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Hereditary spastic paraplegia Type 4 (SPG4): Disease pattern, disease progression and prognostic factors

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Greiner, Judith Theresia, geb. Schöls

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Dekan: Professor Dr. B. Pichler

1. Berichterstatter: Privatdozentin Dr. R. Schüle-Freyer
2. Berichterstatter: Privatdozent Dr. W. S. Gröschel

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List of abbreviations

AAA   ATPase associated with various cellular activities (functional domain of spastin)

aoo   Age of onset of disease

b   Regression coefficient

BMBF   Bundesministerium für Bildung und Forschung

95% CI   95% Confidence interval

CRF   Case Report Form

df   Degrees of freedom

DNA   Deoxyribonucleic acid

DZNE   Deutsches Zentrum für Neurodegenerative Erkrankungen (German Centre of Neurodegenerative Diseases)

ER   Endoplasmic Reticulum

ESCRT III   endosomal sorting complex required for transport III

GeNeMove   German Network of Hereditary Movement Disorders

H   Test statistics for Kruskal-Wallis test

HIH   Hertie Institut für klinische Hirnforschung (Hertie Institute of Clinical Brain Research in Tübingen)

HR   Hydrophobic Region (functional domain of spastin)

HSP   Hereditary Spastic Paraplegia

ICARS   International Cooperative Ataxia Rating Scale

iPSC   Induced pluripotent stem cell

M1   Full-length spastin comprising 616 amino acids

M87   Shorter isoform of spastin lacking the first 86 amino acids of M1 spastin

Mdn   median

MIT   microtubule interacting and trafficking domain (functional domain of spastin)

MMSE   Mini Mental State Examination

mRNA   Messenger ribonucleic acid

MTBD   microtubule binding domain (functional domain of spastin)

NHS	Natural History Study
r	Pearson's correlation coefficient
REEP1	Receptor expression enhancing protein 1
SPAST	Gene mutated in SPG4
spastin	Protein that is encoded by *SPAST*
SPG4	Spastic Paraplegia 4: most common subtype of HSP caused by variants in *SPAST* in gene locus *SPG4*
SPG4	Gene locus of *SPAST*, the gene mutated in SPG4
SPRS	Spastic Paraplegia Rating Scale
t	Test statistics for independent t-test
U	Test statistics for Mann-Whitney test
vus	variance of unknown significance
z	z-score / standard score

Introduction

Hereditary spastic paraplegias (HSP) are a group of diseases characterized by a progressive spastic gait disorder due to the degeneration of corticospinal tract motor neurons. Since the underlying pathology is a length-dependent axonal degeneration the lower extremities are particularly affected [1].

This results in the clinical symptoms of a progressive spasticity and weakness of the legs. Neurogenic bladder disorder also commonly occurs and, in many cases, a mild to moderate impairment of the vibration sense can be measured. If, in addition to this, other neurological or non-neurological systems are involved, the phenotype is "complicated" rather than "pure" [2].

HSP are genetically diverse disorders. More than 80 different genetic loci (spastic gait genetic loci SPG1-83 plus other) including more than 60 different genes have been identified so far [3]. The mode of inheritance can be autosomal dominant, autosomal recessive or X-linked [3]. The most common form of autosomal dominant HSP is SPG4, caused by a variant in the *SPAST* gene, which is located in the *SPG4* gene locus on chromosome 2 (2p22.3) [4].

Prevalence

The prevalence of HSP ranges in epidemiology studies from 0.1 – 9.6 in 100 000 [5]. The most recent and comprehensive European studies were performed in Portugal and Norway and estimated the number of HSP-affected individuals to be 4.1 / 100 000 in Portugal [6] and 7.4 / 100 000 in Norway [7], respectively. They found the mode of inheritance to be autosomal dominant in 5.5 / 100 000 and autosomal recessive in 0.6 / 100 000 [7]. In about 40 percent of autosomal dominant HSP the affected gene is *SPAST* resulting in HSP of the subtype SPG4 [5, 8, 9].

SPG4

SPG4, caused by variants in the SPAST gene, is the most common form of HSP, accounting for about 25% of cases [10, 11]. This disease is present in about 40% of autosomal dominant cases [5, 8, 9]. Furthermore, SPAST variants can be observed in a considerable number of sporadic cases (~12%, [12]) due to incomplete penetrance and occurrence of *de novo* variants. The SPG4 gene *SPAST* codes for the protein spastin [9], a protein involved in axonal transport by modifying the axonal microtubular structure [4]. SPG4 usually manifests as "pure" HSP with progressive spastic paraparesis, bladder problems and impairment of vibration sense as the most frequent presentation. The occurrence of "complicated" phenotypes with additional symptoms due to the affection of other systems has been observed in a small number of cases [8, 13].

Pathology

Spastin-deficient mice show impaired axonal transport and axonal degradation [14, 15]. Focal axonal swellings in the cervical and lumbar spinal tract were found, in which organelles were accumulated. These axonal swellings could already be detected at the pre-sympomatic stage [14], indicating that these changes take place before onset of symptoms. Examinations of microtubule transport revealed defects in both anterograde and retrograde transport [14-16]. In accordance with these findings, axonal swellings containing accumulated axonal transport cargoes and decreased axonal transport was also observed in induced pluripotent stem cell (iPSC)- derived neurons carrying variants in SPAST [1]. The survival of neurons carrying truncating spastin variants was not affected, supporting the hypothesis that SPG4 is an axonopathy rather than a motoneuron disease [15].

In drosophila the postdevelopmental axonal regeneration ability was found to be impaired when spastin gene dosage was reduced [17].

Post mortem studies

Post-mortem studies revealed changes in the distal ends of ascending sensory pathways and the corticospinal tract in the sense of myelin loss of the cervical and thoracic spinal cord as a result of axon degeneration [4, 18-20]. In the cervical spinal cord the degeneration involved mainly fasciculus gracilis fibers, whereas in the thoracic spinal cord degeneration of the lateral corticospinal tracts was paramount [18, 20].

Spastin structure

Isoforms

There are four main cellular isoforms of spastin protein encoded by the *SPAST* gene. The different isoforms are created by use of different initiation codons on the one hand, generating the isoform M1 containing 616 amino acids by use of the initiation codon in position 1, and the isoform M87 containing 530 amino acids by use of the initiation codon at position 87. On the other hand two splice variants exist, generating isoforms containing or missing a stretch of 32 amino-acids encoded by exon 4 [4, 16] .

Examination of the expression patterns of M1 and M87 in mouse and human cells revealed ubiquitous expression of M87 in all tissues (including spinal cord and cortex) and in all stages of development. M1, on the other hand, was only detectable in human adult spinal cord, and absent in cortex and other tissues [4, 21].

Hexamer

6 spastin proteins assemble to form a hexameric ring structure and sever microtubules by pulling the microtubule (-) end through the central pore. Spastin is thus an integral part of microtubule dynamics [4, 22, 23].

Spastin functional domains and cellular function

Microtubule severing: MIT, MTBD, AAA

Spastin is a microtubule severing protein [24]. It contains 3 functional domains that are directly involved in severing activity: an AAA domain (amino acids 342-599), i.e. ATPase associated with various cellular activities, a microtubule binding domain (MTBD: amino acids 270-328) and a microtubule interacting and trafficking domain (MIT: amino acids 116-194) [4].

The microtubule binding domain together with the AAA domain are necessary for hexamer formation and microtubule severing [4, 23].

The microtubules interacting and trafficking domain interacts with 2 different proteins of the endosomal sorting complex required for transport III (ESCRT III), probably assisting the microtubule-severing-process and allowing the fission of recycling tubules from endosomes [4, 16, 25]

Hydrophobic Region (HR)

M1 spastin contains a hydrophobic region (HR, amino acids 49 – 80) which allows M1 to interact with the tubular endoplasmic reticulum (ER) as well as other proteins [4].

Spastin lacking this hydrophobic hairpin domain, because of use of the alternative start codon as well as variants, is not able to interact with the tubular ER or certain other proteins [4, 22, 23].

SPAST variants

The *SPAST* gene is located on chromosome 2 (2p22.3) and contains a total of about 90kb of genomic DNA, of which, distributed on 17 exons, a total of 1848 bases code for the 616 amino acids of the protein spastin [4, 9]. The variant spectrum of SPG4 includes on the one hand missense variants and in frame deletions and insertions, which lead to the exchange, absence or incorporation of individual amino acids, and on the other hand so-called truncating variants, including nonsense variants, small and large deletions and insertions, as well as splice site variants [4].

Variant spectrum of SPG4

In total, more than 200 different variants in *SPAST* have been identified [4, 26]. Missense variants, accounting for about 30% of SPG4 cases, are found almost exclusively in the AAA domain, whereas nonsense variants, splice site variants, insertions and deletions are spread all over the *SPAST* gene [4, 27] . Additionally, in about 20% of cases, large genomic deletions of SPAST can be found, often encompassing several exons [4]. In exon 4, which can be alternatively spliced and therefore is not found in all isoforms of spastin, no missense variants are reported [4].

Clinic

SPG4 is described in the literature as a predominantly "pure" form of HSP [8, 13]. This means that the clinical picture is characterized by the length-dependent degeneration of corticospinal tract motor neurons, leading to a
progressive spastic gait disorder [8, 13, 28]. If, in addition to the pyramidal tract, other parts of the central or peripheral nervous system are affected in a patient, this is referred to as complicated HSP, and in case of a genetically determined SPG4 as complicated SPG4.

The mean age of symptom onset of SPG4 is around 30 years of age, with a wide range from childhood to late adult life [8, 10, 13, 27]. Parodi et al. [27] recently reported earlier age of onset in missense variant carriers than carriers of truncating variants.

In clinical examinations spasticity at rest and gait spasticity interfering with ability to run can be observed [8, 10, 13, 27]. If getting severe, this gait disorder results in the loss of the ability to walk independently [10, 13]. About half of patients present muscle wasting and weakness in legs [8, 13, 27]. Upper limb involvement is paramount in more than every second patient, marked by increased tendon reflexes and less often mild increase in tone [8, 10, 13, 27].

In addition to spasticity of the extremities, many patients have an impairment of bladder function in the sense of a neurogenic bladder disorder with a small-capacity and overactive bladder [8, 13].

Although the pyramidal affection predominates pure HSP, mild impairment of the sense of vibration frequently occurs [13].

Complicating signs and symptoms can be motor symptoms such as limb ataxia, oculomotor disturbances (e.g. cerebellar), dysarthria (e.g. pseudobulbar), dysphagia, extrapyramidal involvement or muscle atrophy, but also non-motor symptoms such as cognitive impairment, psychiatric symptoms, seizures (epilepsy) and hearing impairment [28]. In addition, there are descriptions of patients with visual system involvement who have visual loss, cataract, retinitis pigmentosa or optic atrophy in addition to spastic gait disorder [28].

In the context of SPG4, there are only a few descriptions of complicated forms of progression [8]. Knowledge of the clinical symptoms of SPG4 comes from cross-sectional studies and case reports. However, no longitudinal studies are available on the time course of symptoms and their severity.

Therapy

Causal therapy exists neither for HSP nor for the subtype SPG4 [29, 30]. The treatment therefore concentrates on symptomatic therapy options with a focus on physical therapy, occupational therapy and, if necessary, speech therapy, in order to maintain as much everyday functionality as possible for the longest possible duration. Medical therapeutic options include antispastic drugs which can be applied orally, intrathecally or locally by intramuscular injections (e.g. botulinum toxin) [30].

For the treatment of neurogenic bladder disorders with medication, antimuscarinergics (e.g. Solifenacin), alpha-blockers (e.g. Tamsulosin), vasopressin analogues (Desmopressin) and intravesical botulinum toxin injections are available. Further therapeutic options are disposable catheterisation, suprapubic urinary bladder catheterisation and neuromodulation (chronic stimulation of the sacral root S3).

All these therapies, however, aim only to reduce the daily restrictions caused by the disease, without being able to modulate the disease course and prevent the chronic progression.

For the development of specific drug therapies to treat SPG4 respectively HSP, it is important to better understand the pathophysiological basics, for instance in order to be able to influence the microtubule transport with drugs [31], and also to have reference values for the natural course of the disease along with calculation of sample sizes in order to test the efficiency of new therapies. Therefore, we aim to describe the natural course of disease in this NHS.

Spastic Paraplegia Rating Scale (SPRS): Instrument for measuring the severity of disease

The Spastic Paraplegia Rating Scale (SPRS) was published by Schüle et al. [32] as the result of a multicenter collaboration in the GeNemove network. It is a measure of the disease severity and progression of HSP, enabling studies about the natural course of disease and on this basis clinical trials testing efficiency of new therapies. With the help of a standardized examination procedure, the investigator is able to swiftly record essential clinical aspects of the disease and make differences measurable on the basis of a score.

The standardized clinical characterization of HSP patients includes the recording of gender, family history, variant, age of the patient at the time of disease as well as at time of examination, and age in dependence on walking aids and wheelchair. In addition, differences between asymptomatic carriers of variants and patients with HSP will be distinguished, as well as between patients with pure and complicated HSP on the basis of the additional symptoms registered in the inventory (*see Appendix* Inventory).

What does the SPRS measure

The Spastic Paraplegia Rating Scale comprises 13 items, each of which is graded from 0 to 4, whereby 0 points reflect a normal finding and 4 points are associated with most severe affection (*see Appendix* SPRS). The sum of these 13 items forms the SPRS total score, which is a validated measure for disease severity in HSP. Items 1 – 6 refer to gross motor functions, rating maximal walking distance

and speed, gait quality, quality and speed of stair climbing, and the ability to rise from a chair.

There are 2 items measuring spasticity of hip adductor muscles and knee extension, based on the modified Ashworth Scale [32, 33]. An extra item refers to contractures of lower limbs. Weakness in hip abduction and foot dorsiflexion are recorded in another 2 items based on the Medical Research Council Scale.

Moreover, the pain due to SP related symptoms is recorded in 1 item.

The last item rates bladder and bowel function.

Inventory

In developing the SPRS, the goal was to establish a measurement method that could be applied to any type of HSP. Therefore, complicating signs and symptoms are summarized in the inventory (*see Appendix* Inventory) and are not included in the calculation of the SPRS total score as a measure of disease progress.

Neurological symptoms that are documented in the inventory are epilepsy (seizures), upper limb pyramidal involvement, extrapyramidal involvement, muscle atrophy, fasciculations, sensory deficits, dysphagia and signs of cerebellar involvement like limb and gait ataxia, dysarthria and oculomotor disturbances.

In addition, non-neurological symptoms are registered. Since ophthalmic symptoms are described in complicated HSP visual loss, retinitis pigmentosa, optic atrophy and cataract are included. Further complicating symptoms that are recorded are inter alia cognitive impairment, psychiatric symptoms and hearing impairment.

Validity and Spreading of SPRS

The SPRS developed by Schüle et al. in 2006 [32] was used as a measure of disease severity and progression as it is the only measurement method for disease severity and progression validated for HSP diseases that is known to us. In a cohort of 63 HSP patients (regardless of the molecular genetically confirmed mutant HSP gene), it was validated that 1) SPRS measures what it should

measure (high correlation with landmarks of disability, Barthel index and ICARS (a motor scale developed for rating of ataxia) in the absence of correlation with dementia test MMSE), 2) SPRS increases with disease duration (as far as this can be assessed in the heterogeneous small group), 3) SPRS displays no ceiling-effect (full score is achieved for each item without patients reaching the total score of 52), and 4) SPRS exhibits high interrater reliability (>0.99). According to the definition of HSP, patients have a SPRS total score of >0 due to the spasticity of lower limbs (hip adductor muscles and knee flexion measured by the modified Ashworth Scale as 2 of 13 items of the SPRS).

Moreover, the SPRS score as a measurement of disease severity is proven to correlate significantly with Health-Related Quality of Life in patients with HSP quantified by Mental and Physical Component summary scores [34]. A reduction of disease progression rate measured by SPRS may therefore be able to improve the long-term Health-Related Quality of Life in patients with HSP [34].

The relevance of the SPRS in the scientific research of HSP is reflected in its frequent application in clinical trials and in the over 100 citations (current state of 25.03.2019, e.g. [10, 34-36]) of the publication of Schüle et al. [32] in which the SPRS and its validity as a measurement tool for HSP was published.

Objectives of the SPG4 NHS

The counselling of patients concerning the progression of disease and their risk to become dependent on walking aid and wheelchair requires representative data on the natural course of disease. This data is also essential for the planning of interventional trials which aim to slow down disease progression and help to determine patient numbers needed to prove effectiveness of novel therapies. Therefore, we performed a longitudinal natural history study in a large cohort of 276 patients with genetically proven SPG4 to 1) describe the phenotypic spectrum of SPG4 and the frequency of complicating symptoms, 2) assess variability in age of onset, 3) calculate time to dependency on walking aids, 4) provide prospective data on disease progression, and 5) determine potential disease modifying factors. To this end we analyzed onset of gait difficulties, walking ability, the SPRS, complicating symptoms and potential modifying factors

like sex, type of *SPAST* variant and complicating symptoms in a longitudinal study over 12 years.

Patients and Methods

Clinical HSP networks and recruiting centers

Representatives of different medical disciplines set up the BMBF-funded German Network of Hereditary Movement Disorders (GeNeMove) in 2004 in order to study academical and clinical issues of rare hereditary movement disorders including HSP. A few years later GeNeMove was replaced by the German Centre of Neurodegenerative Diseases (Deutsches Zentrum für Neurodegenerative Erkrankungen DZNE). In this constitution Schüle et al. [32] developed the Spastic Paraplegia Rating Scale (SPRS) as a reliable and valid measure of disease severity. Outpatient clinics for HSP in Bochum, Bonn, Kiel, Magdeburg, Mainz, Munich, Regensburg, Rostock and Tübingen performed examinations based on the SPRS.

HSP registry

The outcomes of these examinations were consolidated in a central database of the Hertie Institute of Clinical Brain Research (Hertie Institut für klinische Hirnforschung HIH) in Tuebingen. Here each case gets a patient ID, family number, master data file, genetic data file and a file with documentation of each visit.

Informed consent

All participating patients were informed in detail about methods and objectives of this study, and gave written consent after adequate time of consideration.

The Ethics Committee of Faculty of Medicine, Eberhard Karls University of Tübingen, gave professional advice and approved this study (reference number: 623/2016BO2).

Inclusion criteria and data selection

The HSP registry was queried for cases with a clinical diagnosis of HSP and genetically suspected or confirmed SPG4. Asymptomatic variant carriers with genetically confirmed SPG4 were inquired as well. Clinical core data (e.g. demographic data, information about genetic status, age of onset etc.) as well as longitudinal data containing the SPRS and clinical examinations performed at each follow-up visit were extracted.

Quality control and missing data

Missing data

Next the missing data was investigated (*see* Appendix table 1*).*

All in all, we had in the beginning 20 examinations from 8 patients that had an incomplete total score due to missing data in at least 1 item, remaining 530 complete SPRS total scores from 186 patients. No case of missing values in the same item and patient in more than one examination could be found.

Completion of data

Missing information was supplemented from medical reports, reports of genetical diagnostic and "source data" (original SPRS scores on paper).

Additionally, the data set was updated by novel examinations, examinations not yet added to the HIH database and examinations of cases with recently genetically diagnosed SPG4. In the end, this led to enhancement of dataset with 811 follow-up examinations from 276 patients.

The completion, quality testing and data manipulation were concluded on July 30, 2016.

Manual control of data plausibility

SPRS

HSP is expected to progress continuously over time; sudden worsening as well as pronounced improvement are unlikely to occur due to the neurodegenerative nature of the disease. To check plausibility of the available SPRS data and identify potential systematic confounders, we therefore manually checked progression outliers. To this end, the Delta^{SPRS} was calculated as the difference between the SPRS total score between two visits divided by the visit interval in months. The 10% most extreme Delta^{SPRS} values (i.e. highest positive Delta^{SPRS} – fast progression; highest negative Delta^{SPRS} – fast improvement) were selected for plausibility control. For these cases, source data was consulted to rule out typos when transferring data from the paper CRF to the electronic database, and medical files were evaluated to identify potential medical confounders (e.g. surgery, other major health related events not related to HSP). No systematic confounders were identified, and all data was therefore included in the analysis.

Inventory

Next, data collected in the inventory was evaluated for plausibility. In some cases, "cognitive impairment" was noted as absent, yet an age of onset of cognitive impairment was given. Consultation of the recruiting physicians revealed that in all of these cases the age of onset of motor symptoms had been erroneously entered instead of the age of onset of cognitive impairment. Similarly, an unexpectedly high number of cases reported with visual loss turned out to be the result of incorrect interpretation of the rating instructions. The data was corrected accordingly.

Independent walking

Medical reports and original paper scores were investigated for information about availability and age of onset of walking aid and wheelchair to assess plausibility in these data.

According to Schüle et al. [10] patients having a SPRS total score of 35 points or more generally use walking device. Walking aid is required, if 3 or 4 points are rated in item 2. Maximum score in item 1 or 2 indicates wheelchair dependency. Consequently, cases meeting these criteria were further investigated.

Data manipulations

Every case was given an anonymous patient ID (from 1 to 276). The inventory coding as well as core data like gender, affection and the mode of inheritance were changed from text fields to a nominal level so it could be used for statistical analysis. The variant type was classified into two categories: "missense" (missense point variants and in frame deletions/insertions) and "truncating" variants (nonsense point variants, frame shift deletions/insertions, exon deletions/insertions).

Definition of a pure HSP phenotype and complicating signs and symptoms

Differentiation into pure and complicated forms of HSP has been suggested by Anita Harding [2, 28] to improve prognostic counselling of HSP patients and to foster homogeneity of cohorts for gene discovery. In her classification Harding regards HSP to be pure if spasticity is accompanied only by neurogenic bladder disturbance, impaired vibration sense, slight impairment of rapid alternating hand movements or mild distal atrophy in the upper limbs. Based on Harding's classification, we considered the following symptoms to be complicating signs: cognitive impairment/mental retardation, psychiatric symptoms, epilepsy/seizures, visual loss, cataract, cerebellar oculomotor disturbances, hearing impairment, dysarthria, dysphagia, limb and gait ataxia, extrapyramidal involvement, muscle atrophy and sensory neuropathy (sensory loss other than vibration sense). To assess the presence of complicating symptoms we analyzed the inventory of complicating signs and symptoms at baseline. For patients with complicating symptoms medical reports were investigated to preserve more detailed information.

Figures

Figures were created using SPSS release 24 and Overleaf (Online LaTeX-Editor). Kaplan Meier curves were created with SPSS (release 24) and with the help of Power Point the curves of total cohort and groups by age of onset of disease were overlaid.

Statistical analysis

Statistical analysis was performed in collaboration with Peter Martus, director of the Institute for Clinical Epidemiology and Applied Biometry, University Hospital of Tübingen. The α-level was 0.05 (two-sided) for significance testing. Adjustment for multiple testing was not carried out over the whole study (i.e. not for all p-values calculated), since it was an exploratory approach. In significance-testing for comparisons of medians and means, Bonferroni-correction was performed. Software used for statistical computations was SPSS (release 24).

Descriptive statistics

For the descriptive statistics of the cohort only the baseline examinations (first examination of each patient) were taken into account, to avoid bias by repeated examinations in same patients.

Explorative statistics were assessed to calculate absolute and relative frequencies, medians, mean values, standard deviations, and ranges (minimum and maximum). To assess whether normal distribution criteria were met, histogram, Q-Q-plot, skew, curtosis, Kolmogorov-Smirnov test and Levene's test were applied. If normal distribution criteria were met, independent t-tests were performed. Otherwise Mann-Whitney tests (comparison of two groups) or Kruskal-Wallis tests (comparison of more than two groups) were analyzed.

In our study cohort, related patients coming from the same family were included. Due to the possibility of bias by inclusion of related patients, the same analyzes were performed in which only the index patients (generally the first family member entered into the study) were included.

Loss of independent walking

The walking abilities were analyzed by Kaplan Meier and cox proportional hazard analyzes. To avoid repeated measurements of single patients, only baseline examination was included in the data analyzes.

Data were censored in case of an event, i.e. at the occurrence of walking aid or wheelchair dependency. The loss of independent walking ability was calculated based on the previous event, i.e. walking aid or in rare cases wheelchair dependency.

Cox proportional hazard analyzes were carried out to investigate the effect of age of onset of disease, variant type and gender on the loss of independent walking ability, as well as on walking aid or wheelchair dependency. To explore the effect of age of onset of disease, the cohort was divided into three groups: less than or equal to 20 years, 21 to 40 years, or at least 41 years of age. Two categories were distinguished regarding variant type (missense vs truncating variant carriers) and gender (female vs male), respectively.

Progression rate

To determine a progression rate of SPG4, the $Delta^{time}$ was calculated in years as the time difference between examinations and the $Delta^{SPRS}$ was calculated as the difference in SPRS total scores between examinations. Afterwards, the rate of progression was computed as the change in SPRS total score per time unit (progression rate = $Delta^{SPRS}$ / $Delta^{time}$).

For comparison and to avoid bias by repeated measurements of single patients with many examinations, we calculated different variants of progression rate likewise: A) including all $Delta^{time}$ (repeated measurements) and B) including only one $Delta^{time}$ per patient (from first to second examination).

Due to the neurodegenerative nature of disease, sudden change in disease severity is unlikely to occur. Therefore, short examination intervals of less than 3 months were excluded from the analysis of progression rate, as they led to large outliers without clinical impact.

Linearity of progression rate and major determinants

In order to identify major determinants of disease progression in SPG4 we performed mixed models. To find out if the disease progression is a steady deterioration over time, or if the rate of disease progression changes over the course of the disease, we performed growth models.

