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Clinical-Genetic Features Influencing Disability in Spastic Paraplegia Type 4

A Cross-sectional Study by the Italian DAISY Network

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Abstract

Background and Objectives

Hereditary spastic paraplegias (HSPs) are a group of inherited rare neurologic disorders characterized by length-dependent degeneration of the corticospinal tracts and dorsal columns, whose prominent clinical feature is represented by spastic gait. Spastic paraplegia type 4 (SPG4, SPAST-HSP) is the most common form. We present both clinical and molecular findings of a large cohort of patients, with the aim of (1) defining the clinical spectrum of SPAST-HSP in Italy; (2) describing their molecular features; and (3) assessing genotype-phenotype correlations to identify features associated with worse disability.

Methods

A cross-sectional retrospective study with molecular and clinical data collected in an anonymized database was performed.

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Glossary

AAA = ATPases Associated with diverse cellular Activities; **AAE** = age at the last available examination; **AAO** = age at onset; **AD** = autosomal dominant; **DD** = disease duration; **HSP** = hereditary spastic paraplegia; **MEP** = motor evoked potential; **SPG** = spastic paraplegia gene; **SPG4** = SPG type 4; **SPRS** = Spastic Paraplegia Rating Scale; **SSEP** = somatosensory evoked potential; **TCC** = thin corpus callosum; **UL** = upper limb.

Results

A total of 723 Italian patients with SPAST-HSP (58% men) from 316 families, with a median age at onset of 35 years, were included. Penetrance was 97.8%, with men showing higher Spastic Paraplegia Rating Scale (SPRS) scores (19.67 ± 12.58 vs 16.15 ± 12.61, p = 0.009). In 26.6% of patients with SPAST-HSP, we observed a complicated phenotype, mainly including intellectual disability (8%), polyneuropathy (6.7%), and cognitive decline (6.5%). Late-onset cases seemed to progress more rapidly, and patients with a longer disease course displayed a more severe neurologic disability, with higher SPATAX (3.61 ± 1.46 vs 2.71 ± 1.20, $p < 0.001$) and SPRS scores (22.63 ± 11.81 vs 12.40 ± 8.83, $p < 0.001$). Overall, 186 different variants in the *SPAST* gene were recorded, of which 48 were novel. Patients with SPAST-HSP harboring missense variants displayed intellectual disability (14.5% vs 4.4%, $p < 0.001$) more frequently, whereas patients with truncating variants presented more commonly cognitive decline (9.7% vs 2.6%, $p = 0.001$), cerebral atrophy (11.2% vs 3.4%, $p = 0.003$), lower limb spasticity (61.5% vs 44.5%), urinary symptoms (50.0% vs 31.3%, $p < 0.001$), and sensorimotor polyneuropathy (11.1% vs 1.1%, $p < 0.001$). Increasing disease duration (DD) and abnormal motor evoked potentials (MEPs) were also associated with increased likelihood of worse disability (SPATAX score>3).

Discussion

The SPAST-HSP phenotypic spectrum in Italian patients confirms a predominantly pure form of HSP with mild-to-moderate disability in 75% of cases, and slight prevalence of men, who appeared more severely affected. Early-onset cases with intellectual disability were more frequent among patients carrying missense *SPAST* variants, whereas patients with truncating variants showed a more complicated disease. Both longer DD and altered MEPs are associated with worse disability.

Hereditary spastic paraplegias (HSPs) are a group of rare inherited neurologic disorders characterized by length-dependent degeneration of the corticospinal tracts and dorsal columns, with spastic gait being their prominent clinical feature.[1,2] Globally, HSPs have a prevalence of 1–5/100,000 individuals worldwide.[3] They can be inherited as autosomal dominant (AD), autosomal recessive, recessive X-linked, or mitochondrial traits, and current genetic classification relies on sequential numbering of loci or genes using a spastic paraplegia gene (SPG) designation.[1] To our knowledge, 85 different disease loci have been identified in 79 known causative genes.[4]

The SPG type 4 (SPG4, also known as SPAST-HSP, OMIM #182601) is the most common form, accounting for about 40% of AD-HSPs and 20% of sporadic HSPs.[3,5] SPAST-HSP has long been considered the prototype of pure HSP, yet other neurologic features may be also part of its phenotype.[6] SPAST-HSP is caused by heterozygous variants in the *SPAST* gene (OMIM# 604277), encoding spastin, a protein of 616 amino acids that is a member of the ATPases Associated with diverse cellular Activities (AAA) protein family.[6] The microtubule-severing activity represents the main function of spastin, which hydrolyzes adenosine tri-phosphate (ATP) to sever microtubules.[7] Four isoforms of spastin are known, comprising the full-length protein (M1), a shorter isoform (M87) lacking the first 86 amino acids of the M1, and 2 splice variants of these, derived from the exclusion of the exon 4.[8,9] Spastin shows a modular structure, including an N-terminal domain, a microtubule interacting and trafficking domain, a microtubule-binding domain, and an AAA ATPase cassette.[10,11]

More than 250 pathogenic variants of the *SPAST* gene have been reported, most of them located in the tripleA protein domain. Missense changes, mostly clustering in the AAA domain, represent the most frequent variants, followed by frameshift, splice site, nonsense variants and deletions, that have been found to be scattered throughout the whole gene.[12]

The characterization of large cohorts of patients with HSP usually offers a significant contribution to clinical research regarding rare disorders. Herein, we present clinical and molecular findings of 723 patients with SPAST-HSP gathered by several neurologic centers throughout Italy, with the aim of (1) defining the clinical spectrum of SPAST-HSP in Italy; (2) describing their molecular characteristics; and (3) assessing genotype-phenotype correlations to identify potential features associated with worse disease severity.

