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Early-Onset and Severe Complex Hereditary Spastic Paraplegia Caused by *De Novo* Variants in *SPAST*

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Abstract

Background: Familial HSP-*SPAST* (SPG4) typically presents with a pure hereditary spastic paraplegia (HSP) phenotype.

Objectives: To delineate the genotypic and phenotypic spectrum of children with *de novo* HSP-*SPAST*.

Methods: A systematic cross-sectional analysis of clinical and molecular features.

Results: We report the clinical and molecular spectrum of 40 patients with heterozygous pathogenic *de novo* variants in *SPAST* (age range: 2.2–27.7 years). We identified 19 unique variants (16/40 carried the same recurrent variant, p.Arg499His). Symptom onset was in early childhood (median: 11.0 months, IQR:6.0) with significant motor and speech delay, followed by progressive ascending spasticity, dystonia, neurogenic bladder dysfunction, gastrointestinal dysmotility, and epilepsy. The mean Spastic Paraplegia Rating Scale score was 32.8±9.7 (SD).

Conclusions: These results confirm that *de novo* variants in *SPAST* lead to a severe and complex form of HSP that differs from classic familial pure HSP-*SPAST*. Clinicians should be aware of this syndrome in the differential diagnosis for cerebral palsy.

INTRODUCTION

The hereditary spastic paraplegias (HSPs) are a heterogeneous group of genetic disorders with common features of progressive spasticity and weakness of the lower limbs[1]. In pure HSP, lower extremity spasticity, weakness and neurogenic bladder dysfunction dominate the clinical picture. Complex forms may additionally present with varying degrees of upper extremity spasticity, intellectual disability, extrapyramidal movement disorders, cerebellar dysfunction, epilepsy, peripheral neuropathy, and structural brain anomalies.

HSP-*SPAST*[2, 3] (SPG4) is caused by pathogenic variants in the *SPAST* gene, which encodes the microtubule-severing ATPase spastin[4, 5]. HSP-*SPAST* is the most common form of autosomal-dominant HSP and accounts for ~30–80% of cases[6–9]. Large cohort studies show that approximately 75% of HSP-*SPAST* cases are inherited, with substantial intra- and interfamilial variability in the age at onset and severity of symptoms[10, 11]. Rarely, individuals with confirmed *de novo* variants have also been identified, but their clinical phenotype is not completely understood. In a recent report, Schieving *et al.* identified 14 previously published *de novo* HSP-*SPAST* cases and further analyzed their own cohort of 13 patients[12]. This analysis showed that six affected individuals had pure HSP, whereas the other seven showed a complex phenotype of early-onset HSP with a motor disorder as well as progressive cognitive dysfunction, loss of verbal skills, and bulbar dysfunction.

This phenotype is notable because individuals with familial HSP-*SPAST*, who, in many cases, carry the same *SPAST* variants as *de novo* cases, do not present with such severe phenotypes.

Here, we systemically characterize the clinical and molecular spectrum of 40 children and young adults with confirmed *de novo* variants in *SPAST*, the largest cohort assembled to date. In a cross-sectional analysis, we confirm that most *de novo* HSP-*SPAST* patients present with an early-onset severe, complex syndrome with progressive spasticity of the limbs, extrapyramidal movement disorders, neurogenic bladder dysfunction, gastrointestinal dysmotility, intellectual disability, and epilepsy. We further employ standardized rating scales to assess disease severity and model the longitudinal progression of motor impairment. Taken together, our data expand the phenotype of HSP-*SPAST* by delineating the distinct core clinical symptoms and disease progression of *de novo* HSP-*SPAST*.

SUBJECTS AND METHODS

Participants

This study was approved by the Institutional Review Board at Boston Children's Hospital (IRB-A00033016-6). Patients were recruited from the Registry and Natural History Study for Early Onset Hereditary Spastic Paraplegia (NCT04712812), the Treat-HSP network (NCT03981276), the Undiagnosed Diseases Program at the NIH, and Scottish Rite Hospital. All cases were confirmed to carry pathogenic or likely-pathogenic (based on ACMG criteria) *de novo* variants in *SPAST* by sequencing the affected individual and both parents.

