

An Update on the Hereditary Spastic Paraplegias: New Genes and New Disease Models

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Abstract: Aims: The hereditary spastic paraplegias (HSPs) are a heterogeneous group of disorders characterized by spasticity in the lower limbs. We provide an overview of HSP with an emphasis on recent developments.

Methods: A PubMed search using the term “hereditary spastic paraplegia” and “hereditary spastic paraparesis” was conducted for a period from January 2012 to January 2015. We discuss and critique the major studies in the field over this 36-month period.

Results: A total of 346 publications were identified, of which 47 were selected for review. We provide an update of the common forms of HSP and include patient videos. We also discuss how next-generation sequencing (NGS) has led to the accelerated discovery of new HSP genes, including *B4GALNT1*, *DDHD1*, *C19orf12*, *GBA2*, *TECPR2*, *DDHD2*, *C12orf65*, *REEP2*, and *IBA57*. Moreover, a single study alone identified 18 previously unknown putative HSP genes and created a model for the protein interactions of HSP, called the “HSPome.” Many of the newly reported genes cause rare, complicated, autosomal recessive forms of HSP. NGS also has important clinical applications by facilitating the molecular diagnosis of HSP. Furthermore, common genetic forms of HSP have been studied using new disease models, such as neurons derived from induced pluripotent stem cells. These models have been used to elucidate important disease mechanisms and have served as platforms to screen for candidate drug compounds.

Conclusion: The field of HSP research has been progressing at a rapid pace. The challenge remains in translating these advances into new targeted disease therapies.

The term “hereditary spastic paraplegia” (HSP) is applied to a group of inherited neurodegenerative disorders characterized by progressive spasticity and weakness in the lower limbs.

There is marked genetic heterogeneity in HSP, with at least 55 genes and 72 loci identified thus far. The mode of inheritance can be autosomal dominant, autosomal recessive, X-linked, or maternal.¹

HSP can also be classified according to the clinical phenotype. In “pure” forms of HSP, the chief clinical feature is spasticity in the lower limbs, although other neurological signs may be present, such as a distal loss of vibration sense or bladder symptoms. In “complicated” forms, major additional manifestations are evident, such as cognitive impairment, seizures, dysarthria, cerebellar signs, and peripheral neuropathy. Thinning of

the corpus callosum (TCC) on cerebral MRI may also be found in patients with HSP. Additionally, some forms of HSP have a characteristic collection of symptoms and signs that can be described as “syndromic,” such as Silver syndrome, which is a form of HSP associated with amyotrophy of the small muscles of the hand.

Currently, the precise mechanisms underlying the HSPs are unknown and there are no disease-modifying treatments available. However, HSP research is advancing at a rapid pace, with major progress being made particularly over the last 3 years, and it is hoped that these advances will translate into new treatments in the future. We will provide an overview of HSP, review the major developments in the field, and discuss the clinical and research implications of these findings.

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Methods

In order to provide an update on HSP, we performed a PubMed search for references using the search terms “hereditary spastic paraplegia” and “hereditary spastic paraparesis.” In order to focus on the most recent developments in the field of HSP research, we restricted our search to articles published during a 36-month period from January 2012 to January 2015. Appropriate articles were critically reviewed for their suitability in relation to clinical relevance or research implications. Only articles published in English were selected, and no abstracts or reports from meetings were included.

Results

A total of 346 articles were identified using the search criteria, of which 47 were included in our review. We grouped the publications into several categories, including gene discovery, advances in molecular diagnosis, and the use of new disease models.

Epidemiology of HSP

A recent systematic review of prevalence studies showed that the global prevalence of autosomal dominant HSP ranged from 0.5 to 5.5 per 100,000, and that of autosomal recessive HSP from 0.0 to 5.3 per 100,000, with pooled averages of 1.8 per 100,000 (95% confidence interval [CI]: 1.0–2.7 per 100,000) and 1.8 per 100,000 (95% CI: 1.0–2.6 per 100,000), respectively.² Prevalence values varied widely across different geographical regions, a finding that was attributed to the different genetic makeup of the populations, as well as methodological heterogeneity of the different studies. In population-based studies, the number of families without a genetic diagnosis after systematic testing ranged from 45% to 67% in the autosomal dominant HSP and 71% to 82% in the autosomal recessive HSP groups, indicating that, at present, the majority of HSP patients do not receive a genetic diagnosis.²

Common Genetic Forms of HSP: An Update on the Clinical and Genetic Features

Despite the genetic heterogeneity of HSP, a small number of genetic forms account for a high proportion of cases. Of the dominant forms, SPG4, SPG3A, and SPG31 are the most frequent; for autosomal recessive disease, the most common causes are SPG11, SPG15, SPG7, and SPG5A. Examples of common forms of HSP can be seen in the patient videos (see Videos 1 and 2).

