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Using Linear and Non-Linear Techniques to Characterize Gait Coordination Patterns of Two Individuals with NGLY1 Deficiency

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Abstract

Individuals with NGLY1 Deficiency, an inherited autosomal recessive disorder, exhibit hyperkinetic movements including athetoid, myoclonic, dysmetric, and dystonic movements impacting both upper and lower limb motion. This report provides the first set of laboratory-based measures characterizing the gait patterns of two individuals with NGLY1 Deficiency, using both linear and non-linear measures, during treadmill walking, and compares them to neurotypical controls. Lower limb kinematics were obtained with a camera-based motion analysis system and bilateral time normalized lower limb joint time series waveforms were developed. Linear measures of joint range of motion, stride times and peak angular velocity were obtained, and confidence intervals were used to determine if there were differences between the patients and control. Correlations between participant and control mean joint waveforms were calculated and used to evaluate the similarities between patients and controls. Non-linear measures included: joint angle-angle diagrams, phase-portrait areas, and continuous relative phase (CRP) measures. These measures were used to assess joint coordination and control features of the lower limb motion. Participants displayed high correlations with their control counterparts for the hip and knee joint waveforms, but joint motion was restricted. Peak angular velocities were also significantly less than those of the controls. Both angle-angle and phase-portrait areas were less than the controls

although the general shapes of those diagrams were similar to those of the controls. The NGLY1 Deficient participants' CRP measures displayed disrupted coordination patterns with the knee-ankle patterns displaying more disruption than the hip-knee measures. Overall, the participants displayed a functional walking pattern that differed in many quantitative ways from those of the neurotypical controls. Using both linear and non-linear measures to characterize gait provides a more comprehensive and nuanced characterization of NGLY1 gait and can be used to develop interventions targeted toward specific aspects of disordered gait.

Keywords

NGLY1, Gait, Disability, Kinematics, Angle-Angle Diagrams, Phase Portraits

1. Introduction

The ability of humans to transport themselves throughout their environment is a critical component of an untold number of goal-directed activities essential to the survival of the species. Many individuals with genetic mutations resulting in developmental disorders have difficulty walking in an efficient and effective manner. Individuals with developmental disorders such as Rett Syndrome, Syngap1 Deficiency, MECP2 Duplication, Fragile X and NGLY1 Deficiency manifest a variety of physiological and motor-based disorders, but all express gait disturbances. NGLY1 Deficiency is an inherited, autosomal, recessive, congenital disorder of deglycosylation (N-linked deglycosylation (NGLY1-CDDG)). This ultra-rare condition results in a decrease in NGLY1 production due to a mutation of the NGLY1 gene. Individuals with NGLY1 deficiency can exhibit a number of myoclonic, dystonic, choreo-athetoid and dysmetric motions during movement [1]. Action tremor has also been documented [2]. Based upon clinical assessments and parental reports, individuals with NGLY1 Deficiency have also been reported to have gait difficulties. However, the gait of NGLY1 Deficient patients has not been documented with laboratory-based motion analysis technology.

Gait has been shown to be associated with the overall severity of neurodevelopmental disorders, e.g. [3], underlining the importance of assessing the gait of individuals with NGLY1 Deficiency. In a recent survey, gait disturbances were reported in over 97% of NGLY1 Deficient patients [1], thereby emphasizing the need for increased knowledge regarding the gait characteristics of these individuals. Having precise documentation of an individual's kinematic gait pattern can provide specific information about how the lower limb joints are coordinated to produce an efficient gait, or conversely, how the discoordination among joint results in impaired gait.

While linear measures such as time-based joint angle waveforms, joint range of motions (ROM), and peak velocities provide important information, non-linear

measures including angle-angle diagrams and phase portraits, can provide complementary information regarding coordination and control, respectively [4] [5]. Angle-angle diagrams represent intersegmental coordination, by plotting the relative motion of two joints throughout a gait cycle [6] [7], while phase portraits provide a direct correlation of a particular joints motion with their associated velocities [8]. Both angle-angle diagrams and phase portraits can be quantified to extract additional information about the dynamics of a particular gait pattern (see Methodology section below).

It is also possible to evaluate the intersegmental coordination between two joints using continuous relative phase (CRP). CRP is calculated by subtracting the phase angles of a distal joint from adjacent, proximal joint throughout the gait cycle in order to understand the relative coordinative structure between the two joints [8] [9]. Simply put, if two body segments rotate in the same direction at the same time, the relative phase angle is considered *in-phase*; conversely, if the segments rotate in the opposite direction, they are demonstrating *out-of-phase* coordination [10]. As with the aforementioned non-linear measures, quantitative measures can also be extracted from basic CRP plots to provide greater insight into intersegmental coordination. Information from both linear and non-linear measures can be used to characterize gait in a more comprehensive manner than if only one or the other category of measures is used [11] [12].

Characterizing the kinematic strategies used by individuals affected by NGLY1 Deficiency and comparing those strategies with those of healthy, neurotypical individuals provides information about any differences between the two participant groups and could offer insights into potential treatments, and/or offer a useful outcome measure for tracking changes from interventions. A full kinematic characterization of gait can be used as an individual's "baseline" and act as a comparison to data collected at a future time for the evaluation of the effectiveness of a therapeutic intervention be it, physical, pharmacological, or genetic. This investigation begins to fill the gap in the literature by providing the first report of laboratory-based kinematic gait assessments of individuals with NGLY1 Deficiency, including comparisons with similarly aged neurotypical controls, using a combination of linear and non-linear measures.

