Gene 988 (2026) 150053

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short Communication

Two pathogenetic intronic variants in SPG4/*SPAST* and expansion of the clinical presentation

Cecilia Evangelisti [a,1,2], Emanuele Panza [a,b,1], Mario Stasi [c], Arun Meyyazhagan [c], Haripriya Kuchi Bhotla [c], Preethi Basavaraju [d], Gustavo Ribas [e], Natalia Fava [b], Sherin Ramadan [b], Ryosuke Miyamoto [f], José Luiz Pedroso [g], Orlando Graziani Povoas Barsottini [g], Hélio Afonso Ghizoni Teive [e], Antonio Orlacchio [c,h,*]

[a] *Medical Genetics Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy*
[b] *Dipartimento di Scienze Mediche e Chirurgiche, Università di Bologna, Bologna, Italy*
[c] *Dipartimento di Medicina e Chirurgia, Università di Perugia, Perugia, Italy*
[d] *Department of Human Genetics and Molecular Biology, Bharathiar University, Coimbatore, TN, India*
[e] *Department of Internal Medicine, Federal University of Paraná (UFPR), Curitiba, PR, Brazil*
[f] *Department of Neurology, Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan*
[g] *Department of Neurology, Federal University of São Paulo (UNIFESP), São Paulo, SP, Brazil*
[h] *Unità di Neurogenetica, Azienda Ospedaliera di Perugia, Perugia, Italy*

ARTICLE INFO

Keywords:
Spastic Paraplegia Type 4 (SPG4)
SPAST
Intronic variants
Minigene assay
Phenotype variability

ABSTRACT

Hereditary Spastic Paraplegia (HSP) is a group of inherited neurodegenerative disorders primarily characterized by progressive lower limb spasticity and weakness. Among the various genetic causes of HSP, pathogenetic variants in SPG4/*SPAST* are the most frequently identified, making them the leading molecular cause of autosomal dominant HSP. The *SPAST* gene encodes spastin, a protein involved in microtubule dynamics. In this study, we focused on the functional characterization of two specific intronic variants in *SPAST* absent or present at a very low frequency in GnomAD database and with conflicting classification of pathogenicity, c.1245 + 5G > A and c.1493 + 2_1493 + 5del respectively. These variants were identified in two independent families, one of Brazilian origin and the other of Japanese descent. Our data shows that the splicing variants impact splicing. Furthermore, through segregation analysis and clinical assessments, we provided a detailed description of the affected individuals, emphasizing the clinical presentation associated with these genetic changes. Notably, in both families, the identified variants co-segregated symptoms consistent with anorexia nervosa, suggesting a potential, previously unrecognized association between *SPAST* pathogenic variants and disordered eating behaviors. Our findings contributed to the expanding clinical spectrum of SPG4-associated HSP and highlighted the importance of characterizing intronic *SPAST* variants. The characterization of intronic pathogenetic variants enhanced our understanding of their potential pathogenic mechanisms, which may have implications for both genetic diagnosis and the broader clinical management of HSP.

Abbreviations: HSP, Hereditary Spastic Paraplegia (HSP); SPG4, Spastic Paraplegia Type 4 (SPG4); OMIM, Online Mendelian Inheritance in Man; AAA, ATPases Associated with diverse cellular Activities; IRBs, Institutional Review Boards; WES, Whole Exome Sequencing; MTBD, MicroTubule-Binding Domain; DSM-5-TR, Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision; SD, Standard Deviation; WGS, Whole Genome Sequencing.

* Corresponding author at: Dipartimento di Medicina e Chirugia, Università di Perugia, Piazza L. Severi – Edificio B, 1° Piano, Sant'Andrea delle Fratte, Perugia 06132, Italy.
E-mail address: antonio.orlacchio@unipg.it (A. Orlacchio).
[1] **These authors contributed equally to this work and shared first authorship.**
[2] *ORCID ID:* 0000-0002-0877-7696 (C. Evangelisti), 0000-0002-2338-1139 (E. Panza), 0009-0005-8706-3139 (M. Stasi), 0000-0002-1934-3906 (A. Meyyazhagan), 0000-0002-9997-6454 (H. Kuchi Bhotla), 0000-0001-7097-2276 (P. Basavaraju), 0000-0003-1997-0552 (G. Ribas); 0009-0007-4577-2935 (N. Fava); 0009-0003-1129-2010 (S. Ramadan); 0000-0001-6434-9541 (R. Miyamoto); 0000-0002-1672-8894 (J.L. Pedroso); 0000-0002-0107-0831 (O.G.P. Barsottini); 0000-0003-2305-1073 (H.A.G. Teive); 0000-0002-2602-3281 (A. Orlacchio).

https://doi.org/10.1016/j.gene.2026.150053
Received 27 October 2025; Received in revised form 6 February 2026; Accepted 11 February 2026
Available online 13 February 2026

1. Introduction

Hereditary Spastic Paraplegia (HSP) is a heterogeneous group of neurodegenerative disorders characterized by slowly progressive spasticity and weakness of the lower limbs. To date, according to Online Mendelian Inheritance in Man (OMIM), 93 subtypes (SPG1-SPG93) were described and in most of them the causative genes was identified. Furthermore, current efforts focus on the investigation of genes function and pathogenetic mechanisms (Meyyazhagan and Orlacchio, 2022; Panza et al., 2022; Martinello et al., 2023).

Many pathways were identified being affected in different forms of HSP. These include, for example, axonal trafficking, mitochondria homeostasis, lipid synthesis, autophagy, endoplasmic reticulum membrane modelling, and amino acids metabolism (Lo Giudice et al., 2014; Magini et al., 2019; Panza et al., 2019; Meyyazhagan et al., 2022; Martinello et al., 2023).

