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Clinical and genetic characterization of hereditary spastic paraplegia



Anders Toft (rgs396)

STUD.MED. 5. SEMESTER KANDIDAT, KØBENHAVNS UNIVERSITET
KANDIDATOPGAVE

VEJLEDERE: KIRSTEN SVENSTRUP OG JØRGEN ERIK NIELSEN

Abstract

Background: Hereditary spastic paraplegia (HSP) is a neurodegenerative disease entity with progressive lower limb spasticity and weakness as the predominant symptoms. Pronounced genetic and clinical heterogeneity characterizes these disorders, given that more than 80 genetic loci have been reported as causative for separate HSP subtypes with differing ages of onset, symptoms, and severity. Phenotypes are divided into pure and complex – the latter with additional system involvement – and every known Mendelian mode of inheritance has been described.

Aim: This study aims to investigate the molecular and clinical spectrum of HSP including the genotype-phenotype correlations in a series of Danish HSP patients treated at Rigshospitalet.

Methods: A total of 101 probands referred to Rigshospitalet with signs of pure or complex HSP and genetically tested for HSP were included. The molecular data were entered into a database and clinical information on each patient added subsequently.

Results: Family history indicated autosomal dominant (AD) inheritance in 35 % of families, autosomal recessive (AR) in 5 %, AD or AR in 6 %, X-linked or AR in 3 %, and 51 % were sporadic. Forty percent of subjects obtained a genetically verified diagnosis, and in 37 % of cases this diagnosis was HSP. Of the 52 disease-causing spastic paraplegia (SPG) gene variants detected, the majority (60 %) had been reported elsewhere and the remainder were novel mutations. In this patient cohort *SPAST* mutations (SPG4) proved most common, followed by SPG7.

Conclusion: In this study, the diverse clinical expression, genetic background, and bridging neuropathology of HSP are carefully described. A database is established, linking the genotypic and phenotypic information of the included probands. Based on this, new and previously observed genetic variations in the most frequent subtypes are discussed. The continuum that exists between HSP and other neurodegenerative diseases – as well as the rapid development in diagnostic technologies – are emphasized, and lastly, the importance of molecularly supported diagnoses is touched on.

Resumé

Baggrund: Hereditær spastisk paraparese (HSP) tilhører gruppen af arvelige, neurodegenerative sygdomme, og er domineret af progredierende spasticitet og kraftnedsættelse i underekstremiteterne. HSP er karakteriseret ved udtalt klinisk og genetisk heterogenitet. Dette fremgår af, at 80 loci hidtil er blevet beskrevet som værende patogene, medførende et tilsvarende antal kliniske undertyper, som hver især udviser varierende debutalder, symptomer og sværhedsgrad. Alle former for mendelsk arvegang er rapporteret. Fænotypisk opdeles HSP i ren og kompleks, hvor sidstnævnte foruden den spastiske paraparese inkluderer yderligere neurologiske symptomer.

Formål: Dette studie har til formål at undersøge HSP-sygdommens molekylære og kliniske spektrum, og herunder analysere genotype-fænotype-korrelationer blandt en række patienter evalueret på Rigshospitalet.

Metoder: 101 probander henvist til Rigshospitalet med henblik på udredning for HSP og efterfølgende genetisk testning blev inkluderet. De molekylære data blev indskrevet i en database, og patienternes respektive kliniske information blev derpå tilføjet.

Resultater: Familieanamnesen indikerede autosomal dominant (AD) arvegang i 35 % af familierne, autosomal recessiv (AR) i 5 %, AD eller AR i 6 % og X-bunden eller AR i 3 %. De resterende 51 % var sporadiske tilfælde. En genetisk verificeret diagnose blev opnået hos 40 % af patienterne, HSP-diagnoser udgjorde 37 %. Størstedelen af de identificerede, sygdomsfremkaldende genvarianter (60 %) var tidligere

beskrevet, mens resten var nyfundne. *SPAST*-mutationer (SPG4) var den hyppigste årsag til HSP i denne patientgruppe, efterfulgt af SPG7.

Konklusion: I dette studie redegøres for det variable kliniske udtryk, den underliggende neuropatologi, samt det genetiske grundlag for HSP. Oprettelsen af en klinisk database muliggør analyse af forholdet mellem patienternes genotyper og fænotyper. Herudfra diskuteres både nye og tidligere observerede genetiske variationer i de hyppigst repræsenterede subtyper. Den uskarpe afgrænsning mellem HSP og andre neurodegenerative sygdomme, samt den hastige teknologiske udvikling inden for diagnostik, understreges, og endelig omtales betydningen for patienten af en molekylært bekræftet diagnose.

Background

Hereditary spastic paraplegia (HSP) comprises a large group of neurodegenerative diseases that share the clinical hallmark of gradual and progressive spastic gait disturbance.

The inheritable forms of spastic paraplegia were first described by Adolph Strümpell and Maurice Lorrain in the late 19th century. Strümpell-Lorrain disease was later renamed hereditary spastic paraplegia and classified into pure and complex forms by Anita Harding in the early 1980s (1). Today, HSP is among the genetically and clinically most heterogeneous human disorders and the spectrum of disease is continuously being widened as novel mutations and phenotypical presentations are reported.

Symptoms

Age of onset

The age of HSP onset is remarkably variable, ranging from early childhood with delayed motor milestones to over 70 years of age (2). The distribution seems bimodal, with peaks in early childhood (under 5 years) and early adulthood (around 40 years) and a mean age of around 30 years (3,4). Although dependent on the underlying genetic cause to a certain degree (3), the age at onset may also differ between family members with identical mutations (5). Accurate determination of age of onset is complicated by the slowly progressive nature of most HSP subtypes: This will often prolong the time it takes for the patient to recognize the signs and symptoms, leading to underestimation of the actual date of onset (5). Therefore, when obtaining the medical history, it is important to inquire about the patients' motor milestones, interest, and participation in physical activity in their youth, as delayed motor milestones as well as exercise avoidance or poor performance may be indicative of an early-onset motor system disorder (5).

Progression

When HSP presents after late childhood, a slow and steady progression over years or decades is usually observed. The symptoms then tend to stabilize over time and patients often reach a functional plateau, from which any further impairment of gait and balance follows normal age-related changes (6). Symptoms occurring in infancy or early childhood can be nearly non-progressive and similar to those seen in spastic diplegic cerebral palsy - a differential diagnostic consideration in early-onset HSP (6). Generally, it was observed by Harding that individuals who are affected early in life (< 35 years) are more likely to experience slow progression and remain ambulant, whereas patients with late-onset HSP (> 35 years) more commonly lose their ability to walk independently, and do so earlier in the course of the disease (1). This finding is in line with recent results, establishing age of onset as a prognostic tool for walking aid and wheelchair dependency (3). In this study by Schüle et al., the ability to walk unassisted was maintained by HSP patients for a median of 22 years, significantly longer in patients with debut at a younger age. However, like the age

of onset, the rate of progression can differ considerably, ranging from fairly static or slowly progressing to accelerated deterioration (5). Despite the unpredictable disease course and degree of disability, life expectancy of HSP patients is considered normal.

