RESEARCH ARTICLE

Specific Gait Changes in Prodromal Hereditary Spastic Paraplegia Type 4: preSPG4 Study

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ABSTRACT: Background: In hereditary spastic paraplegia type 4 (SPG4), subclinical gait changes might occur years before patients realize gait disturbances. The prodromal phase of neurodegenerative disease is of particular interest to halt disease progression by future interventions before impairment has manifested.

Objective: The objective of this study was to identify specific movement abnormalities before the manifestation of gait impairment and quantify disease progression in the prodromal phase.

Methods: Seventy subjects participated in gait assessment, including 30 prodromal *SPAST* pathogenic variant carriers, 17 patients with mild-to-moderate manifest SPG4, and 23 healthy control subjects. An infrared-camera-based motion capture system assessed gait to analyze features such as range of motion and continuous angle trajectories. Those features were correlated with disease severity as assessed by the Spastic Paraplegia Rating Scale, neurofilament light chain as a fluid biomarker indicating neurodegeneration, and motor-evoked potentials.

Results: Compared with healthy control subjects, we found an altered gait pattern in prodromal pathogenic variant carriers during the swing phase in the segmental angle of the foot (Dunn's post hoc test, $q = 3.1$) and heel ground clearance ($q = 2.8$). Furthermore, range of motion of segmental angle was reduced for the foot ($q = 3.3$). These changes occurred in prodromal pathogenic variant carriers without quantified leg spasticity in clinical examination. Gait features correlated with neurofilament light chain levels, central motor conduction times of motor-evoked potentials, and Spastic Paraplegia Rating Scale score.

Conclusions: Gait analysis can quantify changes in prodromal and mild-to-moderate manifest SPG4 patients. Thus, gait features constitute promising motor biomarkers characterizing the subclinical progression of spastic gait and might help to evaluate interventions in early disease stages. © 2022 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society

Key Words: hereditary spastic paraplegia; prodromal; SPG4; motor biomarker; gait analysis

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Hereditary spastic paraplegias (HSPs) are a clinically and genetically heterogeneous group of neurodegenerative disorders resulting in a length-dependent affection of the corticospinal tract (>80 HSP genes described[1]). Pathogenic variants in the *SPAST* gene result in spastic paraplegia type 4 (SPG4), comprising the most common cause of HSP,[2] and manifest as "pure" HSP with hyperreflexia, leg spasticity, pyramidal weakness, and spastic gait resulting in increasing locomotor dysfunction.[3,4]

Because movement disturbance is critical in SPG4, gait features may serve as potential biomarkers to quantify gait abnormalities and disease progression in manifest disease and eventually already in the prodromal stage. Because the therapeutical potential of future interventions is likely most promising in the early stages of HSP,[5] it is crucial to identify and quantify the first changes already in the prodromal phase of genetically stratified cohorts.

Prodromal gait features have already been established in other neurodegenerative movement disorders, such as Parkinson's disease[6] and spinocerebellar ataxia.[7,8] Thus far, gait analyses in HSP have been restricted to patients with clinically manifest gait abnormalities and with heterogeneous genetic backgrounds. In manifest HSP patients, reduced stride length and gait speed were observed as characteristic changes.[9,10] Recent studies identified a decrease in range of motion (RoM) in the sagittal plane of joint and segmental angles.[11,12] Serrao et al[11] clustered manifest HSP patients based on joint RoM values into severity-related groups.[11]

To establish movement biomarkers for characterizing the subclinical progression and natural history for possible therapeutic intervention effects in HSP, detailed knowledge about the gradual gait changes before manifestation of spastic gait is required. The prodromal period of disease provides a unique research opportunity to study such early gait alterations prospectively.

The preSPG4 Study (ClinicalTrials.gov Identifier: NCT03206190) is an observational study that aims to characterize prodromal SPG4-related gait changes in prodromal and mild-to-moderate manifest SPG4 patients for comparison. We hypothesized that digital, objectively measured gait recording could quantify gradual gait changes already in the prodromal state of SPG4 when neither patients nor movement disorder specialists recognize spastic gait in clinical assessment.

Subjects and Methods

Participants

We included 17 patients with SPG4 with manifest spastic gait disturbance from our spastic paraplegia outpatient clinic in Tübingen. All patients were able to walk without walking aids. In addition, 53 participants from the preSPG4 Study (NCT03206190) were included. No sample size calculation was done before study initiation because of the lack of preliminary data. The preSPG4 Study is a single-center, rater-blinded, observational study in individuals at risk for development of SPG4. Descendants and siblings of patients with SPG4 with a 50% risk of inheriting the disease-causing *SPAST* pathogenic variant were included. The respective familial *SPAST* variant was analyzed to identify pathogenic variant carriers without disclosing the genetic result to the clinical examiners. Genetic analysis showed 30 pathogenic variant carriers and 26 participants without a *SPAST* pathogenic variant. SPG4 pathogenic variant carriers were categorized as prodromal if HSP specialists rated their gait within the healthy spectrum (mean scores <2 points in the following scale: 0, normal gait; 1, could be within healthy spectrum; 2, could be within spastic spectrum; 3, definite spastic gait) in a blinded video assessment. The nonpathogenic variant carriers served as healthy control subjects (HCs). Clinical characterization and findings (including reflexes and foot clonus) of the prodromal *SPAST* pathogenic variant carriers and HCs not carrying the *SPAST* pathogenic variant were reported by Rattay et al,[13] and details with regard to our subgroups can be found in Table S2. These mild-to-moderate manifest patients were used to define characteristics in digital, objectively measured gait abnormalities. Three HCs were excluded because of technical problems in the gait assessment resulting in 17 patients with manifest SPG4, 30 prodromal *SPAST* pathogenic variant carriers, and 23 HCs being analyzed. To assess the earliest stages of the disease, we defined a subgroup within the prodromal SPG4 group with a cutoff in the Spastic Paraplegia Rating Scale (SPRS)[14] score <2, analogous to the range in SPRS scores of HCs (average + 2 standard deviations).[14] This prodromal $SPG4_{SPRS<2}$ group included 12 individuals (nine without spasticity identified in the SPRS score). Characteristics of the four groups are shown in Table 1.

