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Genetics in hereditary spastic paraplegias: essential but not enough

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Highlights

- Genetics is the primary driver of disease in hereditary spastic paraplegias
- The identification of mutated genes is not sufficient to predict disease evolution
- Biomarkers may help to predict disease course

Abstract

Hereditary spastic paraplegias consist of a group of rare neurodegenerative diseases characterized by lower-limb spasticity. These inherited Mendelian disorders show high genetic variability associated with wide clinical diversity. Pathophysiological investigations have suggested that mutations in genes affecting the same cellular pathway generally lead to similar clinical symptoms, highlighting the importance of genetic mutation in these diseases. However, phenotype-genotype correlations have failed to explain the observed large inter-individual variability linked to mutations in a single gene, suggesting that genetics alone is not sufficient to explain symptom diversity. The identification of biomarkers, such as neurofilament light chain, could fill the gap and predict disease evolution.

Main text

Hereditary spastic paraplegias (HSPs) consist of a group of rare hereditary neurodegenerative diseases characterized by lower-limb spasticity [1]. They are inherited Mendelian disorders that show high genetic heterogeneity, with more than 80 distinct genetic loci and over 60 gene products identified and named *Spastic Paraplegia Gene* (SPG) 1-83. However, leg spasticity is also present in numerous other genetic diseases, such as leukodystrophies (e.g., X-linked adrenoleukodystrophy) or motoneuron diseases (e.g.,

amyotrophic lateral sclerosis) further extending the genetic heterogeneity of this group of diseases. Such genetic diversity is associated with wide clinical variability, varying from isolated pyramidal symptoms in the legs, i.e., primarily first motoneuron involvement, to a complex combination of first and second motoneuron involvement associated with various other neurological symptoms, such as cognitive impairment, cerebellar ataxia, and peripheral neuropathy [2].

Such pure to complex variability depends, at least partially, on the nature of the mutated gene, suggesting that genetics is the primary driver of the nature of symptoms in HSP patients (Figure 1). However, there is wide inter-individual variability within each genetic entity that is yet to be explained. We propose that biomarkers of disease progression may help to explain some variability and would be of great interest for patient care and trial design.

Genetics is the primary driver of disease in HSP

Despite considerable genetic heterogeneity, the function of proteins encoded by genes mutated in HSPs converge on just a few cellular mechanisms [3]. However, the nature of the biological functions altered by gene mutations strongly influence the symptoms of patients and thus are a strong indicator of disease progression (Figure 1).

One example of functional convergence is the involvement of genes encoding proteins important for myelin maintenance [4]. *PLP1* (SPG2) and *MAG* (SPG75) encode structural myelin protein and a myelin membrane glycoprotein, respectively. *B4GALNT1* (SPG26) is required for the synthesis of complex gangliosides, which may act as neuronal receptors for MAG on axons. *FA2H* (SPG35) encodes an enzyme involved in the synthesis of 2-hydroxylated fatty acids, highly enriched in myelin. Finally, loss-of-function mutations in the kinesin KIF1C (SPG58) are responsible for focal demyelination [5]. Animal models of each of these diseases show normal myelination but later, altered myelin maintenance. Patients with mutations in these genes all show severe disease, with early-onset recessive forms characterized by neurodevelopmental features. Despite the diversity of molecular functions affected by mutations in these five genes, the similarity of symptoms and consistent impaired myelin maintenance observed in animal models highlight the existence of a subclass of HSPs associated with myelin maintenance. Myelin is also altered due to mutations in *GJC2* encoding connexin 47 (SPG44) or in other diseases featuring spasticity, such as X-linked adrenoleukodystrophy, the most frequent form of leukodystrophy, which presents as HSP in forms of adrenomyelonopathy in men and women due to mutations in *ABCD1* [4]. Whether mutations in these genes only affect myelin maintenance is, however, not known.

