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# Diagnostic Yield and Genotype–Phenotype Correlations of Clinical Exome Sequencing in Hereditary Spastic Paraparesis: Experience From Eastern Spain

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## ABSTRACT

**Background:** Hereditary spastic paraparesis (HSP) encompasses a genetically and clinically heterogeneous group of neurodegenerative disorders, primarily characterized by progressive lower limb spasticity and weakness of the lower limbs. Although more than 80 genes have been associated with HSP, achieving definite genetic diagnosis remains challenging, limiting effective patient care, genetic counseling, and understanding of genotype–phenotype correlations. This study aimed to investigate the diagnostic yield of clinical exome sequencing (CES) in a cohort of Spanish individuals with suspected HSP and to explore genotype–phenotype correlations.

**Methods:** A total of 139 non-related Spanish individuals with HSP underwent standardized clinical evaluation and CES. Genetic analyses were performed using a virtual panel containing 129 HSP-associated genes, complemented by phenotype-driven filtering through the Human Phenotype Ontology. Statistical analyses were performed on core clinical and paraclinical features.

**Results:** After clinical review, 108 index cases were included. Male patients were slightly more represented and mean age at onset was 33 years. Pure HSP forms were more prevalent. The most frequent presenting symptoms were gait disturbance and recurrent falls. A genetic diagnosis was achieved in 57 patients (52,8%), with *SPAST* and *SPG7* being the most frequently mutated genes. In total, pathogenic/likely pathogenic variants were identified across 21 genes, including 8 novel variants. HSP with autosomal recessive inheritance was more common than autosomal dominant (29 vs. 25 cases), while dominant/recessive X-linked disease forms were rare (3 cases).

**Conclusions:** CES combined with HPO-based filtering is an effective strategy for achieving genetic diagnosis in patients with suspicion of HSP.

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## 1 | Introduction

Hereditary spastic paraparesis (HSP) comprises a heterogeneous group of genetic neurodegenerative disorders characterized by spasticity, clumsiness and weakness predominantly affecting the lower limbs resulting from axonal degeneration in the corticospinal tracts [1]. Common clinical features include lower limb hyperreflexia and extensor plantar responses. HSP is classified as uncomplicated (pure) or complex forms (HSP plus) [2]. Pure forms are more frequent and typically present with lower limb spasticity accompanied by urinary bladder hypertonia. In contrast, complex forms are less common and involve additional neurological and non-neurological manifestations such as ataxia, seizures, peripheral neuropathy, cognitive impairment, dementia, hypoacusia, optic atrophy and/or skeletal abnormalities. Age of onset may range from congenital forms to late adulthood. In general, early onset forms are related to non-progressive disease, whilst HSP with later onset are usually slowly progressive disorders that steadily impair independent ambulation [3]. Currently, there are no curative or disease-modifying therapies for HSP. Management is symptomatic and focuses on reducing muscle spasticity, preserving mobility, and addressing associated symptoms [4, 5].

There are more than 80 genes described in association with HSP. The majority exhibit an autosomal dominant (AD) inheritance pattern, with *SPAST* and *KIF1A* being the most frequently involved genes [3]. Autosomal recessive (AR) forms are less common, with *CYP7B1*, *SPG7*, and *SPG11* genes accounting for approximately 20% of the cases [3]. X-linked and mitochondrial HSP are rare, comprising 1%–2% of the HSP series [6]. Additionally, other disorders with overlapping clinical features can present as spastic paraparesis, including amyotrophic lateral sclerosis, primary lateral sclerosis, or cerebral palsy, among many others [7–9]. In this clinical scenario, Next-Generation Sequencing (NGS) has emerged as the gold standard, enabling the simultaneous analysis of numerous genes in a single experiment. From custom target panels and clinical exome sequencing (CES) to whole exome sequencing (WES) and whole genome sequencing (WGS), NGS has significantly increased the diagnostic yield, facilitating accurate diagnosis and genetic counseling.

The aim of this work is to analyze the diagnostic yield in a cohort of Spanish patients with HSP using the CES approach and to describe phenotype and genotype characteristics.

## 2 | Methods

This retrospective study evaluated clinical and genetic data from 139 consecutive index patients assessed between 2010 and 2025 at Hospital Universitari i Politècnic La Fe, a national referral center for Hereditary Cerebellar Ataxia and Spastic Paraplegia. The study was approved by the institutional ethics committee at Health Research Institute Hospital La Fe. Informed consent was obtained from all patients and relatives prior to the genetic analysis and genetic counseling was provided before and after the genetic study.

Inclusion criteria consisted of patients presenting with spastic paraparesis as the predominant clinical feature, in whom a genetic etiology was suspected and acquired causes had been ruled out. All patients underwent a comprehensive diagnostic workup to exclude alternative diagnoses, including brain and spinal cord MRI, as well as extensive blood testing to identify potentially treatable metabolic, infectious, or systemic conditions. Additional ancillary tests—such as electrophysiological studies, evoked potentials, and specialized laboratory assessments—were performed based on the clinical presentation. For patients without a definite genetic diagnosis, a minimum follow-up of 5 years was required to rule out other conditions such as primary lateral sclerosis. Patients with other final clinical diagnosis different from HSP were excluded from the analysis.

