diagnosis

The onset of HSP is subtle, with development of leg stiffness or abnormal wear of the shoes.¹ Compared with other causes of spastic paraplegia, such as multiple sclerosis and spinal injury, there is relative preservation of power despite dramatically increased tone in the legs, particularly in patients with early-onset disease.⁶ The important clues to the cause of spastic paraplegia are the age and nature of onset, progression of symptoms, presence of a family history, and other clinical features. Onset in the first years of life with delayed motor milestones is more suggestive of cerebral palsy, particularly if there is a static clinical picture. Clinicians might find it helpful to ask about athletic ability in childhood, because poor performance or lack of interest in sport might indicate a longstanding motor disability. Several other features have also been described under the

rubric of pure HSP and include mild sensory abnormalities of the lower limbs (eg, reduced vibration sense), urinary symptoms (reported in up to 50% of cases in later disease),^{3,7} pes cavus, and mild cognitive decline.^{3,6,8} Upper limbs might show hyper-reflexia, but cranial nerves are rarely involved in HSP.³

Complicated HSPs comprise a large number of conditions in which spastic paraplegia is accompanied by other features, such as ataxia, severe amyotrophy, optic atrophy, pigmentary retinopathy, mental retardation, extrapyramidal signs, dementia, deafness, ichthyosis, peripheral neuropathy, and epilepsy.^{1,3} These forms are often autosomal recessive and are rare, so the finding of additional neurological features with spastic paraplegia should indicate other possible diagnoses.

A sporadic case of spastic paraplegia that develops over the age of 20 years is a fairly frequent clinical problem in neurological practice. In such cases, the absence of a family history means that HSP is a diagnosis of exclusion. The differential diagnoses vary according to age of onset (panel). A significant proportion of cases of undiagnosed spastic paraplegia are likely to be of genetic origin, and detailed family investigations are therefore crucial. This is particularly important for adult-onset cases, because asymptomatic affected individuals and non-penetrant mutation carriers have been described.⁶ The presence of a slowly progressive gait disorder with relatively few sensory symptoms or signs favours a diagnosis of HSP. A family history compatible with autosomal dominant transmission in the context of adult-onset spastic paraplegia is almost always indicative of HSP. Acute or subacute onset of spasticity favours vascular or inflammatory causes, respectively, and in these cases weakness is often more marked. Similarly, spinal cord compression usually has a more aggressive course than HSP, often in association with sensory symptoms and signs plus spinal or referred pain.

The diagnosis of pure HSP in a family in which several members have typical clinical features presents few difficulties. For a patient for whom there is no reliable or verifiable family history, further investigation is required. Investigations include tests for very long chain fatty acids, white cell enzymes, plasma amino acids, serum

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Neuropathobiology Laboratory, Cancer Research UK, London (S Salinas PhD); Department of Clinical Neurosciences, Institute of Neurology, University College London, London (C Proukakis MRCP, T T Warner FRCP); and Department of Medical Genetics, St George's Hospital Medical School, London, UK (A Crosby PhD)

Correspondence to: T T Warner, Department of Clinical Neurosciences, Institute of Neurology, University College London, Royal Free Campus, Rowland Hill Street, London NW3 2PF, UK
t.warner@medsch.ucl.ac.uk

Panel: Differential diagnoses in spastic paraplegia**Childhood onset**

Diplegic cerebral palsy
 Structural (Chiari malformation, atlanto-axial subluxation)
 Hereditary spastic paraplegia
 Leucodystrophy (eg, Krabbe's)
 Metabolic (arginase deficiency, abetalipoproteinaemia)
 Levodopa-responsive dystonia
 Infection (myelitis)
 Multiple sclerosis

Adult onset

Cervical spine degenerative disease
 Multiple sclerosis
 Motor neuron disease
 Neoplasm (primary/secondary spinal tumour, parasagittal meningioma)
 Infection (myelitis)
 Dural arteriovenous malformation
 Chiari malformation
 Adrenoleucodystrophy
 Hereditary spastic paraplegia
 Spinocerebellar ataxias
 Vitamin deficiency (B12 and E)
 Lathyrism
 Levodopa-responsive dystonia
 Infection (syphilis, human T-cell leukaemia virus 1, HIV)
 Copper deficiency

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lipoprotein analysis, vitamin B12 or vitamin E, copper and ceruloplasmin, serum serology for syphilis, human T-cell leukaemia virus 1, and HIV, and neuro-ophthalmological assessment. Positive results on these tests would indicate a cause other than HSP. Age of onset and clinical presentation can help clinicians to determine which tests should be used, particularly for complicated cases in childhood in which metabolic conditions need to be excluded.

In cases of proven HSP, the most common MRI abnormality is thinning of the cervical and thoracic spinal cord.¹⁰ One study of four dominant HSP kindreds also suggested a loss of volume of the corpus callosum, and a higher incidence of cerebral white matter lesions.¹¹ In most cases of pure HSP, nerve conduction studies and electromyography are normal. Central motor conduction times have been reported to be delayed or unrecordable from the lower limbs and lower limb somatosensory evoked potentials are small.¹² CSF analysis is usually normal in HSP.

