Gait Patterns in Patients with Hereditary Spastic Paraparesis

Mariano Serrao, Martina Rinaldi, Alberto Ranavolo, Francesco Lacquaniti, Giovanni Martino, Luca Leonardi, Carmela Conte, Tiwana Varrecchia, Francesco Draicchio, Gianluca Coppola, Carlo Casali, Francesco Pierelli

1 Department of Medico-Surgical Sciences and Biotechnologies, University of Rome Sapienza, Latina, Italy, 2 Rehabilitation Centre, Policlinico Italia, Rome, Italy, 3 Department of Engineering, Roma TRE University, Rome, Italy, 4 Department of Occupational and Environmental Medicine, Epidemiology and Hygiene, INAIL, Monte Porzio Catone, Rome, Italy, 5 Centre of Space Bio-Medicine, University of Rome Tor Vergata, Rome, Italy, 6 Laboratory of Neuromotor Physiology, Istituto Di Ricovero e Cura a Carattere Scientifico Santa Lucia Foundation, Rome, Italy, 7 Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy, 8 Fondazione Don Gnocchi, Milan, Italy, 9 IRCCS, Neuromed, Pozzilli, Isernia, Italy, 10 G.B. Bietti Foundation-IRCCS, Department of Neurophysiology of Vision and Neuroophthalmology, Rome, Italy

These authors contributed equally to this work.
* mariano.serrao@uniroma1.it

Abstract

Background
Spastic gait is a key feature in patients with hereditary spastic paraparesis, but the gait characterization and the relationship between the gait impairment and clinical characteristics have not been investigated.

Objectives
To describe the gait patterns in hereditary spastic paraparesis and to identify subgroups of patients according to specific kinematic features of walking.

Methods
We evaluated fifty patients by computerized gait analysis and compared them to healthy participants. We computed time-distance parameters of walking and the range of angular motion at hip, knee, and ankle joints, and at the trunk and pelvis. Lower limb joint moments and muscle co-activation values were also evaluated.

Results
We identified three distinct subgroups of patients based on the range of motion values. Subgroup one was characterized by reduced hip, knee, and ankle joint range of motion. These patients were the most severely affected from a clinical standpoint, had the highest spasticity, and walked at the slowest speed. Subgroup three was characterized by an increased hip joint range of motion, but knee and ankle joint range of motion values close to control values. These patients were the most mildly affected and had the highest walking speed. Finally, subgroup two showed reduced knee and ankle joint range of motion, and...
hip range of motion values close to control values. Disease severity and gait speed in subgroup two were between those of subgroups one and three.

Conclusions

We identified three distinctive gait patterns in patients with hereditary spastic paraparesis that correlated robustly with clinical data. Distinguishing specific features in the gait patterns of these patients may help tailor pharmacological and rehabilitative treatments and may help evaluate therapeutic effects over time.

Introduction

Hereditary spastic paraparesis is a heterogeneous group of inherited neurodegenerative disorders characterized by retrograde degeneration of the corticospinal axonal fibers [1]. Lower limb spasticity, usually more prominent than muscle weakness, is the key clinical feature in patients with hereditary spastic paraparesis [2] and impairs walking ability, autonomy, and quality of life [3,4]. No treatment is known to reduce disease progression, but antispastic drugs and physiotherapy [5–8] may help reduce the functional impairment of gait. Quantifying and typifying the specific gait disorder in hereditary spastic paraparesis is crucial to designing individual pharmacological and rehabilitative treatments. Most descriptions of paraparetic gait are based on qualitative clinical observations [1,2,5], [9–17]. Some studies have quantitatively evaluated gait impairment in hereditary spastic paraparesis patients, revealing several gait abnormalities of reduced step length, increased step width, reduced range of motion (RoM) at the knee joint [18–20], impaired knee torque and stiffness [19,20], and decreased activity of the rectus femoris muscle [19]. Despite the great relevance of such quantitative assessments, they remain generic without reflecting the wide clinical heterogeneity of gait disorders in hereditary spastic paraparesis patients. Spasticity of the lower limb muscles represents the most important clinical sign of hereditary spastic paraparesis, but it affects different patients to different extents [5,18,20]. Individual differences in spasticity should translate into corresponding biomechanical features of gait; specifically, more spastic patients should have more reduced RoMs during walking [18,20]. We hypothesized that the individual kinematic behavior of patients with hereditary spastic paraparesis could be used to identify distinct subgroups of patients, and that these subgroups would exhibit different levels of limb spasticity. Our aims were as follows: i) to perform a comprehensive analysis of kinematics, kinetics and sEMG (surface electromyography) in adult patients with hereditary spastic paraparesis, and ii) to identify specific gait patterns in subgroups of patients categorized according to their kinematic behavior.

Materials and Methods

Subjects

We recruited fifty patients with hereditary spastic paraparesis (twenty women and thirty men, mean age 47.70 ± 16.06 years, height 1.64 ± 0.11 m, weight: 75.97 ± 18.51 kg, disease duration 17.65 ± 12.50 years). All patients included in the study were able to walk without assistance or walking aids on a level surface. A defined molecular diagnosis of hereditary spastic paraparesis was applied to thirty patients. Of these, twenty-two patients had spastic paraplegia (SPG) type four (mutations in SPAST), two patients had SPG3A (mutations in ATL1), one patient had
SPG5 (mutations in \textit{CYP7B1}), two patients had SPG7 (mutations in the \textit{PGN}), and three patients had SPG31 (mutations in \textit{REEP1}). Twenty patients did not have a molecular diagnosis at the time of examination, but all patients unequivocally showed either a recessive (eight patients) or dominant (twelve patients) inheritance pattern. None of the patients showed any involvement of neurological systems other than the pyramidal one (e.g. cerebellar or sensory deficits). All patients were evaluated independently by two experienced neurologists (C.C. and F.P.) who assessed cognitive functions, cranial nerves, muscle tone, muscle strength, joint coordination, tendon reflexes, and sensory function.