Despite high genetic heterogeneity, few genes account for most of the HSP cases (Panza et al., 2022; Blackstone, 2018). Among them, SPG4/*SPAST* (OMIM 604277), is the most frequent molecular cause for familial autosomal dominant HSP. The *SPAST* gene encodes for the protein spastin. This is a protein belonging to the ATPases Associated with diverse cellular Activities (AAA) family and it has several biological roles especially related to microtubule remodeling and membrane trafficking (Lumb et al., 2012).

HSPs can be divided clinically between pure forms, if only the sign of spastic paraplegia is present, or complicated forms, where spastic paraplegia is accompanied by other signs (Panza and Orlacchio, 2025). Most of the SPG4 patients show a pure form, but phenotypic variations have been reported (Varghaei et al., 2022).

While haploinsufficiency is a recognized pathogenic mechanism for SPG4, (Solowska et al., 2010), it has been shown that long spastin isoforms exhibit a gain of function pathogenic mechanism and may play a central part in HSP pathophysiology (Solowska et al., 2014).

In our study, we identified variants resulting in early stop codons or deletions of part of the SPG4/*SPAST* gene, leading to an impaired microtubule structure assembly (Sandate et al., 2019). Intronic variants or even variants that cause a synonymous aminoacidic change, were associated with splicing defects (Pippucci et al., 2009; Rossi et al., 2023; Benvenuto et al., 2024; Innella et al., 2024). *SPAST* is not an exception, with many findings of altered or deleted spastin protein due to such mechanism (Fonknechten et al., 2000; Meijer et al., 2002; Sauter et al., 2002; de Bot et al., 2010; Klimpe et al., 2011).

Here, we describe in detail the clinical presentation of affected members from two families of middle and high socioeconomic status: one of Brazilian and one of Japanese origin. These variants have already been reported in the literature (Wang et al., 2021; Ferese et al., 2023), but a detailed clinical description of the associated phenotype is missing. Considering the interesting, complicated phenotype associated with these variants, and particularly the intriguing report of anorexia nervosa in both families, we set out to better characterize these variants.

Furthermore, taking into consideration that the variants c.1493 + 2_1493 + 5del and the c.1245 + 5G > A has not yet been assessed by the ClinGen experts' groups, and the latter variant has been reported with conflicting classification of pathogenicity, we set out to characterize the effect of these variants. We tested the effect of these variants confirming their impact on splicing using the mini-gene assay in HEK293T cells and in SH-SY5Y, a neuronal cell line, which confirmed the results in a disease-relevant cell context.

2. Methods

2.1. Participants and clinical assessments

The study was achieved according to a protocol reviewed and accepted by the Ethics Committee of the University of Perugia, Perugia, Italy, in addition to the Azienda Ospedaliera di Perugia, Perugia, Italy, as well as local Institutional Review Boards (IRBs) of the referring institutions. The subjects' consent was obtained agreeing to the Declaration of Helsinki (https://www.wma.net/policies-post/wma-declaration-of-helsinki/). Clinical material was collected, and neurological examinations were carried out by movement disorders specialists (G.R., R.M., J.L.P., O.G.P.B., H.A.G.T., and A.O.).

2.2. Genetic analysis

Genomic DNA of all family members were extracted from peripheral blood using a commercial kit (Qiagen blood mini kit).

Due to the high genetic heterogeneity of HSP and given the increasing number of genes associated with HSP discovered each year, Whole Exome Sequencing (WES) was conducted on all affected individuals to identify potential genetic variants, with a process previously reported (Magini et al., 2019). In brief, alignment to the human reference genome (hg38), detection of sequence variants, and annotation were performed using dedicated programs (Magini et al., 2019). Finally, the identified variants were validated by direct Sanger Sequencing, and segregation analysis was confirmed in each family.

2.3. Bioinformatic prediction of the impact of the intronic variants and literature analysis

Intronic variants were evaluated in terms of impact on the splicing activity by means of bioinformatic tools. We interrogated prediction software to investigate variants pathogenicity, using different software included in the ModiDetails program (SpliceAI, MaxEntScan). Predictions were made using default settings (Table 1).

2.4. Minigene assay

Variants were predicted to affect mRNA splicing. Since a fresh sample of RNA from patients was not available, for each variant a genomic fragment bearing wild-type and mutant DNA was cloned into the exon trapping pSPL3 vector to assess the effect on splicing (Rossi et al., 2023) (see **Supplementary Table S1** for primer sequences). The plasmids were generated and verified through direct sequencing and

Table 1
Results by splicing prediction bioinformatic programs.

Program and score	Mutation c.1245 + 5G > A	Mutation c.1493 + 2–1493 + 5del
SpliceAI https://spliceailookup.broadinstitute.org/	th base variant[+] Δ score*: - Acceptor Loss0.93 - Donor Loss0.98 - Acceptor Gain0.02 - Donor Gain0.02	[+] Δ score*: - Acceptor Loss0.79 - Donor Loss1.00
MaxEntScan	[§] Wild type = 7.77 Mutant = -5.44 (variation −170.01%)	[§] Wild type = 9.46 Mutant = -0.39 (variation −104.12%)
MobiDetails https://mobidetails.chu-montpellier.fr/	MPA score[@] = 10MPA impact = High Impact	MPA score[@] = 10MPA impact = High Impact

[+]**SpliceAI** looks within a window (+/- 500 bp by default) to see how the variant affects the probability of different positions serving as splice acceptors or donors. The offsets in this column represent positions with the biggest change in probability within the window.

*Δ score range from 0 to 1 and can be interpreted as the probability that the variant affects splicing at any position within a window around it (+/- 500 bp by default).