Model

We generated the basic model with dependent variable SPRS total score and predictor time (disease duration up to the time of examination). We applied a scaled identity covariance structure, assuming different covariance structures in random effects. Due to the fact that related patients coming from same families were included in our study, family membership was taken into account for calculations, by adding both family ID and interaction of family ID and patient ID as subjects to the model.

Growth models

We performed growth models by adding to the model described above disease duration as linear (time), quadratic (time*time) and cubic (time*time*time) term. We analyzed growth models for both all disease durations and special disease durations (<10 / <20 / 10 – 20 / ≥10 / ≥20 years), in order to determine whether progression rate is linear in the course of disease.

Mixed models

Next, we performed mixed models to identify major determinants on disease progression. We applied the model described above with disease duration as linear term. Possible factors influencing course of disease were added to the model as additional variables: age of onset of disease (aoo; groups 0 – 20 / 21 – 40 / >40 years), variant type (missense / truncating) and gender (female / male). For the least likelihood of erroneously removing factors from the model that have an impact on disease progression, we decided to use backward selection. Before removing a factor from the model, we tested whether there was an interaction between the factor and the predictor (disease duration). Starting with a mixed model containing all additional variables mentioned above, we removed factors that proved not to be significant stepwise.

The final model was compared for subjects including family ID and patients only (ignoring family membership).

Results

Demographic characteristics of the cohort

276 individuals with a genetically confirmed SPG4 were examined from 2004 to 2016 using the methods of the SPRS. 208 families were included (*see* Table 1). All in all, 811 examinations were done, 791 of which were complete.

In the study cohort we included 156 male and 120 female individuals being 10 to 77 years old, with an average age of 49.5 years (SD 12.9) (*see* Table 2). We had 270 patients with a clinically manifest SPG4 and 6 asymptomatic variant carriers. In 231 patients, coming from 164 families, family history was indicative of autosomal dominant inheritance with individuals in 2 or more generations being affected by a spastic gait disorder. In 38 patients the mode of inheritance was sporadic, and in 1 index patient affection of several individuals in just one generation suggested apparently recessive disease (*see* Appendix Case report of apparently recessive mode of inheritance).

The mean age of onset of disease of the total cohort was 31.6 years (SD 15.7; range 0 - 70). The average age at baseline was 49.5 years (SD 12.9; range 10 – 77) and the disease duration 18.1 years (SD 12.1; range 0 – 63). The average SPRS total score at baseline we found to be 17.2 (SD 9.7; range 0 – 44) (*see* Table 2). Including follow-up visits, the maximum SPRS score was 47.

Evaluating total cohort at baseline, we could find no significant differences in age at study onset, age at onset of disease, disease duration and SPRS total score regarding gender, mode of inheritance and variant type (p≥0.054, see Table 3).

In our cohort we identified 135 cases using walking aid on a regular basis, with a mean age of onset of walking aid dependency of 47.4 years (SD 12.8; range 1 – 73) (see Table 4). In the 50 cases with wheelchair dependency, the mean age of onset of wheelchair dependency was 48.4 years (SD 14.4; range 0 – 72) (see Table 4).

We found a significantly lower age of onset of wheelchair dependency in dependency of variant type: while in missense variant carriers wheelchair dependency occurred at an average age of 38.5 years (SD 19.5; range 0 – 63),

truncating variant carriers became wheelchair dependent in average 12 years later (50.3 years; SD 11.2; range 23 – 72) (t(46) = -2.574, r=0.91, p<0.017; see Table 5). Wheelchair dependency from early childhood occurred only in missense variant carriers (see Table 4).

Further comparisons of the age of onset of walking aid and wheelchair dependency regarding gender, mode of inheritance and variant type revealed no significant differences (p≥0.444, see Table 5).

In the total cohort of 276 individuals, 107 cases had only 1 examination whereas 117 cases had at least 3 follow up examinations (*see* Table 6).

Table 1: families

Number of families	Number of family members
169	1
25	2
8	3
5	4
0	5
1	6
1	7

Table 2: description of the study cohort at baseline

Means, with standard deviations and ranges in brackets. n: frequencies.

	age of study onset (years)	age of onset of disease (years)	disease duration at baseline (years)	SPRS total score at baseline
total cohort (n = 276)	49.5 (12.9; 10 - 77)	31.6 (15.7; 0 - 70)	18.1 (12.1; 0 - 63)	17.2 (9.7; 0 - 44)
gender				
male (n=156)	49.6 (12.1; 12 - 77)	31.5 (15.5; 0 - 65)	18.1 (11.5; 0 - 52)	16.7 (9.6; 0 - 44)
female (n=120)	49.3 (14.0; 10 - 74)	31.8 (16.0; 0 - 70)	18.0 (12.8; 0 - 63)	17.8 (9.9; 0 - 44)
mode of inheritance[1]				
dominant (n=231)	49.5 (12.9; 10 - 77)	30.9 (15.6; 0 - 65)	18.8 (12.5; 0 - 63)	17.1 (9.8; 0 - 44)
sporadic (n=38)	49.2 (13.8; 12 - 73)	34.4 (15.4; 0 - 63)	14.9 (8.7; 3 - 40)	18.1 (9.5; 3 - 43)
variant type[2]				
truncating (n=211)	49.2 (12.9; 10 - 77)	31.6 (15.3; 0 - 65)	18.0 (12.0; 0 - 60)	16.4 (9.7; 0 - 44)
missense (n=47)	48.2 (13.6; 12 - 73)	29.3 (17.9; 0 - 70)	18.5 (13.7; 0 - 63)	19.5 (9.4; 5 - 44)
[1] information missing in 6 cases; 1 case apparently autosomal recessive				
[2] information missing in 18 cases; counts are based on individual cases				

Table 3: test statistics description of the study cohort at baseline

Results from Mann-Whitney tests comparing age at study onset (years), age at onset of disease (years), disease duration at study onset (years) and SPRS total score at study onset for gender (female vs male), mode of inheritance (dominant vs sporadic) and variant type (missense vs truncating). U: test statistic, n=number of total observations, z: z-score, r: estimated effect size, p: significance. Results are significant if p<0.017

		U	n	z	r	p
age of study onset (years)	gender	9353	276	-0.01	-0.00	0.992
	mode of inheritance	4382.5	269	-0.02	-0.00	0.989
	variant type	4785	258	-0.38	-0.02	0.709
age of onset of disease (years)	gender	8054	261	-0.45	-0.03	0.653
	mode of inheritance	3389.5	254	-1.51	-0.09	0.131
	variant type	4068.5	243	-0.73	-0.05	0.465
disease duration at baseline (years)	gender	8069	261	-0.43	-0.03	0.672
	mode of inheritance	3347.5	254	-1.62	-0.10	0.106
	variant type	4360.5	243	-0.04	-0.00	0.967
SPRS total score at baseline	gender	8170	270	-1.23	-0.07	0.220
	mode of inheritance	3913	265	-0.49	-0.03	0.626
	variant type	3897	253	-1.93	-0.12	0.054

Table 4: age of onset of walking aid and wheelchair dependency

Mean age of onset of walking aid and wheelchair dependency for total cohort and comparisons by categories gender (male / female), mode of inheritance (dominant / sporadic) and variant type (missense / truncating). n: frequency, SD: standard deviation.

		Age of onset walking aid dependency				Age of onset wheelchair dependency			
		n	**mean**	SD	range	n	**mean**	SD	range
Total cohort		135	**47.4**	12.8	1 – 73	50	**48.4**	14.4	0 – 72
	male	71	**47.5**	12.8	1 – 73	24	**47.7**	13.0	8 – 69
	female	64	**47.3**	12.9	3 – 72	26	**49.0**	15.9	0 – 72
	dominant	113	**47.3**	11.5	16 – 73	44	**49.3**	11.1	23 – 70
	sporadic	17	**46.2**	19.9	1 – 69	6	**41.5**	30.2	0 – 72
	missense	22	**43.1**	19.1	1 – 70	11	**38.5**	19.5	0 – 63
	truncating	103	**47.7**	11.2	16 – 73	37	**50.3**	11.2	23 – 72

Table 5: test statistics age of onset of walking aid and wheelchair dependency

Comparisons of age of onset (years) of walking aid respectively wheelchair dependency in female vs male, dominant vs sporadic mode of inheritance, and missense vs truncating variant carriers. If normal distribution criteria and homogeneity of variances were met, independent t-tests were analyzed. Otherwise Mann-Whitney tests were performed. t: test statistics for independent t-test, degrees of freedom in brackets. U: test statistic, number of total observations, z: z-score. r: estimated effect size, p: significance. Results are significant if p<0.017.

		t	r	p
Age of onset walking aid dependency	gender	t(133) = 0.091	0.00	0.928
	mode of inheritance	t(128) = 0.333	0.01	0.825
	variant type	U(125) = 1014, z = -0.772	-0.07	0.444
Age of onset wheelchair dependency	gender	t(48) = -0.304	0.01	0.762
	mode of inheritance	U(50) = 126, z = -0.179	-0.03	0.867
	variant type	t(46) = -2.574	0.91	0.013

Table 6: follow-up examinations

follow-up	Frequency of occurrence	Patients with n examinations at all
1	276	107
2	169	52
3	117	40
4	77	24
5	53	18
6	35	8
7	27	6
8	21	5
9	16	5
10	11	6
11	5	3
12	2	0
13	2	2
total	811	276

Bimodal distribution of the age of onset of disease

The age of onset of a spastic gait pattern has impact on the initiated genetic testing. Furthermore, the age of onset of disease is commonly used to determine disease progression, if cross-sectional instead of longitudinal data is analyzed. We therefore aimed to further examine age of onset of disease and possible influencing factors.

We found a bimodal distribution of the age of onset of disease, with a first peak in early childhood at age of 0 – 5 years and a second peak in adulthood around the age of 40 years (see Figure 1).

In order to identify influencing factors on distribution, we compared the distribution of the age of onset of disease in groups missense vs truncating variant carriers

(see Figure 2) and female vs male (see Figure 3). We found no differences in distribution of the age of onset between males and females.

Figure 2 illustrated higher frequencies of age of onset of disease in all age categories, due to the four times larger proportion of truncating than missense variants carriers within our cohort (211 truncating vs 47 missense variant carriers).

If instead of absolute frequencies (indicated by bar heights) relative frequencies are compared, differences of the distribution of age of onset of disease can be found. In missense variant carriers, we find similar frequencies in both peaks of the bimodal distribution, i.e. as many cases with age of onset of disease in early childhood (age 0 – 5 years) as in adulthood (age 36 – 40 years). However, in truncating variant carriers the second peak of the bimodal distribution is about twice as high as the first peak, i.e. among truncating variant carriers most become affected in adulthood and only a few in early childhood.

To summarize, the relative proportion of early onset SPG4 is larger in missense than in truncating variant carriers.
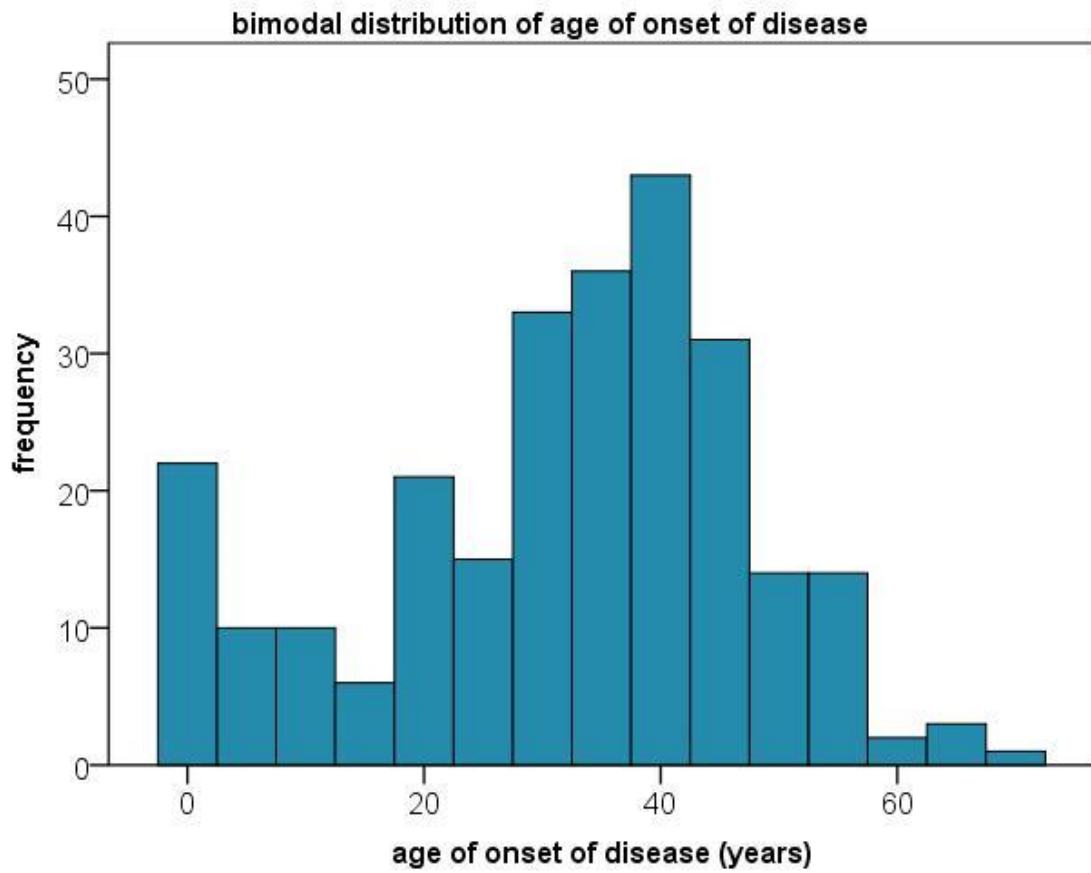
Figure 1: distribution of the age of onset of disease (total cohort)
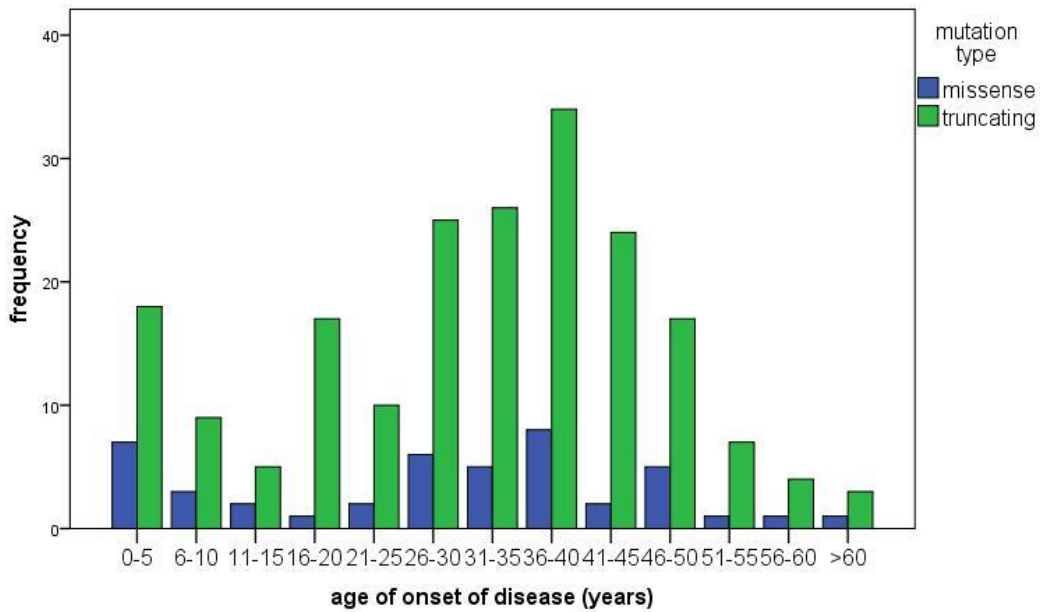
Figure 2: distribution of the age of onset of disease comparing missense and truncating variant carriers
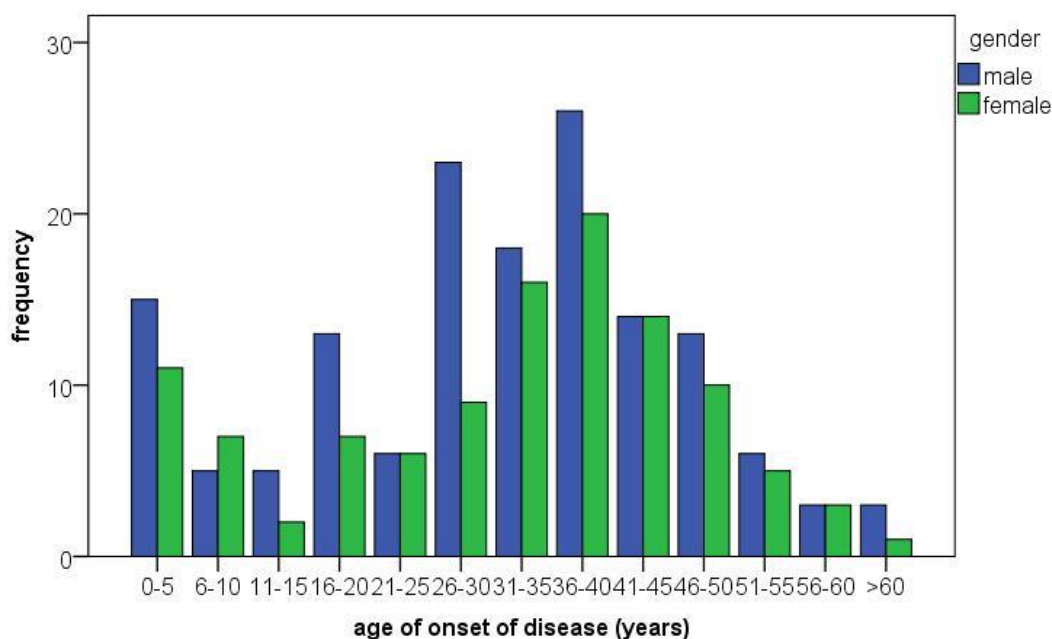
Figure 3: distribution of the age of onset of disease comparing male and female

Intrafamilial variability of the age of onset of disease

The age of onset of SPG4 is variable even within families carrying the same variant. In 35 of the families included in the study cohort, the age of onset was known in more than one family member. Without taking into account the family relationship of the patients and the number of affected family members being included in the cohort, we found the age of onset to range intrafamilial from 1 to 59 years (*see* Appendix table 8), with an average range of 21.0 years.

Variant spectrum of the cohort

The cohort was categorized into two groups by variant type: carriers of missense (missense point variants, inframe deletions and insertions) and truncating variants (nonsense variants, splice site variants, frameshift deletions and insertions, exon deletions and insertions).

The exact genomic variant was reported in 258 cases from 191 families, in the remaining 18 cases only the information "SPG4" was known and these cases were excluded from further analysis concerning variant type. The group containing missense variants included 18.2% (47/258 cases) and the group of truncating variant carriers contained 81.8% of the total cohort (211/258 cases) (see Appendix table 2 - Appendix table 7 *columns patients*).

In Figure 4 the variant spectrum of our cohort is represented in relation to the functional domains and exon borders of spastin. Above the functional domains missense variants are represented, below the exons truncating variants.

The 616 amino acids of M1 spastin are represented by little rectangles and the functional domains are mapped below.

When contemplating the frequency of individual variant types, it is necessary to consider the frequency of several family members and, accordingly, the number of families in which certain variant types occur. Therefore, the following information refers to the number of different families in which certain variants occur (see Appendix table 2 - Appendix table 7 *columns families*).

Missense variants were disease causing in 20.4% of our cohort (39/191 families), whereas 4 of these had inframe deletions respectively insertions and the main proportion missense point variants (see Appendix table 2 and Appendix table 3). Regarding the contribution of these variants on the functional domains of spastin, it is striking that only 4 families had variants concerning amino acids that are not part of the AAA cassette (see Figure 4*)*. 85.7% of the missense variants affected the AAA cassette.

In 79.6% of the cohort (152/191 families) the disease-causing variant was classified as being truncating. These can be broken down to 49 families with nonsense variants (25.7%), 40 with frameshift deletions/insertions (20.9%), 37 exondeletions/duplications (19.4%) and 26 splice-site variants (13.6%) (*see* Appendix table 4 - Appendix table 7).

The most frequent variant in our cohort was the nonsense variant c.1684C>T, which was disease causing in 14 independent families. All other variants occurred in less than five unrelated families.

Figure 4: variant spectrum of the total cohort
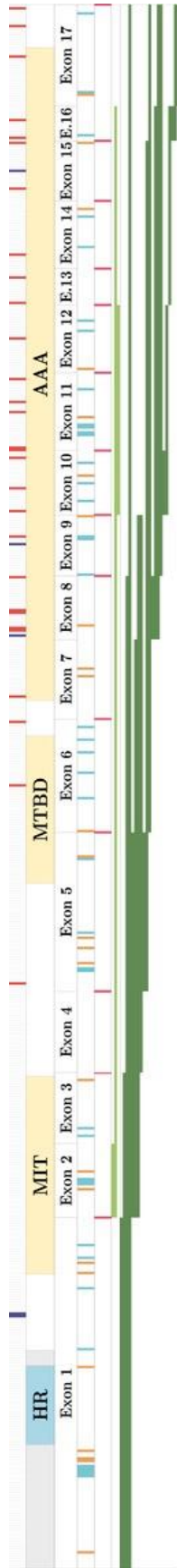
functional domains of spastin

MIT: microtubule interacting and trafficking domain (amino acids 116-194)

MTBD: microtubule binding domain (amino acids 270-328)

AAA: ATPase associated with various cellular activities (amino acids 342-599)

amino acid 1-87 (only present in M1 isoform)

HR: hydrophobic region (amino acids 49-80)

category: missense mutations (rectangles above functional domains represent 616 amino acids of spastin, missense mutations highlined)

missense point mutations

inframe deletions/insertions

category: truncating mutations (represented below functional domains and exons 1-17)

nonsense mutations

frameshift deletions/insertions

splice site mutations (mapped to corresponding exon borders)

exon duplications (bar extending over whole affected region)

exon deletions (bar extending over whole affected region)

The 616 amino acids of M1 spastin are represented by little rectangles, the 17 exons are outlined by bars and the functional domains are mapped in between.

Above the functional domains missense mutations are represented, below the exons truncating mutations.

Exon deletions (darkgreen) and duplications (brightgreen) are represented by bars extending over the whole affected region.

Phenotype

Phenotype studies were performed only on the clinically manifest SPG4 variant carriers. To avoid bias by repeated testing of patients with more than one examination, the following analyzes were performed only for the baseline examinations.

In the examination using SPRS, different symptoms of HSP/SPG4 and their severity are rated in 13 different items, with 0 points corresponding to normal findings and 4 to particularly severe symptoms. Figure 5 presents an overview of the frequency of occurrence of the different degrees of severity of the 13 Items of SPRS if score >0 (corresponding to normal findings) was rated.

The most common symptoms found in our cohort with occurrence in more than 90% of cases were spasticity of knee flexion (item 8) and spastic gait (item 2) as well as reduced maximum gait speed (item 3) and speed of stair climbing (item 5). A spasticity of lower limbs as key symptom of HSP/SPG4 was found in nearly 97%.

We found pain due to spastic paraplegia related symptoms (item 12) and weakness of foot dorsiflexion (item 10) to be the less common symptoms, which were still present in about 50% of our cohort. If, weakness of foot dorsiflexion and hip abduction are summarized as weakness of lower limbs, we found about 70% of our cohort to be affected.

Accordingly, 55-90% showed to be affected in the remaining items, that is (in ascending order of occurrence) arising from chair (item 6), contractures of lower limbs (item 11), weakness of hip abduction (item 9), impairment of bladder and bowel function (item 13), walking distance without pause (item 1), climbing stairs (item 4) and spasticity of hip adductor muscles (item 7).

In addition, half of our cohort (132 patients; 50.4%) presented with impairment of vibration sense.

More detailed information about the frequencies with exact number of cases and percentages as well as examination and evaluation descriptions for each item as in SPRS paper score are outlined in further figures and tables and described below (*see* Figure 6 - Figure 15 *and* Table 7 - Table 13).

item 1: walking distance without pause
item 2: gait quality
item 3: maximum gait speed
item 4: climbing stairs
item 5: speed of stair climbing
item 6: arising from chair
item 7: spasticity of hip adductor muscles
item 8: spasticity of knee flexion
item 9: weakness of hip abduction
item 10: weakness of foot dorsiflexion
item 11: contractures of lower limbs
item 12: pain due to spastic paraplegia related symptoms
item 13: impairment of bladder and bowel function

Figure 5: frequency of occurrence and degrees of severity of SPRS items

For the 13 items of SPRS the frequency of occurrence of the different degrees of severity at baseline are illustrated. The lowest bar (dark blue) represents maximum symptom severity (score 4). Towards the top, the bars represent decreasing symptom severity, with severe manifestation of symptom (score 3) illustrated pale blue, moderate symptomatic (score 2) illustrated dark green, and mild symptomatic (score 1) illustrated bright green. Normal findings (score 0) are represented yellow. Absolute and relative frequencies are reported in respective sections.

Spasticity of lower limbs

Of the 270 cases evaluated as being affected at baseline, 261 patients (96.7%) presented with lower limb spasticity, with score referring to spasticity of the more severely affected side of hip adductor muscles and knee flexion, according to the Ashworth Scale (see Table 7).

Spasticity of hip adductor muscles was found in 84.8 % (229 cases). In more than half of these cases (117 cases) only a slight increase in muscle tone could be found.