Autosomal Dominant Forms

Spastic Paraplegia 4

Spastic paraplegia 4 (SPG4) is the most common cause of autosomal dominant HSP, accounting for 45% of autosomal domi-

nant kindreds,^{3,4} although the prevalence may be higher in specific ethnic groups/geographical regions, such as Sardinia and Taiwan.^{5,6} SPG4 is the result of mutations in the gene *SPAST*, which encodes the protein spastin, a microtubule-severing protein.⁷ Onset is typically in early adulthood, but can vary from infancy to late adulthood. It usually results in a pure phenotype, although complex forms exist, with additional features, including ataxia, cognitive impairment, and seizures. In the majority of cases, patients present with a family history consistent with autosomal dominant inheritance, although *SPAST* mutations can also be found in approximately 10% of apparently sporadic cases.⁴

Spastic Paraplegia 3A

Spastic paraplegia 3A (SPG3A) is the second-most common cause of autosomal dominant HSP, constituting approximately 10% of cases,³ and is the most common form of HSP with onset before 10 years of age.⁸ It is caused by mutations in the *ATL1* gene encoding the atlastin protein.⁹ Most patients with SPG3A present with a pure form of HSP. Homozygous *ATL1* mutations were recently identified in a consanguineous family with HSP, suggesting that SPG3A can also have an autosomal recessive pattern of inheritance.¹⁰

Spastic Paraplegia 31

Spastic paraplegia 31 (SPG31) is the third-most common cause of HSP.¹¹ Mutations in the *REEP1* gene have been identified as the cause of SPG31.¹¹ The phenotype can be pure or complicated. The most common complication is peripheral neuropathy, and less frequent complications include cerebellar ataxia, dementia, and tremor. It can also result in a phenotype resembling Silver syndrome.¹²

Autosomal Recessive Forms

Spastic Paraplegia 11

Spastic paraplegia 11 (SPG11) is the most common cause of autosomal recessive HSP and is the major cause of HSP with a thin corpus callosum (HSP-TCC), accounting for 41% of HSP-TCC in one study.¹³ This form of HSP is the result of mutations in the *SPG11* (or *KIAA1840*) gene, encoding the spatacsin protein.¹⁴ Complicating features include parkinsonism, mental retardation, cognitive decline, lower motor neuron degeneration, and cerebellar signs.¹³ Mutations in *SPG11* have also been found to cause juvenile amyotrophic lateral sclerosis¹⁵ and have been reported to mimic multiple sclerosis.¹⁶

Spastic Paraplegia 15

Spastic paraplegia 15 (SPG15) is the second-most common cause of HSP-TCC after SPG11.¹⁷ The gene causing SPG15 has been identified as *ZFYVE26*, encoding a zinc-finger protein

with a FYVE domain named spastizin.¹⁸ The phenotype of SPG15 is largely indistinguishable from SPG11.¹⁹

Spastic Paraplegia 7

Spastic paraplegia 7 (SPG7) accounts for approximately 5% to 12% of cases of autosomal recessive HSP.²⁰ This form of HSP is the result of mutations in the *SPG7* gene, which encodes the paraplegin protein located in the inner mitochondrial membrane.²¹ It often results in complicated forms of HSP, with features such as cerebellar ataxia/cerebellar atrophy, optic atrophy, a chronic progressive external ophthalmoplegia-like phenotype, and mitochondrial abnormalities on muscle biopsy (Fig. 1). It has been shown that mutations in *SPG7* are an important cause of progressive external ophthalmoplegia with multiple mitochondrial DNA deletions, indicative of a disorder of mitochondrial DNA maintenance.²² Interestingly, there is an overlapping phenotype with the biological counterpart of SPG7, AFG3L2, which is mutated in SCA28.^{23,24} It has also been suggested that there may be a dominant effect for certain *SPG7* mutations.^{25,26}

Spastic Paraplegia 5A

Spastic paraplegia 5A (SPG5A) accounted for 7.3% of autosomal recessive HSP families and 3.3% of sporadic pure HSP patients in one study.²⁷ SPG5A is caused by mutations in the *CYP7B1* gene. This gene encodes a cytochrome P450 protein, implicating a role for impaired cholesterol metabolism in the pathogenesis of HSP.²⁸ It can result in a pure phenotype or a complicated form of HSP with ataxia, optic atrophy, and white matter lesions on MRI. There is a marked accumulation of 27-hydroxycholesterol in the plasma and cerebrospinal fluid of patients with SPG5A, which may be an important pathogenic factor.²⁹ Treatment with statin therapy has been shown to

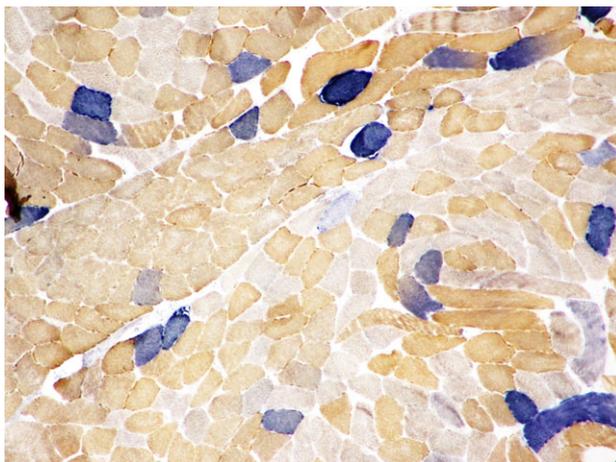


Figure 1 Muscle biopsy from a patient with SPG7 (p.A510V, p.S576W mutations, as previously reported³⁷), demonstrating an abnormal number of cytochrome oxidase negative/succinate dehydrogenase positive fibres consistent with a mitochondrial cytopathy.