[§] **MaxEntScan** report as indicative of altered splicing only variation > 15% as absolute value, and.

a raw score for mutant or wild type of at least 3.

Raw score [0;10], 10: high impact.

[@] Complex evaluation by **MobiDetails**.

nucleofected into HEK293T cells (Lonza). Furthermore, to verify the effect on splicing the same constructs were nucleofected in SH-SY5Y cell line to confirm the effect on splicing in a neuronal derived cell line. In both cases RNA was extracted, and cDNA was synthetized. The amplification of construct specific fragments and size analysis by agarose gel electrophoresis showed bands of different size between wild-type and mutant transfected constructs. Fragment's identity was verified by direct Sanger sequencing.

3. Results

3.1. Identification and characterization of SPAST intronic variants

Two *SPAST* intronic variants were found in six affected members of the Brazilian family identified as BRA53, and in six patients of the pedigree coded as JAP95. The variants co-segregated within the respective families and were observed exclusively in the affected individuals (Fig. 1). The BRA53 variant is in the fifth position at 5′-splice-site within intron 9 (NM_014946.4:c.1245+5G>A), while the JAP95 deletion is located at the 5′-splice-site in intron 12 (NM_014946.3: c.1245+5G>A) (**Supplementary Fig. S1**). The c.1245 + 5G position is highly conserved as it is demonstrated by a score of 8.912 in the 100 vertebrates base-wise conservation by PhiloP value and a score of 8.787 by a PhiloP conservation among 241 Zoonomia representing placental mammals (**Supplementary Fig. S2).** In the case of the c.1493 + 2_1493 + 5del variant, the first base deleted, a T, has a 6.980 score in the 100 vertebrates base-wise conservation by PhiloP value and a score of 6.305 by a PhiloP conservation among 241 Zoonomia representing placental

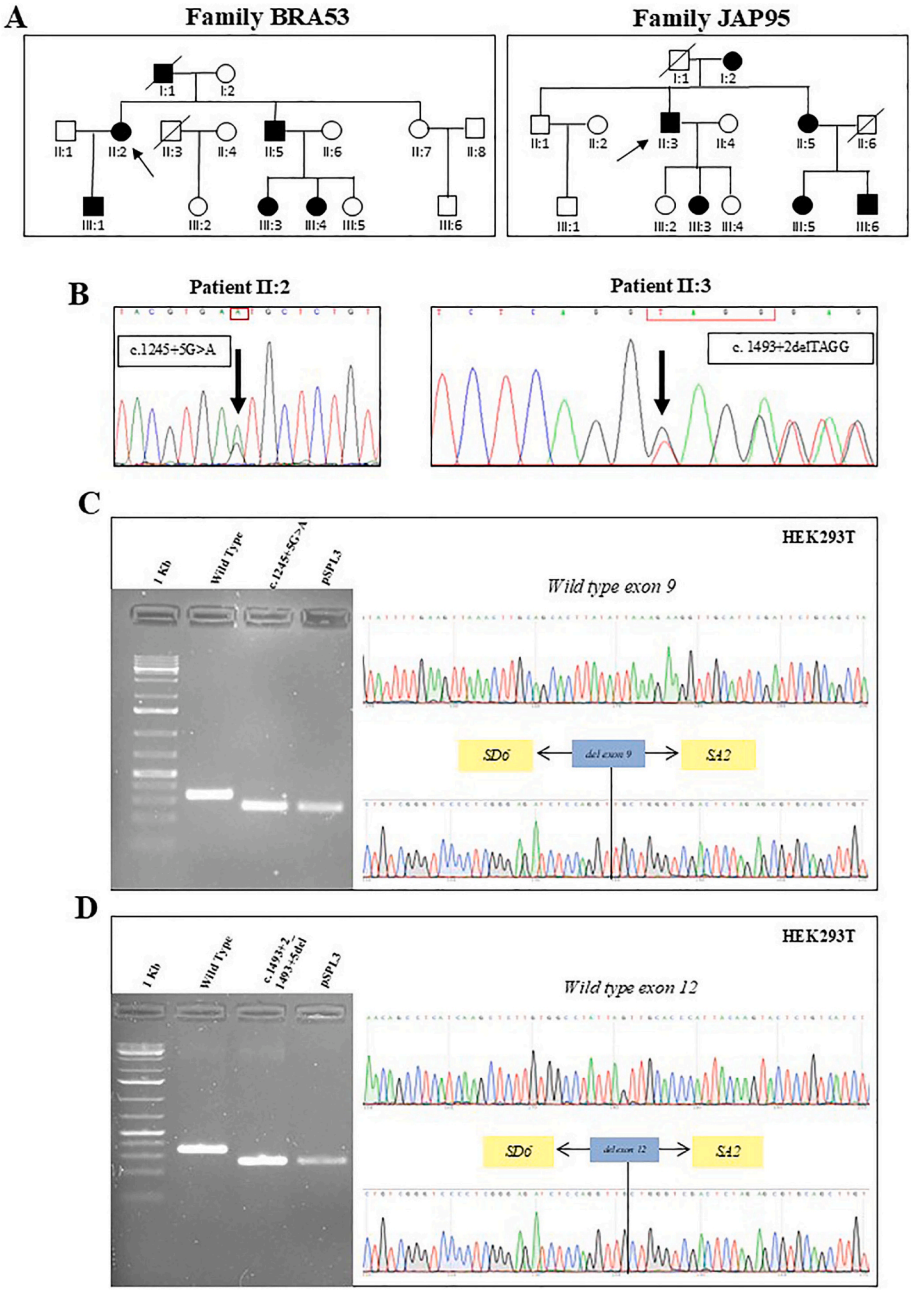

Fig. 1. Analysis of the identified variants. A) Pedigrees of the Brazilian and Japanese families analysed in this study: all family members were available for sampling, containing those deceased. B) Electropherograms showing the presence of variants c.1245 + 5G > A (family BRA53; patient II:2) and c.1493 + 2_1493 + 5del in heterozygous state (family JAP95; patient II:3). C, D) Results of minigene assays for the two variants showing in both cases an abnormal splicing in cells transfected with the constructs bearing the variants in comparison to wild-type constructs.

mammals (**Supplementary Fig. S2**). The c.1493 + 2_1493 + 5del variant was not found in the GnomAD database (V4.1.0) while the c.1493 + 2_1493 + 5del was found at a frequency of 0.000000687 in 1,454,890 control chromosomes in the same database.