### 2.1 | Clinical Evaluation

A standardized questionnaire was used to collect baseline medical history and symptom data. Clinical examination included assessment of the cranial nerves, muscle strength using the standard Medical Research Council (MRC) scale, pinprick, vibratory and positional sensory modalities, reflexes, coordination and gait. Specific attention was given to the presence of urinary symptoms, epilepsy, neuropathy, cognitive impairment, and other neurological features. Probands were classified into pure or complex HSP based on clinical evaluation. Age at symptom onset and ambulatory status at the last visit were also recorded. The clinical Spastic Paraplegia Rating Scale (SPRS) was not performed consistently throughout the series and therefore was not included in the phenotypic description [10]. Depending on the age of symptom onset, five categories were established: infant (0–11 years old), juvenile (12–19 years old), early adulthood (20–30 years old), adulthood (31–64 years old), and late adulthood (> 65 years old). Patients were classified according to their family history as AD (at least one affected individual in the previous generation), AR (at least two affected individuals in the same generation and no affected in the previous), or sporadic (no other affected individual in the family).

### 2.2 | Genetic Study

Genomic DNA was extracted from peripheral blood samples using QIAsymphony DNA Midi Kit (Qiagen) following standard procedures. *Next-Generation* sequencing (NGS) was performed using the Agilent Focused Exome CCP17 clinical exome capture kit (Agilent Technologies, Santa Clara, CA) for Illumina platforms (San Diego, CA) targeting approximately 5200 genes. The library preparation was carried out according to the Bravo NGS SureSelectQXT Automated Target Enrichment protocol as previously reported [11]. Sequences were aligned to the human reference genome (GRCh37/hg19), and variant calling was performed using the Alissa Clinical Informatics Platform. Initially, a virtual panel containing 129 genes associated with HSP and related phenotypes was assembled through literature-based and expert-driven selection and used for analysis (See Table S1). In cases with negative results, a second analysis was performed using Human Phenotype Ontology (HPO: https://hpo.jax.org/app/) terms: *spasticity* (HP:0001257), *spastic gait* (HP:0002064) and *spastic paraparesis* (HP:0002313). The annotated variants were filtered according to a Minor Allele Frequency (MAF) value ≤ 0.02 Exome



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Aggregation Consortium (ExAC) database/gnomAD (https://gnomad.broadinstitute.org/), and 1000genomes (https://www.internationalgenome.org/). The obtained variants were classified according to the guidelines of the American College of Medical Genetics [12]. Variant annotations were cross-referenced with ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and The Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/). Additionally, base conservation was assessed with GERP and in silico analysis was performed with the predictive tools: PROVEAN, SIFT, Polyphen-2, MutationTaster, and Maxent and SpliceAI for variants affecting canonical splice sites. When applicable, Multiplex Ligation–dependent Probe Amplification analysis (MLPA; MRC Holland, Amsterdam, the Netherlands) was used to seek the presence of copy number variants in patients with only one pathogenic/likely pathogenic variant in an AR gene detected by CES, as a complementary analysis to assess for a second pathogenic allele. In one patient with a CES-detected pathogenic single nucleotide variant in *FXN*, the presence of common trinucleotide repeat expansions was evaluated using the ADELLGENE Friedreich's Ataxia kit (BDR Diagnostics). Sanger sequencing was used to perform segregation analysis.

### 2.3 | Statistical Methods

Descriptive analyses were conducted for clinical and paraclinical variables. Categorical variables were compared using Pearson's chi-squared or Fisher's exact tests, as appropriate. Means were compared using independent sample *t*-tests. A $p$-value $< 0.05$ was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.

## 3 | Results

### 3.1 | Demographic Data

A total of 139 index patients with suspected HSP were initially evaluated. Thirty-one patients were excluded due to alternative diagnoses, most commonly primary lateral sclerosis ($n = 10$) and myelopathy ($n = 5$). Additional alternative diagnoses are listed in Table S2. Therefore, 108 index cases were ultimately included in this study (Figure 1). The cohort was predominantly male ($n = 68$, 63%), with a mean age in 2025 of 60 years (range: 12–84). Most patients originated from the Valencian Community ($N = 85$, 79%). The remaining 23 patients were from other regions of Spain or other countries, including Venezuela ($N = 2$), Romania ($N = 1$), Moldova ($N = 1$), Argelia ($N = 1$) and France (N = 1). Consanguinity was reported in 12 cases and considered probable in 4 additional cases due to the small population size of their region of origin. Regarding family history, 28 patients presented as AD cases (26%), 12 as AR (11%) and 68 as sporadic (63%).

### 3.2 | Clinical Assessment

The most common presenting symptoms were gait disturbances ($N = 61$; 56%) and frequent falls ($N = 22$; 20%) Additional symptoms included clumsiness ($N = 9$) or weakness ($N = 5$), and less frequent manifestations were cramps, epilepsy, diplopia, hypotonia, tremor or bladder urgency. The mean age of symptom onset was 33 years [range 0–75]. Adult-onset cases were the most frequent ($n = 50$; 46%), followed by infantile and early adulthood onset, each with 23 cases (21%).