reduce 27-hydroxycholesterol levels; this is a potential therapy that warrants further investigation.²⁹

Recent Discovery of New Causative Genes

The emergence of next-generation sequencing (NGS) technologies has allowed for a rapid acceleration in the discovery of new genes (Fig. 2). Genes discovered within the last 36 months are summarized in Table 1. A detailed listing of known genetic forms of HSP can be found elsewhere.^{30,31}

Many of the recently described genes cause rare autosomal recessive forms of HSP with complex phenotypes. In general, there are no clear diagnostic clues that readily distinguish these recently described genetic forms from other types of HSP.

Multiple HSP-Causing Genes Identified in One Study (Spastic Paraplegia 45, 59–71)

In a recent landmark study, Novarino et al. attempted to identify the cause of autosomal recessive HSP in 55 consanguineous families using whole-exome sequencing (WES).³² Using this approach, the genetic basis of HSP was identified in 75% of cases. Mutations were identified in 13 known disease-causing genes, including *EIF2B5*, *CLN8*, and *ARG1*. Impressively, they also identified 15 putative HSP genes that were unknown at the time of the study: *DDHD2*, *KIF1C*, *USP8*, *WDR48*, *ARL6IP1*, *ERLIN1*, *AMPD2*, *ENTPD1*, *NT5C2*, *ARSI*, *PGAP1*, *FLRT1*, *RAB3GAP2*, *MARS*, and *ZFR*. A candidate HSP gene was reported only if there was a single deleterious variant that segregated in the family or if the gene was identified as mutated in multiple families. Several genes (*ARL6IP1*, *MARS*, *PGAP1*, and *USP8*) were private, that is, mutated in a single family only. In order to validate these genes, the investigators used a knockdown zebrafish model to demonstrate a functional effect.

The investigators also looked to see how HSP-related proteins interacted within a protein network. They first extracted the subnetwork of all previously reported HSP genes from a protein network of all known human proteins (termed the HSP seed network). They then extracted the subnetwork containing all seed genes plus candidate HSP genes to derive the HSP seed plus candidate network. Statistical analysis revealed that both the HSP seed network and the HSP seed plus candidate network were more highly connected than expected by chance. The investigators used these data to identify proximal interactors in the network. These data culminated in the so-called “HSP interactome” or “HSPome,” a network of HSP seeds plus candidates plus proximal interactors.

The “HSPome” provides us with a global overview of HSP and flags other potential genes that may be mutated in HSP patients. The investigators used the putative causal genes to identify major modules involved in the pathophysiology of HSP. This included defective endoplasmic reticulum (ER)-associated degeneration, disruption of the endosomal sorting complexes required for transport, and a novel pathway involving disturbed purine nucleotide metabolism.

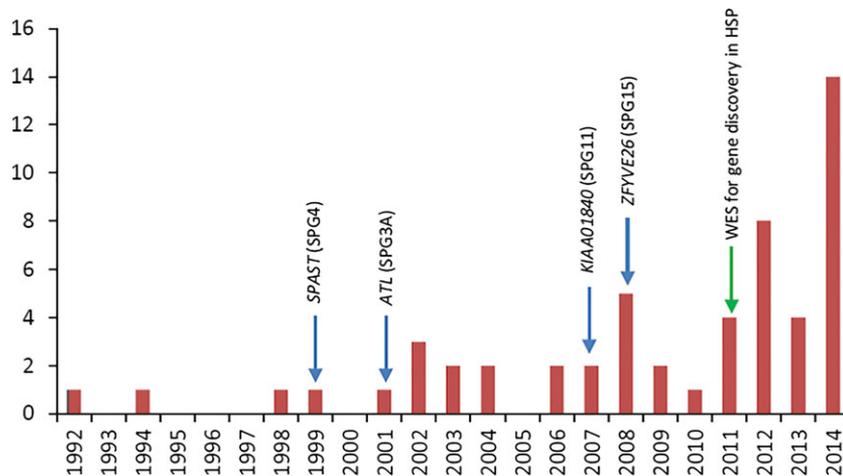


Figure 2 Number of HSP genes discovered per calendar year. The most common HSP genes are indicated (blue arrows). Whole-exome sequencing has been used for gene discovery in HSP since 2011 (green arrow), resulting in a marked increase in the rate of new genes being identified.

The investigators interrogated their exome database for variants in genes emerging from the extended HSPome network. By using this approach, potentially pathogenic variants were identified in three genes (*MAG*, *BICD*, and *REEP2*) found in homozygous linkage intervals in three different families, which appeared to support the usefulness of the HSPome for gene discovery.

