Exploring the relationship between an instrumented walking test and community physical activity in individuals with Congenital Myasthenic Syndrome.

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Abstract

Congenital Myasthenic Syndromes (CMS) are a group of rare genetic disorders affecting the neuromuscular junction structure and function. They are characterised by the presence of fatigable muscle weakness, but the age of onset, presenting symptoms and distribution of weakness differ depending on the genotype and specific mutations affected. The severity of CMS is highly variable amongst individuals, fluctuating and worsening with physical effort, which makes assessments challenging. Currently, there are no validated outcome measures for use in CMS, with most clinicians using outcome measures validated in Myasthenia Gravis and other neuromuscular conditions. The need to establish robust natural history data and validated outcome measures in this rare condition will be increasingly important with emerging novel treatments already in development.

This study looks to answer whether there is a relationship between an instrumented six-minute walking test (6MWT) and community physical activity levels through the use of a wrist-worn physical activity (PA) monitor (AX3, Axivity, UK) over seven days.

40 participants were assessed for PA analysis, with 37 having conducted a corresponding 6MWT test. Participants had a range of CMS subtypes, including the most common (AChR deficiency n=12, DOK7 n=12, RAPSYN n=5). It was identified that participants spent an average of 83.3% of the week in sedentary activity and 12.5% of the week in moderate-vigorous activity (MVPA). Overall, there was a weak correlation between distance walked on the 6MWT (range 25m – 711m) and community PA outcomes. However, participants who spent longer in sedentary activity (≥90% activity/week) all walked less than 500m on the 6MWT.

Further in-depth analysis showed participants with limited walking distance experienced higher levels of walk variability and a greater difference between walking speed at the start and end of the 6MWT. Participants walk ratios (WR) varied, with muscle fatigue resulting in a change in cadence, but not stride length.

This first-of-a-kind study presents the relationship of community PA and in-clinic mobility outcome measurements such as the 6MWT in this heterogeneous CMS population. We have been able to describe with greater detail the features of walking fatigue in this population, by utilising a small patient-worn inertial measurement unit (IMU), alongside the clinic-based instrumented walking test (6MWT). The use of an IMU may prove a useful application in future clinical and research data collection and help guide condition management.

Presentations relevant to this study

Presentations

1. CMS National Patient Day: The development of a Natural History Study in Congenital Myasthenic Syndromes – May 2023
2. Abstract submitted to Faculty Postgraduate Research Symposium – November 2023

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Abbreviations

6MWT	Six-minute walk test
AChR def	Acetylcholine receptor deficiency
bpm	Beat Per Minute
CHAT	Choline Acetyltransferase
CMS	Congenital Myasthenic Syndromes
COL13A1	Collagen 13 with Alpha 1 chain
COLQ	Mutations in the acetylcholinesterase collagen-like tail subunit gene
CoV	Coefficient of Variation
DOK7	Dok-7 protein
GFPT1	Glucosamine-Fructose-6-Phosphate Aminotransferase
HR	Heart Rate
HRmax	Maximum Heart Rate
HSS	Highly Specialist Service
IMU	Inertial Measurement Unit
LPMS	LP-RESEARCH Motion Sensor
MG	Myasthenia Gravis
MVPA	Moderate-Vigorous Physical Activity
PA	Physical Activity
RAPSYN	Receptor Associated Protein of the Synapse
SD	Standard Deviation
SLC5A7	Solute Carrier Family 5 Member 7

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1. Background

Congenital Myasthenic Syndromes (CMS) are a group of rare genetic disorders affecting the neuromuscular junction structure and function.[1,2] The global incidence of CMS is unconfirmed but a recent paediatric study estimated the frequency to be 9.2 per million under the age of 18 years in Great Britian.[3] CMS is characterised by the presence of fatigable skeletal muscle weakness affecting axial, limb, bulbar, ocular (ptosis and ophthalmoplegia) and respiratory muscles. [4,5] However, age at onset, presenting symptoms, distribution of weakness, and response to treatment differ depending on the molecular mechanism that results from the genetic defect [2,6–8] (figure 1), with over 30 genetic subtypes currently identified. [6] The severity of CMS is highly variable amongst individuals and can fluctuate and worsen with physical effort, timing of medication and environment, [7] which makes assessing this population a challenge.

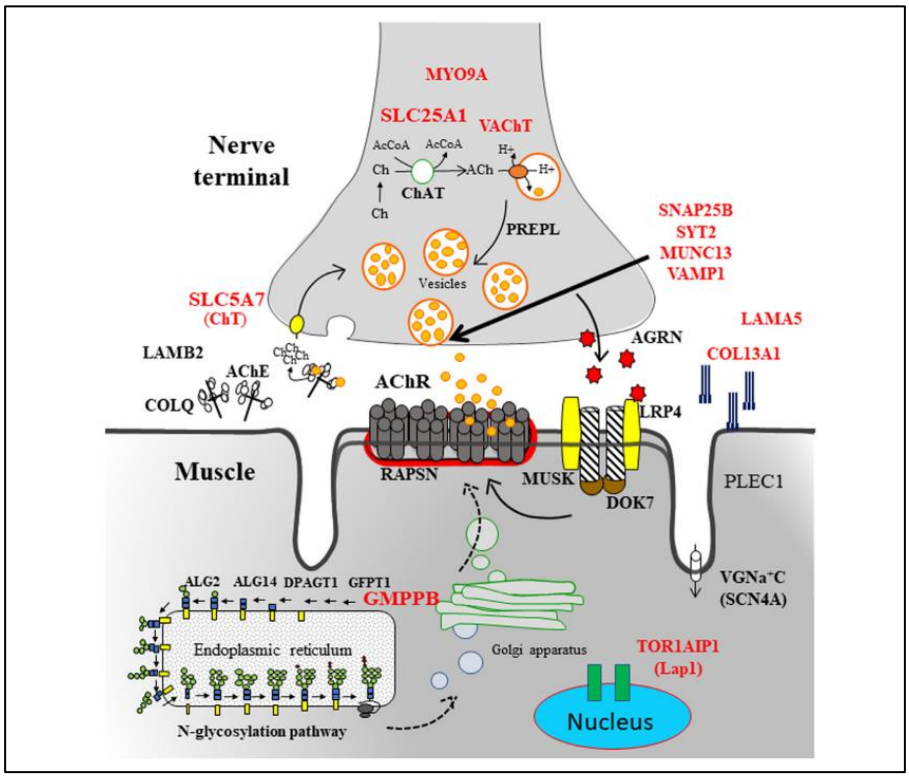

Figure 1. Schematic diagram of the neuromuscular junction identifying the main proteins/genes where mutations lead to impaired neuromuscular transmission.[8]

There are currently no validated outcome measures for use in CMS and many clinical services, including the Oxford CMS Highly Specialist Service, utilise outcome measures validated in Myasthenia Gravis (MG) [9–13] and other broader neuromuscular conditions, to assess and monitor this patient cohort. MG is an autoimmune form of myasthenia, typically presenting

with rapid onset, significant impairment and responds to immunosuppression therapy. As a result, large fluctuations in fatigue can be appropriately captured using MG specific assessments.[12] Conversely individuals with CMS often see small fluctuations in fatigue, and MG specific assessments may not be sensitive enough to pick up change in this population.

However, these assessments completed in clinic, provide a snapshot of an individual's physical ability, and fail to capture the daily fluctuations often seen in this cohort. Additionally, many patients present with CMS symptoms in childhood, for which adult MG assessments are not validated. [14,15]

Exercise induced muscle fatigability is a key symptom of CMS, caused by defect at the neuromuscular junction, and a validated outcome measurement of muscle fatigue in this population has yet to be identified. Muscle fatigue has been described across the literature in many different forms, but the focus of this study is on outcome measures that capture muscle fatiguability and not an individuals' perception of fatigue.

The literature describes several methods for monitoring community physical activity, including direct; such as physical activity monitoring[16] and smartphones,[17] and indirect reporting; such as self-reported diaries and questionnaires.[18] However the evidence-base for the "effectiveness of objective monitors, particularly activity monitors is increasing, with lower levels of variability observed for validity and reliability when compared to subjective measures".[16] Furthermore, there is a growing trend towards wearable activity devices as a "practical and affordable approach to assessing physical activity and sedentary behaviours" [19] in the community and as an additional tool in clinical studies. There is supporting literature of their application in other neuromuscular cohorts [20] including Myotonic Dystrophy [21] and Duchenne Muscular Dystrophy. [22–25]

The six-minute walking test (6MWT) has been validated for clinical and research use in several neuromuscular conditions, [26–29] where fatigue is also a key feature, and is a reasonable outcome measure to adopt in CMS monitoring. Additionally, the application of an instrumented 6MWT that allows reporting of gait parameters over time, would prove valuable in identifying if there is a relationship between fatigable muscle weakness and variability in gait within this population.

However, it is currently not known how the 6MWT reflects community physical activity (PA) in this CMS population. Reporting on the PA levels of a CMS population will provide valuable insights into the fatigue levels of these individuals, with potential future applications to exercise guidance, adjustments to medication plans and clinical trials.

Understanding if there is a relationship between the clinical outcome measures typically completed in a controlled environment (e.g., 6MWT in clinic) and the community PA levels of individuals and subtypes of CMS, may help understand the added value and limitations of these different assessment tools in CMS.

