Hereditary Spastic Paraplegia: Clinicogenetic Lessons from 608 Patients

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Objective: Hereditary spastic paraplegias (HSPs) are genetically driven disorders with the hallmark of progressive spastic gait disturbance. To investigate the phenotypic spectrum, prognostic factors, and genotype-specific differences, we analyzed baseline data from a continuous, prospective cohort.
Methods: We recruited 608 HSP cases from 519 families of mostly German origin. Clinical severity was assessed by the Spastic Paraplegia Rating Scale. Complicating symptoms were recorded by a standardized inventory.
Results: Family history indicated dominant (43%), recessive (10%), and simplex (47%) disease. We observed a significant male predominance, particularly in simplex cases without a genetic diagnosis. Disease severity increased with disease duration. Earlier disease onset was associated with less severe disease. Specific complicating features including cognitive impairment, extrapyramidal or peripheral motor involvement, and ataxia were associated with worse disease severity. Disease severity also depended on the genotype. HSP cases maintained the ability to walk independently for a median disease duration of 22 years. Early onset cases were able to maintain free walking significantly longer and were at less risk to become wheelchair dependent.
Interpretation: This cross-sectional cohort study provides the first large-scale data on disease manifestation, progression, and modifying factors, with relevance for counseling of HSP families and planning of future cross-sectional and natural history studies. Later age of onset, specific complicating features, and the SPG11 genotype are strongly associated with

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more severe disease. Future interventional studies will require stratification for modifiers of disease progression identified in this study. Prospective longitudinal studies will verify progression rates calculated in this baseline analysis.

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Hereditary spastic paraplegias (HSPs) are genetically driven disorders with the clinical hallmark of progressive spastic paraparesis. They can be inherited in an autosomal-dominant, autosomal-recessive, or X-linked manner with >80 published genes or loci.[1,2] Since Anita Harding's classification, HSPs have been divided into pure or complicated forms. An isolated pyramidal syndrome characterizes "pure" HSP, although neurogenic bladder disturbances and impairment of vibration sense may occur. Additional system involvement (cognitive impairment, ataxia, basal ganglia symptoms, visual or auditory disturbances, symptoms of peripheral nerve involvement) defines "complicated" forms of HSP and is assumed to be associated with a more severe disease course.[3,4]

Because HSPs are orphan diseases with a collective prevalence of 2 to 10 per 100,000,[5–7] systematic clinicogenetic studies in large cohorts are missing. However, smaller genotype-specific clinical series have given important insights into phenotypic presentation, frequency, and mutational spectrum.[8–18]

Here we present an in-depth clinical and genetic characterization of a continuous, unselected cohort of 608 HSP patients. Conclusions drawn from this cohort can guide diagnostic testing strategies and improve counseling of HSP patients and their families. Major determinants of disease severity revealed in the current study are of importance for stratification of study cohorts in clinical trials.

Patients and Methods

Cohort and Genetic Workup

A total of 608 HSP patients from 519 families were consecutively enrolled in a continuous series by the GeNeMove and German Center for Neurodegenerative Diseases (DZNE) centers in Bonn, Bochum, Magdeburg, Mainz, Munich, Regensburg, Rostock, Tübingen, and Ulm (Table 1). The vast majority of cases were German and had a nonconsanguineous family background. Patients fulfilling the clinical diagnostic criteria[19] for HSP were included irrespective of their genetic diagnosis. In simplex cases, we excluded structural lesions and inflammatory central nervous system disease by magnetic resonance imaging of brain/spinal cord and cerebrospinal fluid analysis, and examined vitamin B12 levels and human T-cell lymphotropic virus type 1/2 serology.

To delineate the specific HSP syndrome, we performed biochemical and genetic tests. We systematically screened for deficiency of lysosomal enzymes (arylsulfatase A, β-galactosidase, β-hexosaminidase A/B, β-galactocerebrosidase, β-glucocerebrosidase) and elevated levels of very long chain fatty acids. Gene-by-gene genetic diagnostic testing was performed according to the mode of inheritance, age of onset, and phenotypic expression in the family, considering published frequency and phenotypes of genetic subtypes. Furthermore, results from several research screenings were taken into account; details on study populations in these screenings can be found in the respective publications.[13,16,20–31] In addition to single gene testing, all autosomal-dominant HSP genes were screened by a resequencing microarray in 27 families.[27] All known HSP genes and further genes causing spastic phenotypes (98 genes in total) were analyzed in 12 families using a diagnostic grade next generation sequencing-based HSP panel available at the University of Tübingen. In 58 families, whole exome sequencing was performed at the University of Miami using Agilent (Santa Clara, CA) SureSelect 50Mb capture and Illumina (San Diego, CA) HiSeq 2000 and 2500 devices.[32] In summary, we were able to identify the genetic diagnosis in 240 of 519 families. For the remaining 279 index cases, genetic tests are detailed in Table 2.

Written informed consent was obtained from all study participants; the local institutional review boards approved the study.

Clinical History and Neurological Examination

To quantify clinical severity and screen for complicating symptoms, we used the Spastic Paraplegia Rating Scale (SPRS) and a standardized inventory for complicating signs and symptoms.[19] SPRS scores range from zero (no disease manifestation) to a maximum of 52 points (most severe disease manifestation). All investigators were trained in the application of the SPRS. Additional neurophysiological, neuropsychological, or imaging findings are beyond the scope of this study and were not systematically analyzed. For all cases, the first available SPRS examination was selected and age at examination and disease duration are reported according to that time point throughout the article.

Mode of inheritance was classified as dominant when HSP was reported in >1 generation. Families with several affected members in only 1 generation were classified as apparently recessive and cases with negative family history as simplex. In many of the smaller families, family structure did not allow determination of the likelihood of X-linked inheritance. These families were therefore subsumed under the respective autosomal modes of inheritance.

