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ORIGINAL ARTICLE

Quantification of gait in children with mitochondrial disease

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Abstract

Mitochondrial disorders are multisystem conditions that can potentially affect gait in many ways. The aim of this study was to select the optimal protocol to quantify the spatiotemporal parameters of gait in ambulatory children with mitochondrial disorders based on feasibility, test-retest reliability, and the difference between patients and controls. Gait at self-selected pace was quantified in ambulatory children with a genetically confirmed primary mitochondrial disease using the GAITRite electronic walkway. Three protocols were tested: pre-exercise, post-exercise (after a 3-min walking test), and recovery. In 14 ambulatory patients, we showed good to perfect reliability for velocity, cadence, step length, step time, step time variability, and step width in the recovery condition. The difference between patients and 70 individually age- and gender matched healthy controls only became apparent in the post-exercise protocol. In conclusion, measuring spatiotemporal parameters of gait using the GAITRite in ambulatory children with mitochondrial disease is feasible and reliable for most of the parameters measured. When using gait analysis in future studies in children with mitochondrial disease, we advise i) to use an exercise test prior to the gait analysis, ii) to let children practice the test before the actual data collection, and iii) not to use symmetry parameters.

Introduction

Mitochondrial disorders are multisystem conditions that can potentially affect gait in many ways. Since mitochondria are present in almost all cells of the human body, signs and symptoms of all organs may arise, though they are generally most pronounced in the organs with the highest energy consumption (Koopman et al 2016). Mitochondrial disorders are caused by mutations in one of the 1150 nuclear genes encoding proteins involved in oxidative phosphorylation or in mutations in the small circular mitochondrial DNA (Pagliarini et al 2008; Calvo et al 2016). Mitochondrial DNA (mtDNA) mutations are co-existent with healthy mtDNA in variable proportions per cell, called heteroplasmy. With an estimated total prevalence of roughly 1/5000, mitochondrial disease is one of the most common inherited neuromuscular conditions of metabolism (Chinnery and Turnbull 2001).

Currently, there is no definite clinically beneficial treatment for most mitochondrial patients (Gorman et al 2015). Several recent collaborative papers of mitochondrial experts around the globe suggest that validated, clinically meaningful outcome measures should be used to detect clinically relevant effects of treatments (Pfeffer et al 2012; Pfeffer et al 2013). Since many mitochondrial disease patients experience difficulties in ambulation (de Laat et al 2012), we and others previously studied gait analysis with the GAITRite electronic walkway in adults with a mitochondrial disease (Galna et al 2014; Ramakers et al 2017). Both studies showed that the GAITRite was able to quantify subtle changes in the balance and strength of these adults with mitochondrial disease and that reliability of the gait analysis was good to perfect.

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Since 86% of children with mitochondrial disease also experience severe difficulties in walking (Koene et al 2013), we hypothesized that gait analysis is also a widely applicable and reliable outcome measure for children with mitochondrial disease.

The aim of this study was to select the optimal protocol (with good feasibility and the highest test-retest reliability) to quantify the spatiotemporal parameters of gait in ambulatory children with mitochondrial disorders, comparing three different protocols (pre- and post exercise and recovery). Secondly, the gait pattern of each of the mitochondrial patients was individually compared to five age- and gender matched healthy controls to gain more insight in which walking parameters were altered in children with mitochondrial disease. Finally, validity was assessed by correlating the gait parameters with the 3-min walking test (3MWT) distance, a measure for functional walking capacity.

Methods

Study subjects

Children with a genetically confirmed primary mitochondrial disease who were able to walk 30 m five times according to parents were included in this study. Per patient, five individually age- (±0 years) and gender matched healthy controls were recruited via colleagues within the hospital, sports clubs, and social media. Exclusion criteria for this study were: any other disease causing abnormal gait pattern (e.g., orthopedic, other neurological or neuromuscular diseases) and/or severe behavioral problems. The ethics committee of Arnhem-Nijmegen region, The Netherlands, approved this study (NL58062.091.16). Parents and children ≥12 years of age gave written informed consent before participation.