3.2. The SPAST splice site variants resulted in different fragments length as studied by minigene assay

Analysis of the cDNA obtained by RNA extracted by HEK293T cells transfected with the different constructs gave a peculiar pattern in the mutants in comparison to wild-type constructs. The PCR amplification by vector specific primers (SD6 and SA2) (**Supplementary Table S1**) clearly showed a band pattern consistent with exon skipping in both the analysed cases.

For the c.1245 + 5G > A, the wild-type amplicon displayed a size of 335 base pairs (including 71 bp of exon 9), in contrast, the mutant amplicon exhibited a size of 263 bp, significantly smaller than the expected size, but consistent with the amplification of the artificial exons of the pSPL3 plasmid. This size difference is indicative of a skipping of exon 9 due to an aberrant splicing event. For the c.1493 + 2_1493 + 5del variant, the wild-type band observed was a 343 (including the 80 bp exon 12), while the mutant vector gave a band of 263 Bp, again consistent with the skipping on exon 12 and amplification only of the synthetic pSPL3 exons (Fig. 1). The direct sequencing of the fragments unequivocally confirmed the exon skipping events (**Supplementary Table S1**).

Furthermore, these experiments were repeated in a different cell line, SH-SY5Y, of neuronal origin, confirming the same results (**Supplementary Fig. S1**).

3.3. The skipping of the analysed exons alters the open reading frame of SPAST or remove a critical portion of the protein

In both cases, the intronic variants ultimately resulted in skipping of the adjacent 5′ exons (exons 9 and 12). Skipping of exon 9, caused by the c.1245 + 5G > A variant, led to an in-frame deletion removing a portion of the AAA + ATPase domain (IPR003593 InterPro), a region critical for protein function (**Supplementary Fig. S3**). In contrast, skipping of exon 12, also mapping in the same functional domain, due to the c.1493 + 2_1493 + 5del variant resulted in a frameshift, with the generation of a premature stop codon after 13 amino acids (p.Gly471Alafs*13) (**Supplementary Fig. S3**). Considering our data, following ACMG guidelines, the variant c.1245 + 5G > A is classified as “Likely Pathogenic”, while the variant c.1493 + 2_1493 + 5del can be classified as “Pathogenic”.

3.4. Clinical description

An extensive clinical description of the family members is reported in Tables 2 and 3 (family 1 and 2, respectively). In brief, patients presented with spastic paraplegia complicated by other clinical features, remarkably including anorexia nervosa. The diagnosis of anorexia nervosa was established according to the criteria defined in the *Diagnostic and Statistical Manual of Mental Disorders,* Fifth Edition, Text Revision (DSM-5-TR) (American Psychiatric Association, 2022). This peculiar feature was identified in the affected members of both families. While it is recognized that other symptoms can be associated with spastic paraparesis in SPG4 (Rattay et al., 2020), the cooccurrence of anorexia nervosa and SPG4 in these families appeared to be novel. Patients from both families also presented with skeletal abnormalities and bilateral *pes cavus*. No affected individuals exhibited signs of neuropathy, and nerve conduction studies yielded normal results.

The families exhibited an age at onset ranging from the third to the fifth decade, with a mean ± standard deviation (SD) of 46.3 ± 6.5 years in BRA53 and 45.0 ± 6.1 years in JAP95. Furthermore, analysis of age at onset across generations suggested a trend consistent with genetic anticipation. In family BRA53, the mean ± SD ages at onset were 55 years in the first generation, 47.5 ± 1.5 years in the second generation, and 37 ± 1.6 years in the third generation. In family JAP95, the corresponding values were 54 years, 50.5 ± 1.5 years, and 39.3 ± 3.1 years, respectively.

Table 2
Clinical findings of the patients of family BRA53.

	I:1	II:2	II:5	III:1	III:3	III:4
Sex	M	F	M	M	F	F
Age at onset, years[a]	55	46	49	35	39	37
Age at examination	73	61	64	51	53	49
Disease Duration	18	15	15	16	14	12
Disability stage[b]	4	3	4	3	2	2
SPRS[c]	42	32	40	29	22	25
LLH[d]	+	+	+	+	+	+
LLS[e]	+++	++	+++	++	+	+
LLW[f]	+++	++	+++	++	−	+
Babinski sign	B	U	B	U	U	U
Sphincter disturbances	+	−	+	−	−	−
Pes cavus	B	B	B	B	B	B
Anorexia nervosa	+	+	+	+	+	+
Orthostatic hypotension	+	+	+	+	+	+
Bradycardia	+	+	+	+	+	+
Halitosis	+	+	+	+	+	+
Russell's Sign	+	+	+	+	+	+
Insomnia	+	+	+	+	+	+
Lanugo	+	+	+	+	+	+
Scoliosis	+	+	+	+	+	+
Memory impairment	+	+	+	+	+	+
Behavioral/Psychiatric symptoms	D	AD	D	AD	ED	AD

M = male; F = female; U = unilateral; B = bilateral; D = Depression; AD = Anxiety disorders; ED = Emotional dysregulation; + and – indicate the presence or absence of a feature, respectively;
[a]Age at onset was calculated approximately as the time when difficulty walking first appeared in the affected individuals;
[b]Disability stages: 1, no mobility problems or slight stiffness of the legs; 2, moderate gait stiffness; 3, problems running, but able to walk alone; 4, problems walking; 5, wheelchair-bound;
[c]SPRS = Spastic Paraplegia Rating Scale;
[d]LLH, lower limbs hyperreflexia;
[e]LLS, lower limbs spasticity;
[f]LLW, lower limbs weakness.