Pure hereditary spastic paraplegia

In its pure form, HSP is predominantly characterized by a slowly progressive spastic weakness of the lower limbs. Patients complain of difficulty walking or keeping balance, stumbling or abnormal gait (7). In mild cases or early stages, discrete changes may only have been noticed by relatives, but not the patients themselves. Other presenting signs and symptoms include leg stiffness, trouble descending stairs, and increased wear on the front of the footwear. Neurogenic urinary disturbances such as urgency, hesitancy, frequency, nocturia, and urinary incontinence are described in approximately half of patients and become more prevalent with long-lasting disease (1).

Upon clinical examination the key finding is bilateral lower limb spasticity, primarily affecting the quadriceps, hamstrings, adductors, and triceps surae muscles (6). The hypertonicity gives rise to the characteristic scissoring or circumducting gait and toe walking seen in HSP patients due to impeded dorsiflexion of the ankle. This increased muscle tone is most often accompanied by paraparesis of variable severity affecting the iliopsoas, hamstrings and tibialis anterior muscles in a supranuclear pattern (8). Spasticity and paresis may be present in equal measure; however, it is not uncommon to discover mild or even absent weakness in the lower extremities despite extensive spasticity (6). Generally, compared to individuals suffering from other illnesses causing spastic paraparesis (such as primary demyelinating disorders or spinal cord injury) patients with HSP tend to exhibit a remarkable preservation of muscle strength relative to spasticity (7). In the late stages of the disease weakness can become pronounced and more widespread, with a tendency of the muscle affection to progress from distal to more proximal (9). Normally, the spastic paraparesis is symmetrically distributed. In cases with substantial lateralization, compressive or other structural causes should be ruled out (9). Prominent upper motor neuron signs in pure HSP patients also comprise lower limb hyperreflexia, spastic catch, ankle clonus, and extensor plantar responses. A limited number of other neurological findings are commonly included in the pure form of HSP: Deep sensory loss, particularly impaired vibration sensation and occasionally joint position sense affection is found in the distal lower extremities of more than half of patients (3). Pes cavus is also frequently observed, reported by Harding in approximately one third of cases (1). Muscle wasting is not a typical finding, but slight distal atrophy may be noted in some cases. Severe muscle atrophy, however, should prompt further diagnostic clarification. Involvement of the upper extremities can occur and examination may reveal discrete hyperreflexia with Hoffmann's signs, while strength and dexterity are retained (9). Other important negative findings in pure HSP patients are normal cognition, speech and cranial nerve function.

Complex hereditary spastic paraplegia

The syndromic designation of complex HSP covers a multitude of clinical entities in which spastic paraplegia is associated with diverse combinations of additional neurological or non-neurological manifestations. Certain phenotypes are exceedingly rare and have only been documented once (7). Complicating neurological features are: Cognitive dysfunction in the form of intellectual disability or dementia; cerebellar involvement causing ataxia, dysarthria, dysphagia, tremor or nystagmus; axonal or demyelinating peripheral neuropathy; amyotrophy; seizures; extrapyramidal disturbances e.g. parkinsonism, chorea or

dystonia; psychiatric symptoms; and MRI findings such as a thin corpus callosum, white matter changes, leukoaraiosis, spinal cord atrophy, hydrocephalus, or mild cerebral or cerebellar atrophy (5,7,9). Symptoms of complex HSP may include ophthalmological anomalies i.e. optic neuropathy or atrophy, cataract or retinitis pigmentosa; hearing loss; ichthyosis; adrenal insufficiency; and orthopaedic abnormalities and dysmorphic traits such as micro- and macrocephaly, short stature, facial dysmorphisms etc. (7).

Precise delineation of complex HSP is challenging and in some instances more than one underlying disorder can arguably contribute to the clinical presentation. In addition, the complicating symptoms may precede the spastic paraparesis, delaying the diagnostic process. Studies report differing prevalences of complicated HSP from approximately one fourth to more than half of patients (3,4,10-12). As compared to pure HSP, the complex form has been associated with a more severe disease course (3).

Rating of disease severity

Standardized and clinically relevant severity rating scores are not only helpful in assessing the individual patient progress, but an essential tool in evaluating treatment efficacy, identifying predictive and prognostic biomarkers, and comparing patient cohorts for research purposes. Such rating scales have not previously been readily available in HSP. In 2006, Schüle et al. presented and validated the Spastic Paraplegia Rating Scale (SPRS) (13), comprising a 13-item grading system devised to quantify the declining physical functioning of HSP patients. The scale includes variables such as walking distance, gait quality, stair climbing, pain due to spasticity, bladder function, etc. and also contains a non-gradable inventory for recording complicating signs and symptoms. Since its development, the SPRS has been among the most widely used measures of disease severity in HSP (3,4,13-15). Numerous other clinical rating scales have been employed for assessing the status and prognosis of HSP patients. Certain tools and methods are well-established in other medical disorders and some are HSP-specific. They include the Barthell Index (BI), the International Cooperative Ataxia Rating Scale (ICARS), the Mini-Mental State Examination (MMSE), the Modified Ashworth Scale (MAS), the Four-Stage Scale of Motor Disability (4SMD), the Functional HSP Rating Scale (FHSPS), and various walking scales (4,13). Recent studies are utilizing these clinical rating scales to test for potential clinical and paraclinical (e.g. imaging or electrophysical) biomarkers of HSP severity (4,15).

Epidemiology

Previously, prevalence studies in HSP have been scarce and inconsistent in terms of methodology and classification of patients. Systematic epidemiological investigations have been facilitated by the diagnostic criteria suggested by Harding and the advances in genetic research. Still, reported outcomes vary somewhat between studies: McMonagle et al. estimated the prevalence of pure HSP patients in Ireland to be 1.3 per 100.000 individuals (16), while the number of pure HSP patients in Northern Spain is 9.6/100.000 according to Polo et al. (17). A combination of factors may underlie this discrepancy: Different inclusion criteria and diagnostic methods; the handling of sporadic cases, including the extend of differential diagnostic and family investigation; overestimation bias due to disease-clusters in the reporting region; as well as a true geographical distribution based on founder effects or consanguinity in certain populations (5,11). In a systematic review and meta-analysis of the literature, the first of its kind in the field, Ruano et al. estimated the global epidemiology of HSP and hereditary ataxia based on 22 studies from 16 countries (14). Here, the frequency of autosomal dominant (AD) as well as autosomal recessive (AR) HSP was 1.8/100.000.

Etiology

Every case of HSP is caused by genetic changes, either inherited or de novo. The clinical diversity is reflected in the heterogeneous genetic basis. At present, alterations in over 80 genetic loci and more than 60 different spastic paraplegia (SPG) genes have been identified as causative of HSP, and all patterns of Mendelian inheritance (AD, AR, X-linked) along with non-Mendelian mitochondrial transmission are recognized (5,7).