Methods

We retrospectively collected in an anonymized database both molecular and clinical cross-sectional data from a total of 723 Italian patients with SPAST-HSP from 316 families, referred to 23 Italian centers with a specific expertise on neurogenetic

diseases from the Disease Alliance for Italian Spastic Paraplegia centered for You (DAISY) network. Only patients with confirmed molecular diagnosis of SPAST-HSP were included. Data were collected from July 2020 to December 2020. Eight pedigrees included in this study had been already described elsewhere.[13-20] Strengthening the Reporting of Observational Studies in Epidemiology cross-sectional reporting guidelines were used.[21]

DNA Collection and Mutation Analysis

Blood samples were collected at each referral center for both diagnostic and research purposes, according to the protocols of the respective Institutional Ethical Committees, and sent for genomic DNA extraction from peripheral leukocytes and molecular analyses and/or confirmation to 1 of the 2 major diagnostic participating centers (IRCCS Fondazione Stella Maris, Pisa, and Ospedale Pediatrico Bambino Gesù, Rome). Targeted sequencing and Sanger sequencing were used to detect point mutations in *SPAST*. Multiplex ligation-dependent probe amplification analysis was used to identify large deletions and/or duplications.[22] Due to the methods employed, it cannot exclude that deep intronic changes or less typical structural variants could have been missed.

Variant classification was based on the American College of Medical Genetics and Genomics published guidelines.[23] To define the impact of missense variants on protein function, we used an in silico pipeline encompassing 19 prediction tools including Mutation Assessor,[24] FATHMM,[25] LRT,[26] Deo-gen2,[26] Eigen,[27] Eigen PC,[27] Sorting Intolerant From Tolerant (SIFT),[28] SIFT4G,[29] Provean,[30] Minimum Viable Product,[26] Revel,[26] Primate AI,[26] MetaSVM,[26] MetalR,[26] Genomic Evolutionary Rate Profiling,[26] PolyPhen-2 HumDir,[31] PolyPhen-2 HumVar,[31] UMD Predictor,[32] and CADD.[33] Splicing variants and synonymous variants close to splicing sites were also tested using Human Splicing Finder 3.1[34] and NNSPLICE 0.9.[35] We included variants with minor allele frequency <0.1% in the ExAC,[36] gnomAD,[37] 1000 Genomes Project,[38] and dbSNP[39] databases. Nevertheless, synonymous, intronic and other noncoding variants were considered only if they have already been described as pathogenic in previous literature. Whenever possible, segregation of specific variants was performed in family members (affected and not affected).

Clinical Features

Both demographic and phenotypic data were retrospectively collected from medical records for each patient with SPAST-HSP. A multicenter electronic, standardized sheet was used to reduce sources of bias in data entry, that was performed on a shared anonymized database.

Medical history data included family history for the same disorder, age at the last available examination (AAE) (years), age at onset (AAO) (years), and symptom of onset of SPAST-HSP. Findings of brain and spinal cord MRI, nerve conduction studies, and motor evoked (MEP) and somatosensory evoked potentials (SSEP) were recorded. Finally, current use of symptomatic antispastic drugs was also detailed. Overall penetrance was assessed by dividing the number of symptomatic patients by the total number of *SPAST* pathogenic variants' carriers.

Patients were classified as early- or late-onset forms if their AAO was below or above 10 years, respectively. Disease duration (DD) was calculated as AAE-AAO (years). Patients were divided into 2 groups (short- or long-lasting disease forms), using the median value of DD as threshold.

Pure forms were defined for patients manifesting signs of spastic paraplegia, characterized by pyramidal signs and weakness in the LL, and sphincter involvement and/or decreased sense of vibration at the ankles as additional features. Complex phenotype was defined for patients manifesting spastic paraplegia plus at least one other symptom, which could not be otherwise explained, including polyneuropathy, cerebellar ataxia, dysarthria, intellectual disability, parkinsonism, and cognitive decline. Patients were considered affected by cognitive decline if scored below 24 at Mini-Mental State Examination. Intellectual disability was defined if either children scored <70 at Wechsler Intelligence Scale for Children IV or they were delayed at Gross Motor Function Classification System or if they needed support while in school. Pes cavus, presence of Hoffmann sign, and/or brisk deep tendon reflexes in the upper limbs (UL) were considered as part of the pure phenotype, if not accompanied by muscle wasting.[2,40] Disease severity was assessed using the Spastic Paraplegia Rating Scale (SPRS)[41] and disease disability using the SPATAX disability score.[12,42]

Because of the cross-sectional study design, we estimated the rate of disease progression by the disability progression index (current SPATAX disability score divided by DD) and the disease progression index (current SPRS score divided by DD).[43] For patients with available disease progression index values, tertiles were used to identify 3 groups: rapidly, typically, and slowly progressing forms.

To assess whether there was a significant reduction of AAO in subsequent generations, families with at least 2 affected members in the context of longitudinal transmission and with established AAO were included. A pseudo-longitudinal assessment of disease progression was possible in a subset of patients with multiple evaluations and SPRS scores.

Statistical Analysis

The sample was characterized in its clinical and demographic features using descriptive statistics techniques. Quantitative variables were described using mean and SD. Qualitative variables were summarized with absolute and percentage frequency tables. Normality of continuous variables was checked using the Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) was performed to compare AAO among the generations. N-value was specified for each variable. Patients with some missing values have been included in the study and maintained as missing.