2. Methods

2.1. Study Participants

Two individuals diagnosed with NGLY1 Deficiency served as participants in this investigation. Participant 1 was a 15-year-old female (P1) and Participant 2 was an 18-year-old male (P2). NGLY1 Deficiency is an ultra-rare syndrome, defined as an incident rate of less than one per 50,000 per population [13]. In 2022, it was reported that there were 74 identified cases of NGLY1 Deficiency across the world [1]. The opportunity to have two of those individuals participate in the current study was based in no small part on efforts of the Grace Science Foundation, which maintains an extensive registry of patients throughout the world. We chose two

ambulatory individuals in this ultra-rare disorder at an age that would typically show stability with regards of ambulation. Additionally, since both participants were relatively close in age, we were able to use a single set of control data. Both participants provided informed written consent with consent for P1 being provided by her parents and P2 providing his own consent. Both participants are compound heterozygous for the variants c.622 C > T and c.930 C > T in the NGLY1 gene. Both individuals are considered higher functioning individuals based upon clinical assessments. When collectively referring to the NGLY1 participants, P1 and P2 will be referred to as the "participants", throughout the remainder of this report. The Institutional Review Boards of Baylor College of Medicine (H-35835) and the University of Houston (00000855) approved all study procedures.

Neurotypical control data was obtained from the database published by Senden *et al.* [14] which was "designed for comparison [to] pathological gait" as a means of "improving the interpretation of pathological gait and… contributing to better clinical decision making." The data used in this investigation included bilateral sagittal plane hip, knee, and ankle data collected from eight neurotypical individuals walking on a motorized treadmill at a self-selected pace. There were three females and five males included in the data set with an average age of 15 years (SD = 1.70). The kinematic data from this database were collected with a 12-camera VICON motion analysis system and processed with a customized MATLAB script. The data set contained mean kinematic waveforms for each participant which were processed into mean waveforms for individual joints of the lower extremity, as well as numerous spatial and temporal gait measures.

2.2. Study Protocol

Prior to data collection, reflective markers were attached bilaterally with adhesive collars over the hip, knee, shank, ankle, toe, heel, and sacrum. Participants were then asked to begin walking on a motorized treadmill. While on the treadmill, the participants wore a ceiling-mounted safety harness; this harness prevents a participant from falling but does not bear their weight while walking. The participants were provided with an acclimatization period of five minutes, during which time their comfortable walking speed was determined. As highly functioning NGLY1 Deficiency patients, both were verbal and able to indicate when they had obtained their comfortable walking speed. After the acclimation period, participants were given a three-minute rest period. The participants then walked for three minutes at their self-selected speed during which kinematic data was collected using a 16-camera VICON motion analysis system. A 100 Hz sample rate was used to collect the kinematic data and fitted to the VICON Plug-In Gait Model and processed through VICON Nexus software. Bilateral, sagittal plane joint angle data for the hips, knees, and ankles were obtained and data were filtered with a 2nd order Butterworth filter using a 6 Hz cut off frequency via custom MATLAB script.

2.3. Data Processing

The angle waveforms for each joint were partitioned into individual strides using peak knee flexion reference points. Peak knee reference points were chosen because the participants demonstrated a significant number of strides without heel strikes (*i.e.* toe walking) during the acclimatization period. Individual strides were time normalized such that each stride was represented by 100 samples. Over the course of the 3-minute collection period, participants executed a little over 110 total strides. The subsequently analyzed data include strides 11 through 110. The initial 10 steps were discarded as this represented the time it took the motorized treadmill to reach the participants' self-selected speed. The control data, which was also normalized to 100 samples, was arranged such that the initial data sample began at peak knee flexion (see Results section).

2.4. Linear Dependent Measures

Several linear measures were used to characterize the gait features of our two participants and the controls. Initially, mean stride times and standard deviations were calculated. Mean waveforms for each joint were then developed and Pearson r correlations between the participants' and control waveforms were computed. This measure reflects the similarity of the "shape" of the waveforms. Confidence intervals (CI) for each mean joint waveform of the control participants were then calculated to a 95% level. For each participant's waveform, the percentage of samples outside of the control groups' CIs, were computed. This technique was used to assess how similar the amplitudes of the samples across the entire waveforms of the participants were to that of the controls. Linear measures of joint range of motion (ROM), and peak joint velocity, for each stride were calculated along with means, standard deviations, and coefficients of variation (CV).

Potential differences in stride times, ROM, peak joint velocity, between each joint pair were assessed using Welch's t-test to determine if the two lower limbs of each participant performed similarly. This procedure was also applied to the control group's data. Using the control groups' data, 95% CIs were calculated for each variable and the participant's mean values were evaluated to determine if they fell within the control group's CIs. Finally, descriptive statistics were used to report the CVs of each variable to characterize the relative variability of the control groups data compared to the participants data[1].

2.5. Non-Linear Measures

Non-linear measures included angle-angle diagrams [7], phase-portraits for each joint, [15] and continuous relative phase (CRP) using the mean waveforms for the

[1]*As the control database only contained each individual's mean waveform, for each joint, only a single ROM value for each individual could be calculated, hence there was no SD and therefore a CV for each individual could not be calculated. Only a single group CV value could be calculated for each joint from the group mean and SD.*

hip-knee and knee-ankle joint pairs for each leg [8]. The area of the angle-angle and phase phase-portrait diagrams were computed using a customized MATLAB script. CRP analyses can be quantified by two measures, mean absolute relative phase (MARP) and Deviation Phase (DP). MARP is the mean absolute value of CRP curve values. A lower MARP value reflects a more in-phase coordination pattern between the two joints, while greater values indicate more anti-phase coordination patterns. DP is calculated by averaging the standard deviation of CRP curve values. A lower DP value reflects a more stable relationship between two joints compared to a higher DP score [8]. To gain greater insight into intersegmental coordination during the different gait phases, MARP and DP were independently calculated for the stance and swing phase. As all of the non-linear variables were developed using the participants mean waveforms, it is only possible to evaluate their scores relative to the CI of the control groups' data.