AD inheritance is characterized by multiple affected individuals in successive generations; a 50 % risk that any given child will inherit the disease allele; the possibility of male-to-male transmission; and an equal proportion of affected males and females (9). AD HSP most often produces a pure phenotype (2,7,9), and these families may exhibit reduced penetrance, i.e. not all individuals carrying a mutated allele will express the HSP traits (5). The most prevalent type of AD HSP by far is due to a mutation in the SPG4 gene known as *SPAST*, followed by *ATL1* (SPG3A) and *REEP1* (SPG31) mutations (3,7,9).

AR HSP should be suspected in families with few affected members, where healthy parents have multiple affected children, and in consanguineous pedigrees (7,9). Identification of this manner of transmission is typically not as straightforward. Complicated HSP tend to be AR (3,5), the most frequent cause being *SPG11* mutation, especially if MRI has revealed thinning of the corpus callosum – a characteristic of SPG11 HSP and of complicated HSP in general. Pure AR HSP may also be observed, with *SPG7* mutation as the predominant underlying pathology (9).

X-linked and mitochondrial maternal inheritance patterns are rare and associated with complicated neurological syndromes (7). Only a handful of causative genes have been identified for each type.

Finally, a significant number of patients present with absent family history and seemingly sporadic disease. In these instances, HSP is a diagnosis of exclusion, and AD HSP with reduced penetrance, AR HSP, and X-linked HSP should be considered as alternatives to actual de novo mutation (9,10).

Pathophysiology

The progressive symptoms of HSP, including lower limb spasticity and weakness, hyperreflexia, Babinski signs, sensory disturbances, etc., are indicative of spinal cord dysfunction. It is generally accepted that the main pathological mechanism driving neurodegeneration in HSP is a primary axonopathy of fibers in this part of the central nervous system (5,8,9). Vulnerability of the axons seems to increase with fiber length, with the distal segments being most severely affected. Axonal degeneration is therefore maximal in the lumbar part of the descending corticospinal tract and the cervical part of the gracile fasciculus in the ascending dorsal column (8,9) – explaining the preferential targeting of the lower extremities. This simplistic view of HSP pathophysiology, however, is complicated by reports of more widespread neuropathology such as rostral progression from the pyramidal tracts to the level of the internal capsule; lower motor neuron affection with axonal peripheral neuropathy; secondary demyelination; involvement of relatively short CNS neurons; and observations indicating that some HSP abnormalities are more developmental than degenerative (8,9). The extent of these complementary features is far from understood and goes beyond the clinical dissociation of pure and complicated HSP.

At the molecular level, functional characterization of the SPG-genes has revealed proteins that are highly interconnected and involved in a limited number of cellular pathways. The molecular mechanisms include dysfunctional membrane trafficking; axonal transport; and organelle biogenesis as well as mitochondrial, lipid metabolism, and myelination abnormalities (2,5,7). As an example, Spastin – the protein encoded by *SPAST* (SPG4) and member of a large family of ATPases – interacts with other proteins encoded by SPG-

genes: Atlastin-1 (SPG3A), Reticulon-2 (SPG12), and REEP1 (SPG31) in the formation of the tubular endoplasmatic reticulum network (5,7). A molecular defect in one of these proteins generated by the corresponding SPG-gene mutation may cause neuronal degeneration and ultimately an HSP phenotype.

Diagnostics

The diagnosis of HSP is based on the clinical evaluation, demonstrating the characteristic supranuclear affection of the lower limbs; a family history that may or may not disclose a well-known mode of inheritance; exclusion of structural, metabolic, or genetic disorders causing similar presentations; and finally verification of the clinical diagnosis by identifying the particular SPG-gene mutation if possible (5,7). Supplementary diagnostic workup is required to rule out alternative disorders, but is not otherwise necessary to confirm the HSP diagnosis: MRI of the neural axis is usually unremarkable and CSF analyses are normal; as are electromyography (EMG) and nerve conduction studies in pure HSP. Central motor conduction times and somatosensory evoked potentials, however, are often prolonged to and from the lower extremities, respectively, and unaffected in the upper limbs (5,7,9).

Exclusion of other diagnoses is essential, as these may carry a completely different prognosis from HSP and require specific treatment. Important differential diagnoses of HSP include multiple sclerosis, B12 deficiency, adrenomyeloneuropathy, spinocerebellar ataxias such as Friedrich's ataxia, primary lateral sclerosis, dopa-responsive dystonia, structural spinal cord disorders, and many others (6).

Treatment

Currently, no treatment to cure, prevent or even delay HSP is available. Management of this disorder is strictly symptomatic and focused on alleviating the daily inconvenience that HSP patients experience. Spasticity can be reduced medically with muscle relaxants such as baclofen, dantolene, tizandine, or botulinum toxin injections (8), but this treatment is problematic in HSP: Relieving spasticity may suddenly expose the significantly reduced strength in the patients' lower limbs – exacerbating the gait impairment rather than improving it (5). Furthermore, the necessary dose may cause unacceptable side effects in the form of disabling drowsiness. Nevertheless, these are appropriate treatment options in some cases, especially for patients with intolerable spasms or spasticity-related pain. For every HSP patient, physical therapy is recommended in order to maintain muscle strength and range of movement and to avoid complicating pain and contractures (7). Most patients will eventually require assistive devices such as a cane or walker (3), but the majority will avoid wheelchair dependency.

Urinary urgency and bladder hyperactivity is treatable with either anticholinergic drugs, a beta-3 receptor agonist, or detrusor injections of botulinum toxin. Patients with early-onset HSP may benefit from orthopedic options such as ankle-foot orthosis or achilles tenotomy (5). Lastly, complicating features e.g. epilepsy, hydrocephalus, and parkinsonism should be managed as per their respective guidelines.

Aim

The aim of this study is to explore the disease category of hereditary spastic paraplegia and investigate the genotype-phenotype correlations in a series of HSP patients evaluated at Rigshospitalet. To this end, a medical database containing clinical and molecular information on the patients is established, and findings are reviewed in the context of published literature.

Patients and Methods

This study was conducted with approval from the Danish Data Protection Agency (RH-2017-117, 05444). Included patients were diagnosed or managed at Rigshospitalet, a tertiary referral center for HSP patients in Denmark. Only one proband was included from each family and this subject had to have available results from genetic testing for HSP from 2009 to 2017, regardless of the conclusion. These patients were highly selected as described below.