Spasticity of knee flexion was marked in even more cases: 94.0% (254 cases) of our affected cases had at least a slight increase in muscle tone of the quadriceps femoris muscle, whereby only in 13.7% (37 cases) passive movement was difficult or not possible. Though spasticity in knee flexion was found in more cases than spasticity of hip adductor muscles, no case was found with considerable increase in muscle tone with difficulty in passive movement of one joint while the other was completely unaffected.

Table 7: spasticity of lower limbs

		Spasticity – knee flexion *					Σ
		0	1	2	3	4	
Spasticity – hip adductor muscles	0	9	28	4	0	0	41
	1	5	72	37	2	0	116 (117)*
	2	1	20	42	16	0	79
	3	0	4	10	17	0	31
	4	0	0	0	1	1	2
Σ		15	124	93	36	1	269

0: No increase in muscle tone

1: Slight increase in muscle tone, manifested by a catch and release

2: More marked increase in muscle tone through most of the range of motion

3: Considerable increase in muscle tone – passive movement is difficult

4: Limb stiff in adduction

* Information for spasticity knee flexion missing in 1 case

Weakness of lower limbs

In 70.7% (191 cases) a weakness of lower limbs was documented, either in foot dorsiflexion (138 cases; 51.1%) and or hip abduction (164 cases; 60.7%) (see Table 8). If weakness was severe in either foot dorsiflexion or hip abduction, that means no movement was possible against resistance or gravity, at least a mild weakness was found in the other part as well.

When considering the weakness in hip abduction as a function of spasticity in hip adduction, we found a trend of increasing hip weakness with increased hip spasticity (*see* Table 9). Overall, the scores for hip spasticity were usually equal to or slightly higher than those for hip weakness. However, in only one case did a patient present such severe hip weakness, that it was considered plegic. This was not, as expected, associated with a particularly pronounced hip spasticity, but only with a slight increase in muscle tone.

On the other hand, particularly severe spasticity of the hip adductors was accompanied by a moderate (Medical Research Council Scale 3/5) to severe (1-2/5) weakness of the hip abductors.

Table 8: weakness of lower limbs

		Weakness – foot dorsiflexion					Σ
		0	1	2	3	4	
Weakness – hip abduction *	0	78	25	2	0	0	105
	1	47	51	12	1	0	111
	2	7	11	14	6	1	39
	3	0	1	3	9	0	13
	4	0	0	0	0	1	1
Σ		132	88 (89)*	31	16	2	269

0: No weakness

1: Mild weakness (4/5)

2: Moderate weakness (3/5)

3: Severe weakness (1-2/5)

4: Plegia (0/5)

* Information for weakness hip abduction missing in 1 case

Table 9: Spasticity and weakness of hip

		Weakness - hip abduction *					Σ
		0	1	2	3	4	
Spasticity – hip adductor muscles	0	27	13	0	0	0	40 (41)*
	1	59	46	10	1	1	117
	2	17	41	18	3	0	79
	3	2	11	10	8	0	31
	4	0	0	1	1	0	2
Σ		105	111	39	13	1	269

Spasticity – hip adductor muscles	**Weakness - hip abduction**
0: No increase in muscle tone	**0**: No weakness
1: Slight increase in muscle tone, manifested by a catch and release	**1**: Mild weakness (4/5)
2: More marked increase in muscle tone through most of the range of motion	**2**: Moderate weakness (3/5)
	3: Severe weakness (1-2/5)
3: Considerable increase in muscle tone – passive movement is difficult	**4**: Plegia (0/5)
	* Information for weakness hip abduction missing in 1 case
4: Limb stiff in adduction	

Contractures of lower limbs

More than half of our cohort (160 cases; 59.3%) presented Contractures of lower limbs (*see* Figure 6). In 94 cases (34.8%) at least one joint was fixed in abnormal position.
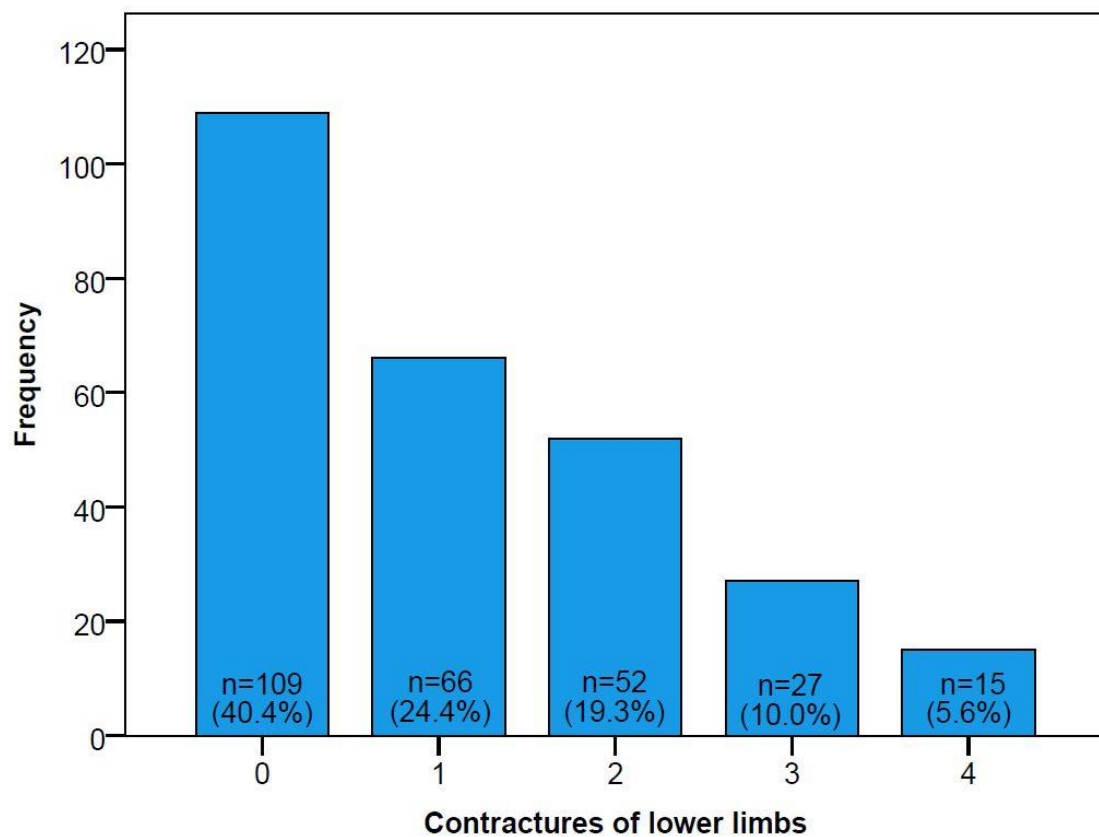
Figure 6: contractures of lower limbs

Score in supine position
- *Hip extension: lumbar spine and thighs touch the underlay;*
- *Hip abduction: angle of >60° between the legs possible*
- *Knee extension: thigh and calf touch the underlay*
- *Ankle dorsal extension: >10° possible;*
- *Ankle pronation: >10° possible*

0: No contractures

1: Mild, not fixed abnormal position of one joint (unilaterally or bilaterally)

2: Fixed contracture of one joint (unilaterally or bilaterally)

3: Fixed contracture of two joints (unilaterally or bilaterally)

4: Fixed contracture of more than two joints (unilaterally or bilaterally)

Movement abilities that are determinant for everyday life

Gait quality, walking distance without pause and maximum gait speed

The gait quality was impaired in 97.4% (263 cases) of the affected cases at baseline (*see* Figure 7). In 74.4% (201 cases) the impairment was interfering with the ability to run.

The maximum gait speed, measured by time to walk a 10m distance including one turn, was reduced in 91.1% (246 cases) to more than 5s to perform task. The majority (178 cases; 65.9%) needed 5-20s to perform task. 10.0% (27 cases) were unable to perform task or required more than 40s (*see* Figure 8).

The walking distance without pause was limited to a distance of less than 500m in 35.9% (97 cases). 5 cases (1.9%) were unable to walk. In 23.3% (63 cases) the walking distance was reported not to be limited due to spasticity (*see* Figure 9).

We found the impairment in the gait quality (item 2) to be prior to the walking distance without pause (item 1). That means, that a stiffness while walking can be observed before the walking distance is reduced due to abnormal exhaustion caused by spasticity of the lower limbs. We found the score for the gait quality, that means for the second item of the SPRS, to be always equal or higher than the score for the walking distance without pause, that means the first item of the SPRS (*see* Table 10).
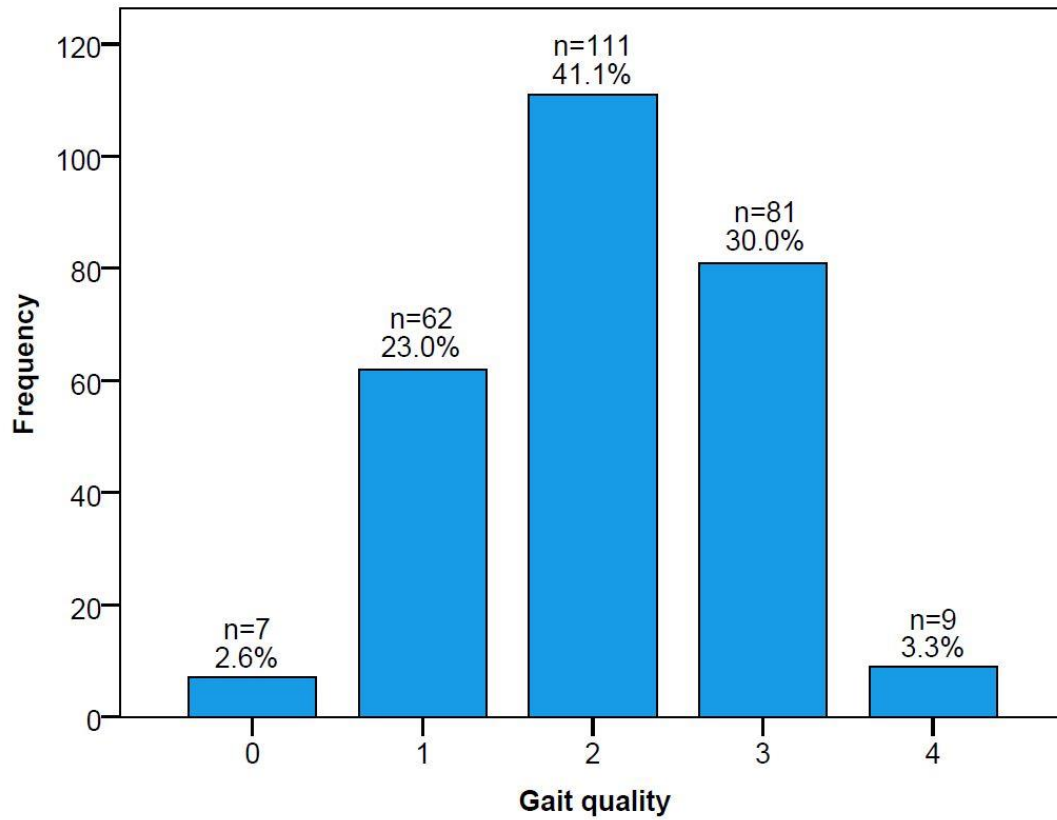
Figure 7: gait quality

Patient is asked to walk as fast as possible a 10 meter distance including one turn
0: Normal
1: Mild stiffness, running still possible
2: Clearly spastic gait, interfering with running
3: Spastic gait requiring use of canes/walker
4: Unable to walk for a 10 meter distance even with maximal support
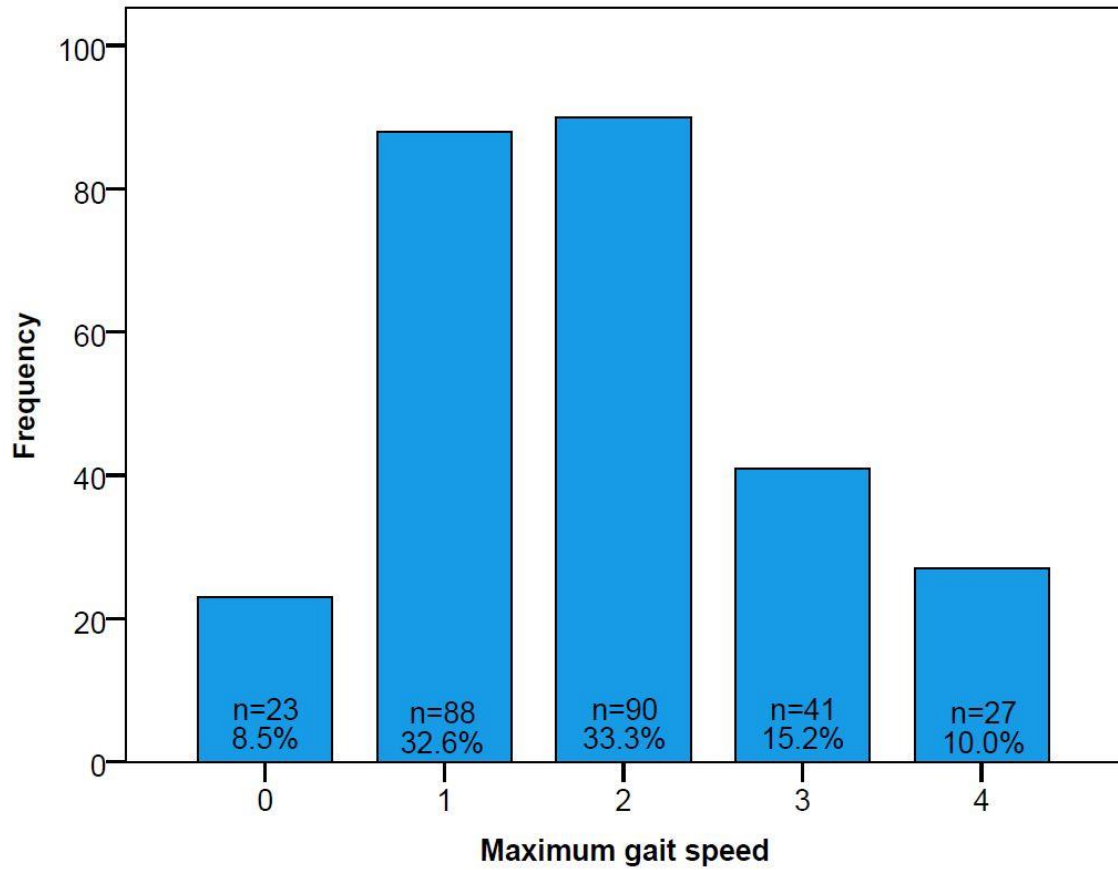
Figure 8: maximum gait speed

Time for a 10 meter distance including one turn
0: Normal
1: Slightly reduced (10m: ≥5s)
2: Moderately reduced (10m: ≥10s)
3: Severely reduced (10m: ≥20s)
4: Unable to walk for a 10 meter distance or time ≥40s
*Information missing in 1 case
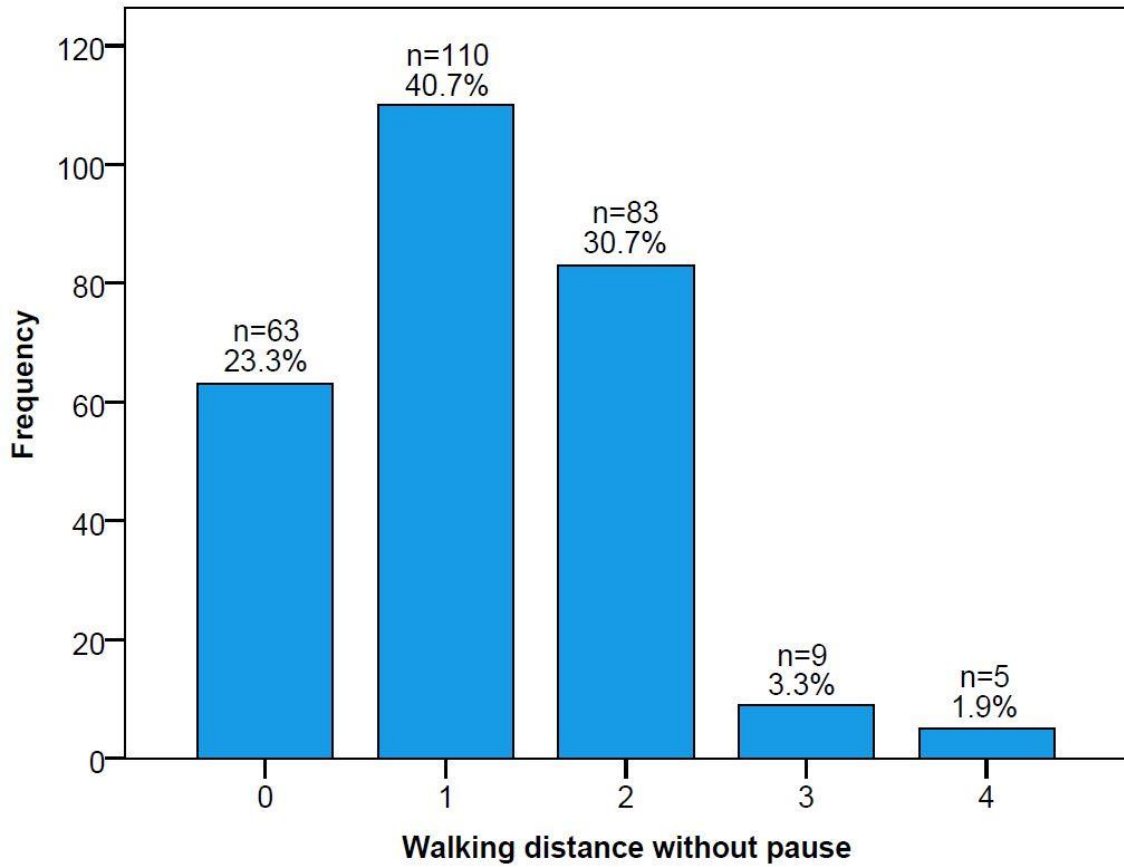
Figure 9: walking distance without pause

0: Normal, unlimited

1: Abnormal exhaustion due to spasticity after more than 500m

2: Walking distance less than 500m

3: Walking distance less than 10m

4: Unable to walk

Table 10: gait

		Walking distance without pause					Σ
		0	1	2	3	4	
Gate quality	0	7	0	0	0	0	7
	1	40	22	0	0	0	62
	2	16	67	28	0	0	111
	3	0	21	55	5	0	81
	4	0	0	0	4	5	9
Σ		63	110	83	9	5	270

Gait quality	**Walking distance without pause**
0: Normal	**0**: Normal, unlimited
1: Mild stiffness, running still possible	**1**: Abnormal exhaustion due to spasticity after more than 500m
2: Clearly spastic gait, interfering with running	**2**: Walking distance less than 500m
3: Spastic gait requiring use of canes/walker	**3**: Walking distance less than 10m
4: Unable to walk for a 10 meter distance even with maximal support	**4**: Unable to walk

Climbing stairs

We found that 79.6% (215 cases) had lost the ability to climb stairs without at least intermittent support of the banister. In 110 cases (40.7%) permanent support of the banister was required. 4.8% (13 cases) were unable to climb stairs (see Figure 10).

The speed of stair climbing was measured by taking the time to climb 5 steps upstairs, turn, and climb 5 steps downstairs. No reduction in the speed of stair climbing could be observed in 9.3% (25 cases). However, in 85.6% (231 cases) the ability to climb stairs was preserved, though the time to perform this task was reduced, that means that more than 5 seconds were needed to perform task. In 20.7% (56 cases) 20 – 40 seconds were needed to perform task (*see* Figure 11).
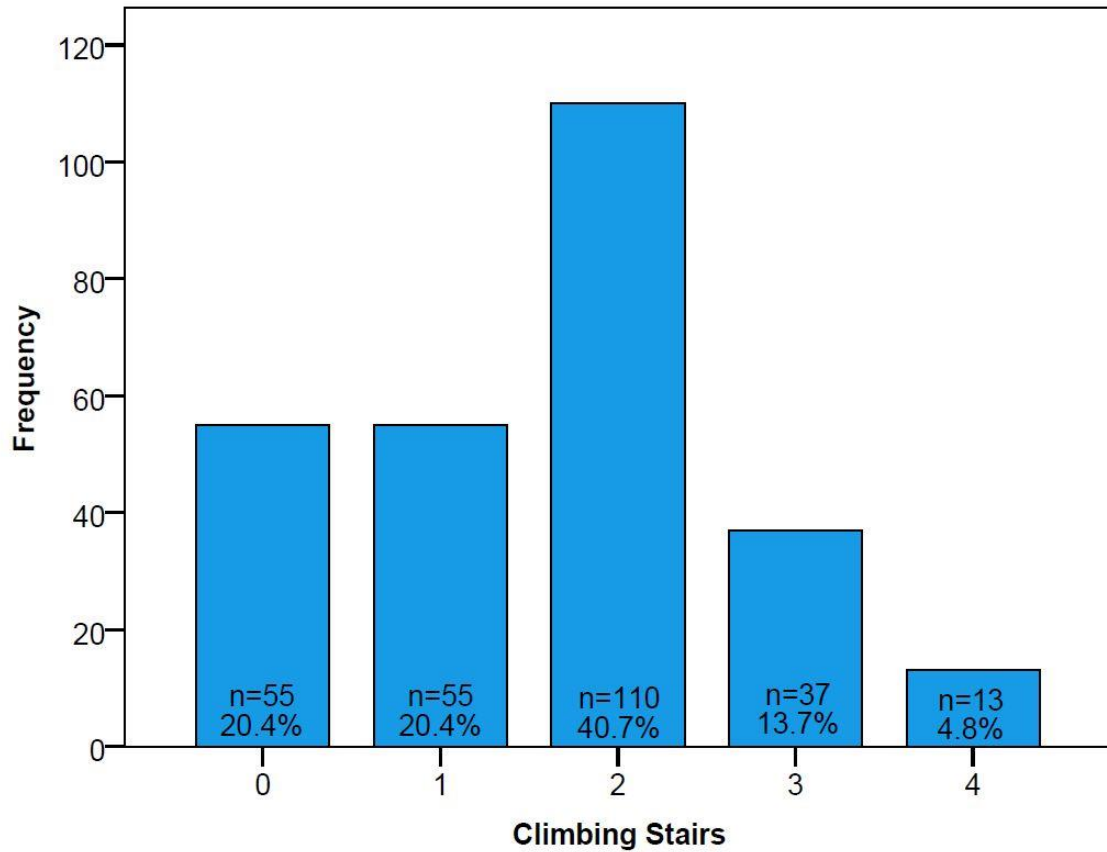
Figure 10: climbing stairs

5 steps upstairs – turn – 5 steps downstairs

0: Normal, needs no support of the banister

1: Mild impairment, needs intermittent support of the banister

2: Moderate impairment, needs permanent support of the banister

3: Severe impairment, needs support of another person or additional walking aid to perform task

4: Unable to climb stairs
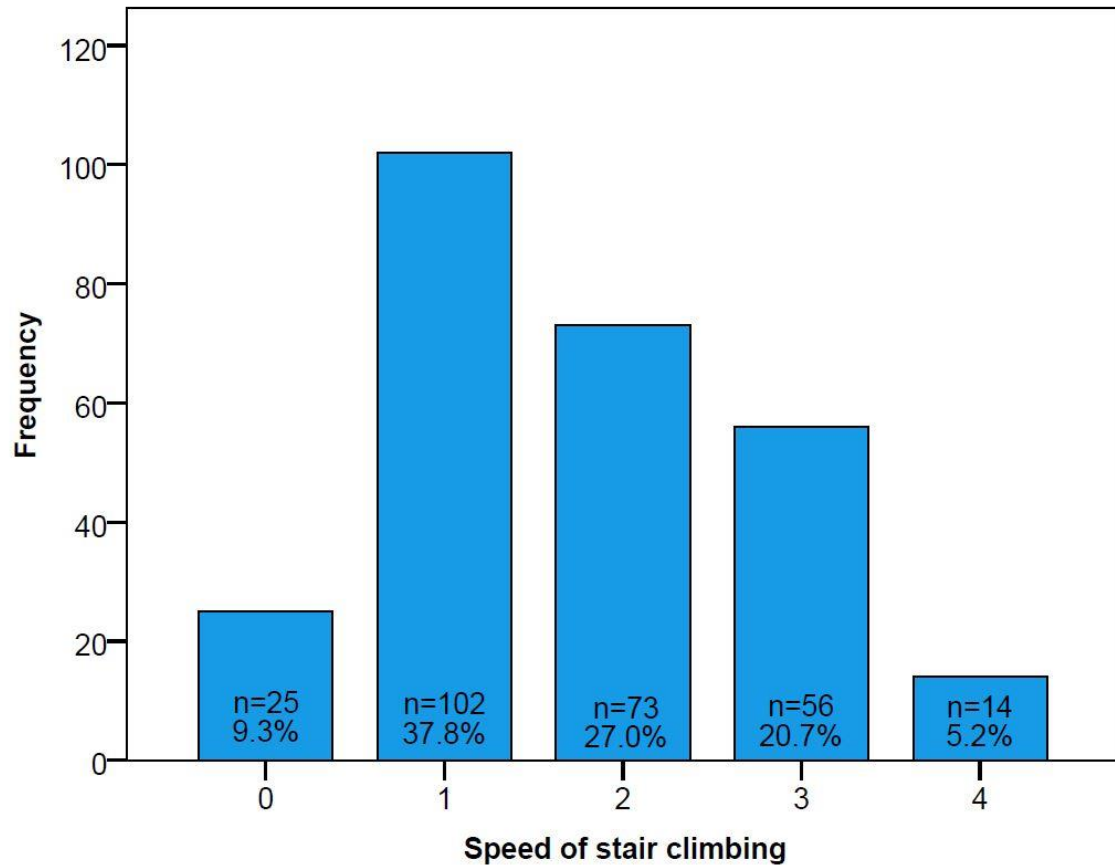
Figure 11: speed of stair climbing

Time for 5 steps upstairs, turn, 5 steps downstairs
0: Normal
1: Slightly reduced (≥5s to perform task)
2: Moderately reduced (≥10s to perform task)
3: Severely reduced (≥20s to perform task)
4: Unable to climb stairs

Speed of walking and climbing stairs

No reduction of speed neither in the consideration of gait nor climbing stairs was found in only 6.3% (17 cases). In the majority of cases (238 cases; 88.1%) both maximum gait speed and speed of climbing stairs was reduced. A reduction of maximum gait speed went along with a reduction of climbing stairs and the other way around, whereas no prediction can be made about which task will be the first to be restricted (see Table 11).