The mapping and cloning of HSP genes has led to specific molecular genetic tests that will allow more focused investigation of potential cases of HSP, thereby precluding more invasive and costly investigations. A molecular genetic diagnosis can now be made in over

50% of cases in autosomal dominant pure HSP by screening the two most common HSP-related genes, *SPAST* (formerly *SPG4*) and *SPG3A*.¹³

Genetic subtypes of HSP

HSP can be inherited as an autosomal dominant, recessive, or X-linked recessive trait, and at least 41 spastic paraplegia gene (*SPG*) loci have been mapped and 17 genes identified to date. Autosomal dominant HSP is the most prevalent form and represents around 70% of cases.¹⁻³ Most cases of pure HSP are autosomal dominant, whereas complicated forms tend to be autosomal recessive.^{1,3} For practical purposes, HSP is divided according to mode of inheritance and presence or absence of complicating features. The table lists the genetic subtypes of HSP that have been identified, and summarises their characteristic clinical features. The following sections describe the clinical phenotypes for the genetic subtypes of HSP, according to mode of inheritance.

Autosomal dominant HSP

SPAST-associated HSP is the most common type of pure autosomal dominant HSP, accounting for 40–45% of such cases,¹⁷ and is the form that has been clinically studied in the most detail. This type of HSP typically has onset from childhood through to late adult life.⁸ Overall, more than half of mutation carriers will not develop symptoms until after the age of 30 years. In most cases, the phenotype is of slowly progressive spasticity in the lower limbs with loss of mobility around two decades after the onset of symptoms.⁸ Symptoms found consistently in a small number of patients, particularly with longer disease duration, include urinary urgency, upper limb hyper-reflexia, decreased vibration sense, and muscle wasting in the lower limbs.^{3,7,8} Complex phenotypes, including cerebellar ataxia,⁴⁸ epilepsy,⁴⁹ thinning of the corpus callosum,⁵⁰ and mental retardation, have been described in several families with a mutation in the *SPAST* gene, which encodes the spastin protein. Progressive cognitive decline has also been reported to become evident by examination from 40 years of age and to progress to clinically evident dementia by the age of 60–80 years,^{51,52} although another study found only subclinical executive dysfunction.⁵³ Severe late-onset dementia with unusual pathological findings has been reported in a patient with a spastin missense mutation.⁵⁴

SPG3A-associated HSP is the second most common cause of autosomal dominant HSP, accounting for approximately 10% of cases.⁵⁵ It usually has a pure phenotype, but earlier onset, often before the age of 10 years. Typically, there is relatively slow progression of symptoms. Mutations in *NIPA1* (formerly *SPG6*) are also a cause of pure HSP that progresses slowly, but can become severe.^{19,56} Penetrance is age dependent and high. Similarly, HSP associated with mutations in *KIAA0196* (formerly *SPG8*) is characterised by more severe spasticity and reduced vibration sense.²¹

| | Inheritance | Locus | Protein | Clinical features | Frequency |
|-------------------------------|-------------|---------------|--|---|-------------------------------------|
| L1CAM (SPG1) ¹⁴ | X-linked | Xq28 | L1 cell adhesion molecule | Mental retardation, hypoplasia of corpus callosum, adducted thumbs, hydrocephalus | Over 100 familial cases |
| PLP1 (SPG2) ¹⁵ | X-linked | Xq21 | Proteolipoprotein 1 | Quadriplegia, nystagmus, mental retardation, seizures | <100 familial cases |
| SPG3A ¹⁶ | AD | 14q12-q21 | Atlastin | Early-onset pure, slow progression HSP | <10% AD HSP |
| SPAST (SPG4) ¹⁷ | AD | 2p22 | Spastin | Variable-onset mainly pure HSP | 40% of pure AD HSP |
| CYP7B1 (SPG5A) ¹⁸ | AR | 8p | Cytochrome P450-7B1 | Variable-onset pure HSP | ~20 families |
| NIPA1 (SPG6) ¹⁹ | AD | 15q11.2-q12 | Non-imprinted in Prader-Willi/ Angelman syndrome region protein 1 | Adult-onset pure HSP | ~10 families |
| SPG7 ²⁰ | AR | 16q | Paraplegin | Variable onset, cerebellar signs, optic atrophy, neuropathy | ~30 families |
| KIAA0196 (SPG8) ²¹ | AD | 8q24 | Strumpellin | Adult-onset pure HSP, marked spasticity | <10 families |
| SPG9 ²² | AD | 10q23.3-q24.2 | .. | Cataracts, motor neuropathy, skeletal abnormalities, gastro-oesophageal reflux | 1 family |
| KIF5A (SPG10) ²³ | AD | 12q13 | Kinesin family member 5A | Early-onset pure HSP, can be complicated with distal amyotrophy | <10 families |
| SPG11 ²⁴ | AR | 15q | Spatacsin | Childhood to early adult onset, thin corpus callosum, cognitive impairment, neuropathy | Many families |
| SPG12 ²⁵ | AD | 19q13 | .. | Early-onset pure HSP | <10 families |
| HSPD1 (SPG13) ²⁶ | AD | 2q24-q34 | Heat shock protein 60 | Adult-onset pure HSP | <10 families |
| SPG14 ²⁷ | AR | 3q27-q38 | .. | Variable onset, motor neuropathy, mental retardation | 1 family |
| ZFYVE26 (SPG15) ²⁸ | AR | 14q | Spastizin | Kjellin syndrome: adolescent onset, pigmented retinopathy, cerebellar signs, mental retardation | <10 families |
| SPG16 ²⁹ | X-linked | Xq11.2 | .. | HSP with onset in infancy, aphasia, sphincter disturbance, mental retardation | 1 family |
| BSCL2 (SPG17) ³⁰ | AD | 11q12-q14 | Seipin | Silver syndrome: variable onset, distal amyotrophy in hands more than in feet | <20 families |
| SPG18 | AD | Reserved | .. | .. | .. |
| SPG19 ³¹ | AD | 9q33-q34 | .. | Adult-onset pure HSP | 1 family |
| SPG20 ³² | AR | 13q | Spartin | Troyer syndrome: childhood onset, amyotrophy, cerebellar signs, developmental delay | Founder mutation in Amish community |
| SPG21 ³³ | AR | 15q | Masparidin | Mast syndrome: early adult onset, thin corpus callosum, cognitive decline, extrapyramidal features, cerebellar signs | Founder mutation in Amish community |
| SPG23 ³⁴ | AR | 1q24-q32 | .. | Lison syndrome: childhood onset, pigmentary abnormalities, facial and skeletal dysmorphism, cognitive decline, tremor | 1 family |
| SPG24 ³⁵ | AR | 13q14 | .. | Childhood-onset pure HSP, pseudobulbar signs | 1 family |
| SPG25 ³⁶ | AR | 6q23-q24 | .. | Adult onset, cataracts, prolapsed intervertebral discs | 1 family |
| SPG26 ³⁷ | AR | 12p11.1-q14 | .. | Adult onset, neuropathy and distal wasting, intellectual impairment | 2 families |
| SPG27 ³⁸ | AR | 10q22.1-q24.1 | .. | Variable onset, cerebellar signs, neuropathy, mental retardation, microcephaly | 2 families |
| SPG28 ³⁹ | AR | 14q21.3-q22.3 | .. | Early-onset pure HSP | 1 family |
| SPG29 ⁴⁰ | AD | 1p31-p21 | .. | Sensorineural deafness, hiatus hernia, pes cavus, hyperbilirubinaemia | 1 family |
| SPG30 ⁴¹ | AR | 2q37 | .. | Adolescent-onset pure HSP, sensory neuropathy | 1 family |
| REEP1 (SPG31) ⁴² | AD | 2p12 | Receptor expression-enhancing protein 1 | Variable-onset pure HSP | 8% of AD pure HSP |
| SPG32 ⁴³ | AR | 14q12-q21 | .. | Childhood onset, mental retardation, thin corpus callosum, pontine dysraphism | 1 family |
| SPG34 | AD | Reserved | .. | .. | .. |
| SPG35 ⁴⁴ | AR | 16q21-q23 | .. | Childhood onset, intellectual decline, seizures | 1 family |
| SPG36 | AD | 12q23-q24 | .. | .. | .. |
| SPG37 ⁴⁵ | AD | 8p21.1-q13.3 | .. | .. | .. |
| SPG38 ⁴⁶ | AD | 4p16-p15 | .. | Distal amyotrophy (Silver syndrome) | 1 family |
| SPG39 ⁴⁷ | AR | 19p13 | Neuropathy target esterase | Childhood onset, marked distal wasting in all four limbs | 2 families |
| SPG41 | AD | 11p14.1-p11.2 | .. | .. | .. |