Disease onset was defined as the onset of the gait disturbance; in cases of conflicting information between personal history, third party history, and medical records, the examiner made a best judgment decision. This was achieved with sufficient certainty in all but 21 cases.

Statistical Analysis

Quantitative features are reported as mean and standard deviation for normally distributed data and median and interquartile range for not normally distributed data. Normal distribution was evaluated by visual inspection of the variable distribution

TABLE 1. Baseline Characteristics of the Hereditary Spastic Paraplegia Cohort

Characteristic	Dominant	Recessive	Simplex	Total
Mode of inheritance				
Cases	293 (48%)	72 (12%)	243 (40%)	608 (100%)
Families	222 (43%)	54 (10%)	243 (47%)	519 (100%)
Genetic etiology				
Solved cases (% solved)	188 (64%)	43 (60%)	68 (28%)	299 (49%)
Solved families (% solved)	139 (63%)	33 (61%)	68 (28%)	240 (46%)
Gender distribution				
Male	153 (52%)	40 (56%)	140 (58%)	333 (55%)
Female	140 (48%)	32 (44%)	103 (42%)	275 (45%)
p	n.s.	n.s.	0.018	0.015
Phenotype[a]				
Pure	159 (54%)	10 (14%)	87 (36%)	256 (42%)
Complicated	134 (46%)	62 (86%)	156 (64%)	352 (58%)
OR, *p*	D vs R: OR = 0.14, $p < 0.0001$		S vs D: OR = 2.13, $p = 0.001$; S vs R: OR = 0.29 $p = 0.002$	
Age of onset, yr				
Mean/SD	29.5/17.0	25.8/17.2	33.9/18.9	30.8/18.0
p	D vs R: n.s.		S vs D: 0.005; S vs R: 0.002	
Age at examination, yr, mean/SD	49.3/13.9	44.7/16.1	48.3/15.5	48.4/14.9
Disease duration, median/IQR	18/18	18/20.5	11/15	14/18
SPRS score, mean/SD	17.4/9.5	22.1/9.2	18.0/9.0	18.2/9.4

Probability values are adjusted for possible clustering effects as detailed in Patients and Methods. To preserve readability of the table, percentage values were not clustered but reflect the actually observed numbers.
[a]Pure versus complicated phenotypes were defined according to the Harding classification.[3]
D = dominant; IQR = interquartile range; n.s. = not significant; OR = odds ratio; R = recessive; S = simplex; SD = standard deviation; SPRS = Spastic Paraplegia Rating Scale.

and its skewness and kurtosis. As most analyses were done for all cases, confirmatory but not descriptive statistical analyses accounted for clustering effects within families. For this, the method of generalized estimating equations (GEE) with independent working correlation matrix and sandwich estimator for standard errors was used. Confidence limits for prevalence based on clustered data were determined using logistic regression models with intercept only. To compare categorical variables across groups we applied logistic regression analysis, to identify predictors for the SPRS score we applied linear regression analysis, and to assess the influence of clinical parameters on walking ability we applied Cox proportional hazard analysis (each adjusted for cluster effects). Descriptive analysis for censored data was done using the Kaplan–Meier method. A *p*-value of < 0.05 was considered statistically significant. SPSSWIN 21.0 (GEE linear and logistic model) and R release 3.1.2 (package survival, GEE for censored data) for Windows were used for statistical calculations and JMP v11 for Mac was used for graph generation. To remove potential bias due to possible family clustering effects, 1 member of each family was randomly selected (random number generator of SPSSWIN) for the generation of graphs in first, third, and fourth figures.

Results

Baseline Demographics

We included 608 patients from 519 families in the study (see Table 1). Family history suggested dominant inheritance in 43%, a recessive trait in 10%, and isolated disease in 47% of families.

TABLE 2. Genetic Testing in Unsolved Cases

Genetic Test	Mode of Inheritances		
	Dominant, n = 83	Recessive, n = 21	Simplex, n = 175
SPG3	54 (65%)	6 (29%)	74 (42%)
SPG4	62 (75%)	9 (43%)	114 (65%)
SPG10	38 (46%)	1 (5%)	41 (23%)
SPG31	42 (51%)	4 (19%)	61 (35%)
SPG5	N/A	5 (24%)	66 (38%)
SPG7	N/A	6 (29%)	73 (42%)
SPG11	N/A	4 (19%)	40 (23%)
SPG15	N/A	4 (19%)	41 (23%)

Number and proportion of index cases tested negatively for the listed genes are given by mode of inheritance. Only index cases for which no genetic diagnosis could be established (n = 279) are included in the analysis.
N/A = not applicable.

Gender Distribution

Gender distribution was unequal, with a significantly larger proportion of males than females in the total cohort (odds ratio [OR] = 1.2, male/female = 0.55/0.45, $p = 0.015$; see Table 1). However, when considering only families with confirmed mutations in autosomal HSP genes, no significant differences in gender distribution were noted. In contrast, males were strongly overrepresented in simplex HSP cases without a genetic diagnosis (OR = 1.5, male/female = 0.60/0.40, $p = 0.009$).

Age of Onset

Age of onset ranged from 0 to 73 years, with a mean of 30.8 years (standard deviation = 18.0). The distribution was bimodal, with a first peak in early childhood (<5 years in 11%) and a second peak around age 40 years (Fig 1A). Gender did not significantly influence the age of onset. Onset in simplex cases was later than in dominant or recessive cases (see Fig 1B, see Table 1).

To determine the influence of the genotype on age of onset, we performed a subgroup analysis for the 5 most frequent genotypes in our cohort (SPG3, SPG4, SPG5, SPG7, SPG11; Fig 2). All genotypes, even those typically considered to be early onset forms of HSP, showed a wide age of onset spectrum ranging from childhood well into adulthood. SPG7 manifested later than all other 4 genotypes. SPG4, although manifesting about 7 years earlier than SPG7, had a significantly later onset than the early onset genotypes SPG3, SPG5, and SPG11 (see Fig 1C). All age of onset comparisons were corrected for possible cluster effects within families using GEE.