Table 1 Demographic, Clinical, Diagnostic, and Molecular Findings of the Whole SPAST–Hereditary Spastic Paraplegias Cohort

	Count (%)	N
Demographics		
Males	419 (58.0%)	723
Family history +ve	619 (86.9%)	712
Probands	316 (43.7%)	723
Clinical features		
Symptomatic	676 (97.8%)	691
Symptom of onset	Gait disorder: 582 (90.9%), motor delay: 43 (6.7%), sphincter disturbances: 8 (1.3%), other: 7 (1.1%): LL pain	642
Spastic gait	619 (93.9%)	659
LL hyperreflexia	636 (98.6%)	645
Babinski sign	461 (71.9%)	641
Rest spasticity	366 (57.1%)	641
LL proximal weakness	345 (54.2%)	637
Pes cavus	181 (29.6%)	612
Hypopallesthesia at ankles	179 (29.6%)	604
UL hyperreflexia	135 (21.3%)	634
Hoffmann sign	57 (9.6%)	595
Saccadic intrusions	11 (1.8%)	596
Nystagmus	27 (4.4%)	617
Urinary disturbances (overall)	271 (43.7%)	620
	Urgency 178 (28.7%), frequency 23 (3.7%), incontinence 81(13.1%), and dysuria 11 (1.8%)	620
Complicated forms	177 (26.6%)	665
	Intellectual disability: 48 (8.0%; n = 600); polyneuropathy: 31 (6.7% n = 466); cognitive decline 39 (6.5% n = 601); other neurologic manifestations 45 (6.9% n = 654): Dysarthria (4), intention tremor (1), parkinsonism (2), depression (3), UL hypotonia (1), ataxia (6), hypoacusia (3), psychosis (1), ptosis (3), epilepsy (18), distal muscle wasting (17), and scoliosis (4)	
Diagnostic findings		
MRI brain alterations (overall)	102 (20.0%)	509
	Cerebral atrophy 41 (8.1%); cerebellar atrophy 7 (1.4%); TCC 7 (1.4%); WMH 32 (6.3%); other 37 (7.3%): cystic pineal gland (2), congenital arachnoid cyst (18), hippocampal sclerosis (14), hippocampal atrophy (1), Arnold-Chiari type 1 malformation (1), and pyramidal tract hyperintensities (1)	509
MRI spinal cord atrophy	13 (3.1%)	420
MEP central altered	340 (73.9%)	460
SSEP central altered	65 (18.2%)	357
Therapy (overall)	325 (66.7%)	487
	Baclofen 270 (83.1%), eperisone 7 (2.2%), cannabinoids 7 (2.2%), botulin toxin 44 (13.5%), gabapentin/pregabalin 4 (1.2%), benzodiazepines 2 (0.6%), and tizanidine 38 (11.7%)	487
Molecular findings		
Variant type	Missense 280 (39.4%), splicing 72 (10.1%), nonsense 103 (14.5%), frameshift 137 (19.3%), deletions 108 (15.2%), and duplications 10 (1.4%)	710

Abbreviations: LL = lover limb; MEP: motor evoked potential; SSEP = somatosensory evoked potential; TCC = thin corpus callosum; UL = upper limb; WMH = white matter hyperintensity.

Table 2 Age at Onset, Age at Examination, Disease Duration, and Severity Scales

	n	Median	min	Max	Mean	SD
Age at onset (y)	634	35	0	80	32.46	17.41
Age at examination (y)	631	47	2	95	45.25	17.70
Disease duration (y)	593	7	0	64	12.87	13.68
SPATAX disability scale	393	3	0	7	3.15	1.59
SPRS score	339	16	0	50	18.22	12.69

Abbreviation: SPRS = Spastic Paraplegia Rating Scale.

The Mann-Whitney *U* test was used to evaluate the presence of any associations between specific features and various SPAST-HSP groups of patients (males/females, probands/family members, early-onset/late-onset cases, short-/long-DD cases, and missense variant carriers/truncating-variant carriers). The Kruskal-Wallis test with pairwise comparisons was used to compare qualitative variables when >2 subgroups of patients were analyzed (slowly vs typically vs rapidly progressing cases). The χ^2 test (or Fisher 2-tailed exact test when required) was used to compare categorical variables between the groups. A logistic regression was performed to identify any factors associated with worse disability (identified by SPATAX score >3) or worse disease severity (SPRS score ≥26). Significance level was set at $p < 0.05$. Statistical analysis was performed by SPSS (Statistical Package for Social Science, IBM SPSS Statistics, Version 24.0. Armonk, NY: IBM Corp).

Standard Protocol Approvals and Patient Consents

The study was conducted according to the criteria set by the Declaration of Helsinki, and each patient or family member, in case of minors, signed an informed consent. The study protocol was approved by the Ethical Committee of the Fondazione Policlinico Universitario A. Gemelli IRCCS (Ethical Board approving number: ID 3998).

Data Availability

Anonymized patients' data are available on reasonable request.

Results

AAO, Clinical Signs, Disease Severity, and Diagnostic Findings

Clinical and demographic characteristics of the DAISY cohort are detailed in Tables 1 and 2. The whole cohort included 723 patients with SPAST-HSP (419 men, 58%) (Table 1) from 316 families, with a male-to-female ratio of 1.38. AAO was reported for 634 symptomatic patients, and it ranged from 0 to 80 years, with a mean age of 32.46 ± 17.41 years and a median of 35 years; the mean AAO was similar in the cohort including only the SPAST-HSP index cases (285/634, 44.95%, 31.91 ± 18.25 years), and it did not differ from the mean AAO of their affected relatives (349/634, 55.05%, 32.91 ± 16.72 years, $p = 0.793$) (eTable 1, links.lww.com/NXG/A516). The AAO followed a bimodal distribution (Figure 1), with the first peak observed in the first decade and the second between the fourth and the fifth decades. Fewer cases (13.1%, 83/634) had AAO ≤8 years.

Considering only families with at least 2 affected relatives from different generations (n = 46), the mean AAO was 39.77 ± 15.7 years (n = 56, median 41.5 years) in the first generation, 25.7 ± 15.6 years (n = 69, median 30 years) in the second generation, and 21.18 ± 15.3 years (n = 28, median 20.5 years) in the third generation. Such differences resulted statistically significant ($p < 0.0005$) by ANOVA (eFigure 1, links.lww.com/NXG/A516). Penetrance of the disease was 97.8%, with complete penetrance at age 80 years (Table 1).