If the SPG gene symbol has been replaced by the HUGO Gene Nomenclature Committee, the new symbol is listed, with the original symbol in parentheses (<http://www.genenames.org>). AD=Autosomal dominant. AR=autosomal recessive. ..=unknown.

Table: Genetic forms of HSP

HSP associated with mutations in the gene for heat shock protein 60 (*HSPD1*; formerly *SPG13*) typically has a late onset without additional features.²⁶ A Gly563Ala missense variant was recently reported to be associated with an earlier age of onset in patients carrying *SPAST* mutations, although this was not pathogenic by itself.⁵⁷ Most intriguingly, another change (Asp29Gly) in the same protein has been reported in an Israeli Bedouin kindred to cause an early-onset fatal neurodegenerative Pelizaeus-Merzbacher-like disease (see *PLP1*-associated HSP) if present in the homozygous state.⁵⁸

Mutations in *BSCL2* (formerly *SPG17*) cause a complicated form of HSP that is characterised by additional amyotrophy of the small muscles of the hands and feet with onset in the early teens to the late thirties (Silver syndrome). Mutations in the *BSCL2* gene also cause hereditary motor neuropathy type V and the autosomal recessive condition Berardinelli-Seip congenital lipodystrophy.³⁰

Mutations in *REEP1* (formerly *SPG31*) lead to a pure form of HSP with a variable age of onset.⁴² It is relatively common, and mutations in the *REEP1* gene have been identified in 3% of a sample of unrelated patients with HSP, which increased to 8–2% in pure HSP if those with *SPG3A* and *SPAST* mutations were excluded.⁵⁹

A base change in *ZFYVE27* (*SPG33*) was reported as causative of pure HSP in a single German family. The protein associates with spastin, and might function in endosomal transport.⁶⁰ However, this base variant has been recently reported in control chromosomes (single nucleotide polymorphism rs35077384) and shown to have a minor allele frequency of 1–7%, dependent on the population studied, which would be consistent with this being a rare neutral sequence variant and not causative of HSP. The original report of a functional effect of the missense change (on intracellular distribution and interaction with spastin) was also questioned.⁶¹ The authors of the original report subsequently accepted that their findings should be interpreted with caution.⁶² *ZFYVE27* is therefore not currently listed on the HUGO database as an HSP-associated gene. Other forms of autosomal dominant HSP have been summarised in the table (associated with *KIF5A*, *SPG9*, *SPG12*, *SPG18*, *SPG19*, *SPG29*, *SPG34*, *SPG36–SPG38*, and *SPG41* genes).