Until 2009, molecular-genetic testing for HSP was performed at the Department of Cellular and Molecular Medicine, University of Copenhagen, on a research basis using consecutive single gene Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA). From 2009 and onwards, molecular-genetic testing was only available abroad and was carried out by CENTOGENE AG, Rostock, Germany. As it was an expensive service the number of genes to be sequenced was considered in each case, and an application for covering of expenses was sent to the National Board of Health. Permission was given in cases with a wish for prenatal diagnostics; if it was essential for treatment; or in cases with undiagnosed progressive neurodegenerative disease resembling HSP. The molecular-genetic analyses performed abroad comprised Sanger sequencing, MLPA, and in 2012 gene panel analysis by next generation sequencing (NGS) was made available. In a number of patients, selected SPG-genes had already been sequenced for research purposes as described. Thus, the analyses were conducted depending on the genes studied in advance and whether the mode of inheritance was AD, AR, or the case sporadic. As novel disease-causing genes continuously have been identified, and the cost of sequencing reduced, applied gene panels have expanded over time. Of note, the specific genes investigated vary between patients, whom have not been screened for mutations in all known SPG-genes. Furthermore, due to the developments in molecular-genetic diagnostics the number of tested genes has increased over time: In the 49 patients tested in 2009 or 2010 the average number of screened genes was 5, compared to a mean of 19 genes in patients tested afterwards.

Classification of the identified variants in terms of potential pathogenicity was based on a range of parameters: Previous reports describing the given variant as disease-causing; the specific type of genetic alteration and whether it can be assumed to disrupt gene function (e.g. nonsense mutations, frameshift mutations, and large deletions); frequency of the variant in control populations in online databases for genetic variation; absence of other SPG-mutations able to explain the observed phenotype; and in silico predictions based on protein structure or function or evolutionary conservation. Other tests of pathogenicity, such as family investigations and functional studies, were carried out when indicated, but are not included in this study.

The outcomes of the molecular-genetic analyses, kept on paper files at Rigshospitalet, were available for 101 patients. These records were converted to a database using Microsoft Excel. By review of medical records, clinical information on each patient was then entered into the database. The clinical variables included: age at onset, current symptoms and progression, family history, findings from the initial clinical examination, paraclinical investigations, treatment, and follow-up. The primary evaluation following the referral of the patient was chosen as a study baseline for assessment of neurological status and HSP severity. Due to insufficient information in patient records, severity rating according to the SPRS was not possible. In its place, the 4SMD was utilized. This rating scale, shown to predict the SPRS with a high statistical significance [11], places patients into one of four categories based on their motor disability.

Results

Clinical findings

A total of 101 individuals were included in the study: Forty-four women and 57 men aged 5-84 years (Table 1). Approximately half (49 patients) had a family history suspicious of HSP. Of patients with affected relatives the mode of inheritance was expected to be AD in 35 families, AR in 5 families, either AD or AR in 6 families, and either X-linked or AR in the remaining 3 families based on the reported pedigree. Age of onset ranged from 0 to 68 years, with a median of 32 years. Fifty-three patients were experiencing urinary urgency or incontinence at this time point, and comorbidity of any kind was present in 61 subjects – the most frequent being hypertension, musculoskeletal disorders, and depression. Eleven and 12 cases, respectively, showed some extent of cognitive affection or findings upon cranial nerve examination (cranial nerves II, III, and VIII), and variable degrees of upper limb involvement (hyperreflexia as a minimum) was demonstrated in 69 individuals. Naturally, all patients presented with lower extremity symptoms in various combinations. The majority was walking unaided at the time of the primary evaluation (71 patients), while only 11 patients were able to run and 5 were confined to a wheelchair. The average time from disease onset to loss of independent gait function could not be calculated as this information was not retrievable from the medical records. MRI revealed abnormalities in 20 cases, mainly non-age-corresponding white matter changes or cerebral or cerebellar atrophy. Based on these findings from the initial examination 73 patients were classified as having pure spastic paraplegia and the remaining 28 as complex. In addition to MRI features, complicating symptoms were: Cognitive impairment in 7 patients (25 % of complex HSP patients); ataxia in 6 (21 %); tremor, polyneuropathy, and upper extremity muscle wasting in 5 patients each (18 %); chronic paresthesia each in 4 patients (14 %); dysdiadochokinesia; epilepsy; dysarthria; optic nerve atrophy; among others. The great majority of complex HSP patients (22 out of 28) presented more than one complicating feature.

In 7 subjects the diagnosis of HSP was subsequently withdrawn in favor of other suspected or confirmed main diagnoses: Two patients who presented with pure HSP developed bulbar symptoms and significant affection of the hands and were re-diagnosed with primary lateral sclerosis. Based on paraclinical findings and symptom development two others were diagnosed with primary progressive multiple sclerosis, which in both cases was considered as a differential diagnosis at the initial evaluation. In three probands non-SPG genetic anomalies were identified as described below.

Table 1. Patient characteristics at the initial evaluation.

Characteristics	No. of patients (%) n = 101
Gender	
Men	57 (56.4)
Women	44 (43.6)
Age	
Range, years (mean ± SD)	5-84 (47 ± 17)
Age of onset	
Range, years (mean ± SD)	0-68 (32 ± 19)
Childhood- or adolescent-onset, < 18 years	29 (28.7)
Family history of HSP	
Most likely	38 (37.6)
Possible	11 (10.9)
Unknown	5 (5.0)
None	47 (46.5)
Suspected mode of inheritance	
AD	35 (34.7)

AR	5 (5.0)
AD or AR	6 (5.8)
XL or AR	3 (3.0)
Sporadic	52 (51.5)
Urological symptoms	53 (52.5)
Non-neurological comorbidity	61 (60.4)
Cognitive impairment	7 (6.9)
Findings on cranial nerve examination	12 (11.9)
Upper extremity involvement	69 (68.3)
Lower extremity involvement	
Spasticity	100 (99.0)
Paraparesis	95 (94.1)
Hyperreflexia	92 (91.1)
Extensor plantar responses/Babinski	87 (86.1)
Sensory involvement	57 (56.4)
HSP phenotype	
Pure	73 (72.3)
Complicated	28 (27.7)
HSP severity (4-stage motor disability rating scale/4SMD)	
Stage 1: Walking without aid and able to run	11 (10.9)
Stage 2: Walking without aid, but unable to run	61 (60.4)
Stage 3: Walking with aid	24 (23.7)
Stage 4: Wheelchair-dependent	5 (5.0)
MRI findings	
Non-age-corresponding white matter changes	11 (10.9)
Cerebellar atrophy	9 (8.9)
Cerebral atrophy	7 (6.9)
Thin corpus callosum	7 (6.9)

Genetic findings

Of the 101 patients tested molecularly a genetic etiology was identified in 40 subjects and a likely pathogenic SPG variant in 37 patients (table 2). In the latter, 52 variants were identified in total, 21 of which were novel, whereas 31 had previously been reported as non-pathogenic in the literature or in commonly used databases for genetic variation. This does not include variants of unknown significance detected in 15 additional patients – 6 of these variants have since been described as benign and the remaining 9 are presented in table 3.