2. Aims of the study

The aim of this study is to explore the relationship between community physical activity (PA) levels, with parameters from a standardised in-clinic instrumented 6-minute walking test, for individuals with Congenital Myasthenic Syndrome (CMS).

To do this, we set out to establish if PA data can be reasonably captured in this population, utilising data cut-offs for PA levels with established parameters. We then evaluated the gait parameters of the same individuals in an instrumented six-minute walking test (6MWT) in a controlled clinic setting and analysed their relationship.

Objectives

1. To assess correlations between clinical assessments (6MWT) and community mobility outcomes (activity monitors) in this CMS cohort.
2. To assess community physical activity levels in this CMS cohort.
3. To provide greater insights through gait analysis into the core symptoms of muscle fatigue in CMS.
4. To understand the benefits and application of mobility outcomes, including physical activity monitoring and gait analysis in this population.

3. Methods

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist [30] has been used to guide reporting of the study results.

3.1 Population

Participants with a genetically confirmed diagnosis of CMS and have their clinical management under the Oxford CMS Highly Specialist Service, were recruited to an established study; *A Natural History Study of Congenital Myasthenic Syndromes, to establish reliable outcome measures suitable for clinical and research assessment*. This study received ethical approval (see Appendix A) from National Health Service Research Ethics Committee in July 2021; REC Ref: 21/LO/0480 and is registered on the public database ISRCTN under reference number 18340272 (https://www.isrctn.com/ISRCTN18340272).

Inclusion criteria were confirmed genetic diagnosis of CMS, age 0-100 years, ability to provide informed consent (Appendix B-D) to the study (or their legal guardian), and able to attend regular study visits (every 6-12 months). 151 potential participants were screened and 101 were excluded due to being unable to commit to regular visits (n=14), unable to travel to study centre during the study time scales (n=8), no confirmed diagnosis of CMS, or other diagnosis confirmed (e.g. MG / Ocular MG) (n=24), did not speak English (n=3), or failed to respond when contacted about the study (n=52).

Demographic outcome measures recorded include age, gender, CMS sub-types confirmed by genetics, height, and weight.

3.2 Study Design

A cohort study with a data cut completed at 12 months following full recruitment, to capture one visit where both physical activity (PA) and the six-minute walk test (6MWT) data were recorded.

3.3 Physical Activity (PA)

Participants who consented to wear an PA monitor over seven days following their study visit were given a wrist worn activity monitor, which contained a three-axis accelerometer device (23 x 32.5 x 7.6 [mm]) attached to the dominant wrist in a non-allergic silicone watch strap. The device was set up to record a seven-day period, starting at 23:59 on the day of provision

(study visit) sampling frequency 100Hz, range 8 (+g). The participants were instructed to wear it on the dominant wrist, at all times (including sleeping, showering and physical activity) and to return it in a pre-paid envelope at the end of the recording period (typically the morning of the 8th day). They were provided with an age-appropriate information booklet (see Appendix E for an example), outlining the use of the device and contact details should they have any issues.

The devices were set up and data downloaded using OmGui Software V1.0.0.43 (https://github.com/digitalinteraction/openmovement/wiki/AX3-GUI).

Physical Activity (PA) data was analysed using software with established PA parameters [31,32] for activity levels – Eslinger (2011), and sub analysis with paediatric parameters – Phillips (2012). The literature recognises the need to flag bouts of motionless data greater than 20 minutes, and seven days of data has been shown to provide reliable PA results, including a minimum of at least five full days of data, with "at least one of those being a weekend day, to allow for the inevitability that some participants will remove the device for at least part of the time". [33] For this study, data was excluded where a total of ≥2 full days of data points were missing or recorded as zero (e.g. sensor wasn't worn), regardless of the time of the day or day of the week. Non-wear time was excluded from the analysis where detected and was defined as a continuous period of >10minutes at zero accelerometer movement detection. The process described above, was programmed in a bespoke programme based in LabVIEW 19.0f, V3.2 (National Instruments, Ireland).

3.4 Gait Analysis

The six-minute walk test (6MWT) is a low-intensity, submaximal exercise test used to assess aerobic capacity and endurance [34,35] and is routinely used in pulmonary rehabilitation to monitor oxygen saturations. It has been widely used in studies for healthy populations [36,37] and in neuromuscular disease [26,27,29,38] thus for our purposes to monitor fatigable muscle weakness and mobility. The 6MWT was completed as the final physical examination (followed only by an optional stair climb) in the series of assessments completed as part of the wider natural history study, as it was anticipated to fatigue participants and may invalidate other assessments within the study if performed earlier.

An instrumented 6MWT included using a 25-metre circuit (see Appendix F for assessment set-up), stopwatch and sacral placement of a triaxial gait analysis device. Instructions given to the participants were to "walk as far as you can in six minutes", starting on the right-hand side of the circuit, walking towards and around the far cone and back down the other side in a

continual loop, repeating for up to 6 minutes. Participants were instructed not to touch the wall whilst walking unless they needed to pause. If the participant needed to pause walking, they were allowed to stop, but should remain standing whilst the timer continued. If the participant needed to sit down, the test was stopped, recorded as early discontinuation and the participant was provided with a chair. Periodically they were encouraged to "keep the pace" but were not instructed to walk faster. Lap times were recorded, along with distance covered at one-minute intervals.

No orthotic devices were allowed other than insoles, and the participants were allowed to walk barefoot if they wished. Where required, the participants wheelchair was used to transport them from the clinic space to the walking area.

A chest worn heart rate sensor (Polar H9) was applied to the participant at least 10 minutes before the start of the 6MWT, in the clinic room. During this time the participant was instructed to rest, and a resting heart rate was taken in the clinic room prior to moving to the 6MWT corridor. Continuous monitoring of the participants heart rate was started alongside the gait analysis recording and the participants maximum heart rate during this time was noted.

Gait analysis was captured through an inertial measurement unit (IMU) which was positioned on the waist of the participant, using an elasticated waist band, so that the device would sit between L4-S1. The IMU used was the Life Performance Motion Sensor (LPMS, Life Performance Research, Japan) which is a matchbox sized, multi-purpose IMU. The sensors used in the LPMS for orientation determination are a 3-axis gyroscope (detecting angular velocity), a 3-axis accelerometer (detecting the direction of the earth's gravity field) and a 3-axis magnetometer to measure the direction of the earth magnetic field. The LPMS recording was started by the assessor within one minute prior to starting the 6MWT, following the instructions given above.

A external high-powered, long-range Bluetooth dongle was used to ensure effective data collection along the corridor (25m distance) and placed halfway along the test, so that the participant and device were always <15m from the dongle.

3.5 Other metrics

The Myasthenia Gravis Activities of Daily Living (MG-ADL) is a patient reported outcome measure of disease burden that is validated for assessment of individuals with MG, with a two point improvement indicating clinical improvement.[9] It correlates well with other MG

outcome measures[9,39] and is useful as a research tool and clinical management. It was performed as part of this study, to identify patient perception of disease severity.

4. Analysis

4.1 Population

Participant data was grouped by gene subtype where possible to allow for evaluation based upon the typical features of each subtype.

4.2 Physical Activity (PA)

To understand the baseline PA levels of this cohort:

a. Analyse data through different data points, accounting for age and height
b. Report percentage of sedentary, light, moderate and vigorous PA per week [%activity/week] across entire cohort
c. Report percentage of sedentary, light, moderate and vigorous PA per week [%activity/week] per subtype where possible
d. Identify any correlation with PA levels and 6MWT data

4.3 Gait Analysis

To understand the reliability of the 6MWT in evidencing fatigable muscle weakness in this CMS cohort, by reporting on the following parameters:

a. Total distance walked in six minutes
b. Average speed [m/s]
c. Change in heart rate (HR) from resting baseline to maximum heart rate (HRmax)
d. Spatial data: stride length [m]
e. Temporal changes: cadence [steps/minute]
f. Change in parameters during the test: walk ratio (stride length[mm]/cadence [steps/min])
g. Relationships between gait analysis and community PA levels

4.4 Statistics

The results of this observational study are described, with trendlines and correlations. However, due to the rarity of the condition and small sample size, no inferential statistics are used. Where possible, results are described by genetic subtype, to assist with understanding difference between different types. Again, due to the small sample sizes, where this is not appropriate a wider cohort result is reported. Where samples are larger than two, we have reported median and mean, to demonstrate if the data is normally distributed.

The following correlation classification will be used when describing the results of this study; 0.0-0.1 = negligible, 0.1-0.39 = weak, 0.4-0.69 = moderate, 0.7-0.89 = strong, 0.9-1.0 = very strong correlation.[40]

5. Results

5.1 Population

Of the 50 participants available via the data cut, 49 participants were identified as having some data from at least one study visit. Of these, 40 participants had adequate PA data for analysis, with reasons for those excluded including n=7 incomplete data sets, n = 1 data more than 7 days (likely error with set up), n = 1 removed overnight resulting in 30% missing data). The full study flow is shown in figure 2.