Ethics Approval

This study was conducted according to the Helsinki Declaration and approved by the Institutional Review Board of the University of Tübingen (reference number: 266/2017BO2) for the preSPG4 Study. In addition, written informed consent was obtained from all study participants.

Gait Assessment and Analysis

All participants underwent an instrumented gait analysis in a movement laboratory using an infrared-camera-based motion capture system (VICON FX with

TABLE 1 *Demographic data of healthy control subjects, prodromal mutation carriers, and patients with manifest SPG4*

Group	Subjects, n	Age (y)	Sex (male/female), n	Height (cm)	SPRS score, mean ± SD [minimum, maximum]	Spasticity (hip/knee), n[a]	Score rating
Healthy control	23	34.7 ± 12.4	12/11	178 ± 9	0.3 ± 0.7 [0, 3]	0/2	0.36 ± 0.36
Prodromal SPG4	30	33.6 ± 12	13/17	176 ± 11	2 ± 1.6 [0, 7]	4/16	0.5 ± 0.43
Prodromal $SPG4_{SPRS < 2}$	12	29.3 ± 6.4	4/8	175 ± 10	0.5 ± 0.5 [0, 1]	0/3	0.25 ± 0.29
Manifest SPG4	17	48.2 ± 8.2	8/9	171 ± 7	11.2 ± 4.1 [2, 17]	12/15	n/a

Shown are average values and SD for mutation carriers and control groups. Reported mean video ratings: 0, normal gait; 1, could be within the healthy spectrum; 2, could be within the spastic spectrum; 3, definite spastic gait.
[a]Items 7 and 8 of SPRS,[14] which represents spasticity of the hip adductors and knee extensors as measured by the mAS,[25] with spasticity recognized as catch/release (mAS > 0 points).
SPG4, spastic paraplegia type 4; SPRS, Spastic Paraplegia Rating Scale; SD, standard deviation; n/a, not applicable; mAS, modified Ashworth scale.

10 cameras). Subjects were instructed to walk normally (self-determined pace) with their own flat shoes for a 10-m distance for several trials. Participants performed an average of 43 gait cycles. Three-dimensional movement trajectories were recorded with 41 reflecting markers, placed according to the Nexus Plug-in Gait model, at a sampling rate of 120 Hz.

Gait cycles were automatically extracted by detecting the heel strike events based on the heel marker positions' vertical components for each foot and then manually verified using a stick figure animation to cross-check for different foot placements. Trials were smoothed with a Savitzky–Golay polynomial filter and resampled equidistantly with 101 data points per gait cycle by linear time interpolation. These procedures of gait analysis were used earlier in several studies on neurodegenerative movement disorders,[15,16] including the quantification of preataxic movement changes in spinocerebellar ataxia.[8]

As standard parameters, we computed gait parameters such as speed, stride length, and the RoM of different segments (see Table 2), which have been identified as sensitive features in manifest HSP in earlier studies.[9,12,17]

In addition, our analysis was focused on the segmental angle trajectories (see Fig. 1 and Supporting Information Explanation 1) of the lower limb (flexion/extension) and leg marker position trajectories in the sagittal plane. Leg marker trajectories were normalized to a zero-ground level over the stance period to correct the individual characteristics of shoes. Each subject's mean gait cycle trajectories were determined by averaging all gait cycles of the left and right legs. Furthermore, gait features were extracted from trajectories, for example, maximum heel ground clearance or RoM of segmental angles.

Clinical Rating Scales and Further Multimodal Biomarkers

All participants were clinically examined by an HSP specialist (T.W.R.), who rated disease severity according to the SPRS.[14] We additionally evaluated the most discriminative SPRS items (7–11) identified by Rattay et al[13] as subscore ($SPRS_{7–11}$). We computed the calculated time to onset/since onset based on the reported manifestation age of the affected parent, as it was used in Rattay et al.[13]

Serum neurofilament light chain (NfL) levels, reported to be increased in manifest SPG4 patients,[18] were measured for all participants within the preSPG4 Study (prodromal subjects and HCs) as referred to by Rattay et al[13] and Wilke et al,[19] and cerebrospinal fluid (CSF) NfL values were conducted from 17 of 30 prodromal SPG4 subjects. The subgroup of manifest patients included in this study was not identical to the manifest group shown as proof of principle in Rattay et al[13] for the NfL measures (serum and CSF). Thus, NfL values were not available for manifest participants. Routine motor-evoked potentials (MEPs) to the leg (tibial nerve, abductor hallucis muscle) for the preSPG4 cohort were determined as previously published in Rattay et al.[13]

Statistics

The nonparametric Kruskal–Wallis test was used to determine between-group differences in movement features. When the Kruskal–Wallis test yielded a significant effect ($P < 0.05$), post hoc analysis was performed using Dunn's test for multiple comparisons. First, Kruskal–Wallis and Dunn's tests were applied to identify differences between HCs, prodromal pathogenic variant carriers, and the manifest SPG4 group. Second,

TABLE 2 *Gait features in healthy control subjects, prodromal mutation carriers, and manifest SPG4 patients*