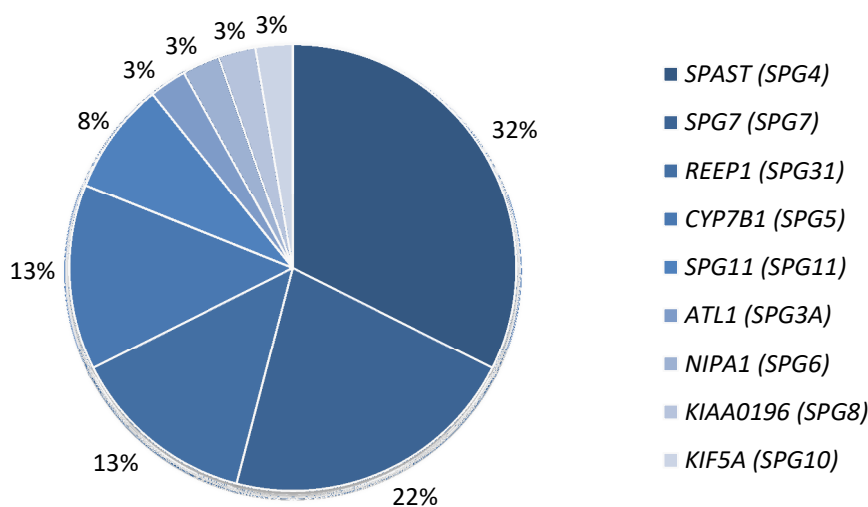
Table 2. Genetic findings in molecularly-confirmed HSP patients (n = 42).

ID	Screened genes	Gene	Variant(s)	HSP Phenotype	Family history	Reference
4	2	<i>SPG5</i>	c.440 G>A (p.G147D), c.945_947dupGGC (p.A316AA)	Pure	None	Both unreported
5	2	<i>SPG5</i>	c.509 delT (p.L170X), c.1456 C>T (p.R486C)	Pure	AR	Unreported, (18)
7	4	<i>SPG5</i>	c.334 C>T (p.R112X), c.1456 C>T (p.R486C)	Pure	None	Unreported, (18)
9	1	<i>SPG7</i>	c.1450-1_c.1457delGGAGAGGCG, c.2097_2098insT (p.D700X)	Pure	None	(19), unreported
15	4	<i>SPG7</i>	c.637C>T (p.R213X), c.1450-1_c.1457delGGAGAGGCG	Pure	AD or AR	Unreported, (19)
21	1	<i>SPG4</i>	Deletion of exons 4-17	Pure	AD	(20)
22	5	<i>SPG31</i>	c.512 delC	Complex	AD	(21)
24	3	<i>SPG7</i>	c.855_856 insC, c.2067 delC	Pure	AR	Both unreported
31	1	<i>SPG4</i>	c.1133dupT (p.L379VfsX15)	Pure	None	Unreported
34	2	<i>SPG7</i>	c.2076 C>G, c.2097 insT	Pure	None	Both unreported

36	4	<i>SPG6</i>	c.316 G>A (p.G106R)	Complex	AD	(22)
38	4	<i>SPG31</i>	c.337 C>T (p.R113X)	Pure	AD	(23)
50	5	<i>SPG10</i>	c.610C>T (p.R204W)	Pure	AD	(24)
52	7	<i>SPG4</i>	c.131C>T (p.S44L)	Pure	AD	(25)
53	1	<i>SPG4</i>	c.1133dupT (p.L379VfsX15)	Pure	AD	Unreported
54	1	<i>SPG4</i>	c.807C>A (p.Y269X)	Pure	None	(26)
55	2	<i>SPG3A</i>	c.1483C>T (p.R495W)	Pure	AD	(27)
56	1	<i>SPG4</i>	c.870+3A>G (skipping of exon 5)	Pure	AD	(28)
58	3	<i>SPG31</i>	c.337 C>T (p.R113X)	Pure	AD	(23)
60	8	<i>SPG5</i>	c.1456T>A (p.R486C) (homozygous)	Pure	None	(18)
62	1	<i>SPG4</i>	c.839_840 delAG	Pure	None	(29)
65	20	<i>SPG31</i>	c.386G>A (p.G129E)	Pure	AD	Unreported
66	9	<i>SPG7</i>	c.637C>T (p.R213*), c.1454_1462del p.R485_E487del	Pure	AR	Unreported, (19)
69	9	<i>SPG7</i>	c.861dupT (p.N288X), c.1672A>T (p.K558X)	Complex	None	(30), (30)
75	10	<i>SPG8</i>	c.1876G>T (p.V626F)	Pure	AD	(31)
77	2	<i>SPG11</i>	c.3493C>T (p.Q1165X), c.5623C>T (p.Q1875X)	Complex	None	Unreported, (32)
79	3	<i>SPG4</i>	c.421C>T (p.Q141X)	Pure	None	(33)
82	65	<i>SPG7</i>	c.1454_1462del (p.Arg485_E487del), c.1672A>T (p.K558*)	Pure	None	(19), (30)
84	50	<i>SPG4</i>	c.160_161del (p.S54Lfs*81)	Pure	None	Unreported
87	24	<i>SPG5</i>	c.1456C>T (p.R486C), c.321_324del (p.K107Nfs*2)	Complex	None	(18), unreported
89	24	<i>SPG7</i>	c.233T>A (p.L78*), c.1045G>A (p.G349S)	Pure	AD	(34), (35)
90	24	<i>SPG4</i>	c.1667C>T (p.A556V)	Pure	AD or AR	(26), (18)
91	15	<i>SPG11</i>	c.1735+1G>A, c.5470C>T (p.R1824*)	Complex	AR or XL	Unreported, (36)
96	11	<i>SPG4</i>	c.1133dupT (p.L379VfsX15)	Pure	AD	Unreported
100	10	<i>SPG4</i>	c.1729-1G>A	Pure	AD	(37)
101	1	<i>SPG31</i>	c.325-1G>A	Complex	AD	Unreported
102	1	<i>SPG11</i>	c.2411_2415del (p.F804) (homozygous)	Complex	None	Unreported

The most frequent genetic cause of HSP in this series was *SPAST* alterations (*SPG4*) in 12 patients (figure 1), followed by 8 *SPG7* (*SPG7*), 5 *REEP1* (*SPG31*), 5 *CYP7B1* (*SPG5*), and 3 *SPG11* (*SPG11*) variants. The remaining 4 patients carried mutations in *ATL1* (*SPG3A*), *NIPA1* (*SPG6*), *KIAA0196* (*SPG8*), and *KIF5A* (*SPG10*). Conversely, no certain pathogenic cause was found in 61 probands, but nonetheless, the clinical diagnosis of HSP was upheld in all but the 7 patients described above.

Figure 1. Distribution of identified genetic etiologies of HSP.



The number of HSP-genes screened in each patient ranged from 1 to 65 (mean of 12.2). This variation is due to a shift in applied genetic technologies as described above and discussed in the following section. Apart from 3 SPG7 mutations described by van Gassen et al. in 2012 all previously reported pathogenic variants were identified before 2010. Thus, mutations in the more rare HSP-causing genes such as *NIP1A* (SPG6) and *KIAA0196* (SPG8) were not identified by gene panels, but conventional sequencing.