Autosomal recessive HSP

A number of families have autosomal recessive HSP that is associated with mutations in the *CYP7B1* (formerly *SPG5A*) gene; this is a pure form with variable age of onset and slow progression.^{18,63} To date, it has been recorded in about 20 families. Mutations in the *SPG7* gene, which encodes paraplegin, account for around 5% of autosomal recessive HSP. This type produces both pure and complicated HSP phenotypes.^{20,64} Cerebellar signs (dysarthria, nystagmus, and ataxia), pale optic discs, and peripheral neuropathy are common complicating features.⁶⁴

SPG11-associated HSP, which is characterised by a thin corpus callosum, is a common and clinically distinct form that is linked to the *SPG11* locus on chromosome 15 in most families.²⁴ Other common features include cognitive impairment and severe axonal neuropathy.^{24,65} HSP associated with mutations in *ZFYVE26* (formerly *SPG15*) has a characteristic autosomal recessive complicated phenotype (Kjellin syndrome), in which spastic paraplegia is accompanied by mental impairment, pigmentary retinopathy, cerebellar signs, and distal amyotrophy.²⁸

SPG20-associated HSP and *SPG21*-associated HSP are two complicated forms that have been identified in members of the Old Order Amish. A single mutation in *SPG20*, which encodes the protein spartin, causes Troyer syndrome,³² whereas a mutation in *SPG21* (encoding the protein maspardin) causes Mast syndrome.³³ Both are due to a founder mutation in this population. Troyer syndrome is characterised by spastic tetraparesis, dysarthria, distal amyotrophy, short stature, and learning difficulty. Mast syndrome is associated with dementia, cerebellar and extrapyramidal signs, and thin corpus callosum.

The remaining recessive forms of HSP (associated with *SPG14*, *SPG23–SPG28*, *SPG30*, *SPG32*, *SPG35*, and *SPG39*) are very rare, and have each been described in only one or two families. Any distinguishing clinical features are listed in the table.

X-linked HSP

HSP caused by mutations in *LICAM* (formerly *SPG1*) is characterised by hydrocephalus, mental retardation, spasticity of the legs, and adducted thumbs.¹⁴ The phenotypic spectrum of L1 syndrome also includes X-linked hydrocephalus with aqueduct of Sylvius stenosis, MASA syndrome (mental retardation, aphasia, spastic paraplegia, and adducted thumbs), and X-linked agenesis of the corpus callosum.

Mutations in the proteolipoprotein gene (*PLP1*; formerly *SPG2*) at Xq21-q22 have been found in families with mainly complicated HSP in which there can be associated peripheral neuropathy and white matter changes on MRI. Mutations (usually duplications) of this gene also give rise to the dysmyelinating condition Pelizaeus-Merzbacher disease, which is characterised by congenital hypotonia, psychomotor deterioration, and progressive pyramidal, dystonic, and cerebellar signs.⁶⁶ Death usually occurs in infancy or childhood. The variation in phenotype between Pelizaeus-Merzbacher disease and *PLP1*-linked HSP is thought to arise from the differential effect that mutations can have on the two isoforms of the protein product, proteolipoprotein 1 (PLP1) and DM20.⁶⁶

One other rare X-linked form of HSP has been described (associated with *SPG16*).²⁹ Affected individuals had quadriplegia, motor aphasia, reduced vision, mild mental retardation, and sphincter disturbance.

For the HUGO gene nomenclature see <http://www.genenames.org>

Pathophysiology of HSP

The main neuropathological finding in HSP is of axonal degeneration of the terminal portions of the long descending (corticospinal tracts) and ascending (dorsal columns) pathways in the spinal cord, although there have also been reports of degeneration of the spinocerebellar tracts and loss of Betz cells in layer V of the motor cortex.^{54,67–69} Any pathophysiological mechanism must explain why the disease involves the longest neurons in the spinal cord.⁶⁷ One current hypothesis, derived from the study of several genes that cause HSP, is that they lead to disruption of the axonal transport of macromolecules, organelles, and other cargoes, which predominantly affects the distal parts of these neurons.^{70,71} As a result of the unique morphology of spinal neurons, the long axons (which can measure up to 1 m in length) are likely to have considerable dependence on membrane trafficking, microtubule-associated transport, and cytoskeletal organisation. They also have additional reliance on mitochondrial function to drive the efficient transport of signals, molecules, and organelles to and from nerve terminals. Thus, membrane trafficking and axonal transport are emerging as potentially important themes in HSP.

Membrane trafficking

All cells have a regulated, dynamic membrane trafficking system that allows interactions between the plasma membrane and other membrane-bound compartments. This trafficking is highly organised and starts with vesicle budding, followed by transport of the vesicle, tethering, and fusion with the target membrane. Endocytosis begins with vesicle formation at the plasma membrane, which contains receptors and/or other transmembrane proteins, and is reliant on the vesicle coat protein, clathrin.⁷² Vesicles are transported along the microtubule cytoskeleton, as described below, and then tethering and fusion of endosomes occurs to deliver cargo to various subcellular locations. These processes depend on families of proteins, such as the Rab family of small GTPases, that mediate the intracellular destination of the vesicles and ESCRT (endosomal sorting complex required for transport)-associated proteins, which sort proteins targeted for ubiquitin-dependent degradation.⁷³ The secretory pathway flows in the opposite direction to endocytosis, from the endoplasmic reticulum (ER) and Golgi apparatus, and allows the delivery of newly synthesised proteins, carbohydrates, and lipids to the cell surface, endosomes, and lysosomes.

