Challenges and Controversies in the Genetic Diagnosis of Hereditary Spastic Paraplegia

Lydia Saputra [1] · Kishore Raj Kumar [2,3,4,5]

Abstract

Purpose of Review The hereditary spastic paraplegias (HSPs) are a group of disorders characterised by progressive lower limb weakness and spasticity. We address the challenges and controversies involved in the genetic diagnosis of HSP.

Recent Findings There is a large and rapidly expanding list of genes implicated in HSP, making it difficult to keep gene testing panels updated. There is also a high degree of phenotypic overlap between HSP and other disorders, leading to problems in choosing the right panel to analyse. We discuss genetic testing strategies for overcoming these diagnostic hurdles, including the use of targeted sequencing gene panels, whole-exome sequencing and whole-genome sequencing. Personalised treatments for HSP are on the horizon, and a genetic diagnosis may hold the key to access these treatments.

Summary Developing strategies to overcome the challenges and controversies in HSP may hold the key to a rapid and accurate genetic diagnosis.

Introduction

The hereditary spastic paraplegias (HSPs) are a group of conditions characterised by progressive weakness and spasticity of the lower limbs [1, 2]. They can have autosomal dominant (AD), autosomal recessive (AR), X-linked and mitochondrial modes of inheritance [3]. The HSPs can be classified as either 'pure' (uncomplicated) or 'complex' (complicated). Pure forms involve lower limb spastic paraplegia and may include bladder involvement and subtle sensory signs such as impaired vibration sense. Complicated forms include additional neurological and non-neurological manifestations, such as cognitive impairment, dysarthria, optic atrophy and peripheral neuropathy [1]. There are also syndromic forms such as Silver syndrome (spastic paraparesis with distal amyotrophy predominantly of the hands). The different genetic forms are assigned spastic paraplegia loci (SPG), although the HSP genes may also be listed according to the new MDSGene nomenclature, e.g. SPAST-HSP for SPG4 [4]. The prevalence of AD HSP ranges from 0.5 to 5.5 per 100,000 and that of AR HSP from 0.3 to 5.3 per 100,000 [5]. Although the HSPs are rare, the progressive and disabling nature of these disorders means that they warrant greater attention from clinicians and researchers.

In this review, we discuss current challenges to reach a genetic diagnosis in HSP. These include (i) the large number of genes involved and the rapid rate of gene discovery, (ii) major phenotypic overlap between HSP and other disorders and (iii) disorders that mimic HSP. Further adding to the complexity is that a single HSP gene can have different patterns of inheritance, for example both autosomal dominant and recessive. Additionally, a single patient with HSP can have

✉ Kishore Raj Kumar
   kkum4618@uni.sydney.edu.au

   Lydia Saputra
   lydia.saputra@health.nsw.gov.au

1 Northern Beaches Hospital, Frenchs Forest, New South Wales, Australia

2 Garvan Institute of Medical Research, Darlinghurst, New South Wales, Australia

3 Molecular Medicine Laboratory, Concord Repatriation General Hospital, Concord, Sydney, New South Wales, Australia

4 Sydney Medical School, University of Sydney, Sydney, New South Wales, Australia

5 Institute of Precision Medicine & Bioinformatics, Sydney Local Health District, Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia

concurrent independent genetic diagnoses. Moreover, pseudodominant inheritance of autosomal recessive disease can occur when an individual with mutations on both copies of the gene has a partner carrying a heterozygous mutation, which may result in an affected offspring, a situation that typically occurs when there is a high carrier frequency in the population. In light of these challenges, we discuss the pros and cons of common genetic testing strategies in HSP such as multi-gene panels, whole-exome sequencing (WES) and whole-genome sequencing (WGS). An accurate, timely genetic diagnosis in HSP may become particularly relevant as new, targeted therapies are on the horizon.

Challenges to a Genetic Diagnosis

Multiple Genes and a Rapidly Increasing Gene List

There are many genes causative of HSP resulting in a high level of genetic heterogeneity. Different forms of HSP are assigned a genetic locus according to the order in which they are discovered (spastic paraplegia loci, SPG). Currently, the Online Mendelian Inheritance in Man (OMIM) lists 81 distinct genetic forms of HSP (Table 1, excluding SPG40 and for SPG65 see SPG45). Of these 81 genetic forms, 13 do not have a specific gene identified. Furthermore, while 55 had been identified in more than 1 family, twenty-six were reported in single families, warranting further confirmation.

Due to the rapid rate of progress of HSP research, new genes are being identified on a regular basis. Examples of recently identified HSP genes include UCHL1 (SPG79), UBAP1 (SPG80), SELENOI (SPG81), PCYT2 (SPG82), HPDL (SPG83), and those not yet assigned a locus (RNF170 and FAR1) [7–16]. Some genes are much rarer than others, and it cannot be excluded that certain mutations may be 'private' to individual families. For example, a SCL33A1 mutation was implicated as a cause of AD HSP (SPG42) in a large Chinese pedigree [17], but mutations in this gene were not identified in a large sample of European HSP cases [18]. In contrast, multiple groups have reported that UBAP1 causes AD HSP with a pure phenotype [8–11]. This suggests that UBAP1 mutations are a relatively frequent cause of HSP, and that UBAP1 warrants inclusion on current HSP gene testing panels.

Overlap with Other Inherited Disorders

There is a large overlap between HSP and other disorders such as inherited forms of hereditary ataxia, peripheral neuropathy, amyotrophic lateral sclerosis (ALS) and Parkinson's disease. Twenty-eight of 81 genetic forms of HSP are assigned alternative phenotypes on OMIM (Table 1) and this presents further diagnostic complexity (Fig. 1). Genetic testing is often performed with gene panels that are tailored to a specific disease category, and therefore an accurate clinical classification becomes a critical step able to significantly influence the diagnostic yield.

Overlap with the Hereditary Cerebellar Ataxias

Inherited ataxias commonly overlap with HSP [19], with a typical example being SPG7 [20, 21]. SPG7 mutations result in mitochondrial dysfunction [22] and may present with ataxia evolving to spastic ataxia phenotypes, as well as other features such as ophthalmoplegia and ptosis. SPG7 accounted for 2.3% of cerebellar ataxia cases in an Italian population [23]. Similarly, mutations in CAPN1 cause HSP with or without ataxia [24–27]. It has been suggested that ataxia and spasticity should not be considered separate phenotypes, but rather as existing on a 'continuous ataxia-spasticity disease spectrum' [19]. KIF1A mutations can cause both HSP and ataxia phenotypes (discussed below) [28•]. Mutations in SACS cause autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS), a disorder characterised by the triad of cerebellar ataxia, peripheral neuropathy, and spasticity; however not all features of the triad may be present and there is a phenotypic overlap with the AR HSP with a thin corpus callosum (AR-HSP-TCC) [29]. VPS13D mutations cause a recessive ataxia-spasticity spectrum movement disorder [30] but have also been reported to cause a pure or complicated form of HSP (Table 1) [31]. Additionally, HSP-like phenotypes can also be caused by expansions in triplet-repeat ataxia loci [32] and thus, may not be detected on a sequencing panel.

Overlap with the Inherited Neuropathies

Many forms of HSP overlap with the inherited neuropathies. Notable examples include mutations in BSCL2, which cause Silver syndrome, a complicated form of HSP in which affected individuals present with early-onset hand muscle wasting and leg spasticity [33]. BSCL2 mutations can also cause a range of phenotypes with lower motor neurone involvement including multifocal motor neuropathy with conduction block, Charcot-Marie-Tooth neuropathy type 2 and distal hereditary motor neuropathy type V [33, 34]. SPG11 mutations are a major cause of AR-HSP-TCC [35], but may also cause AR Charcot-Marie Tooth disease [36]. Mutations in MARS1 cause AR HSP complicated by cognitive impairment and nephrotic syndrome [37], as well as AD Charcot-Marie-Tooth Disease type 2 U [38]. Mutations in REEP1, the cause of SPG31, have been shown to cause distal hereditary motor neuropathy type V (Table 1). Recessive RNF170 mutations have recently been confirmed as a cause of HSP [14, 39]**, but a heterozygous mutation in RNF170 (p.Arg199Cys) was found to cause autosomal dominant late-onset progressive