3. Results

3.1. Linear Results

Mean stride time for the control group was 1.18 seconds with a CI lower boundary of 1.10 and an upper boundary of 1.26. P1's mean was 1.44, thereby falling outside of the upper CI limit. P2's mean value was 1.24, falling just inside the CI's upper boundary. The correlations between the participants' mean waveforms and those of the controls, for both the left and right hip and knee, were extremely high, ranging from 0.95 to 0.99. However, ankle correlation values were only moderately correlated, except for P1's right ankle, which was 0.18 (Table 1).

Table 1. Pearson r correlation values, for each joint, between mean waveforms of controls and participants.

Pearson r values	L Hip	L Knee	L Ankle	R Hip	R Knee	R Ankle
P1 Mean	0.98	0.96	0.56	0.96	0.95	0.18
P2 Mean	0.99	0.97	0.59	0.97	0.99	0.75

Figure 1 displays participants' and control mean joint waveforms (with control CI for each joint).

Table 2 details the percentage of samples in the participants' waveforms that fell within the control CI, for each joint.

Table 2. Percentage of participant samples within the control group's waveforms CIs by joint.

Participant	L Hip	L Knee	L Ankle	R Hip	R Knee	R Ankle
P1	24%	29%	58%	19%	26%	37%
P2	41%	40%	37%	77%	44%	8%

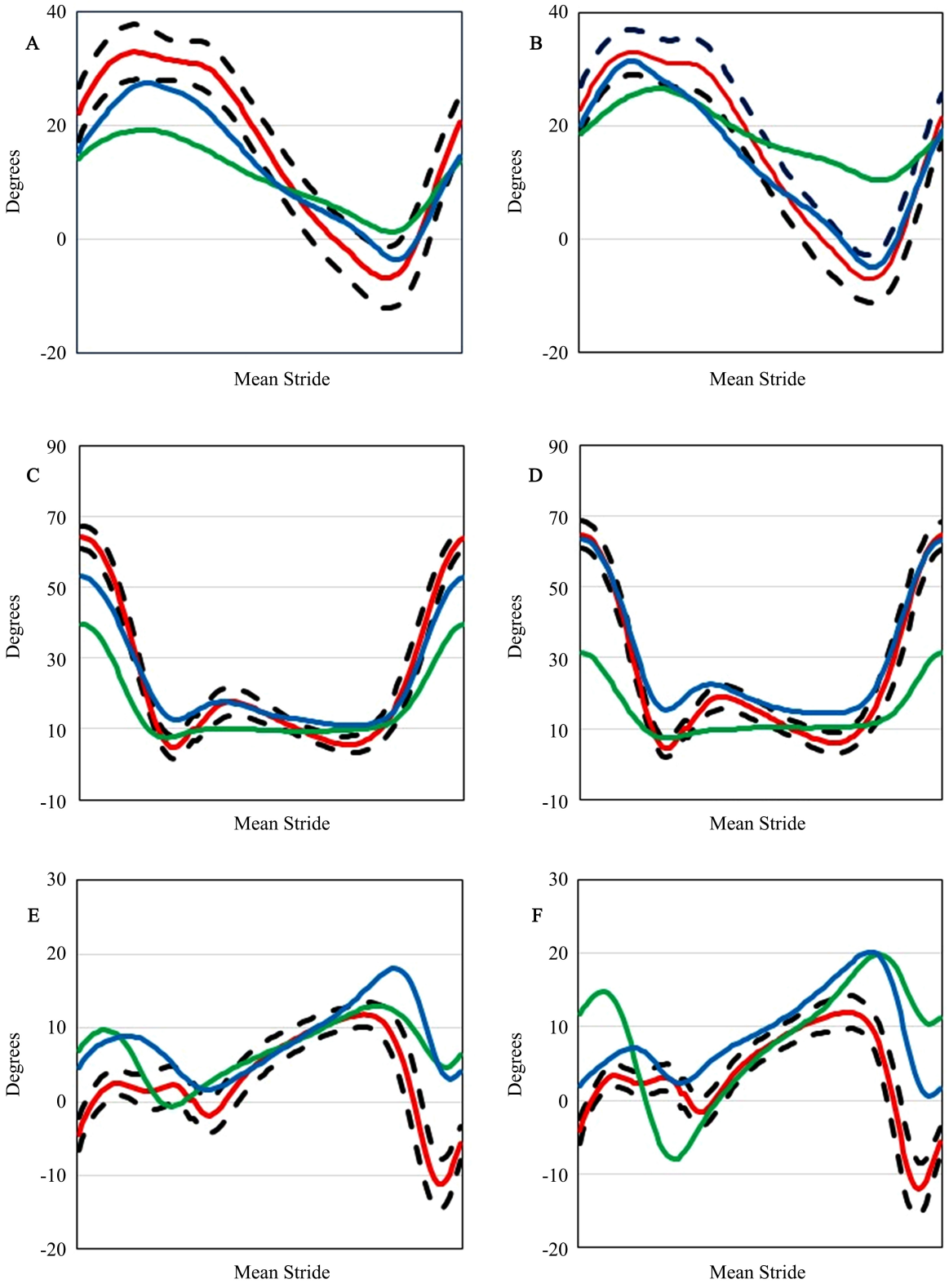

Figure 1. Mean waveforms for the participants and control data for each joint, including the CI for the control data. P1 is in green, P2 is in blue, and the control data are in red. The dashed lines signify the 95% CI of the control group. The left column of panels details the left hip, left knee, and left ankle motion (A, C, E, respectively), while the right panels represent the right hip, right knee, and right ankle (B, D, F, respectively).