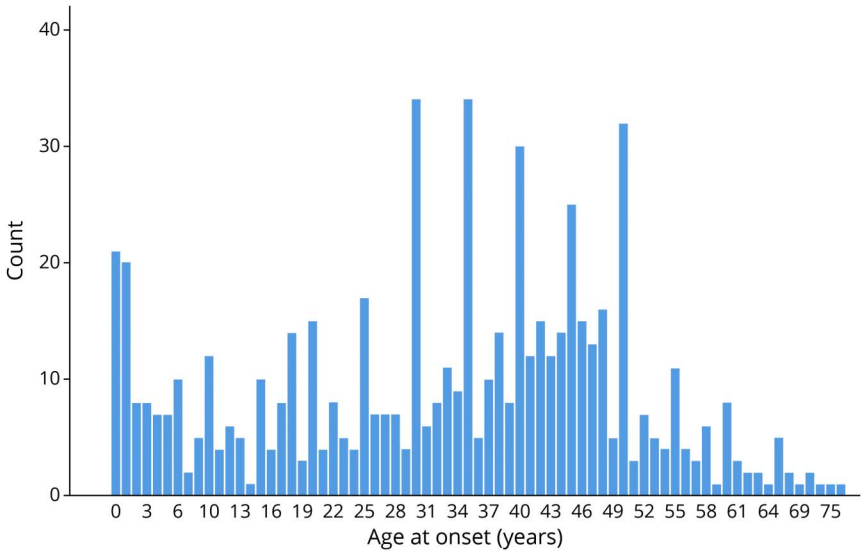
The mean AAE (n = 631) was 45.25 ± 17.70 (range 2–95, median 47) years. AAO and AAE were both available for 599 patients, and the resulting mean DD was 12.87 ± 13.68 (range 0–64, median 7) years (Table 2). Regarding symptoms of onset, the most common presentation was gait difficulties (90.9%), followed by delayed motor milestones (6.7%) and sphincter disturbances (1.3%) (Table 1).

Most common clinical neurologic features included LL hyperreflexia (93.9%), spastic gait (93.9%) and Babinski sign (71.9%), then LL rest spasticity (57.1%), LL proximal weakness (54.2%), and urinary disturbances (43.7%), either presenting as urgency (28.7% of cases), incontinence (13.1%), frequency (3.7%), and/or dysuria (1.8%). Decreased vibration sense at ankles, pes cavus, UL hyperreflexia, Hoffmann sign, and gaze-evoked nystagmus occurred less frequently (Table 1, Figure 2).

In 26.6% of patients with SPAST-HSP (177/665), we observed a complicated phenotype: the most common associated features were intellectual disability (8%), cognitive decline (6.5%), and polyneuropathy (6.7%); other neurologic manifestations, including tremor, parkinsonism, distal muscle wasting, ataxia, and/or epilepsy, were variably present only in a minority of the SPAST-HSP cases (overall 6.9%) (Table 1). In most patients, we assume that the complicating features were related to the underlying *SPAST* variant, as we excluded the most common concurrent diseases (i.e., diabetes in case of polyneuropathy). However, we could not exclude that some rarer associated conditions explaining the complex phenotype, especially in singletons, might have been overlooked.

The mean SPATAX disability score was 3.15 ± 1.59 (n = 393), and the mean SPRS score was 18.22 ± 12.69 (n = 339), respectively (Table 2, Figure 3). Most of the patients with SPAST-HSP assessed by SPATAX had either moderate (SPATAX score = 3–5, 209/393, 53.18%) or mild (SPATAX score = 0–2, 142/393, 36.13%) functional impairment, and

Figure 1 Histogram Showing the Distribution of Age at Onset in the Study Cohort (n = 634)



about 11% of them (42/393) manifested with a very severe motor disability (SPATAX score 6 and 7), causing loss of ambulatory capacity.

Data regarding therapy were available for 487 patients and documented that 66.7% of them assumed at least one of the following antispastic therapies: baclofen (8.1%), botulinum toxin (13.5%), tizanidine (11.7%), eperisone (2.2%), cannabinoids (2.2%), pregabalin/gabapentin (1.2%), and/or benzodiazepines (0.6%) (Table 1). The subjective impression on the efficacy of drug therapies was not recorded in our multicenter proforma sheet.

Globally, 20.0% of the patients with SPAST-HSP who underwent at least 1 brain MRI showed some alterations: some degree of cerebral atrophy was reported in 8.1% of cases and aspecific white matter hyperintensities in 6.3% of cases, whereas

Figure 2 Frequency of Neurologic Features in the Whole SPAST-Hereditary Spastic Paraplegias Cohort