Methods

Clinical and demographic information was collected using a standardized questionnaire[13], the Spastic Paraplegia Rating Scale (SPRS)[14] and SPATAX Disability Score[9]. Demographic data are summarized using frequency counts and percentages for categorical variables and with mean and standard deviation (SD) or median and interquartile range (IQR) for continuous variables. For data presented in Figures 2F,H,I, linear regression analysis (R_{adj}^2) and Spearman's rank correlation coefficient (r) were used. For data shown in Figure 2D, a linear mixed-effects regression model (R_m^2, variance explained by fixed effects only; R_c^2, variance explained by both fixed and random effects) was calculated[15, 16].

RESULTS

Figure 1 summarizes the demographic and molecular data of 40 individuals in this cohort. Core clinical features are summarized in Table 1. Both male and female individuals were present in the cohort (24:16, Figure 1A). Although the median age at onset of symptoms was 0.9 (IQR:0.5) years, the median age at genetic diagnosis was 3.8 years (IQR:3.9, range 1.7–16.3 years), indicating a significant diagnostic delay ($p<0.0001$, Mann-Whitney U test, Figure 1B). The median age at last follow up was 9.6 (IQR:8.4) years.

All *SPAST* (NM_014946.4) variants, except one, were missense variants, and 40% of the cohort (16/40) carried the recurrent c.1496G>A (p.Arg499His) variant (Figure 1C, Supplementary Table 1). The distribution of disease-associated variants along the linear

structure of spastin is depicted in Figure 1C. All missense variants clustered in the AAA domain, containing the functionally important Walker A and Walker B motifs. *In silico* prediction using CADD PHRED scores suggested deleteriousness (CADD PHRED >20) for all missense variants in this cohort. Interestingly, modeling of CADD PHRED scores for all possible missense variants demonstrated particularly low tolerance to genomic variation in the first half of the C-terminal domain. The only truncating variant in our cohort (p.His289ProfsTer2) resided within the microtubule binding domain (MTBD).

The majority of patients did not have ante- or perinatal complications (37/40; one patient was born preterm at 31-weeks gestation, and two pregnancies were complicated by intra-uterine growth restriction and pre-eclampsia, respectively), and there was no increased prevalence of neurological disorders in a three-generation family. All patients presented with motor delay (40/40, Figure 1D), with sitting achieved at a median age of 8.0 months (IQR:4.0), crawling at 12.0 months (IQR:4.0), and assisted walking at 19.5 months (IQR:6.0). Unassisted walking was achieved at 20.5 months (IQR:6.0), but only 21% (8/39) ever achieved this milestone.

On examination, all patients showed lower extremity weakness and spasticity with pyramidal signs, with about half of the cohort showing upper extremity involvement as well (46%, Table 1). This was frequently accompanied by fixed joint contractures (77%), most often at the ankles. Axial hypotonia was common and clinically significant (75%). A subset of patients showed extrapyramidal movement disorders, most commonly focal or segmental dystonia (28%).

The vast majority of patients had intellectual disability (89%), significant speech delay (87%) and a subset remained completely non-verbal (21%). While formal neuropsychological testing was not consistently available, the degree of intellectual disability was rated mild (5/12, 42%) or moderate to severe (7/12, 58%). In many cases there was a pattern of early motor regression (64%, Table 1). Furthermore, about a quarter of patients had epilepsy (26%), including focal-onset epilepsy (2/10, 20%), generalized-onset (4/10, 40%) or epilepsy with both focal and generalized seizures (2/10, 20%). The majority had medically-refractory seizures (6/10, 60%), necessitating treatment with 2 or more appropriately dosed anti-seizure medications. Speech and swallowing dysfunction were present in approximately half of individuals (54% with dysarthria, 46% with dysphagia). Neurogenic bladder dysfunction and gastrointestinal dysmotility were common and often had a substantial impact on quality of life. Gastrointestinal symptoms included gastroesophageal reflux disease (12/39, 31%), sialorrhea (8/36, 22%), significant constipation (defined as regular need for one or more laxatives, 17/39, 44%) and bowel incontinence (18/37, 49%). Cerebellar signs were relatively infrequent (6/39, 15%).