Table 3 reveals that all the ROMs, for all joints, and both participants fell below the lower CI boundary of the controls except for P1's right ankle which exceeded the CI.

Table 3. Mean ROM, CI, and CVs of the control group and participants.

ROM	L Hip	L Knee	L Ankle	R Hip	R Knee	R Ankle
CI Upper Bound	52.2	69.5	26.9	54.0	69.8	28.7
Control Mean	49.1	65.7	24.8	49.7	66.8	26.0
CI Lower Bound	46.4	61.9	22.8	45.3	63.8	23.3
P1 Mean	19.5*	35.8*	16.4*	17.4*	27.9*	33.1*
P2 Mean	32.0*	44.1*	18.8*	37.1*	51.7*	22.7*
Control CV	8.9	8.4	11.9	12.6	6.4	14.9
P1 CV	16.7	12.3	24.2	20.6	20.1	19.5
P2 CV	7.5	4.8	10.4	7.3	4.8	14.7

Peak angular velocities and associated CVs are reported in Table 4. A * denotes that the participant's mean value was outside the confidence interval bounds of the Control data.

Table 4. Mean peak angular velocity, CIs and CVs for control and participants. A * denotes that the participant's mean value was outside the confidence interval bounds of the control data.

Peak Velocity	L Hip	L Knee	L Ankle	R Hip	R Knee	R Ankle
CI Upper Bound	2.53	4.22	1.57	2.64	4.30	1.72
Control Mean	2.35	3.96	1.44	2.44	4.00	1.53
CI Lower Bound	2.16	3.71	1.3	2.16	3.71	1.33
P1 Mean	1.02*	2.21*	0.99*	0.81*	1.77*	1.65
P2 Mean	1.52*	3.08*	0.86*	1.76*	3.22*	0.95*
Control CV	11.3	9.2	13.8	14.6	10.6	18.5
P1 CV	24.2	20.4	35.4	36.4	29.2	42.4
P2 CV	13.9	10.5	19.1	12.1	10.5	27.6

The relative variability (CV values) for both the ROM and peak velocity indicate P1 was more variable than both the Control group and P2. P2's CV was generally quite similar to the controls for both the ROM and velocities with some ROM CV values being less than that of the controls. Only P2's velocity CV for the ankle was noticeably greater than the controls. In general, the results of the linear measures indicate a reduction in the magnitude of these measures, despite similarly shaped

waveforms. These findings suggest that the participant's overall kinematic patterns were altered relative to neurotypical controls. The non-linear measures presented below reveal that both the coordination (angle-phase diagrams and CRP measures) and control (phase portraits) of the lower limbs are disrupted during treadmill walking.

3.2. Non-Linear Results

The areas of the angle-angle diagrams between the hip-knee and the knee-ankle are displayed in Table 5. Not surprisingly, and consistent with the reduced ROMs, all of the values, for both participants, were well below the lower CI values of the controls (Figure 2).

Table 5. Mean angle-angle areas, and CI for the controls and participants.

Angle-Angle areas (degrees^2)	L Hip vs L Knee	L Knee vs L Ankle	R Hip vs R Knee	R Knee vs R Ankle
CI Upper Bound	2249	520	1804	484
Control Mean	2024	632	2084	648
CI Lower Bound	1801	743	2363	812
P1 Mean	372*	123*	215*	123*
P2 Mean	874*	230*	1208*	341*

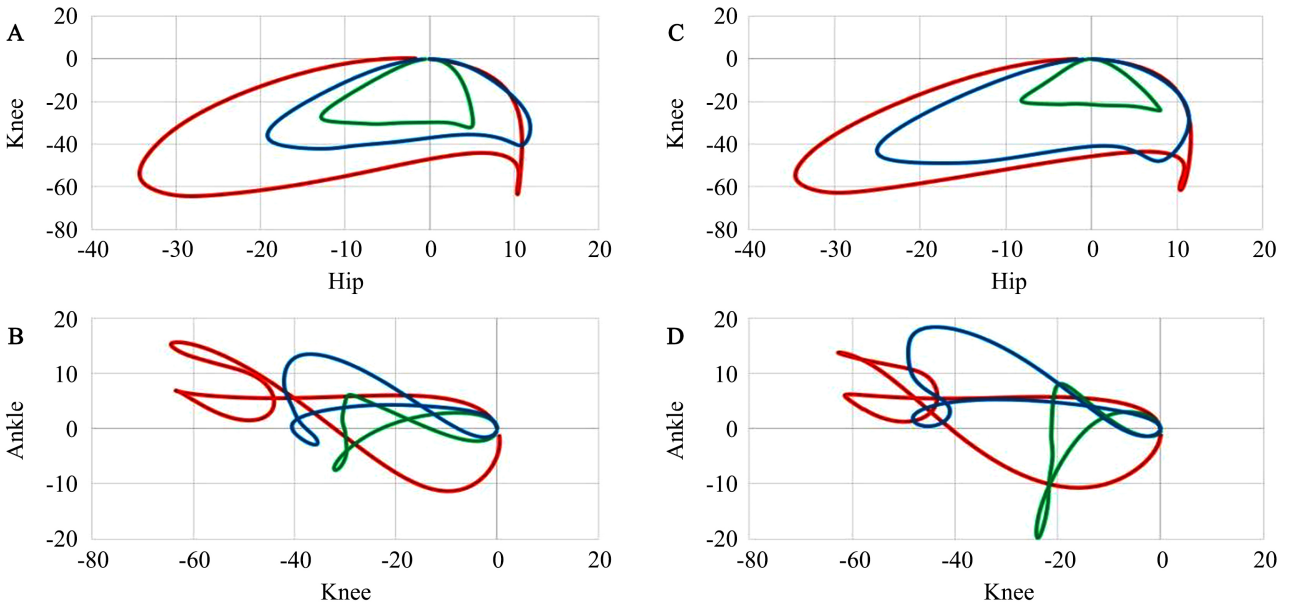

Figure 2. Angle-angle diagrams. P1 in green, P2 in blue, control in red. Panels A and B represent left leg motion, while C and D represent right leg motion.