Gait measure	HC	Prodromal $SPG4_{SPRS < 2}$	Prodromal SPG4	Manifest SPG4	HC vs. prodromal $SPG4_{SPRS < 2}$		HC vs. prodromal SPG4		HC vs. manifest SPG4		Prodromal SPG4 vs. manifest SPG4	
					q	δ	q	δ	q	δ	q	δ
SPRS score	0.3 ± 0.7	0.5 ± 0.52	2 ± 1.64	11.2 ± 4.13	0.8929	−0.26	3.318**	−0.67	6.938***	−1	4.281***	−0.94
Serum NfL	6.54 ± 2.36	7.46 ± 2.35	7.89 ± 3.59	n/a	n.s.	−0.23	n.s.	−0.22	n/a	n/a	n/a	n/a
Standard parameters												
Gait speed (m/s)	1.36 ± 0.12	1.32 ± 0.11	1.28 ± 0.11	1.09 ± 0.21	n.s.	0.09	1.956	0.34	4.284***	0.75	2.727*	0.51
Stride length (mm)	1460 ± 90	1382 ± 127	1369 ± 113	1163 ± 190	1.838	0.36	2.320	0.45	5.047***	0.8	3.199*	0.67
Gait cycle duration (s)	1.08 ± 0.11	1.05 ± 0.06	1.07 ± 0.07	1.08 ± 0.14	n.s.	0.25	n.s.	0.07	n.s.	0.03	n.s.	0.01
Step width (mm)	88 ± 25.9	101 ± 28.9	95 ± 27.8	115 ± 40	n.s.	−0.22	n.s.	−0.1	n.s.	−0.4	n.s.	−0.31
Circumduction (heel) (%)	2.1 ± 2.38	4.2 ± 9.64	3.3 ± 6.61	2.2 ± 2.72	n.s.	−0.04	n.s.	−0.01	n.s.	0.04	n.s.	0.03
Max ground clearance heel (cm)	30.91 ± 2.71	28.43 ± 2.31	27.92 ± 2.35	24.77 ± 4.22	1.913	0.42	2.821*	0.51	4.529***	0.75	2.196	0.46
Max ground clearance toe (cm)	9.98 ± 1.48	8.54 ± 1.26	8.99 ± 1.67	6.68 ± 2.61	2.467*	0.54	2.011	0.36	4.502***	0.77	2.907*	0.56
Segmental angles												
RoM thigh (°)	36.6 ± 2.55	35.1 ± 2.32	35.2 ± 3.08	34.6 ± 6.6	n.s.	0.36	n.s.	0.32	n.s.	0.33	n.s.	0.19
RoM lower leg (°)	75.4 ± 3.48	73.1 ± 2.79	72.5 ± 3.94	60.1 ± 12.79	1.858	0.43	2.297	0.45	5***	0.80	3.171*	0.66
RoM foot (°)	97.7 ± 4.42	91.8 ± 6.1	89.5 ± 7.92	70.8 ± 17.14	2.475*	0.55	3.292**	0.63	5.953***	0.92	3.266*	0.72
Min thigh angle (°)	78.6 ± 3.12	79.9 ± 2.33	79.7 ± 3.39	81 ± 4.23	n.s.	−0.27	n.s.	−0.18	n.s.	−0.38	n.s.	−0.24
Max thigh angle (°)	115.2 ± 2.4	114.9 ± 2.9	114.9 ± 3.26	115.9 ± 3.99	n.s.	0.04	n.s.	0.05	n.s.	−0.17	n.s.	−0.17
Min lower leg angle (°)	34 ± 3.07	35.2 ± 2.01	34.9 ± 2.58	39.8 ± 7.84	n.s.	−0.25	1.111	−0.20	3.32**	−0.58	2.484*	−0.47
Max lower leg angle (°)	109.4 ± 2.83	108.3 ± 2.98	107.5 ± 3.28	99.9 ± 6.76	n.s.	0.22	1.813	0.34	5.025***	0.84	3.639***	0.71
Min foot angle (°)	−70.5 ± 5.25	−66.1 ± 6.15	−63.4 ± 7.64	−52.5 ± 12.06	1.955	−0.43	3.095*	−0.56	5.32***	−0.89	2.779*	−0.57
Max foot angle (°)	27.3 ± 4.18	25.7 ± 3.75	26.2 ± 3.11	18.3 ± 7.94	n.s.	0.21	1.019	0.19	4.039***	0.7	3.325**	0.63

Analyzed gait measures for the four groups: HCs, prodromal SPG4 with SPRS <2, prodromal SPG4, and manifest SPG4 patients. Mean and standard deviation of features, q values of Dunn's post hoc test (*q > 2.3877 [$P < 0.05$], **q > 3.281 Bonferroni-corrected [$P < 0.0031$], ***q > 3.5878 [$P < 0.001$]), and Cliff's delta as effect size (δ) are reported.

SPG4, spastic paraplegia type 4; HC, healthy control; SPRS, Spastic Paraplegia Rating Scale; NfL, neurofilament light chain; n/a, not applicable; n.s., Kruskal-Wallis test not significant; Max, maximum; RoM, range of motion; Min, minimum.