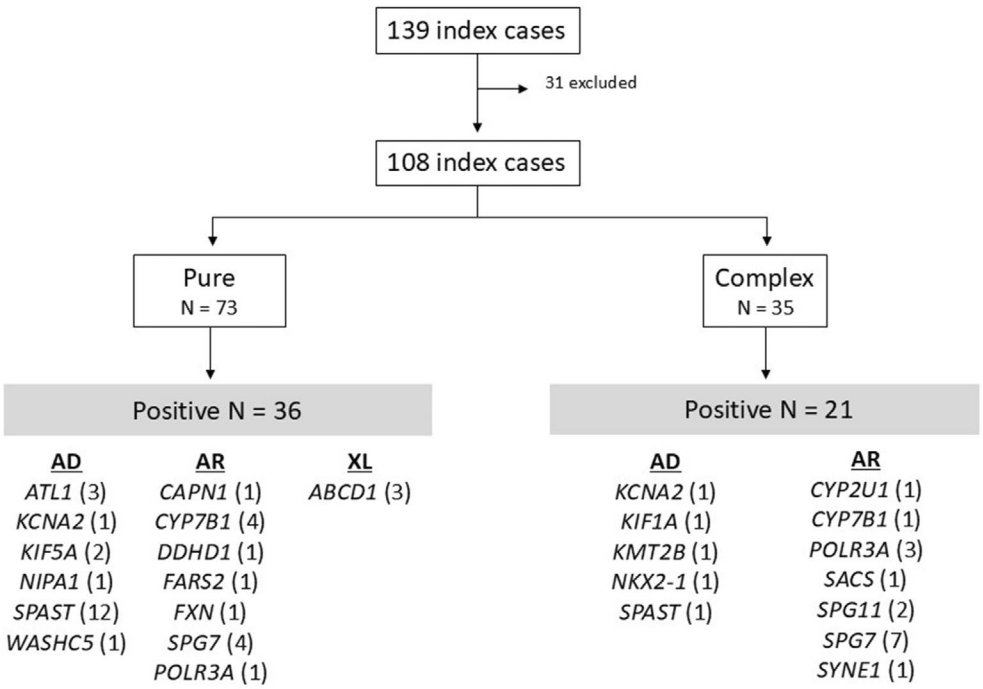


**FIGURE 1** | Flowchart of genetically solved patients included in this study. 139 index cases were clinically suspected of HSP, but 31 were excluded. Among the 108 patients included, 73 presented a pure form of HPS and 35 a complex phenotype. Genetic cause was identified in 36 and 21 patients, respectively. Causing genes are listed for each group. AD, autosomal dominant; AR, autosomal recessive; XLR, recessive X-linked. Numbers in parentheses denote the number of reported cases associated with each gene.



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Juvenile-onset cases were less common ($n=10$), and late-onset cases were rare ($n=2$).

All patients showed varying degrees of lower limb weakness, spastic gait, hyperreflexia, and/or extensor plantar responses on examination. Urinary dysfunction, primarily urgency, was reported in nearly half the cohort ($n=53$; 49%). Neurological signs associated with complex HSP phenotypes included cerebellar ataxia ($N=24$; 22%), cognitive impairment ($N=6$), neuropathy ($N=6$), hypoacusia ($N=6$), optic atrophy ($N=3$) and/or epilepsy ($N=2$). Hence, pure forms of HSP ($N=73$; 68%) were more common than complex ($N=35$; 32%). At the final follow-up (mean follow-up: 9 years, range: 2–25), the mean disease duration was 23.9 years. Independent ambulation was preserved in 39% of patients, 49 (43%) required walking aids, and 20 (18%) were wheelchair-bound.

### 3.3 | Genetics

CES was performed in all 108 index cases, achieving a genetic diagnosis in 57 patients (53%) (Figures 1 and 2). The diagnostic yield was higher in patients with HSP-plus phenotype than in those with pure forms (57% and 49% respectively). We identified 57 different putative disease-causative variants in 21 different genes (Table 1, Figure 2). Ten of these variants were novel and are listed with their associated phenotypes in Table 2. All identified variants were point mutations except three CNVs (Copy Number Variants), thus representing 3.4% of the detected mutated alleles. AR inheritance was more frequent than AD ($N=29$ versus $N=25$ respectively), while recessive X-linked (XLR) forms were found in 3 cases (Figure 1). The most frequent cause of AD HSP in our cohort was pathogenic/likely pathogenic variants in the *SPAST* gene ($N=13$). Ten of them were classified as familial cases with an AD inheritance pattern, whilst three patients presented as sporadic. There was a wide range of age of onset (mean 42; range 13–67), and all presented with a pure HSP phenotype except for patient P67, who developed prominent ataxic gait. Other AD-associated genes were much less common: *ATL1* ($N=3$), *KCNA2* ($N=2$), *KIF5A* ($N=2$), *KIF1A* ($N=1$), *KMT2B* ($N=1$), *NIPA1* ($N=1$), *NKX2-1* ($N=1$), and *WASHC5* ($N=1$).