Table 6 displays the areas of the computed phase-portraits. The values for both participants, for every joint, were well below the lower CI boundary of the controls (Figure 3).

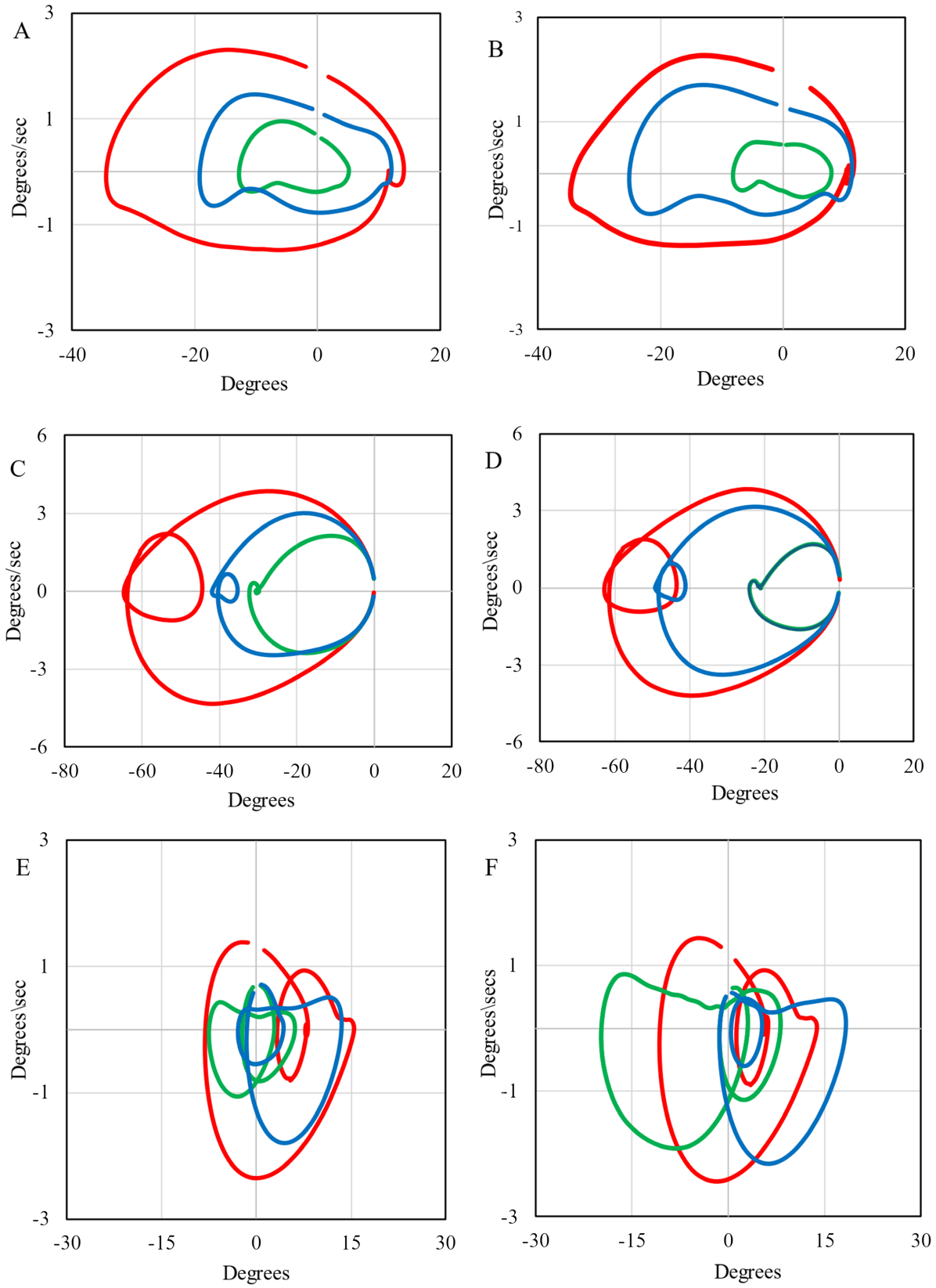

Figure 3. Phase portraits P1 in green, P2 in blue, control in red. panels A and B detail the left and right hips, respectively, C and D are the left and right knees, and E and F the left and right ankles.

Table 6. Mean phase-portrait areas and CIs for the controls and participants.