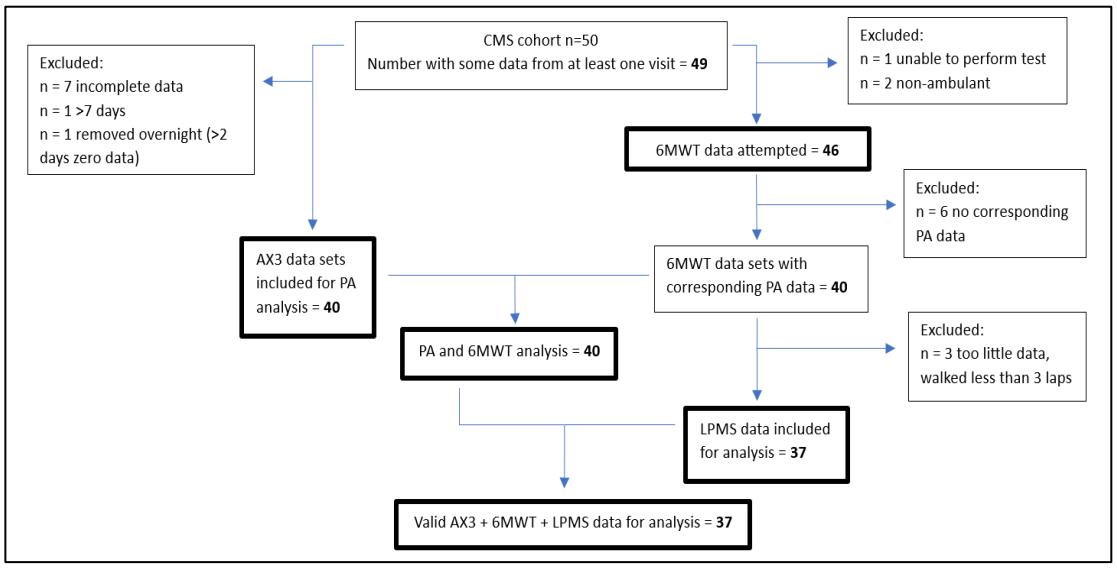

Figure 2. Flow diagram of data included for analysis. Sections in bold depict the final numbers reported for each outcome.

Of the 40 participants included for analysis (sex: 18 female, 22 male), mean age was 32.8 years (range 8 – 72 years), height mean 162.0 cm (range 132.5 cm – 188 cm) and a range of CMS subtypes including; Acetylcholine receptor deficiency (AChR def) n = 12, Choline Acetyltransferase (CHAT) n = 1, COLQ n = 5, Dok-7 protein (DOK7) n = 12, Glucosamine-Fructose-6-Phosphate Aminotransferase (GFPT1) n = 2, Receptor Associated Protein of the Synapse (RAPSYN) n = 5, Solute Carrier Family 5 Member 7 (SLC5A7) n = 1, Slow Channel n = 2. (See table 1 for demographics by disease sub type).

PA was analysed for these 40 participants and are reported as such, to capture the PA levels of the broadest CMS cohort. However, three participants were excluded for further gait analysis, as they failed to walk far enough for robust LPMS data collection (<50 m), leaving 37 data sets available for cross reference analysis of PA and gait analysis.

Type	n =	Age [years] Median (range) *Mean*	Male : Female	Height [cm] Median (range) *Mean*
AChR deficiency	12	27 (13-61) *33.9*	8 : 4	166 (153-176) *164.4*
CHAT	1	27	1 : 0	174
COLQ	5	18.4 (8-26) *19.8*	1 : 4	146 (143-165) *150.8*
DOK7	12	38.5 (10-71) *37.8*	6 : 6	161 (132.5-188) *160.4*
GFPT1	2	41.5 (35-38)	2 : 0	174.5 (172 -177) *174.5*
RAPSYN	5	18 (10-72) *29.4*	3 : 2	166 (141-182) *164*
SLC5A7	1	26	0 : 1	156
Slow-Channel	2	35.5 (26-43)	1 : 1	163.5 (154-173) *163.5*
Total:	40	29.4 (8-72) *32.8*	22 : 18	163.4 (132.5-188) *161.95*

Table 1. Demographics by disease sub type

5.2 Physical Activity (PA)

Sedentary PA levels: Physical Activity (PA) data analysed through Eslinger [31] cut-off levels, identified mean sedentary PA levels [%activity/week] were 83.9% with a range of 55.3% – 94.6%. See table 2 for sub type analysis.

Type	n =	Sedentary [%activity/wk] Median (range) *Mean*	MVPA [%activty/wk] Median (range) *Mean*
AChR deficiency	12	85.05 (71.07 – 94.22) *84.03*	12.47 (4.68 – 18.59) *12.68*
CHAT	1	91.26 (91.26)	7.37 (7.37)
COLQ	5	83.94 (62.96 – 91.35) *81.53*	14.18 (5.90 – 23.90) *13.98*
DOK7	12	83.26 (55.32 – 92.22) *81.77*	13.70 (6.43 – 37.19) *14.48*
GFPT1	2	90.22 (86.60 – 93.85)	8.25 (5.19 – 11.32)
RAPSYN	5	88.43 (80.31 – 94.60) 88.20	10.15 (4.16 – 17.38) *10.27*
SLC5A7	1	77.19 (77.19)	20.87 (20.87)
Slow-Channel	2	83.80 (75.46 – 92.15)	12.19 (6.50 – 17.59)
Total:	40	85.39 (55.32 – 94.60) 83.84	12.40 (2.79 – 37.19) *12.54*

Table 2. Eslinger [31] Physical Activity cut-off levels for Sedentary and MVPA.

A sub analysis with activity parameters identified as suitable for children[32] (10 – 16 years of age) was conducted to establish the variability in cut off parameters and the impact this has on PA level reporting across the cohort. Child specific data parameters account for differences in height and patterns of movement, compared to adults. There was an expected relationship in height versus age in our cohort, with an increase for those under 20 years and then a plateau of height with age thereafter (see figure 3).

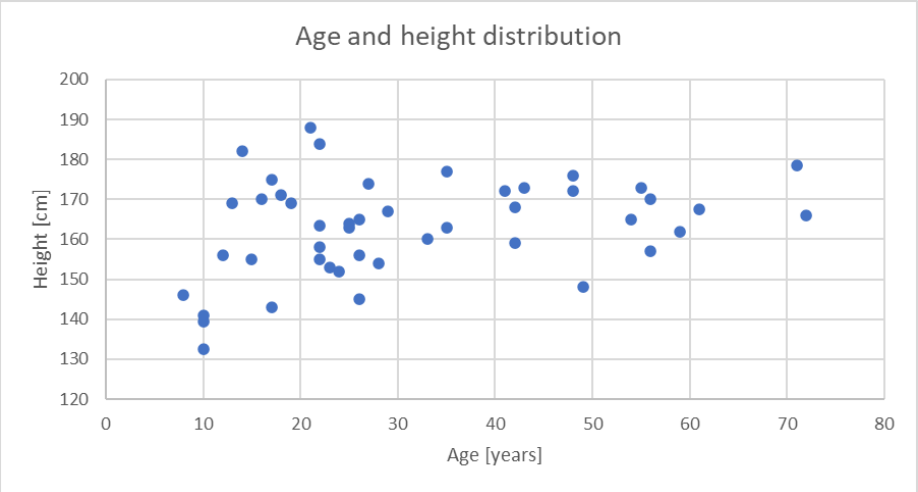

Figure 3. Age and height of cohort

Mean sedentary PA levels [%activity/week] were identified at 81.8% and 79.7% (for Eslinger (adult specific) and Phillips (child specific) data cuts respectively). However, further sub analysis (Appendix G) showed that child specific data cuts (Phillips) score light PA at higher levels (17.9%) versus adult specific data cuts (3.2%), affecting the total MVPA%.

With limited difference in the time spent in sedentary PA between both data cuts it was agreed that Eslinger would be used for PA analysis for the entire cohort and taken forward for further analysis against the instrumented walk, to minimise further variability amongst this already heterogeneous population.

The Myasthenia Gravis Activities of Daily Living (MG-ADL) scores of the 40 participants who had both PA data and 6MWT were analysed, (see figure 4) with a range from 0 – 16 (out of possible 24) and a mean score of 7.1. Subtype analysis revealed; AChR def = 6.9, CHAT = 5.0, COLQ = 10.4, DOK7 = 8.1, GFPT1 = 9.0, RAPSYN = 4.0, SLC5A7 = 1.0, Slow Channel = 4.0.

No participants reported they were unable to stand independently (score of 3 on MD-ADL for rising from a chair).