Table 11: speed of walking and stair climbing

		Speed of stair climbing					Σ
		0	1	2	3	4	
Maximum gait speed *	0	17	6	0	0	0	23
	1	8	69	11	0	0	88
	2	0	26	53	10	1	90
	3	0	0	9	30	2	41
	4	0	0	0	16	11	27
Σ		25	101 *	73	56	14	269

Maximum gait speed: Time for a 10 meter distance including one turn	**Speed of stair climbing**: Time for 5 steps upstairs, turn, 5 steps downstairs
0: Normal	**0**: Normal
1: Slightly reduced (10m: ≥5s)	**1**: Slightly reduced (≥5s to perform task)
2: Moderately reduced (10m: ≥10s)	**2**: Moderately reduced (≥10s to perform task)
3: Severely reduced (10m: ≥20s)	**3**: Severely reduced (≥20s to perform task)
4: Unable to walk for a 10 meter distance or time ≥40s	**4**: Unable to climb stairs
*Information missing in 1 case	

Arising from chair

The ability to arise from chair was measured, by rating the speed, the amounts of attempts and the need of help in order to arise from a straight-back wood or metal chair with arms folded across chest. In nearly half of our cohort (121 cases; 44.8%) no impairment in this task could be found. In 26.3% (71 cases) the task was performed slowly or more than one attempt was needed. The help of arms of seat to push self-up was required in 17.4% (47 cases) (*see* Figure 12).
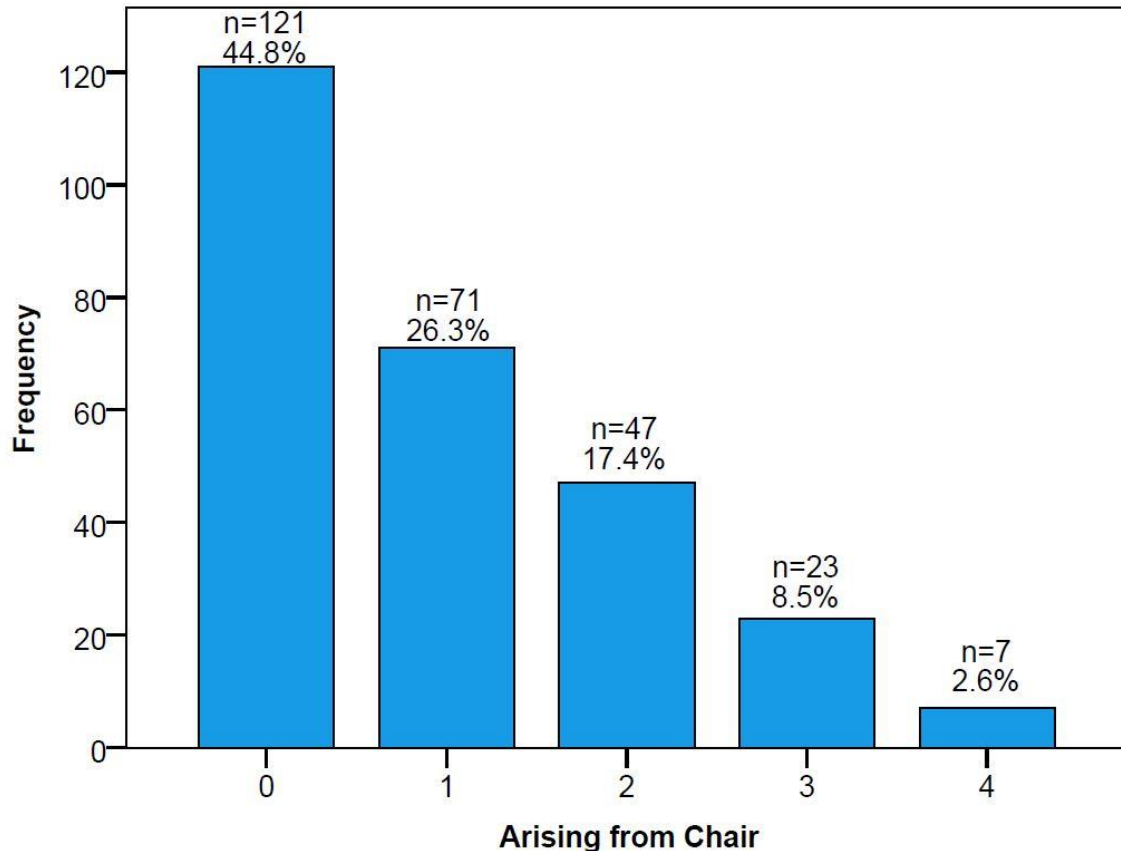
Figure 12: arising from chair

Patient attempts to arise from a straight-back wood or metal chair with arms folded across chest
0: Normal
1: Slow, or may need more than one attempt
2: Pushes self-up from arms of seat
3: Tends to fall back and may have to try more than one time but can get up without help
4: Unable to arise without help

Pain due to spastic paraplegia related symptoms

Pain due to spastic paraplegia related symptoms was reported in about every second case (133 cases; 49.3%). In 33.3% (90 cases) the pain was less than half of waking day present (item 1 + item 2). In 23.3% (63 cases) the intensity of pain was rated to be 4 – 10 points on visual analogue scale (item 2 + item 4) (*see* Figure 13).
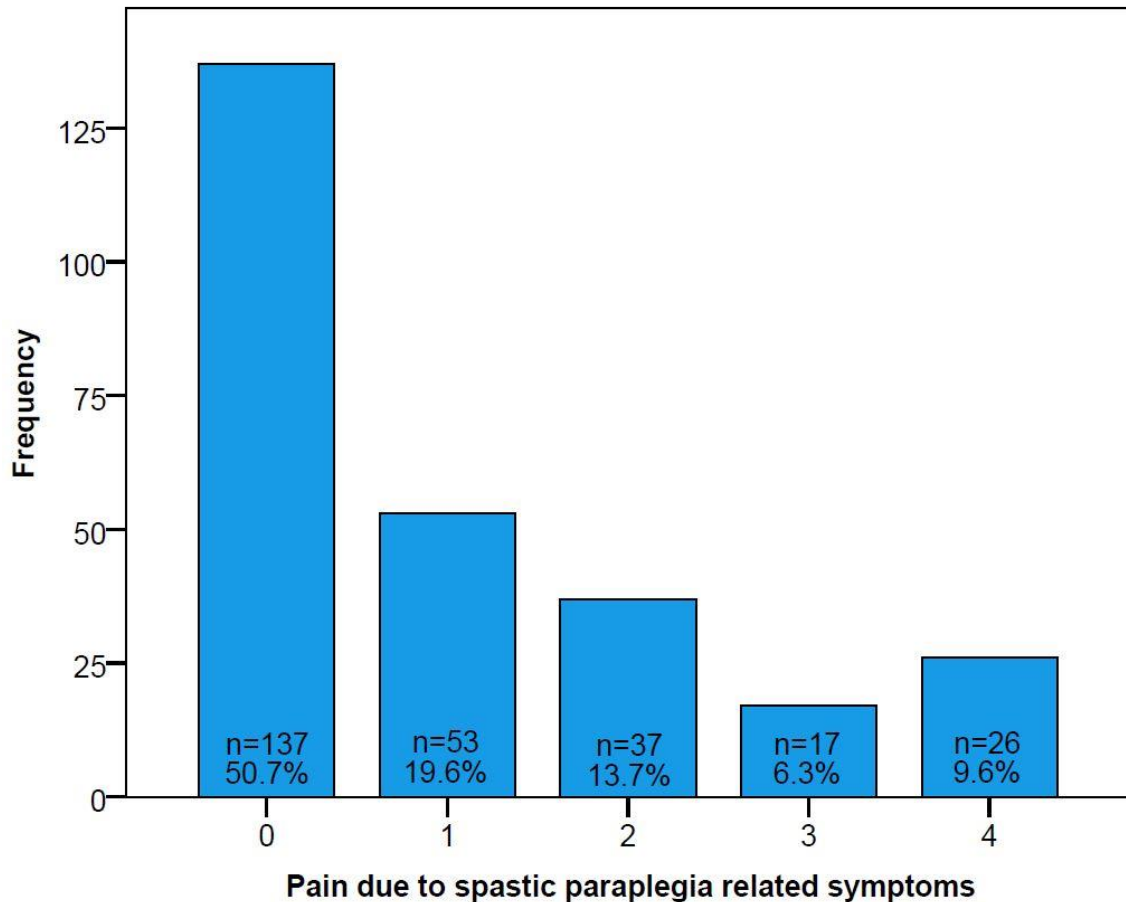
Figure 13: pain due to spastic paraplegia related symptoms

0: None
1: ≤ 50% of waking day present and intensity 0 – 3 points on visual analogue scale
2: ≤ 50% of waking day present and intensity 4 – 10 points on visual analogue scale
3: >50% of waking day present and intensity 0 – 3 points on visual analogue scale
4: >50% of waking day present and intensity 4 – 10 points on visual analogue scale

Bladder and bowel function

An impairment of bladder and bowel function was present in 69.6% (188 cases). In 34.4% (93 cases) the symptomatic was described as an urinary or fecal urgency with difficulties to reach toilet in time. A rare and mild urge incontinence without the necessity of a nappy was found in 21.1% (57 cases). In 10.0% (27 cases) a moderate urge incontinence was present with the need of nappy or catheter when out of the house. In 4.1% (11 cases) permanent catheterization or permanent nappy was required (*see* Figure 14).
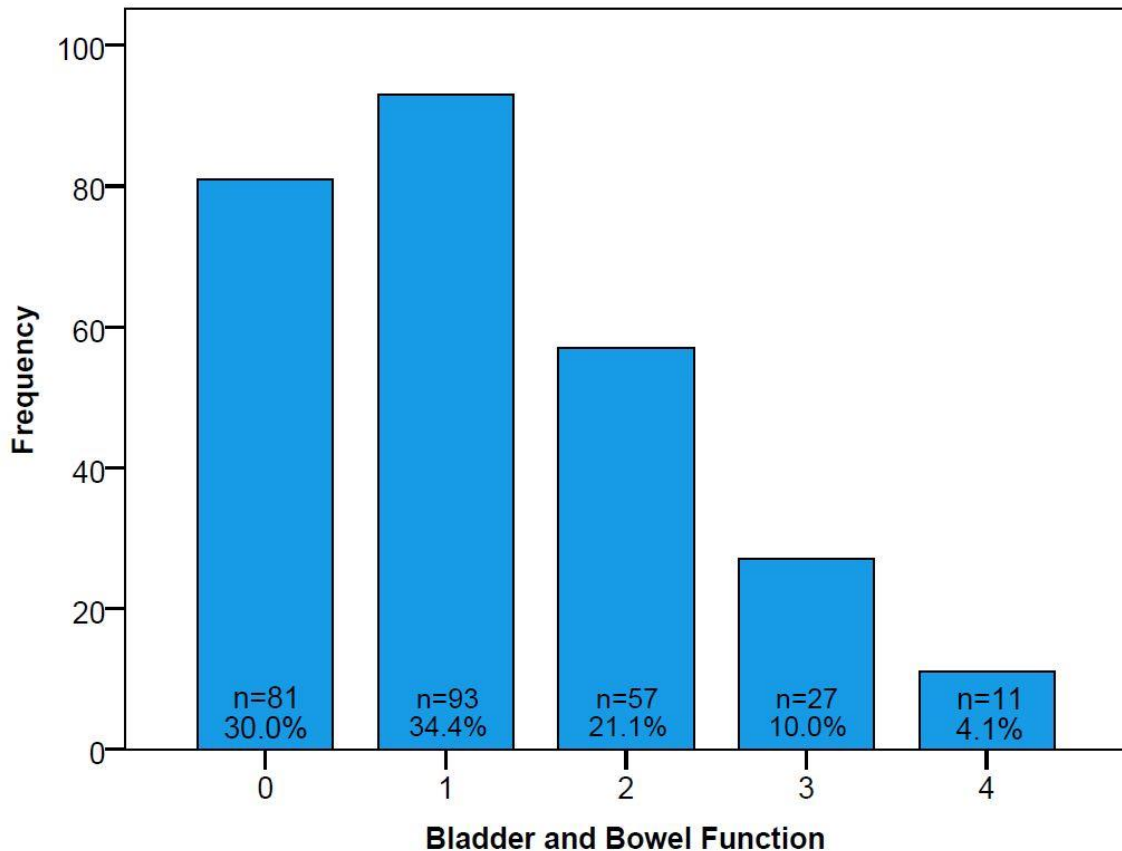
Figure 14: bladder and bowel function

0: Normal bladder and bowel function
1: Urinary or fecal urgency (difficulties to reach toilet in time)
2: Rare and mild urge incontinence (no nappy required)
3: Moderate urge incontinence (requires nappy or catheter when out of the house)
4: Permanent catheterization or permanent nappy

Spasticity of upper limbs in dependency of disease severity

Spasticity of upper limbs was registered with help of the inventory. At baseline, 25 cases (25.3%) presented with spasticity of upper limbs and another 25 (25.3%) cases with increased tendon reflexes. In 49 cases (49.5%) no signs of first motor neuron affection in upper limbs were shown. In the remaining cases no information about signs for spasticity of upper limbs were registered.

We analyzed if spasticity of upper limbs was associated to disease severity measured by SPRS total score (see Figure 15 and Table 12). Since normal distribution criteria were not fulfilled medians are reported.

Comparing SPRS total score in dependency of the spasticity of upper limbs, by criteria none vs increased tendon reflexes only vs spasticity of upper limbs, we

could find no significant differences neither in analyzing Kruskal-Wallis test for comparison of all groups (H(2)=3.97, p=0.138 with 99% CI 0.092 – 0.108) nor in Mann-Whitney tests comparing individual groups (p≥0.036 ; see Table 13). In summary, spasticity of upper limbs does not seem to occur in terms of more severe affection.
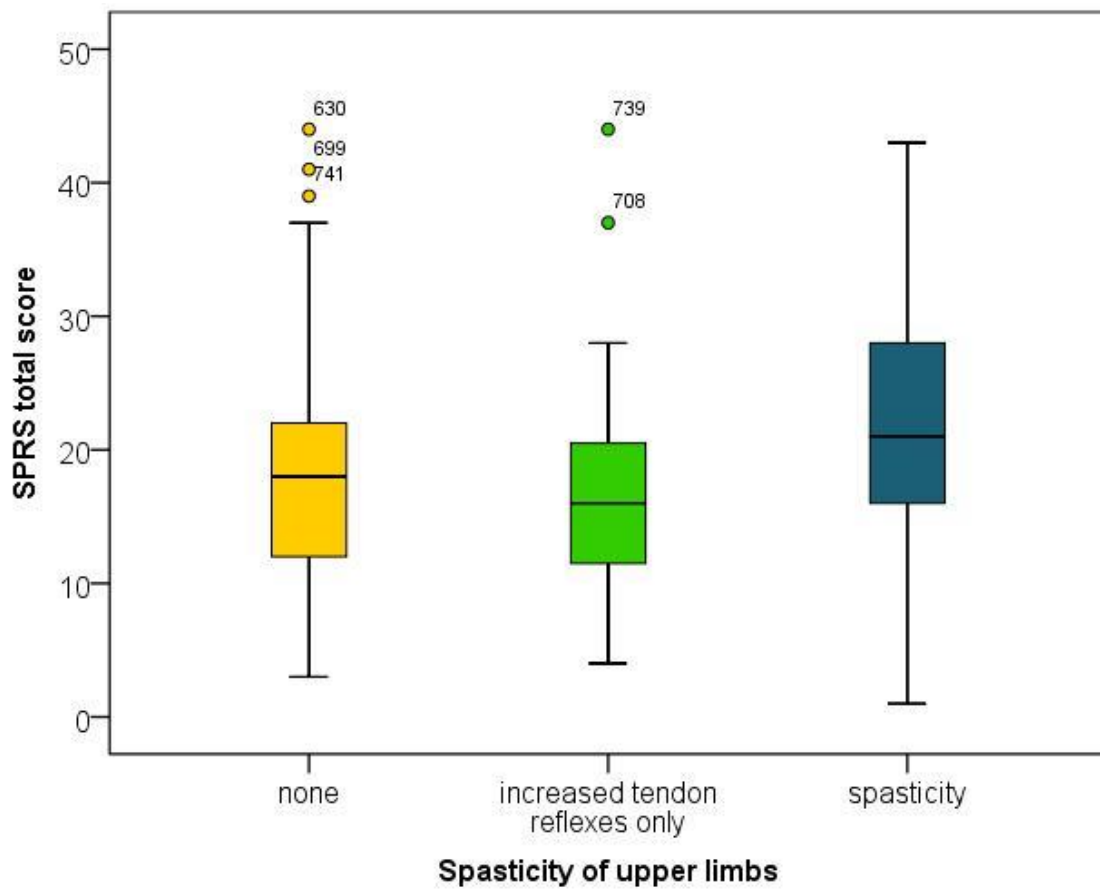
Figure 15: spasticity of upper limbs in dependency of disease severity

Box plot visualizing disease severity measured by SPRS total score at baseline in dependency of spasticity of upper limbs. We found median SPRS total score of 18 / 16 / 21 in patients presenting in upper limbs no spasticity / increased tendon reflexed / spasiticity. Analyzing Kruskal-Wallis test comparing all groups, no significant differences in SPRS total score were found (H(2)=3.97, p=0.138 with 99% CI 0.092 – 0.108).

Table 12: Spasticity of upper limbs in dependency of disease severity

		Spasticity of upper limbs		
		None	Increased tendon reflexes only	Spasticity
Frequencies		49	23	25
SPRS Total score	Median	**18**	**16**	**21**
	Range	3 – 44	4 – 44	1 – 43

Table 13: test statistics spasticity of upper limbs in dependency of disease severity

Results from Mann-Whitney tests comparing disease severity measured by medians of SPRS total score (not normally distributed) in groups no spasticity of upper limbs vs increased tendon reflexes only vs spasticity of upper limbs. Only baseline examinations included. U: test statistic, n=number of total observations, z: z-score, r: estimated effect size, p: significance. Results are significant if p<0.017.

Spasticity of upper limbs	U	n	z	r	p
None vs increased tendon reflexes	518.5	72	-0.54	-0.06	0.295
None vs spasticity	467	74	-1.67	-0.19	0.048
Increased tendon reflexes vs spasticity	200	48	-1.81	-0.26	0.036

Complicating signs and symptoms

Complicating signs and symptoms that may be associated with SPG4 in addition to the core HSP symptoms described above were assessed with the help of the inventory. We found complicating signs and symptoms in one third of our patients at baseline (83 of 234 patients, 35.5%; for further information see Appendix table 9).

Cases with complicated SPG4

Evaluating the clinical symptoms of SPG4 the major share of cases presents with lower limb spasticity, contractures and weakness as well as bladder and bowel dysfunction without additional symptoms. In our cohort we were able to identify 3 cases that presented a completely different clinical overall appearance with significant impairments due to additional complicating signs and symptoms. In the following these cases will be further described.

In **case 52** delayed psychomotor development was observed from birth onwards. He learned to crawl at age 12 month, and started walking with assistance at the age of 2 years. He was never able to walk independently and became wheelchair-dependent for longer distances at the age of 8 years. Follow-up examinations are available at the age of 12, 14, 14, 16, 18 and 19 years with a SPRS total score between 33 and 39. At the time of his last examination he was still able to walk slowly a 100-200m distance using a walking aid. He developed contractures going along with muscle atrophy. In addition to lower limb spasticity he developed slight spasticity of the upper limbs without atrophy of upper limb muscles. Furthermore, dysarthria was noticed over the whole period under review, which was classified as being most likely pseudobulbar. When he was aged 16 years, he developed bradydiadochokinesia as sign of an extrapyramidal involvement. As further complicating symptoms he developed cerebellar limb ataxia, oculomotor disturbances in terms of hypometric saccades and saccadic pursuit, and hearing impairment. Moreover, scoliosis and dystonia of the torso were reported. At his last examination, neuropsychological tests were conducted to further investigate his learning disabilities revealing an IQ of 64.

In addition to SPG4, he was diagnosed with ulcerating colitis when he was 16 years.

In case 52 the HSP-causing missense variant c.1250G>C was identified, which leads to the replacement of the nonpolar amino acid glycine in position 417 (AAA cassette) by alanine (nonpolar). He is the only one in the cohort carrying this variant.

In **case 202** hypotonia of trunk and neck muscles was apparent already in her first year of live as well as impaired psychomotor development. She learned to crawl at age 18 month and never learned to walk independently, though she was able to walk with an assisting device when she was 2-5 years old. When she was 12 years old, she reached 43 points in SPRS total score. She could only walk with maximum support and had already lost her ability to stand independently. Spasticity affected upper limbs to such an extent, that she could hardly eat and drink without assistance of her parents. In addition to spasticity she developed learning disability from early childhood onwards, being able to count till 12 at age of 12 years. She communicated with the help of a speech computer by selecting symbols, since she could hardly say an understandable word due to her pseudobulbar dysarthria/anarthria, which started when she was 5 years old. Her dysarthria was accompanied by dysphagia with choking as well as rhythmic spasm of the pharynx musculature causing fear to her. In addition to SPG4, epilepsy was diagnosed in early childhood with 2-3 absence seizures per day during treatment with ethosuximide and levetiracetam. In addition, a small stature with 134cm of height (<1. Percentile) and 28,5kg of weight (1. Percentile) but normal head circumference (54cm; 63. Percentile) was noted.

In case 202 the genetic testing revealed the *de novo* missense variant c.1496G>A, leading to the replacement of arginine (strong alkaline) by histidine (weak alkaline) in amino acid position 499, which is part of the AAA domain. This variant occurred in a further case of the cohort, in which a pure SPG4 manifested at the age of 38 leading to the use of a walking aid 15 years later and a SPRS total core of 23 after 20 years disease duration.

Case 260 noticed first symptoms at an age of 41 years and was examined at the age of 51 years with a SPRS total score of 31. She was still walking independently. She presented with muscle atrophy of the upper and lower limbs, that was paramount by spasticity to such an extent, that she would not have been able to walk if it had not been for her spasticity, which involves upper and lower limbs as well. Ataxia of gait and limb was observed. On top she showed dysphagia and an extrapyramidal involvement with myoclonus. Additionally, her

eyes were affected by cataract and optic atrophy causing visual loss. SPG4 was caused in her case by a heterozygote deletion of exons 8 to 17. According to her report, 2 siblings that have not been included in our study have the same variant and present a pure HSP being still able to walk independently and having no muscle atrophy. In contrast to her siblings, case 260 was additionally diagnosed with Bechterew's disease. No other case carrying an exon 8_17 deletion was included in our study cohort.

Case reports of asymptomatic variant carriers

6 cases of the cohort (2.2%) proved to be asymptomatic variant carriers that showed no clinical symptoms of HSP in the examination. They were genetically tested for they had at least one affected family member. The asymptomatic variant carriers were examined at 26, 29, 32, 41, 60 respectively 63 years of age.

Independent walking

Loss of the ability to walk independently is a major event in the course of the disease. Hereby, the event was defined as the year in which any kind of walking aid (walking stick, walker, wheelchair) was used on a regular basis. We used Kaplan Meier analysis to analyze the time course of the loss of independent walking and Cox proportional hazard analysis to specify factors influencing loss of independent walking. To avoid bias due to family cluster effects, all analyzes were performed for the total cohort as well as for index cases only.

Loss of independent walking

The loss of independent walking ability occurred after a median disease duration of 23 years (95%CI 20.2 – 25.8 years) regarding total cohort, respectively 22 years (95% CI 19.4 – 24.6 years) including index patients only.

We then evaluated the influence of the following factors on the time course on the ability to walk independently: gender (female vs male), variant type (missense

vs truncating) and age of onset of disease (≤20 years vs 21 – 40 years vs >40 years).

No effect could be proven for gender (p 0.77) and variant type (p 0.85), neither in total cohort nor in index patients only. We found age of onset of disease to have a significant effect (p < 0.05) on disease duration until loss of independent walking ability, regarding total cohort as well as index patients only (see Table 14). This is illustrated in Figure 16. Later age of onset was associated with loss of the ability to walk independently earlier in the disease course. While early onset cases became dependent on a walking aid after a median disease duration of 38 years (95% CI 31.8 – 44.2), late onset cases required a walking aid after a disease duration of 13 years (95% CI 11.6 – 14.4) (see Table 14).