Table 1 Summary of genetic forms of hereditary spastic paraplegia

| Type	Gene	Location	Phenotype MIM number	Inheritance	Identified in more than 1 family with HSP (yes or no)	Allelic disorders/alternative gene-phenotype relationships, MIM number
SPG1	L1CAM	Xq28	MASA syndrome, CRASH syndrome, MIM303350	XLR	Yes	Partial agenesis of the corpus callosum, MIM308840; hydrocephalus due to aqueductal stenosis, hydrocephalus with congenital idiopathic intestinal pseudoobstruction, hydrocephalus with Hirschsprung disease, MIM307000
SPG2	PLP1	Xq22.2	MIM312920	XLR	Yes	Pelizaeus-Merzbacher disease, MIM312080
SPG3A	ATL1	14q22.1	MIM182600	AD	Yes	Hereditary sensory neuropathy type ID, MIM613708
SPG4	SPAST	2p22.3	MIM182601	AD	Yes	
SPG5A	CYP7B1	8q12.3	MIM270800	AR	Yes	Congenital bile acid synthesis defect type 3, MIM613812
SPG6	NIPA1	15q11.2	MIM600363	AD	Yes	
SPG7	SPG7	16q24.3	MIM607259	AR	Yes	
SPG8	KIAA0196 (WSHC5)	8q24.3	MIM603563	AD	Yes	Ritscher-Schinzel syndrome 1, MIM220210
SPG9A, B	ALDH18A1	10q24.1	MIM601162, MIM616586	AD	Yes	AD cutis laxa 3, MIM616603; AR cutis laxa type IIIA, MIM219150
SPG10	KIF5A	12q13.3	MIM604187	AD	Yes	Neonatal intractable myoclonus, MIM617235
SPG11	SPG11	15q21.1	MIM604360	AR	Yes	Juvenile amyotrophic lateral sclerosis 5, MIM602099; axonal Charcot-Marie-Tooth disease type 2X, MIM616668
SPG12	RTN2	19q13.32	MIM604805	AD	Yes	
SPG13	HSPD1	2q33.1	MIM605280	AD	Yes	Hypomyelinating leukodystrophy 4, MIM612233
SPG14	-	3q27-q28	MIM605229	AR	No	
SPG15	ZFYVE26	14q24.1	MIM270700	AR	Yes	
SPG16	-	Xq11.2	MIM300266	XLR	No	
SPG17	BSCL2	11q12.3	Silver spastic paraplegia syndrome, MIM270685	AD	Yes	Progressive encephalopathy with or without lipodystrophy, MIM615924; congenital generalised lipodystrophy type 2, MIM269700; distal hereditary motor neuropathy type VA, MIM600794
SPG18	ERLIN2	8p11.23	MIM611225	AR	Yes	
SPG19	-	9q	MIM607152	AD	No	
SPG20	SPG20	13q13.3	Troyer syndrome, MIM2759002	AR	Yes	
SPG21	SPG21	15q22.31	MAST syndrome, MIM248900	AR	Yes	
SPG22	SLC16A2	Xq13.2	Allan-Herndon-Dudley syndrome, MIM300523	XL	Yes	
SPG23	DSTYK	1q32.1	MIM270750	AR	Yes	Congenital anomalies of kidney and urinary tract 1, MIM610805
SPG24	-	13q14	MIM607584	AR	No	
SPG25	-	6q23-q24.1	MIM608220	AR	No	
SPG26	B4GALNT1	12p11.1-q14	MIM609195	AR	Yes	
SPG27	-	10q22.1-q24.1	MIM609041	AR	No	
SPG28	DDHD1	14q22.1	MIM609340	AR	Yes	
SPG29	-	1p31.1-p21.1	MIM609727	AD	No	
SPG30	KIF1A	2q37.3	MIM610357	AD, AR	Yes	AD mental retardation type 9, MIM 614255; hereditary sensory neuropathy type IIC, MIM614213
SPG31	REEP1	2p11.2	MIM610250	AD	Yes	Distal hereditary motor neuronopathy type VB, MIM614751
SPG32	-	14q12-q21	MIM611252	AR	No	
SPG33	ZFYVE27	10q24.2	MIM610244	AD	No	
SPG34	-	Xq24-25	MIM300750	XLR	No	
SPG35	FA2H	16q23.1	MIM612319	AR	Yes	
SPG36	-	12q23-q24	MIM613096	AD	No	
SPG37	-	8p21.1-q13.3	MIM611945	AD	No	
SPG38	-	4p16-p15	MIM612335	AD	No	

Table 1 (continued)

Type	Gene	Location	Phenotype MIM number	Inheritance	Identified in more than 1 family with HSP (yes or no)	Allelic disorders/alternative gene-phenotype relationships, MIM number
SPG39	PNPLA6	19p13.2	MIM612020	AR	Yes	Laurence-Moon syndrome, MIM245800; Boucher-Neuhauser syndrome MIM215470; Oliver-McFarlane syndrome MIM275400
SPG40	-	-	-	AD	No	
SPG41	-	11p14.1-p11.2	MIM613364	AD	No	
SPG42	SLC33A1	3q25.31	MIM612539	AD	No	Congenital cataracts, hearing loss, and neurodegeneration, MIM614482
SPG43	C19orf12	19p13.11-q12	MIM615043	AR	No	Neurodegeneration with brain iron accumulation 4, MIM614298
SPG44	GJC2	1q42.13	MIM613206	AR	No	Hypomyelinating leukodystrophy 2, MIM608804
SPG45	NT5C2	10q24.3-q25.1	MIM 613162	AR	Yes	
SPG46	GBA2	9p13.3	MIM614409	AR	Yes	
SPG47	AP4B1	1p13.2	MIM614066	AR	Yes	
SPG48	KIAA0415	7p22.1	MIM613647	AR	Yes	
SPG49	TECPR2	14q32.31	MIM615031	AR	Yes	
SPG50	AP4M1	7q22.1	MIM612936	AR	Yes	
SPG51	AP4E1	15q21.2	MIM613744	AR	Yes	Familial persistent stuttering 1, MIM184450
SPG52	AP4S1	14q12	MIM614067	AR	Yes	
SPG53	VPS37A	8p22	MIM614898	AR	Yes	
SPG54	DDHD2	8p11.23	MIM615033	AR	Yes	
SPG55	C12orf65	12q24.31	MIM615035	AR	Yes	Combined oxidative phosphorylation deficiency 7, MIM613559
SPG56	CYP2U1	4q25	MIM615030	AR	Yes	Pseudoxanthoma elasticum
SPG57	TFG	3q12.2	MIM604484	AR	Yes	Hereditary motor and sensory neuropathy, Okinawa type, MIM604484
SPG58	KIF1C	17p13.2	AR spastic ataxia 2, MIM611302	AR	Yes	
SPG59	USP8	15q21.2	-	AR	No	
SPG60	WDR48	3p22.2	-	AR	No	
SPG61	ARL6IP1	16p12.3	MIM615685	AR	Yes	
SPG62	ERLIN1	10q24.31	MIM615681	AR	Yes	
SPG63	AMPD2	1p13.3	MIM615686	AR	No	Pontocerebellar hypoplasia type 9, MIM615809
SPG64	ENTPD1	10q24.1	MIM615683	AR	Yes	
SPG66	ARSI	5q32	-	AR	No	
SPG67	PGAP1	2q33.1	-	AR	No	AR mental retardation 42, MIM615802
SPG68	KLC2	11q13.1	Spastic paraplegia, optic atrophy, and neuropathy, MIM609541	AR	Yes	
SPG69	RAB3GAP2	1q41	-	AR	No	Martsolf syndrome, MIM212720; Warburg micro syndrome 2, MIM614225
SPG70	MARS1	12q13.3	-	AR	No	AD Charcot-Marie-Tooth disease type 2, MIM616280; interstitial lung and liver disease, MIM615486
SPG71	ZFR	5p13.3	-	AR	No	
SPG72	REEP2	5q31	MIM615625	AD, AR	Yes	
SPG73	CPT1C	19q13.33	MIM616282	AD	Yes	
SPG74	IBA57	1q42.13	MIM616451	AR	No	Multiple mitochondrial dysfunctions syndrome 3, MIM615330
SPG75	MAG	19q13.12	MIM616680	AR	Yes	
SPG76	CAPN1	11q13.1	MIM616907	AR	Yes	
SPG77	FARS2	6p25.1	MIM617046	AR	Yes	Combined oxidative phosphorylation deficiency 14, MIM614946
SPG78	ATP13A2	1p36.13	MIM617225	AR	Yes	Kufor-Rakeb syndrome, MIM606693
SPG79	UCHL1	4p13	MIM615491	AR	Yes	?Parkinson disease 5, susceptibility to, MIM613643
SPG80	UBAP1	9p13.3	MIM618418	AD	Yes	
SPG81	SELENOI	2p23.3	MIM618768	AR	Yes	
SPG82	PCYT2	17q25.3	MIM618770	AR	Yes	

Table 1 (continued)

Type	Gene	Phenotype MIM number	Location	Inheritance	Identified in more than 1 family with HSP (yes or no)	Allelic disorders/alternative gene-phenotype relationships, MIM number
SPG83	HPDL	MIM619027	1p34.1	AR	Yes	
Unassigned	RNF170		8p11.21	AR	Yes	AD sensory ataxia 1, MIM608984
Unassigned	FAR1		11p15.3	AR	Yes	Peroxisomal fatty acyl-CoA reductase 1 disorder, MIM616154
Unassigned	NEFL		8p21.2	AR	Yes	Charcot-Marie-Tooth disease, dominant intermediate G, MIM 617882; Charcot-Marie-Tooth disease type 1F, MIM607734; Charcot-Marie-Tooth disease type 2E, MIM607684
Unassigned	VPS13D		1p36.22-p36.21	AR	Yes	Spinocerebellar ataxia, autosomal recessive 4, MIM607317
Unassigned	TUBB4A		19p13.3	AD	Yes	Dystonia 4, MIM128101; hypomyelinating leukodystrophy 6, MIM612438
Unassigned	VCP		9p13.3	AD	Yes	Charcot-Marie-Tooth disease type 2Y, MIM 616687; frontotemporal dementia and/or amyotrophic lateral sclerosis 6, MIM613954; inclusion body myopathy with early-onset Paget disease and frontotemporal dementia 1, MIM167320
Unassigned	POLR3A		10q22.3	AR	Yes	Hypomyelinating leukodystrophy 7 with or without oligodontia and-or hypogonadotropic hypogonadism, MIM607694; Wiedemann-Rautenstrauch syndrome, MIM264090

Information extracted from OMIM [6]

AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive

Fig. 1 Examples of overlapping genes and shared phenotypes with hereditary spastic paraplegia
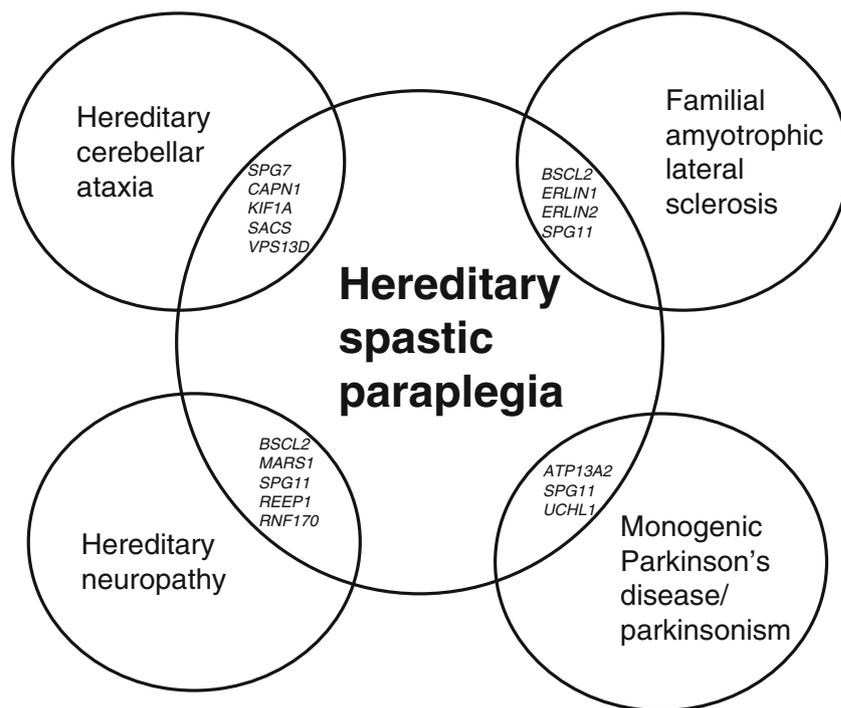
sensory ganglionopathy as a cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS) mimic [40].