A few patients showed exceptionally severe phenotypes: For example, a 4-year-old male patient presented with very limited motor development (attained unsupported sitting at 10 months, but never stood, crawled, or walked), absent speech, bilateral lower extremity spasticity with contractures, and dystonia. Similarly, a 16-year-old female patient developed spasticity in infancy associated with poor motor development (never sat unsupported, crawled, or walked) and contractures, segmental dystonia, generalized epilepsy, moderate

intellectual disability, and speech regression (at best, spoke a few words but is now non-verbal).

Brain and spine MR imaging was available in 38 and 20 cases, respectively, and was normal, with a few exceptions: In four cases note was made of non-specific T2 hyperintensities in the periventricular white matter; in three individuals there was mild thinning of the anterior corpus callosum.

To quantify the degree of motor dysfunction in this cohort, we used the SPRS score, 4-Stage Mobility score, and SPATAX disability stages. About three-quarters of individuals became full time wheelchair users (Figure 1E), at a median age of 36 months (IQR:43.8). Motor disability, as measured by the SPRS score, increased with age, a surrogate for disease duration (*Figure 2C*; R_{adj}^2=0.27, p<0.01, r=0.66, p<0.0001). The mean SPRS score in our cohort was 32.8±9.7 (SD), which is significantly higher than the average SPRS score of 18.2±12.7 (two-tailed p<0.001, Welch's t-test) in large, likely familial HSP-*SPAST* cohorts published previously[17], despite the older age in that cohort (mean age: 32.5±17.4 (SD) years). The SPATAX score similarly increased with age (Figure 1G, R_m^2=0.64, R_c^2=0.64, p<0.0001), with most individuals needing substantial support with ambulation (score of 5 or 6) after approximately 3 years of age. Across our cohort, the average SPATAX score was 5.4±1.0 (SD), which is significantly higher than those in a largely familial cohort of HSP-*SPAST* patients, who averaged a score of 2.95±1.6 (two-tailed p<0.0001, Welch's t-test)[10].

Interestingly, the subset of individuals with the p.Arg499His variant in our cohort, all presented with severe disease including early wheelchair-dependence (100%) and frequent upper limb involvement (11/16 (69%) vs. 7/24 (29%) in patients with other variants). The difference in mean SPRS scores did not reach statistical significance (36.0 vs. 30.4, p=0.12, unpaired t-test), likely because of the limited cohort size and age difference (median age: 6.6 vs. 10.0 years).

We found that health-related quality of life, assessed using the Caregiver Priorities & Child Health Index of Life with Disabilities (CPCHILD)[18, 19], showed an inverse correlation with age and motor disability (Figure 1H&I, r=−0.63, p=0.01 and 0.70, p<0.01 respectively), indicating an increasing burden with disease progression. The CPCHILD questionnaire identified several areas of high priority for caregivers and families with *de novo* HSP-*SPAST* (Figure 1J).

DISCUSSION

Our study confirms that individuals with *de novo* variants in *SPAST* present with an early-onset and severe complex motor disorder associated with progressive motor disability, extrapyramidal movement disorders, intellectual disability, and epilepsy. This phenotype is notably different from the pure HSP phenotype typical of familial HSP-*SPAST*. Using several rating scales, we demonstrate that motor disability is more severe than the typical presentation of familial HSP-*SPAST*.

Our study has limitations: 1) Patient recruitment, though representing multiple centers in North America and Europe, occurred at tertiary care centers only, possibly introducing a bias towards more severely affected cases; 2) Although segregation analyses, i.e. absence of *SPAST* variants in the parents and no family history of HSP, likely confirm the *de novo* status in our cohort, germline mosaicism or mistaken paternity cannot be excluded; 3) While the cross-sectional design allows for a delineation of core clinical features and an approximation of morbidity across the age spectrum, longitudinal natural history data are needed to define disease progression and understand areas of greatest clinical concern.