All frequencies of occurrence included in Kaplan Meier and Cox proportional hazard analyses are reported in Appendix table 11.
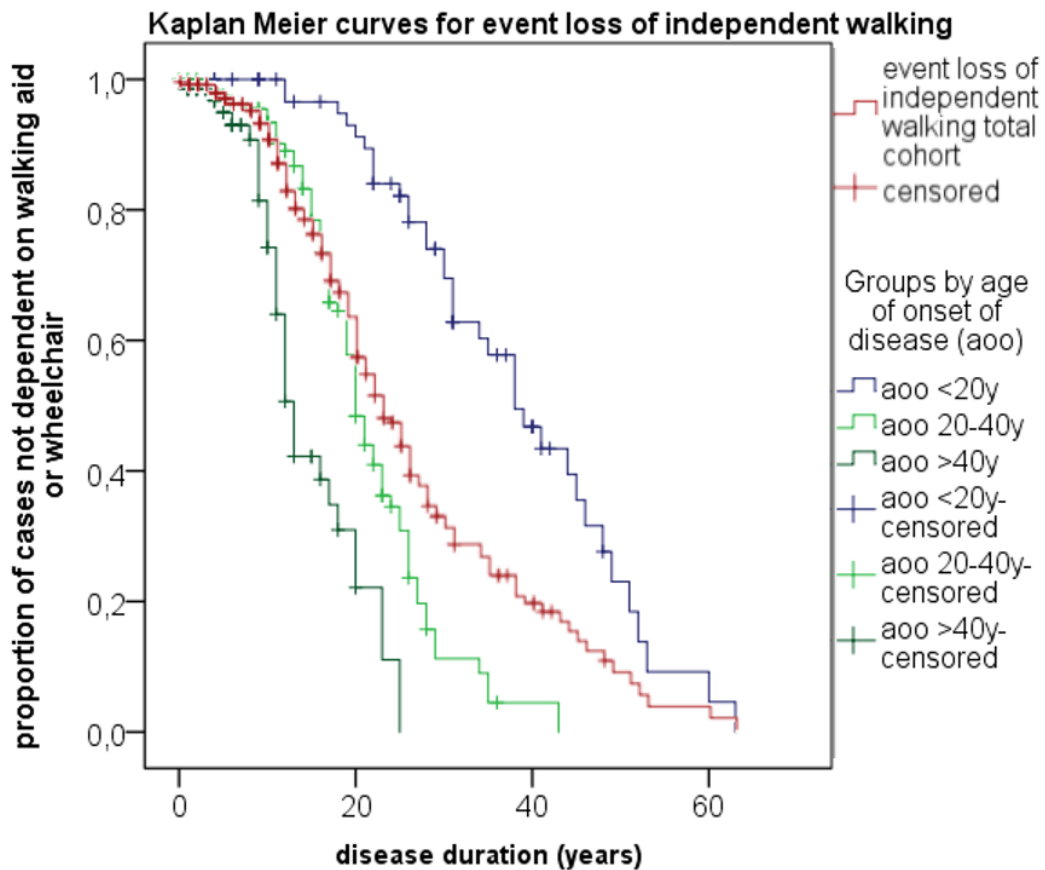
Figure 16: loss of independent walking

The proportion of cases having not yet lost their ability to walk independently is highlined on the Y axis, whereas the X axis maps the disease duration in years. At the beginning of disease all cases are still able to walk independently. The red line represents the proportion of cases being still able to walk independently of the total cohort, whereas the other lines represent the groups formed by age of onset of disease. The figure illustrates, that later age of onset was associated with loss of independent walking ability earlier in the time course of disease.

Table 14: loss of independent walking

Results of Kaplan Meier analyses and Cox proportional hazard analyses for event loss of independent walking and influencing factors, conducted for the total cohort as well as for families (indexes only). In terms of groups by age of onset of disease (aoo) reported hazard, hazard ratio and p-values are results of Cox proportional hazard analyses comparing groupwise (≤20 years and 21 – 40 years in each case with >40 years). P-values noted for group with age of onset of disease >40 years refer to calculations of the whole influence of groups formed by age of onset of disease, reported p-values for age of onset of disease ≤20years and 21 – 40 years refer to this group in comparison to group >40 years. Reported results for variant type and gender are from Cox proportional analysis including all investigated factors.

	All cases					Index				
	median	95%-KI	hazard	Hazard ratio	p	median	95%-KI	hazard	Hazard ratio	p
All cases	23	20.2 – 25.8				22	19.4 – 24.6			
Aoo ≤ 20	38	31.8 – 44.2	-1.9	0.15	0.00	38	29.5 – 46.5	-1.9	0.16	0.00
Aoo 21 – 40	20	18.4 – 21.6	-0.6	0.54	0.00	20	18.2 – 21.8	-0.7	0.48	0.00
Aoo > 40	13	11.6 – 14.4			0.00	13	11.5 – 14.5			0.00
Gender			.05	1.06	0.77			-0.4	0.96	0.96
Variant type			.04	1.05	0.85			0.1	1.08	1.1

Walking aid dependency

The median disease duration until walking aid dependency proved to be 23 years (95% CI 20.3 – 25.7) in total cohort, respectively 22 years (95% CI 19.4 – 24.6) taking into account index patients only.

No effect could be proven for gender (p 0.71) and variant type (p 0.70). We found age of onset of disease to have a significant effect (p < 0.05) on disease duration until walking aid dependency (see Table 15). Figure 17 illustrates, that later age of onset was associated with walking aid dependency earlier in the disease course. While early onset cases became dependent on a walking aid after a median disease duration of 39 years (95% CI 32.8 – 45.2), late onset cases required a walking aid after a disease duration of 13 years (95% CI 11.6 – 14.4) (see Table 15).

Figure 17: walking aid dependency

The proportion of cases being not yet dependent on walking aid is highlined on the Y axis, whereas the X axis maps the disease duration in years. At the beginning of disease all cases are still able to walk without walking aid. The red line represents the proportion of cases of the total cohort being not dependent on walking aid, whereas the other lines represent the groups formed by age of onset of disease. The figure illustrates, that later age of onset was associated with walking aid dependency earlier in the time course of disease.

Table 15: walking aid dependency

Results of Kaplan Meier analyses and Cox proportional hazard analyses for event walking aid dependency and influencing factors, conducted for the total cohort as well as for families (indexes only). In terms of groups by age of onset of disease (aoo) reported hazard, hazard ratio and p-values are results of Cox proportional hazard analyses comparing groupwise (≤20 years and 21 – 40 years in each case with >40 years). P-values noted for group with age of onset of disease >40 years refer to calculations of the whole influence of groups formed by age of onset of disease, reported p-values for age of onset of disease ≤20years and 21 – 40 years refer to this group in comparison to group >40 years.   Reported results for variant type and gender are from Cox proportional analysis including all investigated factors.

	All cases					Index				
	median	95%-KI	hazard	Hazard ratio	p	median	95%-KI	hazard	Hazard ratio	p
All cases	23	20.3 – 25.7				22	19.4 – 24.6			
Aoo ≤ 20	39	32.8 – 45.2	-1.9	0.14	0.00	38	29.5 – 46.5	-1.8	0.16	0.00
Aoo 21 – 40	20	18.4 – 21.6	-0.6	0.55	0.00	20	18.2 – 21.8	-0.7	0.49	0.00
Aoo > 40	13	11.6 – 14.4			0.00	13	11.5 – 14.5			0.00
Gender			0.1	1.07	0.71			-0.1	0.94	0.76
Variant type			0.1	1.10	0.70			0.1	1.08	0.77

Wheelchair dependency

Wheelchair dependency occurred after a median disease duration of 41 years (95%CI 34.8 – 47.2 years) regarding total cohort, respectively 44 years (95% CI 35.2 – 52.8 years) including index patients only.

No effect could be proven for gender (p 0.50) and variant type (p 0.64). We found age of onset of disease to have a significant effect (p < 0.05) on disease duration until wheelchair dependency (see Table 16). In Figure 18 it is illustrated, that later age of onset was associated with wheelchair dependency earlier in the disease course. While early onset cases became dependent on wheelchair after a median disease duration of 48 years (95% CI 39.2 – 56.8), late onset cases required wheelchair after a disease duration of 25 years (95% CI 18.4 – 31.6) (see Table 16).
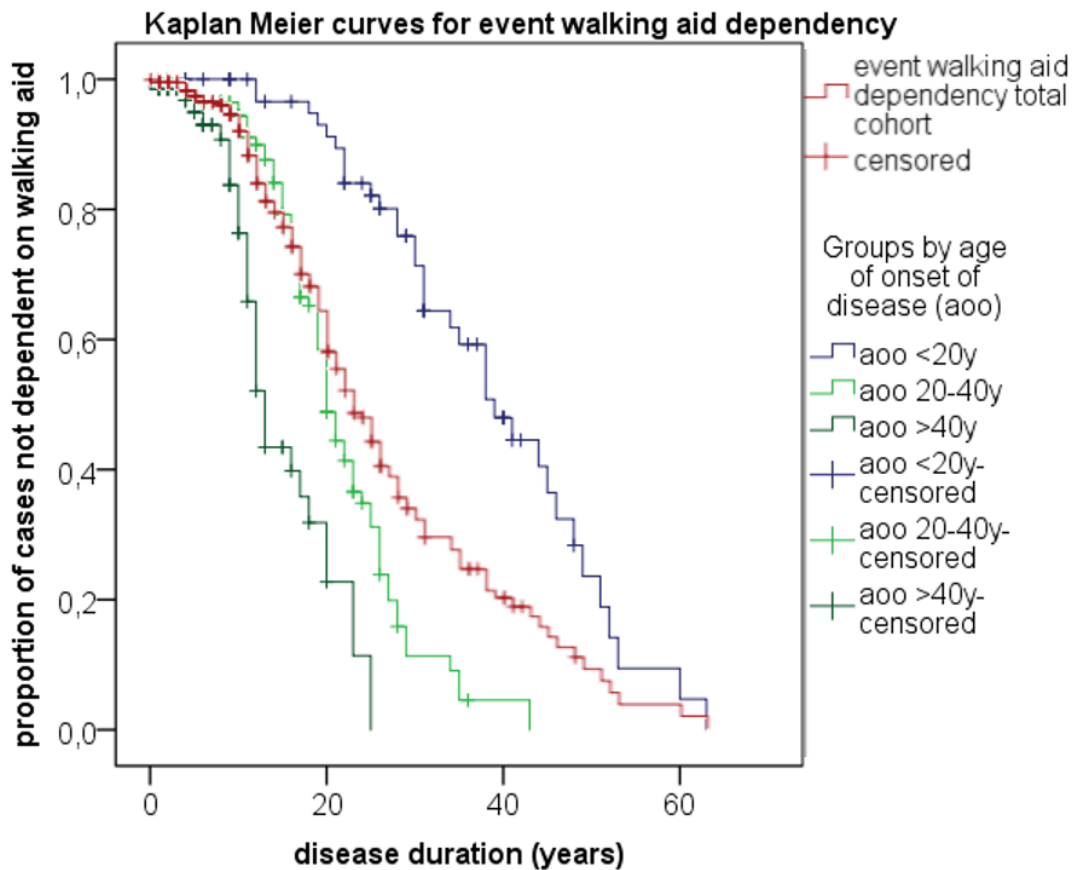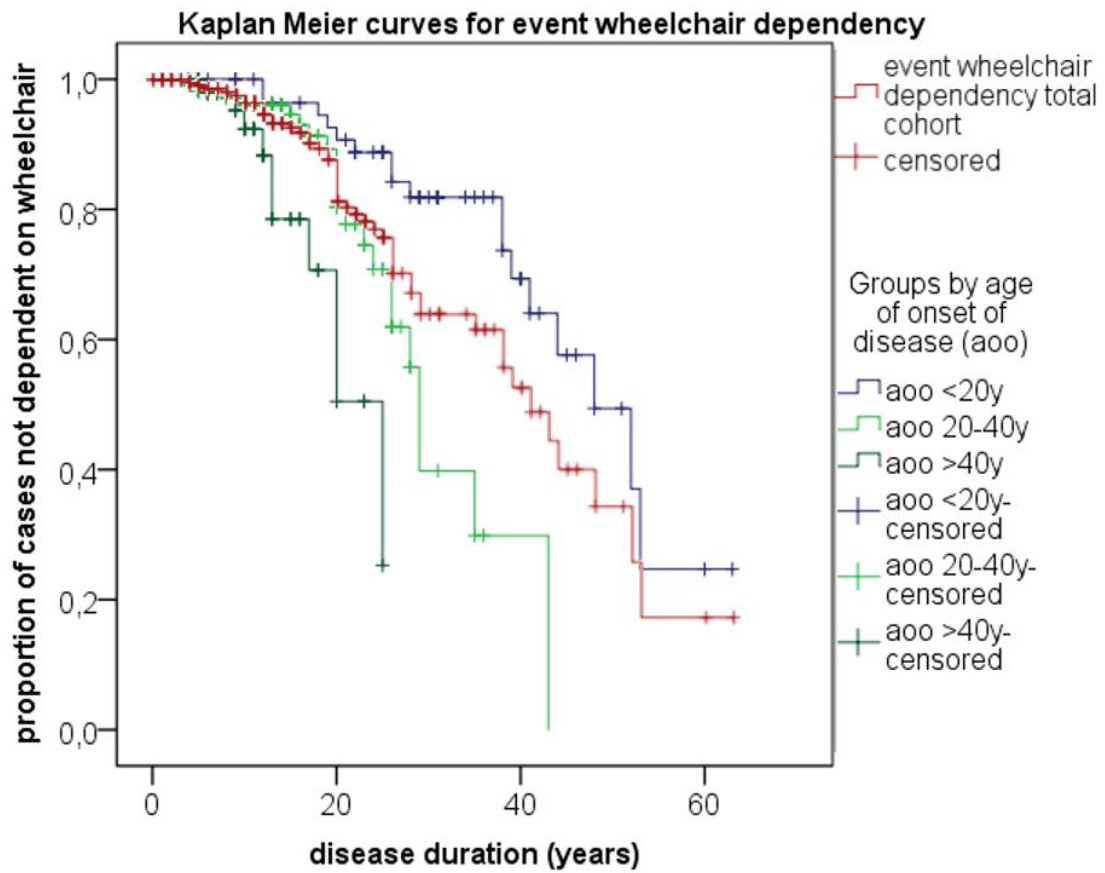
Figure 18: wheelchair dependency

The proportion of cases being not yet dependent on wheelchair is highlined on the Y axis, whereas the X axis maps the disease duration in years. At the beginning of disease, no case is dependent on wheelchair. The red line represents the proportion of cases of the total cohort being not dependent on wheelchair, whereas the other lines represent the groups formed by age of onset of disease. The figure illustrates, that later age of onset was associated with wheelchair dependency earlier in the time course of disease.

Table 16: wheelchair dependency

Results of Kaplan Meier analyses and Cox proportional hazard analyses for event wheelchair dependency and influencing factors, conducted for the total cohort as well as for families (indexes only). In terms of groups by age of onset of disease (aoo) reported hazard, hazard ratio and p-values are results of Cox proportional hazard analyses comparing groupwise (≤20 years and 21 – 40 years in each case with >40 years). P-values noted for group with age of onset of disease >40 years refer to calculations of the whole influence of groups formed by age of onset of disease, reported p-values for age of onset of disease ≤20years and 21 – 40 years refer to this group in comparison to group >40 years. Reported results for variant type and gender are from Cox proportional hazard analysis including all investigated factors.

	All cases					Index				
	median	95%-KI	hazard	Hazard ratio	p	median	95%-KI	hazard	Hazard ratio	p
All cases	41	34.8 – 47.2				44	35.2 – 52.8			
Aoo ≤ 20	48	39.2 – 56.8	-1.7	0.19	0.000	52	34.8 – 69.2	-1.8	0.17	0.001
Aoo 21 – 40	29	27.6 – 30.4	-0.8	0.45	0.045	29	23.5 – 34.5	-1.4	0.24	0.002
Aoo > 40	25	18.4 – 31.6			0.002	20	16.0 – 24.0			0.001
Gender			0.2	1.22	0.500			0.2	1.23	0.569
Variant type			-0.2	0.84	0.639			-0.1	0.79	0.787

Progression

SPG4 is known to be a progressive neurodegenerative disease. However, known to us no data is available on disease progression, determinants of disease progression and if progression changes in course of disease or in dependency of age.

In order to provide prospective data on disease progression, we analyzed longitudinal SPRS data and calculated the progression rate. In 169 cases we had the results of 2 or more examinations (see Table 6), so that we could include 511 SPRS total scores in our calculations. The median disease duration in between examinations was 1.0 year (range 0.0 – 11.8 years; see Figure 19).

Short examination intervals of less than 3 months were excluded from calculations, due to lack of clinical impact on natural course of disease and statistical bias resulting from large outliers (for exemplification results with all examinations are reported nevertheless in Table 17, grey background). Figure 20 and Figure 22 illustrate that the change of the SPRS total score per time unit reveals a large variance with short examination intervals and that this variance decreases as the time interval increases, even though examination intervals of less than 3 months are excluded (outliers could not be depicted in scale).

Progression rate was calculated as Delta^{SPRS} per Delta^{time} (change in SPRS total score per time difference in between examinations ins years).

For comparison, and to avoid bias by repeated measurements of single patients with many examinations, we calculated different variants of progression rate likewise: A) including all Delta^{time} (repeated measurements) and B) including only one Delta^{time} per patient (from first to second examination). Normal distribution criteria were not fulfilled in the calculated variable for progression rate. Therefore, medians are reported in progression rate analyzes.

We found the median progression rate to be 1.0 points increase of SPRS total score per year (variant A), respectively 0.7 points in variant B (see Table 17). Figure 20 (variant A) and Figure 21 (variant B) illustrate progression rate in dependency of time interval between examinations.

Examination of Delta^{SPRS} from first to second examination revealed improvement in SPRS total score of 1 -11 points in 28.9% of cases (46 out of 159).

Table 17: Progression rate

SPG4 Progression measured by change of SPRS total score depending on the time interval between examinations. Results are computed by difference of SPRS total score between two examinations divided by time interval in years between examinations. Under "all examinations" all examinations of cases with at least two examinations are included if time intervals of respective column are matched, cases having several examinations are therefore analyzed repeatedly. In "1st to 2nd examination" are no repeated analyses of different examinations in one patient, but only variation of the SPRS total score depending on the time interval between first and second examination. Row frequencies reflects numbers of calculated changes in SPRS total scores. SD: Standard deviation, y: years.

	All examinations						1st to 2nd examination	
Time (months) between examinations	>3	6 – 12	12 – 18	18 – 24	≥0	<3	>3	≥0
Frequencies	477	153	172	40	511	34	151	159
Median	1.0	1.0	0.9	1.1	1.0	0.0	0.7	0.6
Minimum	-17.8	-9.4	-10.9	-2.4	-57.7	-57.7	-10.9	-32.5
Maximum	20.1	16.7	14.9	4.4	730.5	730.5	16.7	730.5
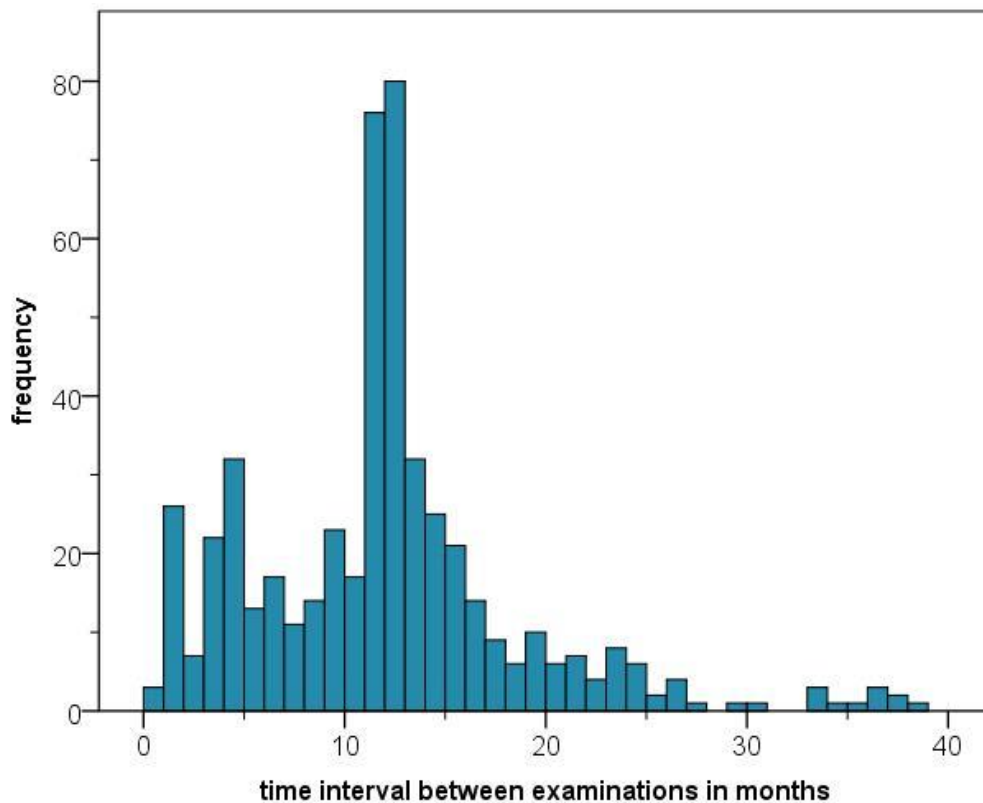
Figure 19: time interval between examinations in months

Frequencies of occurrence of time intervals between examinations (Delta^{time} in months). Longest time intervals (upper 5%) are not depicted to allow better overview of common time intervals.
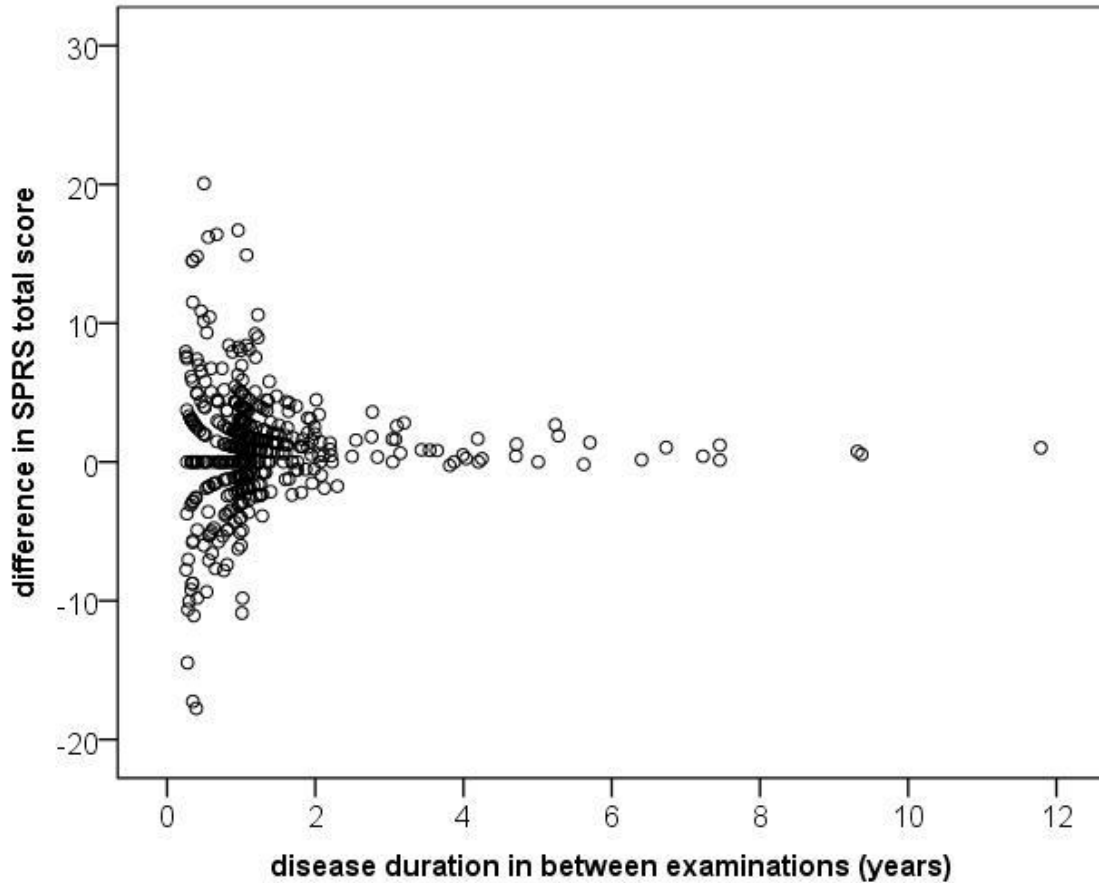
Figure 20: progression rate analyzed over all examinations

SPG4 Progression measured by change of SPRS total score depending on the time interval between examinations (Delta^SPRS/Delta^time variant A). Results are computed by difference of SPRS total score between two examinations divided by time interval in years between examinations. Examination intervals <3 months were not displayed due to outliers that could not be depicted in this scale. All examinations of cases with at least two examinations are included. Cases having several examinations are therefore analyzed repeatedly. Respective results in table progression rate (columns all examinations).
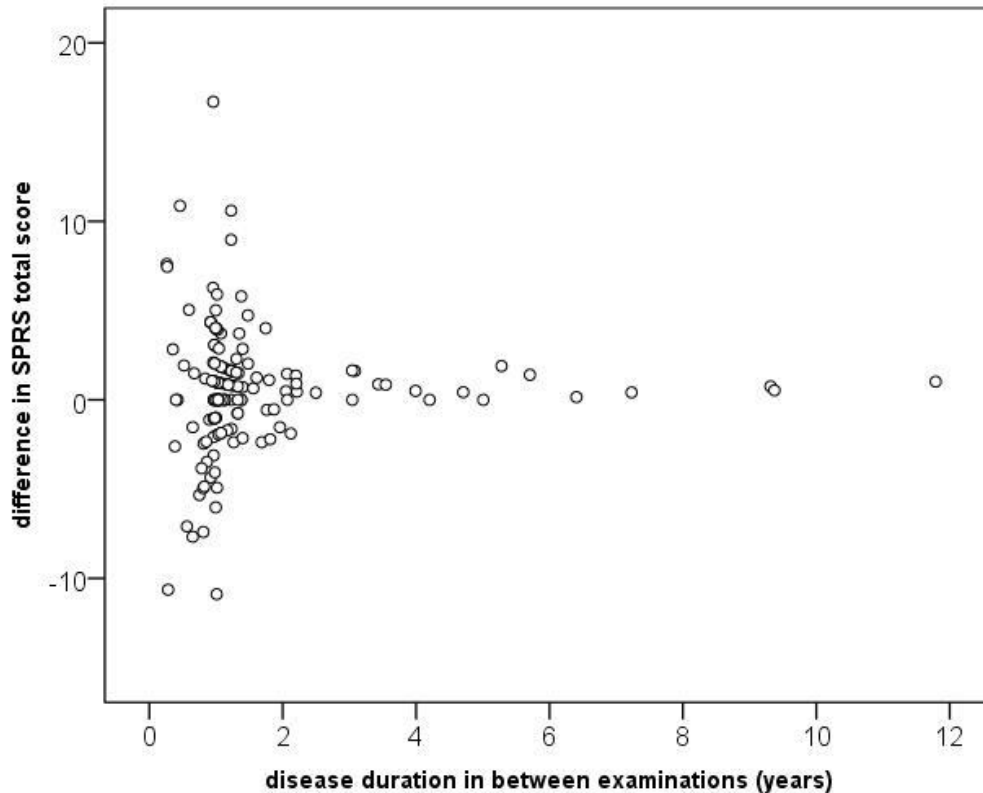
Figure 21: progression rate baseline examinations

SPG4 Progression measured by change of SPRS total score depending on the time interval between examinations (Delta^{SPRS}/Delta^{time} variant B). Results are computed by difference of SPRS total score between two examinations divided by time interval in years between examinations. Examination intervals <3 months were not displayed due to outliers that could not be depicted in this scale. Only variation of the SPRS total score depending on the time interval between first and second examination is depicted, and therefore no case is included repeatedly. Respective results in table progression rate (column 1st and 2nd examination).