Overlap with Hereditary Amyotrophic Lateral Sclerosis

There are many shared genes between HSP and ALS. For example, mutations in *ERLIN1* have been implicated in SPG62, but may also be the cause of a slowly progressive early-onset ALS [41]. *ERLIN2* mutations, causing SPG18, can evolve into rapidly progressive ALS [42] or cause juvenile primary lateral sclerosis [43]. Notably, Erlin1 and erlin2 are highly homologous endoplasmic reticulum membrane proteins that assemble into a ring-shaped complex [44]. Other examples of HSP genes implicated as causing ALS phenotypes include *SPG11* [45] and *BSCL2* [34].

Overlap with Monogenic Parkinson Disease

SPG11 has been linked with parkinsonism or dystonia-parkinsonism. This is highlighted by a recent study which showed that disruption of presynaptic dopaminergic pathways was a widespread phenomenon in individuals with *SPG11* mutations, even without clinical manifestations of parkinsonism [46]. Of note, patients were unresponsive to levodopa, a finding which may relate to post-synaptic damage [46].

Recently, *ATP13A2* mutations have been described as a cause of HSP complicated by cognitive impairment, cerebellar ataxia, and axonal motor and sensory polyneuropathy (SPG78) [47]. Mutations in this gene were first reported as a cause of an AR form of early-

onset parkinsonism with pyramidal degeneration and dementia known as Kufor-Rakeb syndrome [48].

There has been a suggestion of a link between *UCHL1* and Parkinson's disease [49], although this association has not been confirmed. Mutations in *UCHL1* have subsequently been implicated in an early-onset neurodegenerative syndrome, which may be considered HSP complicated by optic atrophy, cerebellar ataxia, seizures, myotonia, fasciculations, dorsal column signs, facial dysmorphism, myopathic facies, microcephaly and fasciculations [50, 51].

HSP Mimics: Other Mendelian Causes and Management Implications

HSP may be due to mutations in many other genes outside of the SPG loci, typically causing complicated phenotypes. For example, pathogenic variants in *OPA3* can cause an optic atrophy plus syndrome, characterised by optic atrophy and lower limb spasticity [52]. Mutations in *PEX16* have been shown to cause HSP complicated by cerebellar ataxia and dystonia [53, 54]. *TUBB4A* mutations have been initially described as a cause of whispering dysphonia (DYT4 dystonia) [55], but have subsequently been reported as a cause of HSP [56].

Several of the HSP mimics may be neurometabolic disorders with whose timely diagnosis has relevant implications for therapeutic strategies and management [57]. These disorders may have distinctive clinical features and biochemical findings (Table 2). Important examples include mutations in *ABCD1*, the gene associated with adrenoleukodystrophy and

Table 2 Examples of 'treatable' inherited mimics in HSP

Disorder	Genetic basis	Mode of inheritance	Additional clinical features	Biochemical findings	Treatment	References
Adrenoleukodystrophy, MIM 300100; adult adrenomyeloneuropathy, MIM 300100	*ABCD1*	XLR	Sphincter disturbances, sexual dysfunction, adrenocortical dysfunction	Elevated very long chain fatty acids	Corticosteroid replacement therapy for adrenal insufficiency	Kim et al. [24•], Raymond et al. [62]
Argininemia, MIM 207800	*ARG1*	AR	Dystonia, dementia, peripheral neuropathy, epilepsy	Newborn screening, elevation of plasma arginine concentration	Measures to reduce ammonia, such as protein-restricted diet, branched-chained amino acids supplement and sodium benzoate.	Tsang et al. [63]
Biotinidase deficiency, MIM 253260	*BTD*	AR	Seizures, hypotonia, limb weakness, ataxia, developmental delay, visual impairment, hearing loss, cutaneous abnormalities	Newborn screening or deficient biotinidase enzyme activity in serum/plasma	Treatment with biotin	Wolf [64], Wolf [65]
Primary coenzyme Q10 deficiency 8, MIM	*COQ7*	AR	Primary coenzyme Q10 (CoQ10) deficiency is usually associated fatal neonatal encephalopathy with hypotonia, multiple-system atrophy-like phenotype, dystonia, spasticity, seizures, intellectual disability, sensorineural hearing loss, steroid-resistant nephrotic syndrome, hypertrophic cardiomyopathy	Reduced levels of CoQ10 in skeletal muscle or reduced activities of complex I+III and II+III of the mitochondrial respiratory chain on frozen muscle homogenates	2,4-Dihydroxybenzoate bypass treatment, high-dose oral CoQ_{10} supplementation	Wang et al. [66], Salviati et al. [67]
Cerebrotendinous xanthomatosis, MIM 213700	*CYP27A1*	AR	Cerebellar signs, intellectual impairment, seizures, peripheral neuropathy, cataract, tendon xanthomas	Elevated levels of cholestanol and bile alcohols in serum and urine	Chenodeoxycholic acid	Nicholls et al. [61], Verrips et al. [68]
DOPA-responsive dystonia, MIM 128230	*GCH1*	AD, AR	Foot dystonia, later development of parkinsonism, diurnal variation in symptoms, dramatic and sustained response to levodopa	Reduced concentrations of total biopterin and total neopterin in the cerebrospinal fluid	Levodopa/decarboxylase inhibitor	Fan et al. [58]
Methylmalonic aciduria and homocystinuria cblC type MIM 277400	*MMACHC*	AR	Cognitive impairment (5/8), spastic dysuria (3/8), personality change and depression (3/8), ataxia (2/8), seizures (2/8), limb numbness (2/8) and developmental delay (2/8). When patients were diagnosed, the mean serum homocysteine level, the methylmalonic acid level in urine, the serum propionylcarnitine (C3) level and the ratios of	Elevated urine methylmalonic acid and serum homocysteine levels	Intramuscular cobalamin, oral betaine and folate	Wei et al. [59]

Table 2 (continued)

Disorder	Genetic basis	Mode of inheritance	Additional clinical features	Biochemical findings	Treatment	References
			C3-to-acetylcarnitine (C2) and free carnitine (C0) were all dramatically elevated. Cranial MRIs showed nothing remarkable except mild brain atrophy.			
Homocystinuria due to MTHFR deficiency, MIM 236250	MTHFR	AR	Polyneuropathy, behavioural abnormalities, cognitive impairment, psychosis, seizures, leukoencephalopathy	Severe hyperhomocysteinemia associated with the characteristic amino acid profile	Betaine and vitamins	Lossos et al. [60]
Phenylketonuria, MIM 261600	PAH	AR	Cognitive impairment	Serum phenylalanine concentrations	Classic phenylketonuria diet/protein restricted diet	Kasim et al. [69]
Dystonia 9, MIM 601042; GLUT1 deficiency syndrome 1, MIM 606777; GLUT1 deficiency syndrome 2, MIM 612126; Stomatin-deficient cryohydrocytosis with neurologic defects, MIM 608885	SLC2A1	AD	Seizures, delayed neurologic development, acquired microcephaly, intermittent ataxia, paroxysmal exercise-induced dyskinesia, choreo-athetosis, alternating hemiplegia	Cerebrospinal fluid analysis for hypoglycorrhachia	Ketogenic diet	Verrotti et al. [70]

AD, autosomal dominant; *AR*, autosomal recessive; *XLR*, X-linked recessive

adrenomyeloneuropathy, which can cause spastic paraplegia in males and carrier females [24•]. Dopa-responsive dystonia may be misdiagnosed as HSP and is typically responsive to levodopa therapy [58]. Recently, combined homocysteinaemia with methylmalonic aciduria due to pathogenic recessive variants in the *MMACHC* gene has been highlighted as a treatable cause of HSP [59]. Testing urine methylmalonic acid and serum homocysteine levels and sequencing the *MMACHC* gene is critical when this rare condition is suspected [59]. Severe 5,10-methylenetetrahydrofolate reductase deficiency has also been reported as a cause of a complicated HSP phenotype, responsive to treatment with betaine and vitamins [60]. Additionally, cerebrotendinous xanthomatosis may mimic HSP and is treatable with chenodeoxycholic acid [61].

HSP Mimics: Overlap with Disorders Without Clear Mendelian Inheritance

Several disorders that do not have a readily recognisable monogenic cause may be difficult to differentiate from HSP, such as primary lateral sclerosis (PLS). PLS is a degenerative, mainly sporadic neuronopathy with primarily upper motor neurone features [71]. PLS frequently presents with spastic paraplegia, affects older, predominantly male patients and invariably progresses to involve cervical and bulbar regions [71]. However, the disease often remains as an isolated spastic paraplegia for many years and bulbar symptoms can appear after 10 years in up to 20% of patients [71]. Consequently, in the absence of family history, PLS and HSP may be clinically indistinguishable for longer than a decade [71]. However, cortical excitability studies may be used to differentiate these two conditions in a clinical setting [72], and genetic testing for HSP genes may also help [73].