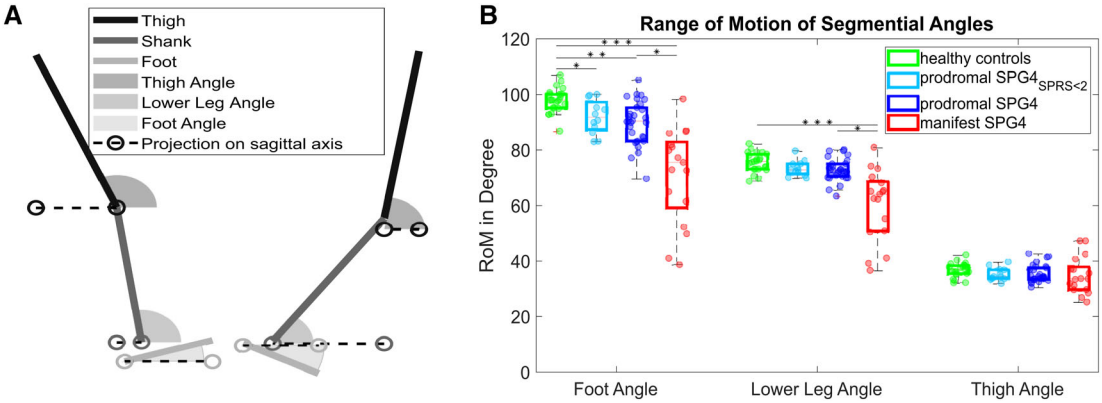

FIG. 1. Range of motion (RoM) of segmental angles in healthy control subjects, prodromal pathogenic variant carriers, and manifest spastic paraplegia type 4 (SPG4) patients. (A) Calculation of segmental flexion angles in relation to the floor. Angles are always measured in reference to their projection on the sagittal axis, corresponding to the ground. Left side displays a positive foot angle, while the right side shows a negative foot angle. (B) RoM of segmental angles of the four groups healthy controls, prodromal $SPG4_{SPRS<2}$, prodromal SPG4, and manifest SPG4. Black bars indicate significance between groups with asterisks indicating: *q > 2.3788; **q > 3.281 Bonferroni-corrected; ***q > 3.5878. [Color figure can be viewed at wileyonlinelibrary.com]

we tested for differences between HCs, prodromal $SPG4_{SPRS<2}$, and prodromal pathogenic variant carriers. We report three significance levels: (1) uncorrected, *q > 2.3788 ($P < 0.05$); (2) Bonferroni-corrected for multiple feature comparisons, **q > 3.281 ($P < 0.05/16 = 0.0031$), n = 16 (number of analyzed features); and (3) ***q > 3.5878 ($P < 0.001$). Effect sizes are reported by Cliff's delta (δ)[20] as small if $\delta < 0.28$, medium if $0.28 < \delta < 0.43$, and large if $\delta \geq 0.43$, according to Vargha and Delaney.[21] Linear regression was fitted to stride length, gait speed, and each segmental angle's RoM of prodromal SPG4 individuals to investigate the effect of stride length and gait speed on segmental RoMs. We report coefficients of determination (R^2) for comparison for the foot, lower leg, and thigh segmental RoM for prodromal SPG4.

Kruskal–Wallis test and Dunn's post hoc analyses were applied on each of the 101 time steps within one gait cycle for leg marker trajectories and segmental angles to estimate group differences.

We assessed four prominent gait cycle events: heel strike, heel-off, toe-off, and maximum heel ground clearance. Group differences for the segmental angles and heel and toe ground clearance were analyzed. We identified the most discriminative event for each trajectory based on the group differences—(1) foot angle and (2) heel ground clearance at maximum heel ground clearance, (3) lower leg angle and (4) toe ground clearance at heel strike, and (5) thigh angle at toe-off—and correlated these features together with the segmental angles RoM with the disease severity measured by SPRS score[14] and $SPRS_{7-11}$ subscore. Identified gait features in the prodromal group were also correlated to serum and CSF NfL, MEPs of the leg (amplitude and central motor conduction time [CMCT]), and estimated time to onset. We report Pearson correlation coefficients and different significance levels: (1) uncorrected, *$P < 0.05$; (2) Bonferroni-corrected for multiple feature comparisons, **$P < 0.05/8 = 0.0063$, n = 8 (number of analyzed features); and (3) ***$P < 0.001$.

Results

Demographics and Clinical Findings

The manifest SPG4 group was significantly older than HCs and prodromal SPG4 patients (q = 3.4397 and q = 3.8788, respectively). There were no group differences between HCs and prodromal SPG4 in age and sex. For demographic details, see Table 1. Significantly more prodromal pathogenic variant carriers have spasticity in the lower extremity, brisk reflexes, and pyramidal weakness than HCs ($P < 0.001$); see Table S2 for more details. The disease severity measured by the SPRS score differed significantly among all three groups (see Table 2 for more details).

Gait Changes in Mild-to-Moderate Manifest SPG4 Versus HCs

Gait speed (***q = 4.284, $\delta = 0.75$), stride length (***q = 5.047, $\delta = 0.8$), and maximum heel and toe ground clearance (***q = 4.529, $\delta = 0.75$ and ***q = 4.502, $\delta = 0.77$, respectively) were significantly reduced in mild-to-moderate manifest SPG4 compared with HCs. RoMs of the foot (***q = 5.953, $\delta = 0.92$) and lower leg (***q = 5, $\delta = 0.8$) were also reduced significantly (Fig. 1B). Further, maximum and minimum angles in the foot ($\delta = 0.7$, $\delta = -0.89$) and lower leg ($\delta = 0.84$, $\delta = -0.58$) were reduced/increased significantly (**q > 3.281); see Table 2 and Fig. 2 for more details.