The severity of the disease was rated using the Spastic Paraplegia Rating Scale (SPRS). The spasticity of hip and knee joint muscles was scored by the Modified Ashworth scale included in SPRS as a spasticity-related subscale [21]. Table 1 summarizes the clinical features and genotypes of all patients. Twelve out of fifty patients were assuming oral antispastic drugs (baclofen or tizanidine) since 4–6 years. All patients were clinically stable at the time of the study evaluation. Indeed, their clinical assessment (SPRS) did not change over the last six months prior to the study. At the time of the evaluation, all patients were undergoing physical therapy, which included lower limb and stretching exercises, balance, and gait training.

The control group was fifty healthy subjects (twenty-three women and twenty-seven men, mean age 49.12 ± 11.76 years, height 1.68 ± 0.07 m, weight 70.83 ± 13.22 kg).

All participants provided written informed consent before taking part in the study, which complied with the Helsinki Declaration and had local ethics committee approval (ICOT-Sapienza, Polo Pontino).

**Gait analysis**

Kinematic data were recorded at 300 Hz using an optoelectronic motion analysis system (SMART-D System, BTS, Milan, Italy) consisting of eight infrared cameras spaced around the walkway. In accordance with a validated biomechanical model, twenty-two reflective spherical markers (15 mm in diameter) were attached on the anatomical landmarks in accordance with a validated biomechanical model [22], using double-adhesive tape in such a way as to prevent them from falling out of place during the test. In detail, the markers were placed over the cutaneous projections of the spinous processes of the seventh cervical vertebra and sacrum and bilaterally over acromion, anterior superior iliac spine, great trochanter, lateral femoral condyle, fibula head, lateral malleoli and metatarsal head. In addition to markers directly applied to the skin, sticks, or wand, varying in length from 7 to 10 cm, placed at 1/3 of the length of the body segment (femur and leg) were used. Anthropometric data were collected for each subject [23].

Ground reaction forces were acquired by two dynamometric platforms (Kistler 9286B, Winterthur, Switzerland), attached to each other in the longitudinal direction but displaced by 0.2 m in the lateral direction (sampling rate 1200 Hz).

Surface myoelectric signals were recorded at 1000 Hz using a 16-channel wireless system (FreeEMG300 System, BTS, Milan, Italy). After skin preparation, bipolar Ag/AgCl surface electrodes (H124SG Kendall ARBO, Donau, Germany) were placed over the muscle belly in the direction of the muscle fibers according to the European Recommendations for Surface Electromyography [24] and the atlas of muscle innervation zones [25]. Bipolar electrodes, eight in total, were placed on the right side of the body of each subject on the tibialis anterior (TA), gastrocnemius lateralis (LG), gastrocnemius medialis (MG), vastus lateralis (VL), vastus medialis (VM), rectus femoris (RF), biceps femoris (BF), and semitendinosus (ST). Acquisition of kinematic, kinetic, and electromyographic data was integrated and synchronized.
### Table 1. Patients’ characteristics.