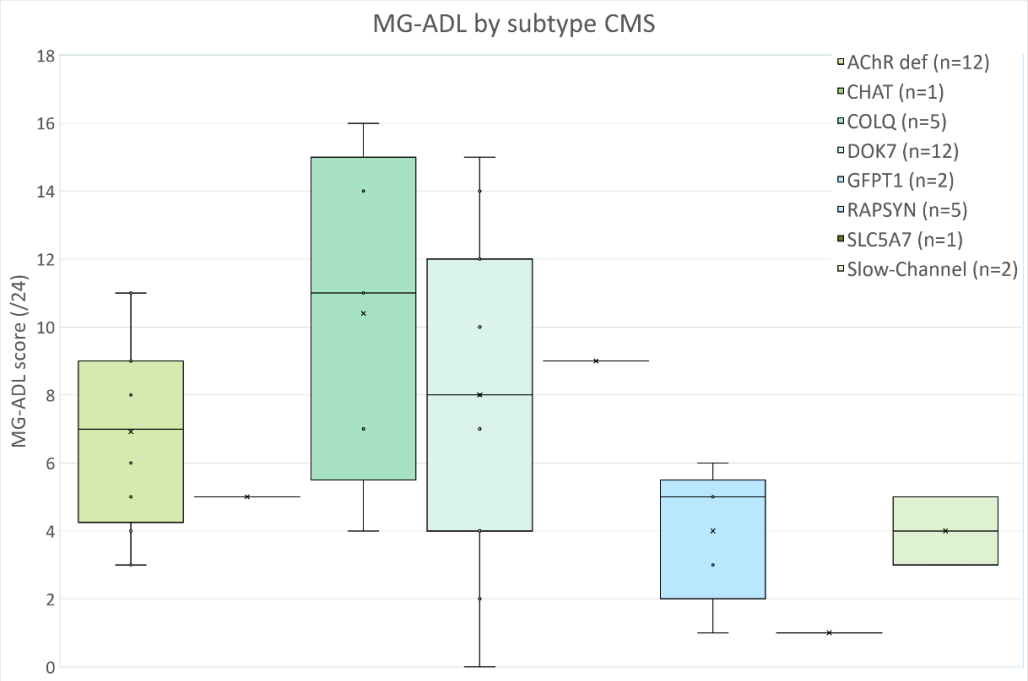

Figure 4. Myasthenia Gravis Activities of Daily Living (MG-ADL) by sub type

There was negligible correlation between sedentary PA levels in the community and individual MG-ADL score, see figure 5 (R^2 = 0.056, p=0.646).

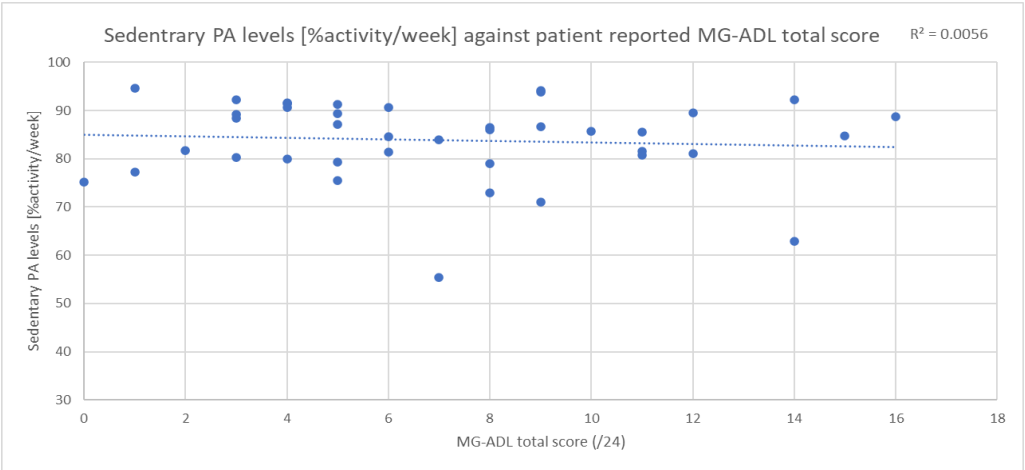

Figure 5. Sedentary Activity % against MG-ADL total

However, 6/8 of the questions in the MG-ADL score relate to ocular, bulbar and respiratory function (appendix H), with only two questions specific to upper and lower limb ability. A closer look at items specific to movement ability (figure 6 & figure 7), shows that the MG-ADL does not correlate with PA captured in the community, with some participants reporting no issues with rising to stand from a chair (MG-ADL score 0) but have high rates of sedentary

activity levels. Inversely some who stating they always need to use their arms to rise from a chair, had similar MVPA levels as those with no issues.

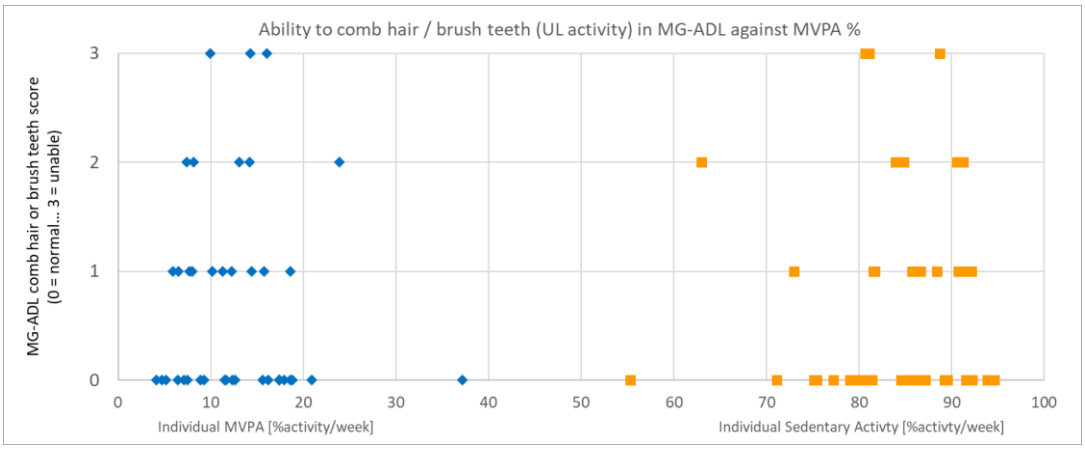

Figure 6. MG-ADL item 5 - ability to comb hair / brush teeth against sedentary and moderate-vigorous PA levels.

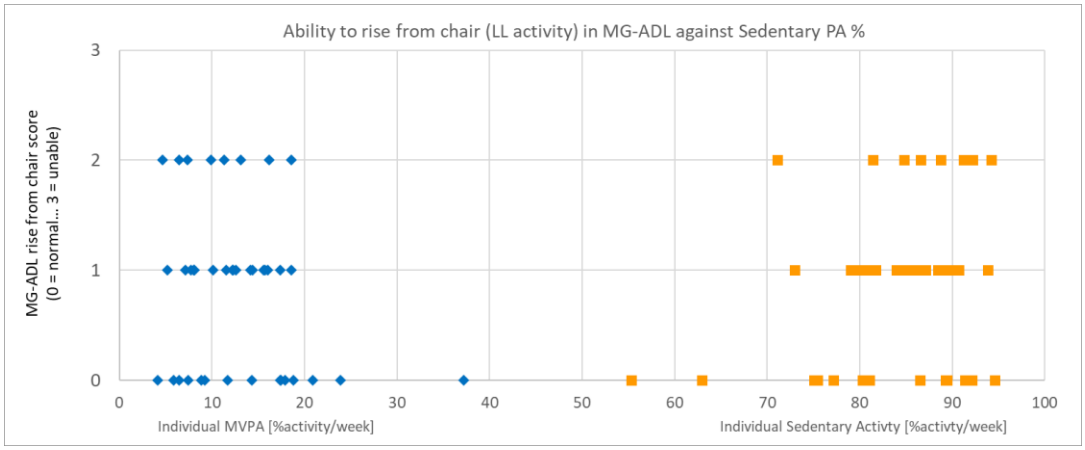

Figure 7. MG-ADL item 6 - ability to rise to stand from a chair against sedentary and moderate-vigorous PA levels.

5.3 Gait Analysis

A total of 46 participants attempted the 6MWT, 40 of these with a corresponding PA data set were taken forward for further analysis.

Seven (17.5%) of those 40 patients failed to walk the full six minutes (range 1min 28s – 4min 25s) and their gene subtypes included AChR deficiency (n = 1, 2.5%), GFPT1 (n = 1, 2.5%), DOK7 (n = 3, 7.5%), CHAT (n = 1, 2.5%), and COLQ (n = 1, 2.5%). These seven were still included in the 6MWT analysis, but IMU gait analysis was not possible where the participant was unable to walk for more than three laps (n = 3). See table 3 for details of the 40 participants included in 6MWT analysis.

Total walking distance: mean walking distance of the 40 participants 6MWT was 405.1m with a range of 25m – 711m, and median 451.2m.

Type	n =	Total distance walked [m] Median (range) *Mean*	Difference min1 & min6 distance [m] Median (range) *Mean*	Difference lap time first to last lap [s] Median (range) *Mean*	Speed [m/s] across total 6MWT Median (range) *Mean*
AChR deficiency	12	486 (100 – 625) *448.3*	-5 (-53 to 1) *-8.3*	2 (-3 to 49) *8.7*	1.35 (0.50 – 1.74) *1.27*
CHAT	1	188	-92	17.0	0.98
COLQ	5	405 (180 – 445) *357.8*	-12 (-71 to -9) *-23.2*	3 (2 to 51) *12.6*	1.13 (0.68 – 1.24) *1.03*
DOK7	12	395 (25 – 605) *348.9*	-15 (-50 to 5) *-15*	3 (-1 to 57) *10.45*	1.10 (0.16 – 1.68) *1.0*
GFPT1	2	215.5 (50 – 381)	-23 (-44 to -2)	33.5 (7 to 60)	0.81 (0.57 – 1.06)
RAPSYN	5	468 (342 – 711) *510.3*	-2 (-8 to 4) *-1.4*	1 (0 to 1) *0.6*	1.30 (0.95 – 1.98) *1.41*
SLC5A7	1	567	-8	2	1.58
Slow-Channel	2	466 (391 – 541)	-21 (-42 to 0)	2.5 (0 to 5)	1.29 (1.09 – 1.50)
Total:	40	451.2 (25 – 711) *405.1*	-18.1 (-71 to 5) *-14.8*	-5.6 (-3 to 60) *9.6*	1.19 (0.50 – 1.98) *1.17*

Table 3. 6MWT data by CMS subtype

Difference in distance walked and relevant lap times were recorded:

- Difference in distance walked from at the first and last minute ranged from +25m (gaining speed) to -75m (reduction in three laps), with a mean of 10 metres less in minute six compared to minute one.
- Difference in lap time between the first and last lap (25m) ranged from zero (no change) to +50 seconds (slowed down), with a mean increase in lap time of 9.7 seconds in the final lap completed.
- Participants with RAPSYN walked further and faster overall, whilst DOK7 had some of the slowest walking speeds and shortest distances.