Measurements

Anthropometric data were collected for each subject before gait assessment. Leg length was measured as true leg length, from the Anterior Superior Iliac Spine (ASIS) toward the medial malleolus on both sides. Genetic and clinical data were collected from the charts.

Gait analysis and data processing

A portable GAITRite electronic walkway system was used to quantify gait patterns (Platinum model GAITRite, software version 4.8.5, CIR systems, USA). The system was set up in a laboratory setting and consisted of a 7 m long walkway with 2 m free walking space at both ends for acceleration (Fig. 1a). All spatiotemporal gait parameters were captured by the GAITRite software. Incomplete footfalls and scrubs were removed. We have focused our gait analysis based on the model introduced by Lord et al in 2013 (Lord et al 2013). This model consists of five domains for assessing gait including pace (step length and step velocity), rhythm (step time), variability (step length and step time variability), asymmetry (step time asymmetry), and postural stability (step width, step width variability and step length asymmetry) (Lord et al 2013). Mean velocity, step length, step time, and step width were calculated for each protocol. Variability of step length, time, and width was calculated as the root of the mean variance of the left and right foot. Step time- and step length-asymmetry was defined as the absolute difference between both feet. Velocity, cadence and average step length were normalized for leg length according to Stansfield et al (Stansfield et al 2003).

Gait assessment

Participants were instructed to refrain from walking for long distances at the day of the measurements and to come to the clinic using the hospital transportation or a wheelchair. All patients rested for at least ten minutes while the study was explained and informed consent was given.

Children were instructed to walk across the GAITRite-mat barefoot at their self-selected pace ("as if you were in your house"). All subjects started 1 m before the mat and stopped at least 1 m after the mat (Fig. 1a) to minimize the effect of acceleration and deceleration.

We have tested three protocols: normal walking at self-selected pace before the 3MWT (pre-exercise protocol; test 1 and 2), and after the 3MWT to test the influence of exercise (post-exercise protocol; test 3 and 4) and a protocol to assess the recovery after a 10-min break in which the child sat on a chair (recovery protocol; test 5), see also Fig. 1b. Each of the five tests consisted of three walks (trials) across the mat without breaks in between. There was a one-minute break between test 1 and 2 and between 3 and 4 in which the child sat on a chair. The order and timing of the protocol was fixed to prevent influences of the exercise-induced exhaustion with the pre-exercise protocol.

Subjects who were not able to understand the instructions were encouraged by a parent or investigator standing on the other side of the mat with a motivational toy or object. If participants were not able to complete three valid trials within 3 min, the test was noted as "missing". Cooperation was noted on a self-developed 1–5 scale by the researcher, where 1 is no cooperation, 2 is cooperation with distraction and/or lack of understanding of the instructions leading to >2 repeats of trials, 3 is limited cooperation leading to 1–2 repeats of trials, 4 cooperation with little distraction not obviously affecting the gait, and 5 is complete and direct cooperation.