Axonal transport

Axonal transport mainly depends on microtubule tracks, and is powered by two distinct classes of molecular motors, namely dynein (retrograde transport) and kinesins (mainly anterograde transport).⁷⁴ Cytoplasmic dynein is a ubiquitous motor of the AAA (ATPase-associated with various cellular activities) family, and

comprises many subunits that are responsible for attachment to microtubules and cargo recruitment.⁷⁵ Dynein is necessary for a wide variety of cellular processes, such as cell division and Golgi maintenance, and neurons are highly dependent on the proper function of this molecular complex for their axonal transport. Indeed, dynein has been shown to be involved in the axonal transport of numerous cargoes, such as neurotrophin-signalling endosomes,⁷⁶ mitochondria,⁷⁷ injury-generated signals,⁷⁸ and RNA-associated proteins.⁷⁹

The kinesin family comprises several members, some of which are responsible for the delivery of material to nerve terminals. Kinesin 1 is composed of two kinesin heavy chains and two kinesin light chains.⁷⁴ The heavy chains contain both ATPase and microtubule-binding domains, whereas the light chains are thought to be selective for cargo recognition.⁷⁴ Early studies in *Drosophila* showed the crucial role of kinesin 1 in axonal maintenance,^{80,81} because mutations in *Khc* and *Klc* genes led to accumulation of mitochondria and synaptic vesicles and swellings due to the impairment of axonal transport.⁸² The maintenance of the cytoskeletal tracks also depends on molecular motors, which are responsible for the transport of short microtubules and neurofilaments to where they are required for growth and repair.^{83–85} Important components of the process of microtubule remodelling are those proteins that sever microtubules into short lengths.⁸⁵

Abnormal axonal transport and membrane trafficking in HSP

This section summarises the current evidence to support the role for eight HSP proteins (kinesin heavy chain, spastin, atlastin, NIPA1, spatacsin, spastizin, spartin, and maspardin) in axonal transport and membrane trafficking.

Kinesin heavy chain (KIF5A)

The most direct evidence to support the hypothesis of abnormal axonal transport in HSP comes from the finding that mutations in the gene for the KIF5A subunit of kinesin 1 (formerly *SPG10*) are associated with an early-onset, pure form of HSP.²³ Kinesin heavy chain is an integral part of the motor protein involved in fast anterograde microtubule-dependent axonal transport.^{23,86} In-vitro studies have shown that mutant forms of *KIF5A* lead to reduced gross cargo flux along microtubules due to reduced microtubule affinity or gliding velocity,⁸⁷ and a deficiency in kinesin-dependent cargo is thus likely to underlie the terminal degeneration of axons.

Spastin (SPAST)

SPAST encodes the protein spastin, a member of the AAA family. Spastin is present in different isoforms depending on the translation initiation codon (ATG) used and on splicing of the exons, in particular exon 4.⁸⁸ It has

a predicted size of up to 616 amino acids. Experimental work has suggested that the second ATG, which gives rise to a shorter isoform, is the main initiation codon,^{88,89} although a recent study in rats found that the longer isoform was expressed at higher concentrations in spinal cord neurons than in other neurons.⁹⁰ Spastin possesses two main structural domains, an MIT (microtubule interacting and trafficking) domain in the N-terminus⁹¹ and a catalytic AAA domain in the C-terminus.^{88,91} Over 150 mutations have been described in *SPAST*-associated HSP in all exons, except in exon 4. These mutations, mainly missense and truncating, affect the AAA domain of spastin, suggesting a loss of function in the pathogenesis of HSP.⁹² More recently, gene rearrangements, in particular exon deletions, have been shown to be a common cause of the disease.⁹³ Neurons seem to be unusually sensitive to haploinsufficiency of spastin because splice-site mutations that result in both normal and aberrant splice forms of the protein are sufficient to produce the full clinical picture.^{94,95}

Early studies in cultured cells showed that mutant spastin localised with microtubules and overexpression of wild-type spastin led to microtubule disassembly.⁹⁶ In motor neurons, spastin is enriched in cellular regions where dynamic microtubules are found, including the distal axon.⁹⁷ Comparative studies in primary neurons on spastin and katanin, a related AAA microtubule-severing enzyme, suggest that spastin-mediated microtubule severing is particularly involved in axonal branching. Overexpression of spastin in rat hippocampal neurons caused a dramatic enhancement of axonal branch formation associated with an increase in numbers of severed, short microtubules.⁹⁸ Depletion of spastin led to neurons with shorter axons with fewer branches.⁹⁸ These findings are consistent with the growing evidence that spastin has a role in microtubule turnover, and the microtubule-severing activity of spastin has been confirmed by in-vitro assays.^{88,99,100} AAA mutations also abolish this microtubule-severing activity of spastin.^{96,99} Spastin might have an additional microtubule-bundling activity, as it can bundle polymerised microtubules in vitro, and mutant forms might induce microtubule stabilisation when overexpressed in cell lines.^{88,92} However, the physiological relevance of these observations remains to be addressed.