4. Discussion

We reported the characterization of two splice site donor variants in the SPG4/*SPAST* gene at the, c.1245 + 5G and c.1493 + 2_1493 + 5del in two families, one Brazilian and one Japanese.

The evidence collected strongly suggested that the variants are associated with the disease. In fact, a) the c.1493 + 2_1493 + 5del variant is not found in GnomAD (V4.1.0) while the c.1493 + 2_1493 + 5del was found at a frequency of 0.000000687 in 1,454,890 control chromosomes in the same database, confirming a low frequency of these variants; b) segregate in the families in the affected members only; c) the variant bases have a high PhiloP conservation; d) bioinformatic tools predict a pathogenetic effect; e) minigene assay performed in two different cell lines, show alteration of the splicing.

While patient’s cells fibroblasts are a reliable material to investigate even neurological conditions (Evangelisti et al., 2024), in our case, individual’s biological material was not available. Thus, we investigated and confirmed the effect of this variant in HEK293T cells by minigene assay and expanded the molecular analysis by transfecting our constructs in a neuronal cell line, SH-SY5Y, confirming the same results.

In our work, we described the clinical presentation of the affected members of the two families. Interestingly, in both families anorexia nervosa was present in all patients with spastic paraplegia. This observation is particularly interesting since no reports described this association with SPG4 before. While it is possible that this association is not strictly correlated with *SPAST* pathophysiology, nevertheless, it is

Table 3
Clinical findings of the patients of family JAP95.

	I:2	II:3	II:5	III:3	III:5	III:6
Sex	F	M	F	F	F	M
Age at onset, years[a]	51	52	49	41	42	35
Age at examination	76	63	63	57	58	47
Disease Duration	25	11	14	16	16	12
Disability stage[b]	4	2	3	3	3	2
SPRS[c]	40	21	37	35	32	18
LLH[d]	+	+	+	+	+	+
LLS[e]	+++	+	++	++	++	+
LLW[f]	+++	+	++	++	++	+
Babinski sign	B	U	U	U	U	U
Sphincter disturbances	+	–	–	–	–	–
Pes cavus	B	B	B	B	B	B
Anorexia nervosa	+	+	+	+	+	+
Orthostatic hypotension	+	+	+	+	+	+
Bradycardia	+	+	+	+	+	+
Halitosis	+	+	+	+	+	+
Russell's Sign	+	+	+	+	+	+
Insomnia	+	+	+	+	+	+
Lanugo	+	+	+	+	+	+
Scoliosis	+	+	+	+	+	+
Memory impairment	+	+	+	+	+	+
Behavioral/Psychiatric symptoms	D	ED	AD	AD	ED	AD

M = male; F = female; U = unilateral; B = bilateral; D = Depression; AD = Anxiety disorders; ED = Emotional dysregulation; + and – indicate the presence or absence of a feature, respectively;
[a]Age at onset was calculated approximately as the time when difficulty walking first appeared in the affected individuals;
[b]Disability stages: 1, no mobility problems or slight stiffness of the legs; 2, moderate gait stiffness; 3, problems running, but able to walk alone; 4, problems walking; 5, wheelchair-bound;
[c]SPRS = Spastic Paraplegia Rating Scale;
[d]LLH, lower limbs hyperreflexia;
[e]LLS, lower limbs spasticity;
[f]LLW, lower limbs weakness.

plausible that the paraplegia symptoms have been worsened by a debilitated state in anorexic patients. Indeed, anorexia nervosa is associated with profound impairments in both muscular and nervous system function because of malnutrition and insufficient protein and caloric intake. Compared with other organs and tissues, the nervous system is particularly vulnerable to damage, making it plausible that this condition, superimposed on the presence of a causative *SPAST* mutation for HSP, could only further exacerbate the clinical presentation. Moreover, the families involved had middle to high socioeconomic status, making it unlikely that environmental or psychosocial factors alone could account for the anorexia nervosa phenotype independently of *SPAST* mutations.

Furthermore, a recent line of research proposed a link between spastin and lipid droplet dynamics and between spastin and metabolism. It was shown that spastin deletion affects metabolic properties and organelle characteristics in different model organisms, confirmed in human cells. These studies showed that spastin regulates lipid droplet formation and dispersion through the reorganization of the endoplasmic reticulum along microtubules (Arribat et al., 2020). This is a pathway where spastin and other HSP-related proteins synchronize the shaping of the endoplasmic reticulum and microtubules stability to determine the dispersion of lipid droplets in the cell, converging in comparable clinical features in case of alteration. In this view, spastin might modulate appetite or energy balance, linking anorexia nervosa to this form of HSP (Arribat et al., 2020).

In the literature, these variants were described (Wang et al., 2021; Ferese et al., 2023), but, at the best of our knowledge, a detailed clinical description of the patients bearing these variants has not been reported, leaving the riddle of the association of anorexia nervosa and spastic paraparesis unsolved.