The investigators of this study were appropriately careful to label the new genes as “candidates” only, and the results need to be interpreted with a degree of caution until mutations in these genes are identified in unrelated families. Notably, several candidate genes have been subsequently confirmed in independent studies, including *REEP2*,³³ *DDHD2*,^{34,35} and *KIF1C*.³⁶

The investigators also used the network to examine the similarity of HSP genes (seed plus candidate) to other common neurological disorders. They found that there was a significant overlap with sets of genes previously implicated in three neurodegenerative diseases: amyotrophic lateral sclerosis; Alzheimer’s disease; and Parkinson’s disease.

This study was able to identify a large number of novel HSP genes that appear to converge on several key biological pathways. Some of these forms of HSP may be potentially treatable, such as those involving metabolic defects (e.g., purine nucleotide metabolism) that could be potentially bypassed. The HSPome could be a useful tool for HSP researchers to discover new genes in the future, particularly in families with private mutations.

Further Evidence that HSP Genes are Allelic with Other Disorders

It is clear from recent reports that HSP genes can be allelic with other disorders (Table 2), further emphasizing the overlap between HSP and other clinical phenotypes. It can be hard to distinguish between these disorders according to the clinical manifestations alone. For example, HSP complicated by neuropathy can be difficult to differentiate from a hereditary neuropathy with pyramidal features, and HSP complicated by

ataxia can be difficult to distinguish from a type of spastic ataxia (SPAX). It can be argued that these disorders are not truly distinct, but rather that they exist on a clinical spectrum. This also makes it challenging to determine whether or not a disorder should be included under the category of HSP.³¹

Approaches to the Genetic Diagnosis of HSP

A molecular diagnosis in HSP can be advantageous because it provides for diagnostic certainty, avoids further unnecessary investigations, assists genetic counseling, enables a preimplantation or prenatal genetic diagnosis, and facilitates recruitment into future clinical trials.

The advent of NGS has provided clinicians with new approaches for identifying a molecular diagnosis in HSP (Fig. 3). This technology is proving to be practical, timely, and cost-efficient and avoids a long diagnostic odyssey involving numerous individual genetic tests ordered in series. A disadvantage of NGS is that it frequently detects variants of uncertain pathogenicity, requiring further investigations, including family studies, expression studies, and so on. Furthermore, there are ethical issues related to the identification of “incidental findings,” such as the detection of mutations in cancer-predisposing genes, highlighting the importance of careful pre- and post-test genetic counseling.

NGS Gene Panels

One approach is to use targeted NGS to screen a large panel of genes that would otherwise have been prohibitively expensive and time-consuming to achieve using conventional Sanger sequencing. This approach allows for good coverage of the genes of interest and was able to identify the genetic cause of HSP in 40% of individuals with autosomal recessive or sporadic inheritance in one study.³⁷ A disadvantage of this directed approach is that NGS panels are limited to known HSP-causing genes and can rapidly become out of date.

TABLE 1 List of recently discovered HSP genes (last 36 months)

SPG Loci	Inheritance	Gene	Molecular Insights	Clinical Phenotype	Number of Families Identified Worldwide, to Date (Ethnicity)
SPG12	AD	<i>RTN2</i>	Abnormal ER morphogenesis	Pure HSP ⁵²	4 (multiethnic)
SPG26	AR	<i>B4GALNT1</i>	Disturbance in ganglioside biosynthesis	Early-onset form of HSP complicated by cognitive impairment, psychiatric illness, cerebellar ataxia, peripheral neuropathy, and cortical atrophy and white matter hyperintensities on neuroimaging. ⁵³ Affected subjects may have absent GM2 (ganglioside) and increased levels of its precursor GM3 ⁵⁴	12 (multiethnic)
SPG28	AR	<i>DDHD1</i>	Altered lipid metabolism leading to mitochondrial dysfunction	Initially associated with a pure form of HSP onset 6 to 15 years of age, although complicating features have been described, including axonal neuropathy, distal sensory loss, cerebellar eye movement disturbances, subtle neuropsychological deficits, and impairment of brain and muscle energy metabolism on MRS ^{55,56}	5 (multiethnic) Found in 1 of 96 index patients from AR HSP families in a follow-up study ⁵⁶
SPG43	AR	<i>C19orf12</i>	The cellular functions of <i>C19orf12</i> are unclear	Gait difficulty, spasticity, and peripheral neuropathy, age of onset 7 and 12 years. ⁵⁷ <i>C19orf12</i> mutations have also been found to cause NBIA (see Table 2).	1 (Malian)
SPG46	AR	<i>GBA2</i>	Connects glucosylceramide metabolism and HSP	HSP with infantile or childhood onset and a phenotype complicated by cognitive impairment, cataract, TCC, cerebellar atrophy, hypogonadism in males, no residual glucocerebrosidase activity of <i>GBA2</i> in blood cells of one affected individual. ⁵⁸ Also causes an AR cerebellar ataxia phenotype (see Table 2)	5 (multiethnic) In a follow-up study, found in 1 of 46 patients with AR or sporadic complicated HSP ⁵⁹
SPG47	AR	<i>AP4B1</i>	The AP4 complex is ubiquitously expressed in neurons and is involved in vesicle formation, trafficking, and sorting processes.	Progressive spastic paraplegia, intellectual disability, seizures, periventricular white matter changes, and TCC ⁶⁰	4 (multiethnic)
SPG49	AR	<i>TECPR2</i>	Disturbance of autophagy	HSP with onset in childhood and a phenotype complicated by severe intellectual disability, fluctuating central hypoventilation, wake apnea, gastroesophageal reflux disease, areflexia, distinct dysmorphic features, and TCC ⁶¹	3 (Jewish Bukharian)
SPG54	AR	<i>DDHD2</i>	Impaired lipid metabolism	Early-onset (<2 years) spastic paraplegia, intellectual disability, short stature, and dysgenesis of the corpus callosum. ^{34,35} Patients may also present with a spastic ataxia phenotype. An abnormal lipid peak may be detected on MRS. ³⁴	9 (multiethnic) In a follow-up study, found in 1 of 150 probands with AR or sporadic complicated HSP ⁵⁹
SPG55	AR	<i>C12orf65</i>	Implicates dysfunction of mitochondria	Childhood-onset HSP; key clinical features include the clinical triad of optic atrophy, axonal neuropathy, and spastic paraparesis. ⁶² Mutations in this gene can cause other distinct phenotypes (Table 2).	2 (multiethnic)
SPG56	AR	<i>CYP2U1</i>	Impaired lipid metabolism	Early-onset complicated HSP phenotype, frequently involving the arms, rarely causing dystonic postures and cognitive impairment, infraclinical axonal neuropathy on nerve conduction studies, with MRI findings of a TCC, white matter abnormalities, and/or calcification of the basal ganglia ⁵⁵	6 (multiethnic) In a follow-up study, found in 1 of 150 probands with AR or sporadic complicated HSP ⁵⁹