Loss-of-function mutations in *SPG11*, *ZFYVE26* (SPG15), *AP5Z1* (SPG48), and *ATP13A2* (SPG78) have been associated with the accumulation of membranes or lipofuscin-like material in lysosomes [6–10], reminiscent of phenotypes observed in certain lysosomal-storage disorders. Mutations in these genes are associated with early-onset spasticity, intellectual deficiency and later cognitive impairment, peripheral neuropathy, and Parkinsonism, highlighting the convergence of the affected cellular functions with a particular clinical HSP phenotype. Somewhat related to this function, mutations in *VPS37A* (SPG53) and *UBAP1* (SPG80), encoding subunits of the ESCRT-I complex, may affect endosomal sorting. However, there is a certain lack of convergence, as mutations in SPG53 result in a severe form of HSP associated with neurodevelopmental delay, whereas SPG80 mutations lead to relatively pure spasticity in the lower limbs, although the description of the clinical symptoms needs to be refined due to the low number of patients. SPG53 is autosomal recessive, leading to a loss of function of VPS37A [11], whereas *UBAP1* mutations are truncating mutations that affect only one allele [12,13]. This difference may underlie the clinical divergence between these two forms of HSP that affect the same cellular pathway.

Another set of HSP genes encoding proteins involved in the maintenance of endoplasmic reticulum (ER) morphology are associated with relatively pure pyramidal forms of autosomal dominant HSPs. Atlastin-1 (SPG3) is a GTPase involved in homotypic fusion of the ER. REEP1 (SPG31), REEP2 (SPG72), and reticulon-2 (SPG12) harbor hydrophobic domains that form hairpins and insert into the ER, contributing to the high membrane curvature of this organelle. A similar hairpin domain is also present in the long isoform of spastin encoded by *SPAST* (SPG4), allowing its association with the ER. Loss of the ER morphogens spastin, atlastin-1, or REEP1 are associated with lower levels of triglycerides and a decrease in the number or size of lipid droplets (LDs) [14–16] tightly associated with the ER, consistent with the role of the ER in LD formation [17]. It is not currently known whether the alteration of ER morphology or LD formation is responsible for the onset of symptoms in SPG3, SPG4, and SPG31 patients. These rather-purely pyramidal forms of HSP are all associated with a decrease in the number or size of LDs. By contrast, the accumulation of LDs and triglycerides has been observed in *Ddhd2* (SPG54) and *Spartin* (SPG20) knockout models [18,19], both of which are complex forms of HSP. These observations suggest that mutations leading to high LD formation are associated with complex HSPs, whereas mutations associated with lower LD formation lead to relatively pure forms of HSPs, highlighting the need for thorough pathophysiological studies to identify reliable clusters of genes associated with certain HSP subgroups (Figure 1).

Finally, several genes associated with HSPs encode mitochondrial proteins. An interesting feature in this subgroup is the high variability of symptoms observed in patients, varying from relatively pure spasticity to very severe neurodevelopmental disorders. This has been notably observed with mutations in *FARS2* (SPG77), *C12orf55* (SPG55), *IBA57*, and *HPDL* [20,21]. Patients with mutations in these genes also frequently show optic atrophy, as observed in patients with mutations in *SPG7*, the most frequent form of HSP associated with mitochondria, in which patients can show symptoms varying from spasticity to ataxia or neuropathy [22]. Such variable presentation of symptoms highlights that although genetic mutations are responsible for the disease, identification of the mutated gene is not, on its own, sufficient to predict disease evolution.

Identification of the mutated gene is not sufficient to predict disease evolution

The identification of mutated genes in HSP patients is critical, as the mutation is a strong driver of the pathology. However, patients with mutations in the same gene can show a very different course of symptom progression, or sometimes even different diseases, suggesting that identification of the gene mutation is not sufficient to fully predict disease evolution.

Wide clinical divergence is observed, but may be explained, in patients with mutations in genes encoding proteins involved in the degradation of IP3 receptors (*ERLIN1*/SPG62, *ERLIN2*/SPG18, *RNF170*). RNF170 is a E3-ubiquitin ligase that is recruited by Erlin1/2 complexes to active IP3 receptors to promote their degradation [23]. Patients with autosomal recessive diseases present a severe form of HSP, associated with neurodevelopmental delay, that is likely due to the loss of function of encoded proteins. By contrast, patients with autosomal dominant point mutations in *ERLIN2* and *RNF170* show a relatively pure form of HSP or pure sensory ataxia, respectively [24]. Both types of mutations impair the degradation of IP3 receptors but the mechanisms underlying the phenotypic difference between severe autosomal recessive HSP and the less severe autosomal dominant disease are yet to be elucidated.