Pathogenic genetic changes that are characteristically inherited in an AD manner accounted for approximately 55 % of findings, while 45 % were AR defects. In one patient, genetic testing identified a SACS mutation causing AR spastic ataxia of Charlevoix-Saguenay (ARSACS), a differential diagnosis of HSP most commonly found in people with recent ancestors from Quebec, Canada. This conclusion was supported by characteristic pontine hypointensities on MRI. Similarly, two patients tested positive for accumulation of very long chain fatty acids, which led to the testing and discovery of mutation in the *ABCD1* gene and a diagnosis of adrenoleukodystrophy.

Most pathogenic variants in this series were heterozygous point mutations (48 %) in the form of nonsense (52 %) or missense (48 %) mutations. One proband carried a homozygous missense mutation in exon 6 of the *CYB7B1* gene (SPG5) causing pure AR HSP as reported by Goizet et al. (18). The remainder were deletions (23 %), duplications (11 %), splice site mutations (8 %), and insertions (6 %).

Table 3. Variants of unknown significance.

#	Screened genes	Gene(s)	Variant(s)	Variant type	HSP Phenotype	Family history
10	7	<i>SPG13</i>	c.1685G>T (p.G562V)	ht	Pure	AD
28	9	<i>SPG6</i>	c.45_47dupGGCGGC (p.A15_A16dup)	ht	Pure	None
39	3	<i>SPG4</i>	c.1742G>C (p.R581P)	ht	Pure	None
51	3	<i>SPG31</i>	c.230T>C (p.L77P)	ht	Pure	AD
80	65	<i>SPG11, SPG8</i>	c.2620+4A>G, c.265A>G (p.I89V)	hm, ht	Pure	AR
83	48	<i>SPG15, SPG30</i>	c.685G>A (p.A229T), c.3259C>T (p.P1087S)	ht, ht	Complex	None
92	23	<i>SPG15</i>	c.677G>A (p.R226H), c.-73G>T	compound ht	Pure	None
93	23	<i>SPG7</i>	c.1345C>T (p.P449S)	ht	Pure	None
97	23	<i>SPG11</i>	c.28G>A (p.A10T)	ht	Complex	None

ht: heterozygous, hm: homozygous

Review of selected patients

SPAST mutation (SPG4) was identified as the most frequent genotype (32 % of the genetically verified and 12 % of the entire cohort). In accordance with the literature (7), all 12 SPG4 patients demonstrated a pure phenotype and presented with adult onset HSP in the range of 20 to 59 years of age (mean of 40 years). Seven could account for a dominant family history and 5 were sporadic. Previously described genetic changes were present in 8 patients, while 4 variants with high probability of pathogenicity were unreported. The most frequently detected SPG4-gene alteration was p.Leu379ValfsX15 in 3 seemingly unrelated probands (#31, #53, and #96 in table 2). This heterozygous frameshift mutation in exon 8, predicted to cause a truncated protein or loss of protein production, has only been encountered in these three Danish males. Ages of onset in these individuals were 31, 42, and 40 years followed by slowly progressing gait disturbance. Two had a father with similar symptoms and several affected paternal family members, while the relatives of the remaining index patient showed no signs of spastic paraplegia. They all had healthy children, some with healthy children of their own. In addition to spasticity and weakness all three complained of urinary urgency, two additionally of erectile dysfunction. By their late sixties the two oldest patients received walking aids and the youngest of the three is still walking unaided at the age of 60.

Of note, one patient did express symptoms of late onset cognitive impairment with declining MMSE score, a trait reported on multiple occasions in SPG4 patients (39), and could be classified as having complex HSP. Genetic analyses revealed two more unreported SPG4-gene variants, namely p.Arg581Pro and p.Ser54LeufsX81, the first a missense mutations in exon 17 and the latter a truncating deletion of two bases in exon 1. Both of these patients presented with sporadic HSP in their fifties with spastic paraparesis, pes cavus, and decreased sensation distally in the lower limbs. A quite rapid progression was observed in the individual carrying the p.R581P mutation, whom in the course of 3-5 years developed a somewhat uncharacteristic gait pattern with stooping posture, very short strides, and internally-rotated, dragging feet. In one female subject (# 90) a known disease-causing heterozygous *SPAST* mutation p.Ala556Val (26) was detected. This anomaly leads to pure AD HSP and corresponded very well with findings in the patient. Reduced penetrance and variable expression, frequent phenomena in SPG4 (7), would explain the pedigree with evident symptoms in the great-grandmother and uncertain affection of the father and grandfather. Interestingly, a second heterozygous mutation in the *CYP7B1* gene, p.Arg486Cys, was identified in the patient. This variant has been described as causative for AR SPG5 HSP (18). Whether it is able to modify an HSP phenotype caused by SPG4-gene mutation is currently unknown.

The second most common genetic etiology in this study was *SPG7* in 8 probands (22 % of confirmed cases). This (generally) AR, pure or complicated form of HSP is due to homozygous or compound heterozygous mutations in the *SPG7* gene on chromosome 16 (7). All but one of these patients demonstrated a pure phenotype. In the remaining patient (#69), who carried two previously described truncating mutations (30), clinical examination revealed complicating bilateral optic disc pallor as well as signs of spinocerebellar affection, accompanied by cerebellar atrophy on MRI. These findings are highly characteristic of SPG7 HSP and have been observed in more than half of SPG7 patients in some studies (5). This individual presented without any family history of disease, as did three other SPG7 patients. One proband has a monozygotic twin (#15), but they are otherwise the only affected members of their family. The first of their identical mutations is a novel missense mutation in exon 7 (c.637C>T p.Arg213X) which results in a frameshift and a truncated protein, while the second is an exon 11 deletion reported by McDermott et al. (19). From the age of 30 the sisters developed fairly slowly progressing gait abnormality and mild urinary urgency. Clinical investigation showed upper extremity involvement in addition to impaired sensation and discrete ataxia in the lower limbs. One SPG7 patient with two known mutations (#89) described a family history with similar gait disturbance in at least three successive generations. As mentioned, AD SPG7 has been observed in several studies (8), and although this might be the case in this family, the patient may also carry an unidentified AD mutation in another SPG-gene. Unfortunately, the possibility of family analysis was limited. A diagnosis of SPG31 was made in 5 patients. In accordance with the literature (5,7), they all expressed an AD pattern of inheritance; an early age of onset (mean of 15 years); and most (3/5) a pure HSP phenotype. Complicating symptoms were distal muscular atrophy in the upper extremity of two patients – a feature that has previously been observed in this HSP-category (5,21). One of these patients (#101), with a disease onset of 12 years, experienced problems using her hands from an early age and had bilateral pes cavus upon clinical examination, mimicking a Silver Syndrome.

The genetically confirmed diagnoses of the remaining 12 patients were distributed across 6 types of HSP, of which SPG5 (*CYP7B1*) and SPG11 (*SPG11*) accounted for most. 7 of the 16 genetic variants identified in these patients have been encountered elsewhere, while the rest are novel (table 2).