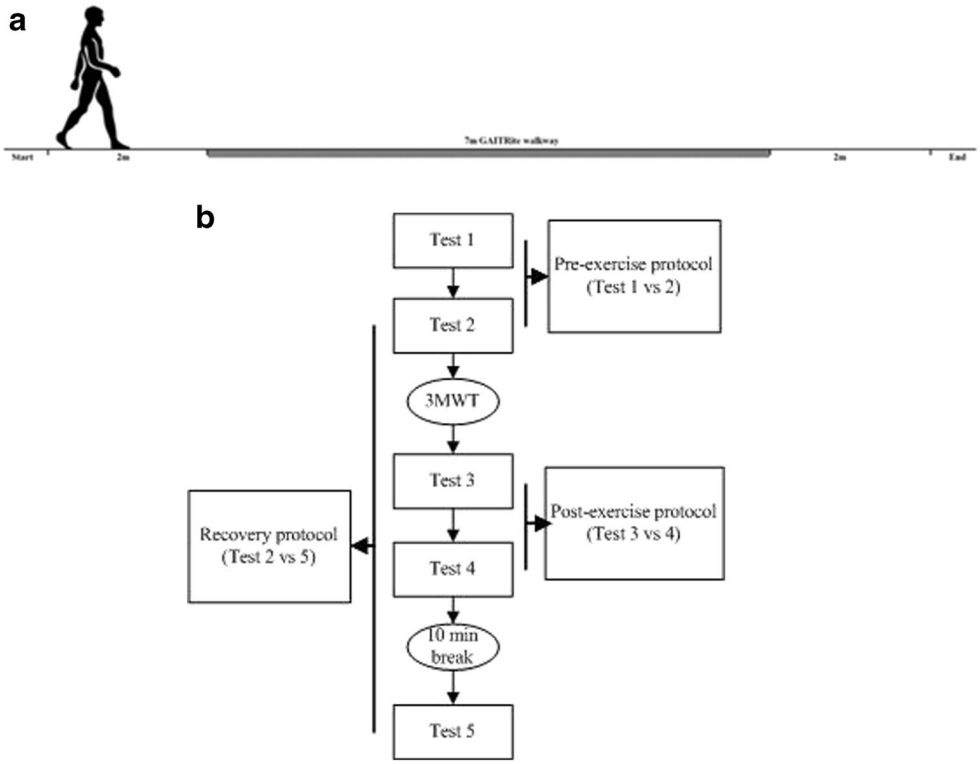

Fig. 1 a. Schematic representation of the GAITRite walkway setup. Children started 2 m in front of and ended 2 m behind the actual GAITRite walkway to ensure they would have a constant walking speed. Reproduced with permission of Biomed Central
b. Gait assessment flowchart. All subjects completed the same rotation of trial conditions and were given the same resting period. Each condition consisted of three walks across the GAITRite walkway

3-min walking test

Between test 2 and 3, the 3-min walking distance test (3MWT; pylons 25 m apart, standardized every-minute encouragements "One minute, two more minutes to go, you're doing well") was performed to assess their functional ambulation capacity and to induce muscular tiredness. The 3MWT instead of the 6-min walking test was selected because of the higher feasibility and lower burden to the children. When the child was not able to comply with the instructions of the 3MWT, muscular fatigue was induced by playing for 3 min and the distance of the 3MWT was not noted.

Validity

The correlation between gait parameters and the 3MWT was assessed to test the validity of the spatiotemporal parameters.

Statistical analysis

All data are presented as median and range unless otherwise specified. To test the reliability of the gait parameters measured with the GAITRite, Intraclass Correlation Coefficients for agreement (ICCs) were calculated for each study group, variable and protocol. ICCs above 0.7 were defined as 'acceptable', above 0.8 were defined as 'good' and above 0.9 'perfect'. Furthermore, we tested differences between patients and their healthy age- and gender matched controls at the pre-exercise protocol (second test) and the post exercise protocol (third test) using the paired non-parametrical Friedman test in non-Gaussian distribution; statistical significance has been defined as $p < 0.05$ (two-tailed). Correlation analysis was carried out between the 3MWT and the different gait characteristics for the pre-exercise protocol (second test) only. Since this is an explorative study, we did not correct for multiple testing.

Results

Study subjects

Fourteen ambulatory children with genetically-confirmed mitochondrial disease and 70 age- and gender matched controls were included in this study (Table 1). Because two 10-year-old boys with mitochondrial disease were included and insufficient 10-year-old controls could be recruited, they were matched to three or four 10-year-old and two or one 11-year-old boys each. The physical characteristics of the patients and controls are present in Table 1 and the disease specific characteristics of the patients are presented in Table 2. The most common signs and symptoms of the patients were exercise intolerance ($n = 10$) and psychomotor retardation ($n = 5$). Six out of 14 children carried the m.3243A > G mutation (heteroplasmy percentages in urinary epithelial cells 61–96%). Patients had significantly shorter leg length compared to controls, but height, weight, and BMI showed no statistically significant differences (Tables 1