As illustrated above, autosomal dominant mutations in genes encoding ER morphogens are associated with pure HSPs. However, amongst the ER morphogens, ARL6IP1 (SPG61) may be an outlier, as it is associated with complex childhood-onset HSP. This form of the disease is not dominant, but autosomal recessive, and thus likely due to a loss of function of ARL6IP1, which impairs continuity of the ER tubular network and locomotor activity in a Drosophila model [25]. Of note, autosomal recessive mutations in the ER

morphogen *REEP1* cause spinal muscular atrophy [26]. Replicated in a mouse model, the complete knockout of *Reep1* led to a more severe phenotype than inactivation of only one allele [27]. The number of mutated alleles, and thus haploinsufficiency or loss of function of the ER morphogens, have an impact on disease evolution that is not explained by the molecular function of the mutated gene products. In addition, both autosomal dominant and recessive mutations in SPG72 lead to a similar phenotype [28] caused by the loss of REEP2 function. These examples highlight the requirement of thorough cellular studies of the pathophysiological mechanisms to understand varying disease severity in HSP patients. They also illustrate that identification of the mutated gene is not sufficient to predict disease evolution, and functional characterization of the consequences of mutations are required to establish a prognosis.

In further support of the need to precisely study gene variations, another illustration of the insufficient knowledge provided solely by the identification of the causative gene comes from a study of a large cohort of SPG4 patients. These patients show involvement of the corticospinal tract and posterior columns, rarely associated with other neurological signs. Despite a mean age at onset of 35 years and a slow disease course, individual disease trajectories are highly variable [29]. The age at onset ranges from birth to almost 80 years and there is incomplete age-dependent penetrance, highlighting the high inter-individual variability in the most frequent genetic entity in HSPs. We gathered more than 800 patients to confirm the bimodal distribution of the age at onset and show, for the first time, that a younger age at onset is associated with missense mutations and an older age at onset with truncating mutations [30]. There was high intrafamilial variability, suggesting that modifying genetic or environmental factors could affect the age at onset. Amongst known genetic modifiers, the presence of the non-pathogenic S44L variant in *SPAST* [31], together with a pathogenic variant in *SPAST*, decreased the age at onset. As the type of mutation has an impact on disease progression, it is possible that the location of the truncating or missense mutation could also have an impact, but this is difficult to prove in a rare disease. For a different genetic entity, we were able to show that SPG7 patients carrying the A510V variant present with a cerebellar ataxia phenotype, rather than HSP [22].

Overall, these observations show that identification of the mutated gene responsible for the pathology is important, but it is still not sufficient to predict the phenotype. Similarly, environmental factors or modifier genes could also affect the disease. However, the identification of such factors that may influence disease progression require very large cohorts, which are unlikely to be assembled, except for SPG4.

Can biomarkers help predict disease course?

A critical question in patient care is the evolution of the disease, which is sometimes only poorly explained by the mutated gene. The longitudinal analysis of biomarkers could circumvent this issue by objectively placing each patient within the theoretical evolution of the disease. Such markers could also be useful to evaluate therapeutic responses in clinical trials. The search for biomarkers in HSP patients has identified several markers. However, most are diagnostic markers, and only a few can currently be used as predictive markers.

Many genes responsible for HSP encode enzymes involved in various metabolic pathways. Measurement of the enzyme substrates or products is thus used in certain cases as a diagnostic strategy, even before genetic validation (Table 1). The best example is *CYP7B1* (SPG5), which encodes a cytochrome P450 7α-hydroxylase responsible for the degradation of oxysterols. Loss of CYP7B1 leads to the accumulation of oxysterols, such as 25-hydroxycholesterol (25-OHC), 27-hydroxycholesterol (27-OHC), and 3β-hydroxy-5-cholestenoic acid, in the serum and cerebrospinal fluid (CSF) of SPG5 patients [32,33]. In a cross-sectional study, the levels of 27-OHC correlated with symptom severity and disease duration in SPG5 patients [32] and we showed that 25-OHC, 27-OHC, and their ratio to total cholesterol discriminated between SPG5 patients and healthy controls with 100% sensitivity and specificity [33]. A phase II trial that tested atorvastatin, chenodeoxycholic acid, and resveratrol showed that only the first drug lowered 27-OHC by 30%, whereas chenodeoxycholic acid restored an abnormal bile acid profile. This study showed that 25-OHC and 27-OHC are robust biomarkers for therapeutic intervention. Other HSP genes encode enzymes, which could lead to the identification of biomarkers, even though they are rarely used for diagnosis. Instead, they are used to validate the deleterious effects of variants identified in patients (Table 1). No specific pathway biomarkers have been identified thus far. Such biomarkers would be of critical importance, as they could be the signature of common pathophysiological pathways that are likely to exist for certain forms of HSP (Figure 1).