Regarding HSP with AR inheritance pattern, *SPG7* was the most frequently involved ($N=12$). Details of four of these *SPG7* patients have been previously published [13] (Table 2). Seven of these patients presented complicated HSP forms with cerebellar ataxia. The common p.(Ala510Val) pathogenic variant in the *SPG7* gene was found in 10 patients either in homozygosity or compound heterozygosity ($N=6$ and $N=4$, respectively). Consanguinity was only reported in only one of the homozygous cases. Other common AR forms of HSP were caused by deleterious variants in *CYP7B1* ($N=5$, 4%) and *POLR3A* (N = 4, 3.5%). All five *CYP7B1* cases were homozygous for four different known damaging variants, although consanguinity was only reported in two patients. Only patient P44, who harbored the p.(Glu321Lys) mutation in homozygosity in *CYP7B1*, developed a complicated form of HSP including ataxic gait. Onset in *CYP7B1* cases ranged from 12 to 36 years, and none retained independent ambulation at the end of follow-up (disease duration: 17–39 years). All *POLR3A* patients carried the intronic c.1909 + 22G>A recurrent mutation in compound heterozygosity; two of these patients (P6 and P14) have been previously reported [11]. All of them presented a complicated form with ataxic gait in combination with skeletal anomalies such as *pes cavus* or scoliosis ($N=2$), dental abnormalities ($N=2$), dysarthria ($N=1$), or epileptic crisis during infancy ($N=1$). Finally, other less common AR genes in our cohort were *SPG11* ($N=2$)

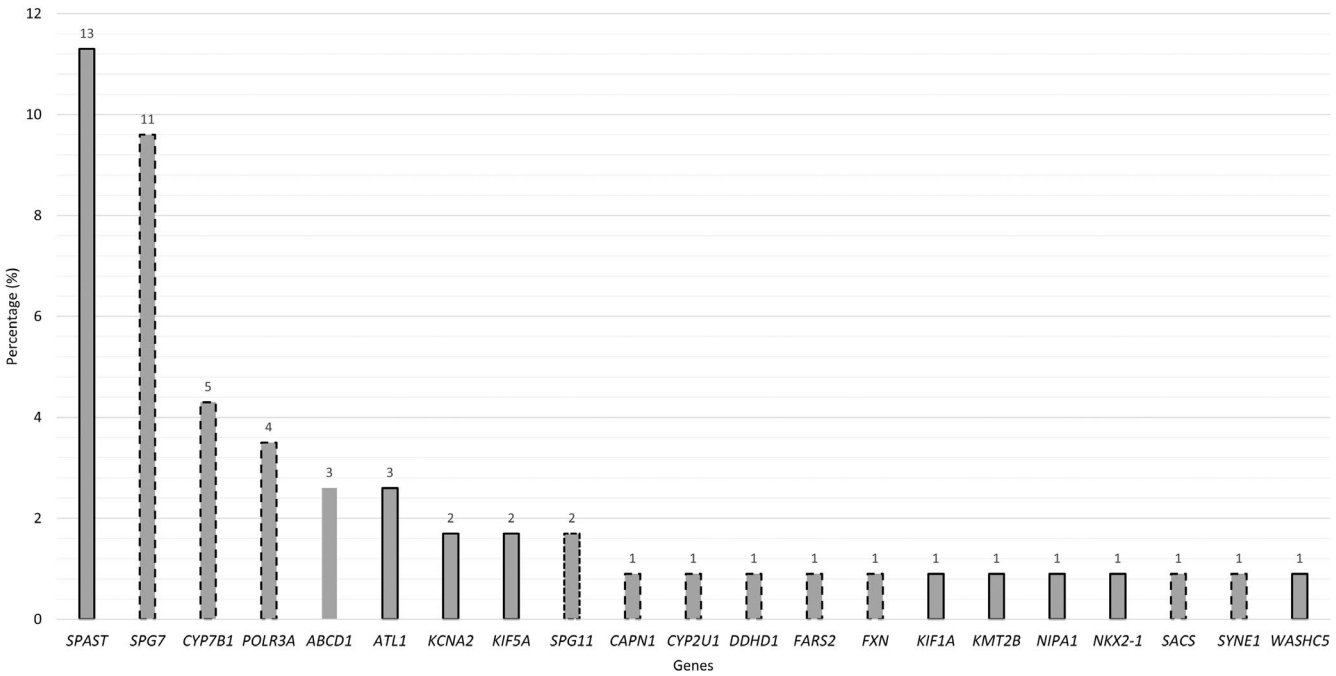


**FIGURE 2** | Frequency of the different genes found to be the cause of spastic paraparesis in our cohort. Percentage of each gene is represented in the Y axis and total number of patients is indicated at the top of each bar. Different inheritance patterns are also identified: Black framed bars represent autosomal dominant fashion; discontinuous framed bars represent autosomal recessive and non-framed bars define recessive X-linked genes.



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**TABLE 1** | List of variants found in the cohort.