Participants with RAPSYN had a greater mean walking distance (figure 8), those with AChR deficiency and DOK7 had the greatest variability, with 50% of DOK7 participants walking less than 400m.

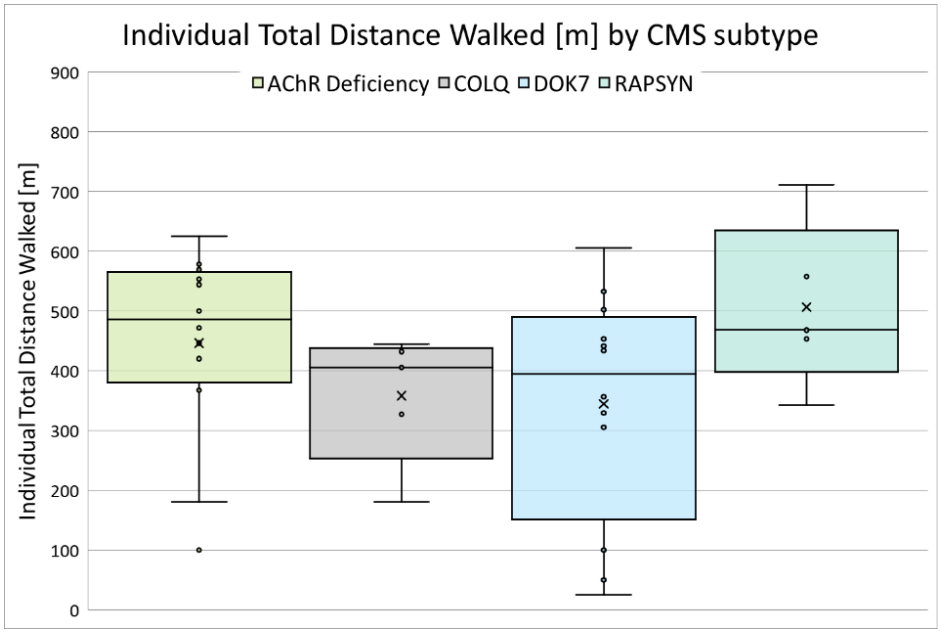

Figure 8. Individual total distance walked by CMS subtype.

Heart Rate: Mean resting heart rate (HR) of these 40 participants, taken prior to the 6MWT was 86 beats per minute (bpm) with a median of 88bpm and range of 61bpm – 111bpm. This elevated to a maximum heart rate (HRmax) mean of 147bpm, median of 142 and range of 111bpm – 215bpm during the 6MWT. An overall cohort mean elevation of 61.5bpm, median 62bpm and range 25bpm – 107bpm, demonstrating an increase in participant effort during the 6MWT. However, there was weak but significant correlation between heart rate elevation and distance walked ($R^2 = 0.1812$, $p=0.009$) suggesting that HR elevation cannot be used as a predictor of effort in this cohort whilst completing the 6MWT (see figure 9).

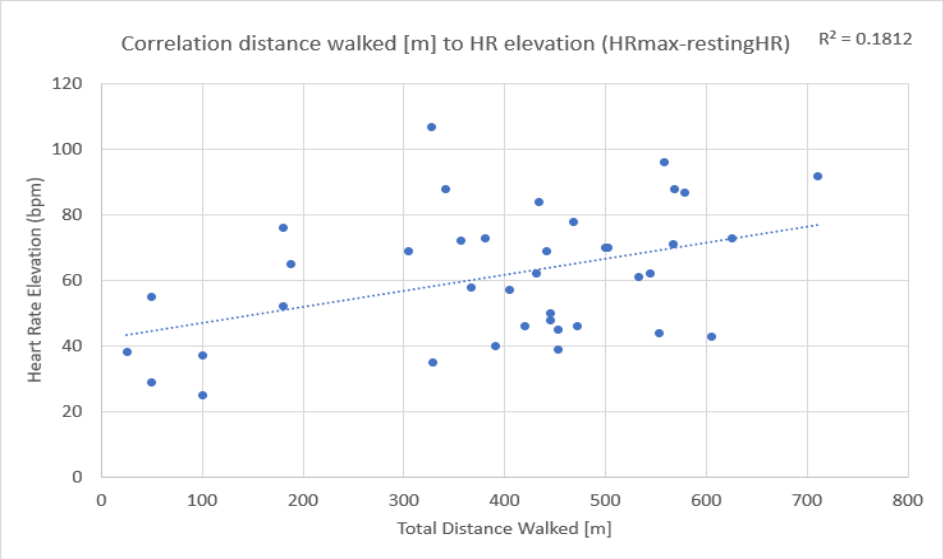

Figure 9. HR elevation [bpm] and total distance walked [m] on the 6MWT.

The following results are of the 37 data sets of participants that reached the minimum IMU gait analysis threshold, cross-referenced with their PA data.

Walking time and velocity: Mean lap time was 23.5 seconds (s), with a median of 20.3 and range of 12.7s – 68.9s. Mean walking speed was 1.16 meters per second [m/s] with a median of 1.22m/s and range 0.4m/s – 2.0m/s. Both the AChR deficiency and DOK7 sub types show outliers (figure 10), which correspond with participants who paused walking during the test due to fatigue, resulting in a significant lap time increase.

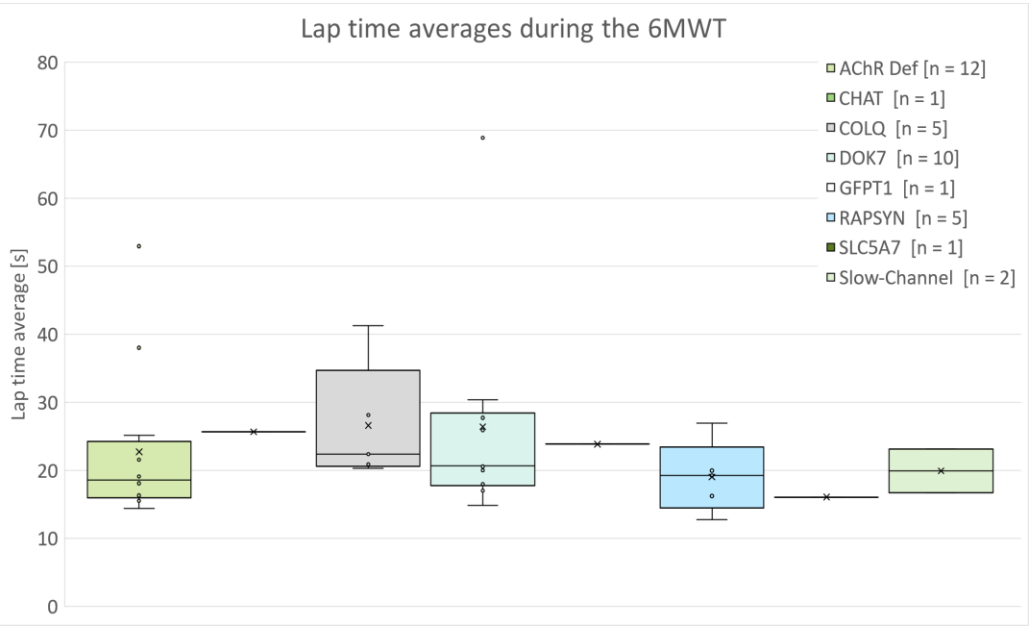

Figure 10. Lap time mean distribution by CMS subtype

Difference between laps: Figure 11 shows the total difference in walking distance achieving between the first minute of walking and the sixth minute of walking in the 6MWT. ($R^2 = 0.5408$, p=<0.001).