Imaging findings in pure forms of HSP to visualize corticospinal degeneration are poor. Atrophy of the corpus callosum in SPG11 or SPG15 and cerebellar atrophy in SPG7 are used for diagnosis but are not indicative of severity. White matter changes (“ear of the lynx” in SPG15) do not reflect clinical evolution. Thalamic atrophy observed by MRI in a small cohort of SPG4 patients correlated with the clinical score [34] but the pathophysiological meaning of such a change is still unclear. A systematic longitudinal follow-up with high-field MRI in large cohorts of HSP patients with the same genotype would be helpful to better characterize genetic entities.

An overarching biomarker could be neurofilament light chain (NfL), a subunit of the neuronal cytoskeleton that is released into CSF and blood as a sign of axonal damage. Its concentration reflects the severity and progression of various neurological diseases [35]. Ultra-sensitive single molecule array allows the measurement of NfL in blood, with a close correlation with CSF concentrations [36–38]. In HSP, two studies in a heterogeneous cohort of spastic patients confirmed NfL as a diagnostic biomarker in CSF and serum but without genotype correlations [39,40]. NfL levels correlated with cross-sectional disease progression, but showed relative stability longitudinally. Interestingly, there was a sex-related difference for CSF, with higher NfL concentrations in men. This has already been reported for other neurodegenerative diseases, but to a lesser degree [41]. In X-linked adrenoleukodystrophy, plasma NfL correlated with clinical scores but was not changed at the one and two-year follow-ups, even for patients with more rapid clinical progression [42]. However, patients at risk of converting to a life-threatening inflammatory brain demyelination were spotted by higher NfL levels, which were responsive to treatment [43], highlighting the importance of biomarkers for clinical care. Neuroimaging surveillance, as well as correlations between NfL and imaging, should be assessed to predict and follow the conversion to cerebral adrenoleukodystrophy [44].

Perspectives

The gap between genetic cause and clinical phenotype in HSP is large. A number of unifying pathophysiological pathways have emerged, such as ER morphology, lysosomal dysfunction, and myelin maintenance. However, these studies do not explain inter-individual variability, even with the same pathogenic variant. Understanding disease modifiers, whether genetic or environmental, associated with pathophysiological events would be crucial but may be challenging for rare forms of HSP. Alternatively, the identification and subsequent integration of markers that reflect biological processes will aid in the characterization of progression and the care of patients (Figure 2), as recently illustrated for those with X-linked adrenoleukodystrophy [43], and would also be an important tool for the evaluation of clinical trials.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

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Figure legends

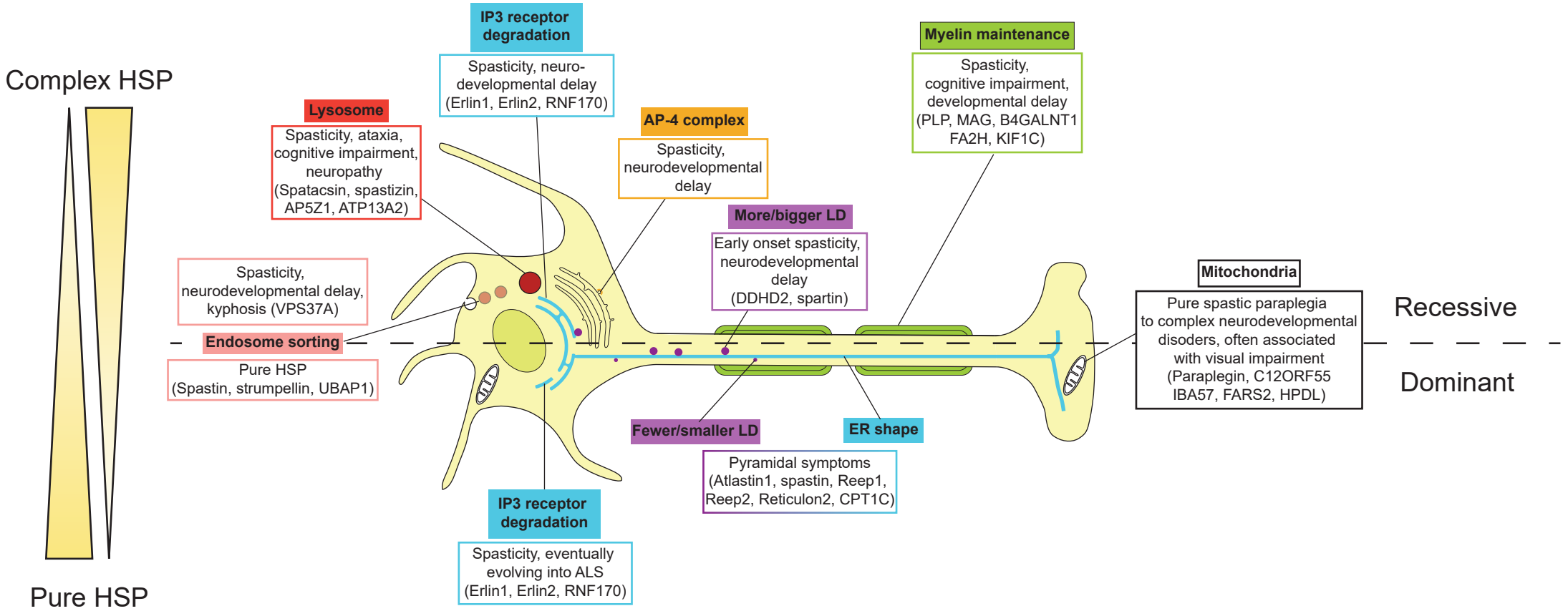
Figure 1. Classification of the main HSP entities into subgroups according to the cellular function affected by the mutated gene and the main clinical features observed in patients.