| Patient | Gene | Variant 1/reference | Variant 2/reference |
|---|---|---|---|
| 13 | *ABCD1* | c.565C>T;(p.Arg189Trp)/rs1131691916 | — |
| 49 | *ABCD1* | c.584A>G;p.(Gln195Arg)/rs2522266647 | — |
| 59 | *ABCD1* | c.1975C>T;p.(His659Tyr)/rs2091774102 | — |
| 17 | *ATL1* | c.488 T>C;p.(Val163Ala)/rs1595600383 | — |
| 24 | *ATL1* | c.650G>A;(p.Arg217Gln)/rs119476049 | — |
| 40 | *ATL1* | c.470 T>G;p.(Leu157Trp)/rs119476051 | — |
| 52 | *CAPN1* | **c.1A>T;p.(Met1?)** | c.463C>T;(p.Gln155*)/PMID: 27320912 |
| 113 | *CYP2U1* | **c.852del;p.(Phe284Leufs*4)** | **c.852del;p.(Phe284Leufs*4)** |
| 32 | *CYP7B1* | c.187C>T;p.(Arg63*)/rs746979262 | c.187C>T;p.(Arg63*)/rs746979262 |
| 44 | *CYP7B1* | c.961G>A;p.(Glu321Lys)/rs866340497 | c.961G>A;p.(Glu321Lys)/rs866340497 |
| 58 | *CYP7B1* | c.889A>G;p.(Thr297Ala)/rs587777222 | c.889A>G;p.(Thr297Ala)/rs587777222 |
| 60 | *CYP7B1* | c.187C>T;p.(Arg63*)/rs746979262 | c.187C>T;p.(Arg63*)/rs746979262 |
| 112 | *CYP7B1* | c.321_324del;p.(Lys107Asnfs*2)/rs770285398 | c.321_324del;p.(Lys107Asnfs*2)/rs770285398 |
| 71 | *DDHD1* | c.1729C>T;p.(Arg577*)/rs776191400 | c.1729C>T;p.(Arg577*)/rs776191400 |
| 7 | *FARS2* | c.874G>A;p.(Gly292Arg)/rs747278433 | c.407C>A;p.(Pro136His)/rs199863563 |
| 29 | *FXN* | EXP > 200REP | c.389G>T;p.(Gly130Val)/rs104894107 |
| 62 | *KCNA2* | c.881G>A;p.(Arg294His)dn/rs886041761 | — |
| 93 | *KCNA2* | c.881G>A;p.(Arg294His)dn/rs886041761 | — |
| 100 | *KIF1A* | c.32G>A;p.(Arg11Gln)/rs1575654528 | — |
| 70 | *KIF5A* | **c.2274G>C;p.(Glu758Asp)** | — |
| 75 | *KIF5A* | c.751G>A;p.(Glu251Lys)/rs387907285 | — |
| 106 | *KMT2B* | **c.439C>T;p.(Arg147*)** | — |
| 25 | *NIPA1* | c.126C>A;p.(Asn42Lys)dn/PMID: 35464835 | — |
| 3 | *NKX2-1* | c.434C>A;p.(Ser145*)/rs863225300 | — |
| 6* [10] | *POLR3A* | c.1909 + 22G>A/rs191875469 | c.1628A>C;p.(Gln548Pro)/PMID: 36530930 |
| 14* [10] | *POLR3A* | c.1909 + 22G>A/rs191875469 | c.3688G>A;(p.Asp1230Asn)/PMID: 36530930 |
| 85 | *POLR3A* | c.1909 + 22G>A/rs191875469 | **c.1030C>T;p.(Arg344Cys)** |
| 114 | *POLR3A* | c.1909 + 22G>A/rs191875469 | c.685C>T;p.(Arg229*)/rs1384567912 |
| 68 | *SACS* | c.7205_7206del; p.(Leu2402Argfs*6)/rs773182375 | deletion exon 5 |
| 8 | *SPAST* | c.1133 T>G;p.(Leu378Arg)/PMID: 20932283 | — |
| 26 | *SPAST* | c.1741C>T;p.(Arg581*)/rs778023258 | — |
| 28 | *SPAST* | **c.1614delTinsCACC; p.(Ala538_Arg539insThr)** | — |
| 30 | *SPAST* | c.734C>G;p.(Ser245*)/rs1553314896 | — |
| 31 | *SPAST* | Deletion exon 17 | — |
| 34 | *SPAST* | c.1642G>A;p.(Asp548Asn)/PMID: 19875132 | — |
| 42 | *SPAST* | **c.1322-3 T>A** | — |
| 45 | *SPAST* | c.1196C>T;p.(Ser399Leu)/rs1553317025 | — |
| 54 | *SPAST* | c.734C>A;p.(Ser245*)/rs1553314896 | — |

(Continues)



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**TABLE 1** | (Continued)

| Patient | Gene | Variant 1/reference | Variant 2/reference |
|---|---|---|---|
| 57 | *SPAST* | c.1413 + 2dup/rs587777756 | — |
| 67 | *SPAST* | c.870 + 3A>G/rs1553314979 | — |
| 72 | *SPAST* | c.1738dup;p.(Ile580Asnfs*5)/PMID: 30476002 | — |
| 74 | *SPAST* | c.1553 T>C;p.(Leu518Pro)/rs1553319290 | — |
| 88 | *SPG11* | c.2278 T>C;p.(Cys760Arg)/rs2505563283 | c.2444G>A;p.(Arg815Lys)/rs312262742 |
| 94 | *SPG11* | **c.3842A>G;p.(Tyr1281Cys)** | **c.6845A>T;p.(Asp2282Val)** |
| 10* | *SPG7* | c.1529C>T;p.(Ala510Val)/rs61755320 | c.1529C>T;p.(Ala510Val)/rs61755320 |
| 19* [12] | *SPG7* | c.1529C>T;p.(Ala510Val)/rs61755320 | **c.935C>A;p.(Ala312Glu)/[12]*** |
| 27* [12] | *SPG7* | c.1529C>T;p.(Ala510Val)/rs61755320 | c.1529C>T;p.(Ala510Val)/rs61755320 |
| 38* [12] | *SPG7* | c.1529C>T;p.(Ala510Val)/rs61755320 | c.1529C>T;p.(Ala510Val)/rs61755320 |
| 46* [12] | *SPG7* | c.806G>A;p.(Trp269*)/rs1235945505 | c.806G>A;p.(Trp269*)/rs1235945505 |
| 47* [12] | *SPG7* | c.1529C>T;p.(Ala510Val)/rs61755320 | deletion exon 17 |
| 55* [12] | *SPG7* | c.806G>A;p.(Trp269*)/rs1235945505 | c.1529C>T;p.(Ala510Val)/rs61755320 |
| 73 | *SPG7* | c.233 T>A;p.(Leu78*)/rs121918358 | c.1529C>T;p.(Ala510Val)/rs61755320 |
| 84 | *SPG7* | c.1529C>T;p.(Ala510Val)/rs61755320 | c.1529C>T;p.(Ala510Val)/rs61755320 |
| 89 | *SPG7* | **c.839G>A;p.(Arg280Lys)** | c.1045G>A;p.(Gly349Ser)/rs141659620 |
| 95 | *SPG7* | c.1529C>T;p.(Ala510Val)/rs61755320 | c.1529C>T;p.(Ala510Val)/rs61755320 |
| 97 | *SYNE1* | c.21148C>T;p.(Arg7050*)/rs763325410 | c.21148C>T;p.(Arg7050*)/rs763325410 |
| 50 | *WASHC5* | c.1772C>T;p.(Ser591Phe)/rs1554593899 | — |