TABLE 1 (Continued)

SPG Loci	Inheritance	Gene	Molecular Insights	Clinical Phenotype	Number of Families Identified Worldwide, to Date (Ethnicity)
SPG57	AR	<i>TFG</i>	Disturbance of morphology and function of the ER	Early-onset spastic paraplegia, optic atrophy, and an axonal demyelinating motor neuropathy ⁶³	1 (Indian)
SPG72	AD and AR	<i>REEP2</i>	Disturbance of ER morphology and trafficking activity	Pure, with an early age of disease onset ³³	3 (multiethnic)
HSP loci not assigned	AR	<i>IBA57</i>	Disturbance in mitochondrial [4Fe-4S] protein maturation	A combination of spastic paraplegia, optic atrophy, and peripheral neuropathy (SPOAN) ⁶⁴	1 (Arab)
SPG45, 59–71	AR	Discussed separately in the text			

AD, autosomal dominant; AR, autosomal recessive; ER, endoplasmic reticulum; HSP, hereditary spastic paraplegia; MRS, magnetic resonance spectroscopy; NBIA, neurodegeneration with brain iron accumulation; TCC, thin corpus callosum.

WES and Whole-Genome Sequencing as Diagnostic Tools in HSP

Another approach is to search for mutations in known disease genes using WES.³⁸ There are several advantages to this approach, including the identification of unexpected disease genes that would otherwise be missed using a directed approach. For example, genome-wide linkage analysis and exome sequencing was recently used to identify a mutation in the *LYST* gene, known to cause Chédiak-Higashi syndrome, as the cause of autosomal recessive, complicated HSP.³⁹ Furthermore, a patient with a phenotype resembling HSP was unexpectedly found to have a *GCH1* mutation on WES, leading to the diagnosis of dopa-responsive dystonia and successful treatment with levodopa.⁴⁰ However, the disadvantages of using an exome sequencing approach is that compared to a “targeted panel,” the coverage of known HSP genes may not be as complete.³⁷

Whole-genome sequencing (WGS) offers several advantages over WES, including more-consistent coverage and better detection of noncoding variants, copy number variants, and chromosomal modifications. WGS is likely to offer a greater diagnostic yield, compared to WES, and will perhaps be the genetic investigation of choice in the future.

DNA-Microarray-Based Diagnosis

Recently, investigators designed a high-throughput bead array genotyping chip using the reported common hotspot HSP mutations.⁴¹ This technique could be a useful method for preliminary genetic screening for HSP patients, but it can only be used to test for 96 common point mutations.

A Conventional Genetic Testing Strategy in HSP

A series of directed genetic tests targeting the most common genetics forms of HSP is a good option when other strategies are unavailable.

In those with autosomal dominant inheritance, approximately half will have SPG4, and so these patients should undergo

sequencing of the *SPAST* gene as the initial genetic investigation. Additional methods, such as multiplex ligation-dependent probe amplification, may also be required owing to the high frequency of partial deletions of the *SPAST* gene in autosomal dominant HSP. SPG3A and SPG31 should also be considered given that they are relatively common causes of autosomal dominant HSP.