Table 1 Study population demographics

	Patients ($n = 14$)		Controls ($n = 70$)		Difference (p-value)
	median	range	median	range	
Gender (% female)	43		43		
Age (years)	11	(5–16)	11	(5–16)	0.05
Height (cm)	147	(101–168)	154	(109–187)	0.11
Weight (kg)	38.2	(14.9–57.0)	41.2	(17.7–108.5)	0.63
BMI (kg/m2)	17.8	(13.9–21.2)	18	(12.8–33.3)	0.75
Leg length (mean)	75	(46–85)	82	(53–100)	0.02
3-min walking test (distance, m)	215	(131–283)	235	(180–370)	0.03
Cooperation first test (1–5 scale)	5	(2–5)	5	(3–5)	0.03
Cooperation second test (1–5 scale)	5	(3–5)	5	(3–5)	0.05
Cooperation third test (1–5 scale)	5	(3–5)	5	(3–5)	0.04
Cooperation forth test (1–5 scale)	5	(2–5)	5	(3–5)	0.05
Cooperation fifth test (1–5 scale)	5	(2–5)	5	(4–5)	<0.001
Cooperation 3MWT (1–5 scale)	5	(2–5)	5	(4–5)	0.06

and 2). Patients had a significantly lower 3-min walking distance compared to their healthy peers (215 m versus 235 m, respectively; $p = 0.03$).

Feasibility

All participants completed the full protocol. Three patients showed low cooperation (<4, meaning that the child was easily distracted or did not understand the instructions leading to one or more repeats of trials), one patient cooperated, but with no full understanding of the tasks, and ten patients completed the protocol fully cooperatively. Of the healthy controls, only one out of 70 children was not always cooperative, three controls cooperated with no full understanding of the tasks, and 66 controls were fully cooperative. Patients were significantly less cooperative in almost all protocols (Table 1).

Gait characteristics

Comparisons of the gait characteristics per group for each protocol are shown in Table 3. Patients and controls showed no statistically significant differences for the pre-exercise protocol. In the post-exercise protocol, only step length variability and step width variability reached significance (3.8 cm (patients) versus 3.2 cm (controls); $p = 0.02$ and 3.5 cm versus 2.7 cm; $p = 0.05$, respectively). When comparing the spatiotemporal parameters of pre- and post-exercise gait, patients have an increased step time asymmetry (5.0 ms (pre-exercise) versus 13.0 ms (post-exercise); $p = 0.008$). While healthy controls increase their step length and decrease step width (3.1 cm (pre-exercise) versus 3.2 cm (post-exercise); $p = 0.02$ versus 9.0 cm (pre-exercise) and 8.5 cm (post-exercise); $p = 0.008$, respectively), these parameters did not reach statistical significance in patients.

Reliability

ICCs of the gait parameters for the different protocols are presented in Table 4. In patients, good to perfect reliability was found for velocity, step length, and step width for all protocols (ICC > 0.8). When only cooperative patients were assessed in the recovery protocol, six out of ten parameters showed good or perfect reliability. Although the ICCs in the clinically more homogeneous control group tended to be lower, especially for step width, a similar pattern of the reliability of the parameters in the various protocols was found in the healthy control group.

Validity

In patients, none of the spatiotemporal gait parameters correlated significantly to the 3MWT distance. In healthy controls, we only found statistically significant correlations between the 3MWT distance and normalized velocity ($r = -0.33$; $p = 0.006$), normalized step length ($r = 0.43$; $p < 0.001$), and step width ($r = 0.29$; $p = 0.03$) in the pre-exercise protocol.