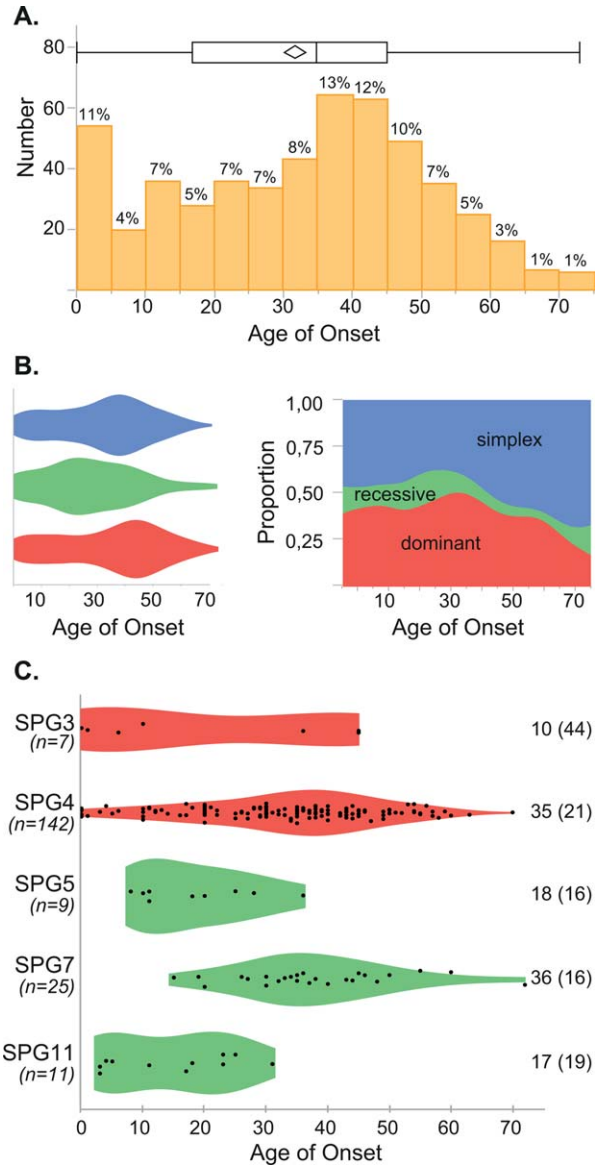


FIGURE 1: Age of onset distribution in hereditary spastic paraplegia (HSP). (A) Age of onset distribution in HSP patients followed a bimodal distribution with a first peak in early childhood (<5 years in 11%) and a second peak around age 40 years. The horizontal boxplot on top illustrates the age of onset distribution, with minimum, first quartile, median, third quartile, and maximum. The mean diamond indicates the mean (30.8 years) and the upper and lower 95% confidence interval of the mean. (B) Age of onset varied in dependence on mode of inheritance, with later onset in simplex cases than in dominant or recessive cases. The Y-extent of the irregular shapes indicates the proportion of cases with a particular mode of inheritance. The frequency of dominant and recessive inheritance decreases with increasing age of onset (right). In contrast, negative family history is more frequent with later age of onset. All modes of inheritance, however, can be observed across the whole age of onset spectrum. (C) Age of onset is influenced by the genotype. Age of onset varies in dependence on the genotype. Median age of onset in years and the interquartile range are given on the right. One affected family member from each family was randomly selected for preparation of this figure to avoid potential bias due to family clustering effects. [Color figure can be viewed in the online issue, which is available at www.annalsofneurology.org.]

Genotype Distribution

Among 519 index patients, the underlying genotype was identified in 46% (240 families; see Fig 2). Thirty-seven percent (83 of 222) of dominant families, 39% (21 of 54) of recessive index cases, and 72% (175 of 243) of simplex cases remained without a genetic diagnosis (see Tables 1 and 2).

SPG4 was by far the most common genotype and was diagnosed in 196 cases from 149 families. When used as a first-line diagnostic test in dominant cases, the diagnostic yield was 61% (121 of 197 dominant index cases tested for SPG4), including 25 families with macrodeletions of the *SPAST* gene diagnosed by multiplex ligation-dependent probe amplification (21% of our autosomal-dominant SPG4 cohort). In simplex cases, *SPAST* mutations were found in 15% (24 of 161 simplex index cases tested for SPG4), with a similar proportion of genomic deletions (17%, 4 of 24) as in dominant families. Four apparently recessive cases carried pathogenic SPG4 mutations, demonstrating reduced penetrance in the parent generation.

The overall diagnostic yield for SPG3 in SPG4-negative dominant index cases was 7% (5 of 68; 8 SPG4-negative dominant index cases were not tested for SPG3). In previous series, SPG3 has been identified in ~40% of autosomal index cases with a disease onset before the age of 10 years.[33] In our cohort, 41 dominant index patients had an onset in the first decade. In 27 of these, SPG3 was tested and revealed mutations in 5 (5 of 27, 19%) families. *SPAST* mutations were considerably more frequent in this subgroup (13 of 34 index cases tested for SPG4, 38%).