It may also be challenging to differentiate between HSP and cerebral palsy. HSP may be distinguishable from spastic diplegic cerebral palsy by the absence of perinatal risk factors for brain injury and normal brain imaging, or specific findings indicative of an HSP syndrome, such as thinning of the corpus callosum [74]. Genetic testing may also be helpful, for example, a patient with childhood onset, non-progressive, spastic diplegia with no previous family history of HSP was long considered as affected by cerebral palsy, until his son also developed the same phenotype: genetic testing in these patients disclosed a heterozygous pathogenic variant in *ATL1* (SPG3A) which had arisen de novo in the affected parent [75].

There may also be diagnostic uncertainty in differentiating HSP from multiple sclerosis. A personal observation is that patients may be referred to the neurogenetics clinic with HSP, only to find evidence of demyelinating lesions consistent with MS on upon repeating brain or spinal cord MRI. Conversely, mutations in HSP genes may be identified in individuals formerly diagnosed with MS. For example, SPG2 has been shown to mimic MS [76], and rare variants in genes including *KIF5A* and *REEP1* were identified in patients with primary progressive MS [77].

When Should a Complex Disorder Be Diagnosed as HSP?

It may be difficult to decide when to categorise a disorder as HSP when the phenotype is complex. A chief consideration should be whether lower extremity weakness and spasticity are the *predominant* clinical manifestations [78]. For example, *ATP13A2* mutations are known to cause Kufor-Rakeb syndrome [48], neuronal ceroid lipofuscinosis [79] and neurodegeneration with brain iron accumulation (NBIA) [80]. More recently, *ATP13A2* mutations have been described as a cause of HSP complicated by cognitive impairment, cerebellar ataxia, and axonal motor and sensory polyneuropathy (SPG78) [47]. However, there is debate over whether an HSP predominant phenotype is a clinical outlier and if a new HSP locus was warranted [81]. Similarly, hypomorphic mutations in *POLR3A* were reported as a cause of HSP and ataxia [82], however, other authors considered that this condition should be defined as a 'POLR3-related disorder' instead [83].

HSP Genes with Different Modes of Inheritance

Variants in some HSP genes may be inherited with different modes of transmission, adding further complexity to the interpretation of genetic findings. As an example, biallelic mutations in *KIF1A* cause spastic paraplegia, distal wasting, peripheral neuropathy and mild cerebellar signs (AR SPG30) [84]. *KIF1A* mutations can also cause hereditary sensory and autonomic neuropathy type 2 with AR inheritance (Table 1). However, de novo dominant *KIF1A* mutations may result in a phenotypic spectrum overlapping with AR SPG30 including mental retardation, speech delay, epilepsy, optic nerve atrophy, thinning of the corpus callosum, periventricular white matter lesion and microcephaly [85–88]. A recent study showed that heterozygous mutations in *KIF1A* may result in two distinct phenotypes, a pure to complex HSP phenotype and a congenital or early-onset ataxia phenotype [28•]. Additionally, mutations in *REEP2* have been identified in families with both AD and AR inheritance [37, 89]. A mutation in *REEP2* has been found to cause AD HSP with a pure, early-onset phenotype [89], while the AR form is characterised by early-onset HSP with delayed motor milestones and normal cognition [37]. Similarly, *ATL1* mutations are usually associated with dominant HSP (SPG3A), but recessive mutations in *ATL1* have been shown to cause both pure and complex forms of HSP [90, 91].

Individuals with Concurrent Independent Genetic Diagnoses

Individuals presenting with HSP may have concurrent independent genetic diagnoses, further complicating genetic testing. As an example, a recent study showed two possible genetic diagnoses in a non-consanguineous family with 3 affected siblings: two brothers with intellectual impairment and spastic paraplegia, and a sister with behavioural disturbance and pes cavus. All affected siblings carried a maternally inherited interstitial 15q duplication and a paternally inherited *REEP1* variant [92•]. In this case, it was thought that the 15q duplication was causing intellectual impairment and behavioural abnormalities, with supportive evidence from methylation and functional studies. On the other hand, the dominant HSP phenotype was attributed to the *REEP1* variant. This in keeping with a large study of 7374 consecutive unrelated patients referred to a clinical diagnostic laboratory for WES, which demonstrated multiple molecular diagnoses in 4.9% of cases in whom WES was informative [93]. The results of these studies suggest that perhaps we too often claim a 'phenotypic expansion' to explain a phenotype that is different or more complicated than previously reported for a given gene, while in some of these cases the reason would be a 'double hit' and not a phenotypic expansion.

Pseudodominant Inheritance and Intronic Variants

In a recent study, a patient with spastic paraplegia and ataxia was investigated with WES, revealing a novel missense variant in *SPG7* (c.2195T>C; p.Leu732Pro) [94•]. To seek a second variant, WGS was performed, revealing an unreported, deep intronic variant (c.286 + 853A>G), shown to activate a cryptic splice site [94•]. The deep intronic variant would not have been identified with WES alone, highlighting the usefulness of WGS to increase diagnostic yield [94•]. Furthermore, it sheds light on the apparent dominant pattern of inheritance of SPG7 [95], which may be due to the mutation on the other allele being missed [94•]. Another report highlights the importance of an intronic variant in *POLR3A*, a gene previously associated with hypomyelinating leukodystrophy type 7 (Table 1), as a frequent cause of HSP and cerebellar ataxia [82]. Compound heterozygous mutations in *POLR3A* were found in approximately 3.1% of index cases of HSP and cerebellar ataxia, with over 80% carrying the same intronic mutation (c.1909+22G>A) which activates a cryptic splice site [82]. This suggests that non-coding DNA variants may account for a substantial number of unsolved cases of HSP.

Strategies for a Genetic Diagnosis

There are several options for reaching a genetic diagnosis in individuals with HSP and it can be challenging for the clinician to decide upon which approach to adopt. Different strategies include targeted sequencing gene panels, whole-exome sequencing (WES), or whole-genome sequencing (WGS) (Table 3). Targeted sequencing gene panels are commonly used but will overlook a diagnosis if the mutation is in a gene that is outside the panel. Furthermore, gene panels also are not reliable in detecting copy number variants (CNVs), structural variants (SVs) and intronic variants. WES can be a useful approach but again may not be reliable for CNVs, SVs, and will fail to detect deep intronic variants. WGS may be the most complete approach [24•, 54, 96], with uniformity of coverage that allows for the accurate detection of CNVs, SVs [97, 98], in addition to the detection of non-coding variants. However, this approach is limited by the expense and difficulty processing, storing, and interpreting the large amounts of genomic

data. Both WES and WGS allow for testing of many genes and so may not be restricted to single panel, e.g. patients can be tested for both 'ataxia' and 'HSP' genes in a single test [24•]. The WES and WGS data can be used in several ways. For example, a panel of relevant HSP genes may be analysed, such as those listed in Table 1. A larger, less specific panel of genes can also be interrogated, such as the TruSight One 'clinical exome'—a panel of 4813 genes that have been associated with human disease [99]. WES or WGS family studies may provide valuable additional information regarding segregation of genetic variants with the disease phenotype. For example, parent-child trios of healthy parents and an affected child may facilitate the detection of homozygous, compound heterozygous or de novo variants.

It is critical to remember that CNVs (e.g. exonic deletions in *SPAST* [100]) are important to consider and may require a separate test (e.g. multiplex ligation probe amplification or MLPA), unless using a method that provides reliable detection such as WGS. Furthermore, testing for repeat expansion disorders will often require a separate test such as a fluorescent repeat-primed PCR assay. However, a recent study suggests that long repeat expansions may be detectable from PCR-free WGS data using a software tool called ExpansionHunter [101]. Furthermore, a homoplasmic m.9176 T>C mutation in the mitochondrial *ATP6* gene has been found to cause HSP. WES may allow for the detection of mitochondrial point mutations using 'off-target reads', providing additional diagnoses [102]. WGS provides exceptionally high coverage of the mitochondrial genome, allowing for accurate detection of mitochondrial point mutations even at low levels of heteroplasmy [103]. Hypothesis-free methods such as WES and WGS may also detect multiple concurrent genetic defects, as described above [92•].

Table 3 Comparison of different approaches for the genetic diagnosis of hereditary spastic paraplegia

Technique	Pros	Cons
Targeted sequencing panels	- Less expensive* - Reduce incidental findings	- Gene list may be restrictive, missing unexpected findings or mutations in genes implicated in overlapping phenotypes - Inadequate coverage of CNVs, SVs; MLPA may be required - Inadequate coverage of deep non-coding variants
Whole-exome sequencing	- Gene panel not restrictive - Less expensive compared to whole-genome sequencing*	- Inadequate coverage of CNVs, SVs; MLPA may be required s- Inadequate coverage of deep non-coding variants - Challenge of incidental findings
Whole-genome sequencing	- Gene panel not restrictive - Detection of CNVs, SVs (e.g. deletions in *SPAST*) - Detection of non-coding variants (see example of deep intronic variants reported in *SPG7*)	- Expensive* - Challenge of processing, storing and analysing large amounts of data - Challenge of incidental findings

*Note that to our knowledge, a cost-effectiveness study for genetic testing in hereditary spastic paraplegia comparing the different approaches has not yet been performed.

Benefits of a Genetic Diagnosis in HSP

There are numerous benefits of a genetic diagnosis in HSP which may prompt the decision to undertake genetic testing. As an example, it may provide for prognostic information and facilitate genetic counselling and family planning. It may also allow for a prenatal diagnosis/ preimplantation genetic diagnosis.

A genetic diagnosis rarely leads to findings with direct management implications (as discussed earlier, see Table 2). However, it may hold future value in that it could be used to enrol patients in clinical trials that target the disease mechanism. A targeted, disease-modifying treatment appears most likely for two forms of HSP—SPG4 and SPG5.