The mean age at onset in each family was consistent with the typical clinical presentation of SPG4 (Solowska and Baas, 2015). Although genetic anticipation has been reported in several SPG4 families (Orlacchio et al., 2002; Orlacchio et al., 2004; Kawarai et al., 2017; Kamada et al., 2018), its occurrence remains a matter of debate (Munhoz, 2022). In the present SPG4 pedigrees (BRA53 and JAP95), we observed a general trend toward earlier onset across successive generations. Should this pattern be confirmed in additional families with similar clinical profiles, several mechanisms could be considered. One hypothesis is that genetic or environmental modifiers might influence disease onset in certain individuals. However, the most likely explanation is that the apparent decrease in onset age reflects a diagnostic artifact arising from greater clinical awareness and improved detection in younger generations.

Intra and inter-familial phenotypic variations are well known features associated with SPG4/*SPAST* pathogenic variants (Santorelli et al., 2000; Orlacchio et al., 2005). Our observation contributes to add to the variable clinical presentation of SPG4 patients.

The variants cause an in-frame skip of exon 9 affecting the AAA domain, and an out-of-frame skipping of exon 12, with the formation of a premature stop codon affecting the AAA ATPase domain. Indeed, different pathogenetic variants causing alteration at the level of *SPAST* exon 9 have been reported (Magariello et al., 2010); at the same time, bigger deletions involving exon 10 to 12 have been already described (Beetz et al., 2006; Depienne et al., 2007), supporting a mechanistic causal effect of the alteration of the AAA domain.

Furthermore, mutant *SPAST* transcripts escaping from nonsense-mediated decay pathway might be translated into shorter proteins, affecting the functional domains, MTBD and AAA (Fig. 1). Even though we did not conduct SPG4/*SPAST* protein analysis due to technical and material limitations, it is well known that truncated SPG4 proteins may be biologically ineffective, even though they are produced (Beetz et al., 2006; Depienne et al., 2007).

It has been suggested that the decreased microtubule-severing activity by pathogenetic variants in *SPAST* would cause different events including the production of longer and less dynamic microtubules, resulting in a disorganized microtubule structure (McNally and Roll-Mecak, 2018), and an increased number of dynamic plus-ends of microtubules, event that would alter endosomal tubulation and fission events (Solowska and Baas, 2015).

The consistent presence of atypical clinical features across patients with HSP, including anorexia nervosa, orthostatic hypotension, bradycardia, halitosis, Russell's sign, insomnia, lanugo, scoliosis, memory impairment, and affective symptoms such as depression, anxiety disorders, and emotional dysregulation, suggests a possible contribution of additional genetic factors associated with anorexia nervosa. However, some of these features, particularly halitosis and insomnia, may represent secondary manifestations of anorexia nervosa and its metabolic and nutritional consequences rather than independent clinical features. Further investigation will be necessary to explore the potential contribution of modifying variants across the genome, for instance by integrating Whole Genome Sequencing data.

5. Conclusions

In summary, we provided evidence of additional allelic heterogeneity in SPG4 and observed phenotypic variations in the two families studied. We also provide evidence for splicing alterations, and we describe further genotype-phenotype correlation evidence. Additionally, functional analysis of mutant spastin, and proteomic analysis are necessary to enhance our understanding of SPG4 pathogenesis.

CRediT authorship contribution statement

Cecilia Evangelisti: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Emanuele Panza:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision,

Investigation, Formal analysis, Data curation. **Mario Stasi:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Arun Meyyazhagan:** Writing – review & editing, Formal analysis, Data curation. **Haripriya Kuchi Bhotla:** Writing – review & editing, Formal analysis, Data curation. **Preethi Basavaraju:** Writing – review & editing, Formal analysis, Data curation. **Gustavo Ribas:** Writing – review & editing, Resources. **Natalia Fava:** Writing – review & editing, Data curation. **Sherin Ramadan:** Writing – review & editing, Formal analysis. **Ryosuke Miyamoto:** Writing – review & editing, Resources, Investigation. **José Luiz Pedroso:** Writing – review & editing, Resources, Investigation. **Orlando Graziani Povoas Barsottini:** Writing – review & editing, Resources, Investigation. **Hélio Afonso Ghizoni Teive:** Writing – review & editing, Resources, Investigation. **Antonio Orlacchio:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Funding

This work was supported by the Department of Medicine and Surgery of the University of Perugia (*Fondo Ricerca di Base*, Grants n. DSCH_BASE19_ORLACCHIO and RICERCABASE_2020_ORLACCHIO to A.O.), and the University of Perugia (*Progetto di Ricerca di Ateneo*, Grant n. RICERCA_ATENEO_ALIMENTAZIONE to A.O.).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank the patients and their family members for taking part in this study. We are extremely grateful to the Genetic Bank, Department of Medicine and Surgery, University of Perugia, Perugia, Italy, for the service provided and we show gratitude to the Associazione Italiana Vivere la Paraparesi Spastica Ereditaria (A.I.Vi.P.S. onlus) for the great support.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2026.150053.

Data availability

Data will be made available on request.

The data supporting the findings of this study are available within the article and its supplementary materials. Further information can be obtained from the corresponding author upon request.

References

American Psychiatric Association, 2022. Diagnostic and statistical manual of mental disorders (5th Edition-Text Revision). Arlington: American Psychiatric Association.

Arribat, Y., Grepper, D., Lagarrigue, S., Qi, T., Cohen, S., Amati, F., 2020. Spastin mutations impair coordination between lipid droplet dispersion and reticulum. PLoS Genet. 16, e1008665.

Beetz, C., Nygren, A.O., Schickel, J., Auer-Grumbach, M., Bürk, K., Heide, G., Kassubek, J., Klimpe, S., Klopstock, T., Kreuz, F., Otto, S., Schüle, R., Schöls, L., Sperfeld, A.D., Witte, O.W., Deufel, T., 2006. High frequency of partial *SPAST* deletions in autosomal dominant hereditary spastic paraplegia. Neurology 67, 1926–1930.