Phase-portrait areas (degrees/sec)	L Hip	L Knee	L Ankle	R Hip	R Knee	R Ankle
CI Upper Bound	162.1	512.8	85.4	180.5	537.8	95.7
Control Mean	142.9	459.6	74.3	150.5	475.4	81.5
CI Lower Bound	123.9	400.9	63.2	120.5	413.1	67.3
P1 Mean	17.9*	106.9*	19.9*	13.0*	57.0*	59.7*
P2 Mean	50.8*	176.9*	32.4*	68.6*	247.4*	43.1*

The continuous relative phase measures of MARP and DP are presented in Table 7. For P1, the right leg's stance, swing, and full stride Hip-Knee MARP fell outside the controls' CI boundaries. The stance phase demonstrates more in-phase coordination than that of the swing phase. Across the entire stride, P1's MARP is more out of phase, relative to the controls. Conversely, none of P1's left leg Hip-Knee MARPs exceeded the controls' CIs. During stance, P2's left and right Hip-Knee MARPs were significantly more in-phase than the controls. The right swing MARP was also less while the left swing MARP was not significantly different. Across the entire stride, only the right Hip-Knee MARP was significantly different, displaying more in-phase coordination than the controls. For both participants, all the Knee-Ankle MARP comparisons values exceed that of the lower CI boundary of the controls, thereby displaying greater in-phase coordination within both the stance and swing phase.

Of the 24 DP comparisons between the two participants and the control data, 16 fell outside of the controls' CIs (Table 7). The left knee-ankle DP during the stance phase, for both participants, fell within the control's CI. For the left swing phase both participants displayed greater variability for the knee-ankle across the entire stride. Interestingly, all the significantly different DP values for the participants' hip-knee were less than the values for the controls. These data indicate that, overall, the participants displayed a less variable relationship between the knee and hip joints than did the neurotypical controls. Notably, P1 displayed greater variability than the controls for the left knee-ankle DP during both the swing phase and across the entire stride, but no differences for the right leg. P2 displayed significantly greater variability than the controls during both the swing and stride phases for both legs. Overall, the data suggests a trend towards lower inter-joint coordination variability between the hip and knee across all phases of the gait cycle and greater variability in the knee-ankle relationships during the swing and across the entire stride, relative to the controls.

Figure 4 displays the control and participants' mean CRP waveforms and control CI. P1 in green, P2 in blue, Control in red. Panels A and B represent left and right hip-knee CRP, respectively while C and D represent left and right knee-ankle CRP waveforms.

Table 7. MARP and DP scores for swing, stance, and complete stride for the controls and participants.

MARP Scores	Gait Phase	Side	−CI	Mean	+CI	P1	P2
Hip-Knee	Stance	L	68.8	72.5	76.2	71.9	67.7*
		R	69.8	72.5	75.1	59.7*	64.5*
	Swing	L	94.1	99.0	103.8	103.6	99.0
		R	97.3	100.4	103.5	117.3*	95.8*
	Stride	L	80.6	82.9	85.3	84.4	81.2
		R	81.1	83.5	85.9	89.1*	76.8*
Knee-Ankle	Stance	L	130.4	144.0	160.9	113.6*	85.4*
		R	132.4	146.6	160.7	97.8*	109.8*
	Swing	L	104.0	120.8	142.7	55.0*	84.7*
		R	119.0	131.4	143.8	37.8*	99.0*
	Stride	L	123.7	134.8	149.4	90.5*	85.4*
		R	130.2	140.6	151.0	68.2*	105.5*
DP Scores	**Gait Phase**	**Side**	**−CI**	**Mean**	**+CI**	P1	P2
Hip-Knee	Stance	L	35.0	38.7	40.2	31.3*	28.5*
		R	35.6	39.3	41.9	20.5*	26.2*
	Swing	L	35.0	38.1	42.2	38.5	33.8*
		R	36.5	39.4	42.2	39.5	32.0*
	Stride	L	37.6	40.2	42.8	37.5*	35.0*
		R	38.8	40.9	43.0	41.3	32.3*
Knee-Ankle	Stance	L	26.2	36.1	46.1	34.7	44.4
		R	25.1	37.1	48.6	23.0*	36.6
	Swing	L	25.4	38.1	51.6	62.0*	75.4*
		R	27.2	36.1	45.0	36.3	65.8*
	Stride	L	29.9	40.4	51.0	55.2*	58.3*
		R	30.2	38.5	46.9	41.9	50.1*