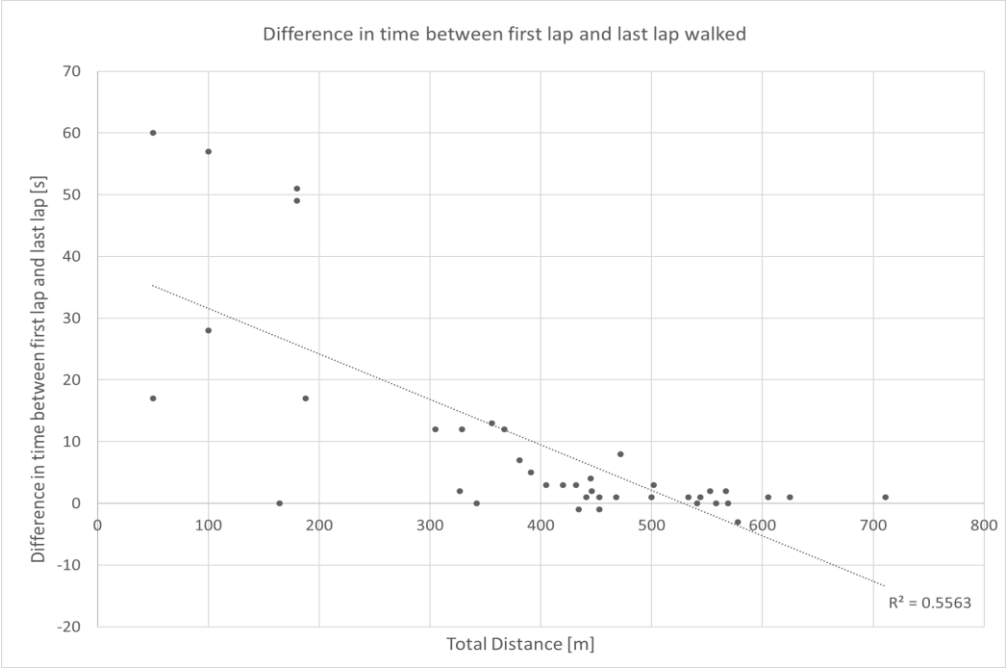

Figure 11. Difference in distance walked between first minute of walking and minute 6 of the 6MWT.

A negative value denotes a reduction in distance walked, for example, the participant identified in the red circle walked a total of 391m, but walked 42m less in their last minute, compared to their first. 59% of participants walked a shorter distance (≥5m) in their last minute and those with the greatest difference, walked a shorter distance overall.

Figure 12 shows the difference in lap times between the first lap and last, e.g. first 25m walked and final 25m walked, irrespective of total distance walked ($R^2 = 0.5563$, p=<0.001). Individuals with the smallest difference in time taken to complete first lap to last lap, walked the greatest distance overall, and suggests a better ability to pace their gait throughout the 6MWT. Inversely those with the largest difference in time between laps had a reduced overall walking distance.

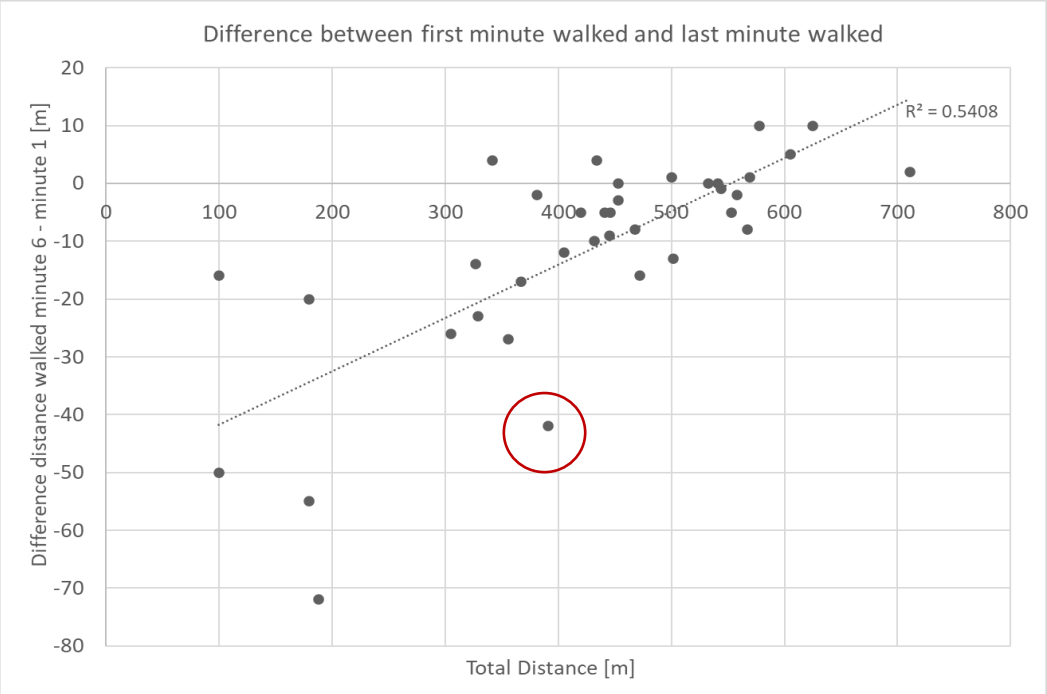

Figure 12. Difference in time between first lap and last lap completed (25m lap) in the 6MWT.

In-depth gait analysis was conducted and appendix I gives an example of how this raw data looks, demonstrating clear evidence of the fatigue with increasing lap times for individual participants.

Stride length & cadence: Figure 13 & figure 14 show that neither stride length (R^2=0.0021, p=0.788) nor cadence (R^2=0.0164, p=0.450) showed any statistically significant relationship to total distance walked, as measured over the 6-minute walk test.

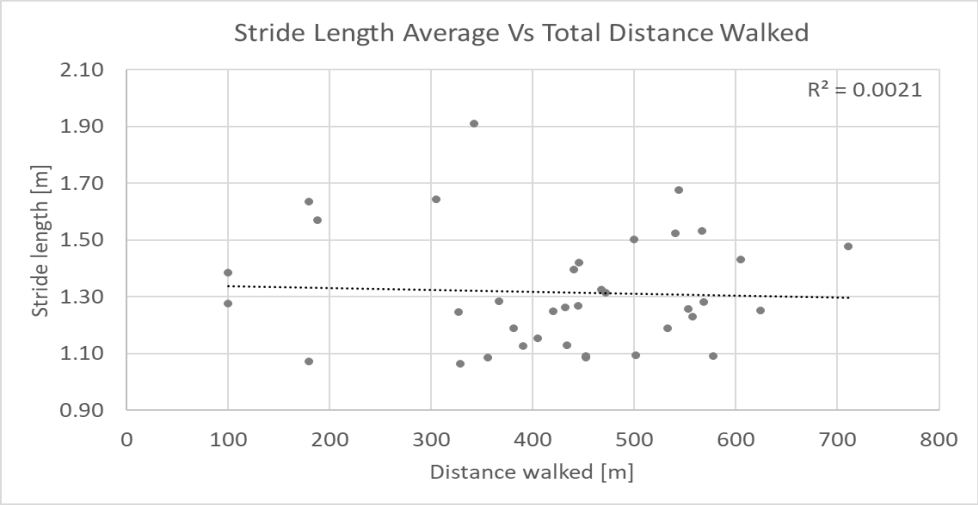

Figure 13. Mean stride length v total distance walked for individual participants in the 6MWT.

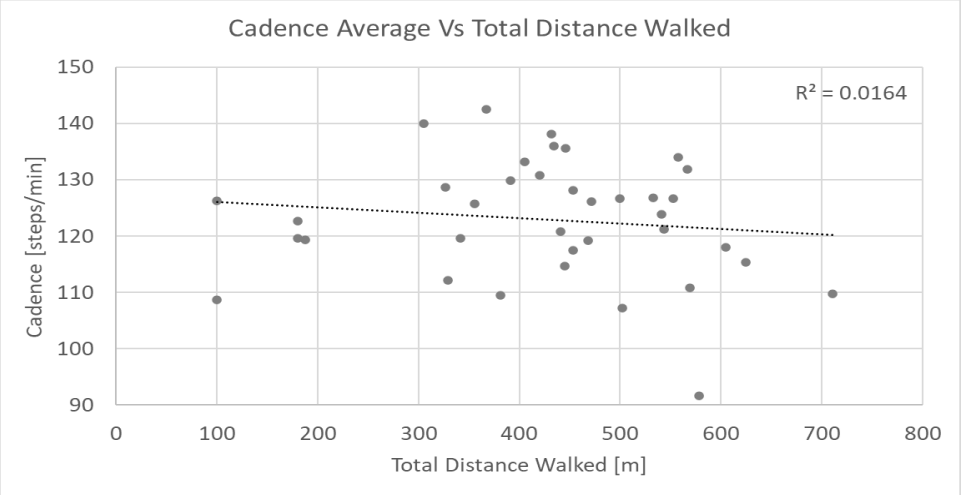

Figure 14. Mean cadence v total distance walked for individual participants in the 6MWT.

From this data a Walk Ratio (WR), which describes the relationship between step length and cadence (WR = step length [mm] / cadence [steps/min]) could be derived (figure 15) (R^2=0.002, p=<0.793).