Discussion

To our knowledge, this is the first study to investigate the feasibility and same day test-retest reliability of gait analysis in children with genetically-confirmed mitochondrial disease. This exploratory study in 14 ambulatory patients showed good feasibility of the GAITRite in quantifying the spatiotemporal parameters in this population. We found good to perfect

Table 2 Dummy

Gender	Age	Gene	Mutation	Heteroplasmy (%)	Mitochondrial syndrome	Myopathy, encephalopathy or encephalomyopathy?	Wheelchair for long distances?	Psychomotor retardation?	Ataxia	Exercise intolerance	Muscle weakness	Cardiomyopathy	Hearing loss	Vision loss	Contracture of achilles tendon	Scoliosis	Cooperative
M	5	*TAZ*	c.788_794del		Barth	M	+			+	+	+					+
M	9	mt deletion	m.08649_16084del7435bp	30 (U)	CPEO	M					+						+
M	13	*MT-TL1*	m.3243A>G	96 (U)		M				+	+						+
F	14	*RARS*	c.442A>G; c.1519-G>A			E		+	+							+	+
M	10	*MT-TL1*	m.3243A>G	61 (U)		M				+							+
M	10	*MT-TL1*	m.3243A>G	81 (U)		M				+					+		+
F	12	*MT-TL1*	m.3243A>G	93 (U)		EM		+		+	+				+		+
F	13	*MT-TL1*	m.3243A>G	71 (U)		M				+							+
M	8	*MT-ATP6*	m.09185t>C		Leigh	EM	+	+	+	+							+
M	15	*MTFMT*	c.626C>T; c.766C>T		Leigh	EM	+	+									
M	16	*MT-TL1*	m.3243A>G	87 (U)	MELAS	EM	+	+		+	+		+				+
F	10	*MT-TI*	m.04300A>G	100 (M)	MICM	M	+	+		+					+		+
F	5	*NDUFS7*	c.364A>G		Leigh-like	E		+									
F	15	*AGK*	c1131+5G>A		Sengers	M				+	+	+		+		+	+

Table 3 Gait characteristics for the pre-exercise protocol and the post-exercise protocol for patients and their age- and gender matched controls

		Pre-exercise protocol (test 2)				Post-exercise protocol (test 3)				Difference (p-value)			
		Patients (n = 14)		Controls (n = 70)		Patients (n = 14)		Controls (n = 70)		Patients versus controls		Test 2 vs 3	
		median	range	median	range	median	range	median	range	Test 2	Test 3	Patients	Controls
Velocity	(cm/s)	103	(75–149)	124	(83–157)	108	(75–157)	124	(63–163)	0.05/0.25#	0.20/0.32#	0.09	0.26
Cadence	(step/min)	120	(100–157)	120	(97–167)	120	(102–198)	120	(95–165)	0.70/0.49#	0.47	0.09	0.70/0.49#
Step length	(cm)	52	(36–70)	62	(33–78)	54	(36–73)	63	(30–80)	**0.006**/0.14#	**0.007**/0.10#	0.11	**0.03**
Step length variability	(cm)	3.7	(2.0–6.9)	3.1	(1.6–7.9)	3.8	(2.3–8.0)	3.2	(1.2–10.6)	0.29	**0.02**	0.59	0.47
Step length asymmetry	(cm)	1.6	(0.2–3.1)	1.5	(0.04–4.7)	1.2	(0.22–4.9)	1,0	(0.03–4.1)	0.92	0.86	0.29	0.34
Step time	(ms)	499	(382–599)	498	(358–619)	502	(303–589)	499	(363–630)	0.54	0.7	0.33	0.15
Step time variability	(ms)	30	(14–94)	25	(14–67)	29	(17–80)	24	(12–86)	0.36	0.06	1.00	0.34
Step time asymmetry	(ms)	5,0	(<0.01–24)	9,0	(<0.01–51)	13	(<0.01–33)	8.5	(<0.01–42)	0.13	0.65	**0.008**	0.81
Step width	(cm)	9.8	(5.1–22.1)	8.8	(3.6–14.7)	9.8	(4.0–19.1)	8.4	(3.9–14.5)	0.18	0.06	0.11	**<0.001**
Step width variability	(cm)	3.3	(2.3–5.2)	2.5	(1.1–5.1)	3.5	(2.4–6.5)	2.7	(1.4–6.5)	0.07	**0.05**	0.29	0.06