A link to the involvement of spastin in the endocytic pathway came from the finding of spastin binding to chromatin modifying protein 1B, a protein associated with the endosomal sorting complex, probably mediated by the MIT domain.¹⁰¹ A partial co-localisation of spastin with an endosomal and an ER marker was also reported, leading to the suggestion that spastin might function in locally regulating microtubules responsible for the axonal movement of membranous organelles.¹⁰² Recent biochemical studies have shown that spastin can form hexamers that bind to polymerised tubulin and induce a

conformational change that is responsible for microtubule breakage.^{103,104} Furthermore, disease-related mutations were shown to interfere with pore loops in this hexameric structure.^{103,104}

To investigate the effect of spastin activity and mutations in vivo, studies have been done in various animal models of HSP that have shown an effect of spastin on axonal growth and trafficking. Knockdown of wild-type spastin in *Drosophila* and zebrafish has led to abnormal axonal development and synaptic function.^{105,106} In a mouse model with a deletion mutation in *SPAST* that leads to a premature stop codon (mimicking a human pathogenic mutant), axonal degeneration and accumulation of mitochondria in abnormal swellings close to the axonal growth cone were described.¹⁰⁷ Interestingly, these findings might have a human correlate because abnormal mitochondrial distribution, thought to indicate defective transport, was reported in post-mortem spinal motor neurons from an individual with *SPAST*-associated HSP.¹⁰⁸

Although spastin mutations, especially large deletions, truncating mutations, and nonsense changes, act through a loss of function, a recent study has indicated that this might not always be the case.⁹⁰ The investigators suggested that the pathogenic forms of mutant spastin were initiated from the first ATG codon. This full-length isoform was found in higher concentrations in rat spinal cord neurons during adulthood, but not in other neurons.⁹⁰ Experimental expression of a short dysfunctional peptide in neurons, comprising the N-terminal region (amino acids 1–273) expressed from the first ATG codon, was deleterious to axonal growth. Expression of this peptide in squid giant axons inhibited fast axonal transport, whereas expression of a similar short peptide translated from the second ATG codon did not have these deleterious effects.⁹⁰

These studies of spastin have implicated disruption of processes that maintain the microtubule cytoskeleton, which in turn might adversely affect axonal transport and lead to abnormal axonal growth or degeneration. However, the precise defect, including the involvement of the endocytic pathway, remains to be determined.

Atlastin 1 (SPG3A)

The *SPG3A* gene encodes the protein atlastin 1.¹⁶ On the basis of its similarity to proteins in the dynamin superfamily of large GTPases and studies in heterologous cell-culture systems, atlastin 1 was implicated in neurite outgrowth and intracellular membrane trafficking, especially at the ER-to-Golgi interface.¹⁰⁹ However, more recent work has shown that the atlastin family functions mainly in ER and Golgi morphogenesis, but does not seem to be required for anterograde ER-to-Golgi trafficking.¹¹⁰ Abnormal morphogenesis of ER and Golgi might interfere with correct membrane distribution or polarity of corticospinal neurons. In cultured neurons, atlastin 1 was found to be enriched in growth cones

and promoted axon elongation during neuronal development.¹¹¹ These findings suggest a problem during development and might help to explain the very early onset of *SPG3A*-associated HSP. Interestingly, atlastin has also been shown to interact with spastin, suggesting a common mechanism for pathogenesis.^{102,112}

Non-imprinted in Prader-Willi/Angelman syndrome region protein 1 (NIPA1)

Mutations in the *NIPA1* gene, which encodes the non-imprinted in Prader-Willi/Angelman syndrome region protein 1 (NIPA1), have been identified in an adult-onset, pure form of HSP.^{19,56} NIPA1 is thought to be an Mg²⁺ transporter that associates with early endosomes and the cell surface in various neuronal and epithelial cells.¹¹³ Loss-of-function mutations seem to lead to abnormal trafficking of the protein and/or Mg²⁺ transport across membranes. The *Drosophila* orthologue, spichthyn, shows preferential localisation on early endosomes and was recently reported to have a role in maintenance of microtubules and axonal transport.¹¹⁴ The investigators found that spichthyn had a functional role in bone morphogenetic protein signalling, which leads to upregulation of several genes, including some with potent axonal inhibitory function. Depletion of spichthyn in *Drosophila* was also found to lead to synaptic overgrowth at the neuromuscular junction, and bone morphogenetic protein signalling also regulated microtubule architecture in axons.¹¹⁴

Spatacsin (SPG11)

Frameshift, nonsense, and splice mutations in the gene for spatacsin have been identified in many families with *SPG11*-associated HSP, suggesting loss of function.^{24,65} Spatacsin is ubiquitously expressed in the nervous system, particularly in the cerebellum, cerebral cortex, and hippocampus. It has at least one transmembrane domain, and immunofluorescence experiments have shown diffuse cytosolic expression, with slight colocalisation with mitochondria and the ER, but no association with the Golgi or microtubules.²⁴ Thus, the pathogenesis of this form of HSP is unknown, although the finding of accumulation of pleiomorphic membranous material in unmyelinated axons of a sural nerve biopsy from a patient with *SPG11*-associated HSP was interpreted as compatible with disturbed axonal transport.¹¹⁵

Spastizin (ZFYVE26)

Truncating mutations in the *ZFYVE26* gene, which encodes a zinc finger protein with FYVE domain, have recently been found.²⁸ The protein was named spastizin, and its mRNA was found to be widely distributed in tissues, with similar expression in the rodent brain to spatacsin. Initial cell studies suggest localisation of spastizin in the ER and endosomes, again raising the possibility of a role in membrane trafficking at these sites.

Spartin (SPG20) and maspardin (SPG21)

The mutation found in *SPG20*-associated HSP results in a truncated form of the protein spartin, implying a loss of function.^{32,116} Spartin contains the MIT domain also found in spastin, therefore linking it with a transport function, but there are conflicting data concerning its subcellular localisation, with one study showing mitochondrial localisation,¹¹⁷ whereas another study has suggested both nuclear and cytoplasmic distribution.¹¹⁸ Spartin has been shown to be involved in the degradation of the epidermal growth factor receptor, and mutations are thought to affect trafficking of this receptor and endocytosis.¹¹⁶ Before its involvement in HSP was identified, maspardin had been shown to co-localise with vesicles of the endosomal/trans-Golgi apparatus,¹¹⁹ and also with transferrin-positive vesicles.³³

Abnormal mitochondrial function in HSP

This section summarises the current evidence to support the role for three HSP proteins (paraplegin, heat shock protein 60, and receptor expression-enhancing protein 1) in mitochondrial dysfunction.