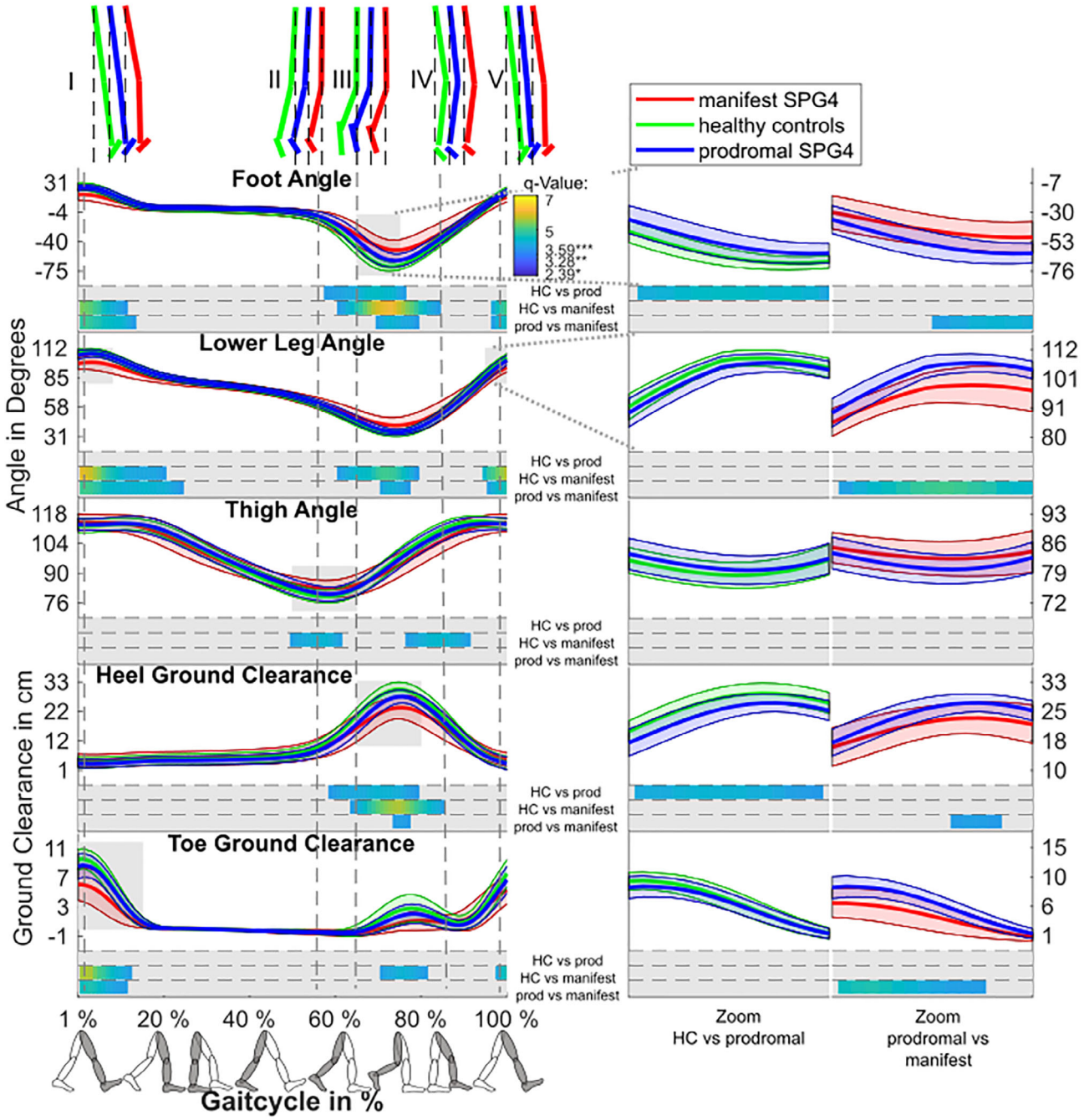

FIG. 2. Gait trajectories and segmental angles during a gait cycle of healthy control subjects (HCs), prodromal SPAST pathogenic variant carriers, and manifest spastic paraplegia type 4 (SPG4) patients. Individual trajectories and segmental angles during a gait cycle (from heel strike to heel strike) for groups of HCs (green), prodromal SPG4 patients (blue), and manifest SPG4 patients (red). On the left side of the figure, the whole gait cycle is displayed for the three segmental angles and the heel and ankle ground clearance. In the upper part, the thigh, lower leg, and foot segments are plotted based on the mean angles to the given time point (dashed line). Kruskal–Wallis test and post hoc Dunn's test were applied to each percent step of the gait cycle to compare group differences, and significant results ($q > 2.3788$) are displayed color-coded below each feature. Group-wise comparison is displayed as HCs versus prodromal SPG4 patients, HCs versus manifest SPG4 patients, and prodromal SPG4 versus manifest SPG4 patients. On the right side, outtakes of the gait cycle around the most characteristic time points are displayed separately for HCs versus prodromal SPG4 patients and prodromal SPG4 versus manifest SPG4 patients. The corresponding gait period is shaded gray on the left side. [Color figure can be viewed at wileyonlinelibrary.com]

Gait Changes in Prodromal *SPAST* Pathogenic Variant Carriers Versus HCs

Prodromal pathogenic variant carriers compared with HCs revealed reduced maximum ground clearance of the heel (*$q = 0.2821$, $\delta = 0.51$). RoMs of the foot (**$q = 3.292$, $\delta = 0.63$) segmental angles were significantly reduced, as shown in Fig. 1B, driven mainly by the increase of the minimum plantarflexion (*$q = 3.095$, $\delta = -0.56$).