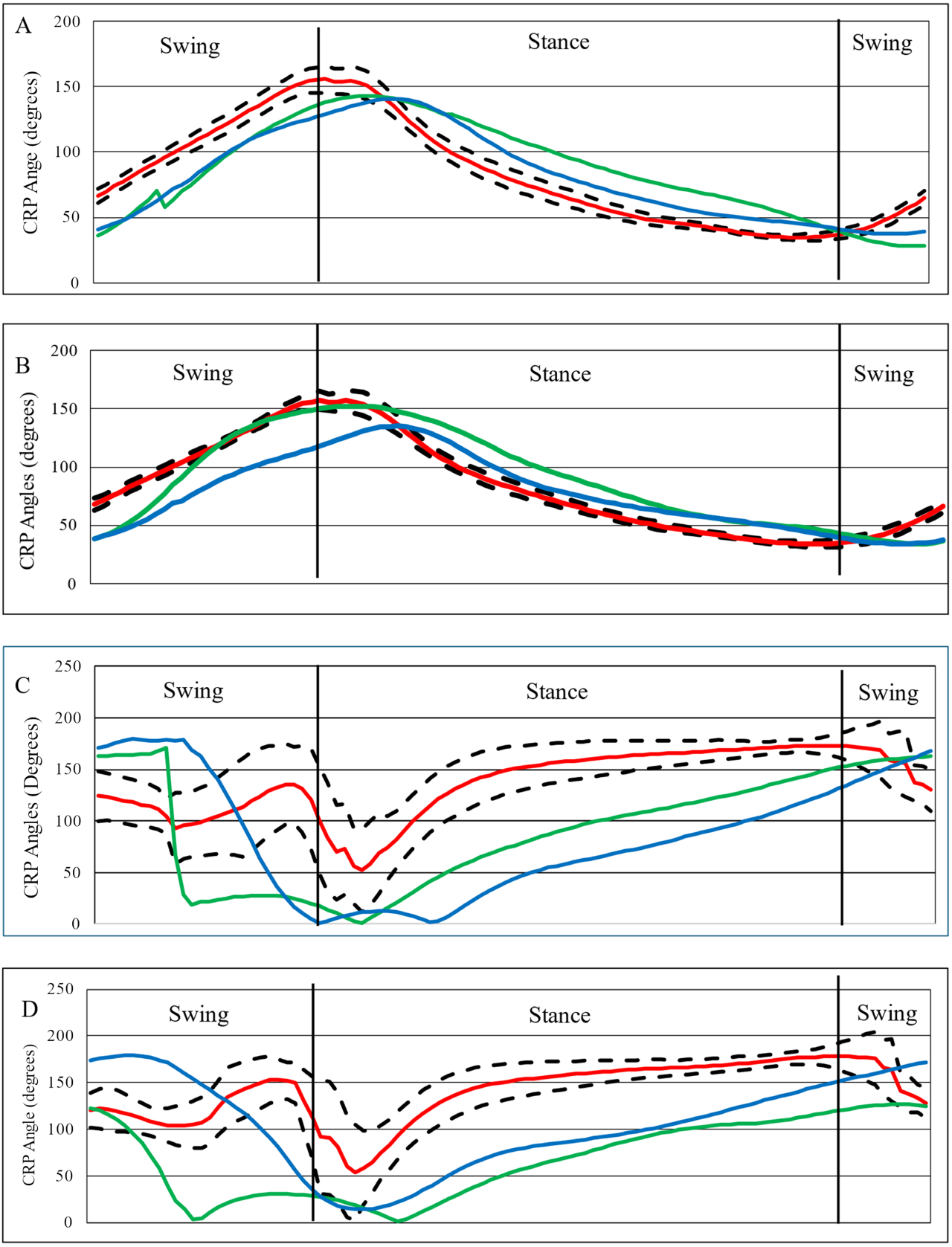

Figure 4. CRP angles during swing and stance phases.

4. Discussion

Quality of gait is a reliable indicator of overall health status in neurotypical individuals and has been closely linked with the severity of neurodevelopmental disorders [3]. Accurately assessing gait metrics of individuals with NGLY1 may provide crucial insight into their overall health status. It is crucial then to accurately assess the gait metrics of individuals with NGLY1 Deficiency using a comprehensive set of measures. These measures can be assessed alongside traditional motor abilities to further detail the overall motor abilities and clinical severity of individual patients.

The complementary use of linear and non-linear gait measures provides deeper insights into specific gait features that can be modified to produce a more efficient gait. Such information can be used to detect subtle changes in gait characteristics over time, or in response to therapeutic interventions, be they traditional forms of physical therapy, pharmacological, or genetic interventions.

In the present study, the gait metrics of two individuals with NGLY1 Deficiency were compared to that of aged-matched neurotypical individuals obtained from a published database. This report is the first to provide laboratory-based gait measures obtained for individuals with NGLY1 Deficiency and the authors hope this data will serve as an initial comparative point for future investigations.

Figure 1 displays the very high correlations of the hip and knee joint waveforms between the controls and participants, suggesting overall similar strategies of locomotion in the hip and knee. In contrast, the ankle waveforms for both participants are only moderately correlated with the control waveforms. Table 2 displays the percentage of the participants' waveform samples fall within the controls CI. P2 displayed a greater percentage of samples within the CIs for both hips and knees than P1, but the opposite pattern is observed in the ankle joint. This finding appears to be driven by the fact that P2 displays a greater degree of flexion throughout the mean stride than both the controls and P1. Overall, this quantitatively suggests that the gait variability amongst individuals with NGLY1 mutations may be moderate, and care should be taken in generalizing pattern of gait across individuals. Similarly, this highlights the importance of establishing baseline locomotive and coordinative values for these individuals to serve as their own comparators.

The participants exhibited decreased joint ROMs in most cases (all, in fact, save P1's right ankle). This suggests the participants had restricted motion of the lower limbs as represented by the difference between the maximum and minimum joint angles across the waveforms. The data in Table 5 (angle-angle areas) corroborates the discrete ROM data, but also suggests that paired joint motion is suppressed across the entire stride. Despite P1's ankle having a greater ROM, both ankle angle-angle areas are significantly less than the control values, suggesting an overall narrower range of available behaviors. This finding points to the value of using multiple measures to characterize lower limb kinematics during gait.

Figure 2 illustrates the decrease in overall lower limb motion exhibited by the participants, but also provides insight into the coordination between these joints.

It is evident that the coordination between the hip and knee (for both participants) closely resembles that of the controls, despite the differences across traditional measures of gait. Conversely, the shape of both participants' knee and ankle diagrams vary greatly from the control, reflecting significantly different coordination patterns. Additionally, both participants have adopted patterns that are different from each other, possibly suggesting they have been able to adapt to their individual restrictions to form an assembly of joint motions to produce reasonably effective gait. As the relationship between the hip and knee generally mirror the control data, despite restricted overall motion, this suggests the knee is well controlled. However, as coordination pattern between the knee and ankle is disrupted, this suggests the possibility that individuals with NGLY1 Deficiency gait difficulties may primarily stem from the inability to control the more distal ankle joint.

The phase portraits (**Figure 3**) are consistent with the reduction in the ROM and velocities presented in **Table 3** and **Table 4**, but further illustrate that these reductions are across the entire gait cycle. These reductions are quantified by the significant decreases in phase portrait areas reported in **Table 6**. In general, the shapes of the participants' hip and knee phase portraits are similar to those of the controls, despite the quantitative difference. Consistent with the disrupted coordination between the knee and ankle being primarily the result of altered ankle motion, the ankle phase portraits of the participants also reflect altered control features relative to the controls.