Autosomal recessive HSP should be tested according to whether there is evidence of a TCC on neuroimaging.⁴² Those with a TCC should be tested for SPG11 and SPG15, whereas those without TCC should be testing for SPG5A and SPG39 (childhood-onset) or SPG7 (adult-onset). As mentioned above, SPG4 can be found in sporadic cases, and so *SPAST* mutations should be excluded even in those patients without a family history.

Investigating Disease Mechanisms

The corticospinal tract is one of the longest projection pathways in the central nervous system, with axons up to 1 meter in length and the axonoplasm comprising more than 99% of the total cell volume.⁴³ This length is necessary to permit the rapid relay of actions potentials, enabling timely voluntary movements. Complex intracellular machineries are required to sort and distribute intracellular cargoes over these long distances. This includes microtubules, which form part of the cytoskeletal scaffolding and provide platforms for intracellular transport. Furthermore, a variety of mechanoenzymes within the kinesin, dynein, and myosin protein superfamilies facilitate much of the anterograde and retrograde transport specificity through selective cargo interactions.⁴³ Various adaptor proteins allow for additional specificity and regulatory control. These processes allow for the tightly controlled delivery of intracellular cargoes to the growth cones during axonal development and to specialized axon domains.⁴³ HSP results from a disturbance in these cellular processes, leading to a length-dependent impairment of axon development and maintenance. Neuropathological studies show degeneration of corticospinal tract axons (chiefly in the thoracic spinal cord) and degeneration of *fasciculus gracilis* fibers (predominantly in the cervicomedullary region), supporting the hypothesis that HSP is a length-dependent, “dying back” axonopathy.^{31,44} The common clinical and pathological features of the different HSPs

TABLE 2 Recent examples of HSP genes implicated in other clinical phenotypes (last 36 months)

Alternate Clinical Phenotype		Gene	SPG Loci
Neuropathies	Hereditary sensory neuropathy type 1 ⁶⁵	<i>ATL1</i>	SPG3A
	Distal hereditary motor neuropathy type 5 ⁶⁶	<i>REEP1</i>	SPG31
	Charcot-Marie-Tooth disease type 2 (hereditary motor and sensory neuropathy 2) with pyramidal features ⁶⁷	<i>KIF5A</i>	SPG10
	Charcot-Marie-Tooth disease type 6 (hereditary motor and sensory neuropathy 6) ⁶⁸	<i>C12orf65</i>	SPG55
	Hereditary motor and sensory neuropathy with proximal dominant involvement (HMSN-P) ⁶⁹	<i>TFG</i>	SPG57
Cerebellar ataxia	Autosomal recessive cerebellar ataxia (ARCA) with spasticity ⁷⁰	<i>GBA2</i>	SPG46
	Two clinically defined syndromes in which early-onset autosomal recessive cerebellar ataxia occurs with hypogonadotropic hypogonadism: (1) Boucher-Neuhäuser syndrome, which is also associated with chorioretinal dystrophy, and (2) Gordon Holmes syndrome, which may be accompanied by brisk reflexes ⁷¹	<i>PNPLA6</i>	SPG39
Mitochondrial disease phenotypes	Combined oxidative phosphorylation deficiency type 7 (COXPD7) resulting in optic atrophy and Leigh syndrome-like disease ⁷²	<i>C12orf65</i>	SPG55
	Progressive external ophthalmoplegia with spastic paraplegia ²²	<i>SPG7</i>	SPG7
	Multiple mitochondrial dysfunction syndrome 3 ⁷³	<i>IBA57</i>	No HSP loci assigned
NBIA	Neurodegeneration with brain iron accumulation 4 ⁷⁴	<i>C19orf12</i>	SPG43
IBMPFD	Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia ⁷⁵	<i>VCP</i>	No HSP loci assigned

appear to converge on a small number of functional groups involving membrane trafficking and organelle shaping, mitochondrial regulation, myelination and lipid/sterol modification, and axon pathfinding.⁴³ For example, SPG4 is the result of mutations in *SPAST*, which encodes spastin, a microtubule severing protein. Mutations in *SPAST* may lead to a disturbance in the functions of the spastin protein involving the formation of the microtubule cytoskeleton and in the shaping of the ER network.⁴³

Key disease pathways in HSP can be recapitulated using a variety of different disease models. By pharmacologically manipulating these disease pathways, researchers can identify potential novel therapeutic agents.

Established Disease Models

Several animal models have been used to study HSP, including bovine, murine, zebrafish, *Drosophila*, and *Caenorhabditis elegans*.³¹ For instance, investigators used a drosophila model of SPG4 using neural knockdown of drosophila spastin.⁴⁵ They were able to demonstrate HSP-like phenotypes and recapitulate important molecular mechanisms, such as excessive stabilization of microtubules in the neuromuscular junction synapse. They also provided evidence that administration of the microtubule-disrupting drug, vinblastine, significantly attenuates these phenotypes in vivo. Another group studied SPG7 using paraplegin-deficient mice and showed that intramuscular viral delivery of paraplegin rescues peripheral axonopathy.⁴⁶ This study provided proof of principle that gene transfer may be an effective therapeutic option for patients with paraplegin deficiency.