De novo variants are known to increase with paternal age[20], including in genes associated with intellectual disability or epilepsy[21]. We found that the median paternal age at birth of an affected child in our cohort was 33.5 years (IQR: 6.9), which could possibly convey an increased risk for *de novo* variants. Investigations in larger cohorts, and comparisons to the median paternal age in familial cases of HSP-*SPAST*, could test this hypothesis in the future.

Severe phenotypes are thought to be enriched in neurological disorders caused by *de novo* variants because of both selection through reduced reproductive fitness, and intrinsic properties of the respective genes[22]. One of several molecular mechanisms may be contributing to the more severe phenotype associated with *de novo SPAST* variants described herein. For example, in cohorts of individuals with a peripheral neuropathy phenotype, it has been shown that the severity and age of onset of the neuropathy (which is driven by a highly penetrant, ultrarare or *de novo* variant) may be modified by additional missense variants that alone are not penetrant for disease[23, 24]. Another possibility is that non-coding variants may influence disease penetrance and expression, as has been shown for example in *TBX6*-associated congenital scoliosis[25] and some forms of lethal lung developmental disorders[26]. These molecular mechanisms warrant further exploration in the context of *de novo SPAST* variants.

Notably, out of 40 individuals in our cohort, all but one carry missense variants with 19 harboring the recurrent Arg499His variant. Prior studies have linked missense variants, including p.Arg499His, to an earlier age of onset[11, 27, 28] but these studies did not specify *de novo* cases versus familial cases. Our study raised the possibility that a subset of severely affected individuals with p.Arg499His and other missense mutations in prior studies could potentially have been *de novo* cases. It has been hypothesized that Arg499His may lead to a younger age of onset because of its effect on the SPAST protein, including its hydrophobicity and ability to cleave microtubules[28]. This remains to be tested experimentally.

Further studies are also necessary to explore additional genotype-phenotype correlations in both familial and *de novo* HSP-*SPAST*. In addition, the early disease onset, prominent developmental delay, speech difficulties, bowel/bladder symptoms, extrapyramidal movements, and epilepsy in *de novo* HSP-*SPAST* point to: 1) a neurodevelopmental impact of the variants; and 2) more widespread involvement of CNS areas beyond dysfunction of the pyramidal system.

Of clinical importance, the constellation of motor delay, spasticity, extrapyramidal symptoms, seizures, and intellectual disability in *de novo* HSP-*SPAST* can resemble the clinical presentation of cerebral palsy (CP), and many patients in our cohort received this diagnosis before evaluation in a subspeciality neurology or movement disorders clinic. This emphasizes that HSP should be part of the differential diagnosis for any child with spastic paraparesis[29].