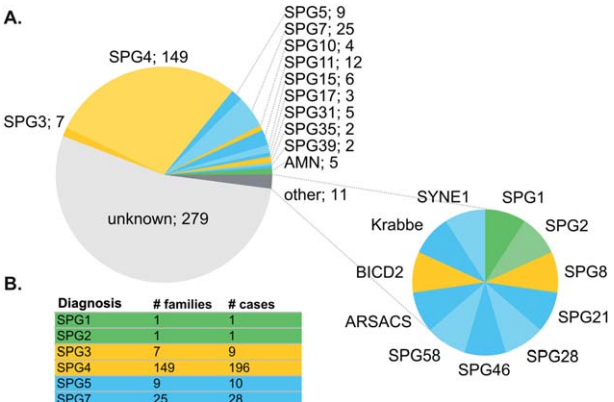
In 63 families (28 with recessive family history and 35 simplex cases), we identified causal variants in recessive genes. This group demonstrated high genetic heterogeneity, with mutations in 13 different genes. The most common genotype was SPG7 (25 families), followed by SPG11 (12 families), and SPG5 (9 families; see Fig 2).

Interestingly, 9 index cases had pathogenic mutations in genes not listed as SPG genes by the Human Gene Organization.[34] We identified 5 cases with adrenomyeloneuropathy and 1 case each with Krabbe disease, mutations in *BICD2*,[29,35] *SACS*, and *SYNE1*.

In 279 families, the genetic etiology remained unknown. Table 2 gives an overview of the genetic tests performed in this subgroup.

Frequency of Complicating Signs and Symptoms

Three quarters of patients (75%) had an involvement of neurological systems exceeding upper motor neuron involvement. Sensory involvement was present in >50% of all patients, ataxia in almost one-third (28%), and



Diagnosis	# families	# cases
SPG1	1	1
SPG2	1	1
SPG3	7	9
SPG4	149	196
SPG5	9	10
SPG7	25	28
SPG8	1	1
SPG10	4	5
SPG11	12	15
SPG15	6	7
SPG17	3	3
SPG21	1	2
SPG28	1	1
SPG31	5	5
SPG35	2	2
SPG39	2	2
SPG46	1	1
SPG58	1	1
AMN	5	5
ARSACS	1	1
BICD2	1	1
Krabbe disease	1	1
SYNE1	1	1

FIGURE 2: Genotype distribution in the study cohort. (A) Genotype distribution in 519 hereditary spastic paraplegia (HSP) families. Of the 519 HSP families, the diagnosis was genetically confirmed in 240. The number of families for each genotype is listed separated by a semicolon. Autosomal dominant genes are depicted in shades of yellow, autosomal recessive genes in shades of blue, and X-linked genes in shades of green. (B) Diagnosis distribution in HSP cases and families. The table lists the number of cases and families included for each genotype. The total number of solved families/cases is 240/299.

peripheral motor involvement (ie, loss of stretch reflexes or muscle atrophy) in 19% (Fig 3A). Fifty-eight percent had additional symptoms other than impairment of vibration sense and/or bladder disturbances and were therefore classified as complicated HSP (see Table 1).[3]

Next we compared the frequency of complicated versus pure HSP in dependence on mode of inheritance. Complicated disease manifestations were most common in recessive cases, followed by simplex cases (see Table 1). In contrast, pure disease manifestations predominated in dominant cases. This was true for the total cohort of 608 cases as well as for the subgroup of genetically confirmed cases (n = 299).

We then analyzed whether the frequency of specific complicating symptoms differs across the most common genotypes, SPG3, SPG4, SPG5, SPG7, and SPG11. As the genotype was identical for patients from the same family and presence or absence of complicating symptoms was nearly identical within families, the generalized estimating approach was not feasible. Thus, we analyzed families instead of single patients. In the rare cases where symptoms differed between members of the same family,