Microtubule-targeting drugs hold great promise for HSP due to *SPAST* mutations (SPG4). Supporting this concept, vinblastine has been shown to ameliorate the disease phenotype in a *Drosophila* model of SPG4 [104]. Additionally,

microtubule-targeting drugs have been shown to rescue axonal swellings in cortical neurons in a mouse model of SPG4 [105]. In human patient-derived olfactory neurosphere-derived cells, *SPAST* mutations result in decreased levels of acetylated α-tubulin, a marker of stabilised microtubules, as well as reduced speed of peroxisome trafficking [106]. Tubulin binding drugs such as taxol, vinblastine, epothilone D and noscapine may increase acetylated alpha tubulin and thereby restore axonal transport, directly targeting the mechanism involved in SPG4 [106, 107].

Several genes associated with HSP phenotypes disturb lipid metabolic pathways as a potential therapeutic target, including *CYP7B1*, *EPT1*, *PCYT2*, *DDHD1*, *DDHD2*, *PNPLA6*, *B4GALNT1*, *CYP2U1*, *FA2H*, *GBA2*, *PLA2G6*, *ATP13A2*, *BSCL2*, *C19orf12*, *ERLIN2*, *SPART*, *SPAST*, *SPG11*, *SPG15*, *ATL1* and *REEP1* [108]. SPG5 is a recessive cause of HSP due to mutations in the *CYP7B1* gene encoding a distinct microsomal oxysterol-7α-hydroxylase. This enzyme is involved in the degradation of cholesterol into primary bile acids. CYP7B1 deficiency results in accumulation of neurotoxic oxysterols, with elevation of 25-hydroxycholesterol (25-OHC) and 27-hydroxycholesterol (27-OHC) in the plasma and a much higher increase of 27-OHC in the CSF [109, 110]. Two recent studies have explored the use of drugs to lower cholesterol biomarkers in HSP. A study by Marelli and colleagues used atorvastatin, chenodeoxycholic acid and resveratrol in 21 patients with SPG5A and assessed 25-OHC and 27-OHC as diagnostic biomarkers [111••]. Treatment with atorvastatin decreased plasma 27-OHC but did not change the 27-OHC to total cholesterol ratio or 25-OHC levels. Marelli and colleagues also identified an abnormal bile acids profile in patients with SPG5, with a reduction in total serum bile acids and a decrease of ursodeoxycholic and lithocholic acids in comparison to deoxycholic acid. Treatment with chenodeoxycholic acid restored the bile acid profile. The authors concluded that atorvastatin and chenodeoxycholic acid may be worth considering for the treatment of SPG5A. A randomised placebo control trial by Schols and colleagues found that atorvastatin treatment reduced 27-OHC and 25-OHC in the serum, although 27-OHC was not significantly reduced in the cerebrospinal fluid [109••]. It is important to note that both these trials have demonstrated a reduction in cholesterol/bile acid biomarkers, but without benefit in terms of clinical, imaging, or electrophysiological outcome measures.

A more recent study explored the use of intravenous formulated mouse and human CYP7B1 mRNA in mice lacking the endogenous *Cyp7b1* gene mutated in SPG5A. Results indicated that the treatment was safe and demonstrated a reduction in neurotoxic oxysterols in the liver, serum and to some degree in the brain, suggesting that this may be a valid strategy for the treatment of this condition [112].

Conclusion

The genetic diagnosis of HSP is complex and can represent a major challenge for clinicians. The complexity arises in part because of the high degree of genetic heterogeneity, with over 80 different genetic forms, and a growing number of genes being identified. Furthermore, there is a high level of phenotypic complexity, with HSP clinically and genetically overlapping with a variety of neurological phenotypes, including inherited forms of cerebellar ataxia, ALS, Parkinson's disease, and peripheral neuropathy. There are many conditions that mimic HSP that the clinician should be alert for, and it may be particularly important to detect the rare HSP mimics that have management implications.

An understanding of the genetic and phenotypic complexity underlying HSP is essential to guide genetic testing strategies. Gene panels are commonly used, but the gene panel itself needs to be comprehensive to encompass the large number of genes involved. Panels must be regularly curated given that the rapid rate of gene discovery as they can quickly become obsolete. Furthermore, gene panels are typically based on a specific phenotypic category, and a genetic diagnosis may be missed if the responsible mutation is in a gene outside of that disease category. Thus, directed testing approaches such as gene panels may miss unanticipated findings [54].

Hypothesis-free approaches such as clinical WES, WES and WGS somewhat overcome the potential problems of gene panels by allowing for the potential interrogation of many relevant genes. However, clinical WES and WES may miss certain mutation types such as CNVs, SVs and repeat expansions, potentially detectable with WGS. In fact, WGS may be the most comprehensive method for coverage and detection of mutation types but is unfortunately limited by cost.

Next-generation sequencing has greatly improved our ability to detect a genetic diagnosis in HSP. Yet, large studies have shown that the diagnostic rate for HSP is still only about 45–50% of cases [113, 114]. The genetic diagnosis of HSP still represents a great challenge for clinicians, and there are no clear guidelines available about which approach to choose. However, it will become increasingly important to identify a genetic diagnosis in a rapid and accurate manner for enrolment in clinical trials and as targeted treatments become available.

Compliance with Ethical Standards

Conflict of Interest Lydia Saputra and Kishore Raj Kumar have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any original studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. Kumar KR, Blair NF, Sue CM. An update on the hereditary spastic paraplegias: new genes and new disease models. Mov Disord Clin Pract. 2015;2(3):213–23. https://doi.org/10.1002/mdc3.12184.

2. Fink JK, Heiman-Patterson T, Bird T, Cambi F, Dube MP, Figlewicz DA, et al. Hereditary spastic paraplegia: advances in genetic research. Hereditary Spastic Paraplegia Working group. Neurology. 1996;46(6):1507–14. https://doi.org/10.1212/wnl.46.6.1507.

3. Finsterer J, Loscher W, Quasthoff S, Wanschitz J, Auer-Grumbach M, Stevanin G. Hereditary spastic paraplegias with autosomal dominant, recessive, X-linked, or maternal trait of inheritance. J Neurol Sci. 2012;318(1-2):1–18. https://doi.org/10.1016/j.jns.2012.03.025.

4. Marras C, Lang A, van de Warrenburg BP, Sue CM, Tabrizi SJ, Bertram L, et al. Nomenclature of genetic movement disorders: recommendations of the international Parkinson and movement disorder society task force. Mov Disord. 2016;31(4):436–57. https://doi.org/10.1002/mds.26527.

5. Ruano L, Melo C, Silva MC, Coutinho P. The global epidemiology of hereditary ataxia and spastic paraplegia: a systematic review of prevalence studies. Neuroepidemiology. 2014;42(3):174–83. https://doi.org/10.1159/000358801.

6. McKusick VA. Mendelian inheritance in man and its online version, OMIM. Am J Hum Genet. 2007;80(4):588–604. https://doi.org/10.1086/514346.

7. Das Bhowmik A, Patil SJ, Deshpande DV, Bhat V, Dalal A. Novel splice-site variant of UCHL1 in an Indian family with autosomal recessive spastic paraplegia-79. J Hum Genet. 2018;63(8):927–33. https://doi.org/10.1038/s10038-018-0463-6.

8.• Farazi Fard MA, Rebelo AP, Buglo E, Nemati H, Dastsooz H, Gehweiler I, et al. Truncating mutations in UBAP1 cause hereditary spastic paraplegia. Am J Hum Genet. 2019;104(4):767–73.

https://doi.org/10.1016/j.ajhg.2019.03.001\ **UBAP1 is an important new gene causing autosomal dominant HSP. It has been identified by numerous groups and probably warrants widespread clinical testing.**

9. Gu S, Chen CA, Rosenfeld JA, Cope H, Launay N, Flanigan KM, et al. Truncating variants in UBAP1 associated with childhood-onset nonsyndromic hereditary spastic paraplegia. Hum Mutat. 2019;41:632–40. https://doi.org/10.1002/humu.23950.

10. Lin X, Su HZ, Dong EL, Lin XH, Zhao M, Yang C, et al. Stop-gain mutations in UBAP1 cause pure autosomal-dominant spastic paraplegia. Brain. 2019;142(8):2238–52. https://doi.org/10.1093/brain/awz158.

11. Nan H, Ichinose Y, Tanaka M, Koh K, Ishiura H, Mitsui J, et al. UBAP1 mutations cause juvenile-onset hereditary spastic paraplegias (SPG80) and impair UBAP1 targeting to endosomes. J Hum Genet. 2019;64(11):1055–65. https://doi.org/10.1038/s10038-019-0670-9.

12. Ahmed MY, Al-Khayat A, Al-Murshedi F, Al-Futaisi A, Chioza BA, Pedro Fernandez-Murray J, et al. A mutation of EPT1 (SELENOI) underlies a new disorder of Kennedy pathway phospholipid biosynthesis. Brain. 2017;140(3):547–54. https://doi.org/10.1093/brain/aww318.

13. Vaz FM, McDermott JH, Alders M, Wortmann SB, Kolker S, Pras-Raves ML, et al. Mutations in PCYT2 disrupt etherlipid biosynthesis and cause a complex hereditary spastic paraplegia. Brain. 2019;142(11):3382–97. https://doi.org/10.1093/brain/awz291.

14.• Wagner M, Osborn DPS, Gehweiler I, Nagel M, Ulmer U, Bakhtiari S, et al. Bi-allelic variants in RNF170 are associated with hereditary spastic paraplegia. Nat Commun. 2019;10(1): 4790. https://doi.org/10.1038/s41467-019-12620-9 **RNF170 has now been independly confirmed as a cause of autosomal recessive HSP. Of note, heterozygous mutations in this gene can cause autosomal dominant sensory ataxia 1.**

15. Husain RA, Grimmel M, Wagner M, Hennings JC, Marx C, Feichtinger RG, et al. Bi-allelic HPDL variants cause a neurodegenerative disease ranging from neonatal encephalopathy to adolescent-onset spastic paraplegia. Am J Hum Genet. 2020;107(2):364–73. https://doi.org/10.1016/j.ajhg.2020.06.015.

16. Ferdinandusse S, McWalter K, Te Brinke H, Lodewijk IJ, Mooijer PM, Ruiter JPN, et al. An autosomal dominant neurological disorder caused by de novo variants in FAR1 resulting in uncontrolled synthesis of ether lipids. Genet Med. 2020. https://doi.org/10.1038/s41436-020-01027-3.