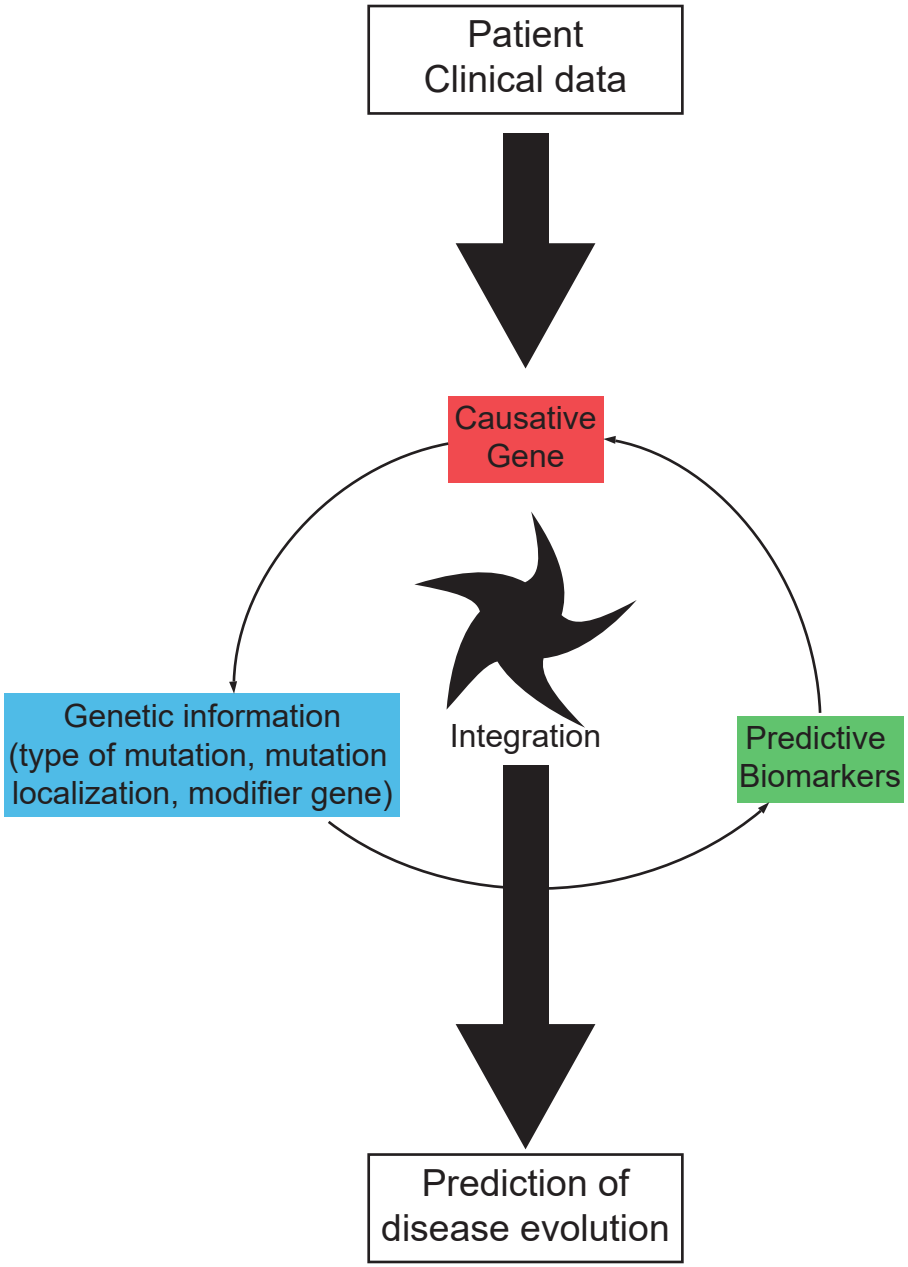
Figure 2. Diagram showing the need for multimodal integration of clinical, genetic, and biomarker data to help predict disease evolution for each patient.