*Note:* Previously reported variants are referenced with the ClinVar SNP ID, or the corresponding PMID when no ClinVar entry exists. *These patients have been previously reported by the authors in the cited articles. Bold: unreported variants.

and *CAPN1*, *CYP2U1*, *SACS*, *DDHD1*, *FARS*, and *FXN*, which were found in a single patient each.

Pathogenic/likely pathogenic variants in only one X-linked gene, *ABCD1*, were identified in three independent patients, representing the 2,7% of the total series. All had adult-onset pure HSP phenotypes (ages 30–52 years), without cognitive impairment, white matter abnormalities, or other features typical of adrenoleukodystrophy.

Four genes in our series—*ABCD1*, *FXN*, *KMT2B*, and *NKX2-1*—are known to be associated with neurodegenerative diseases in which spasticity is part of the clinical manifestations. Causative variants in these genes were found in 7 patients (6%).

Segregation analysis was possible in 15 (26%) families and confirmed two de novo variants: *NIPA1* c.126C>A;p.(Asn42Lys) in patient P25, and *KCNA2* c.881G>A;p.(Arg294His) in patient P62. The same *KCNA2* variant was also found in patient P94, although familial testing was not feasible.

Among the 51 patients with negative CES results, demographic characteristics (65% male; mean age 57 years; geographic origin) were comparable to the solved group. There were no statistically significant differences in age at onset (mean: 30.7 years) or HSP phenotype (72% pure forms). Regarding inheritance pattern, unsolved cases were more frequently sporadic ($n = 37$; 73%) than AD ($n = 10$; 20%) or AR ($n = 4$; 8%), but this distribution did not significantly differ from the solved cases.

## 4 | Discussion

Hereditary spastic paraplegia comprises a group of neurodegenerative genetic disorders characterized by spasticity and weakness in the lower limbs. The associated phenotype is highly heterogeneous, often combining neurological and non-neurological impairments and frequently overlapping with other neurological conditions such as amyotrophic lateral sclerosis, primary lateral sclerosis, and cerebral palsy. Nonetheless, accurate differentiation is essential for appropriate therapeutic management and genetic counseling. The genetic landscape of HSP is complex, with more than 80 causative genes identified to date and different inheritance patterns. Notably, some genes implicated in HSP can also cause other neurodegenerative diseases (e.g., *KIF5A* variants in the tail domain usually cause ALS, SPG7 variants can cause spinocerebellar ataxias, etc.). This clinical and genetic overlap reflects the complexity of the diagnostic scenario and has been significantly impacted by the emergence and widespread use of next generation sequencing technologies.

In this study we analyzed a cohort of 108 HSP patients, highlighting the broad clinical and genetic heterogeneity of this condition. Pure forms of HSP were more common than complex,



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**TABLE 2** | Phenotype of patients with previously unreported variants.

| Patient | Gender | Phenotype | Age at onset (ys) | Age at the end of follow-up (ys) | Presenting symptom | Neurological sings | Non neurological signs | Ambulation | Gene | Transcript | Novel variants [ACMG criteria] In silico predictions | Previously reported variants (for disorders with AR inheritance) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 28 | M | Pure | 40 | 78 | Gait disturbance | LLW, HLL, US | SK | Cane | *SPAST* | NM_014946 | c.1614delTinsCACC;p.(Ala538_Arg539insThr)[PM2, PM4, PP1, PP4]— | — |
| 42 | F | Pure | 67 | 76 | Gait disturbance | LLW, HLL, US | SK | Cane | *SPAST* | NM_014946 | c.1322-3 T>A [PM2, PM4, PP1, PP4] MaxEnt: var. −46.16%; SpliceAI: 0.30 AL | — |
| 52 | F | Pure | 25 | 52 | Unknown | LLW, HLL, US, DA | — | 2 canes | *CAPN1* | NM_005186 | c.1A>T;p.(Met1?) [PVS1, PM2, PM3] SIFT: 0; MT:1 | c.463C>T;p.(Gln155[a]) |
| 70 | M | P | 55 | 70 | Gait disturbance | LLW, HLL, US, DA | — | Wheelchair | *KIF5A* | NM_004984 | c.2274G>C;p.(Glu758Asp) [PM2 + PP1 + PP2][a] SIFT: 0.151; MT: 1 | — |
| 85 | M | Plus | 57 | 69 | Frequent falls | LLW, HLL, PCS | Teeth anomalies | Cane | *POLR3A* | NM_007055 | c.1030C>T;p.(Arg344Cys) [PM2, PM3, PP2, PP3] SIFT: 0; Polyphen2: 1; MT:1 | c.1909 + 22G>A |
| 89 | F | Plus | 50 | 66 | Gait disturbance | LLW, HLL, US, A, CI, DM, T | SK | Cane | *SPG7* | NM_003119 | c.839G>A;p.(Arg280Lys) [PM2, PM3][a] SIFT:0.882; MT:1 | c.1045G>A;p.(Gly349Ser) |