17. Lin P, Li J, Liu Q, Mao F, Li J, Qiu R, et al. A missense mutation in SLC33A1, which encodes the acetyl-CoA transporter, causes autosomal-dominant spastic paraplegia (SPG42). Am J Hum Genet. 2008;83(6):752–9. https://doi.org/10.1016/j.ajhg.2008.11.003.

18. Schlipf NA, Beetz C, Schule R, Stevanin G, Erichsen AK, Forlani S, et al. A total of 220 patients with autosomal dominant spastic paraplegia do not display mutations in the SLC33A1 gene (SPG42). Eur J Hum Genet. 2010;18(9):1065–7. https://doi.org/10.1038/ejhg.2010.68.

19. Synofzik M, Schule R. Overcoming the divide between ataxias and spastic paraplegias: Shared phenotypes, genes, and pathways. Mov Disord. 2017;32(3):332–45. https://doi.org/10.1002/mds.26944.

20. Kang C, Liang C, Ahmad KE, Gu Y, Siow SF, Colebatch JG, et al. High degree of genetic heterogeneity for hereditary cerebellar ataxias in Australia. Cerebellum. 2019;18(1):137–46. https://doi.org/10.1007/s12311-018-0969-7.

21. Kumar KR, Blair NF, Vandebona H, Liang C, Ng K, Sharpe DM, et al. Targeted next generation sequencing in SPAST-negative hereditary spastic paraplegia. J Neurol. 2013;260(10):2516–22. https://doi.org/10.1007/s00415-013-7008-x.

22. Wali G, Kumar KR, Liyanage E, Davis RL, Mackay-Sim A, Sue CM. Mitochondrial function in hereditary spastic paraplegia: deficits in SPG7 but not SPAST patient-derived stem cells. Front Neurosci. 2020;14:820. https://doi.org/10.3389/fnins.2020.00820.

23. Mancini C, Giorgio E, Rubegni A, Pradotto L, Bagnoli S, Rubino E, et al. Prevalence and phenotype of the c.1529C>T SPG7 variant in adult-onset cerebellar ataxia in Italy. Eur J Neurol. 2019;26(1): 80–6. https://doi.org/10.1111/ene.13768.

24.• Kim A, Kumar KR, Davis RL, Mallawaarachchi AC, Gayevskiy V, Minoche AE, et al. Increased diagnostic yield of spastic paraplegia with or without cerebellar ataxia through whole-genome sequencing. Cerebellum. 2019. https://doi.org/10.1007/s12311-019-01038-0 **This study suggests it may be helpful to investigate spastic paraplegia and ataxia as a single disease spectrum or common entity, using WGS to interrogate both HSP and ataxia genes.**

25. Wang Y, Hersheson J, Lopez D, Hammer M, Liu Y, Lee KH, et al. Defects in the CAPN1 gene result in alterations in cerebellar development and cerebellar ataxia in mice and humans. Cell Rep. 2016;16(1):79–91. https://doi.org/10.1016/j.celrep.2016.05.044.

26. Shetty A, Gan-Or Z, Ashtiani S, Ruskey JA, van de Warrenburg B, Wassenberg T, et al. CAPN1 mutations: expanding the CAPN1-related phenotype: from hereditary spastic paraparesis to spastic ataxia. Eur J Med Genet. 2018;62:103605. https://doi.org/10.1016/j.ejmg.2018.12.010.

27. Gan-Or Z, Bouslam N, Birouk N, Lissouba A, Chambers DB, Veriepe J, et al. Mutations in CAPN1 cause autosomal-recessive hereditary spastic paraplegia. Am J Hum Genet. 2016;98(5): 1038–46. https://doi.org/10.1016/j.ajhg.2016.04.002.

28.• Nicita F, Ginevrino M, Travaglini L, D'Arrigo S, Zorzi G, Borgatti R, et al. Heterozygous KIF1A variants underlie a wide spectrum of neurodevelopmental and neurodegenerative disorders. J Med Genet. 2020. https://doi.org/10.1136/jmedgenet-2020-107007 **Recent study showing that heterozygous *KIF1A* variants can cause both HSP and ataxia phenotypes.**

29. Synofzik M, Soehn AS, Gburek-Augustat J, Schicks J, Karle KN, Schule R, et al. Autosomal recessive spastic ataxia of Charlevoix Saguenay (ARSACS): expanding the genetic, clinical and imaging spectrum. Orphanet J Rare Dis. 2013;8:41. https://doi.org/10.1186/1750-1172-8-41.

30. Seong E, Insolera R, Dulovic M, Kamsteeg EJ, Trinh J, Bruggemann N, et al. Mutations in VPS13D lead to a new recessive ataxia with spasticity and mitochondrial defects. Ann Neurol. 2018;83(6):1075–88. https://doi.org/10.1002/ana.25220.

31. Koh K, Ishiura H, Shimazaki H, Tsutsumiuchi M, Ichinose Y, Nan H, et al. VPS13D-related disorders presenting as a pure and complicated form of hereditary spastic paraplegia. Mol Genet Genomic Med. 2020;8(3):e1108. https://doi.org/10.1002/mgg3.1108.

32. Bettencourt C, Quintans B, Ros R, Ampuero I, Yanez Z, Pascual SI, et al. Revisiting genotype-phenotype overlap in neurogenetics: triplet-repeat expansions mimicking spastic paraplegias. Hum Mutat. 2012;33(9):1315–23. https://doi.org/10.1002/humu.22148.

33. Ito D BSCL2-related neurologic disorders/seipinopathy. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K et al., editors. GeneReviews((R)). Seattle (WA)1993.

34. Musacchio T, Zaum AK, Uceyler N, Sommer C, Pfeifroth N, Reiners K, et al. ALS and MMN mimics in patients with BSCL2 mutations: the expanding clinical spectrum of SPG17 hereditary spastic paraplegia. J Neurol. 2017;264(1):11–20. https://doi.org/10.1007/s00415-016-8301-2.

35. Stevanin G, Santorelli FM, Azzedine H, Coutinho P, Chomilier J, Denora PS, et al. Mutations in SPG11, encoding spatacsin, are a major cause of spastic paraplegia with thin corpus callosum. Nat Genet. 2007;39(3):366–72. https://doi.org/10.1038/ng1980.

36. Montecchiani C, Pedace L, Lo Giudice T, Casella A, Mearini M, Gaudiello F, et al. ALS5/SPG11/KIAA1840 mutations cause autosomal recessive axonal Charcot-Marie-Tooth disease. Brain. 2016;139(Pt 1):73–85. https://doi.org/10.1093/brain/awv320.

37. Novarino G, Fenstermaker AG, Zaki MS, Hofree M, Silhavy JL, Heiberg AD, et al. Exome sequencing links corticospinal motor neuron disease to common neurodegenerative disorders. Science. 2014;343(6170):506–11. https://doi.org/10.1126/science.1247363.

38. Gonzalez M, McLaughlin H, Houlden H, Guo M, Yo-Tsen L, Hadjivassilious M, et al. Exome sequencing identifies a significant variant in methionyl-tRNA synthetase (MARS) in a family with late-onset CMT2. J Neurol Neurosurg Psychiatry. 2013;84(11): 1247–9. https://doi.org/10.1136/jnnp-2013-305049.

39. de Sainte Agathe JM, Mercier S, Mahe JY, Pereon Y, Buratti J, Tissier L, et al. RNF170-related hereditary spastic paraplegia: confirmation by a novel mutation. Mov Disord. 2020. https://doi.org/10.1002/mds.28371.

40. Cortese A, Callegari I, Curro R, Vegezzi E, Colnaghi S, Versino M, et al. Mutation in RNF170 causes sensory ataxic neuropathy with vestibular areflexia: a CANVAS mimic. J Neurol Neurosurg Psychiatry. 2020;91(11):1237–8. https://doi.org/10.1136/jnnp-2020-323719.

41. Tunca C, Akcimen F, Coskun C, Gundogdu-Eken A, Kocoglu C, Cevik B, et al. ERLIN1 mutations cause teenage-onset slowly progressive ALS in a large Turkish pedigree. Eur J Hum Genet. 2018;26(5):745–8. https://doi.org/10.1038/s41431-018-0107-5.

42. Amador MD, Muratet F, Teyssou E, Banneau G, Danel-Brunaud V, Allart E, et al. Spastic paraplegia due to recessive or dominant mutations in ERLIN2 can convert to ALS. Neurol Genet. 2019;5(6):e374. https://doi.org/10.1212/NXG.0000000000000374.

43. Al-Saif A, Bohlega S, Al-Mohanna F. Loss of ERLIN2 function leads to juvenile primary lateral sclerosis. Ann Neurol. 2012;72(4):510–6. https://doi.org/10.1002/ana.23641.

44. Pednekar D, Wang Y, Fedotova TV, Wojcikiewicz RJ. Clustered hydrophobic amino acids in amphipathic helices mediate erlin1/2 complex assembly. Biochem Biophys Res Commun. 2011;415(1):135–40. https://doi.org/10.1016/j.bbrc.2011.10.032.

45. Daoud H, Zhou S, Noreau A, Sabbagh M, Belzil V, Dionne-Laporte A, et al. Exome sequencing reveals SPG11 mutations causing juvenile ALS. Neurobiol Aging. 2012;33(4):839 e5–9. https://doi.org/10.1016/j.neurobiolaging.2011.11.012.

46. Faber I, Martinez ARM, Martins CR Jr, Maia ML, Souza JP, Lourenco CM, et al. SPG11-related parkinsonism: clinical profile, molecular imaging and l-dopa response. Mov Disord. 2018;33(10):1650–6. https://doi.org/10.1002/mds.27491.

47. Estrada-Cuzcano A, Martin S, Chamova T, Synofzik M, Timmann D, Holemans T, et al. Loss-of-function mutations in the ATP13A2/PARK9 gene cause complicated hereditary spastic paraplegia (SPG78). Brain. 2017;140(2):287–305. https://doi.org/10.1093/brain/aww307.