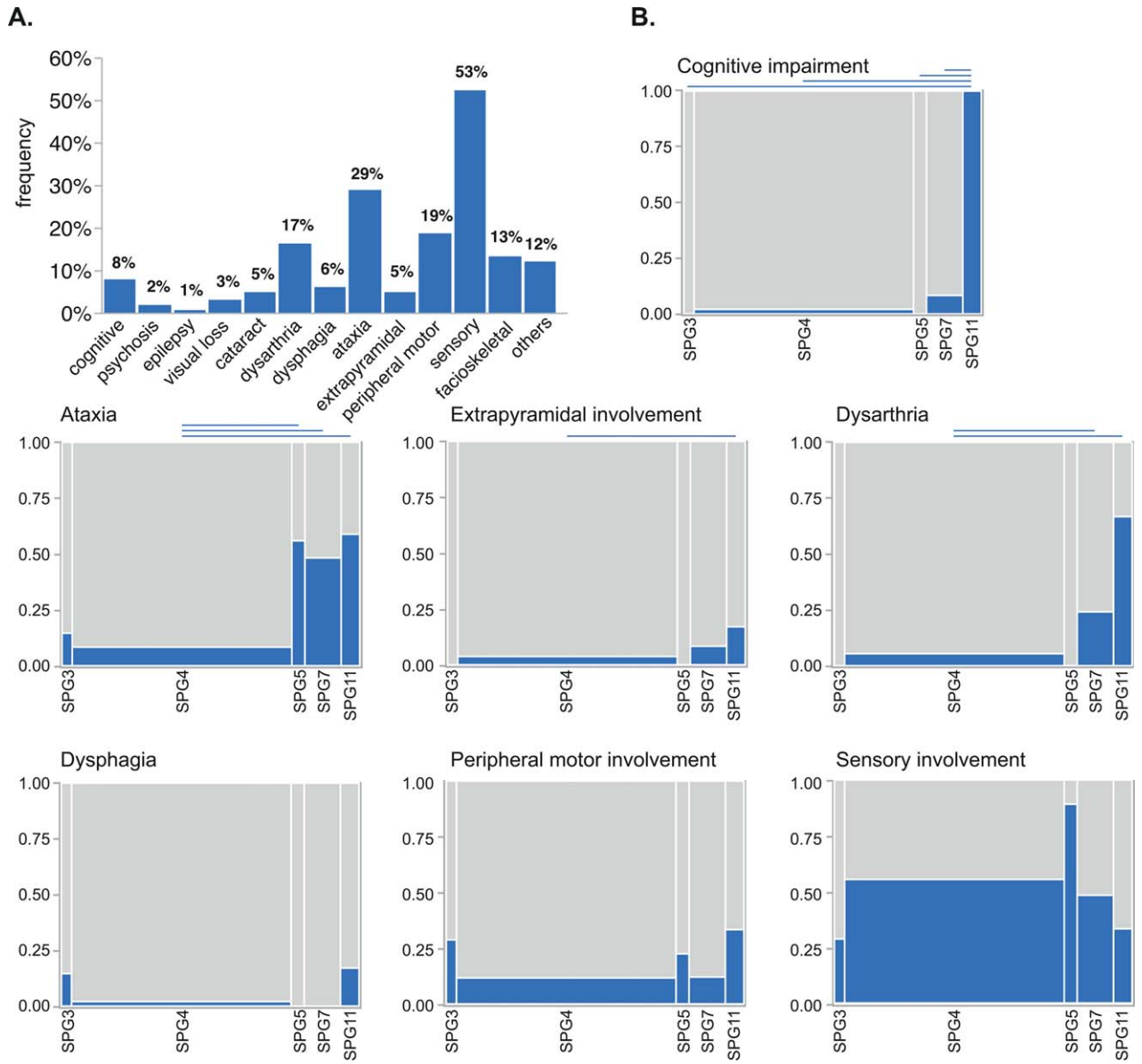


FIGURE 3: Frequency of complicating signs and symptoms. (A) Frequency of complicating signs and symptoms. Bars indicate the proportion of patients with a given sign or symptom of the total (n = 519). One affected family member from each family was randomly selected for preparation of this figure to avoid potential bias due to family clustering effects. (B) Complicating signs and symptoms in selected genotypes. Mosaic plots demonstrating the frequency of selected complicating signs and symptoms in the 5 most common genotypes in our cohort (SPG3, SPG4, SPG5, SPG7, SPG11) are shown. The area of the tiles is proportional to the number of cases within that category. Pairwise comparisons were performed between genotypes. Significant differences (adjusted $p < 0.0001$) are indicated by horizontal lines on top of each plot; all other comparisons were not significant. [Color figure can be viewed in the online issue, which is available at www.annalsofneurology.org.]

we rated a family positive if at least 1 member of the family showed the respective sign or symptom.

Significant associations between genotypes and complicating symptoms were identified for cognitive impairment, dysarthria, cerebellar involvement, and extrapyramidal involvement (Pearson chi-square, adjusted $p = 0.005$). We then performed pairwise comparisons for each genotype and complicating symptoms. Frequency of complicating symptoms for each genotype and significant differences (adjusted $p < 0.0001$) are detailed in Figure 3B and Table 3.

Use of Gait Assistive Devices and Loss of Ambulation

At the time of investigation (median disease duration = 14 years), 582 participants (96%) were still able to walk for at least 10m. Three hundred eighty-five (63%) were able to walk independently, whereas 197 (32%) needed canes or

TABLE 3. Factors Associated with Disease Severity

Factor	Estimate	Standard Error	2-Sided *p*
Complicated HSP	3.424	0.6854	<0.001
Dysphagia	8.364	1.9817	<0.001
Cognitive impairment	7.916	1.9883	<0.001
Extrapyramidal involvement	6.495	1.7007	<0.001
Peripheral motor involvement	6.215	1.1064	<0.001
Dysarthria	5.707	1.1027	<0.001
Sensory involvement	1.847	0.7209	0.010
Ataxia	1.898	0.7837	0.015
Psychosis	7.469	3.1123	0.016
Epilepsy	6.973	5.5454	n.s.
Visual loss	5.919	3.2839	n.s.
Cataract	2.307	1.7632	n.s.
Facioskeletal abnormalities	1.162	1.0837	n.s.

Generalized linear model with Spastic Paraplegia Rating Scale total score as dependent variable and disease duration, disease duration squared, and age of onset as independent variables. The above-listed variables were independently entered into the model. Generalized estimating equations were used to account for possible clustering effects within families.
HSP = hereditary spastic paraplegia; n.s. = not significant.

walkers; only 26 participants (4%) had completely lost their ability to walk. Seventy participants (12%) reported use of a wheelchair on a regular basis.

To assess the risk of becoming dependent on a walking aid or wheelchair, we performed a Kaplan–Meier analysis. After disease durations of 10/20/30/40 years, respectively, 25/48/64/72% of patients regularly used a walking aid. The median disease duration until loss of independent walking was 22 years (Fig 4A).

The proportion of patients using a wheelchair was much smaller, that is, at 10/20/30/40 years into the disease, 5/12/18/29% of patients depended on a wheelchair in daily life. After a disease duration of 37 years, only one-quarter of patients regularly used a wheelchair (see Fig 4B). Of patients using a walking aid, the median time until they moved on to use a wheelchair was 16 years.

Factors Associated with Earlier Loss of Independent Walking

We analyzed whether age of onset or gender are associated with the risk of becoming walking aid or wheelchair dependent. Later age of onset was strongly associated with loss of independent walking earlier in the disease course (hazard ratio $[HR]^{10years} = 1.676$, confidence interval [CI] = 1.538–1.842, $p < 0.001$). To rule out that this effect was driven by the possible presence of alternative diagnoses especially in cases without genetically confirmed HSP, we repeated the analysis in the subgroup of cases with confirmed mutations in HSP genes. The same association between late disease onset and earlier loss of independent walking ability was observed in this subgroup ($HR^{10years} = 1.583$, CI = 1.397–1.774], $p < 0.001$; see Fig 4A, middle gray line). Finally, we considered whether the underlying genotype rather than the age of onset per se contributes to this effect. As SPG4 is the only genotype frequent enough in our cohort for a genotype-specific subgroup analysis, we performed a clustered survival analysis in the SPG4 subcohort (n = 196), whereof 96 patients became walking aid dependent during the observation timeframe. Even in this genotype-specific subgroup, later disease onset was associated with earlier walking aid dependency ($HR^{10years} = 1.583$, CI = 1.357–1.842, $p < 0.001$).