Benvenuto, M., Cesarini, S., Severi, G., Ambrosini, E., Russo, A., Seri, M., Palumbo, P., Palumbo, O., Castori, M., Panza, E., Carella, M., 2024. Phenotypic description of a patient with ODLURO syndrome and functional characterization of the pathogenetic role of a synonymous variant c.186G>A. Genes (Basel) 15, 430.

Depienne, C., Fedirko, E., Forlani, S., Cazeneuve, C., Ribaï, P., Feki, I., Tallaksen, C., Nguyen, K., Stankoff, B., Ruberg, M., Stevanin, G., Durr, A., Brice, A., 2007. Exon deletions of SPG4 are a frequent cause of hereditary spastic paraplegia. J. Med. Genet. 44, 281–284.

Evangelisti, C., Ramadan, S., Orlacchio, A., Panza, E., 2024. Experimental cell models for investigating neurodegenerative diseases. Int. J. Mol. Sci. 25, 9747.

Ferese, R., Scala, S., Suppa, A., Campopiano, R., Asci, F., Zampogna, A., Chiaravalloti, M. A., Griguoli, A., Storto, M., Pardo, A.D., Giardina, E., Zampatti, S., Fornai, F., Novelli, G., Fanelli, M., Zecca, C., Logroscino, G., Centonze, D., Gambardella, S., 2023. Cohort analysis of novel *SPAST* variants in SPG4 patients and implementation of *in vitro* and *in vivo* studies to identify the pathogenic mechanism caused by splicing mutations. Front. Neurol. 14, 1296924.

Fonknechten, N., Mavel, D., Byrne, P., Davoine, C.S., Cruaud, C., Bönsch, D., Samson, D., Coutinho, P., Hutchinson, M., McMonagle, P., Burgunder, J.M., Tartaglione, A., Heinzlef, O., Feki, I., Deufel, T., Parfrey, N., Brice, A., Fontaine, B., Prud'homme, J. F., Weissenbach, J., Dürr, A., Hazan, J., 2000. Spectrum of SPG4 mutations in autosomal dominant spastic paraplegia. Hum. Mol. Genet. 9, 637–644.

Innella, G., Coccia, E., Cristalli, C.P., Zacchi, E., Calabrese, S., Bacchi, I., Palombo, F., Taormina, S., Evangelisti, C., Lanzoni, G., Carelli, V., Diquigiovanni, C., Ferrari, S., Panza, E., Rossi, C., Vaisfeld, A., Bonora, E., Turchetti, D., 2024. Effectiveness and impact of transcript analysis in clinical genetics daily practice. Clin. Genet. 107, 570–575.

Kamada, M., Kawarai, T., Miyamoto, R., Kawakita, R., Tojima, Y., Montecchiani, C., D'Onofrio, L., Caltagirone, C., Orlacchio, A., Kaji, R., 2018. Spastic paraplegia type 31: a novel *REEP1* splice site donor variant and expansi,on of the phenotype variability. Parkinsonism Relat. Disord. 46, 79–83.

Kawarai, T., Montecchiani, C., Miyamoto, R., Gaudiello, F., Caltagirone, C., Izumi, Y., Kaji, R., Orlacchio, A., 2017. Spastic paraplegia type 4: a novel *SPAST* splice site donor mutation and expansion of the phenotype variability. J. Neurol. Sci. 380, 92–97.

Klimpe, S., Zibat, A., Zechner, U., Wellek, B., Shoukier, M., Sauter, S.M., Pantakani, D.V., Mannan, A.U., 2011. Evaluating the effect of spastin splice mutations by quantitative allele-specific expression assay. Eur. J. Neurol. 18, 99–105.

Lo Giudice, T., Lombardi, F., Santorelli, F.M., Kawarai, T., Orlacchio, A., 2014. Hereditary spastic paraplegia: clinical-genetic characteristics and evolving molecular mechanisms. Exp. Neurol. 261, 518–539.

Lumb, J.H., Connell, J.W., Allison, R., Reid, E., 2012. The AAA ATPase spastin links microtubule severing to membrane modelling. BBA 1823, 192–197.

Magariello, A., Muglia, M., Patitucci, A., Ungaro, C., Mazzei, R., Gabriele, A.L., Sprovieri, T., Citrigno, L., Conforti, F.L., Liguori, M., Gambardella, A., Bono, F., Piccoli, T., Patti, F., Zappia, M., Mancuso, M., Iemolo, F., Quattrone, A., 2010. Mutation analysis of the SPG4 gene in italian patients with pure and complicated forms of spastic paraplegia. J. Neurol. Sci. 288, 96–100.

Magini, P., Marco-Marin, C., Escamilla-Honrubia, J.M., Martinelli, D., Dionisi-Vici, C., Faravelli, F., Forzano, F., Seri, M., Rubio, V., Panza, E., 2019. P5CS expression study in a new family with *ALDH18A1*-associated hereditary spastic paraplegia SPG9. Ann. Clin. Transl. Neurol. 6, 1533–1540.

Martinello, C., Panza, E., Orlacchio, A., 2023. Hereditary spastic paraplegias proteome: common pathways and pathogenetic mechanisms. Expert Rev. Proteomics 20, 171–188.

McNally, F.J., Roll-Mecak, A., 2018. Microtubule-severing enzymes: from cellular functions to molecular mechanism. J. Cell Biol. 217, 4057–4069.

Meijer, I.A., Hand, C.K., Cossette, P., Figlewicz, D.A., Rouleau, G.A., 2002. Spectrum of SPG4 mutations in a large collection of north American families with hereditary spastic paraplegia. Arch. Neurol. 59, 281–286.