| Patients | Gender | Height(cm) | Body Wt.(Kg) | Age(yr) | Diagnosis | Onset(yr) | Duration(yr) | SPRS | ASHhip | ASHknee | Tot. |
|----------|--------|------------|--------------|---------|-----------|-----------|--------------|------|--------|---------|------|
| p1       | M      | 166        | 80           | 67      | _AR       | 30        | 37           |      | 3      | 3       | 21   |
| p2       | F      | 158        | 48           | 39      | _AD       | 17        | 22           | 1    | 2      | 1       | 16   |
| p3       | F      | 156        | 66           | 57      | SPG5      | 36        | 21           | 2    | 2      | 2       | 20   |
| p4       | M      | 178        | 84           | 37      | —         | —         | —            | 0    | 0      | 0       | 0    |
| p5       | F      | 145        | 76           | 50      | _AD       | 30        | 20           | 3    | 3      | 3       | 21   |
| p6       | F      | 146        | 70           | 54      | _AD       | 45        | 9            | 1    | 1      | 1       | 6    |
| p7       | F      | 160        | 62           | 72      | _AD       | 60        | 12           | 2    | 2      | 2       | 6    |
| p8       | F      | 154        | 57           | 66      | SPG4      | 30        | 36           | 0    | 1      | 1       | 6    |
| p9       | M      | 183        | 75           | 35      | _AD       | 13        | 22           | 1    | 1      | 1       | 7    |
| p10      | M      | 164        | 75           | 56      | SPG4      | 45        | 11           | 1    | 1      | 1       | 12   |
| p11      | F      | 163        | 58           | 21      | SPG4      | 3         | 18           | 1    | 1      | 2       | 2    |
| p12      | M      | 162        | 63           | 66      | SPG4      | 34        | 32           | 3    | 3      | 3       | 35   |
| p13      | F      | 152        | 78           | 40      | SPG4      | —         | —            | 0    | 0      | 0       | 0    |
| p14      | M      | 160        | 57           | 34      | SPG4      | 1–2       | 33           | 3    | 3      | 3       | 25   |
| p15      | M      | 174        | 87           | 47      | SPG4      | 35        | 12           | 0    | 1      | 1       | 7    |
| p16      | M      | 150        | 62           | 67      | _AD       | 56        | 11           | 1    | 2      | 6       | 2    |
| p17      | M      | 164        | 76           | 67      | _AR       | 45        | 22           | 3    | 3      | 3       | 21   |
| p18      | M      | 170        | 73           | 58      | SPG4      | 45        | 13           | 1    | 2      | 2       | 27   |
| p19      | M      | 177        | 104          | 24      | SPG4      | 14        | 10           | 1    | 2      | 2       | 11   |
| p20      | M      | 170        | 88           | 48      | _AR       | 10        | 38           | 1    | 2      | 2       | 13   |
| p21      | M      | 180        | 85           | 25      | _AD       | 13        | 12           | 0    | 0      | 0       | 3    |
| p22      | M      | 162        | 62           | 24      | _AR       | 12        | 12           | 3    | 3      | 3       | 21   |
| p23      | M      | 182        | 109          | 49      | SPG4      | 37        | 12           | 2    | 2      | 2       | 21   |
| p24      | F      | 170        | 69           | 43      | SPG4      | 38        | 5            | 0    | 0      | 5       | 5    |
| p25      | F      | 158        | 69           | 72      | SPG4      | 40        | 32           | 1    | 3      | 3       | 31   |
| p26      | F      | 162        | 58           | 43      | SPG4      | 5         | 38           | 1    | 2      | 2       | 7    |
| p27      | F      | 142        | 56           | 78      | SPG4      | 45        | 33           | 2    | 3      | 3       | 28   |
| p28      | M      | 170        | 70           | 49      | _AD       | 24        | 25           | 3    | 3      | 3       | 22   |
| p29      | F      | 159        | 73           | 56      | _AR       | 35        | 21           | 1    | 3      | 3       | 20   |
| p30      | F      | 158        | 61           | 64      | SPG31     | 15        | 49           | 1    | 0      | 1       | 12   |
| p31      | F      | 149        | 77           | 72      | SPG31     | 16        | 56           | 2    | 3      | 2       | 23   |
| p32      | M      | 157        | 87           | 59      | _AR       | 30        | 29           | 1    | 2      | 2       | 28   |
| p33      | F      | 150        | 54           | 47      | SPG7      | 30        | 17           | 1    | 0      | 0       | 7    |
| p34      | M      | 164        | 76           | 32      | _AR       | 14        | 18           | 4    | 4      | 4       | 26   |
| p35      | M      | 170        | 104          | 39      | _AD       | 36        | 3            | 1    | 2      | 2       | 12   |
| p36      | F      | 145        | 43           | 22      | _AD       | 12        | 10           | 3    | 3      | 3       | 21   |
| p37      | M      | 172        | 51           | 17      | SPG3A     | 13        | 4            | 1    | 1      | 1       | 10   |
| p38      | M      | 181        | 81           | 28      | SPG4      | 13        | 15           | 2    | 2      | 2       | 12   |
| p39      | M      | 161        | 78           | 58      | SPG4      | 43        | 15           | 2    | 3      | 3       | 17   |
| p40      | M      | 177        | 103          | 70      | SPG4      | 60        | 10           | 2    | 2      | 2       | 23   |
| p41      | M      | 165        | 69           | 28      | _AD       | 20        | 8            | 2    | 3      | 3       | 16   |
| p42      | M      | 186        | 136          | 39      | SPG3A     | 20        | 19           | 2    | 2      | 2       | 27   |
| p43      | F      | 161        | 80           | 56      | SPG4      | 35        | 21           | 2    | 2      | 2       | 22   |
| p44      | M      | 161        | 84           | 62      | SPG4      | 40        | 22           | 0    | 1      | 5       | 5    |
| p45      | M      | 183        | 78           | 38      | SPG4      | 30        | 8            | 4    | 4      | 4       | 27   |
| p46      | M      | 175        | 68           | 46      | SPG7      | 40        | 6            | 1    | 2      | 1       | 15   |

(Continued)
Experimental Procedure

Patients and controls were asked to walk barefoot at a comfortable, self-selected speed along a walkway approximately 10 m in length while looking forward. Because we were interested in natural locomotion, only general, qualitative instructions were provided. Before the recording session, subjects practiced for a few minutes to familiarize themselves with the procedure. The starting position was adjusted to ensure that the right foot always landed at least on one of the two force platforms embedded in the middle of the pathway. Given that typical walking speeds were slow in these patients, we instructed the control subjects to also walk at a low but comfortable speed. In this way, the general characteristics of gait could be compared between the groups without any potential bias due to speed differences (see below speed matching procedure).

Ten trials per patient were recorded, instead healthy subjects were evaluated for a total of 15 trials (10 trials self selected speed and 5 trials slow walking). To avoid muscle fatigue, blocks of three trials were separated by a one-minute rest period.

Speed matching procedure

Walking speed was matched between groups as follows: we considered only those control group subjects whose mean walking speed fell within the range identified by patients’ mean walking speed ± SD [26]. Unpaired two-sample t-test was used to investigate differences in walking velocity between patients and controls. In this way, the mean speed values were not statistically different between groups (patients 2.40 ± 1.29 km/h; controls 2.63±0.71 km/h, p = 0.283).

Data Analysis

Kinematic, kinetic and electromyographic data were normalized to the duration of the gait cycle and interpolated to 201 samples using a polynomial procedure. Gait cycle was defined as the time between two successive foot contacts of the same leg. In this study, heel strike and toe-off events were determined by maximum and minimum of limb angle excursions. Limb angle was calculated as the angle between a vertical axis from the greater trochanter and a vector drawn from the greater trochanter to lateral malleolus projected on the sagittal plane: a 0° limb angle means that the leg was positioned vertically under the body; positive angles denote flexion (i.e. limb positioned in front of the vertical axis) and negative angles denote extension (i.e. limb positioned behind the vertical axis) [27–29]. When subjects stepped on the force platforms, these kinematic criteria were verified by comparison with foot strike and lift-off measured from a threshold crossing event in the vertical force: stance phase was defined as the interval during which the vertical reaction force exceeded 7% of body weight. In general, the

Table 1. (Continued)

| Patients | Gender | Height(cm) | Body Wt.(Kg) | Age(yr) | Diagnosis | Onset(yr) | Duration(yr) | SPRS | ASHhip | ASHknee | Tot. |
|----------|--------|------------|--------------|---------|-----------|-----------|-------------|------|--------|---------|------|
| p47      | M      | 172        | 86           | 43      | SPG31     | 30        | 13          | 0    | 0      | 0       | 2    |
| p48      | F      | 170        | 65           | 23      | _AR       | 20        | 3           | 0    | 0      | 0       | 1    |
| p49      | M      | 162        | 73           | 51      | _AD       | 46        | 5           | 0    | 2      | 19      |      |
| p50      | F      | 159        | 62           | 47      | SPG4      | 45        | 2           | 0    | 0      | 3       |      |