In summary, our results confirm that *de novo* variants in *SPAST* lead to a childhood-onset and severe form of complex HSP that differs from classic familial HSP-*SPAST*. Further studies are needed to investigate differential disease mechanisms in *de novo* versus familial HSP-*SPAST*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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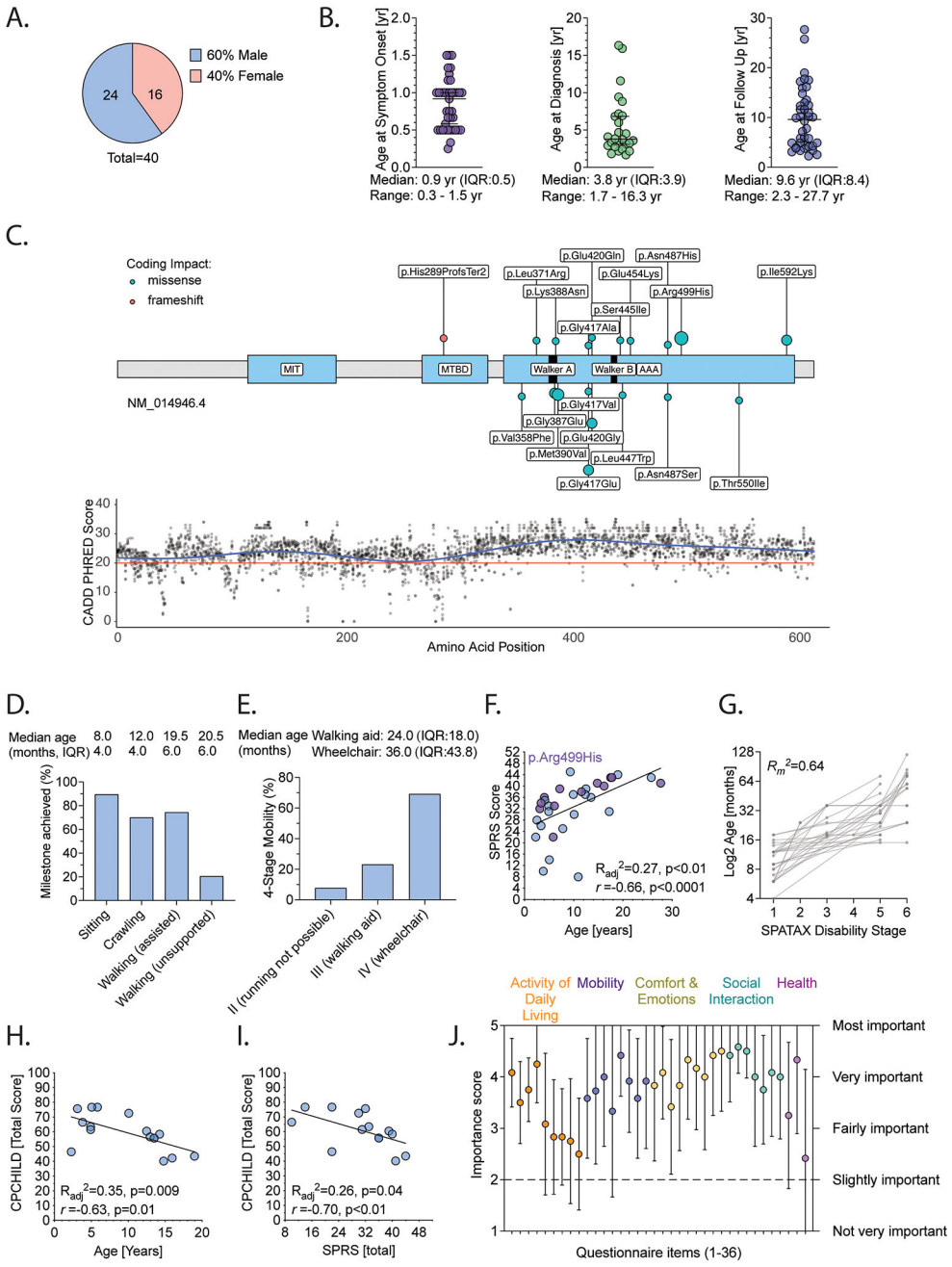

Figure 1. Molecular and clinical spectrum of 40 patient with *de novo* SPAST variants.

(A) This cohort includes 40 patients with HSP-*SPAST* (SPG4) caused by pathogenic *de novo* variants in *SPAST*. The male to female ratio was 24:16. (B) The median (+95% confidence interval) and range for the age at onset of symptoms, age at genetic diagnosis and age at last follow up are shown. The median diagnostic delay (time between onset of symptoms and a molecular diagnosis) was 3.1 years (IQR:4.3). Most of the patients in this cohort are children (33/40). (C) Schematic of the spastin protein structure. Information on functional domains was adapted from Solowska *et al*[5]. Coding impacts are depicted by colored points. Allele frequencies in this cohort are represented by the size of each point.