The MARP CRP values highlight that the relationship between the hip and knee, for both the controls and the participants, function more in-phase during the stance phase than during the swing phase. This finding is consistent with other investigators who have also reported the swing phase exhibits more out-of-phase relationships between the hip and knee than the stance phase **[16]**.

All of the participants' MARP knee-ankle scores are significantly less than those of the controls. While the neurotypical individuals were able to affect a degree of independent control of the two joints that produced an effective kinematic walking pattern, the greater in-phase relationships between these joints demonstrated by the participants suggest that they were unable to control the relationship between the knee and ankle in such a way as to produce neurotypical gait. The greater in-phase relationship exhibited by the participants reflects a more static relationship between the knee-ankle that has been labeled as "freezing". Originally proposed by Bernstein [17] as a process to reduce the number of kinematic degrees of freedom utilized when first learning a movement, the concept of freezing out possible kinematic coordination patterns was initially demonstrated by Vereijken *et al.*, [18]. These authors proposed that freezing out certain combinations of joint motions is accomplished by increased co-contraction of relevant muscles across of given joint, thereby decreasing their ability to be controlled independently. Increased co-contraction necessarily results in a more in-phase relationship between given joints. This "freezing" can be beneficial when first learning a skill but can also serve to hinder efficient movement patterns if high levels of co-

contraction are maintained. Increased co-contraction is responsible for increased stiffness which carries a higher metabolic cost and is associated with inefficient movement [19]. Individuals with NGLY1 deficiencies are known to suffer from contractures and other orthopedic ailments [20], and while the role these contractures play in decreasing motor demands is unknown, they could play a substantial role in the locomotive characteristics of these individuals.

Although the current study did not investigate muscle activation patterns that may have revealed increases in co-contraction, previous work has suggested increased co-contraction is associated with spastic or stiff-legged gait patterns [21]. Spastic gait is characterized by reduced peak knee flexion and velocity and overall reduced gait velocity, [22], all of which were demonstrated by our participants. Additionally, increased stiffness can result in toe walking, was occasionally observed in our participants, which itself can contribute to reduced knee flexion [23].

Finally, although only two individuals participated in this preliminary investigation, the data suggests other than reduced gait velocity, and the associated reduced joint ROMs and velocities, there is no compelling evidence of a gait "pattern" than can be considered as "stereotypical" of those with NGLY1 Deficiency. In contrast, individuals with Parkinson's often demonstrate what is referred to as Parkinsonian gait, characterized by shuffling and freezing episodes [24]. Similarly, individuals with cerebellar deficiencies demonstrate stereotypical gait features associated with ataxia, including a wide-based stance and irregular lurching steps [25]. Both Parkinsonian and cerebellar ataxic gait would be readily recognized by a clinician through observation. Conversely, our two participants displayed differences in joint coordination patterns even from one another, in addition to significant differences from the controls. In other words, there were no stereotypical behaviors that both of our participants demonstrated that could be used to characterize their gait patterns of being explicitly associated with NGLY1-Deficiency. Critically, however, the participants did utilize self-similar strategies of gait. This again highlights the importance of establishing baseline values of gait for individuals suffering from NGLY1 disorders and using these data as future comparators for interventions and health changes.

5. Limitations and Future Directions

As a case study this investigation only examined two individuals with NGLY1 Deficiency, the generalizability of the findings is limited. Though two individuals do represent a sizable portion of the known number of diagnosed individuals [1] estimates suggest a higher incidence and thus more data is nonetheless needed. The evaluation of the participant's gait solely during treadmill walking may also limit generalizability to overground gait. In particular, the use of a harness during treadmill gait to prevent falls could impact postural control or influence gait characteristics. However, a recent review of 22 studies comparing treadmill and overground gait concluded that despite minor differences in some measures, "the findings indicated that the spatiotemporal parameters of TW (treadmill walking) and

OW (overground walking) did not have any significant differences that must be considered when performing clinical protocols." [26] Therefore, we concluded that the assessment of treadmill walking was an appropriate protocol to begin to characterize the gait quality of our two NGLY1 Deficient participants.

Future work should include a larger number of participants as well as exploring overground walking, where the most relevant daily-life improvements are likely to be gleaned, in this population. Additionally, EMG of the lower limb could be obtained to assess musculature activation patterns and the degree of co-contraction to determine what role neuromuscular activation has in the stiff-legged gait observed in the participants.

6. Summary

This initial report of the gait characteristics of NGLY1 deficient individuals using laboratory-based techniques revealed many significant differences from that of neurotypical controls. There were significantly reduced ROMs and peak angular velocities at the hip, knee, and ankle, despite similar stride times. The reduction in limb motion and velocity was supported by decreased areas in angle-angle and phase portrait diagrams. CRP analyses revealed significant differences in coordination patterns between the participants and controls, with greater overall variability. The participants also exhibited distinct locomotive strategies from each other, suggesting that NLGY1 deficiency may not exhibit a characteristic gait type. While the participants' control of the hip and knee was relatively similar to those of neurotypical walkers, the evidence suggests that ankle control may be deficient. This is most evident in the angle-angle and phase portrait diagrams. The use of non-linear techniques enabled a more nuanced evaluation of our participants' gait, which revealed significant differences in their coordination patterns that were not evident if only classical linear measures had been employed. Therapists and clinicians can take advantage of a range of kinematic assessment measures that can provide them with information that can be used to guide targeted therapeutic applications.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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