AD = autosomal dominant; AR = autosomal recessive; F = female; M = male; SPRS = Spastic Paraplegia Rating Scale; ASH = Ashworth scale of muscle spasticity; _ = molecular diagnosis still not available. The table lists the SPRS scores; higher scores indicate higher disease’s severity.

doi:10.1371/journal.pone.0164623.t001
difference between the time events measured from kinematics and kinetics was no more than 3% [29] and kinematic criterion proved to be very robust in both healthy subjects [27] and neurological patients [28]. The raw sEMG signals were band-pass filtered using a zero-lag third-order Butterworth filter (20–450 Hz), rectified, and low-pass filtered with a zero-lag fourth-order Butterworth filter (10 Hz). For each individual, the sEMG signal from each muscle was normalized to its peak value across all trials [29].

Time-distance, kinematic, kinetic and sEMG parameters were evaluated after preprocessing procedures.

**Time-distance parameters.** The following time-distance gait parameters were calculated for each subject: walking speed (km/h), stance duration (% gait cycle), swing duration (% gait cycle), first and second double support duration (% gait cycle), cadence (step/s), step length (% limb length), and step width (% limb length).

**Kinematic parameters.** We computed the anatomical angles for hip, knee, and ankle joints (in the sagittal plane), and trunk and pelvis (frontal, sagittal, and transverse plane). From these variables, we derived the RoM at each joint or segment, defined as the difference between the maximum and minimum value during the gait cycles.

**Kinetic parameters.** Net internal joint moments (Moment\textsubscript{Ankle}, Moment\textsubscript{Knee}, Moment\textsubscript{Hip}) were calculated with an inverse dynamics approach [30] and were normalized to the subject’s body weight. Joint moment curves were used to calculate the angular impulse (AI), i.e., the area under the joint moment curve within a specific time interval [31,32].

Angular impulse quantifies the total contribution of a joint moment to the production of movement and accounts for different gait adaptations (e.g., changes in walking speed) more accurately than peak moment values and it is defined as:

\[
\frac{\int M \, dt}{\Delta t}
\]

where \( M \) is the flexor-extensor moment of the joint of interest, and \( \Delta t \) is the time interval used to calculate the integral. These angular impulses were hip extensor angular impulse during the first double support subphase (AI\textsubscript{1stDS_Hip}); hip flexor angular impulse during the second double support subphase (AI\textsubscript{2ndDS_Hip}); knee first and second extensor angular impulse (AI\textsubscript{1st_Knee} and AI\textsubscript{2nd_Knee} respectively) during the stance phase; ankle dorsiflexor angular impulse during the first double support subphase (AI\textsubscript{1stDS_Ankle}); ankle plantar flexor angular impulse during the mid-stance subphase (AI\textsubscript{MidStance_Ankle}); and ankle planter flexor angular impulse during the second double support subphase (AI\textsubscript{2ndDS_Ankle}) (S1 Fig). We also evaluated the moment of support (MS) as follow:

\[
MS = MH + MK + MA
\]

calculated as the sum of the moments MH, MK, and MA, which refer to the total curves of Moment\textsubscript{Hip}, Moment\textsubscript{Knee}, and Moment\textsubscript{Ankle}, respectively.

Specifically, we considered the area (MS\textsubscript{Area}) within the gait cycles and the values of the two peaks of the curve (MS\textsubscript{Peak1} and MS\textsubscript{Peak2}).

sEMG parameters. From the processed EMG signals, we calculated the simultaneous activation by considering the time-varying multi-muscle co-activation function (TMCf) proposed by Ranavolo and colleagues [33]:

\[
TMCf(d(t), t) = \left( 1 - \frac{1}{1 + e^{-12(d(t) - 0.5)}} \right) \cdot \left( \sum_{i=1}^{N} \frac{EMG_i(t)}{\max_{i=1...N} EMG_i(t)} \right)^2
\]

where d(t) is the mean of the differences, N is the number of muscles considered in the analysis,
and $EMG_i$ is the sEMG signal of $i_{th}$ muscle. For each subject, data over individual strides were calculated and then averaged across cycles.

As co-activation indices, we considered the area of the TMCf (TMCf$_{Area}$) within the gait cycles. We calculated the TMCf and TMCf$_{Area}$ by considering knee (RF-VL-VM vs BF-ST) and ankle (MG-LG vs TA) antagonistic muscles (TMCf$_{Knee}$, TMCf$_{Area,Knee}$, TMCf$_{Ankle}$, TMCf$_{Area,Ankle}$, respectively).

Patients’ subgroups classification. In order to classify patients according to their kinematic behavior, we used a z-score with a one tailed z-test for statistical significance [34]. Thus, we chose a z-score of mean±1.5 SD (93% percentile) of the joint RoMs of the control group as the threshold for subgrouping patients with hereditary spastic paraparesis. This z-score threshold is considered as a fairly selective score used in several research fields [35–38]. According to this criterion, each patient joint RoM could be either reduced (below threshold), increased (above threshold), or not significantly different from the values of the healthy controls. Thus, three subgroups of patients were identified. Subgroup one was patients with a statistically significant reduction of RoM at hip, knee, and ankle joints; subgroup two was patients with knee and ankle joint RoMs significantly reduced, but hip joint RoM not significantly different from the control value; and subgroup three was patients with hip joint RoM significantly increased, but ankle and knee joint RoMs not significantly different from the control values (Fig 1).