New Disease Models

New human neuronal models of disease have potential advantages over traditional animal models. For example, animal models may

not be fully representative of human disease pathophysiology, owing to differences in brain development and signaling pathways. Studies using these new disease models have focused on the most prevalent HSP genes.

Neurons Derived from Induced Pluripotent Stem Cells

Recently, Denton et al. successfully generated a human neuronal model of SPG4 by establishing induced pluripotent stem cells (iPSCs) from a patient with SPG4 and then differentiating these cells into telencephalic glutamatergic neurons.⁴⁷ They were able to demonstrate evidence of axonal swellings in keeping with an accumulation of axonal transport cargoes. They also showed increased levels of acetylated tubulin in SPG4 neurons, consistent with impaired function of spastin as a microtubule severing protein. Notably, they were able to rescue the axonal swelling phenotype using vinblastine.

SPG3A is caused by mutations in the *ATL1* gene, which encodes the atlastin 1 GTPase protein. This protein mediates homotypic fusion of ER tubules to form the polygonal ER network. Investigators recently modeled SPG3A using skin fibroblasts from a young girl with a novel *ATL1* mutation.⁴⁸ The fibroblasts were used to produce iPSCs, which were then differentiated into forebrain neurons. The SPG3A neurons displayed markedly reduced axon outgrowth and also showed alterations in mitochondrial motility. The axon growth defects could be rescued by treatment with the microtubule-destabilizing drugs, vinblastine and taxol, indicating an important role for ER interactions with dynamic microtubules. The results suggest that abnormal axonal development plays a key role in SPG3A pathogenesis. These findings are discordant with the notion that HSP is a progressive disorder characterized by distal dying-back axonal degeneration, rather than being the result of a primary defect in the growth of axons. This was reconciled by the fact that SPG3A is the most common early-onset

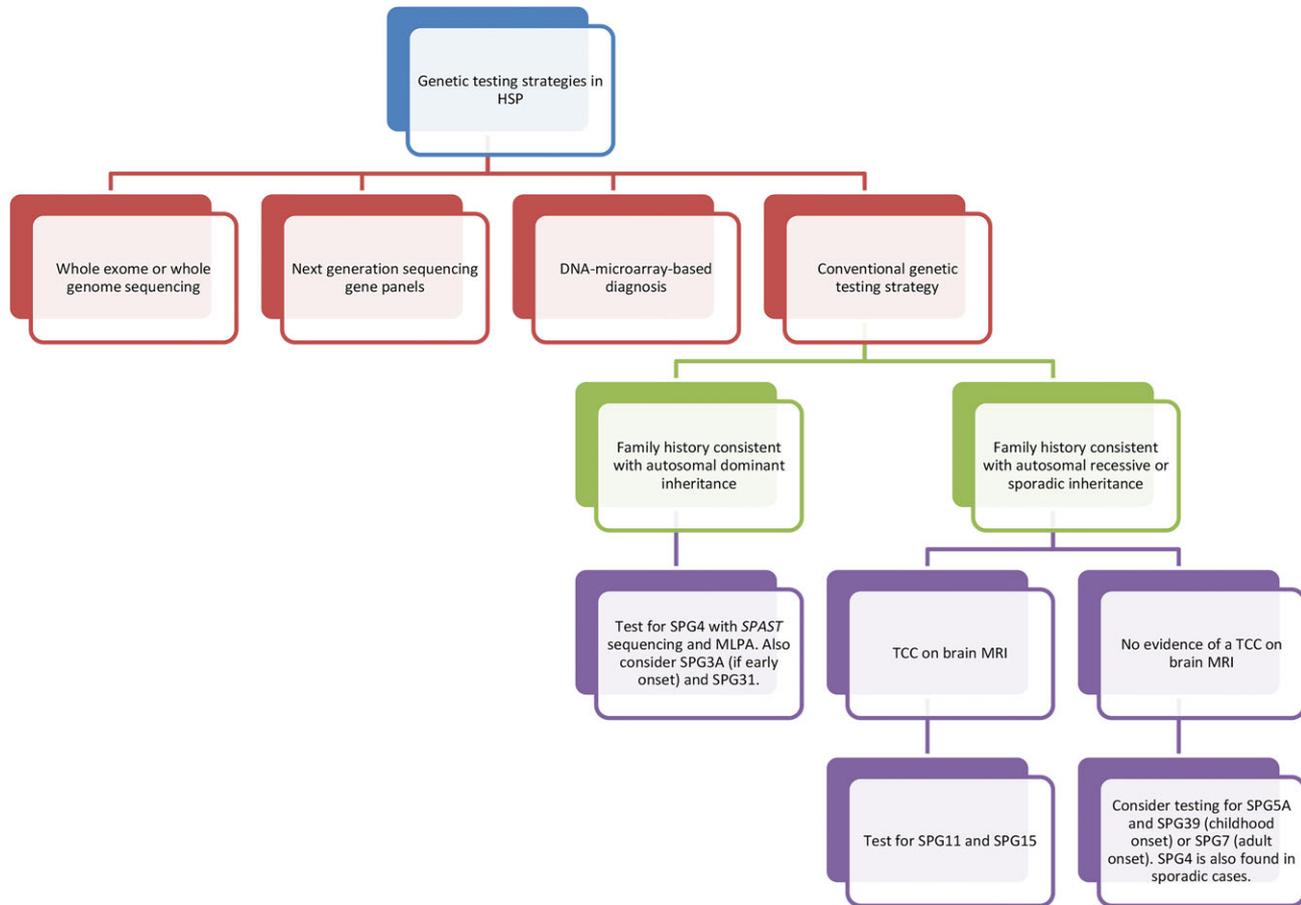


Figure 3 Genetic testing strategies in HSP. MLPA, multiplex ligation probe amplification.