48. Ramirez A, Heimbach A, Grundemann J, Stiller B, Hampshire D, Cid LP, et al. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. Nat Genet. 2006;38(10):1184–91. https://doi.org/10.1038/ng1884.

49. Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, et al. The ubiquitin pathway in Parkinson's disease. Nature. 1998;395(6701):451–2. https://doi.org/10.1038/26652.

50. Bilguvar K, Tyagi NK, Ozkara C, Tuysuz B, Bakircioglu M, Choi M, et al. Recessive loss of function of the neuronal ubiquitin hydrolase UCHL1 leads to early-onset progressive neurodegeneration. Proc Natl Acad Sci U S A. 2013;110(9):3489–94. https://doi.org/10.1073/pnas.1222732110.

51. Osaka H, Wang YL, Takada K, Takizawa S, Setsuie R, Li H, et al. Ubiquitin carboxy-terminal hydrolase L1 binds to and stabilizes monoubiquitin in neuron. Hum Mol Genet. 2003;12(16):1945–58. https://doi.org/10.1093/hmg/ddg211.

52. Arif B, Kumar KR, Seibler P, Vulinovic F, Fatima A, Winkler S, et al. A novel OPA3 mutation revealed by exome sequencing: an example of reverse phenotyping. JAMA Neurol. 2013;70(6):783–7. https://doi.org/10.1001/jamaneurol.2013.1174.

53. Kumar KR, Wali G, Davis RL, Mallawaarachchi AC, Palmer EE, Gayevskiy V, et al. Expanding the spectrum of PEX16 mutations and

novel insights into disease mechanisms. Mol Genet Metab Rep. 2018;16:46–51. https://doi.org/10.1016/j.ymgmr.2018.07.003.

54. Kumar KR, Wali GM, Kamate M, Wali G, Minoche AE, Puttick C, et al. Defining the genetic basis of early onset hereditary spastic paraplegia using whole genome sequencing. Neurogenetics. 2016;17(4):265–70. https://doi.org/10.1007/s10048-016-0495-z.

55. Lohmann K, Wilcox RA, Winkler S, Ramirez A, Rakovic A, Park JS, et al. Whispering dysphonia (DYT4 dystonia) is caused by a mutation in the TUBB4 gene. Ann Neurol. 2013;73(4):537–45. https://doi.org/10.1002/ana.23829.

56. Kancheva D, Chamova T, Guergueltcheva V, Mitev V, Azmanov DN, Kalaydjieva L, et al. Mosaic dominant TUBB4A mutation in an inbred family with complicated hereditary spastic paraplegia. Mov Disord. 2015;30(6):854–8. https://doi.org/10.1002/mds.26196.

57. Shribman S, Reid E, Crosby AH, Houlden H, Warner TT. Hereditary spastic paraplegia: from diagnosis to emerging therapeutic approaches. Lancet Neurol. 2019;18(12):1136–46. https://doi.org/10.1016/S1474-4422(19)30235-2.

58. Fan Z, Greenwood R, Felix AC, Shiloh-Malawsky Y, Tennison M, Roche M, et al. GCH1 heterozygous mutation identified by whole-exome sequencing as a treatable condition in a patient presenting with progressive spastic paraplegia. J Neurol. 2014;261(3):622–4. https://doi.org/10.1007/s00415-014-7265-3.

59. Wei Y, Zhou Y, Yuan J, Ni J, Qian M, Cui L, et al. Treatable cause of hereditary spastic paraplegia: eight cases of combined homocysteinaemia with methylmalonic aciduria. J Neurol. 2019;266(10):2434–9. https://doi.org/10.1007/s00415-019-09432-8.

60. Lossos A, Teltsh O, Milman T, Meiner V, Rozen R, Leclerc D, et al. Severe methylenetetrahydrofolate reductase deficiency: clinical clues to a potentially treatable cause of adult-onset hereditary spastic paraplegia. JAMA Neurol. 2014;71(7):901–4. https://doi.org/10.1001/jamaneurol.2014.116.

61. Nicholls Z, Hobson E, Martindale J, Shaw PJ. Diagnosis of spinal xanthomatosis by next-generation sequencing: identifying a rare, treatable mimic of hereditary spastic paraparesis. Pract Neurol. 2015;15(4):280–3. https://doi.org/10.1136/practneurol-2015-001117.

62. Raymond GV, Moser AB, Fatemi A. X-linked adrenoleukodystrophy. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K et al., editors. GeneReviews((R)). Seattle (WA)1993.

63. Tsang JP, Poon WL, Luk HM, Fung CW, Ching CK, Mak CM, et al. Arginase deficiency with new phenotype and a novel mutation: contemporary summary. Pediatr Neurol. 2012;47(4):263–9. https://doi.org/10.1016/j.pediatrneurol.2012.06.012.

64. Wolf B. Biotinidase deficiency should be considered in individuals exhibiting myelopathy with or without and vision loss. Mol Genet Metab. 2015;116(3):113–8. https://doi.org/10.1016/j.ymgme.2015.08.012.

65. Wolf B. Biotinidase deficiency. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K et al., editors. GeneReviews((R)). Seattle (WA)1993.

66. Wang Y, Smith C, Parboosingh JS, Khan A, Innes M, Hekimi S. Pathogenicity of two COQ7 mutations and responses to 2,4-dihydroxybenzoate bypass treatment. J Cell Mol Med. 2017;21(10):2329–43. https://doi.org/10.1111/jcmm.13154.

67. Salviati L, Trevisson E, Doimo M, Navas P. Primary coenzyme Q10 deficiency. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K et al., editors. GeneReviews((R)). Seattle (WA)1993.

68. Verrips A, Hoefsloot LH, Steenbergen GC, Theelen JP, Wevers RA, Gabreels FJ, et al. Clinical and molecular genetic characteristics of patients with cerebrotendinous xanthomatosis. Brain. 2000;123(Pt 5):908–19. https://doi.org/10.1093/brain/123.5.908.

69. Kasim S, Moo LR, Zschocke J, Jinnah HA. Phenylketonuria presenting in adulthood as progressive spastic paraparesis with dementia. J Neurol Neurosurg Psychiatry. 2001;71(6):795–7. https://doi.org/10.1136/jnnp.71.6.795.

70. Verrotti A, Di Francesco L, Striano P. GLUT1 deficiency and pediatric-onset hereditary spastic paraplegia: a new association. Eur J Paediatr Neurol. 2019;23(2):233–4. https://doi.org/10.1016/j.ejpn.2019.02.010.

71. Vazquez-Costa JF, Bataller L, Vilchez JJ. Primary lateral sclerosis and hereditary spastic paraplegia in sporadic patients. An important distinction in descriptive studies. Ann Neurol. 2016;80(1):169–70. https://doi.org/10.1002/ana.24671.

72. Geevasinga N, Menon P, Sue CM, Kumar KR, Ng K, Yiannikas C, et al. Cortical excitability changes distinguish the motor neuron disease phenotypes from hereditary spastic paraplegia. Eur J Neurol. 2015;22(5):826–31, e57-8. https://doi.org/10.1111/ene.12669.

73. Brugman F, Veldink JH, Franssen H, de Visser M, de Jong JM, Faber CG, et al. Differentiation of hereditary spastic paraparesis from primary lateral sclerosis in sporadic adult-onset upper motor neuron syndromes. Arch Neurol. 2009;66(4):509–14. https://doi.org/10.1001/archneurol.2009.19.

74. Pearson TS, Pons R, Ghaoui R, Sue CM. Genetic mimics of cerebral palsy. Mov Disord. 2019;34(5):625–36. https://doi.org/10.1002/mds.27655.

75. Rainier S, Sher C, Reish O, Thomas D, Fink JK. De novo occurrence of novel SPG3A/atlastin mutation presenting as cerebral palsy. Arch Neurol. 2006;63(3):445–7. https://doi.org/10.1001/archneur.63.3.445.

76. Rubegni A, Battisti C, Tessa A, Cerase A, Doccini S, Malandrini A, et al. SPG2 mimicking multiple sclerosis in a family identified using next generation sequencing. J Neurol Sci. 2017;375:198–202. https://doi.org/10.1016/j.jns.2017.01.069.

77. Jia X, Madireddy L, Caillier S, Santaniello A, Esposito F, Comi G, et al. Genome sequencing uncovers phenocopies in primary progressive multiple sclerosis. Ann Neurol. 2018;84(1):51–63. https://doi.org/10.1002/ana.25263.

78. Fink JK. Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms. Acta Neuropathol. 2013;126(3):307–28. https://doi.org/10.1007/s00401-013-1115-8.

79. Bras J, Verloes A, Schneider SA, Mole SE, Guerreiro RJ. Mutation of the parkinsonism gene ATP13A2 causes neuronal ceroid-lipofuscinosis. Hum Mol Genet. 2012;21(12):2646–50. https://doi.org/10.1093/hmg/dds089.

80. Schneider SA, Paisan-Ruiz C, Quinn NP, Lees AJ, Houlden H, Hardy J, et al. ATP13A2 mutations (PARK9) cause neurodegeneration with brain iron accumulation. Mov Disord. 2010;25(8):979–84. https://doi.org/10.1002/mds.22947.

81. de Bot S, Kamsteeg EJ, Van De Warrenburg BPC. Complicated hereditary spastic paraplegia due to ATP13A2 mutations: what's in a name? Brain. 2017;140(12):e73. https://doi.org/10.1093/brain/awx280.

82. Minnerop M, Kurzwelly D, Wagner H, Soehn AS, Reichbauer J, Tao F, et al. Hypomorphic mutations in POLR3A are a frequent cause of sporadic and recessive spastic ataxia. Brain. 2017;140(6):1561–78. https://doi.org/10.1093/brain/awx095.

83. Gauquelin L, Tetreault M, Thiffault I, Farrow E, Miller N, Yoo B, et al. POLR3A variants in hereditary spastic paraplegia and ataxia. Brain. 2018;141(1):e1. https://doi.org/10.1093/brain/awx290.