Statistical analysis

The Kolmogorov–Smirnov and Shapiro-Wilk tests were used to analyze the normal distribution of the data. Unpaired two-sample t-test or the Mann-Whitney test (two-tailed) were used for between-group differences in the demographic characteristics, time-distance parameters, joint kinematics, joint kinetics and sEMG values. Cohen’s $d$ values were also evaluated to estimate the effect size for the comparison between the two means. A multivariate ANOVA was used to compare demographic and clinical parameters (age, gender, disease onset and duration, Ashworth and SPRS scores) between subgroups of patients. One-way ANOVA was used to evaluate the differences in gait variables between the subgroups. Post hoc analyses (with Bonferroni’s corrections) were performed when significant differences were found with the ANOVA. Descriptive statistics included means ± SD, and significance level was set at $p<0.05$. Lower (LB) and upper (UB) bound of 95% confidence interval are reported for SPRS.

Results

Demographic and clinical characteristics

There were no significant differences between the group of patients with hereditary spastic paraparesis and the control group regarding gender, age, weight and height (all $p>0.05$). Multivariate analysis showed a significant main effect of the subgroup ($F_{(14,84)} = 4.468, p<0.001$). Specifically, we observed significantly lower values of hip-spasticity Ashworth-score in both subgroups two (mean ± SD: 1.25 ± 0.86) and three (0.82 ± 0.88) as compared with subgroup one (2.23 ± 1.15) ($p = 0.017$ and $p<0.001$, respectively); significantly lower values of knee-spasticity Ashworth-score in subgroup three (0.88 ± 0.93) than both subgroup one (2.47 ± 1.12) and subgroup two (2.06 ± 0.68) ($p<0.001$ and $p = 0.002$, respectively); significantly lower values of total SPRS score in both subgroup two (16.07 ± 6.48, LB: 12.62, UB: 19.52) and subgroup three (5.64 ± 5.36, LB: 2.89, UB: 8.40) than subgroup one (21.33 ± 7.17, LB: 17.65, UB: 25.02), as well as in subgroup three than subgroup two ($p = 0.049, p<0.001, p<0.001$, respectively). No significant differences between subgroups were found for any other variable.
Time-distance parameters

When comparing the whole sample of patients with the healthy participants, no significant differences were found in any time-distance parameters, except for step width and step length, whose values were significantly increased and reduced, respectively, in patients compared with controls (Table 2). A significant effect of patients' subgroup was found, using one-way ANOVA, on most of the time-distance parameters. These were the main effect of walking speed ($F_{(2,47)} = 6.703, p = 0.003$); stance duration ($F_{(2,47)} = 4.923, p = 0.011$); swing duration ($F_{(2,47)} = 4.900, p = 0.012$); second double support duration ($F_{(2,47)} = 4.551, p = 0.016$); and step length ($F_{(2,47)} = 11.173, p < 0.001$). Post-hoc analysis revealed significantly higher values of walking speed in subgroup three than in subgroup one, lower stance duration in subgroup three than in subgroup one, higher swing duration in both subgroups two and three than in subgroup one, lower second double support duration in subgroup three than in subgroup one and higher step length in subgroup three than in both subgroups one and two and in subgroup two than in subgroup one (Fig 2A).

Kinematic parameters

Significant lower values in knee and ankle RoMs and significant higher values in trunk lateral bending, flexion-extension, and rotation RoMs and pelvis rotation RoM were found in patients than in controls (Table 2). A significant main effect of the subgroup was found, using one-way ANOVA, on hip ($F_{(2,47)} = 33.747, p < 0.001$), knee ($F_{(2,47)} = 38.555, p < 0.001$), ankle ($F_{(2,47)} = 14.043, p < 0.001$), and pelvis tilt ($F_{(2,47)} = 4.328, p = 0.019$) RoMs. Post-hoc analysis revealed significant higher values in hip RoM in both subgroups two and three than in subgroup one,
higher values of knee RoM in both subgroups two and three than in subgroup one and in subgroup three than in subgroup two, higher values of ankle RoM in subgroup three than in both subgroups one and two, and lower values of pelvis tilt RoM in both subgroups two and three than in subgroup one (Fig 2B and 2C).

**Kinetic parameters**

Significant differences were found only for AI_{1stKnee} whose value was higher in patients than controls (Table 2). A significant effect of the subgroup was found, using one-way ANOVA, on
Fig 2. Time-distance and joint and trunk kinematic parameters in HSP subgroups. (A) Mean values (±SD) of time distance parameters. (B) Mean (with SDs in light colors) kinematic plot of joint angular displacements during the gait cycle. (C) Mean values (±SD) of range of angular motion (RoM). Mean values of healthy controls for both time-distance and kinematic parameters, are reported in each bar graph (dotted line) and plot (black line). Asterisks indicate significant differences among the three subgroups at post hoc analysis (⁎ p<0.05, ⁎⁎ p<0.001).

doi:10.1371/journal.pone.0164623.g002
AI_{1stDS.Hip} (main effect, F(2,47) = 3.517, p = 0.043). Post-hoc analysis showed lower values of this parameter in subgroup three than subgroup one (Fig 3A and 3B).

sEMG parameters

Significant higher values in TMCf_{Area.Amkle} were found in patients than controls (Table 2). No significant effect of the subgroup was found, using one-way ANOVA, on sEMG parameters (Fig 3C and 3D).