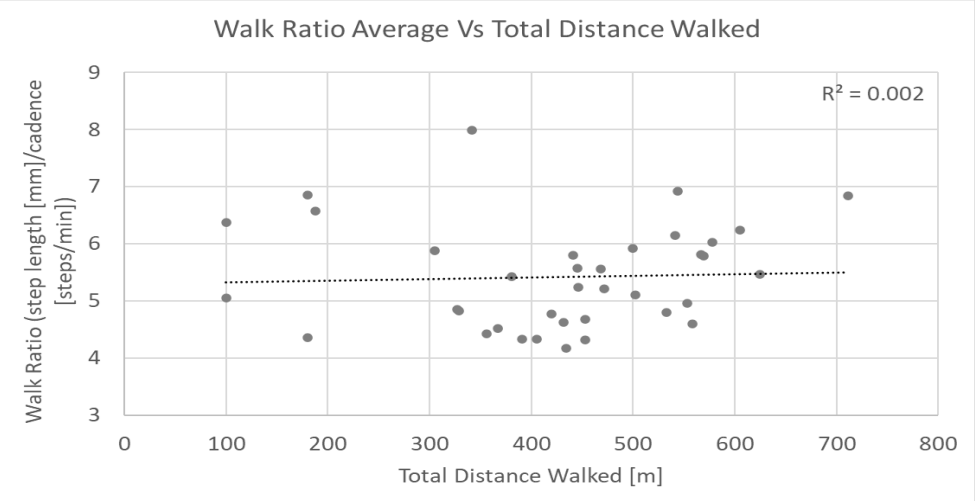

Figure 15. Walk Ratio (WR) v total distance walked for individual participants in the 6MWT.

Lap time coefficient: a lap time correlation coefficient across the 6MWT was captured for each participant, with a positive value reflecting an overall increase in lap time and therefore a reduction in walking speed. Conversely a negative value, indicated a participant increasing speed with a reduction in lap time. Figure 16 shows individual lap time correlation coefficient, denoted by each point on the graph, and indicates a decline in walking speed in both median (0.43) and mean (0.34) across the cohort.

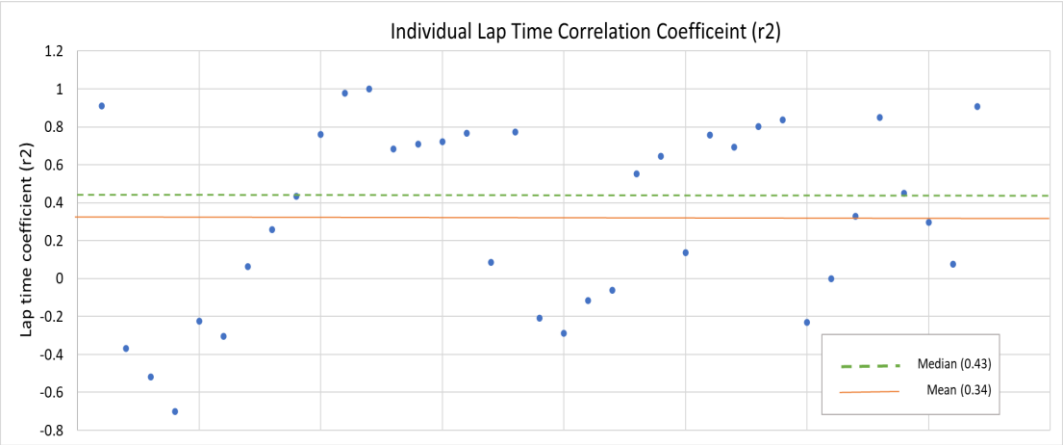

Figure 16. Lap time correlation coefficient for individuals completing the 6MWT.

Coefficient of variation (CoV) is the ratio of the standard deviation to the mean and was plotted for stride length (figure 17), cadence (figure 18) and walk ratio (figure 19), against total distance walked. The higher the CoV, the greater the level of dispersion around the mean, leading to greater variability and a reduced model fit. No significant and or strong relationship was found between total distance walked and any variation outcomes for stride length (R^2=0.0196, p=0.409), cadence (R^2=8E-07, p=0.987) or walk ration (R^2=7E-07, p=<0.961).

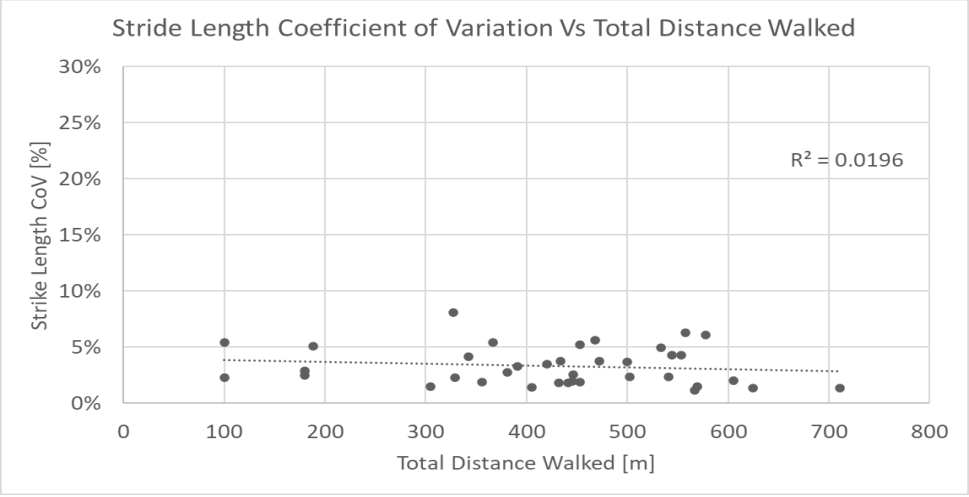

Figure 17. Stride length Coefficient of Variation (CoV) [%] Vs Total Distance Walked [m].

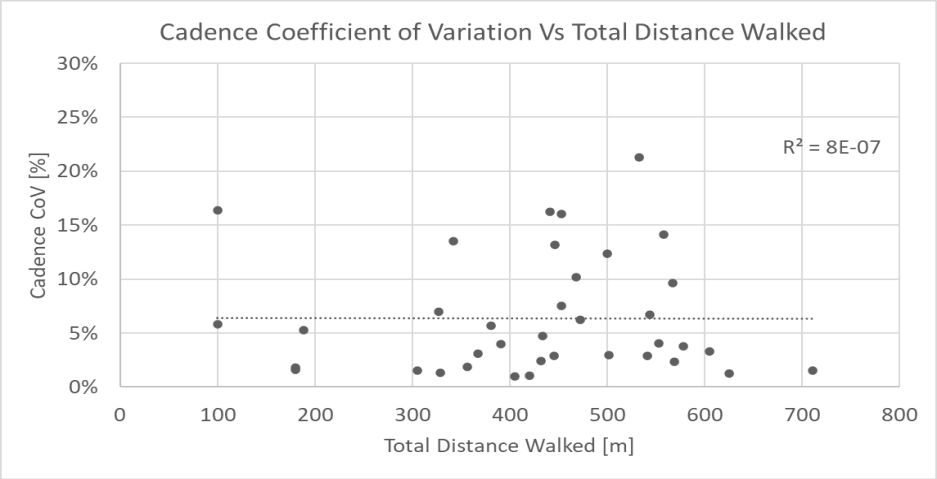

Figure 18. Cadence Coefficient of Variation (CoV) [%] Vs Total Distance Walked [m]

However, changes observed in walk ratio (WR) can be explained by changes in cadence, rather than stride length. Meaning that participants maintained a reasonably stable stride length, but changed the number of steps they take per minute when fatigued.

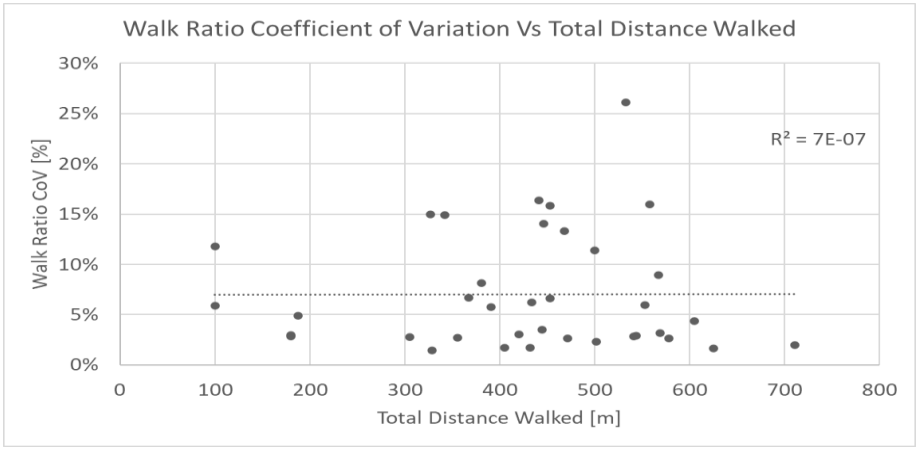

Figure 19. Walk Ration Coefficient of Variation (CoV) [%] Vs Total Distance Walked [m]

Correlations of PA data and Gait Data

Figure 20 shows there was weak but statistically significant correlation between community based moderate-vigorous physical activity (MVPA) time and total distance achieved on a 6MWT (R^2=0.1416, p=0.017) using the Eslinger parameters, with one adult participant achieving a total 453m with an MVPA of 4.16%.

There was negligible correlation between community based sedentary activity time and total distance achieved on a 6MWT (R^2=0.0792, p=0.081), however participants who spent longer in sedentary activity (≥90% activity/week) all walked less than 500m on the 6MWT.