Paraplegin (SPG7)

Paraplegin is part of the metalloprotease AAA complex, an ATP-dependent proteolytic complex on the inner mitochondrial membrane that controls protein quality and regulates ribosomal assembly.^{120,121} Loss of the metalloprotease AAA complex in fibroblasts from patients with *SPG7*-associated HSP caused reduced activity of complex I in the mitochondrial respiratory chain and increased sensitivity to oxidative stress.^{64,122} Paraplegin-deficient mice develop axonal swellings caused by accumulations of organelles and neurofilaments, similar to those seen in spastin-deficient mice, which precede axonal degeneration and correlate with onset of motor impairment.¹²³ This again implicates an axonal transport problem, which might be secondary to mitochondrial dysfunction. A recent study of AFG3-like protein 2 (AFG3L2), another mitochondrial metalloprotease homologous to paraplegin, which forms a supracomplex with paraplegin to control protein quality control in mitochondria, found that null or missense *Afg3l2* mouse models had marked impairment of axonal development leading to neonatal death.¹²⁴ The mice developed a severe early-onset tetraparesis and were found to have reduced myelinated fibres in the spinal cord, and impaired respiratory chain complex I and III activity. The phenotype was reported to be more severe than that seen in paraplegin-deficient mice due to the higher neuronal expression of AFG3L2, but also serves to link mitochondrial function with HSP.

Heat shock protein 60 (HSPD1)

HSPD1 encodes a mitochondrial protein, heat shock protein 60, which is thought to assist in the folding of a

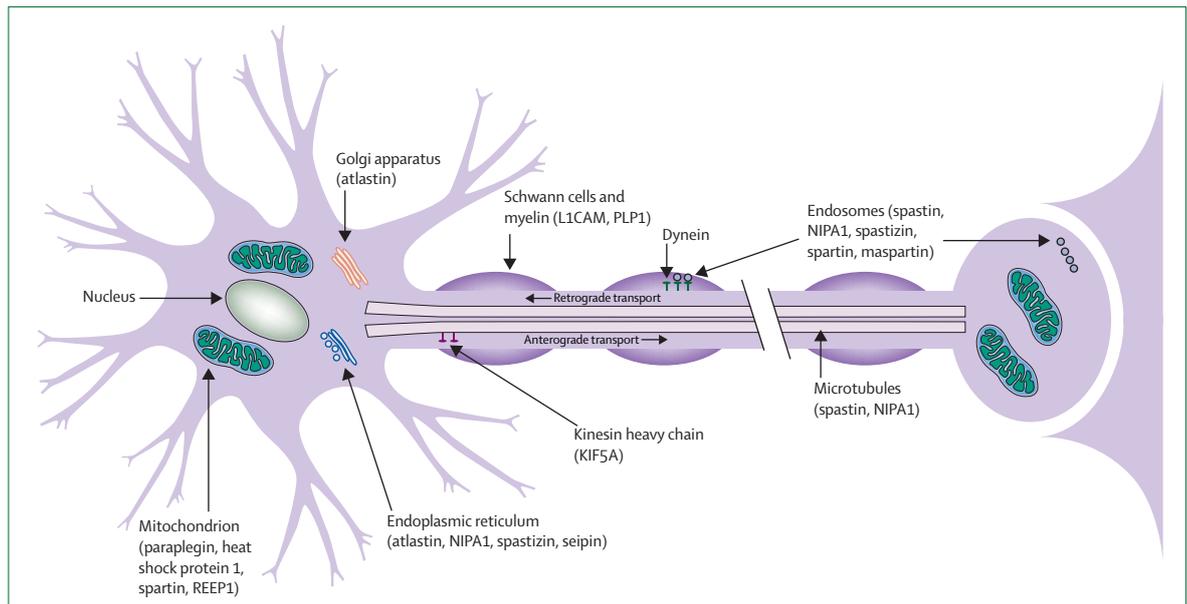


Figure: Schematic representation of a neuron indicating sites of potential pathogenic mechanisms of mutant spastic paraplegia proteins

For the OMIM database see
<http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim>

subset of proteins located in mitochondria.^{26,125} A study of cells from a patient with the Val98Ile mutation showed that there was decreased expression of the mitochondrial quality control proteases Lon (ATP-dependent protease La) and ClpP (ATP-dependent Clp protease proteolytic subunit) at both mRNA and protein levels.¹²⁵ A reduction in degradative activity of the protein quality control system in mitochondria in *HSPD1*-associated HSP is thought to lead to subsequent mitochondrial dysfunction.

Receptor expression-enhancing protein 1 (REEP1)

REEP1 encodes receptor expression-enhancing protein 1, which is found in mitochondria and, because of its conserved protein-domain structure, might be involved in chaperone-like activities.⁴² Although mitochondrial localisation of REEP1 is apparent, its function in this or other organelles remains to be elucidated.

Other protein abnormalities in HSP

Autosomal recessive HSP

A recent development has been the identification of mutations in the gene that encodes cytochrome P450-7B1 (*CYP7B1*), which causes autosomal recessive pure HSP.¹⁸ In the liver, *CYP7B1* offers an alternative pathway for cholesterol degradation and also provides the primary metabolic route for the modification of dehydroepiandrosterone neurosteroids in the brain. These findings provide the first direct evidence of a potential role for altered cholesterol metabolism in the pathogenesis of a motor neuron degenerative disease.