form of HSP, with many individuals presenting with nonprogressive forms.⁴⁸

Another group analyzed the expression and function of the spatacsin protein (mutated in SPG11) in human forebrain neurons derived from human iPSCs, including patient and control lines.⁴⁹ The SPG11 patient-derived neurons exhibited downregulation of specific axonal-related genes, a decrease in neurite complexity, and an accumulation of membranous bodies within axonal processes. These findings pointed toward axonal dysfunction in human neurons with SPG11 mutations. The investigators also showed evidence of axonal pathology in mouse cortical neurons.

Olfactory Neurosphere-Derived Cells

Neural progenitor cells derived from biopsies of the olfactory mucosa were used as a model to compare healthy controls and patients with SPG4, in order to identify cell functions altered in HSP.⁵⁰ Patient-derived cells were smaller than control cells, had altered intracellular distributions of peroxisomes and mitochondria, and had slower-moving peroxisomes. There was also a major dysregulation of gene expression. Compared to control cells, patient-derived cells had 50% spastin, 50% acetylated α -tubulin (a marker of stable microtubules), and 150% stathmin (a microtubule-destabilizing enzyme). The paradoxical finding

of reduced acetylated α -tubulin was ascribed by the investigators to increased stathmin expression as a compensatory mechanism, leading to a reduction in stabilized microtubules and altered organelle trafficking (this result is contradictory to the findings of Denton et al., perhaps reflecting the fact that they used a different disease model¹⁷). Cells were exposed to subnanomolar doses of two microtubule inhibitors, paclitaxel and vinblastine. This resulted in increased acetylated α -tubulin levels in patient cells, compared to control levels, which appeared to indicate the utility of this cell model for screening candidate compounds for drug therapies.

Conclusion

Our understanding of HSP is progressing at a rapid pace. The complexity of the disorder and the vast number of underlying genetic etiologies make developing a diagnostic approach to HSP particularly challenging.

The advent of NGS technology has seen a large number of new HSP genes identified. Many of the new disease genes cause rare, early-onset, autosomal recessive forms of HSP with complex phenotypes. Therefore, these findings may be of limited clinical relevance to Western societies, where pure forms of HSP with autosomal dominant inheritance predominate. However, the gene discoveries have provided insights into the

pathophysiological mechanisms underpinning HSP, reinforcing the fact that several key molecular pathways may be involved, including defects in endomembrane trafficking, mitochondrial function, and lipid metabolism.

Several new disease models have allowed researchers to interrogate the molecular pathways further in the common, established genetic forms of HSP. Of these new models, neurons derived from iPSCs promise to be the most biologically relevant model available. There are inherent difficulties using stem cells to model human disease, as outlined by others.⁵¹ There may be specific difficulties studying HSP using these techniques, given that HSP affects the longest motor axons, a feature that is difficult to recapitulate in a laboratory dish. Despite these hurdles, researchers appear to have successfully used these new disease models as drug discovery platforms. Several candidate drugs have emerged, particularly microtubule–destabilizing drugs, such as vinblastine and taxol.

The goal is to eventually test the candidate therapies in human clinical trials. It is likely that the side effects of chemotherapeutic agents, such as vinblastine, will be unacceptable to most patients with HSP, although perhaps smaller doses of these agents can be used that will avoid toxicity.⁵⁰ Novel treatments should perhaps be targeted to patients who are presymptomatic or have early-stage disease, given that major neurodegenerative changes are yet to take place. It will be interesting to determine whether candidate therapies are broadly effective for all forms of HSP or whether the benefits will be limited to certain HSP genotypes. A further difficulty will be recruiting enough patients for randomized, control trials, and this will require large multicenter collaborative efforts.

There have been major advances in the field of HSP research over the last few years. The challenge remains to convert these advances into new disease-modifying therapies that have meaningful benefits for patients.

Author Roles

(1) Research Project: A. Conception, B. Organisation, C. Execution; (2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; (3) Manuscript Preparation: A. Writing of the First Draft, B. Review and Critique.

K.R.K.: 1A, 1B, 1C, 3A, 3B

N.F.B.: 1A, 1B, 1C, 3A, 3B

C.M.S.: 1A, 1B, 1C, 3B

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Supporting Information

Video accompanying this article are available in the supporting information here.

Video 1. Typical features of SPG4 and SPG31.

Video 2. Eye signs in SPG7 and a family with SPG5A.³⁷