84. Klebe S, Lossos A, Azzedine H, Mundwiller E, Sheffer R, Gaussen M, et al. KIF1A missense mutations in SPG30, an autosomal recessive spastic paraplegia: distinct phenotypes according to the nature of the mutations. Eur J Hum Genet. 2012;20(6):645–9. https://doi.org/10.1038/ejhg.2011.261.

85. Cheon CK, Lim SH, Kim YM, Kim D, Lee NY, Yoon TS, et al. Autosomal dominant transmission of complicated hereditary spastic paraplegia due to a dominant negative mutation of KIF1A, SPG30 gene. Sci Rep. 2017;7(1):12527. https://doi.org/10.1038/s41598-017-12999-9.

86. Citterio A, Arnoldi A, Panzeri E, Merlini L, D'Angelo MG, Musumeci O, et al. Variants in KIF1A gene in dominant and sporadic

forms of hereditary spastic paraparesis. J Neurol. 2015;262(12): 2684–90. https://doi.org/10.1007/s00415-015-7899-9.

87. Ylikallio E, Kim D, Isohanni P, Auranen M, Kim E, Lonnqvist T, et al. Dominant transmission of de novo KIF1A motor domain variant underlying pure spastic paraplegia. Eur J Hum Genet. 2015;23(10):1427–30. https://doi.org/10.1038/ejhg.2014.297.

88. Ohba C, Haginoya K, Osaka H, Kubota K, Ishiyama A, Hiraide T, et al. De novo KIF1A mutations cause intellectual deficit, cerebellar atrophy, lower limb spasticity and visual disturbance. J Hum Genet. 2015;60(12):739–42. https://doi.org/10.1038/jhg.2015.108.

89. Esteves T, Durr A, Mundwiller E, Loureiro JL, Boutry M, Gonzalez MA, et al. Loss of association of REEP2 with membranes leads to hereditary spastic paraplegia. Am J Hum Genet. 2014;94(2):268–77. https://doi.org/10.1016/j.ajhg.2013.12.005.

90. Khan TN, Klar J, Tariq M, Anjum Baig S, Malik NA, Yousaf R, et al. Evidence for autosomal recessive inheritance in SPG3A caused by homozygosity for a novel ATL1 missense mutation. Eur J Hum Genet. 2014;22(10):1180–4. https://doi.org/10.1038/ejhg.2014.5.

91. Willkomm L, Heredia R, Hoffmann K, Wang H, Voit T, Hoffman EP, et al. Homozygous mutation in Atlastin GTPase 1 causes recessive hereditary spastic paraplegia. J Hum Genet. 2016;61(6):571–3. https://doi.org/10.1038/jhg.2016.6.

92.• Aguilera-Albesa S, de la Hoz AB, Ibarluzea N, Ordonez-Castillo AR, Busto-Crespo O, Villate O, et al. Hereditary spastic paraplegia and intellectual disability: clinicogenetic lessons from a family suggesting a dual genetics diagnosis. Front Neurol. 2020;11:–41. https://doi.org/10.3389/fneur.2020.00041 **A recent study showing concurrent genetic diagnoses in a family with HSP.**

93. Posey JE, Harel T, Liu P, Rosenfeld JA, James RA, Coban Akdemir ZH, et al. Resolution of disease phenotypes resulting from multilocus genomic variation. N Engl J Med. 2017;376(1): 21–31. https://doi.org/10.1056/NEJMoa1516767.

94.• Verdura E, Schluter A, Fernandez-Eulate G, Ramos-Martin R, Zulaica M, Planas-Serra L, et al. A deep intronic splice variant advises reexamination of presumably dominant SPG7 Cases. Ann Clin Transl Neurol. 2020;7(1):105–11. https://doi.org/10.1002/acn3.50967 **This report highlights the utility of WGS in detecting deep intronic variants in the *SPG7* gene.**

95. Sanchez-Ferrero E, Coto E, Beetz C, Gamez J, Corao AI, Diaz M, et al. SPG7 mutational screening in spastic paraplegia patients supports a dominant effect for some mutations and a pathogenic role for p.A510V. Clin Genet. 2013;83(3):257–62. https://doi.org/10.1111/j.1399-0004.2012.01896.x.

96. Kumar KR, Cowley MJ, Davis RL. Next-generation sequencing and emerging technologies. Semin Thromb Hemost. 2019;45: 661–73. https://doi.org/10.1055/s-0039-1688446.

97. Meienberg J, Bruggmann R, Oexle K, Matyas G. Clinical sequencing: is WGS the better WES? Hum Genet. 2016;135(3): 359–62. https://doi.org/10.1007/s00439-015-1631-9.

98. Gross AM, Ajay SS, Rajan V, Brown C, Bluske K, Burns NJ, et al. Copy-number variants in clinical genome sequencing: deployment and interpretation for rare and undiagnosed disease. Genet Med. 2019;21(5):1121–30. https://doi.org/10.1038/s41436-018-0295-y.

99. Kirk EP, Barlow-Stewart K, Selvanathan A, Josephi-Taylor S, Worgan L, Rajagopalan S, et al. Beyond the panel: preconception screening in consanguineous couples using the TruSight One "clinical exome". Genet Med. 2018;21:608–12. https://doi.org/10.1038/s41436-018-0082-9.

100. Beetz C, Nygren AO, Schickel J, Auer-Grumbach M, Burk K, Heide G, et al. High frequency of partial SPAST deletions in autosomal dominant hereditary spastic paraplegia. Neurology. 2006;67(11): 1926–30. https://doi.org/10.1212/01.wnl.0000244413.49258.f5.

101. Dolzhenko E, van Vugt J, Shaw RJ, Bekritsky MA, van Blitterswijk M, Narzisi G, et al. Detection of long repeat expansions from PCR-free whole-genome sequence data. Genome Res. 2017;27(11):1895–903. https://doi.org/10.1101/gr.225672.117.

102. Wagner M, Berutti R, Lorenz-Depiereux B, Graf E, Eckstein G, Mayr JA, et al. Mitochondrial DNA mutation analysis from exome sequencing-a more holistic approach in diagnostics of suspected mitochondrial disease. J Inherit Metab Dis. 2019;42(5):909–17. https://doi.org/10.1002/jimd.12109.

103. Puttick C, Kumar KR, Davis RL, Pinese M, Thomas DM, Dinger ME et al. mity: a highly sensitive mitochondrial variant analysis pipeline for whole genome sequencing data. bioRxiv. 2019:852210. 10.1101/852210.

104. Orso G, Martinuzzi A, Rossetto MG, Sartori E, Feany M, Daga A. Disease-related phenotypes in a Drosophila model of hereditary spastic paraplegia are ameliorated by treatment with vinblastine. J Clin Invest. 2005;115(11):3026–34. https://doi.org/10.1172/JCI24694.

105. Fassier C, Tarrade A, Peris L, Courageot S, Mailly P, Dalard C, et al. Microtubule-targeting drugs rescue axonal swellings in cortical neurons from spastin knockout mice. Dis Model Mech. 2013;6(1):72–83. https://doi.org/10.1242/dmm.008946.

106. Fan Y, Wali G, Sutharsan R, Bellette B, Crane DI, Sue CM, et al. Low dose tubulin-binding drugs rescue peroxisome trafficking deficit in patient-derived stem cells in hereditary spastic paraplegia. Biol Open. 2014;3(6):494–502. https://doi.org/10.1242/bio.20147641.

107. Wali G, Sue CM, Mackay-Sim A. Patient-derived stem cell models in SPAST HSP: disease modelling and drug discovery. Brain Sci. 2018;8(8):142. https://doi.org/10.3390/brainsci8080142.

108. Rickman OJ, Baple EL, Crosby AH. Lipid metabolic pathways converge in motor neuron degenerative diseases. Brain. 2020;143(4):1073–87. https://doi.org/10.1093/brain/awz382.

109.•• Schols L, Rattay TW, Martus P, Meisner C, Baets J, Fischer I, et al. Hereditary spastic paraplegia type 5: natural history, biomarkers and a randomized controlled trial. Brain. 2017;140(12): 3112–27. https://doi.org/10.1093/brain/awx273 **One of two clinical trials investigating the use of cholesterol-lowering drugs to treat SPG5. SPG5 may prove to be the most treatable form of HSP and so in the future, it may be particularly important to identify individuals with *CYP7B1* mutations.**

110. Schule R, Siddique T, Deng HX, Yang Y, Donkervoort S, Hansson M, et al. Marked accumulation of 27-hydroxycholesterol in SPG5 patients with hereditary spastic paresis. J Lipid Res. 2010;51(4): 819–23. https://doi.org/10.1194/jlr.M002543.

111.•• Marelli C, Lamari F, Rainteau D, Lafourcade A, Banneau G, Humbert L, et al. Plasma oxysterols: biomarkers for diagnosis and treatment in spastic paraplegia type 5. Brain. 2018;141(1):72–84. https://doi.org/10.1093/brain/awx297 **The other clinical trial investigating the use of cholesterol-lowering drugs to treat SPG5.**

112. Hauser S, Poenisch M, Schelling Y, Hoflinger P, Schuster S, Teegler A, et al. mRNA as a novel treatment strategy for hereditary spastic paraplegia type 5. Mol Ther Methods Clin Dev. 2019;15:359–70. https://doi.org/10.1016/j.omtm.2019.10.011.

113. Kara E, Tucci A, Manzoni C, Lynch DS, Elpidorou M, Bettencourt C, et al. Genetic and phenotypic characterization of complex hereditary spastic paraplegia. Brain. 2016;139(Pt 7): 1904–18. https://doi.org/10.1093/brain/aww111.

114. Schule R, Wiethoff S, Martus P, Karle KN, Otto S, Klebe S, et al. Hereditary spastic paraplegia: clinicogenetic lessons from 608 patients. Ann Neurol. 2016;79(4):646–58. https://doi.org/10.1002/ana.24611.