(Continues)



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**TABLE 2** | (Continued)

| Patient | Gender | Phenotype | Age at onset (ys) | Age at the end of follow-up (ys) | Presenting symptom | Neurological sings | Non neurological signs | Ambulation | Gene | Transcript | Novel variants [ACMG criteria] In silico predictions | Previously reported variants (for disorders with AR inheritance) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 94 | F | Plus | 29 | 74 | Lower limb weakness | LLW, HLL, N, T | — | Wheelchair | *SPG11* | NM_025137 | c.3842A>G;p.(Tyr1281Cys) [PM2, PM3, PP3][a] SIFT: 0.018; MT:0.76<br>c.6845A>T;p.(Asp2282Val) [PM2, PM3, PP3] SIFT:0.001; MT:1; MaxEnt: var. + 17.62% | — |
| 106 | M | Plus | 4 | 19 | Gait disturbance | LLW, HLL, DA, CI, Dystonia | FDF, SK | Cane | *KMT2B* | NM_014727 | c.439C>T;p.(Arg147[a]) [PVS1, PM2] MT:1 | — |
| 114 | M | Plus | 2 | 25 | Gait disturbance | CI, US | Teeth anomalies | Wheelchair | *CYP2U1* | NM_183075 | c.852del;p.(Phe284Leufsa4) homozygosity[PVS1, PM2]— | — |

*Note:* Only in silico prediction tools that provided a result for each variant are shown. SIFT and MT scores range from 0 to 1. Deleterious prediction is interpreted by values closer to 0 in SIFT and closer to 1 in Polyphen2 and MT.
Abbreviations: A, ataxia; AL, acceptor loss; AR, autosomal recessive; CI, cognitive impairment; DA, dysarthria; DM, dysmetria; F, female; FDF, facial dysmorphic features; HLL, hyperreflexia lower limbs; LLW, lower limb weakness; M, male; MT, mutation taster; N, neuropathy; PCS, posterior cord syndrome; SK, skeletal anomalies; T, tremor; US, urinary signs; Var, variation score.
[a]These variants are classified as variants of uncertain significance.



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and adult onset was the most frequent presentation. Among patients with complex HSP, cerebellar ataxia was the most frequent additional neurological feature, while epilepsy, peripheral neuropathy, and cognitive impairment were less common.

A molecular diagnosis was established in 57 families, yielding a diagnostic rate of 53%. Among the AD genes, *SPAST* was the most frequently mutated, followed by *ATL1*, which is consistent with previously reported HSP series in the Caucasian population [3, 14]. Interestingly, we have not detected any variant in the *REEP1* gene, which is typically reported as the third most common AD HSP gene [3]. Regarding AR forms, the most commonly reported genes in the literature are *CYP7B1*, *SPG7*, and *SPG11* [3, 14]. In our cohort, *SPG7* was the most frequently observed AR gene, followed by *CYP7B1*. Only two unrelated patients were found to carry biallelic pathogenic variants in the *SPG11* gene. In contrast, *POLR3A* emerged as the third most frequent AR gene in this cohort, accounting for four HSP cases. Interestingly, all of them harbored the intronic c.1909 + 22G>A variant in compound heterozygosity. This variant has an allele frequency of 0.001365 in the general population (gnomAD v2.1.1) and has increasingly been reported in the literature in association with ataxic presentations [15, 16].

Although sporadic cases are typically assumed to result from autosomal recessive inheritance, other mechanisms—such as X-linked transmission, incomplete penetrance, anticipation, de novo mutations, and pathogenic mitochondrial variants—must also be considered [3]. De novo mutations, in particular, are increasingly recognized in neurogenetic disorders [17, 18]. Consequently, genetic family analysis is especially recommended in sporadic cases in order to offer proper genetic counseling. However, obtaining parental DNA can be challenging in diseases with late onset of symptoms, as can be the case of HSP, limiting segregation analyses. For example, incomplete penetrance and anticipation have been described for the *SPAST* gene [19, 20], which is linked to SPG4 (Spastic Paraplegia 4; OMIM #182601) with an AD inheritance pattern. In our series, three cases initially considered sporadic carried pathogenic mutations in the *SPAST* gene. Parental samples were unavailable for segregation analysis, precluding confirmation of de novo origin or incomplete penetrance.