Meyyazhagan, A., Kuchi Bhotla, H., Pappuswamy, M., Orlacchio, A., 2022. The puzzle of hereditary spastic paraplegia: from epidemiology to treatment. Int. J. Mol. Sci. 23, 7665.

Meyyazhagan, A., Orlacchio, A., 2022. Hereditary spastic paraplegia: an update. Int. J. Mol. Sci. 23, 1697.

Munhoz, R.P., 2022. Anticipation in genetic diseases: moving beyond nucleotide repeat disorders. Can. J. Neurol. Sci. 5, 627–628.

Orlacchio, A., Kawarai, T., Gaudiello, F., Totaro, A., Schillaci, O., Stefani, A., Floris, R., St George-Hyslop, P.H., Sorbi, S., Bernardi, G., 2005. Clinical and genetic study of a large SPG4 italian family. Mov. Disord. 20, 1055–1059.

Orlacchio, A., Kawarai, T., Rogaeva, E., Song, Y.Q., Paterson, A.D., Bernardi, G., St George-Hyslop, P.H., 2002. Clinical and genetic study of a large italian family linked to SPG12 locus. Neurology 59, 1395–1401.

Orlacchio, A., Kawarai, T., Totaro, A., Errico, A., St George-Hyslop, P.H., Rugarli, E.I., Bernardi, G., 2004. Hereditary spastic paraplegia: clinical genetic study of 15 families. Arch. Neurol. 61, 849–855.

Panza, E., Martinelli, D., Magini, P., Dionisi Vici, C., Seri, M., 2019. Hereditary spastic paraplegia is a common phenotypic finding in ARG1 deficiency, P5CS deficiency and HHH syndrome: three inborn errors of metabolism caused by alteration of an interconnected pathway of glutamate and urea cycle metabolism. Front. Neurol. 10, 131.

Panza, E., Meyyazhagan, A., Orlacchio, A., 2022. Hereditary spastic paraplegia: genetic heterogeneity and common pathways. Exp. Neurol. 357, 114203.

Panza, E., Orlacchio, A., 2025. Biomarkers in Hereditary Spastic Paraplegias. Int. J. Mol. Sci. 26, 1950.

Pippucci, T., Panza, E., Pompilii, E., Donadio, V., Borreca, A., Babalini, C., Patrono, C., Zuntini, R., Kawarai, T., Bernardi, G., Liguori, R., Romeo, G., Montagna, P., Orlacchio, A., Seri, M., 2009. Autosomal recessive hereditary spastic paraplegia with thin *corpus callosum*: a novel mutation in the SPG11 gene and further evidence for genetic heterogeneity. Eur. J. Neurol. 16, 121–126.

Rattay, T.W., Boldt, A., Völker, M., Wiethoff, S., Hengel, H., Schüle, R., Schöls, L., 2020. Non-motor symptoms are relevant and possibly treatable in hereditary spastic paraplegia type 4 (SPG4). J. Neurol. 267, 369–379.

Rossi, C., Ramadan, S., Evangelisti, C., Ferrari, S., Accadia, M., Toydemir, R.M., Panza, E., 2023. Case report: Functional characterization of a novel *CHD7* intronic variant in patients with CHARGE syndrome. Front. Genet. 14, 1082100.

Sandate, C.R., Szyk, A., Zehr, E.A., Lander, G.C., Roll-Mecak, A., 2019. An allosteric network in spastin couples multiple activities required for microtubule severing. Nat. Struct. Mol. Biol. 26, 671–678.

Santorelli, F.M., Patrono, C., Fortini, D., Tessa, A., Comanducci, G., Bertini, E., Pierallini, A., Amabile, G.A., Casali, C., 2000. Intrafamilial variability in hereditary spastic paraplegia associated with an SPG4 gene mutation. Neurology 55, 702–705.

Sauter, S., Miterski, B., Klimpe, S., Bönsch, D., Schöls, L., Visbeck, A., Papke, T., Hopf, H. C., Engel, W., Deufel, T., Epplen, J.T., Neesen, J., 2002. Mutation analysis of the spastin gene (SPG4) in patients in Germany with autosomal dominant hereditary spastic paraplegia. Hum. Mutat. 20, 127–132.

Solowska, J.M., Garbern, J.Y., Baas, P.W., 2010. Evaluation of loss of function as an explanation for SPG4-based hereditary spastic paraplegia. Hum. Mol. Genet. 19, 2767–2779.

Solowska, J.M., D 'Rozario, M., Jean, D.C., Davidson, M.W., Marenda, D.R., Baas, P.W., 2014. Pathogenic mutation of spastin has gain-of-function effects on microtubule dynamics. J. Neurosci. 34, 1856–67.

Solowska, J.M., Baas, P.W., 2015. Hereditary spastic paraplegia SPG4: what is known and not known about the disease. Brain 138, 2471–2484.

Varghaei, P., Estiar, M.A., Ashtiani, S., Veyron, S., Mufti, K., Leveille, E., Yu, E., Spiegelman, D., Rioux, M.F., Yoon, G., Tarnopolsky, M., Boycott, K.M., Dupre, N., Suchowersky, O., Trempe, J.F., Rouleau, G.A., Gan-Or, Z., 2022. Genetic, structural and clinical analysis of spastic paraplegia 4. Parkinsonism Relat. Disord. 98, 62–69.

Wang, C., Zhang, Y.J., Xu, C.H., Li, D., Liu, Z.J., Wu, Y., 2021. The investigation of genetic and clinical features in patients with hereditary spastic paraplegia in central-Southern China. Mol. Genet. Genomic Med. 9, e1627.