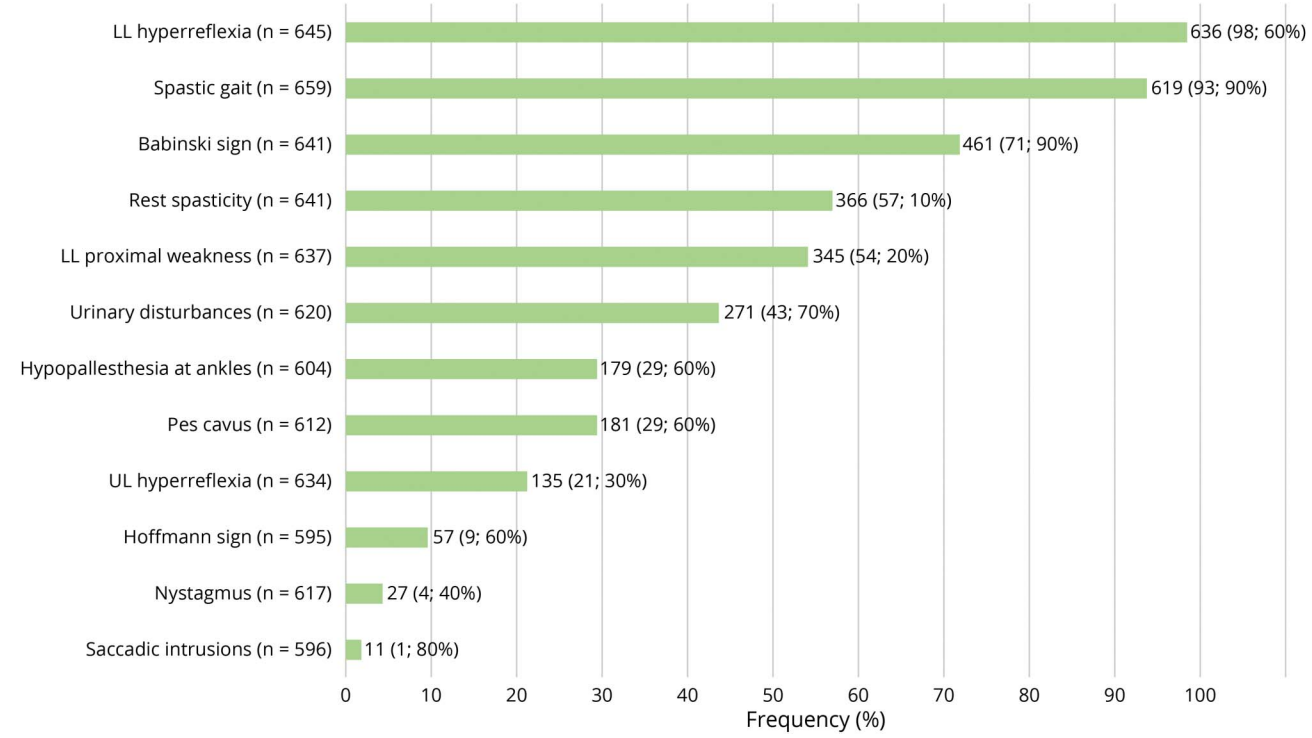
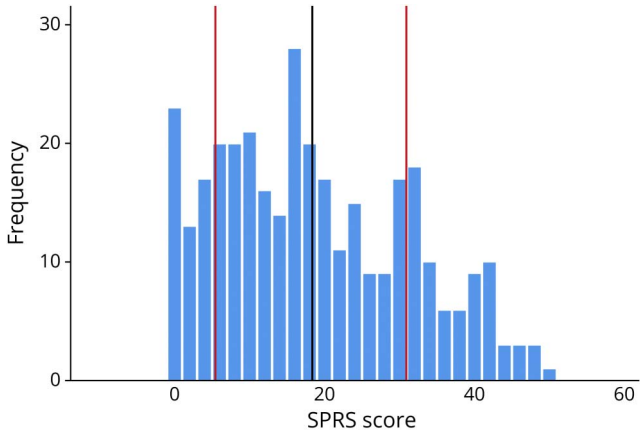


Figure 3 Histogram Showing the Distribution of the SPRS Score in the Study Cohort (n = 339)



Marked vertical black line shows the mean value, whereas red lines represent standard deviation values. SPRS = Spastic Paraplegia Rating Scale.

cerebellar atrophy and thin corpus callosum (TCC) were observed in 1.4% of cases each. Only 13 of 420 patients (3.1%) showed mild to moderate spinal cord atrophy at MRI (Table 1). Of note, peculiar brain MRI findings included 2 distinct complicated SPAST-HSP pedigrees previously reported. In the first family, all the affected carriers (c.1841C>T/p.T614I) showed a congenital pontocerebellar arachnoid cyst,[15] whereas in the second (c.961dupG/p.D321Gfs*6), all patients with SPAST-HSP had signs of frontotemporal atrophy associated with temporal lobe epilepsy.[17]

Peripheral nerve involvement was reported only in 6.7% of patients with SPAST-HSP (31 of 466), mainly with features of sensory-motor axonal polyneuropathy. MEP displayed impaired central conduction time in 73.9% of examined patients, whereas SSEP revealed altered central sensory pathway conduction in 18.2% of cases (Table 1).

Sex Differences and Factors Associated With AAO, Severity, and Progression of the Disease

In our cohort, males with SPAST-HSP showed more frequently spastic gait (96.6%, 373/386 vs 90.1%, 246/273, $p = 0.001$) and higher SPRS score (19.67 ± 12.58 vs 16.15 ± 12.61, $p = 0.009$, eFigure 2, eTable 2). Antispastic treatments also resulted more frequent among male compared with female patients (71.6%, 204/285 vs 59.9%, 121/202, $p = 0.007$) (eTable 2). Any of these sex differences neither depended on the AAO (32.75 ± 17.11 vs 32.04 ± 17.87, $p = 0.855$) nor on the DD (12.98 ± 13.38 vs 12.70 ± 14.15, $p = 0.307$). Penetrance was also slightly higher in males, yet not reaching the statistical significant values (98.8%, 396/401 vs 96.6%, 280/290, $p = 0.064$) (eTable 2).

To assess if AAO could influence the severity of the neurologic phenotype, we divided the whole SPAST-HSP cohort in early (≤10 years-old) and late-onset (>10 years-old) cases according to the age of patients at their initial symptoms. Both neurologic disability and disease severity, assessed by SPATAX and by SPRS scores, respectively, were similar in the 2 groups (eTable 3, links.lww.com/NXG/A516); however, late-onset cases seemed to progress more rapidly. This observation is supported by both their higher disability progression (0.38 ± 0.49 vs 0.26 ± 0.27, $p = 0.014$) and disease progression indices (1.87 ± 2.13 vs 1.18 ± 1.18, $p < 0.001$) (eTable 3). Moreover, late-onset patients were more likely undergoing antispastic treatment (72.2% vs 39.4%, $p < 0.001$) (eTable 3).