Discussion

We investigated the gait patterns in patients with hereditary spastic paraparesis by performing a comprehensive analysis of all time-distance, kinematic, kinetic, and sEMG parameters. In particular, our study was aimed at identifying specific subgroups of patients according to their kinematic behavior. Our assumption herein was that the decrease in the joint RoMs reflected the presence and extent of spasticity, and thus the primary deficit characterizing the gait of patients with hereditary spastic paraparesis. Few studies have previously investigated the gait in adults or children with hereditary spastic paraparesis [18–20]. In line with these previous studies, we found an abnormal gait pattern characterized by reduced step length, increased step width, and reduced RoM at the knee joint in the whole sample of patients as compared with the control group. Furthermore, we found increased trunk RoM in all three spatial planes, increased pelvic tilt, increased hip joint torques (AI_{1st.Knee}), reduced ankle joint RoM, and increased co-activation of muscles acting at the ankle joint. In addition to these general biomechanical characteristics of gait, one would expect some differential characteristics in distinct subgroups of patients according to clinical involvement of the pyramidal tract, given that patients with hereditary spastic paraparesis exhibit different degrees of severity both within and between families [1]. Thus, some specific biomechanical features may not emerge because they are hidden within their global walking strategy. Compared to previous studies, we enrolled a greater sample of patients with HSP (fifty in our study compared with twenty-two [18], nine [19] and twenty [20]) and performed an overall analysis of time-distance, kinematic (upper and lower body), kinetic and sEMG parameters. This allowed us to identify subgroups of patients and to define a global picture of walking strategies adopted by them. When subgrouping patients according to the hip, knee and ankle joint kinematic behavior, three clear gait patterns emerged. The gait pattern of subgroup one was characterized by reduced RoMs at hip, knee and ankle joints. Patients of this subgroup were the most severely affected (highest SPRS score) (Fig 1), and walked at the slowest speed. The gait pattern was characterized by the highest stance and second double support durations and the shortest swing duration and step length (Fig 2A). Such gait pattern reflects on one hand the reduced gait speed; on the other hand, the attempt to increase the most stable configuration duration (bipedal support), aimed at maintaining the dynamic balance. Furthermore, in these patients, we observed increased values of RoM for pelvis tilt and hip extensor angular impulse during the first double support subphase (Figs 2C and 3A and 3B). The former result might be due to spasticity and contracture of hip muscles as reported in neurological disorders with lower limb spasticity [20], [39–41]. The last result indicates that HSP patients, although they have reduced hip RoMs, need to greatly involve the hip joint for weight acceptance increasing the internal torques. Interestingly, this finding further reinforces the notion that spasticity predominates on muscle weakness in the most severely involved patients [42,43]. The gait pattern of subgroup three was characterized by increased hip joint RoM and knee and ankle joint RoMs close to control values. These patients were the most mildly affected (lowest SPRS score) (Fig 1) and showed the highest walking speed. Their gait pattern included the highest swing duration and step length, the
shortest stance and second double support duration values (Fig 2A) and the lowest pelvis tilt RoM and hip extensor angular impulse (during the first double support sub phase) than the other subgroups (Figs 2C and 3A and 3B). From Fig 2, it is possible to note that patients of this subgroup showed a gait pattern which was close to that of healthy controls in terms of time-distance parameters. It is also important to note that, with respect to healthy subjects, this subgroup of patients showed increased trunk RoM in all three spatial planes. Considering the very low SPRS score in this subgroup, this result suggests that the compensatory mechanisms represented by the increased trunk movements and hip RoM are the most important biomechanical
features characterizing the gait disorders from the early phase of the disease. Patients of sub-
group two had characteristics between those of subgroups one and three, in terms of disease
severity (Fig 1) and gait speed, and showed hip joint RoM close to controls but decreased knee
and ankle joint RoMs. In particular, their gait pattern was characterized by intermediate values
with respect to the other two subgroups in terms of step length, swing duration and pelvis tilt
RoM (Fig 2).

As regards the sEMG, we observed significantly increased co-activation of antagonist mus-
cles acting at the ankle for the whole group of HSP patients compared with healthy controls
(Table 2). When analyzing patients’ subgroups, a trend, although not statistically significant,
for higher co-activation values of knee and ankle antagonist muscles was also observed (Fig 3C
and 3D). Such a finding reflects the inability of the CNS to selectively activate lower limb joint
muscles and may be explained by inefficient mechanisms of reciprocal inhibition [44] and the
supraspinal and spinal plastic neuronal changes associated with the development of spasticity
[45,46]. In general the different patterns of gait disturbance seem to correlate fairly well with
the different degree of disease’s severity among patients, as measured by the SRPS score, with
mild (SPRS < ten), moderate (SPRS < twenty) and more severe presentation (SPRS > twenty).
In addition, since we chose to study only patients with pure pyramidal signs, regardless of the
 genetic form (namely SPG3A, 4, 5, 7 and 31), we can safely assume that the identified three dif-
ferent gait patterns based on lower limb kinematic behavior, also reflect the different degree of
pyramidal tract involvement in individual patients. We think that identifying specific gait pat-
terns [41] in patients with hereditary spastic paraparesis may be useful in: i) improving our
understanding on gait disorder in hereditary spastic paraparesis by sorting out the most mean-
ingful gait features from the complexity of locomotion; ii) recognizing specific abnormalities
and their impact on clinical decision-making; and iii) individualizing rehabilitative treatment
and better evaluating its effects over the time.

Supporting Information

S1 Data. Main data underlying the findings described in the manuscript.

(XLSX)

S1 Fig. Method of angular impulse evaluation on hip, knee, and ankle joint moments
curves.

(TIF)

Author Contributions

Conceptualization: MS MR AR.

Data curation: MR MS AR.

Formal analysis: MR MS GM.

Funding acquisition: FP.

Investigation: MR MS.

Methodology: MS MR AR C. Conte GC.

Project administration: FP FL.

Resources: MS MR LL C. Casali FP.

Software: MR GM.
Supervision: MS C. Casali FP
Validation: MS AR FL.
Visualization: MS C. Casali FP.
Writing – original draft: MS MR AR.
Writing – review & editing: MS MR AR GM TV C. Casali FP FL FD.