Differences in gait characteristics between patient and healthy controls, significant *p*-values below p = 0·05 are shown in **bold**, # *p*-value for non-normalized/normalized values

Table 4 Intra class correlations coefficients (ICCs) of the gait parameters for each walking protocol

		Pre-exercise protocol (test 1 versus 2)					
		Patients (n = 14)		Controls (n = 70)		Cooperative patients (*n* = 11)	
		ICC	95%CI	ICC	95%CI	ICC	95%CI
Velocity	(cm/s)	**0.900**	(0.721–0.967)	0.775	(0.662–0.854)	**0.941**	(0.807–0.984)
Cadence	(step/min)	0.583	(0.132–0.841)	**0.908**	(0.857–0.942)	**0.940**	(0.788–0.984)
Step length	(cm)	**0.971**	(0.911–0.990)	**0.960**	(0.937–0.975)	**0.946**	(0.813–0.985)
Step length variability	(cm)	0.625	(0.145–0.863)	0.336	(0.109–0.529)	0.547	(−0.082–0.857)
Step length asymmetry	(cm)	0.162	(−0.432–0.634)	0.426	(0.213–0.600)	0.211	(−0.438–0.712)
Step time	(ms)	0.580	(0.127–0.893)	**0.934**	(0.895–0.958)	**0.917**	(0.724–0.977)
Step time variability	(ms)	0.726	(0.357–0.902)	0.394	(0.177–0.575)	0.739	(0.265–0.923)
Step time asymmetry	(ms)	0.543	(0.0.63–0.824)	0.252	(0.018–0.460)	0.471	(−0.084–0.818)
Step width	(cm)	**0.980**	(0.914–0.994)	**0.851**	(0.771–0.905)	**0.969**	(0.811–0.993)
Step width variability	(cm)	0.618	(0.135–0.860)	0.463	(0.256–0.629)	0.529	(−0.101–0.850)
Post-exercise protocol (test 3 versus 4)							
Velocity	(cm/s)	*0.835*	(0.568–0.944)	*0.809*	(0.710–0.877)	**0.984**	(0.934–0.996)
Cadence	(step/min)	0.209	(−0.327–0.650)	**0.935**	(0.897–0.959)	**0.970**	(0.893–0.992)
Step length	(cm)	**0.972**	(0.916–0.991)	**0.950**	(0.959–0.984)	**0.957**	(0.855–0.988)
Step length variability	(cm)	0.699	(0.277–0.893)	0.223	(−0.14–0.435)	0.698	(0.180–0.910)
Step length asymmetry	(cm)	0.747	(0.372–0.912)	0.543	(0.356–0.689)	0.718	(0.233–0.916)
Step time	(ms)	0.403	(−0.114–0.756)	**0.946**	(0.915–0.966)	**0.953**	(0.839–0.987)
Step time variability	(ms)	*0.870*	(0.645–0.956)	0.500	(0.301–0.658)	*0.841*	(0.508–0.955)
Step time asymmetry	(ms)	0.411	(−0.062–0.753)	0.430	(0.222–0.601)	0.361	(−0.128–0.755)
Step width	(cm)	**0.974**	(0.917–0.992)	*0.893*	(0.833–0.932)	**0.960**	(0.863–0.989)
Step width variability	(cm)	0.742	(0.315–0.913)	0.451	(0.241–0.620)	0.709	(0.103–0.919)
Recovery protocol (test 2 versus 5)							
Velocity	(cm/s)	**0.977**	(0.930–0.992)	0.766	(0.601–0.860)	**0.973**	(0.904–0.993)
Cadence	(step/min)	**0.918**	(0.701–0.975)	*0.880*	(0.717–0.940)	**0.960**	(0.863–0.989)
Step length	(cm)	**0.945**	(0.837–0.982)	**0.939**	(0.903–0.961)	**0.913**	(0.719–0.976)
Step length variability	(cm)	0.765	(0.416–0.918)	0.277	(0.044–0.481)	0.722	(0.238–0.917)
Step length asymmetry	(cm)	0.165	(−0.370–0.624)	0.212	(−0.016–0.422)	0.082	(−0.536–0.633)
Step time	(ms)	**0.933**	(0.735–0.980)	*0.888*	(0.731–0.944)	**0.938**	(0.785–0.983)
Step time variability	(ms)	*0.855*	(0.618–0.951)	0.389	(0.173–0.570)	*0.889*	(0.658–0.968)
Step time asymmetry	(ms)	0.380	(−0.125–0.741)	0.519	(0.323–0.671)	0.263	(−0.325–0.722)
Step width	(cm)	**0.969**	(0.904–0.990)	**0.782**	(0.672–0.859)	**0.954**	(0.847–0.987)
Step width variability	(cm)	0.608	(0.122–0.857)	0.348	(0.125–0.537)	0.626	(0.081–0.884)