Coefficients of determination for the foot ($R^2_{\text{stride length}} = 0.23$; $R^2_{\text{gait speed}} = 0.29$), the lower leg ($R^2_{\text{stride length}} = 0.31$; $R^2_{\text{gait speed}} = 0.38$), and the thigh ($R^2_{\text{stride length}} = 0.14$; $R^2_{\text{gait speed}} = 0.29$) segments indicate that the decrease of the segmental angles RoMs was not predominantly driven by stride length or gait speed.

The RoM effect size of the foot angle ($\delta = 0.63$) was comparable with the effect size of the SPRS score ($\delta = 0.67$). Further results and q values are shown in Table 2.

The in-depth analysis of differences within a gait cycle showed specific movement changes of prodromal SPG4: differences occurred in the swing phase and around the heel strike event. During the swing phase, foot angle and heel ground clearance magnitudes were reduced (Fig. 2III,IV). At the heel strike, the toe ground clearance was significantly reduced in prodromal pathogenic variant carriers (Fig. 2I,V).

First Gait Signs in Prodromal $SPG4_{SPRS < 2}$

Remarkably, differences were identified even for the prodromal subgroup $SPG4_{SPRS < 2}$ compared with HCs. RoM in the segmental angle of the foot ($^*q = 2.475$, $\delta = 0.55$) was significantly reduced, as well as maximum toe ($^*q = 2.467$, $\delta = 0.54$) ground clearance. In the prodromal $SPG4_{SPRS < 2}$ subgroup, effect sizes in all significant gait features were substantially larger (up to 210%) than for the SPRS score ($\delta = -0.26$).

Differences in heel strike, heel-off, toe-off, and maximum ground clearance of the heel are shown in Fig. 3 and depicted for all four groups.

Correlations of Gait Features with Severity of Disease

We chose the three segmental angles (foot, lower leg, thigh) and ground clearance (toe and heel) at prominent events within the gait cycle (heel strike, heel-off, toe-off, and maximum heel ground clearance). Correlations between these gait features and disease severity (SPRS score) in patients with manifest SPG4, prodromal pathogenic variant carriers, and HCs are shown in Fig. S1. Within the whole range of disease severity (SPRS range, 0–17), all chosen gait features correlated significantly with the SPRS score (eg, foot angle at maximum heel ground clearance: $^{***}P < 0.001$).

In patients with manifest SPG4, the foot ($r = -0.57$, $^*P = 0.016$), lower leg ($r = -0.55$, $^*P = 0.023$), and thigh RoM ($r = -0.6$, $^*P = 0.012$); the foot angle at maximum heel ground clearance ($r = 0.63$, $^{**}P = 0.006$); the lower leg angle at heel strike ($r = -0.49$, $^*P = 0.04$); and thigh angle at toe-off ($r = 0.59$, $^*P = 0.012$) correlated with the SPRS score.

In the prodromal group, gait characteristics were shifted with disease progression gradually toward the abnormalities seen in mild-to-moderate manifest SPG4 patients, as illustrated in Fig. 2 by the blue line (prodromal pathogenic variant carriers) passing between those of HCs (green line) and manifest SPG4 patients (red line). The foot angle at maximum heel ground clearance ($r = 0.58$, $^{***}P < 0.001$) and the foot RoM ($r = -0.49$, $^{**}P = 0.005$) of prodromal SPG4 correlated significantly with the SPRS score. The $SPRS_{7–11}$ subscore ($r = -0.37$) correlated significantly with the toe ground clearance at heel strike. Correlations with calculated time to onset/since onset did not indicate significant results. For more details, see Table S1.

Correlations of Gait Features with Multimodal Biomarker

Of the kinematic items, the foot angle at maximum heel ground clearance ($r = 0.48$, $P = 0.0075^*$) and the foot RoM ($r = -0.46$, $P = 0.01^*$) correlated significantly with NfL levels in serum (n = 30) for prodromal pathogenic variant carriers. CSF NfL levels (n = 17) did not correlate with gait features. HCs showed no correlation (foot angle: $r = 0.18$, $P = 0.58$; foot RoM: $r = 0.12$, $P = 0.52$).

The foot, lower leg, and thigh RoMs ($r = -0.529$, $^{**}P = 0.0046$; $r = -0.48$; $^*P = 0.0114$; and $r = -0.484$; $^*P = 0.011$, respectively) and foot angle at maximum heel ground clearance ($r = 0.478$, $^*P = 0.012$) correlated with MEP CMCT[13] (mean of left and right leg) (see Fig. S2). The toe ground clearance at heel strike correlated with the MEP amplitude ($r = 0.445$, $^*P = 0.0176$). For more details, see Table S1.

Discussion

This study analyzed gait in the prodromal and mild-to-moderate manifest stage of SPG4, the most common subtype of HSP. We found characteristic gait abnormalities in patients with mild-to-moderate manifest SPG4 and proved significant changes in gait parameters already in the prodromal stage of the disease before movement specialists recognized gait abnormalities and before clinical assessment (SPRS) shows discriminative results from HCs ($SPG4_{SPRS < 2}$ subgroup). The quantified changes showed large effect sizes, essential for motor biomarkers in rare diseases like SPG4 to achieve high statistical power despite small cohorts in upcoming clinical trials.