We have identified two cases with de novo variants in our cohort. The c.126C>A variant in the *NIPA1* gene was found in a male patient with a pure HSP phenotype, symptom onset at 33 years, and preserved independent ambulation after 14 years of disease. This variant has only been previously reported once in the literature, in a Chinese patient with an earlier onset (5 years old) and also a pure HSP phenotype [21]. Interestingly, this case was reported to be de novo too. Hence, this is the second patient reported to date with the c.126C>A variant, but with a milder clinical course. The other de novo variant found in our cohort was the c.881G>A;p.(Arg294His) in the *KCNA2* gene, a well-described pathogenic mutation [22]. This gene is related to SPG6 (OMIM #616366), and the de novo mutational mechanism has been documented in several cases including the variant reported here [22].

CES analysis using HPO terminology enables the expansion of genetic screening. Using this strategy enabled us to detect causative variants in four genes in which spastic paraparesis was the only or predominant manifestation of a neurological condition other than HSP: *ABCD1*, *FXN, KMT2B* and *NKX2-1*. The *ABCD1* gene is associated with adrenoleukodystrophy (OMIM #300100), a progressive neurodegenerative disorder in which spasticity is one of the hallmark clinical features [23–25]. In fact, it has explained a small proportion of patients in other HSP cohorts [26, 27]. The *FXN* gene is linked to Friedreich ataxia (OMIM #229300), a complex neurological disease, which may present with predominant lower limb spasticity in late- or very-late-onset cases. Our patient with *FXN* biallelic mutations initially presented with lower limb spasticity, gait disturbance, and urinary symptoms, later developing dysarthria, gait instability, and dysmetria [28]. Regarding *KMT2B*, this gene is commonly associated with childhood-onset dystonia 28 (OMIM #617284) that typically begins in the lower limbs. Our patient, with a novel variant, consulted at the age of 4 because of gait disturbance, but examination revealed spastic paraparesis, as well as dysmorphic traits such as micro and brachycephaly, broad neck, low-set ears, microtia, down-turned palpebral fissures and carpal mouth. He developed delayed motor development, precocious puberty, language regression at the age of 9, mild cognitive impairment and dystonia. At the age of 20, he remained ambulant but experienced frequent falls. Finally, *NKX2-1* is associated with a complex neurodevelopmental disorder that includes choreoathetosis and hypothyroidism with or without neonatal respiratory distress (OMIM #610978). Although spastic paraparesis is not a common feature, our patient presented with mild neonatal hypotonia and delayed motor milestones, followed by the development of spastic paraparesis in early childhood. During late infancy, additional features emerged, including mild ataxia, choreoathetosis of the upper limbs, and hypothyroidism.

The absence of statistically significant differences in gender, age of onset, or HSP phenotype between genetically diagnosed and undiagnosed patients underscores the considerable clinical heterogeneity of HSP. Several factors may explain the lack of genetic diagnosis. First, considering the overlapping phenotype between HSP and other neurological entities such as primary lateral sclerosis, it is possible that patients with negative genetic test develop other symptoms or signs in the course of their disease, which change the clinical diagnosisIn our view, patients with adult-onset progressive spastic paraparesis and negative genetic testing should retain a provisional diagnosis of spastic paraplegia for at least 15 years after the onset of symptoms, to rule out a primary lateral sclerosis [29]. Secondly, the existence of variants of uncertain significance (VUS) complicates accurate genetic interpretation. The biologic effect of these variants should be characterized in order to reclassify them as benign or pathogenic. Thirdly, CES has technical limitations and may miss certain pathogenic mutations, including those in newly annotated disease-causing genes, as well as deep intronic variants, CNVs, or large chromosomal rearrangements. The use of powerful techniques as Whole Exome Sequencing or Whole Genome Sequencing could be applied as a second-tier approach to overcome these CES limitations [30, 31]. Moreover, mitochondrial genes have not been explored in our series and may represent a source of missed diagnoses. Finally, it seems possible that new genes linked to HSP may be described in the future.



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In summary, HSP encompasses a broad clinical spectrum and shares overlapping features with other neurological diseases, complicating diagnosis. The genetic variability further contributes to diagnostic delays. Nonetheless, clinical exome sequencing, particularly when combined with HPO-based filtering, represents a valuable tool to improve diagnostic yield and facilitate accurate genetic counseling.

### Author Contributions

**Lidón Carretero-Vilarroig:** data curation, formal analysis, writing-original draft preparation. **Rafael Sivera:** data curation, formal analysis, supervision, writing – review and editing. **Raquel Baviera:** data curation, formal analysis, writing – review and editing. **Carmen Espinós:** data curation, writing – review and editing. **Nuria Muelas:** data curation, formal analysis, writing – review and editing. **Juan Francisco Vazquez-Costa:** data curation, formal analysis, writing – review and editing. **Elena Aller:** data curation, writing – review and editing. **Luis Bataller:** conceptualization, data curation, formal analysis, writing – review and editing, funding acquisition. **Teresa Jaijo:** conceptualization, data curation, formal analysis, supervision, writing – review and editing.

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This study was approved by the Research Ethics Committee from Hospital Universitari I Politècnic La Fe (2021-435-1). The authors confirm that they have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** List of the 129 genes included in the virtual panel of the CES analysis. **Table S2:** Diagnosis in excluded patients.



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