ICCs in *italic* are good (above 0·80) and ICCs in **bold** are perfect (above 0·90)

reliability for six out of ten gait parameters in the recovery protocol. Reliability was highest in the recovery protocol, suggesting that practice is required before the first walk is registered. The difference between patients and healthy controls, however, only became apparent in the post-exercise protocol. In this small cohort, none of the spatiotemporal parameters correlated significantly to the distance walked at the 3MWT.

Gait is an extremely complex process, involving the interaction between the musculoskeletal system and central and peripheral nervous system. Larger studies in adults with mitochondrial disease showed discrete but statistically significant differences for velocity, step length, step length variability, step time variability, and step width variability between patients and controls (Galna et al 2014; Ramakers et al 2017). Despite matching the patients individually to five age- and gender matched controls, we could only detect statistically significant differences between patients and controls in step length and step width variability after exercise as performed in the 3MWT.

However, the gait parameters deviating most markedly from the age- and gender matched were found in the most severely affected patients, namely the two patients with Leigh syndrome and the patient with MELAS syndrome. The two ambulatory patients with ataxia had much milder gait abnormalities. In other pathologies like Parkinson's disease, it is known that the variability parameters of gait may be more sensitive to pathology compared to routine spatiotemporal measures such as velocity (Hausdorff 2007). The lack of differences between patients and controls is probably due to the low sample size and the relatively mild disease severity in our cohort, since a trend toward wider step width and longer step time variability in the post-exercise protocol and a trend towards higher step width variability in the pre-exercise protocols was observed.

We found good to perfect repeatability of velocity, cadence, step length, step time, step time variability, and step width in our patients. Our reliability data are comparable to the data obtained in children with neurological gait disorders and adult patients with the m.3243A > G mutation, with respect to the reliability of these spatial gait parameters (Graser et al 2016; Ramakers et al 2017). In other studies in children with various other neurological conditions, the test repeatability was generally good (Wondra et al 2007), but tended to be variable between parameters (Thorpe et al 2005; Sorsdahl et al 2008) and populations (Thorpe et al 2005; Morrison et al 2012). Since both symmetry parameters showed low repeatability in our study, we advice not to use these parameters for longitudinal evaluations, as recommended by Graser et al previously (Graser et al 2016).

Gait analysis is also used to quantify subtle changes in walking patterns of children and adults with various protocols (Galna et al 2013; Hagmann-von Arx et al 2015; Gilchrist and Tanner 2016; Hollands et al 2016; Lim et al 2016; Manicolo et al 2016) and was able to obtain insight in the effects of a therapeutic intervention (Koopman et al 2012; Lord et al 2013). In our patient cohort, same-day repeatability of several spatiotemporal parameters was good to perfect in the recovery protocol. However, in the healthy controls, the same-day repeatability of the selected spatiotemporal parameters in the recovery protocol was only acceptable for two out of six parameters and low for step time variability. This suggests that the heterogeneity in our patient population may have falsely increased the ICCs, as ICCs are dependent on the between-subject variance (larger between subject variance leads to larger ICCs).