Conversely, early-onset cases more often presented with complicated forms (46% vs 24.8%, $p < 0.001$) and mainly associated with intellectual disability (35.6% vs 3.4%, $p < 0.001$) (eTable 3, links.lww.com/NXG/A516). Moreover, missense variants were more frequent among early-onset cases (56.7% vs 40.7%, $p = 0.005$).

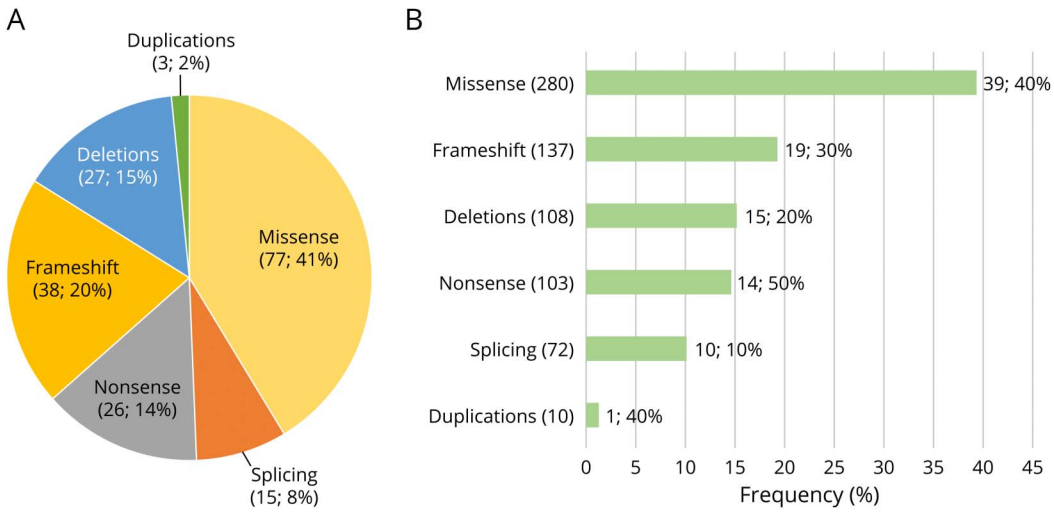
Patients with SPAST-HSP were also compared according to their DD, and the median value of 7 years was the cutoff to classify them into short- and long-duration cases, respectively. Long-DD SPAST-HSP patients displayed a more severe neurologic disability, with higher SPATAX (3.61 ± 1.46, n = 252 vs 2.71 ± 1.20, n = 91, $p < 0.001$) and SPRS scores (22.63 ± 11.81, n = 211 vs 12.40 ± 8.83, n = 73, $p < 0.001$), and as expected, they had a lower AAO (29.39 ± 17.32, n = 294 vs 36.15 ± 17.04, n = 299, $p < 0.001$) (eTable 4, links.lww.com/NXG/A516). Of interest, they were also more frequently carriers of truncating variants (63.1%, 154/244 vs 51.2%, 144/281, $p = 0.006$) and showed more often associated signs of UL involvement (eTable 4). Finally, long-DD SPAST-HSP patients more frequently displayed MRI alterations and cognitive decline (eTable 4).

Based on tertiles of the values of disease progression index (0.83 and 1.7 points/y), available for 278 patients, we identified the group of slowly (≤0.83 points/y), typically (>0.83 and ≤1.7 points/y), and rapidly evolving (>1.7 points/y) patients with SPAST-HSP (eTable 5, links.lww.com/NXG/A516). Rapidly evolving patients had later AAO (38.91 ± 18.88 years vs 32.94 ± 16.61 years in typically evolving patients and 23.09 ± 17.52 years in slowly progressing patients, $p < 0.0005$), more often displayed impaired central MEPs (62.3% vs 43.6% in typically evolving vs 34.0% in slowly progressing patients, $p = 0.012$), and more frequently were complicated forms (36.7% vs 16.2% in typically evolving vs 12.6% in slowly progressing patients, $p < 0.0005$). No substantial differences regarding class of SPAST-HSP pathogenic variant were found between slowly and rapidly evolving SPAST-HSP cases in our cohort (eTable 5).

Type and Distribution of Variants and Genotype-Phenotype Correlations

Overall, 186 different variants in *SPAST* were recorded among 316 SPAST-HSP families included in this study (Figure 4). Forty-eight variants (25.8%) have not been reported elsewhere, and 8 of 186 were present in the gnomAD polymorphic database, though all at a very low frequency (<0.1%) (eTable 6, links.lww.com/NXG/A516). The most common types were

Figure 4 Schematic Representation of the Molecular Data



The pie chart (A) illustrates the relative prevalence of different variants found (total number: 186). The histogram (B) schematizes their relative prevalence in the whole SPAST-HSP cohort (n = 710).

missense (41.40%, 77/186), followed by frameshift (20.43%, 38/186), deletions (14.52%, 27/186), nonsense (13.98%, 26/186), splice site (8.06%, 15/186), and duplications (1.61%, 3/186). Most of the missense variants (87.01%, n = 67/77) clustered in the AAA domain, between amino acids 342 and 599.

The c.131C>T/p.S44L, previously reported as a modifier sequence variation,[64] was reported in a single male individual with symptom onset at the age of 45 years, who manifested a moderately severe disease (SPRS of 23 after 12 years of disease) and showed an associated cognitive decline. This patient harbored the c.1634C>G/p.S545* on the other allele. Of interest, the 131C>T/p.S44L sequence variation was the unique variant detected in a 84-year old woman displaying a very-late-onset (74 years) spastic paraparesis, associated with TCC and signs of cerebellar atrophy at the brain MRI. However, we did not include her in the study cohort, as pathogenic *SPAST* variants were not found in association with such modifier.

The prevalence of individual neurological manifestations was compared between SPAST-HSP carriers of truncating (namely frameshift, nonsense, splice site, and small deletions) vs missense variants (eTable 7, links.lww.com/NXG/A516).