Similarly, cases with a later age of onset became wheelchair dependent earlier in the disease course; this association was significant in the total cohort ($HR^{10years} = 1.195$, CI = 1.020–1.411, $p = 0.029$) as well as in the subgroups of genetically confirmed cases ($HR^{10years} = 1.243$, CI = 1.000–1.538, $p = 0.047$) and SPG4 cases ($HR^{10years} = 1.452$, CI = 1.072–1.967, $p = 0.016$), despite the small sample sizes in these subcohorts (eg, 39 of 22 events in genetically confirmed/SPG4 cases).

Gender was not associated with the risk of becoming walking aid or wheelchair dependent during the

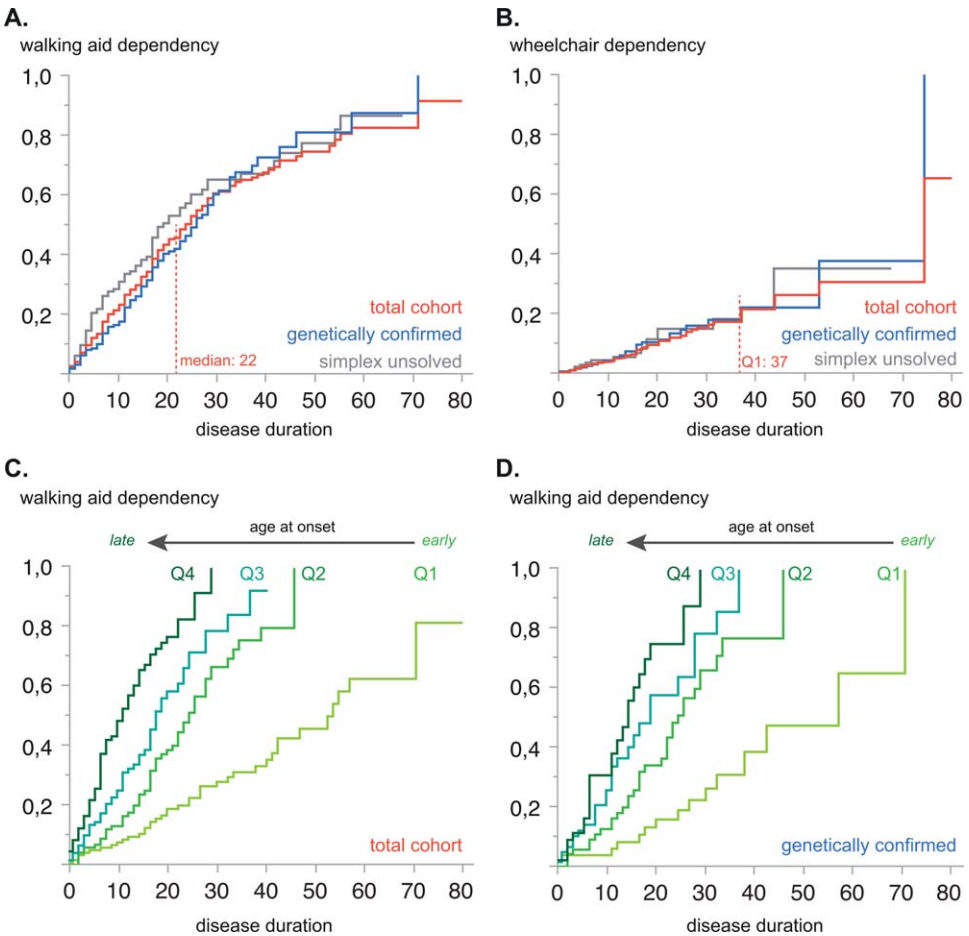


FIGURE 4: Kaplan–Meier analysis. (A) Loss of independent walking. Hereditary spastic paraplegia (HSP) cases lose the ability to walk independently after a median disease duration of 22 years. For the subgroups of HSP cases with genetically confirmed diagnosis and simplex cases without a genetic diagnosis, the time course of walking aid dependency is virtually indistinguishable from the total cohort. (B) Wheelchair dependency. After a disease duration of 37 years, one-quarter of patients are dependent on a wheelchair. Again, the subgroups of genetically confirmed cases and simplex unsolved cases are indistinguishable from the total cohort. (C) Influence of age of onset on independent walking (total cohort). The total cohort was divided into 4 subgroups of equal size according to their age of onset quartiles (Q1 < 17 years, Q2 < 35 years, Q3 < 45 years, Q4 ≥ 45 years). Later onset is associated with a higher risk of becoming walking aid dependent earlier in the disease course. (D) Influence of age of onset on independent walking (genetically confirmed cases). The effect demonstrated in C was confirmed when only the cases with confirmed genetic diagnoses were included in the analysis. One affected family member from each family was randomly selected for preparation of this figure to avoid potential bias due to family clustering effects. [Color figure can be viewed in the online issue, which is available at www.annalsofneurology.org.]

course of the disease, neither in the total cohort, nor in the subgroups of genetically confirmed or SPG4 cases.

Factors Associated with More Severe Disease

ASSOCIATION OF AGE OF ONSET, DISEASE DURATION, AND GENOTYPE WITH DISEASE SEVERITY. To explore factors associated with disease severity, we performed a multivariate linear regression with disease severity (total SPRS score) as the dependent variable and gender, disease duration, and age of onset as independent variables. The best model was reached after adding disease duration squared as an additional independent variable, yielding a combined regression coefficient r^2 of 0.163 ($p < 0.001$). Disease duration had the strongest effect on disease severity (B [nonstandardized coefficient of linear regression] = 0.404, $p < 0.001$), followed by age of onset (B = 0.104, $p < 0.001$). Longer disease durations as well as later age of onset were hereby associated with more severe disease. Women were slightly more severely affected than men (B = 1.504, $p = 0.041$).