Progression rate in dependency of time interval between examinations

Figure 22 illustrates the decrease in variance of progression rate with longer time interval in between examinations, grouped by time intervals of 6-12 vs 12-18 vs 18-24 months (see Figure 22 and Table 17, corresponding colored background). Medians of progression rate did not differ significantly, neither in Kruskal-Wallis test comparing all groups (H(2)=0.65, p=0.726) nor in Mann-Whitney-tests comparing individual groups (p≥0.459; see Table 18). No indication was found of bias in the calculation of the progression rate, due to the inclusion of examination intervals that were not representative.

Figure 22: progression rate in dependency of time interval between examinations

SPG4 Progression measured by change of SPRS total score depending on the time interval between examinations. Results are computed by difference of SPRS total score between two examinations divided by time interval in years between examinations. Changes in SPRS total score are shown as a function of the time interval between examinations: 6-12 / 12-18 / 18-24 months. Short examination intervals were not displayed due to outliers that could not be depicted in this scale. Respective results in table progression rate.

Table 18: test statistics for progression rate in dependency of time interval in between examinations

Results from Mann-Whitney tests comparing progression rates (Delta^{SPRS} / Delta^{time}) in dependency of time interval in months (groups 6 – 12, 12 – 18 and 18 – 24 months). U: test statistic, n=number of total observations, z: z-score, r: estimated effect size, p: significance. Results are significant if p<0.017

Time interval (months)	U	n	z	r	p
6 – 12 vs 18 - 24	2888.5	193	-0.55	-0.039	0.587
6 – 12 vs 12 - 18	12533.5	325	-0.74	-0.041	0.459
12 – 18 vs 18 - 24	3403	212	-0.11	-0.007	0.916

Progression rate in dependency of age

In order to analyze if progression rate differs in between younger and older patients, medians of progression rate were compared for patients being aged 10 – 50 years (Mdn 1.0, range -14.5 – 16.7) vs ≥ 51 years (Mdn 0.9, range -17.8 – 20.1). We did not find significant differences between groups (U=27930, z = -0.239, r=-0.011, p=0.811).

Progression rate in dependency of disease duration

To find out whether disease progression changes during the course of the disease, we analyzed progression rates for disease duration 0 – 10 vs 11 – 20 vs 21 – 30 vs ≥31 years (see Figure 23 and Table 19). Non-parametric tests were conducted, since the assumption of normal distribution was violated. In order to analyze, if groups differed significantly, we performed the Kruskal-Wallis test finding no significant differences in between groups (H(3)=1.42, p=0.700). Additionally, medians of short disease duration (0 – 10 years; Mdn 0.6, range -7.4 – 16.7) and long disease duration (≥31 years; Mdn 0.9, range -17.8 – 20.1) were compared with the help of the Mann-Whitney test, showing no significant differences of progression rate in between groups, as well (U=3841, z=-0.49, r=-0.036, p=0.628).
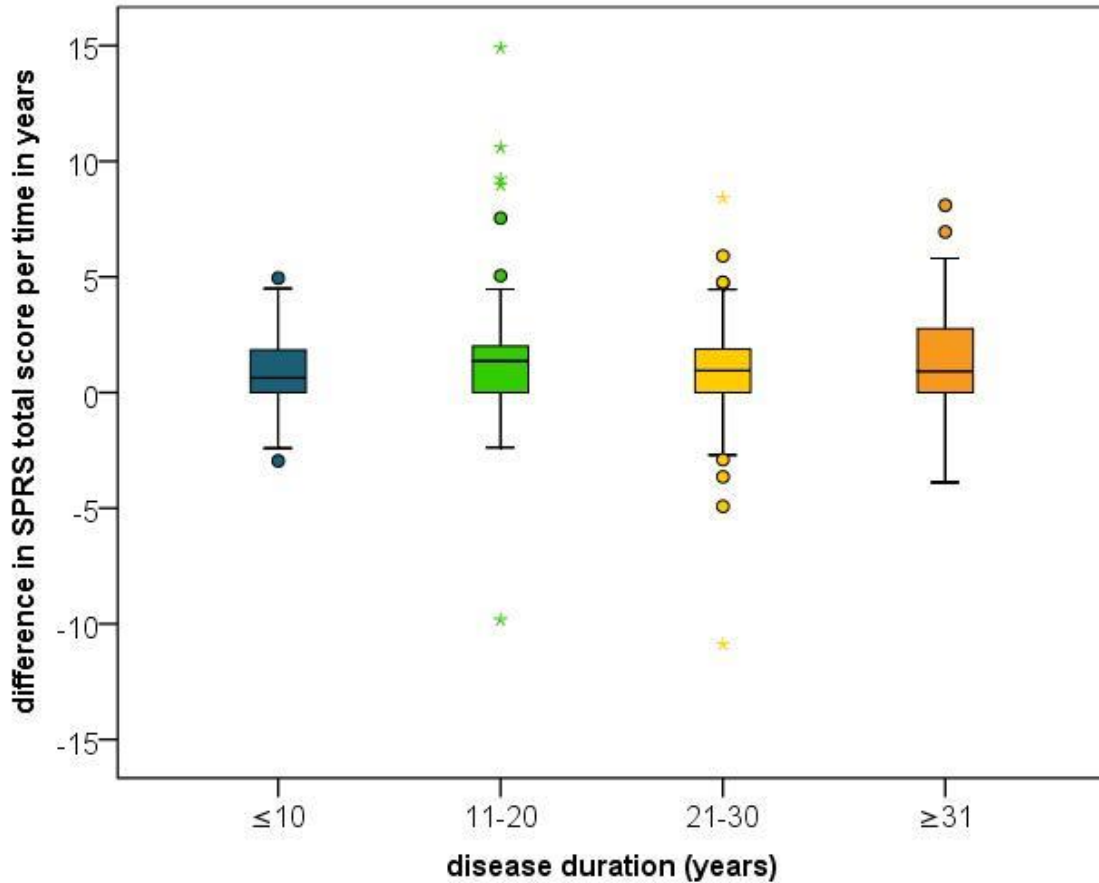
Figure 23: Progression rate in dependency of disease duration

SPG4 Progression measured by change of SPRS total score depending on the time interval between examinations. Results are computed by difference of SPRS total score between two examinations divided by time interval in years between examinations. 4 groups are formed by disease duration measured in years. In this figure only time intervals between examinations of 1 year and more are included, to achieve a better overview and less spikes due to SPRS changes in short intervals. All results are reported in table progression rate in dependency of disease duration.
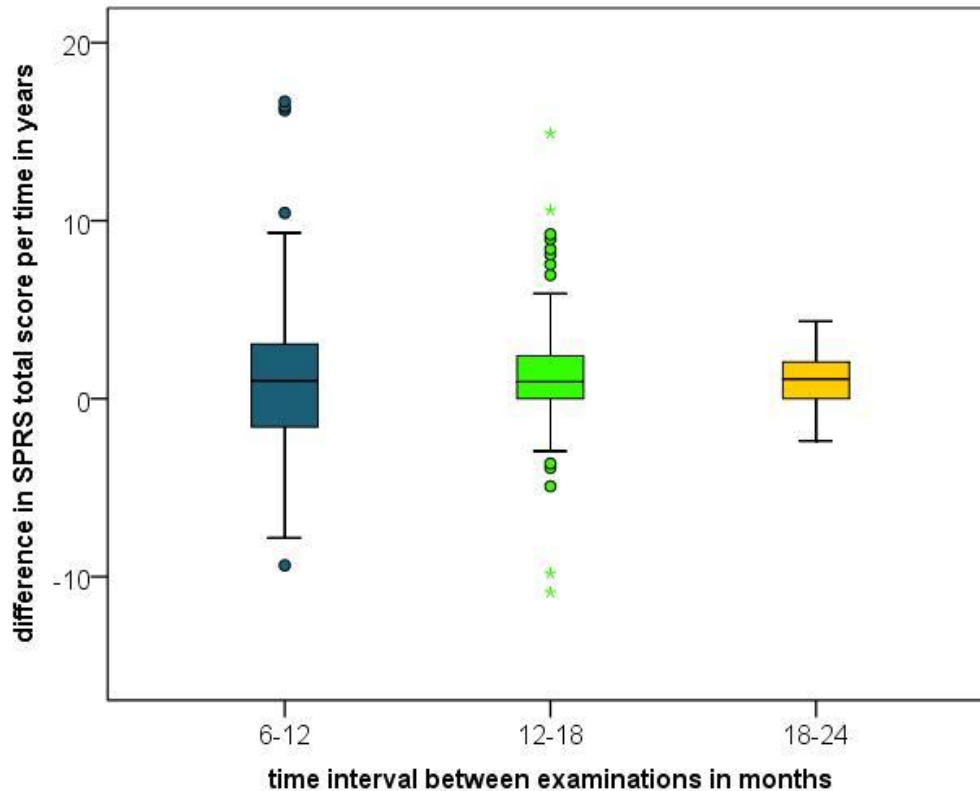
Table 19: Progression rate in dependency of disease duration

SPG4 Progression measured by change of SPRS total score depending on the time interval between examinations. Results are computed by difference of SPRS total score between two examinations divided by time interval in years between examinations. Row frequencies reflects numbers of calculated changes in SPRS total scores. SD: Standard deviation, y: years.

	Disease duration			
	≤10y	**11 – 20y**	**21 – 30y**	**≥31y**
Frequencies	99	150	142	81
Median	**0.6**	**1.3**	**1.0**	**0.9**
Range	-7.4 – 16.7	-17.3 – 16.4	-14.5 – 14.5	-17.8 – 20.1

Growth models of progression rate

In order to optimize the model describing the progression rate of SPG4 measured by SPRS total score, we analyzed growth models of progression rate. The predictor was the time, meaning disease duration at the time of the examination. SPRS total score was the dependent variable. Family membership was taken into account in our calculation, because of the possibility of bias due to related patients, so we tested for families and interaction of related patients. We applied a scaled identity covariance structure, assuming different covariance structures in random effects.

We compared models with linear (time), quadratic (time*time) and cubic (time*time*time) terms, to determine which curve shape describes best disease progression in SPG4 (see first model in Appendix table 12). The assumption of disease duration as cubic term did not improve the model. Adding disease duration as quadratic term revealed significant improvement of the model (p 0.006 for quadratic term), though the regression coefficient b was 0.01 (95% CI 0.00 – 0.01) was very small and indicated therefore little impact on progression rate (see Appendix table 12). In the model including disease duration as linear term, we found for disease duration a regression coefficient b of 0.69 (SE 0.05, 95% CI 0.59 – 0.79, p<0.001).

Growth models in dependency of disease duration

To analyze if progression rate changes in course of disease growth models were conducted in the same manner as described above including only examinations if disease duration amounted <10 years, <20 years, 10 – 20 years, ≥10 years or ≥20 years, respectively (see Appendix table 12).

The addition of disease duration as quadratic term revealed significant improvement if disease durations of less than 10 years were excluded from analysis (p<0.05, see disease duration ≥10 years in Appendix table 12). In all other the other calculations no significant improvement was found by adding quadratic or cubic term. Since the quadratic term proved to be significant in disease durations ≥10 years, though not in other disease durations, especially

not in disease duration 10 – 20 years and ≥20 years, it was removed from the model for further analysis.

In summary, growth models indicate a linear progression rate in SPG4.

Influencing factors on progression rate

There is little known about factors that influence the course of SPG4. In order to identify major determinants influencing progression rate of SPG4, we analyzed mixed models with additional variables gender (male / female), variant type (missense / truncating) and age of onset of disease (aoo; groups 0 – 20 years / 21 – 40 years / >40 years). The basic model contained SPRS total score (measuring device of disease progression) as dependent variable with predictor time (disease duration at time of examination) and random intercept. Family membership was taken into account in our calculation, because of the possibility of bias due to related patients, so we tested for families and interaction of related patients. We applied a scaled identity covariance structure, assuming different covariance structures in random effects. We conducted a backward selection, starting with a model including all additional variables, removing factors that proved to be not significant (neither alone nor in interaction with disease duration) step by step (see Appendix table 13).

Gender and variant type were removed from model, since they proved to be not significant neither alone not in interaction with time (p≥0.080;see step 1/1*/2/2* in Appendix table 13). In consequence, age of onset of disease was the only remaining additional variable. Next, we compared models with interaction of age of onset with disease duration and without (step 3/3*). In addition, we tested if the model was robust if family membership was not taken into account, i.e. only patients but not families were included in the model (step 4/4*). In both models including interaction of age of onset and disease duration (i.e. step 3* and step 4*) the group with age of onset of disease 21 – 40 years did not longer have significant impact (p≥0.873) and 95% CI surrounded value zero (see Appendix table 13). Therefore, the interaction was not included in the final model.

In summary, we found a model of best fit for progression rate with disease duration at examination as predictor for SPRS total score including age of onset

of disease as influencing factor (see Table 20). Progression rate was lower in early onset cases (age of onset of disease 0 – 20 years) in comparison to cases with age of onset of disease 21 – 40 years. Steepest progression rate was found in late onset cases (age of onset of disease >40 years).

Table 20: final model influencing factors on progression rate

Final model from mixed models analysis with backward selection on factors influencing progression rate. Dependent variable SPRS total score, predictor time, remaining additional variable is age of onset of disease (aoo; groups 0 – 20 / 21 – 40 / >40years). b: regression coefficient; SE: standard error; CI: 95% confidence interval; lower: lower bound; upper: upper bound; y: years; significant if p≤0.05

parameter	b	SE	95% CI		p
			lower	upper	
Intercept	8.2	0.6	7.1	9.4	0.000
Disease duration	0.8	0.1	0.7	0.9	0.000
Aoo 0 – 20y	-8.0	1.3	-10.6	-5.4	0.000
Aoo 21 – 40y	-1.6	0.7	-3.1	-0.2	0.029
Aoo >40y	Parameter redundant				

Discussion

In this natural history study, we aimed to describe the natural disease progression of SPG4 with the help of longitudinal data of standardized and validated examination procedures. We recruited 276 patients with a total of 811 examinations based on SPRS over a time period of 12 years. This data is important for a better understanding of clinical phenotype, calculation of time until loss of independent walking, identification of influencing factors and determination of a progression rate of disease. This knowledge is required to calculate sample sizes necessary for clinical trials [10, 30, 32, 37].

Results

Age of onset of disease

The mean age of onset of disease in our cohort was 31.6 years. This is similar to findings of a large French cohort with age of onset 29.3 years [27].

We found a bimodal distribution of age of onset of disease, with a first peak at age 0 – 5 years and a second peak around the age of 40. These findings confirm the bimodal distribution of age of onset also seen by Parodi et al. [27]. In both our cohort and the French cohort the relative proportion of patients with age of onset of disease in early childhood compared with onset in adulthood was larger among carriers of missense than truncating variant carriers. Nonetheless, we could not confirm the earlier age of onset in missense variant carriers reported by Parodi et al. [27], although we see the same trend.

This is of interest since the association of early onset SPG4 with missense variants has been regarded as a hint for a dominant negative effect of mutant SPAST in the pathophysiology of these cases [4] in contrast to haploinsufficiency that is suggested by truncating variants and large genomic deletions in the majority of SPG4 patients [38].

If the variant type is the critical driver of age of onset, one would expect that age of onset is similar within families and patients carrying the same variant. However, this obvious assumption is refuted by our findings in families with reported age of onset in at least two affected members. In 35 families we found the age of onset to vary between 1 and 59 years with an average variability of 21.0 years between family members. This result is in line with 27 years as the average difference in the age of onset of disease found by Parodi et al. [27]. Therefore, no reliable prediction of the age of onset of the disease can be made neither from the underlying variant nor from the age of onset of disease of further family members. The determination of age of onset is a major issue that depends largely on the awareness of the patient or his relatives and the definition on symptoms that define onset of disease. E.g. spouses may have noticed an altered gait pattern several years before the patient himself became aware of a relevant gait problem or a physiotherapist may realize abnormal gait patterns much earlier than a person not used to sensitively realize movement disorders. Similarly, a gait problem may disclose earlier in patients with high demands on locomotion like sprinters/runners than a physically inactive person. In our study we decided to define age of onset by the onset of abnormal/spastic gait as reported by patients and their relatives. This largely depends on the recall of the families and may become less precise after long disease duration. This problem may only be solved in longitudinal studies with regular examinations of asymptomatic variant carriers.

Variant spectrum

The variant spectrum of the SPG4 cohort investigated in this study constitutes mainly of truncating variants (80%) and includes only 20% missense variants. Unbiased comparative data is rare. In a large French cohort Parodi et al found 30% of missense variants [27]. This number has also been reported in a recent review on SPG4 [4]. As there are no obvious reasons for these differences, this may reflect ethnical differences in our cohort with mostly German background. While the truncating variants are distributed over the entire gene, the missense variants accumulate especially in the AAA cassette. The amino acids of the AAA

cassette comprise 41.7% (275 amino acids of a total of 616 amino acids) of amino acids of the entire spastin protein. As 85.7% of missense variants affect the AAA cassette, variants in this domain of the SPAST gene are statistically twice as frequent as expected from a random distribution. Since the AAA domain is responsible for the microtubule severing function of spastin, this distribution of missense variants supports the hypothesis that microtubule severing is relevant for SPG4 pathogenesis.

There is no other region in spastin where missense variants accumulate. On the contrary, the 6 missense variants outside the AAA cassette are distributed all over the remaining protein. However, no missense variants have been observed in amino acid positions 1-87, which are constituting the M1 isoform and lacking in the M87 isoform [4].

According to the literature, the majority of SPAST variants are private variants or occur only in few families. We found the c.1684C>T nonsense variant in 14 independent families. Otherwise no variantal hotspots were paramount in our cohort. This has relevant implications for genetic testing: for diagnostic purposes, the whole gene needs to be analyzed. In addition, due to the considerable proportion of genomic deletions and duplications in about 20% of SPG4 families either Multiplex ligation-dependent probe amplification (MLPA) or whole exome sequencing with copy number of variants (WES-based evaluation of CNVs) are essential to detect these structural gene variants.

Clinical presentation

The vast majority of SPG4 patients (151 of 234 patients with full clinical information, 64.5%) in our cohort presented with a "pure" phenotype of HSP restricted to spasticity predominant in lower limbs, urinary symptoms and impaired vibration sense. This is in good accordance with SPG4 cohorts published earlier [8, 13, 27].

Spread of pyramidal signs to the upper limbs is discussed in the literature as complicating symptom or indicator of primary lateral sclerosis [39]. We found brisk reflexes of upper limbs to be a very common feature of SPG4 that was found in about half of our cases. As we did not see a correlation between spasticity of

upper limbs and severity of disease (by means of the SPRS total score), upper limb affection was not regarded as an indicator of a more severe course of disease. A high frequency of upper limb involvement in HSP was also seen in an electrophysiological assessment of the corticospinal tract using motor evoked potentials [40]. In this study, more than 30% of HSP patients showed abnormal motor evoked potentials.

Of importance for therapeutic considerations, about half of SPG4 patients in our cohort reported pain related to HSP (item 12 of the SPRS). This finding matches well with a recent study on non-motor symptoms in SPG4 that found pain in more than 41% of patients by the use of a validated pain questionnaire [41].

In about one third of patients (83 of 234 patients, 35.5%) additional symptoms like muscle atrophy (25 patients), impaired touch sense (24 patients), impaired joint position sense (14 patients), impaired thermesthesia (21 patients) or impaired pinprick sensation (14 patients) were observed. Detailed studies of the medical records of these patients revealed only mild manifestations of these additional symptoms that did not cause relevant handicap and therefore may be still regarded as "pure" HSP according to the classification of Harding [2, 28]. In contrast, three patients in our series (1.3%) showed severe complicating symptoms including intellectual disability (2 patients), epilepsy, severe ataxia and optic atrophy (1 patients each) (for details see Results Cases with complicated SPG4 and next chapter). In the literature SPG4 is referred as a mainly pure form of HSP. Nevertheless, complicated cases of SPG4 have been reported who presented with intellectual disability, epilepsy, cerebellar ataxia and psychiatric disorders as complicating symptoms [42-46]. Chelban et al. recently reported complicating symptoms indicative of complex phenotype in 25% of SPG4 cohort, finding inter alia psychiatric disorders, seizures, intellectual disability, dysarthria and dysphagia [42]. Findings by Chelban et al. and in our cohort raise the question, whether complicating symptoms in SPG4 are underrepresented due to the assumption of a pure phenotype. In order to verify the actual frequency of complicating symptoms in SPG4, additional signs and symptoms need to be recorded in detail and with differentiation of severity. The challenge here is that not only a detailed assessment of neurological symptoms is required, but also a

detection and severity assessment of diseases from other disciplines (autoimmune diseases, psychological disorders, etc.).

Cases with complicated SPG4

To understand better the development of complicated phenotypes in SPG4 we compared the patients from this series with patients from the literature carrying the same variant. For the c.1496G>A missense variant, that results in an exchange of the arginine to histidine at amino acid position 499 of spastin, four additional cases with the same variant have been reported [47-49]. All cases including our patient shared onset in early childhood and gross motor delay with severe spasticity of lower and in most cases upper limbs. All patients presented speech problems ranging from delay in articulation and expressive language at the age of 3 years to severe dysarthria and even anarthria at the age of 12 years. Three patients developed dysphagia. Two patients showed learning disability whereas intellectual ability was reported to be normal in two others and the fifth patient was delayed in self-help skills at the age of 3 years.

In contrast to this rather homogenous phenotype of complicated HSP, we and others observed SPG4 patients with the identical c.1496G>A variant but a pure type of HSP. Whereas two cases from the literature had onset in childhood [12, 50], one case from our series had onset in adulthood. Further patients with the c.1495C>T variant resulting in the exchange of the same amino acid at position 499 (Arg499Cys) had onset in early childhood as well. This indicates on the one hand a propensity to early onset and complicated HSP resulting from exchange of amino acid 499, but shows on the other hand that additional (genetic or non-genetic) factors modify the variantal effect and the phenotypic expression in SPG4.

The variant c.1250G>C found in case 52 with complicated and early onset HSP has not been described in the literature before. We still believe that this variant is causing the disease as segregation analysis proved this variant to be absent in both parents and therefore to have occurred *de novo* in *SPAST.*

The deletion of exons 8-17, causing SPG4 in case 260, has been reported recurrently in the literature [38, 51, 52]. Whereas these cases were reported to

display a pure type of HSP starting between 6 and 52 years of age, case 260 from our series presented with ataxia, dysphagia, myoclonus, cataract and optic atrophy. The reason for the phenotypical difference remains unclear, but differences in phenotype point to additional genetic or extra-genetic factors causing the more severe course of SPG4 in our patient.

In all these cases it would be interesting to perform further genetic analyzes by exome or genomes sequencing to gain more insight into potential variants in other HSP-related proteins like atlastin, REEP1 or reticulon2 that are known to interact with spastin.

Loss of independent walking / wheelchair dependency

To assess disease progression, we used two complementary approaches. In a first cross-sectional analysis we assessed the loss of independent walking ability and the age at wheelchair dependency as two striking events that could be remembered rather precisely by the vast majority of patients and mark important landmarks of mobility as a key feature of SPG4. In a second longitudinal approach we analyzed data of disease severity as determined by the SPRS in follow-up visits.