Eight of the total 37 verified HSP patients (22 %) exhibited a complex phenotype, and as expected, they were overrepresented in the AR subgroup (27 % compared to 14 % of AD): All 3 patients with confirmed

SPG11 showed signs of cognitive impairment, a frequent trait in this subtype (32). In one sporadic proband (#77), the most severely affected in this series, two nonsense mutations were detected, one of them known (32). This person developed an early onset spastic paraplegia and had a near-tetraparalysis at the time of the initial examination (age of 42). Complicating findings in this patient included cognitive impairment, bladder dysfunction, polyneuropathy, thin corpus callosum, cortical atrophy, and white matter lesions. She went on to develop dysphagia and progressive weight loss, refused treatment of pneumonia, and died at the age of 46.

Nine patients harbored variants of unknown significance: Three had heterozygous missense mutations in SPG-genes associated with AR HSP. By themselves these changes are insufficient to explain the observed phenotype as an additional mutation is required. One of these patients did, however, carry a second mutation in another AR SPG-gene. Still, as no digenic inheritance has been described for the *ZFYVE26* and *KIF1A* genes a diagnosis of HSP could not be genetically confirmed. Five probands carried AD SPG-gene variants without sufficient evidence of pathogenicity. In one patient, a potentially disease-causing homozygous splice site mutation was detected in the *SPG11* gene (c.2620+4A>G). This alteration was predicted to have a possible effect on splicing and was undescribed in several online databases. A co-occurring *KIAA0196* missense mutation was identified in the patient as well, and molecular-genetically it was concluded that the changes might be consistent with both SPG11 and SPG8. Co-segregation analyses and functional assays such as qPCR or western blotting – that may help determine pathogenicity – were not included in this study as described.

Discussion

General discussion

Delineation of hereditary spastic paraplegia

Since the first characterization and subsequent classification of HSP this neurodegenerative syndromic entity has been ever-expanding, both genetically and clinically. Considerable advances in molecular diagnostics over the last decade have led to the identification of numerous disease-causing genomic regions which in turn have broadened the range of phenotypic expressions.

At first glance, the pronounced genetic heterogeneity of HSP appears to produce a quite stereotypical phenotype dominated by progressive, spastic weakness of the lower extremities. Variation and indefiniteness are, however, keywords in HSP regarding clinical manifestation, familial presentation, and delineation from other disorders: Age of onset may range from early childhood through senescence; progression can be unnoticeable for decades or rapidly invalidating; symptoms are often characteristic, but potentially widespread as described, and each of these features varies to the point of unpredictability in a family with the same pathogenic mutation. This illustrates the limited genotype-phenotype association in HSP (9), but is also indicative of genetic or environmental modifiers affecting this correlation (5). There is a significant clinical overlap between HSP and other diseases of the central nervous system such as dystonia-parkinsonism or spinocerebellar ataxias, and as with other clinical syndromes it is problematic to determine if a particular disorder belongs to the HSP category or not. For instance, Friedrich's ataxia and ARSACS – AR diseases where ataxia and spasticity are often accompanied by corticospinal deficits – are not considered types of HSP, whereas the *SPG7* mutation that frequently causes ataxia in addition to spastic paraparesis is recognized as HSP (7,8). Similarly, SPOAN syndrome and mitochondrial *ATP6* gene mutation are examples of non-HSP hereditary disorders with lower limb spasticity and weakness as the predominant symptoms. Conversely, anomalies in certain spastic paraplegia genes have been discovered in other conditions than

HSP: *ATL1* mutation (SPG3A) in AD hereditary sensory neuropathy (HSN1); *SPG11* mutation in juvenile parkinsonism and ALS; and *BSCL2* mutation (SPG17) and *SPG20* hypermethylation in other diseases as well (8). Neither the segregation of genetic subtypes into pure and complex HSP phenotypes is clear-cut. In fact, many forms of HSP have been described in both pure and complex versions: SPG4 and SPG3A, previously regarded as prototypical of pure HSP, have both been associated with muscle wasting, thin corpus callosum, and several other complicating symptoms (5,8). On the contrary, *SPG7* and *SPG11* mutations, respectively associated with ataxia and mental retardation, can often present as pure phenotypes. Almost every type of AD HSP has originally been reported as pure, but as the number of families with AD inheritance has increased over time, the existence of complicated manifestations has been disclosed in most cases. Apart from modifying factors, the explanation may be that in some individuals the phenotype progresses from pure to complex during the course of the disease (5). A final note on the diversity of HSP is the finding that at least one subtype may be inherited in both an AD and recessive manner: Initially, homozygous or compound heterozygous mutations in the *SPG7* gene was identified as causative of AR HSP. Studies have subsequently detected heterozygous mutations in this gene leading to pure or complicated HSP phenotypes with comparable features (7,8). Naturally, this trait has consequences for genetic counseling, and whether any additional forms of HSP can be transmitted similarly remains to be determined.

Thus, HSP does not seem to represent a single disease entity, but numerous disorders that may at best share a final common neurodegenerative pathway. In the same vein, post-mortem studies have consistently demonstrated axonal degeneration of corticospinal tract and dorsal columns as described previously. However, neuropathological studies in HSP are to be interpreted with caution: The number of autopsies performed on HSP patients has been limited and the selection of patients tends to favor cases with undetermined genetic cause or abnormal clinical presentation (8). Furthermore, since HSP does not, as a rule, shorten lifespan post-mortem studies may reveal structural changes that are related to aging or due to other late onset disorders such as Alzheimer's disease. Collectively, it can be challenging to conclude whether a given symptom or pathology is a consequence of an HSP gene mutation, and while the first step of encountering a potential HSP patient remains to exclude other diagnoses, the recent progress in genetic testing is rapidly facilitating precise molecular-genetic diagnostics.

Developments in molecular-genetic diagnostics

Discovery of the SPG-genes began approximately 30 years ago by way of genome-wide linkage analysis and subsequent positional cloning of the candidate region, a time-consuming approach taking several years (5). In a number of cases, the genomic region has been identified, but not the specific disease-causing gene, why more HSP subtypes than causative genes have been reported up till now (7). Sanger sequencing, comprising sequential SPG-gene screening based on candidate gene prioritization then became the most widely used method of locating SPG point mutations. Here, the selection of target genes for molecular testing is based on clinical information regarding age of onset, mode of inheritance, and a pure or complex phenotype (5,7). As an example, a patient with pure HSP and AD family history should initially be tested for *SPAST*-mutation if the age of onset exceeds 10-15 years, and *ATL1* if it is below. Negative results would then prompt further sequencing of AD HSP genes (e.g. *REEP1*, *KIF5A*, *NIPA1*, etc.). Similar consensus guidelines are available for AR HSP, but the diagnostic process is based entirely on the individual presentation, as exhaustive guidelines are unattainable. Naturally, this technique does carry some disadvantages: The ill-defined genotype-phenotype associations in HSP complicate the selection of target genes; sporadic cases

without family history may be particularly challenging; and copy-number variants are not detected, why complementary MLPA analysis is recommended. Testing of all candidate genes by Sanger sequencing may be tedious and costly (10). The recent introduction of next generation sequencing (NGS) has significantly improved genetic diagnostics in HSP and is increasingly replacing Sanger sequencing as the method of choice (5). NGS enables analysis of all relevant genes simultaneously and has facilitated the detection of both common pathogenic mutations and genetic modifiers, as well as uncovered several unique causes of HSP. Sporadic cases and small families constitute less of a problem with the NGS approach and so do patients with mutations in known genes who express unusual phenotypes (5). Mutations deep within introns or in promotor and other regulatory sequences, however, are not detected using this method; neither are repeats or large deletions. Another drawback of high-throughput sequencing in the clinical practice is the greater number of variants of unknown significance. Eventually, these may prove to expand the genetic basis of HSP, but until validated they represent interpretive difficulties for clinicians in the counseling of patients (2).