All variants, except one, were missense variants, and 40% of the cohort (16/40) carries the c.1496G>A (p.Arg499His) variant. The lower panel shows CADD PHRED scores computed for all possible missense variants in *SPAST* (NM_014946.4) aligned to the linear protein structure. A generalized additive model was used to predict the tolerance for genetic variation across the protein (blue line). The recommended cut off value for deleteriousness (CADD PHRED = 20) is marked by a red line. (D) Developmental delay is a universal feature of patients with *de novo SPAST* variants and motor milestones are often prominently delayed. Bar graphs indicate the proportions of patients who achieved a given motor milestone. The median age at which each milestone was achieved is shown above the graph. (E) Level of ambulation at last follow up, measured on the 4-Stage Mobility Scale (1 = mild symptoms walking without an aid; 2 = walking without aid but unable to run; 3 = walking with aid; and 4 = wheelchair dependent). Most patients depend on walking aids or are wheelchair-dependent. The median age at which walking aids and/or wheelchair-dependency was reached is shown above the graph. (F) SPRS scores correlate with age as an indicator of disease duration. The SPRS ranges from 0 to 52 measuring speed of gait, stair climbing, quality of gait, arising from a chair, degree of leg spasticity, weakness, contractures, and bladder dysfunction, with higher scores indicating more severe disability. The black line indicates a linear regression model (R_{adj}^2 = 0.27, p= 0.0019). Spearman's rank correlation analysis shows a positive correlation between age and motor function (r = 0.66, p< 0.0001). Patients with the p.Arg499His variant are highlighted in purple. (G) Longitudinal assessment of SPATAX disability stages (0 = no disability; 1 = no functional handicap but signs at examination; 2 = mild, able to run, 3 = moderate, unable to run, limited walking without aid; 4 = severe, walking with one stick; 5 = walking with two sticks or walker, 6 = unable to walk, requiring wheelchair; 7 = confined to bed (not shown)). A general trend towards higher scores, indicating greater motor disability, with increasing age becomes apparent using a mixed-effects linear regression model (R_m^2 = 0.64, R_c^2 = 0.64, p<0.0001). (H) Lower CPCHILD scores, indicating lower health-related quality of life, correlate with age as a surrogate for disease duration (R_{adj}^2=0.35, p=0.009, r=−0.63, p=0.01). (F) Lower CPCHILD scores, indicating lower health-related quality of life, correlate with SPRS scores as an indicator for disease severity (R_{adj}^2=0.26, p=0.04, r=−0.70, p<0.01). (G) Rating of importance for each item on the CPCHILD questionnaire. No items were rated below the threshold level of importance (2.0 = slightly important). Items relating to positioning, transferring, and mobility, communication and social interaction, and comfort and emotions, are, on average, considered the most important contributors to quality of life.
Abbreviations: CPCHILD (Caregiver Priorities & Child Health Index of Life with Disabilities); IQR (interquartile range); SD (standard deviation); SPRS (Spastic Paraplegia Rating Scale); yr (years)

Table 1.

Core clinical symptoms in patients with *de novo SPAST* variants. Human phenotype ontology term identifiers are provided in brackets.

Neurological symptoms	
Delayed motor development (HP:0001270)	100%, 40/40
Delayed speech development (HP:0000750)	87%, 33/39
Non-verbal (HP:0001344)	21%, 8/39
Intellectual disability (HP:0100543)	89%, 24/27
Motor regression (HP:0002376)	64%, 25/39
Epilepsy (HP:0001250)	26%, 10/39
Dysarthria (HP:0001260)	54%, 21/39
Dysphagia (HP:0002015)	46%, 18/39
Bladder and bowel symptoms	
Urinary urgency (HP:0000012)	51%, 20/39
Urinary retention (HP:0000016)	15%, 6/39
Urinary incontinence (HP:0000020)	67%, 26/39
Bowel incontinence (HP:0002607)	51%, 20/39
Motor examination	
Spastic diplegia (HP:0002061)	54%, 21/39
Spastic tetraplegia (HP:0002510)	46%, 18/39
Lower extremity hyperreflexia (HP:0002395)	95%, 37/39
Babinski sign (HP:0003487)	97%, 30/31
Contractures (HP:0003121)	77%, 30/39
Axial hypotonia (HP:0008936)	75%, 21/28
Ataxia (HP:0001251)	5%, 2/39
Focal or segmental dystonia (HP:0001332)	28%, 11/39
Rigidity (HP:0002063)	5%, 2/39