We found 50% of our SPG4 patients to be walking aid dependent at a median of 23 years after onset of disease. Wheelchair dependent was only a minority of our cohort (50 of 270 patients with manifest SPG4, 18.5%). Wheelchair dependency was reached after a median of 41 years after onset of disease. Of importance, patients with early onset had a longer duration of disease before loss of independent walking (see Figure 17 and Figure 18). Comparing groups with age of onset of disease ≤20 years to patients with age of onset >40 years, the earlier onset group requires walking aids after a disease duration three times longer than the late onset group (38 vs 13 years). Similarly, wheelchair dependency occurred in the early onset group after twice the disease duration as in the late onset group (25 vs 48 years). Pathophysiological factors that drive these age dependent differences in disease progression are not yet known. Some effect on wheelchair dependency seem to derive from the variant type. We found significant earlier use of a wheelchair in patients carrying missense variants compared to patients

with truncating variants (see Table 4). In addition, we found wheelchair dependency in early childhood only in missense variant carriers.

Comparable data on progression of HSP is limited to two studies from the Tübingen group. Schüle et al [10] found rather similar results in a mixed cohort including patients with different genotypes and HSP patients without a known genetic cause. As 196 SPG4 patients from the Schüle paper have been included in our cohort, these findings do not represent independent observations. In SPG5, a rare genotype of HSP that often goes along with afferent ataxia as a complicating symptom of HSP, Schöls et al. found a similar duration until walking aid dependency of 23 years but a shorter duration of 33 years until wheelchair dependency [36]. This difference is likely to represent genotype specific differences with a more severe course of disease in SPG5 probably due to additional handicap deriving from afferent ataxia as complicating symptom.

Longitudinal disease progression

In the longitudinal analysis of disease severity using prospective follow-up data of the SPRS this study revealed an average progression rate of 1 SPRS point per year. We restricted our analysis to follow-up visits of more than 3 months, as in a slowly progressive disease like SPG4 short term changes are more likely to reflect variability in daily condition, reproducibility of the score or acute effects of special therapies (e.g. botulinum toxin injections) than real changes in disease. This notion is supported by our finding that short examination intervals reveal calculated annual changes of > 50 SPRS points improvement and > 700 points worsening in some patients (see Table 17). This is implausible given that the SPRS has a maximum total score of 52 points.

In our analysis we found improvement in SPRS scores between the first and the second visit in 46 of 159 patients (29%) with longitudinal follow-up data. This high frequency may derive from optimized treatment initiated at the first visit to the specialized HSP outpatient clinic like physiotherapy or antispastic, analgesic or spasmolytic medication influencing SPRS items.

To analyze whether disease progression is linear within the course of disease we first assessed the progression rate in dependency of disease duration. We found similar progression rates in all groups independent from duration of disease (see Figure 23). In a second approach we analyzed growth models of disease progression including disease duration as linear, quadratic or cubic terms. We found the linear model to result in the best fit.

To assess factors that constitute major determinants of disease progression we performed mixed models including age of onset, gender and variant type as additional variables. We found only age of onset as an influencing factor with a lower progression rate in early onset cases compared to late onset cases (see Table 20). This is important information that needs to be considered in the planning of clinical trials to assure comparability of group performance. Groups need to be randomized according to age of onset to prevent that apparently slower progression occurs in the group with more early onset patients. Comparable data is missing in the literature as no prospective longitudinal natural history studies have been performed. Parodi et al. just state from their cohort that they are seeing more severe cases in the late onset than in the young onset group [27]. Our finding from the cross-sectional analysis that SPG4 progresses faster (after a shorter disease duration) to walking aid dependency and wheelchair dependency in late onset cases is in line with the higher progression rate we found in the late onset cases calculated from longitudinal data (see Table 20).

Outlook

Results from this NHS of SPG4 enable not only a deeper understanding of disease progression and more accurate patient information about the course of disease and prognosis, but also predictions of disease progression for designing and conducting of therapy studies. Therapeutic trials can be based on the measurement of disease severity by SPRS and the knowledge of progression rates in merely symptomatic therapy with its limited options.

Taking into account that SPG4 is a slowly progressive and rare disease, observation periods of at least 2 years would be advisable.

Assuming an observation period of at least 2 years, a remarkably lower variance of individual progression rates is obtained, as depicted in Figure 22. However, it must be taken into account, that the evaluation of progression rate for time intervals of 2 years between examinations was based on only 40 measuring points and therefore provided considerably less data than the examination of the shorter time intervals. This may enhance the effect of lower variance in progression rate with longer examination intervals.

Furthermore, given the anticipated slow progression rate of 1 point in the SPRS total score per year, the observation period must provide sufficient time for a measurable progression to be expected.

The required number of cases to confirm a significant effect of a new therapy depends on the anticipated effect size. Data from this NHS of SPG4 can be used to determine required sample sizes.

Summary

Hereditary spastic paraplegias (HSP) are a group of rare neurodegenerative diseases characterized by a progressive spastic gait disorder and caused by variants in a wide variety of genes. The most common subtype is Spastic paraplegia 4 (SPG4). We performed a longitudinal natural history study in a large cohort of 276 patients with genetically proven SPG4 to 1) describe the phenotypic spectrum and frequency of complicating symptoms, 2) assess variability in age of onset, 3) calculate time to dependency on walking aids, 4) provide prospective data on disease progression using the Spastic Paraplegia Rating Scale (SPRS), and 5) determine potential disease modifying factors.

Patients were recruited via HSP outpatient clinics of the German Center for Neurodegenerative Diseases (DZNE) as well as the BMBF-funded GeNeMove network. Longitudinal data available in the HSP Registry, including detailed demographic, phenotypic and mutation data as well as the SPRS were analyzed. Average self-reported disease onset in SPG4 is at 31.6 years; in addition to a spastic gait pattern, lower limb spasticity, lower limb weakness, contractures, neurogenic bladder disorder and impairment of vibration sense were the most frequently observed features. Pyramidal motor affection of the upper limbs was observed in half of our cohort.

Loss of independent walking is perceived as a negative milestone by many HSP patients. Half of our cohort regularly used a walking aid at baseline (average loss of independent walking ability at 47.4 years). 20% were wheelchair dependent (median age of onset 48.4 years). Hereby, missense variant carriers used a wheelchair at a significantly younger age than carriers of truncating SPAST variants (38.5 vs 50.3 years). Additionally, disease duration until walking aid and wheelchair dependency was influenced by the age of onset. In early onset SPG4 (<20 years) walking aid and wheelchair dependency occurred after longer disease duration (39/48 years) than in late onset SPG4 (>40 years; 13/25 years). We used longitudinal SPRS follow-up examinations and linear mixed models to determine the disease progression. SPG4 progresses with an average progression rate of 1 point in SPRS total score per year. Age of disease onset

significantly determined disease progression with slower progression in early onset cases.

In conclusion we here establish progression rates of SPG4 using both patient-centered outcomes (walking aid/wheelchair use) as well as the clinician-reported SPRS. Age of onset significantly influences disease progression and therefore needs to be considered in clinical trials. Based on our results trial sample sizes and trial duration can be determined that are required to demonstrate effectiveness of new therapies in clinical trials.

Zusammenfassung

Hereditäre Spastische Paraplegien (HSP) sind eine Gruppe von seltenen neurodegenerativen Erkrankungen, die durch eine fortschreitende spastische Gangstörung gekennzeichnet sind. Mutationen in über 80 verschiedenen Genen können zu einer HSP führen. Der häufigste genetische Subtyp ist die Spastische Paraplegie Typ 4 (SPG4). Wir haben eine longitudinale Verlaufsstudie an einer großen Kohorte von 276 Patienten mit genetisch gesicherter SPG4 durchgeführt, um 1) das phänotypische Spektrum und die Häufigkeit komplizierender Zusatzsymptome zu beschreiben, 2) die Variabilität des Erkrankungsalters zu untersuchen, 3) die Zeit bis zum Verlust der freien Gehfähigkeit zu bestimmen, 4) prospektive Daten zum Krankheitsverlauf zu liefern und 5) mögliche krankheitsmodifizierende Faktoren zu identifizieren.

Die Patienten wurden aus HSP-Spezialambulanzen des Deutschen Zentrums für Neurodegenerative Erkrankungen (DZNE) sowie des BMBF-geförderten GeNeMove-Netzwerkes rekrutiert. Wir analysieren statistisch die Längsschnittdaten von Untersuchungen auf Grundlage der Spastic paraplegia rating scale (SPRS).

Der durchschnittliche Erkrankungsbeginn liegt bei 31,6 Jahren. Neben der spastischen Gangstörung sind Spastik und Schwäche der unteren Extremitäten, Kontrakturen, neurogene Blasenstörungen und Beeinträchtigung des Vibrationsempfindens häufige Symptome. Wir fanden in der Hälfte unserer Kohorte Anzeichen für eine motorische Beeinträchtigung auch der oberen Extremität.

Ein Meilenstein der Beeinträchtigung bei SPG4 ist der Verlust der freien Gehfähigkeit. Die Hälfte unserer Kohorte war angewiesen auf Gehhilfen (Beginn durchschnittlich mit 47,4 Jahren). 20% waren auf einen Rollstuhl angewiesen (Beginn durchschnittlich mit 48,4 Jahren); Träger von Missensmutationen nutzten einen Rollstuhl hierbei in signifikant jüngerem Alter als Träger trunkierender Mutationen (38,5 vs. 50,3 Jahre). Des Weiteren trat der Verlust der freien Gehfähigkeit und die Rollstuhlabhängigkeit bei früh einsetzender SPG4 (< 20

Jahre) nach längerer Krankheitsdauer (39/48 Jahre) auf als bei spät einsetzender SPG4 (>40 Jahre; 13/25 Jahre).

Wir setzen longitudinale SPRS-Verlaufsuntersuchungen und lineare gemischte Modelle ein, um die Erkrankungsprogression zu bestimmen. Die SPG4 nimmt hierbei um durchschnittlich einen Punkt auf der SPRS-Skala pro Jahr zu. Das Erkrankungsalter bestimmt signifikant die Krankheitsprogression mit einer langsameren Progressionsrate bei früh-beginnender SPG4.

Zusammenfassend berichten wir hier die Progressionsrate der SPG4 unter Verwendung Patienten-zentrierter Outcomes (Abhängigkeit von Gehhilfe/Rollstuhl) und sog. „clinician-reported" Outcomes (SPRS). Das Alter bei Erkrankungsbeginn beeinflusst signifikant die Erkrankungsprogression und muss daher bei der Planung klinischer Studien berücksichtigt werden. Basierend auf unseren Ergebnissen sind Kalkulation von Fallzahlen sowie Studiendauer für künftige Therapiestudien möglich.

References

1. Denton, K.R., et al., *Loss of spastin function results in disease-specific axonal defects in human pluripotent stem cell-based models of hereditary spastic paraplegia.* Stem Cells, 2014. **32**(2): p. 414-23.
2. Harding, A.E., *Classification of the hereditary ataxias and paraplegias.* Lancet, 1983. **1**(8334): p. 1151-5.
3. Blackstone, C., *Converging cellular themes for the hereditary spastic paraplegias.* Curr Opin Neurobiol, 2018. **51**: p. 139-146.
4. Solowska, J.M. and P.W. Baas, *Hereditary spastic paraplegia SPG4: what is known and not known about the disease.* Brain, 2015. **138**(Pt 9): p. 2471-84.
5. Ruano, L., et al., *The global epidemiology of hereditary ataxia and spastic paraplegia: a systematic review of prevalence studies.* Neuroepidemiology, 2014. **42**(3): p. 174-83.
6. Coutinho, P., et al., *Hereditary ataxia and spastic paraplegia in Portugal: a population-based prevalence study.* JAMA Neurol, 2013. **70**(6): p. 746-55.
7. Erichsen, A.K., et al., *Prevalence of hereditary ataxia and spastic paraplegia in southeast Norway: a population-based study.* Brain, 2009. **132**(Pt 6): p. 1577-88.
8. McDermott, C.J., et al., *Clinical features of hereditary spastic paraplegia due to spastin mutation.* Neurology, 2006. **67**(1): p. 45-51.
9. Hazan, J., et al., *Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia.* Nat Genet, 1999. **23**(3): p. 296-303.
10. Schule, R., et al., *Hereditary spastic paraplegia: Clinicogenetic lessons from 608 patients.* Ann Neurol, 2016. **79**(4): p. 646-58.
11. Burguez, D., et al., *Clinical and molecular characterization of hereditary spastic paraplegias: A next-generation sequencing panel approach.* J Neurol Sci, 2017. **383**: p. 18-25.
12. Depienne, C., et al., *Spastin mutations are frequent in sporadic spastic paraparesis and their spectrum is different from that observed in familial cases.* J Med Genet, 2006. **43**(3): p. 259-65.
13. Salinas, S., et al., *Hereditary spastic paraplegia: clinical features and pathogenetic mechanisms.* Lancet Neurol, 2008. **7**(12): p. 1127-38.
14. Kasher, P.R., et al., *Direct evidence for axonal transport defects in a novel mouse model of mutant spastin-induced hereditary spastic paraplegia (HSP) and human HSP patients.* J Neurochem, 2009. **110**(1): p. 34-44.
15. Tarrade, A., et al., *A mutation of spastin is responsible for swellings and impairment of transport in a region of axon characterized by changes in microtubule composition.* Hum Mol Genet, 2006. **15**(24): p. 3544-58.
16. Blackstone, C., C.J. O'Kane, and E. Reid, *Hereditary spastic paraplegias: membrane traffic and the motor pathway.* Nat Rev Neurosci, 2011. **12**(1): p. 31-42.
17. Stone, M.C., et al., *Normal spastin gene dosage is specifically required for axon regeneration.* Cell Rep, 2012. **2**(5): p. 1340-50.
18. Fink, J.K., *Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms.* Acta Neuropathol, 2013. **126**(3): p. 307-28.

19. Wharton, S.B., et al., *The cellular and molecular pathology of the motor system in hereditary spastic paraparesis due to mutation of the spastin gene.* J Neuropathol Exp Neurol, 2003. **62**(11): p. 1166-77.

20. Deluca, G.C., G.C. Ebers, and M.M. Esiri, *The extent of axonal loss in the long tracts in hereditary spastic paraplegia.* Neuropathol Appl Neurobiol, 2004. **30**(6): p. 576-84.

21. Solowska, J.M., et al., *Quantitative and functional analyses of spastin in the nervous system: implications for hereditary spastic paraplegia.* J Neurosci, 2008. **28**(9): p. 2147-57.

22. Roll-Mecak, A. and R.D. Vale, *Structural basis of microtubule severing by the hereditary spastic paraplegia protein spastin.* Nature, 2008. **451**(7176): p. 363-7.

23. White, S.R., et al., *Recognition of C-terminal amino acids in tubulin by pore loops in Spastin is important for microtubule severing.* J Cell Biol, 2007. **176**(7): p. 995-1005.

24. Evans, K.J., et al., *Linking axonal degeneration to microtubule remodeling by Spastin-mediated microtubule severing.* J Cell Biol, 2005. **168**(4): p. 599-606.

25. Reid, E., et al., *The hereditary spastic paraplegia protein spastin interacts with the ESCRT-III complex-associated endosomal protein CHMP1B.* Hum Mol Genet, 2005. **14**(1): p. 19-38.

26. Shoukier, M., et al., *Expansion of mutation spectrum, determination of mutation cluster regions and predictive structural classification of SPAST mutations in hereditary spastic paraplegia.* Eur J Hum Genet, 2009. **17**(2): p. 187-94.

27. Parodi, L., et al., *Spastic paraplegia due to SPAST mutations is modified by the underlying mutation and sex.* Brain, 2018. **141**(12): p. 3331-3342.

28. Harding, A.E., *Hereditary spastic paraplegias.* Semin Neurol, 1993. **13**(4): p. 333-6.

29. Bellofatto, M., et al., *Management of Hereditary Spastic Paraplegia: A Systematic Review of the Literature.* Front Neurol, 2019. **10**: p. 3.

30. Trummer, B., D. Haubenberger, and C. Blackstone, *Clinical Trial Designs and Measures in Hereditary Spastic Paraplegias.* Front Neurol, 2018. **9**: p. 1017.

31. Fassier, C., et al., *Microtubule-targeting drugs rescue axonal swellings in cortical neurons from spastin knockout mice.* Dis Model Mech, 2013. **6**(1): p. 72-83.

32. Schule, R., et al., *The Spastic Paraplegia Rating Scale (SPRS): a reliable and valid measure of disease severity.* Neurology, 2006. **67**(3): p. 430-4.

33. Bohannon, R.W. and M.B. Smith, *Interrater reliability of a modified Ashworth scale of muscle spasticity.* Phys Ther, 1987. **67**(2): p. 206-7.

34. Klimpe, S., et al., *Disease severity affects quality of life of hereditary spastic paraplegia patients.* Eur J Neurol, 2012. **19**(1): p. 168-71.

35. Lindig, T., et al., *Gray and white matter alterations in hereditary spastic paraplegia type SPG4 and clinical correlations.* J Neurol, 2015. **262**(8): p. 1961-71.

36. Schols, L., et al., *Hereditary spastic paraplegia type 5: natural history, biomarkers and a randomized controlled trial.* Brain, 2017. **140**: p. 3112-3127.

37. Chrestian, N., et al., *Clinical and genetic study of hereditary spastic paraplegia in Canada.* Neurol Genet, 2017. **3**(1): p. e122.

38. Beetz, C., et al., *High frequency of partial SPAST deletions in autosomal dominant hereditary spastic paraplegia.* Neurology, 2006. **67**(11): p. 1926-30.

39.   Fink, J.K., *Hereditary spastic paraplegia.* Curr Neurol Neurosci Rep, 2006. **6**(1): p. 65-76.

40.   Karle, K.N., et al., *Electrophysiological characterisation of motor and sensory tracts in patients with hereditary spastic paraplegia (HSP).* Orphanet J Rare Dis, 2013. **8**: p. 158.

41.   Rattay, T.W., et al., *Non-motor symptoms are relevant and possibly treatable in hereditary spastic paraplegia type 4 (SPG4).* J Neurol, 2020. **267**(2): p. 369-379.

42.   Chelban, V., et al., *Truncating mutations in SPAST patients are associated with a high rate of psychiatric comorbidities in hereditary spastic paraplegia.* Journal of Neurology Neurosurgery and Psychiatry, 2017. **88**(8): p. 681-687.

43.   Gigli, G.L., et al., *Spastic paraplegia, epilepsy, and mental retardation in several members of a family: a novel genetic disorder.* Am J Med Genet, 1993. **45**(6): p. 711-6.

44.   Nielsen, J.E., et al., *Hereditary spastic paraplegia with cerebellar ataxia: a complex phenotype associated with a new SPG4 gene mutation.* Eur J Neurol, 2004. **11**(12): p. 817-24.

45.   Vahter, L., et al., *The prevalence of depression in hereditary spastic paraplegia.* Clin Rehabil, 2009. **23**(9): p. 857-61.

46.   Webb, S., et al., *Autosomal dominant hereditary spastic paraparesis with cognitive loss linked to chromosome 2p.* Brain, 1998. **121 (Pt 4)**: p. 601-9.

47.   de Souza, P.V., et al., *Infantile-onset ascending spastic paraplegia phenotype associated with SPAST mutation.* J Neurol Sci, 2016. **371**: p. 34-35.

48.   Gillespie, M.K., et al., *Association of Early-Onset Spasticity and Risk for Cognitive Impairment With Mutations at Amino Acid 499 in SPAST.* J Child Neurol, 2018. **33**(5): p. 329-332.

49.   Ogasawara, M., et al., *A p.Arg499His Mutation in SPAST Is Associated with Infantile Onset Ascending Spastic Paralysis Complicated with Dysarthria and Anarthria.* Neuropediatrics, 2019. **50**(6): p. 391-394.

50.   Polymeris, A.A., et al., *A series of Greek children with pure hereditary spastic paraplegia: clinical features and genetic findings.* J Neurol, 2016. **263**(8): p. 1604-11.

51.   Depienne, C., et al., *Exon deletions of SPG4 are a frequent cause of hereditary spastic paraplegia.* J Med Genet, 2007. **44**(4): p. 281-4.

52.   Wang, K. and G. Zhao, *Exon 8-17 deletions of SPAST in a Chinese family with hereditary spastic paraplegia: a case report and literature review.* J Neurol Sci, 2015. **357**(1-2): p. 282-4.

Erklärung zum Eigenanteil der Dissertationsschrift

Die Arbeit wurde in der Klinik für Neurologie Tübingen und dem Hertie Institut Tübingen unter Betreuung von PD Dr. Rebecca Schüle-Freyer durchgeführt.

Die Konzeption der Studie erfolgte durch PD Dr. Rebecca Schüle-Freyer, Prof. Dr. rer. nat. Peter Martus, Prof. Dr. Ludger Schöls und mich.

Die Datenerhebung erfolgte durch verschiedene Untersucher in Bochum, Bonn, Kiel, Magdeburg, Mainz, München, Regensburg, Rostock und Tübingen. Die Untersucher sind in der folgenden Tabelle mit jeweiliger Anzahl erhobener Untersuchungen aufgelistet.

Untersucher	Anzahl SPRS		Zentrum
Gallenmüller	15	1,81%	München
Haack	1	0,12%	München
Heck	2	0,24%	München
Hengel	11	1,32%	Tübingen
Henkel	28	3,37%	Magdeburg
Karle (Eckstein)	85	10,23%	Tübingen
Klebe	47	5,66%	Kiel
Klimpe	88	10,59%	Mainz
Klopstock	13	1,56%	München
Kohl	36	4,33%	Regensburg
Küpper	4	0,48%	München
Kurzwelly	14	1,68%	Bonn
Lohmann	6	0,72%	Tübingen
Marxreiter	1	0,12%	Regensburg
Neuhofer	4	0,48%	München
Otto	32	3,85%	Bochum
Petersen	3	0,36%	München

Untersucher	Anzahl SPRS		Zentrum
Rattay	68	8,18%	Tübingen
Rimmele	41	4,93%	Rostock
Schöls	26	3,13%	Tübingen
Schüle	193	23,23%	Tübingen
Stendel	14	1,68%	München
Stolze	8	0,96%	Kiel
Strigl-Pill	3	0,36%	München
Synofzik	1	0,12%	Tübingen
Vogt	5	0,60%	Bonn
Weiland	2	0,24%	Tübingen
Wiethoff	58	6,98%	Tübingen
Winkler	8	0,96%	Regensburg
Winner	1	0,12%	Tübingen
Wolf	13	1,56%	Tübingen

Die Aufbereitung der Daten erfolgte durch mich nach Anleitung von PD Dr. Rebecca Schüle-Freyer.

Prof. Dr. rer. nat. Peter Martus (Institutsleiter Institut für Klinische Epidemiologie und angewandte Biometrie Tübingen) bestimmte die Auswertungsmethoden für die verschiedenen statistischen Analysen. Die statistische Auswertung erfolgte durch mich.

Ich versichere, das Manuskript selbständig (nach Anleitung durch PD Dr. Rebecca Schüle-Freyer) verfasst zu haben und keine weiteren als die von mir angegebenen Quellen verwendet zu haben.

Tübingen, den

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Appendix

Inventory

SPRS: Inventory of Complicating Signs & Symptoms

Code	Date	Rater

Max. walking distance (m)

Cognitive impairment (CI)[1]
- ☐ yes
- ☐ no
- ☐ unknown

Age at onset of CI
- ☐ childhood (< 10y)
- ☐ adolescence (< 20y)
- ☐ adulthood (20-65y)
- ☐ old age (> 65y)

highest academic degree: _____

Psychiatric symptoms[2]
- ☐ psychosis
- ☐ personality disorder
- ☐ bipolar disorder
- ☐ other
- ☐ none
- ☐ unknown

Visual loss (c.c. < 0.8)
- ☐ yes
- ☐ no
- ☐ unknown

Retinitis pigmentosa[3]
- ☐ yes
- ☐ no
- ☐ unknown

Optic atrophy[3]
- ☐ yes
- ☐ no
- ☐ unknown

Cataract[3]
- ☐ yes
- ☐ no
- ☐ unknown

Oculomotor disturbances
- ☐ cerebellar
- ☐ other
- ☐ none
- ☐ unknown

Dysarthria
- ☐ cerebellar
- ☐ bulbar
- ☐ pseudobulbar
- ☐ unclassified
- ☐ none
- ☐ unknown

Dysphagia
- ☐ yes
- ☐ no
- ☐ unknown

Upper limb pyramidal involvement
- ☐ increased tendon reflexes only
- ☐ spasticity
- ☐ none
- ☐ unknown

Muscle atrophy
- ☐ upper limbs
- ☐ lower limbs
- ☐ none
- ☐ unknown

Fasciculations[4]
- ☐ 1 region
- ☐ 2 regions
- ☐ 3 regions
- ☐ 4 regions
- ☐ none
- ☐ unknown

Limb ataxia
- ☐ yes
- ☐ no
- ☐ unknown

Gait ataxia
- ☐ yes
- ☐ no
- ☐ unknown

Extrapyramidal involvement
- ☐ hypo-/akinesia
- ☐ rigor
- ☐ tremor
- ☐ myoclonus
- ☐ dystonia
- ☐ other
- ☐ none
- ☐ unknown

Impaired vibration sense[5]
- ☐ yes
- ☐ no
- ☐ unknown

Other sensory deficits
- ☐ touch
- ☐ joint position
- ☐ temperature
- ☐ pinprick
- ☐ none

Other signs and symptoms
- ☐ hearing impairment
- ☐ seizures
- ☐ skin abnormalities
- ☐ pes cavus
- ☐ scoliosis
- ☐ diabetes mellitus
- ☐ anemia
- ☐ other
- ☐ none
- ☐ unknown

[1] rate by clinical impression
[2] do not rate unipolar depression
[3] diagnosed by an ophthalmologist
[4] 4 regions: cranial, cervical, thoracic, lumbo-sacral
[5] Impaired vibration sense is defined as a vibration perception threshold < 6/8 measured at the mallealus medialis using a Rydel-Seiffer tuning fork (128 Hz)

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SPRS

Spastic Paraplegia Rating Scale (SPRS)

Code	Date	Rater

(1) Walking distance without pause
Due to history, walking aids allowed
0: Normal, unlimited
1: Abnormal exhaustion due to spasticity after more than 500m
2: Walking distance less than 500m
3: Walking distance less than 10 m
4: Unable to walk

(2) Gait quality
Patient is asked to walk as fast as possible a 10 meter distance including one turn
0: Normal
1: Mild stiffness, running still possible
2: Clearly spastic gait, interfering with running
3: Spastic gait requiring use of canes/walker
4: Unable to walk for a 10 meter distance even with maximal support

(3) Maximum gait speed _____ sec
Time for a 10 meter distance including one turn, taken by stop watch
0: Normal
1: Slightly reduced (10m: >= 5s)
2: Moderately reduced (10m: >= 10s)
3: Severely reduced (10m: >= 20s)
4: Unable to walk for a 10m distance or time >= 40s

(4) Climbing stairs
5 steps upstairs - turn - 5 steps downstairs
0: Normal: needs no support of the banister
1: Mild impairment: needs intermittent support of the banister
2: Moderate impairment: needs permanent support of the banister
3: Severe impairment: needs support of another person or additional walking aid to perform task
4: Unable to climb stairs

(5) Speed of stair climbing _____ sec
Time for 5 steps upstairs - turn - 5 steps downstairs, taken by stop-watch
0: Normal
1: Slightly reduced (>= 5s to perform task)
2: Moderately reduced (>=10s to perform task)
3: Severely reduced (>= 20s to perform task)
4: Unable to climb stairs

(6) Arising from chair
Patient attempts to arise from a straight-back wood or metal chair with arms folded across chest
0: Normal
1: Slow, or may need more than one attempt.
2: Pushes self up from arms of seat.
3: Tends to fall back and may have to try more than one time but can get up without help.
4: Unable to arise without help.