For many neurological conditions, it has been shown that the spatiotemporal parameters of gait are representative of disease severity (Coker et al 2010; Stephenson et al 2015; Gilchrist and Tanner 2016). The two studies in adult mitochondrial disease previously showed that velocity, step length, step length variability, step time variability, and step width variability correlate to the presence and severity of gait stability, exercise tolerance, myopathy, and/or cerebellar ataxia (Galna et al 2014; Ramakers et al 2017). We found no correlations between any of the spatiotemporal parameters of gait and the anchor we used, namely functional ambulation (distance walked at the 3MWT). This may again be due to the complex interplay between growth, development, maturation of gait, and disease severity in this small sample (Di Nardo et al 2017). Importantly, the impact of these growth and maturation factors should be taken into account when intervention studies with a longer duration using gait analysis are designed, illustrating the importance of natural history studies.

We used the 3MWT as a "stress test" to enlarge the difference between patients and healthy controls. Parents report that children have an impaired balance or trip over more frequently after walking a long distance, and we hypothesized the 3MWT would unmask their compensatory mechanisms to maintain normal gait. We did observe statistically significant increased step length and step width variability between both groups post-exercise (3.8 cm (patients) versus 3.2 cm (controls); $p = 0.02$ and 3.5 cm (patients) versus 2.7 cm (controls); $p = 0.05$, respectively), which were not observed before the 3MWT (pre-exercise). When comparing the post- and pre-3MWT gait changes in children with mitochondrial disease to their healthy peers, healthy children tend to decrease their step width after the 3MWT (post-exercise protocol), whereas patients with mitochondrial disease do not. Since we could only detect differences between children with mitochondrial disease and their healthy peers in the post-exercise protocol, we recommend using an exercise protocol before the gait measurement to induce neuromuscular fatigue and diminish compensatory mechanisms to maintain normal gait.

We did not include time for practice in our study. A reliability study in children with ambulatory CP allowed children to perform two to four trials before data collection (Wondra et al 2007). This might lead to better reliability, as shown by the increase in the ICCs between the first (test 1 versus 2) and the last (test 2 versus 5) comparison in our study. We advise letting the child perform at least one well-performed walk before starting the actual gait measurements, to induce a more stable measurement.

Although repeatability may be lower in young or non-typically developing children (Thorpe et al 2005; Guffey et al 2016), we found no obvious differences in the ICC when less-cooperative patients were excluded, which probably reflects that walking is an automated process which requires little cooperation. We therefore argue that spatiotemporal parameters, especially those on the variability of gait are promising outcome measures that are universally applicable to all ambulatory patients with mitochondrial disease.

This study has several other limitations. We assessed test-retest reliability on the same day, within the same hour for most subjects. Because of logistical and ethical reasons it was not suitable to let patients visit the hospital for a second measurement within the same week or month. Secondly, we

included a very heterogenous sample of children who were able to walk 30 m five times. This may enhance the generalizability of our results to all ambulatory children with mitochondrial disease without severe behavioral problems, although the enormous heterogeneity seen in mitochondrial diseases may require a new reliability study for each separate population. More importantly, the heterogeneity may have positively influenced the reliability calculations, as illustrated by the lower ICCs in the control group. Thirdly, we did not determine responsiveness in our cohort, since no effective intervention is available yet. In children with CP, gait parameters showed good responsiveness in two small short-time intervention studies, (Kelly et al 2008; Coker et al 2010) but since the mechanism of action of this intervention is probably not comparable to the interventions that will be used in mitochondrial disease, responsiveness should still be confirmed in future intervention studies.

In conclusion, measuring the spatiotemporal parameters of gait using the GAITRite in ambulatory children with mitochondrial disease is feasible and reliable for most of the parameters measured. When using gait analysis in future studies, we advise i) to use a (standardized) exercise test prior to the gait analysis, ii) to let children practice the test before the actual data collection, and iii) to not use symmetry parameters to increase repeatability. Longitudinal natural history studies should incorporate gait analyses in this growing and developing population to facilitate the interpretation of the data from future intervention studies using this outcome measure.

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Compliance with ethical standards

Conflict of interests This study was not industry sponsored.

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