Two families with an autosomal recessive form of complicated HSP with childhood onset and associated wasting have been reported to be caused by mutations in

the neuropathy target esterase gene (listed in OMIM as *SPG39*).⁴⁷ This was of interest because the phenotype has similarities to that seen in organophosphate poisoning, and neuropathy target esterase is involved in organophosphate compound toxicity.

A single consanguineous family with autosomal recessive HSP associated with severe mutilating sensory neuropathy has been described and found to be caused by missense mutations in the gene for epsilon-subunit of the cytosolic chaperonin-containing t-complex peptide 1 (*CCT5*). However, the mechanism of axonal degeneration that affects both central and peripheral neurons is unclear.¹²⁶

Autosomal dominant HSP

BSCL2 encodes the ER protein seipin, the function of which is unknown but it is thought to act at the interface of the ER with lipid droplets.¹²⁷ In one study, mutant seipin in *BSCL2*-associated HSP seemed to accumulate in the ER and to increase the concentration of ER stress-mediated molecules, which induced apoptosis in cultured cells.¹²⁸

Mutations in the *KIAA0196* gene, which encodes the protein strumpellin, underlie an adult-onset, pure form of HSP.²¹ In zebrafish, knockdown of strumpellin or transfection with disease-associated mRNA led to shorter motor neuron axons with abnormal branching when compared with controls, although the underlying mechanism is unknown.²¹

X-linked HSP

Mutations in the *L1CAM* gene, which encodes the L1 cell adhesion molecule at the Xq28 locus, are responsible for one of the more common forms of complicated HSP. *L1CAM* is a transmembrane

glycoprotein expressed mainly in neurons and Schwann cells, and seems to play an important part in the development of the nervous system and is involved in guidance of neurons in the developing CNS.¹²⁹ Different mutations in this gene also cause other neurological phenotypes.¹⁴

PLP1 mutations cause a complicated form of HSP and Pelizaeus-Merzbacher disease. *PLP1* and its smaller isoform *DM20* are the most abundant myelin proteins in the CNS. A link with trafficking has been made in the study of a mouse model with a null mutation of *PLP1*, in which impairment of fast anterograde and retrograde transport was shown.¹³⁰ At autopsy, patients with null mutations of *PLP1* have length-dependent axonal loss in the CNS.¹³¹

Overview of HSP pathogenetic mechanisms

The best-characterised genetic mechanisms in HSP support a view that defects at different cellular sites lead to impairment of transport of macromolecules and organelles, disturbance of mitochondrial function, or abnormalities of the developing axon. The figure shows the various intracellular sites where spastic paraplegia proteins have been found, suggested to reside, or function. Long spinal axons are likely to be susceptible to perturbations of membrane trafficking and axonal transport, and this might lead to abnormal axonal development, growth, and maintenance, and eventually to degeneration.

In *SPAST*-associated HSP, spastin seems to be involved in microtubule remodelling of the cytoskeleton. Mutations are thought to affect the transport of cargoes using this network by loss of function, and have been shown to affect axonal growth and branching. In *KIF5A*-associated HSP, *KIF5A* directly affects one of the molecular motors that powers fast anterograde axonal transport along microtubules. Other spastic paraplegia proteins might influence transport of endosomes and traffic between the ER and Golgi or plasma membrane. Another theme, identified through the study of *SPG7*, *HSPD1*, and *REEP1*, is of mitochondrial dysfunction due to disruption of protein quality control systems. This might disrupt mitochondrial function in general, but particularly oxidative phosphorylation and ATP synthesis, which might then affect axonal transport, an ATP-dependent process. Mitochondria are implicated in the pathogenesis of various neurodegenerative disorders, and the CNS seems to be disproportionately sensitive to mitochondrial dysfunction.¹³²

The recent finding of mutations in the gene that encodes *CYP7B1*¹⁸ is tantalising because cholesterol is a vital component of neuronal cells, in particular myelin. It is possible that abnormalities in the metabolism of cholesterol could influence the early development of axons (*CYP7B1* is associated with early-onset HSP) prior to degeneration, in a similar manner to that seen in *PLP1*-associated HSP.

Search strategy and selection criteria

References for this Review were identified through searches of PubMed from 1985, to August, 2008, by use of the terms “hereditary spastic paraplegia OR paraparesis”, “axonal degeneration”, and “SPG”. Articles were also identified from the authors’ own files. Only papers published in English were considered.

Conclusions

The increasing number of genes identified to be associated with HSP has, at face value, complicated the classification of the disorder. However, for clinicians it has led to several available genetic tests to simplify the diagnosis of both familial and sporadic cases of spastic paraplegia, with service testing available for *SPG3A*, *SPAST*, and *REEP1*, and many others available on a research basis. For the neuroscientist, the increasing number of proteins identified that can cause axonal degeneration in the spinal cord has led to novel insights into the processes that maintain these axons, and several pathogenetic themes are emerging, including those involving axonal transport and membrane trafficking. The unravelling of the processes that are vital to axonal homeostasis and the pathogenetic mechanisms underlying HSP should help us to identify potential therapeutic solutions. Importantly, it might also shed light on the early axonal pathology of other, more devastating, neurodegenerative disorders, such as motor neuron disease, Alzheimer’s disease, and Huntington’s disease.

Contributors

TTW coordinated the Review and prepared the first draft. All authors then contributed to revising and modifying this and subsequent drafts.

Conflicts of interest

We declare that we have no conflicts of interest.

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