References
1. Lo Giudice T, Lombardi F, Santorelli FM, Kawarai T, Orlaccio A. Hereditary spastic paraplegia: clinical-genetic characteristics and evolving molecular mechanisms. Exp Neurol. 2014; 261:518–539. doi: 10.1016/j.expneurol.2014.06.011 PMID: 24954637
2. Faber I, Serveheere KR, Martinez AR, D’Abreu A, Lopes-Cendes I, França MC Jr. Clinical features and management of hereditary spastic paraplegia. Arq Neuropsiquiatr. 2014; 72(3):219–226. PMID: 24676440
3. Klimpe S, Schüle R, Kassubek J, Otto S, Kohl Z, Klebe S, et al. Disease severity affects quality of life of hereditary spastic paraplegia patients. Eur J Neurol. 2012; 19(1):168–171. doi: 10.1111/j.1468-1331.2011.03443.x PMID: 21631647
4. Orsucci D, Petrucci L, Ienco EC, Chicco L, Simi P, Fogli A, et al. Hereditary spastic paraparesis in adults. A clinical and genetic perspective from Tuscany. Clin Neurol Neurosurg. 2014; 120:14–19. doi: 10.1016/j.clineuro.2014.02.002 PMID: 24731568
5. Fink JK. Hereditary spastic paraplegia. Curr Neurol Neurosci Rep. 2006; 6(1):65–76. PMID: 16469273
6. Zhang Y, Roxburgh R, Huang L, Parsons J, Davies TC. The effect of hydrotherapy treatment on gait characteristics of hereditary spastic paraparesis patients. Gait Posture. 2014; 39(4):1074–1079. doi: 10.1016/gaitpost.2014.01.010 PMID: 24566467
7. Heetla HW, Halbertsma JP, Dekker R, Staal MJ, van Laar T. Improved gait performance in a patient with hereditary spastic paraplegia after a continuous intrathecal baclofen test infusion and subsequent pump implantation: a case report. Arch Phys Med Rehabil. 2015; 96(6):1166–1169. doi: 10.1016/j.apmr.2015.01.012 PMID: 25626112
8. Bertolucci F, Di Martino S, Orsucci D, Ienco EC, Siciliano G, Rossi B, et al. Robotic gait training improves motor skills and quality of life in hereditary spastic paraplegia. NeuroRehabilitation. 2015; 36(1):93–99. doi: 10.3233/NRE-141196 PMID: 25547770
9. Adams RD, Victor M, Ropper AH, Samuels MA, Klein J, editors. Principles of Neurology, 10th ed, New York, McGraw-Hill Education Medical, 2015.
10. Rowland LP, Pedley TA, editors. Neurologia de Merritt, 12nd ed, Nederland, Wolters Kluwer Health, 2011.
11. Martinuzzi A, Montanaro D, Vavla M, Paparella G, Bonanni P, Musumeci O, et al. Clinical and Paraclinical Indicators of Motor System Impairment in Hereditary Spastic Paraplegia: A Pilot Study. PLoS One. 2016; 11(4). doi: 10.1371/journal.pone.0153283 PMID: 27077743
12. Henson BJ, Zhu W, Hardaway K, Wetzel JL, Stefan M, Albers KM, et al. Transcriptional and post-transcriptional regulation of SPAST, the gene most frequently mutated in hereditary spastic paraplegia. PLoS One. 2012; 7(5). doi: 10.1371/journal.pone.0036505 PMID: 22574173
13. Wedding IM, Koht J, Tran GT, Misceo D, Selmer KK, Holmgren A, et al. Spastic paraplegia type 7 is associated with multiple mitochondrial DNA deletions. PLoS One. 2014; 9(1). doi: 10.1371/journal.pone.0086340 PMID: 24460038
14. Beetz C, Schüle R, Deconinck T, Tran-Viet KN, Zhu H, Kremer BP. REEP1 mutation spectrum and genotype/phenotype correlation in hereditary spastic paraplegia type 31. Brain. 2008; 131(4):1076–86. doi: 10.1093/brain/awn026 PMID: 18321925
15. Goizet C, Boukhris A, Durr A, Beetz C, Truchetto J, Tesson C. CYP7B1 mutations in pure and complex forms of hereditary spastic paraplegia type 5. Brain. 2008; 132(6):1589–600. doi: 10.1093/brain/awp073 PMID: 19439420
16. Solowska JM, Baas PW. Hereditary spastic paraplegia SPG4: what is known and not known about the disease. Brain. 2015; 138(9):2471–64. doi: 10.1093/brain/awv178
17. Wilkinson PA, Crosby AH, Turner C, Bradley LJ, Ginsberg L, Wood NW. A clinical, genetic and biochemical study of SPG7 mutations in hereditary spastic paraplegia. Brain. 2004; 127(5):973–80. doi: 10.1093/brain/awv125 PMID: 14985266
18. Klebe S, Stolze H, Kopper F, Lorenz D, Wenzelburger R, Volkmann J, et al. Gait analysis of sporadic and hereditary spastic paraplegia. J Neurol. 2004; 251(5): 571–578. doi: 10.1007/s00415-004-0366-7 PMID: 15164190

19. Piccinini L, Cimolini V, D’Angelo MG, Turconi AC, Crivellini M, Galli M. 3D gait analysis in patients with hereditary spastic paraparesis and spastic diplegia: a kinematic, kinetic and EMG comparison. Eur J Paediatr Neurol. 2011; 15(2): 138–145. doi: 10.1016/j.ejpn.2010.07.009 PMID: 20829081

20. Marsden J, Ramdharry G, Stevenson V, Thomson A. Muscle paresis and passive stiffness: key determinants in limiting function in Hereditary and Sporadic Spastic Paraparesis. Gait Posture. 2012; 35(2): 266–271. doi: 10.1016/j.gaitpost.2011.09.018 PMID: 22050971

21. Schüle R, Holland-Letz T, Klimpe S, Kassubek J, Klopotock T, Mall V, et al. The Spastic Paraplegia Rating Scale (SPRS): a reliable and valid measure of disease severity. Neurology. 2006; 67(3): 430–434. doi: 10.1212/00055394.2006.004227 PMID: 16894103

22. Davis RB III, Öunpuu S, Tyburski D, Gage JR. A gait analysis data collection and reduction technique. Hum Mov Sci. 1991; 10: 575–587. doi: 10.1016/0167-9457(91)90046-Z

23. Winter DA, editor. Biomechanics of Human Movement, 2nd ed, New York, Wiley and Sons, 1979.

24. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. J Electromyogr Kinesiol. 2000; 10(5): 361–374. doi: 10.1016/S1050-6411(00)00027-4 PMID: 11018445