(7) Spasticity - hip adductor muscles
Score more severely affected side
0: No increase in muscle tone
1: Slight increase in muscle tone, manifested by a catch and release
2: More marked increase in muscle tone through most of the range of motion
3: Considerable increase in muscle tone - passive movement is difficult
4: Limb stiff in adduction

(8) Spasticity - knee flexion
Score more severely affected side
0: No increase in muscle tone
1: Slight increase in muscle tone, manifested by a catch and release
2: More marked increase in muscle tone through most of the range of motion
3: Considerable increase in muscle tone - passive movement is difficult
4: Limb stiff in flexion or extension

(9) Weakness - hip abduction
0: No weakness
1: Mild weakness (4/5)
2: Moderate weakness (3/5)
3: Severe weakness (1-2/5)
4: Plegia (0/5)

(10) Weakness - foot dorsiflexion
0: No weakness
1: Mild weakness (4/5)
2: Moderate weakness (3/5)
3: Severe weakness (1-2/5)
4: Plegia (0/5)

(11) Contractures of lower limbs
Score in supine position
- Hip extension: lumbar spine and thighs touch the underlay. Hip abduction: angle of >60° between the legs possible
- Knee extension: thigh and calf touch the underlay
- Ankle dorsal extension: > 10° possible; ankle pronation: > 10° possible

0: No contractures
1: Mild, not fixed abnormal position of one joint (unilaterally or bilaterally)
2: Fixed contracture of one joint (unilaterally or bilaterally)
3: Fixed contracture of two joints (unilaterally or bilaterally)
4: Fixed contracture of more than two joints (unilaterally or bilaterally)

(12) Pain due to SP related symptoms
0: None
1: <= 50% of waking day present AND intensity 0 - 3 points on visual analogue scale
2: <= 50% of waking day present AND intensity 4 - 10 points on visual analogue scale
3: > 50% of waking day present AND intensity 0 - 3 on visual analogue scale
4: > 50% of waking day present

(13) Bladder and bowel function
0: Normal bladder and bowel function
1: Urinary or fecal urgency (difficulties to reach toilet in time)
2: Rare and mild urge incontinence (no nappy required)
3: Moderate urge incontinence (requires nappy or catheter when out of the house)
4: Permanent catheterisation or permanent nappy

Total Score _____

Missing information was supplemented from medical reports, reports of genetical diagnostic and "source data" (original SPRS scores on paper). Additionally, the data set was updated by novel examinations, examinations not yet added to the HIH database and examinations of cases with recently genetically diagnosed SPG4. vus: variant of unknown significance.

	Information of data query from HSP registry				After completion of data			
	Available		missing		available		missing	
	cases	examinations	cases	examinations	cases	examinations	cases	examinations
Patient ID	194	550	0	0	276	811	0	0
Family number	125	540	8	10	276	811	0	0
Year of birth	131	428	63	122	276	811	0	0
gender					276	811	0	0
genotype	177	505	17 vus	45 vus	276	811	0	0
variant	101	331	93	219	261	789	15	22
Mode of inheritance	181	531	13	19	270	801	6	10
status	193	548	1	2	276	811	0	0
diagnosis	194	550	0	0	276	811	0	0
Age of onset	181	532	13	18	261	791	15	20
Walking aid	181	535	13	15	258	774	18	37
Wheelchair	180	531	14	19	250	721	26	90
Examination date	193	549	1	1	276	811	0	0
Item 1	*193*	548	*2*	2	276	811	0	0
Item 2	*193*	547	*3*	3	275	810	1	1
Item 3	*190*	546	*4*	4	274	809	2	2
10m walking in seconds	*181*	493	*57*	84	232	747	44	64
Item 4	*192*	543	*7*	7	270	805	6	6
Item 5	*192*	546	*4*	4	273	808	3	3
Climbing 5 stairs up and down in seconds	*164*	457	*62*	90	222	730	54	81
Item 6	*192*	543	7	7	273	808	3	3
Item 7	*193*	548	2	2	276	811	0	0
Item 8	*193*	548	2	2	275	810	1	1
Item 9	*192*	546	4	4	273	808	3	3
Item 10	*193*	547	3	3	274	809	2	2
Item 11	*193*	546	4	4	271	806	5	5

	Information of data query from HSP registry				After completion of data			
	Available		missing		available		missing	
	cases	examinations	cases	examinations	cases	examinations	cases	examinations
Item 12	*192*	544	*6*	6	272	807	4	4
Item 13	*192*	546	*4*	4	274	809	2	2
Total Score	*186*	530	*8*	20	258	791	18	20

Appendix table 2: variant spectrum of the cohort – missense variants

Transkript ID ENST00000315285.3

Regions	cDNA	Protein	Number of patients	Number of families
	692C>T	A231V	1	1
MTBD	926G>A	R309H	1	1
	1000G>A	D334N	1	1
AAA	1031T>C	I344T	1	1
	1109G>A	G370E	2	2
	1111C>G	L371V	1	1
	1130G>A	G377E	1	1
	1132C>G	L378V	1	1
	1172T>C	L391P	2	1
	1219A>G	S407G	1	1
	1250G>C	G417A	1	1
	1276C>T	L426F	1	1
	1313T>C	I438T	1	1
	1322A>G	D441G	1	1
	1325A>T	E442V	1	1
	1367A>G	D456G	3	1
	1378C>T	R460C	3	3
	1405T>G	F469V	1	1
	1453G>C	A485P	1	1
	1496G>A	R499H	2	2
	1526C>G	P509R	2	1
	1553T>C	L518P	2	1
	1631A>G	Y544C	1	1
	1685G>A	R562Q	2	2
	1691T>C	L564P	1	1
	1710G>T	K570N	1	1
	1785C>A	S595R	1	1
	1821G>C	W607C	1	1
	1844C>T	T615I	1	1
Σ			41	35

Appendix table 3: variant spectrum of the cohort – inframe deletions / insertions

Transkript ID ENST00000315285.3

Regions	cDNA	Protein	Number of patients	Number of families
	297_302dupTGCCTC	A100_S101insSA	1	1
AAA	1101_1103delGTT	L367del	1	1
	1210_1212delTTT	F403del	1	1
	1651_1652delinsTA	A551Y	3	1
∑			6	4

Appendix table 4: variant spectrum of the cohort – nonsense variants

Transkript ID ENST00000315285.3

Regions	cDNA	Number of patients	Number of families
	19C>T	1	1
	127G>T	1	1
	131C>A	1	1
	139A>T	4	2
HR	238C>T	4	1
MIT	349C>T	1	1
	361A>T	2	1
	448A>T	1	1
	469G>T	1	1
	577C>T	7	1
	716T>G	1	1
	734C>G	1	1
	746C>G	1	1
MTBD	841G>T	1	1
	870G>A	1	1
AAA	1054C>T	2	2
	1063C>T	1	1
	1114A>T	4	4
	1242A>G	1	1
	1291C>T	4	3
	1360G>T	1	1
	1417C>T	2	1
	1606C>T	1	1
	1684C>T	19	14
	1741C>T	5	5
∑		68	49

Appendix table 5: variant spectrum of the cohort – frameshift deletions / insertions

Transkript ID ENST00000315285.3

Regions	cDNA	Number of patients	Number of families
	110_123delGGCCGGCCCCTCCG	1	1
	261_261delG	2	1
	330_331delCG	1	1
MIT	367_367delinsTA	1	1
	382_382delT	1	1
	455_459delGTATT	1	1
	456_460delTATTG	5	3
	510_511delGT	1	1
	520_521insA	1	1
	706_710delAAAGA	1	1
	707_708delAA	4	1
	752_753delCA	2	2
MTBD	839_840delAG	6	2
	911_911delC	2	1
	940_941delTT	1	1
	965_966insA	1	1
	978_981delCCTT	1	1
	995_995delT	1	1
AAA	1174_1174delG	1	1
	1215-1219delTATAA	2	2
	1260_1261delGA	1	1
	1282_1283insGCTCTTTTTTGGTGAGG	1	1
	1306_1307delinsG	1	1
	1340_1344delTGTGT	2	1
	1348-1352delAGAAG	1	1
	1392_1393insA	1	1
	1463_1463delinsTTA	1	1
	1474_1474delC	1	1
	1560_1561delTC	1	1
	1596_1597insA	2	2
	1695_1695delA	1	1
	1745_1745delT	3	2
	1838_1839insA	1	1
Σ		53	40

Appendix table 6: variant spectrum of the cohort – exon deletions / duplications

Transkript ID ENST00000315285.3

cDNA	Number of patients	Number of families
Ex1_del	8	6
Ex1-3_del	2	1
Ex1-7_del	3	1
Ex1-17_del	1	1
Ex2_dup	1	1
Ex2-5_del	1	1
Ex2-7_del	2	2
Ex2-9_del	2	2
Ex2-16_dup	2	1
Ex4-9_del	4	1
Ex5_del	2	1
Ex5-15_del	1	1
Ex6-17_del	2	1
Ex8_del	3	3
Ex8-16_del	2	2
Ex8-17_del	1	1
Ex9-17_del	1	1
Ex10_del	1	1
Ex10-12_del	2	2
Ex10-12_dup	1	1
Ex13-16_del	2	2
Ex16_del	2	1
Ex16-17_del	2	1
Ex17_del	4	2
Σ	52	37

Appendix table 7: variant spectrum of the cohort – splice site variants

Transkript ID ENST00000315285.3

Exon borders	cDNA	Number of patients	Number of families
1 / 2	415+1G>A	1	1
3 / 4	586+9delTAAT	1	1
4 / 5	683-1G>A	1	1
5 / 6	870+1G>A	1	1
6 / 7	1004+5G>T	1	1
7 / 8	1098+2T>C	1	1
8 / 9	1173+1G>A	1	1
9 / 10	1245+1G>A	4	4
	1245+1G>T	1	1
	1245+6T>G	1	1
10 / 11	1322-14T>A	2	1
	1322-9T>G	1	1
11 / 12	1414-1G>A	1	1
	1414-2A>G	2	1
12 / 13	1492_1493+2delAGGT	2	1
	1493+1G>A	6	1
	1493+2T>A	1	1
13 / 14	1536+1G>A	1	1
	1536+1G>T	1	1
	1536+5_1536+7delGTA	1	1
14 / 15	1616+1G>A	1	1
15 / 16	1687+2T>A	4	1
17	1853+1G>T	1	1
∑		37	26

Case report of apparently recessive mode of inheritance

In our cohort 1 case (case 175) was included in whose family tree the mode of inheritance appeared to be recessive.

Case 175 was the first family member diagnosed with HSP/SPG4, though his sister (43 years of age) already developed similar symptoms. His parents, their siblings (1 each) and the siblings' children (3 respectively 1) were reported to have no neurological symptoms. Therefore, the mode of inheritance was rated to be apparently recessive. Case 175 affected when he was 40 years old. 8 years later he was still able to walk independently and improved within 1 year from a

SPRS total score of 8 to 4 points. Genetic diagnostics revealed a truncating variant with a deletion of the first exon (Ex1_del).

Appendix table 8: intrafamilial variability of the age of onset of disease

Family ID	Patient ID	Age of onset	Mean age of onset	Range age of onset	Variant
4	4	37	46.0	18	Ex6-17_del*
	51	55			
5	18	10	23.3	30	Ex17_del
	126	20			
	5	40			
8	63	30	38.5	17	Ex16-17_del
	9	47			
12	13	16	40.0	48	1745del
	16	64			
16	20	6	13.0	14	Ex1_del
	19	20			
18	22	20	23.0	6	1414-2A>G
	44	26			
22*	26	1	20.7	59	839_840delAG
	27	1			
	28	60			
24	32	38	38.5	1	1553T>C
	33	39			
29	85	0	15.0	30	839_840delAG
	41	30			
30	49	18	31.3	22	577C>T
	48	23			
	43	32			
	141	32			

Family ID	Patient ID	Age of onset	Mean age of onset	Range age of onset	Variant
	129	34			
	42	40			
	47	40			
32	46	29	36.0	11	1684C>T
	158	39			
	159	40			
33	53	12	26.0	28	261_261delG
	50	40			
37	56	0	7.0	14	Ex1-3_del
	73	14			
39	75	33	39.3	13	707_708delAA
	177	34			
	74	44			
	76	46			
41	61	0	8.0	14	1651_1652delinsTA
	62	10			
	60	14			
44	67	3	26.0	46	1492_1493+2delAGGT
	66	49			
48	72	42	45.5	7	361A>T
	71	49			
69	98	6	15.3	24	1367A>G
	99	10			
	100	30			
71	148	30	32.5	5	1684C>T
	104	35			
73	106	15	23.0	16	911delC
	107	31			

Family ID	Patient ID	Age of onset	Mean age of onset	Range age of onset	Variant
76	115	0	37.3	50	1493+1G>A
	113	38			
	111	43			
	114	44			
	112	49			
	110	50			
85*	124	20	25	15	Ex4-9_del
	192	20			
	31	35			
86	35	34	47.3	31	139A>T
	57	43			
	127	65			
87	37	1	25.0	36	1687+2T>A
	128	30			
	40	32			
	214	37			
90	131	10	20.7	30	456_460delTATTG
	133	12			
	194	40			
97	168	34	37.0	6	Ex2-16_dup
	139	40			
118	170	17	21.0	8	1417C>T
	171	25			
152	208	28	32.0	8	716T>G
	209	36			
158	217	30	35.5	11	1684C>T
	216	41			
178	239	25	34.5	19	Ex5_del

Family ID	Patient ID	Age of onset	Mean age of onset	Range age of onset	Variant
	238	44			
180	241	1	3.5	5	1340_1344delTGTGT
	242	6			
182	245	18	23.5	11	Ex1_del
	244	29			
186	249	12	23.5	23	1526C>G
	250	35			
192	257	27	37.0	20	Ex16_del
	256	47			
201	268	2	20.7	38	1684C>T
	266	20			
	267	40			
*age of onset of disease missing in 1 family member					

Appendix table 9: Complicating signs and symptoms at baseline according to inventory

Random verification of the information given in the inventory about the presence of complicating signs and symptoms revealed little consistency with examination results in medical reports. Since source data (medical reports) were not available for all cases, no information on the actual frequency of complicating signs and symptoms can be given. Valid percentages indicated.

Complicating signs and symptoms	Frequency of occurrence (baseline)		Frequency of missing data
Cognitive impairment	**10**	3.8%	15
Psychiatric symptoms (not specified)	**4**	1.6%	18
Visual items — Visual loss	**6**	2.3%	15
Visual items — Cataract	**6**	2.4%	25
Visual items — Retinitis pigmentosa*	**0**	0%	189
Visual items — Optic atrophy*	**2**	2.3%	190
Oculomotor disturbances (cerebellar)	**10**	3.8%	16
Dysarthria	**9** — 1 pseudobulbar / 8 unclassified	3.4%	12
Dysphagia	**5**	1.9%	11
Muscle atrophy	**25**	9.6%	15
Fasciculations*	**2**	2.1%	179
Limb ataxia	**6**	2.3%	14
Gait ataxia	**15**	5.8%	17
Extrapyramidal involvement	**5**	1.9%	14
Sensory items — Impaired touch sense	**24**	9.3%	18
Sensory items — Impaired joint position	**14**	5.4%	18
Sensory items — Impaired sense of temperature	**21**	8.1%	18
Sensory items — Impaired pinprick	**14**	5.4%	18
Hearing impairment	**7**	2.7%	16
Seizures	**1**	0.4%	16
not included in calculation of complicating signs and symptoms at baseline due to missing values in >50% of all patients			

Appendix table 10: Frequencies complicating signs and symptoms

Sum of complicating signs and symptoms in individuals at baseline examination. Missing values in 42 cases (15.2%). Valid percentages given.

Sum of complicating signs and symptoms	Patients with n complicating symptoms at baseline	
0	151	64.5%
1	49	20.9%
2	18	7.7%
3	3	1.3%
4	4	1.7%
5	4	1.7%
6	2	0.9%
7	1	0.4%
8	2	0.9%
total	234	100%

Appendix table 11: frequencies of events and censored data for loss of independent walking, walking aid dependency and wheelchair dependency

		Loss of independent walking		Walking aid dependency		Wheelchair dependency	
		All cases	Index	All cases	Index	All cases	Index
Event yes	**All cases**	**137**	**108**	**134**	**107**	**49**	**34**
Age of onset of disease (years)	≤ 20	36	27	35	27	17	12
	21 – 40	68	51	67	51	22	12
	> 40	33	30	32	29	10	10
gender	male	73	62	71	62	24	16
	female	65	47	64	46	26	19
Variant type	missense	23	19	22	19	11	8
	truncating	105	80	103	79	37	25
No event (censored)	**All cases**	**112**	**84**	**115**	**85**	**191**	**149**
Age of onset of disease (years)	≤ 20	29	17	30	17	45	29
	21 – 40	50	40	51	40	92	75
	> 40	33	27	34	28	54	45
gender	male	70	53	72	53	114	94
	female	50	31	51	32	86	56
Variant type	missense	21	18	22	18	32	28
	truncating	92	60	94	61	154	109

Appendix table 12: growth models of progression rate

Growth models analysis on progression rate, analyzed including all examinations and separate disease durations only (<10 / <20 / 10 – 20 / >10 / >20 years). Dependent variable SPRS total score, predictor time. b: regression coefficient; SE: standard error; CI: 95% confidence interval; low: lower bound; upp: upper bound; y: years; significant if p≤0.05

filter			b	SE	95% CI		p
					low	upp	
	Linear	time	0.69	0.05	0.59	0.79	0.000
	Quadratic	time	0.50	0.09	0.32	0.67	0.000
		time*time	0.01	0.00	0.00	0.01	0.006
	Cubic	time	0.51	0.13	0.24	0.77	0.000
		time*time	0.01	0.01	-0.01	0.02	0.346
		time*time*time	0.00	0.00	-0.00	0.00	0.902
Disease duration <10y	Linear	time	0.70	0.21	0.28	1.13	0.001
	Quadratic	time	-0.05	0.59	-1.23	1.12	0.927
		time*time	0.08	0.056	-0.03	0.19	0.175
	Cubic	time	-0.67	1.40	-3.47	2.12	0.632
		time*time	0.23	0.33	-0.42	0.89	0.482
		time*time*time	-0.01	0.02	-0.06	0.03	0.630
Disease duration ≥10y	Linear	time	0.73	0.06	0.60	0.85	0.000
	Quadratic	time	0.41	0.15	0.12	0.71	0.005
		time*time	0.01	0.00	0.00	0.01	0.017
	Cubic	time	0.40	0.30	-0.18	0.98	0.176
		time*time	0.01	0.01	-0.01	0.03	0.440
		time*time*time	-0.00	0.00	-0.00	0.00	0.953

filter			b	SE	95% CI		p
					low	upp	
Disease duration 10 – 20y	Linear	time	1.05	0.12	0.82	1.29	0.000
	Quadratic	time	0.79	0.85	-0.89	2.46	0.355
		time*time	0.01	0.03	-0.05	0.07	0.751
	Cubic	time	8.80	6.19	-3.42	21.02	0.157
		time*time	-0.54	0.42	-1.37	0.29	0.202
		time*time*time	0.01	0.01	-0.01	0.03	0.193
Disease duration <20y	Linear	time	0.85	0.09	0.67	1.03	0.000
	Quadratic	time	0.52	0.23	0.08	0.96	0.021
		time*time	0.02	0.01	-0.00	0.04	0.108
	Cubic	time	-0.36	0.51	-1.37	0.65	0.487
		time*time	0.13	0.06	0.01	0.24	0.030
		time*time*time	-0.00	0.00	-0.01	0.00	0.058
Disease duration ≥20y	Linear	time	0.76	0.07	0.62	0.90	0.000
	Quadratic	time	0.46	0.25	-0.03	0.95	0.063
		time*time	0.00	0.00	-0.00	0.01	0.203
	Cubic	time	0.88	0.84	-0.77	2.53	0.296
		time*time	-0.01	0.02	-0.05	0.04	0.773
		time*time*time	0.00	0.00	-0.00	0.00	0.601

	parameter	b	SE	95% CI low	95% CI upp	p
Step 1	Time	0.75	0.05	0.65	0.86	0.000
	Aoo 0 – 20y	-7.58	1.36	-10.25	-4.91	0.000
	Aoo 21 – 40y	-1.32	0.77	-2.83	0.19	0.086
	Aoo >40y	parameter redundant				
	Missense	1.69	0.97	-0.22	3.59	0.082
	Truncating	parameter redundant				
	Male	-0.60	0.72	-2.02	0.82	0.404
	Female	parameter redundant				
Step 1*	Time	0.78	0.08	0.63	0.93	0.000
	Aoo 0 – 20y	-7.61	1.36	-10.28	-4.93	0.000
	Aoo 21 – 40y	-1.35	0.77	-2.87	0.16	0.080
	Aoo >40y	parameter redundant				
	Missense	1.70	0.97	-0.21	3.60	0.080
	Truncating	parameter redundant				
	Male	-0.27	1.01	-2.25	1.71	0.788
	Female	parameter redundant				
	Male*time	-0.05	0.10	-0.24	0.15	0.635
	Female*time	parameter redundant				
Step 2	Time	0.75	0.05	0.65	0.86	0.000
	Aoo 0 – 20y	-7.63	1.36	-10.30	-4.96	0.000
	Aoo 21 – 40y	-1.40	0.76	-2.90	0.09	0.066
	Aoo >40y	parameter redundant				
	Missense	1.67	0.97	-0.22	3.59	0.082
	Truncating	parameter redundant				

	parameter	b	SE	95% CI		p
				low	upp	
Step 2*	Time	0.76	0.06	0.64	0.87	0.000
	Aoo 0 – 20y	-7.62	1.36	-10.30	-4.95	0.000
	Aoo 21 – 40y	-1.40	0.76	-2.90	0.10	0.067
	Aoo >40y	parameter redundant				
	Missense	1.84	1.33	-0.76	4.44	0.165
	Truncating	parameter redundant				
	Missense*time	-0.02	0.14	-0.30	0.25	0.863
	Truncating*time	parameter redundant				
Step 3	Time	0.76	0.05	0.66	0.86	0.000
	Aoo 0 – 20y	-7.99	1.34	-10.62	-5.37	0.000
	Aoo 21 – 40y	-1.63	0.74	-3.10	-0.17	0.029
	Aoo >40y	parameter redundant				
Step 3*	Time	1.00	0.11	0.79	1.21	0.000
	Aoo 0 – 20y	-4.78	1.82	-8.53	-1.21	0.009
	Aoo 21 – 40y	-0.08	1.09	-2.22	2.06	0.939
	Aoo >40y	parameter redundant				
	Aoo 0 – 20y *time	-0.39	0.14	-0.66	-0.12	0.005
	Aoo 21 – 40y *time	-0.26	0.13	-0.51	-0.01	0.039
	Aoo >40y *time	parameter redundant				
Step 4	Time	0.74	0.05	0.64	0.84	0.000
	Aoo 0 – 20y	-7.76	1.37	-10.45	-5.08	0.000
	Aoo 21 – 40y	-1.53	0.76	-3.01	-0.04	0.045
	Aoo >40y	parameter redundant				

	parameter	b	SE	95% CI		p
				low	upp	
Step 4*	Time	0.97	0.11	0.76	1.18	0.000
	Aoo 0 – 20y	-4.28	1.88	-7.96	-0.60	0.023
	Aoo 21 – 40y	-0.18	1.10	-2.34	1.99	0.873
	Aoo >40y	parameter redundant				
	Aoo 0 – 20y *time	-0.40	0.14	-0.68	-0.12	0.005
	Aoo 21 – 40y *time	-0.23	0.13	-0.49	0.02	0.068
	Aoo >40y *time	parameter redundant				