Carriers of missense and truncating changes did not differ regarding the AAO (eTable 7, links.lww.com/NXG/A516). Patients with SPAST-HSP with missense variants had shorter DD (10.16 ± 12.34, n = 227 vs 14.31 ± 14.54, n = 298, $p < 0.001$), but higher SPATAX score (3.56 ± 1.69, n = 114 vs 3.05 ± 1.49, n = 204, $p = 0.009$, eTable 7). Also, they displayed more frequently intellectual disability (14.5%, 34/243 vs 4.4%, 13/297, $p < 0.001$) (eTable 7).

Conversely, patients with truncating variants more commonly presented cognitive decline (9.7%, 29/299 vs 2.6%, 6/232, $p = 0.001$) and showed cerebral atrophy at the brain MRI (11.2%, 29/259 vs 3.4%, 6/178, $p = 0.003$) (eTable 7, links.lww.com/NXG/A516). Moreover, they more frequently had LL spasticity at rest (61.5%, 198/322 vs 44.5%, 106/238, $p < 0.001$), LL proximal weakness (59.6%, 190/319 vs 44.1%, 105/238, $p < 0.001$), urinary symptoms (50.0%, 157/314 vs 31.3%, 73/233, $p < 0.001$), polyneuropathy (11.1%, 25/226 vs 1.1%, 2/177, $p < 0.001$) and pes cavus (36.1%, 110/305 vs 23.1%, 54/234, $p = 0.001$) (eTable 7, links.lww.com/NXG/A516).

Factors Associated With Worse Disability and Disease Severity

A binomial logistic regression was performed to ascertain the effects of DD, phenotype (pure or complicated), presence of altered MEPs, intellectual disability or cognitive impairment, and class of variant on the likelihood that patients had worse disability (SPATAX score >3). The logistic regression model was statistically significant, $\chi^2(2) = 31689$, $p < 0.0005$. The model explained 19.8% (Nagelkerke R2) of the variance in SPATAX and correctly classified 64.0% of cases. Sensitivity was 46.3%, and specificity was 76.3%. Of the 6 predictor variables, only 2 were statistically significant: DD and impaired MEPs (as shown in Table 3). Increasing DD was associated with an increased likelihood of exhibiting SPATAX>3. Patients with increased central motor conduction time at MEPs had 2.72 times higher odds to exhibit a SPATAX score >3 than patients with normal MEPs. Longer DD was also associated with an increased likelihood of exhibiting a worse disease severity, i.e., SPRS ≥26 (eTable 8, links.lww.com/NXG/A516).

Pseudo-longitudinal assessment of disease progression was feasible in 28 patients from 16 families in whom more than 4 clinical evaluation and SPRS scoring were possible over on average a 3.4 ± 2.2 year period. Median disease progression was 0.5 (range −1 to −6.35, mean 0.78 ± 1.40) point/yr.

Table 3 Binomial Logistic Regression to Identify Variables Associated With SPATAX Score >3

	B	SE	Wald	df	Sig	Exp (B)	95% CI for EXP (B) lower	95% CI for EXP (B) upper
Disease duration	0.065	0.014	20.707	1	**<0.001**	1.067	1.038	1.098
MEP central impaired	−0.937	0.391	5.739	1	**0.017**	0.392	0.182	0.843
Complicated phenotype	−0.362	0.497	0.530	1	0.467	0.697	0.263	1.844
Intellectual disability	−0.864	0.673	1.647	1	0.199	0.422	0.113	1.577
Cognitive impairment	−0.202	0.588	0.118	1	0.731	0.817	0.258	2.587
Class of variant	—	—	4.615	5	0.465	—	—	—
Missense	0.783	1.249	0.392	1	0.531	2.187	0.189	25.319
Spice site	0.814	1.396	0.340	1	0.560	2.257	0.146	34.794
Nonsense	0.208	1.395	0.022	1	0.882	1.231	0.080	18.944
Frameshift	−0.059	1.298	0.002	1	0.964	0.943	0.074	12.007
Deletion	0.839	1.304	0.414	1	0.520	2.314	0.180	29.784
Constant	−0.503	1.398	0.129	1	0.719	0.605	—	—

Significant *p* values are highlighted in bold.

Discussion

This study provides a detailed clinical and genetic characterization of a wide Italian SPAST-HSP cohort, which, to our knowledge, is one of the largest cohorts reported.[12,40,44,45] This work represents the first achievement of the DAISY network, created to support collaborative research on HSPs in Italy. Our data confirm a predominant association of SPAST-HSP with pure HSP forms,[12] often characterized by mild or moderate motor disability, supporting the relatively benign course of SPAST-HSP when compared with other HSPs.[1]

In patients with SPAST-HSP manifesting with complicated forms, peripheral nerve and/or cognitive involvement were prevalently associated with spasticity: in this regard, our SPAST-HSP cohort shows a mildly higher prevalence of cognitive involvement than others,[12,40,45,46] whereas psychiatric manifestations were rare. Our findings are supported by the results of a small cross-sectional study assessing cognitive involvement in SPAST-HSP, evidencing signs of cognitive impairment in more than 80% of the 31 adults and low average IQ scores in 60% of the 5 children tested.[47]

Regarding potential neuroanatomic correlates of cognitive involvement in SPAST-HSP, routine brain MRI is often normal,[48,49] with peculiar abnormalities anecdotically reported only in some families.[50-52] In our cohort, similar to what previously reported,[45] signs of cortical atrophy and/or WMH were detected in about 20% of patients with available brain MRI data. Nonetheless, more advanced MRI techniques have allowed to reveal widespread signs of CNS involvement also in patients with SPAST-HSP,[53,54] and accordingly data from a knock-down zebrafish SPAST-HSP model support a role for spastin on maintenance not only of the longest central axons but also of other CNS pathways.[55] Advanced neuroimaging studies on larger patients' cohorts using standardized protocols are needed to better define the pattern of brain involvement in SPAST-HSP and the functional role of spastin in the CNS.