We then repeated the analysis within the subgroup of cases with genetically confirmed diagnosis. Again, disease duration remained the strongest determinant of disease severity, followed by age of onset. Gender effects were no longer significant; they were, however, observed at about the same magnitude as for the total cohort (B = 1.672, $p = 0.126$).

Finally, we added the 5 most common genotypes in our cohort to the model. SPG11 cases were much more severely affected than the other 4 genotypes, and SPG5

cases also showed a tendency toward a more unfavorable disease course ($B = 5.368$, $p = 0.051$). In particular, SPG5 cases were significantly more severely affected than SPG3 cases ($B = 7.763$, $p = 0.001$).

ASSOCIATION OF COMPLICATING SIGNS/SYMPTOMS WITH DISEASE SEVERITY. Next, we analyzed whether complicating signs and symptoms are associated with more severe disease. As we have shown that disease duration and age of onset are strongly associated with disease severity, we generated a multiple linear model in analogy to the model above, with SPRS score as the dependent variable and disease duration, disease duration squared, and age of onset as independent variables, again using GEE to control for cluster effects within families. Presence of complicating symptoms according to the criteria proposed by Anita Harding[3] was strongly associated with more severe disease ($B = 3.424$, $p < 0.001$). When added independently to the model, dysphagia, cognitive impairment, extrapyramidal involvement, peripheral motor involvement, dysarthria, sensory involvement, ataxia, and psychosis were associated with higher SPRS scores, indicating more severe disease (ordered by decreasing effect size; see Table 3).

Discussion

Since Anita Harding's landmark paper on classification of HSPs and hereditary ataxias in 1983,[3] we have derived our clinical knowledge mostly from small genotype-specific studies and anecdotal expert testimonies transmitted via clinical and genetic reviews. This has led to 2 major shortcomings: (1) some clinical observations from smaller-scale studies are insufficiently substantiated by statistical evidence; and (2) as hardly any of the existing clinical studies on HSP use standardized and validated outcome measures, conclusions from these studies are of limited use for planning and execution of clinical trials. The need for trial readiness, however, is increasingly recognized in rare diseases. Results and conclusions from this large continuous cohort of HSP cases characterized by standardized and validated measures to capture phenotype and major determinants of disease severity are an essential first step toward trial readiness and represent the baseline data for an ongoing natural history study in HSP.

Mode of Inheritance

HSPs are generally perceived as Mendelian diseases. Similar to existing studies in cohorts of simplex HSP cases[26,36,37] with negative family history, we identified mutations in known autosomal-dominant, autosomal-recessive, and X-linked genes in 28% of simplex cases in our cohort. However, 72% of simplex cases remained without genetic diagnosis, including 35 (of 47) cases where whole exome sequencing was performed. Although mutations missed by whole exome sequencing or mutations in as yet unknown HSP genes and the presence of phenocopies in our cohort might contribute to this high number of genetically unsolved simplex cases, we believe that non-Mendelian modes of inheritance in HSP may also be hidden contributors. In the same vein, complex or digenic modes of inheritance have recently been described in several diseases with predominantly Mendelian inheritance, including Alport syndrome, facioscapulohumeral muscular dystrophy, hereditary optic atrophy, and hereditary ataxias.[38–41]

Age of Onset

Age of onset is highly variable in HSP and ranged from the first to the eighth decade in genetically confirmed cases of our series. This wide spectrum observed in all genotypes and all modes of inheritance hampers phenotype–genotype predictions in individual cases. Mutation-specific effects and contribution of genetic and nongenetic modifiers to phenotypic expression may explain this variability but have hardly been studied in HSP so far. Childhood onset does not reliably predict autosomal-recessive inheritance in HSP—a notion that is believed to be true for other hereditary neurological disorders like cerebellar ataxias.[42] Almost half of the cases presenting as childhood onset HSP report a dominant family history, and three-quarters of genetically solved childhood onset cases are explained by mutations in autosomal-dominant HSP genes.

Gender Distribution

We observed a significant male predominance in our cohort that was mainly driven by a high proportion of males (60%) in the subgroup of simplex cases without a genetic diagnosis. We consider it most likely that as yet unknown X-linked genetic factors strongly contribute to this effect, as mutations in the 4 known X-chromosomal HSP genes (*L1CAM, PLP1, SLC16A2, ABCD1*) typically cause characteristic complicated phenotypes, at least in males. We therefore hypothesize the presence of further X-chromosomal HSP genes with relevant proportional contribution to HSP.

Interestingly, a trend toward male preponderance was also observed in dominant cases and has been reported previously for SPG4.[43] It has been discussed whether higher disease penetrance in males, as has been reported for some specific *ATL1* and *SPAST* mutations,[44] may cause this gender imbalance. However, our finding that gender is equally distributed among the subgroup of

cases with confirmed mutations in autosomal genes argues against this notion.

Genotype Distribution

In accordance with earlier studies,[45,46] SPG4 was found to be by far the most common form of HSP regardless of inheritance mode, followed by SPG7 and SPG11.[10,11,15,36,47,48]

Previous studies in selected cohorts suggest that SPG3 is the main cause of childhood onset dominant HSP.[17] In our unbiased continuous cohort, we found SPG4 to be about twice as common in this subgroup as SPG3. Different genotype distributions among tested nationalities (French vs German) may contribute to these discrepant findings.

Despite our extensive efforts, almost 50% of HSP patients remained genetically unsolved, with a particularly low diagnostic success rate in simplex cases (28%). Even in the 97 patients in whom all relevant known genes were examined by panel or whole exome sequencing, 72 remained without a diagnosis. The portion of unsolved cases in this group is particularly high due to prior exclusion of common HSP genes by single-gene testing. Together, these findings suggest that further HSP genes remain to be identified.