Gait Characteristics in Mild-to-Moderate Manifest SPG4

Characteristic gait changes in mild-to-moderate manifest SPG4 patients (SPRS 2–17) were a significant reduction of foot and lower leg RoMs resulting from both increased minimum and a decreased maximum of the segmental angles. The reduction in RoM of segmental angles,[9,11,12] step height (heel ground clearance),

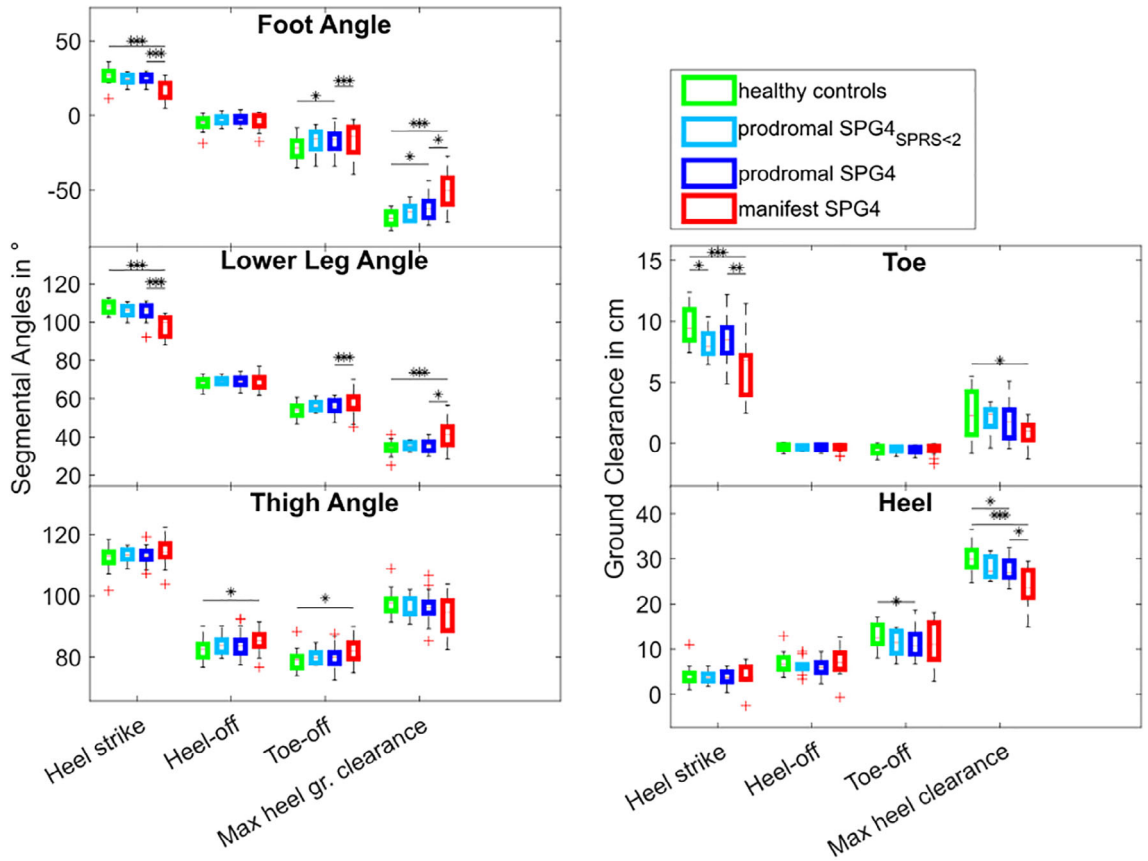

FIG. 3. Gait features within the gait cycle of healthy control subjects (HCs), prodromal *SPAST* pathogenic variant carriers, and patients with manifest spastic paraplegia type 4 (SPG4). Boxplots of foot angle, lower leg angle, thigh angle, toe marker, and heel marker at the heel strike, heel-off, toe-off, and at maximum heel ground clearance. Displayed are the four groups: HCs, prodromal $SPG4_{SPRS<2}$, prodromal SPG4, and manifest SPG4. Black bars indicate significance between groups with asterisks indicating *q > 2.3788; **q > 3.281 Bonferroni-corrected; ***q > 3.5878. [Color figure can be viewed at wileyonlinelibrary.com]

gait speed, and stride length are in line with previous reports.[10,22] The reduced RoMs in lower limb segments are supposed to be caused by an interplay of lower limb spasticity and pyramidal weakness, two primary HSP symptoms. We found reduced foot ground clearance during the swing phase of a gait cycle, which supports Kerstens et al's[23] qualitative findings that stumbling is a crucial feature in manifest HSP locomotion, and gait measures correlate with fear of falling.[10]

Besides identifying gait characteristics that distinguished mild-to-moderate manifest patients from HCs, we searched for gait measures that showed sensitivity to disease severity change. In line with disease severity correlations in the study of Martino et al,[12] the SPRS score correlated with the RoMs of the foot and lower leg angle in our study. The reduced levels of joint RoM were previously used by Serrao et al[11] to cluster three different groups of manifest HSP patients. Our observation of reduced RoMs in the foot and lower leg correspond to the intermediate severe group in Serrao et al[11] (SPRS score: 16.07 ± 6.48).

Regarding specific events in the gait cycle, we found correlations of the SPRS score with foot angle at maximum heel ground clearance ($r = 0.63$, $^{**}P = 0.006$), the lower leg angle at the heel strike event ($r = -0.49$, $^{*}P = 0.04$), and the thigh angle at the toe-off event ($r = 0.59$, $^{*}P = 0.012$). These events highlight features immediately after fast joint flexion/extension periods during the gait cycle (see Fig. 2, instances III and IV for foot angle, I for lower leg angle, and III for thigh angle). These observations would fit the hypothesis that fast muscle contraction during swing (eg, gastrocnemius and soleus) leads to spasticity and thus to reduced mobility in the foot angle. The reduced segmental angles correlated with the SPRS score; this may be explained by the velocity-dependent stiffness of the HSP joints.[24] The reductions of the segmental angles and RoMs in manifest patients are supposed to be driven by muscle paresis, especially of the dorsiflexor and hip flexor muscles, combined with severe stiffness of plantar flexor and knee extensor muscles,[24] which are symptoms that are also assessed by the SPRS.[14] For all manifest patients, spasticity and/or pyramidal weakness was documented in at least one joint in the SPRS. These results indicated that the progression of mild-to-moderate manifest SPG4 patients who can still walk freely (without

walking aids) could be quantified because of spasticity-dependent outcomes in the gait cycle.