As in previous similar studies, we observed and documented an age-related penetrance and a bimodal distribution regarding AAO in our SPAST-HSP cohort too,[12,44,45,56] with 1 peak in the first decade and the second during mid-adulthood. However, the mean AAO of our patients (about 32 years) was slightly higher compared with other SPAST-HSP cohorts,[12,40,46,57-59] likely because the DAISY network mostly involves neurologic centers for adult patients (19/23 centers).

Moreover, as already observed by some authors,[40,56] we assessed a pseudo-anticipation of parental transmission in some SPAST-HSP pedigrees included in our study. This might actually result from earlier detection of clinical HSP signs in children of adult probands assessed for genetic counseling. Yet, the occurrence of pseudo-anticipation has been proposed in other genetic disorders not caused by microsatellite expansions, possibly due to still unknown genetic or epigenetic mechanisms.[60] Therefore, we are planning to conduct prospective studies on available large SPAST-HSP pedigrees with affected probands of 2 or more generations to definitely clarify this issue.

Differently from previous studies documenting either a slight male excess[12] or no specific sex prevalence,[44] we found a definite higher male prevalence in our SPAST-HSP cohort (M:F ratio ~1.4). Two systematic literature reviews[59,61] also indicated a higher male prevalence in SPAST-HSP, supporting our findings. Furthermore, our SPAST-HSP male group manifested worse

motor disability, i.e., showed higher mean SPRS score and more frequent use of antispastic drugs compared with the female group. These findings might support a protective effect of estrogens in female carriers of *SPAST* pathogenic variants, as proposed by some authors,[61] and, in our opinion, this topic should be further addressed in experimental SPAST-HSP models.

According to the literature,[12,40,44] our study confirmed that longer duration and faster disease progression were both associated with worse functional motor outcome; of further note, pathologic MEP responses were also associated with worse motor disability, suggesting the potential role of MEPs as prognostic predictor in SPAST-HSP. A recent systematic review about MEP studies in HSPs[62] confirmed a similar prevalence of MEP abnormalities in patients with SPAST-HSP (~60%–70% vs 74% of our cohort), but it could not draw definite conclusions about their prognostic role. In this regard, longitudinal studies on large patients' cohorts could help to definitively address the prognostic value of MEPs in SPAST-HSP.

By comparing early- and late-onset SPAST-HSP forms within our cohort, both groups showed a similar disease burden, as we did not find differences regarding either degree of disability or global disease severity. These findings are in contrast with those obtained in a large French-German SPAST-HSP cohort[12] and, conversely, agree with those obtained in another research study[40]: such discrepancies could be either related to different criteria used to classify early and late-onset forms[40] or to a bias due to the retrospective design of the studies.[12,40]

Revision of genetic data evidenced 186 distinct *SPAST* pathogenic variants in our families, of which 48 have never been reported. As in other SPAST-HSP cohorts, we found predominance of truncating variants[12,40,45,58,63] and overall comparable frequency of missense pathogenic variants.[40,45]

In one of our patient with SPAST-HSP, we also found the heterozygous S44L (p.Ser44Leu) variant, a known genetic modifier in SPAST-HSP, in association with a pathogenic *SPAST* variant. This specific variant, with an allele frequency between 0.6%[64] and 3.1%[65] in controls, was reported to negatively affect AAO and/or disease severity when associated with pathogenic *SPAST* variants, likely by downregulating the expression of the M87 isoform or increasing the stability of the M1 isoform.[6,64] Of interest, a patient with sporadic pure HSP not included in this SPAST-HSP cohort, who had a very late symptom onset, only resulted carrier of the heterozygous S44L at *SPAST* molecular testing. It is debated whether the S44L might play per se a detrimental role on upper motor neurons function under specific conditions.[45] However, given the evidence of age-related penetrance of SPAST-HSP pathogenic variants, this case suggests that it could also exert a milder yet detrimental effect on the longest pyramidal axons manifesting only at a very advanced age.

Genotype-phenotype correlations confirmed that intellectual disability was more frequent among carriers of *SPAST* missense variants,[12] but instead, no associations were found with disease severity. The association of missense *SPAST* variants with both early-onset cases and the occurrence of intellectual disability in SPAST-HSP suggests that these class of pathogenic variants might affect brain development possibly through a dominant negative gain of function.[7] Indeed, microtubule-severing spastin activity is also relevant during neurodevelopment.[6]

On the other hand, signs or symptoms of a more widespread neurologic involvement, such as polyneuropathy, cognitive decline and/or cerebral atrophy, were more frequently associated with truncating variants, supporting that reduced levels of spastin expression could also affect other pathways besides the dorsal columns and corticospinal axons.[40]

The main limitation of our study consists in the retrospective and largely cross-sectional design, which might have yielded bias in data collection. Some data, as approximation of penetrance, unquestionably need more accurate family studies to be clearly established. Moreover, some discrepancies between our results and those of previous similar retrospective studies on SPAST-HSP might depend either on the differences in the sample size or in the applied diagnostic protocols.

In conclusion, we have characterized in detail the clinical and molecular spectrum of a wide Italian cohort of patients with SPAST-HSP, being among the largest described in the literature. Our results point out to DD, evidence of MEP alterations, and possibly male sex, as predictors of worse disability.

A recent study involving both animal- and patient-derived SPAST-HSP neuronal cell models supported the feasibility of spastin-elevating therapeutic approaches to rescue neurite defects[8,66]: thus, the establishment of this Italian network will hopefully carry on contributing in the HSP research field sharing large patients' cohorts for further collaborative studies aiming to assess reliable biomarkers of disease severity and/or progression in SPAST-HSP, a still unmet need in view of forthcoming clinical trials to test disease-modifying therapies.

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Continued

Appendix *(continued)*

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Appendix *(continued)*

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