Mutations in Non-SPG Genes

Despite stringent inclusion criteria, we found several patients with mutations in non-SPG genes whose frequency may be underestimated by our approach, because these genes have not been investigated systematically. However, not all hereditary diseases causing spasticity are registered with an SPG number by the Human Gene Organization.[34] X-linked adrenomyeloneuropathy, Krabbe disease, and Alexander disease as well as spastic variants of hereditary ataxias (e.g. autosomal-recessive spastic ataxia Charlevoix Saguenay,[49] late onset Friedreich ataxia,[50,51] and autosomal-dominantly inherited spinocerebellar ataxias type 1, 3, or 7)[52] may mimic HSP.[53] These genes therefore need to be considered in the genetic differential diagnosis of HSP.

Progression of Gait Disturbance

To our knowledge, the question regarding in what timeframe and at what proportion of cases HSP leads to walking aid and wheelchair dependency has never been addressed in a systematic way in a large cohort before. This contrasts with the impact of loss of ambulation on the lives of affected patients. We demonstrate that even after a disease duration of 40 years, about one-quarter of HSP patients are still walking without walking aids and less than one-third depend on a wheelchair in daily life. The earlier the age of onset, the longer the ability to walk independently is retained over the course of the disease. That this association of early disease onset and later walking aid or wheelchair dependency is also present within the subgroup of SPG4 cases demonstrates that this association is not merely a consequence of the underlying genotype but reflects a more general characteristic of disease evolution in HSP. Although these data need validation in prospective longitudinal cohort studies, they might prompt counseling of patients toward a more favorable disease course than previously assumed.

Complicating Symptoms

Almost 60% of patients in our cohort presented with a complicated phenotype according to Harding.[3] The clinical classification of HSP as proposed by Anita Harding and adapted in numerous genotype–phenotype studies over time[8–18] follows the assumption that reliable prediction of a genotype based on specific phenotypic features is possible. Although limited sample size and the need to correct for multiple comparisons limit our power to support the frequency distribution of complicating signs and symptoms across genotypes (see Fig 3) statistically, our data support rather typical disease presentations for SPG11 and to some extent SPG5 and SPG7. For the majority of genetic subtypes, however, age of onset and phenotypic expression are extremely broad. Not a single genotype in our study presented exclusively with pure HSP.

Factors Associated with More Severe Disease

Objective measures of disease progression are valuable tools to plan cohort size and duration of clinical trials. Data from larger cohorts using measures validated for HSP are essentially missing. To our knowledge, there is only 1 study evaluating cross-sectional and longitudinal disease progression in HSP.[54] The informative power of this study, however, is limited due to the small sample size ($n = 64$) and the choice of outcome parameters, none of which is validated in HSP. Using the SPRS, a clinical rating scale validated to measure disease severity in pure and complicated forms of HSP,[19] we here present the baseline data of an ongoing natural history study. These data allow us to identify factors associated with higher SPRS scores and thus more severe disease.

Age of onset and disease duration showed the strongest effect on disease severity, whereby late age of onset and longer disease duration were associated with higher SPRS scores. These findings thus corroborate our findings on the favorable influence of early disease onset on the ability to walk unaided. These data for HSP are, however, in contrast to studies in other neurodegenerative

diseases including spinocerebellar ataxias,[55] Friedreich ataxia,[56,57] and Huntington disease[58] that all demonstrate faster disease progression in earlier onset cases. Future longitudinal studies will clarify the role of potentially underlying neurodevelopmental defects in early onset cases and whether progression rate of HSPs remains linear over the disease course.

As suspected, complicated HSP is associated with a more severe disease. Nonetheless, not all complicating signs and symptoms were found to contribute to this effect. Cognitive impairment, dysphagia, dysarthria, and extrapyramidal and peripheral motor involvement were found to be most strongly associated with more severe disease. It is, however, likely that not the complicating symptom per se but the pathomechanism associated with a specific genotype is the true driving force behind disease progression. Complicating features may therefore be indicators of this underlying pathomechanism. We were able to demonstrate an association of genotype with more severe disease in SPG11 and SPG5 compared to SPG3, SPG4, and SPG7. Stratification for phenotypic expression in larger cohorts with a specific genotype will be needed to study this effect.

Limitations

The cross-sectional design of this study does not allow reliable calculation of disease progression rates. This is mostly due to the multiple biases influencing the retrospective determination of age of onset and thus disease duration in HSP. Longitudinal studies will therefore be needed to establish progression rates in HSP and determine whether the nonlinear increase in disease severity in later disease stages reflects true disease evolution in HSP or represents an artifact of the cross-sectional study design, for example, due to recall bias or imprecise determination of age of onset. Furthermore, our study is limited by the large proportion of genetically unsolved cases, which results partly from incomplete screening of all >50 known HSP genes and partly from the likely contribution of yet unidentified HSP genes.

Conclusion

In this large-scale cross-sectional cohort study, we demonstrate that the majority of HSP patients do not become wheelchair dependent even decades into the disease. Early age of onset was associated with a more favorable prognosis. In addition to disease duration, age of onset, genotype, and specific complicating symptoms were identified as main factors associated with disease severity. Our data provide guidance for future interventional studies that require stratification for modifiers of disease severity and potentially progression identified in this study. Longitudinal studies are on the way to verify progression rates and provide prospective natural history data in a representative cohort of HSP patients.

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Author Contributions

R.S., S.W., P.M., S.Z., and L.S. were responsible for the concept and design of the study. All authors contributed to data acquisition, data analysis, and drafting the manuscript. R.S., S.W., and L.S. drafted the figures. R.S. and S.W. contributed equally to the work.

Potential Conflicts of Interest

Nothing to report.

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