Table 1. Biomarkers identified in HSP entities

	Mutated gene	Biomarker	Use of biomarker and evolution
Biochemical biomarkers			
SPG5	*CYP7B1*	High 27-hydroxycholesterol and 25-hydroxycholesterol levels	Diagnosis ++, Unclear association with disease severity
SPG9	*ALDH18A1*	Low ornithine, citrulline, arginine, and proline levels	Diagnosis/validation of pathogenic variant
SPG26	*B4GALNT1*	Absence of GM2/GD2 and complex ganglioside	Diagnosis/validation of pathogenic variant
SPG46	*GBA2*	High glucosylceramide levels	Diagnosis/validation of pathogenic variant
SPG82	*PCYT2*	Accumulation of PC etherphospholipid	Diagnosis/validation of pathogenic variant
X-ALD	*ABCD1*	High levels of very long chain fatty acids	Diagnosis ++
MEDGEL syndrome/HSP	*SERAC1*	Change in the ratio of phosphatidylglycerol 34:1 to phosphatidylglycerol 36:1	Diagnosis/validation of pathogenic variant
Imaging biomarkers			
SPG4	*SPAST*	Thalamus atrophy	Correlated with disease severity
SPG7	*SPG7*	Optical coherence tomography	Diagnosis
X-ALD	*ABCD1*	Spinal cord area and DTI	Change with follow up in longitudinal study
Magnetic resonance spectroscopy			
SPG54	DDHD2	Abnormal lipid peak by H^+ MRS	Diagnosis
Neurofilament light chain (NfL)			
X-ALD	*ABCD1*	Plasma NfL	Correlated with clinical score but not disease evolution, except during conversion to brain

			demyelination
Spastic patients	No specific genotype	Serum and cerebrospinal fluid NfL	Correlated with disease severity but not disease evolution
Other biomarkers			
SPG35	*FA2H*	Bristly hair, presenting longitudinal groove by scanning electron microcopy	Diagnosis
X-ALD	*ABCD1*	GFAP	No evolution with that of the disease


Complex HSP
Pure HSP
IP3 receptor degradation
Spasticity, neuro-developmental delay (Erlin1, Erlin2, RNF170)
Lysosome
Spasticity, ataxia, cognitive impairment, neuropathy (Spatacsin, spastizin, AP5Z1, ATP13A2)
AP-4 complex
Spasticity, neurodevelopmental delay
Myelin maintenance
Spasticity, cognitive impairment, developmental delay (PLP, MAG, B4GALNT1 FA2H, KIF1C)
More/bigger LD
Early onset spasticity, neurodevelopmental delay (DDHD2, spartin)
Spasticity, neurodevelopmental delay, kyphosis (VPS37A)
Endosome sorting
Pure HSP (Spastin, strumpellin, UBAP1)
Mitochondria
Pure spastic paraplegia to complex neurodevelopmental disoders, often associated with visual impairment (Paraplegin, C12ORF55 IBA57, FARS2, HPDL)
Recessive
Dominant
Fewer/smaller LD
ER shape
Pyramidal symptoms (Atlastin1, spastin, Reep1, Reep2, Reticulon2, CPT1C)
IP3 receptor degradation
Spasticity, eventually evolving into ALS (Erlin1, Erlin2, RNF170)


Patient
Clinical data
Causative
Gene
Genetic information
(type of mutation, mutation
localization, modifier gene)
Integration
Predictive
Biomarkers
Prediction of
disease evolution