Gait Characteristics in Prodromal SPG4

In prodromal SPG4, the RoM of the foot segment (Fig. 1) and maximum heel ground clearance were reduced. In comparison with the mild-to-moderate manifest SPG4 patients, the lower leg angle was not reduced for prodromal SPG4. These changes represent the gradual appearance of those seen in manifest SPG4 patients and were visible during the gait cycle at the toe-off event and during the swing phase (Fig. 2, instances II and III), showing the importance of identifying relevant time points within the gait cycle. In prodromal SPG4, only changes in the foot angle kinematics were identified, supporting that distal muscles are more affected[24] because of length-dependent affection of the corticospinal tract.

Importantly, significant gait changes could already be quantified for the prodromal subgroup $SPG4_{SPRS < 2}$, affecting the foot RoM and the maximum toe ground clearance. The changes in this subgroup (ie, foot RoM with effect size $\delta = 0.55$) highlight the relevance of instrumented gait analyses in the very early phase of SPG4 because it quantifies the earliest changes that were not visible to movement disorder specialists nor identified by the clinical SPRS score. This insensitivity of the SPRS could be caused by the lack of a spasticity test of the calf muscle. In contrast, increased Achilles tendon reflexes were seen in prodromal pathogenic variant carriers, reflecting the most distal location of a length-dependent axonal mechanism in HSP (see Table S2). This also reflects why changes in the foot segmental angle were seen as the earliest quantifiable changes in the prodromal subgroup $SPG4_{SPRS < 2}$.

Gait Features as a Biomarker in the Prodromal SPG4

In the prodromal disease stage, quantifiable biomarkers are essential as possible study endpoints for therapeutic endeavors. Our results suggest that gait features can fulfill these requirements by showing gradual progression during the prodromal phase of SPG4, with the foot RoM and foot segmental angle at the maximum heel ground clearance and minimum plantarflexion correlating with first disease symptoms (measured by the SPRS). The hypothesis that the identified gait changes are indeed an expression of incipient changes in SPG4 with specific neural degeneration is strengthened by the correlation of gait parameters with NfL values in the prodromal phase. NfL is a fluid biomarker indicating axonal degeneration processes[18,25] and is increased in prodromal SPG4 and manifest SPG4 patients.[13] Two of our most sensitive gait measures in the prodromal phase showed significant correlations to NfL levels in serum (foot angle at maximum heel ground clearance: $r = 0.48$, $^*P = 0.0075$; foot RoM: $r = -0.46$, $^*P = 0.01$) for the prodromal group. Thus, these gait measures present a promising performance measure associated with neural degeneration in the prodromal and mild-to-moderate manifest phase of SPG4, including subjects without visible spastic gait. Interestingly, the foot, lower leg, and thigh RoMs and foot angle at maximum heel ground clearance also correlated with the mean motor-evoked CMCT of the legs despite no differences when comparing prodromal versus HCs on a group level.[13] Slower CMCTs relate to a decreased foot RoM and may constitute the length-dependent affection of the corticospinal tract. Because the MEPs are limited regarding the amplitudes, we could not show the primarily axonal characteristic of the disease. Other techniques such as corticomuscular coherence analysis[26] would be needed to quantify axonal damage in prodromal SPG4.

Study Limitations

The relatively small cohort size limits our findings inherent to studies on rare diseases. Thus, larger future studies may be needed, including, in particular, a higher number of prodromal subjects, to further validate the promises of gait measures and relate gait changes to corresponding changes in molecular (such as CFS NfL) and imaging biomarkers. Another limitation is the thus far cross-sectional analysis. Detected changes need to be validated in longitudinal observations to prove their capability as disease progression markers. The upcoming follow-up observations will address this in the 10-year observational period of the preSPG4 Study.

In addition, it would be interesting to examine different gait speeds. Subtle gait changes may occur in faster locomotion because of the velocity-dependent stiffness of the joints. This fits the clinical impression that spastic gait changes are earlier depicted while running, with walking still considered unremarkable. Furthermore, future analyses and modeling studies on the kinetics level are highly warranted to better understand the underlying mechanisms of the here observed subtle kinematic changes. Neuromusculoskeletal models might help to explain gradual changes in kinetics and kinematics based on neurodegeneration of the spinal cord.

Conclusion

This study was able to quantify subclinical gait impairment in *SPAST* pathogenic variant carriers even without any clinical sign of pyramidal affection. Objectively measured gait features indicate disease progression over the prodromal and mild-to-moderate manifest phase and showed larger effect sizes than clinical scores for the mildest affected subgroup, potentially reducing sample size in future interventional trials. Thus,

quantitative movement features represent promising candidates for motor biomarkers in future therapeutic interventions in homogenous cohorts of prodromal SPG4.

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Data Availability

The datasets for this manuscript are not publicly available because raw data regarding human subjects (eg, genetic raw data, personal data) are not shared freely to protect the privacy of the human subjects involved in this study; no consent for open sharing has been obtained. Requests to access an anonymous data set should be directed to Dr. Winfried Ilg and Dr. Tim W. Rattay.

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Supporting Data

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3. Manuscript Preparation: A. Writing of the first draft, B. Review and Critique;
C.L.: 1B, 1C, 2A, 2B, 3A.
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