25. Barbero M, Merletti R, Rainoldi A, editors. Atlas of Muscle Innervation Zones: Understanding Surface Electromyography and Its Applications, New York, Springer, 2012. doi: 10.1007/978-88-470-2463-2

26. Mari S, Serrao M, Casali C, Conte C, Martino G, Ranavolo A, et al. Lower limb antagonist muscle co-activation and its relationship with gait parameters in cerebellar ataxia. Cerebellum. 2014; 13: 226–236. doi: 10.1007/s12311-013-0533-4 PMID: 24170572

27. Vasudevan EV, Torres-Oviedo G, Morton SM, Yang JF, Bastian AJ. Younger is not always better: development of locomotor adaptation from childhood to adulthood. J Neurosci. 2011; 31(8): 3055–3065. doi: 10.1523/JNEUROSCI.00275-2014 PMID: 25185815

28. Martino G, Ivanenko YP, Serrao M, Ranavolo A, d’Avella A, Draicchio F, et al. Locomotor patterns in cerebellar ataxia. J Neurophysiol. 2014; 112: 2810–2821. doi: 10.1152/jn.00275.2014 PMID: 25185815

29. Vaughan C, Brian D, O’Connor J. Dynamics of Human Gait, 2nd ed. Champaign, IL: Human Kinetics Publishers; 1992.

30. De Vita P, Lassiter T Jr, Horta-Bagyi T, Tony M. Functional knee brace effects during walking in patients with anterior cruciate ligament reconstruction. Am J Sports Med. 1998; 26: 778–784. PMID: 9850778

31. Don R, Serrao M, Vinci P, Ranavolo A, Cacchio A, Ioppolo F, et al. Foot drop and plantar flexion failure determine different gait strategies in Charcot-Marie-Tooth patients. Clin Biomech. 2007; 22(8): 905–916. doi: 10.1016/j.clinbiomech.2007.06.002 PMID: 17686557

32. Ranavolo A, Mari S, Conte C, Serrao M, Silvetti A, Iavicoli S, et al. New muscle co-activation index for biomechanical load evaluation in work activities. Ergonomics. 2015; 58(5): 863–879. doi: 10.1111/ergo.12539

33. van der Waal JM, Terwee CB, van der Windt DA, Bouter LM, Dekker J. The impact of non-traumatic hip and knee disorders on health-related quality of life as measured with the SF-36 or SF-12. J Rheumatol. 2001; 28(6): 906–917. doi: 10.1007/S00034-001-0656-8

34. Meyer AC, Boscardin WJ, Kwasa JK, Price RW. Is it time to rethink how neuropsychological tests are used to diagnose mild forms of HIV-associated neurocognitive disorders? Impact of false-positive rates on prevalence and power. Neuroepidemiology. 2013; 41(3–4): 208–216. doi: 10.1159/000354629 PMID: 24157543

35. Wasser K, Pilgram-Pastor SM, Schnaudigel S, Stojanovic T, Schmidt H, Knauf J, et al. New brain lesions after carotid revascularization are not associated with cognitive performance. J Vasc Surg. 2011; 53(1): 61. doi: 10.1016/j.jvs.2010.07.061 PMID: 20875716

36. France CR, Rhudy JL, McGlone S. Using normalized EMG to define the nociceptive flexion reflex (NFR) threshold: further evaluation of standardized NFR scoring criteria. Pain. 2008; 145(1–2): 211–218. doi: 10.1016/j.pain.2009.06.022 PMID: 19595510

37. Weinstein G, Beiser AS, Decarli C, Au R, Wolf PA, Seshadri S. Brain imaging and cognitive predictors of stroke and Alzheimer disease in the Framingham Heart Study. Stroke. 2013; 44(10): 2787–2794. doi: 10.1161/STROKEAHA.113.009947 PMID: 23920020
39. Mao Y, Chen P, Li L, Li L, Huang D. Changes of pelvis control with subacute stroke: A comparison of body-weight-support treadmill training coupled virtual reality system and over-ground training. Technol Health Care. 2015; 23 Suppl 2: S355–S364. doi: 10.3233/THC-150972 PMID: 26410502

40. De Quervain IA, Simon SR, Leurgans S, Pease WS, McAllister D. Gait pattern in the early recovery period after stroke. J Bone Joint Surg Am. 1996; 78(10): 1506–1514. PMID: 8876578

41. Roche N, Pradon D, Cosson J, Robertson J, Marchiori C, Zory R. Categorization of gait patterns in adults with cerebral palsy: a clustering approach. Gait Posture. 2014; 39(1): 235–240. doi: 10.1016/j.gaitpost.2013.07.110 PMID: 23948331

42. McDermott C, White K, Bushby K, Shaw P. Hereditary spastic paraparesis: a review of new developments. J Neurol Neurosurg Psychiatry. 2000; 69(2):150–160. Review. doi: 10.1136/jnnp.69.2.150 PMID: 10896685

43. Schüle R, Wiethoff S, Martus P, Karle KN, Otto S, Klebe S, et al. Hereditary Spastic Paraplegia -clinical-genetic lessons from 608 patients. Ann Neurol. 2016. doi: 10.1002/ana.24611 PMID: 26856398

44. Meunier S, Pol S, Houeto JL, Vidalhiet M. Abnormal reciprocal inhibition between antagonist muscles in Parkinson’s disease. Brain J Neurol. 2000; 123(5): 1017–1026. PMID: 10775546

45. Simmons RW, Richardson C. Peripheral regulation of stiffness during arm movements by coactivation of the antagonist muscles. Brain Res. 1988; 473: 134–140. doi: 10.1016/0006-8993(88)90324-1 PMID: 3208115

46. Falconer K, Winter DA. Quantitative assessment of co-contraction at the ankle joint in walking. Electromyogr Clin Neurophysiol. 1985; 25: 135–149. PMID: 3987606