In the future, as the cost of NGS is reduced and the quality improved, whole genome sequencing will provide an opportunity to evaluate molecular-genetic datasets retrospectively as novel SPG-genes are discovered.

Discussion of study findings

In this study, clinical and genetic data from 101 patients referred to Rigshospitalet with symptoms of pure or complex HSP was collected and reviewed. Clinically, the considerable variability in age of onset (from 0 to 68 years) and the distribution of HSP phenotypes (72 % pure and 28 % complicated) fall within previously reported ranges (3,4,10-12). Approximately half of patients presented with family history indicative of HSP, the great majority (35 % of the total population) describing AD inheritance. An equal number could not inform of any affected family members and this amount of sporadic cases is not uncommon in HSP (3,11).

Molecular analyses – including Sanger, MLPA and NGS – identified a causative SPG-gene alteration in 37 % of patients, while alternative genetic etiologies were detected in 3 additional probands. This outcome is in line with results from other studies, in which the proportion of genetically confirmed diagnoses typically ranges from one third to half of patients (3,10-12,38). Plausible disease-causing SPG-gene changes were found in 49 % of subjects with a positive family history, compared to 33 % of sporadic cases, emphasizing the aforementioned diagnostic difficulties in this patient group. Several reasons may contribute to the large quantity of genetically unsolved probands: Patients with AD family history may previously have been tested for specific AD SPG-gene mutations on a research basis, and if a pathogenic variant was identified the particular patient would not be included in this study. Patients may also harbor mutations in unscreened genes. One would suspect this factor to be of increasing importance the further back patients have been tested as additional HSP genes have continuously been added to the molecular-genetic analyses. Furthermore, there is a possibility that an examined gene carries an alteration that is missed by the molecular technique applied. This is especially relevant when SPG-gene sequences have not been analyzed for copy number variants, which was the case in 10 patients in this series. Copy number analysis by MLPA, in turn, rarely covers all genes in a gene panel. As described, disease-causing HSP genes are rapidly being uncovered these days and some of the unsolved cases are potentially due to mutations in unknown SPG-genes. Non-mendelian modes of inheritance such as mosaicism, di- or oligogenic transmission have also been suggested as a contributor to the lack of genetic diagnoses (2,3), particularly regarding subjects

without family history. Finally, the differential diagnoses mimicking HSP might of course account for a part of the genetically unverified patients.

In accordance with other studies (3,10-12,14), *SPG4 (SPAST)* was identified as the most common etiology. For this reason, sequencing of the *SPAST* gene is often recommended as the initial analysis, except in the case of apparent non-AD inheritance (10). *SPG7* variants constituted the second most frequent cause of HSP as observed elsewhere (3), followed by mutations in *REEP1* (SPG31) and *CYP7B1* (SPG5). Finally, AD HSP caused by molecular-genetic alterations in *ATL1* (SPG3A), *NIPA1* (SPG6), *KIAA0196* (SPG8), or *KIF5A* (SPG10) was observed in one patient each. In addition to SPG-gene variants two patients carried an *ABCD1* mutation causing X-linked adrenoleukodystrophy and one patient was diagnosed with ARSACS. The *SACS* mutation is increasingly being detected worldwide, but has not been reported in a Danish patient up till now. As this variant has not been screened for previously in patients with clinical HSP, its frequency in this patient population is currently unknown, as is the founder capabilities of the particular ARSACS mutation in this study.

A significant number of previously undescribed mutations were identified in this patient cohort, amounting to 21 variants in the genetically verified HSP patients. As with all previously undescribed genetic changes these findings come with a degree of uncertainty regarding whether they are truly pathogenic, i.e. the actual source of the patient's symptoms. In this case, analyses such as segregation studies in the probands' family will help determine if the given mutation underlies the observed HSP phenotype and will also provide information on the mode of inheritance. Conversely, unaffected family members that harbor the same genetic variation as the proband will contradict a disease-causing significance. If no relatives are available for testing one has to rely on other markers of pathogenicity e.g. in silico predictions, functional studies, and occasionally amino acid changes at the same position will have been reported previously.

Regarding the treatment of HSP, no disease-modifying therapy is presently available and pharmacological intervention is limited to the use of muscle relaxants to relieve symptoms of spasticity. Research groups, however, are aiming and working to uncover therapeutic approaches. Development of stem cell and mouse models is used to further explore the neuropathology of HSP and test experimental treatments. One strategy among several that has been pursued in recent years is modulation of the cytoskeleton: A working hypothesis here is the potential rescue of neurons with dysfunctional microtubule dynamics due to loss of Spastin function (encoded by *SPAST*) by use of microtubule-stabilizing drugs (40). Undoubtedly, the ongoing elucidation of the molecular defects underlying HSP is a prerequisite for future personalized treatment. Currently, identification of the particular disease-causing mutation in HSP patients holds no implications for their management. Importantly, this does not mean that a genetic diagnosis is without consequences for the individual patient. Psychologically, patients acquire a definitive explanation for the symptoms they have been experiencing, and do so to a greater extent than a clinical diagnosis alone would offer. Knowing the exact molecular-genetic defect also implies that the hope for a future cure is closer than it would be without the specific molecular-genetic diagnosis. Furthermore, it will bring closure to patients that have been misdiagnosed initially or in whom differential diagnoses have not been excluded. The more practical aspects of obtaining an exact molecular diagnosis include higher quality patient counseling: The prognosis of the proband in question may be more accurately appraised, and the mode of inheritance established – defining the probability of transmission to the offspring. If so desired, predictive molecular testing can be carried out in relatives at-risk and the option of prenatal diagnostics becomes available. The hope is that, as treatments of HSP subtypes start to emerge, genetic testing will allow early identification of asymptomatic carriers, and thus initiation of therapy prior to the age of onset.

In conclusion, this study explores the genetic and clinical features of hereditary spastic paraplegia - a continuously expanding and highly heterogeneous disease entity. The shared symptoms and pathology of the subtypes, as well as their characteristic distinctions are presented, as is the continuum that exists between HSP and other neurodegenerative disorders. The analyses of the included patients revealed a relatively wide genotypic and phenotypic spectrum in the Danish HSP population, and while an exhaustive review of all cases was beyond the scope of this project, several known and novel mutations were characterized and findings generally consistent with published literature. New subtypes and underlying pathologies will unquestionably be identified in upcoming years and the rapidly evolving experimental techniques heralds a new era in the management of HSP patients.

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