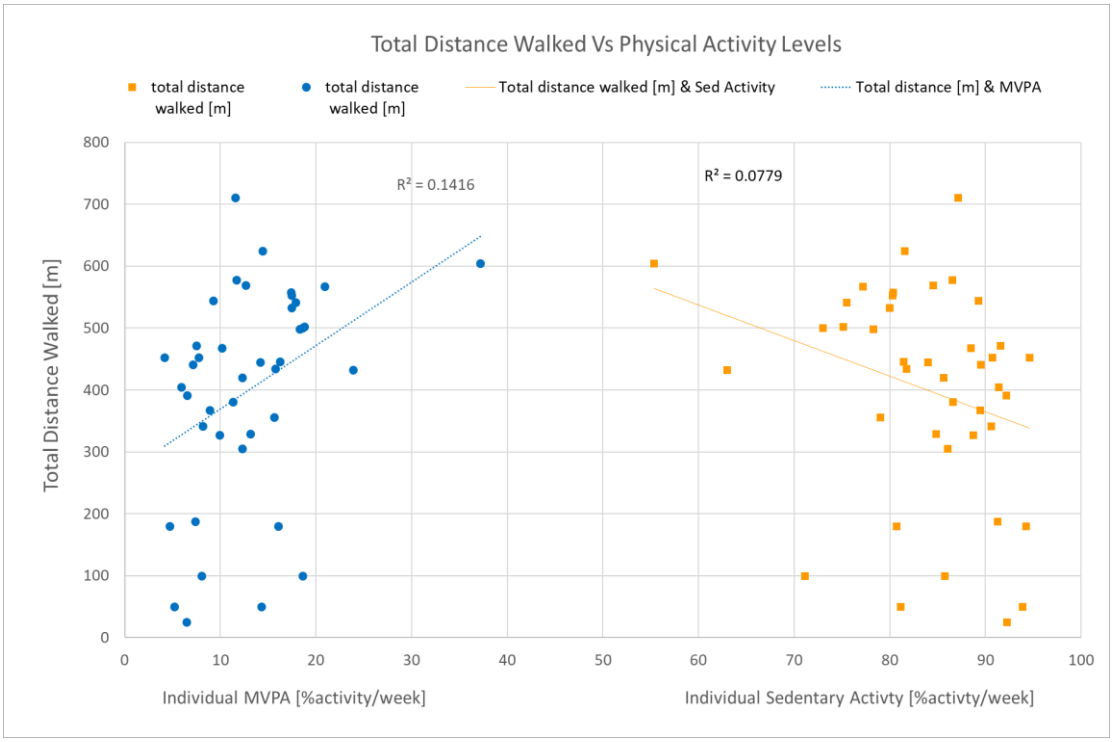

Figure 20. Communtiy physical activity (PA) levels and total distance walked in 6MWT [m]

6. Discussion

This study found that…

1. There is weak correlation between community activity levels and distance walked in an instrumented 6MWT, identifying several limitations in the use of PA monitors in this population.
2. There was variability between CMS subtypes both in walking distance and speed in the 6MWT; with RAPSYN subtype participants walking furthest and fastest, whilst DOK7 participants had some of the slowest walking speeds and shortest distances.
3. There was moderate correlation between the difference in distance walked from minute one to minute six, against total distance walked. And there was moderate correlation between first and last lap time difference, against total distance walked. This means our model explains some variability observed, yet both were found to be highly significant.
4. Changes in stride length, cadence and walk ratio all had negligible correlation to total distance walked in the 6MWT, but any changes observed in walk ratio were driven by a change in cadence, meaning participants maintained a stable stride length, but reduced the number of steps taken per minute when fatigued.
5. Myasthenia Gravis Activities of Daily Living (MG-ADL) score does not correlate with community based physical activity (PA) levels in CMS.

The Quantitative Myasthenia Gravis (QMG) scale is a 13-item scale that objectively measures disease severity in MG, by assessing muscle strength and fatigability; including diplopia, ptosis, facial muscles, dysphagia, dysarthria, proximal limb, hand muscles, neck muscles and respiratory function.[12,41] It is not currently validated for use in congenital myasthenic syndromes. Due to the limited validity of the QMG in our cohort and that six of the 13-items assess respiratory, facial, and bulbar function, we chose not to explore what relationship the QMG has to physical activity and gait in this study.

6.1 Population

We describe here the community physical activity (PA) levels, patient reported activities of daily living (MG-ADL) and data from an instrumented six-minute walk test (6MWT) in a cohort of 40 individuals with congenital myasthenic syndrome (CMS).

The population in this study ranged from 8 – 72 years of age, with a range of CMS subtypes, including the most common, AChR deficiency, DOK7 and RAPSYN. We have reported the results with both median and mean values, to evaluate data distribution, and identified a high proportion of younger participants with RAPSYN compared to the remaining sub types. This may reflect a selection bias of individuals consenting to research, as individuals with RAPSYN can present with respiratory crisis, which can be life-threatening in infancy and early childhood, and milder phenotypes present with a later disease onset. [8] It is reasonable to assume that adults with RAPSYN might see little burden from their CMS and therefore be less inclined to participate in a natural history study.

We also failed to capture data on some of the most severe individuals with CMS in this study for several reasons; including those with a high disease burden may be less able to travel to a national centre for regular study visits, and they may experience higher levels of fatigue and be less able or willing to complete the walking test required as part of this analysis. Furthermore, in a post COVID environment, there is a risk of bias towards individuals with milder subtypes willing to attend regular hospital visits, and those with more severe symptoms preferring instead the benefits of virtual consultations. We did however include four participants who were predominately wheelchair users (e.g., for at least all outdoor mobility or greater) which allows us to draw some conclusions about those most severely affected.

The rarity of this condition is reflected in the small sample size, and the heterogeneity of this cohort limits the strength of our analysis, and ability to draw broader themes across the wider population. We have not reported any comorbidities that this cohort may have in addition to CMS, which could further impact our analysis and conclusions drawn.

6.2 Physical Activity (PA)

Individuals with neuromuscular weakness often experience limited participation in physical activity (PA) resulting from muscle weakness and fatigue [20]. Increasingly researchers and policy makers have shifted towards activity-based measurements (accelerometery) to provide

objective estimates of different activity levels in a given population. [42–44] The World Health Organisation's (WHO) [19] updated 2020 "guidelines on physical activity and sedentary behaviour" highlights the need to "accelerate advancements in sensor technology to ensure it provides a practical and affordable approach to assessing physical activity and sedentary behaviours" for population monitoring and research.

There is an assumption that individuals with myasthenia, who experience pathophysiological muscle fatigue with increasing levels of activity, should be overly cautious when embarking on exercise. However, there is currently minimal evidence on what baseline PA levels are for this population, to help guide safe exercise prescription. We therefore report in what we believe this to be the first of its kind analysis of wrist worn accelerometery data to capture the community PA levels of a small (n=40) CMS cohort.

Our results identified that this cohort spent a mean of 81% of the time (analysed across seven days) in sedentary activity. Defined data cuts for sedentary, light, moderate and vigorous PA vary across the literature, with some overlap in healthy populations. Metabolic equivalent of task (MET) is the amount of energy used to complete a given physical activity and are often referenced in the literature in PA guidelines.[45,46] As a general consensus across the literature[47–51] the following can be considered a fair representation of PA in each category:

1. Sedentary: (<1.5 METs) lying, sleeping sitting watching television or using computer, driving the car, and standing still
2. Light: (1.5 – 3 METs) slow walking, cooking food, washing dishes
3. Moderate: (3-6 METs) fast paced walking, climbing the stairs, housework – e.g., hoovering
4. Vigorous: (≥6 METs) running, jumping, physical exercise

We further explored the variability in outcomes for both adult [33] and paediatric [32] populations, and our results highlight a bias towards capturing higher levels of moderate PA in adult data parameters, with paediatric specific parameters more likely to identify higher levels of light activity. This shift is driven by the difference in movement patterns and lifestyles, of healthy adults compared with healthy children, with the later moving differently, "using more frequent and agitated upper limb movements". [44]

In addition to the risks associated with low levels of moderate-vigorous physical activity (MVPA) levels, total time spent in sedentary activity is also felt to be a risk factor for adverse health outcomes.[51] The WHO 2020 guidelines [19] recommend MVPA for child and adolescence (7-15years) to be 60min/day (≥ 4%) and that adults should do at least 150–300 min of moderate-intensity aerobic physical activity, or at least 75–150 min of vigorous-intensity

aerobic physical activity per week (approximately 1.5-3% MVPA). Our results found a mean sedentary PA time of 81.8%, with variability between individuals and subtypes and mean MVPA at 15.0%. Overall, this data appears to identify that our population are far exceeding the WHO MVPA guidelines, which raises question to the validity of the data points applied, and concerns about the validity of the meaningful outputs from the data gathered. There are several lessons to learn from the literature about interpretation of our findings and conclusions that can be drawn.

Data cuts from healthy populations: As discussed, the availability of different data cuts is a key limitation to drawing robust conclusions from our data. Data cut parameters are established from lab-based analysis in healthy individuals, with variability across different age groups, one study identifying MVPA levels of healthy 6-11 year olds to be up to 21%.[52] There is a clear need to establish more robust free-living/community PA level data points in different populations[53] and this is likely to be further limited when analysing PA in rare conditions.

Instructions for device wear: A recent study, of 17 individuals aged 10-18 years with a variety of neuromuscular conditions (Charcot Marie Tooth disease; congenital myopathy and muscular dystrophy) concluded that "none of the participants registered time in vigorous-intensity PA (6-9 MET)" over a four day weekend period.[54] However this study did not disclose what data cut reference values were utilised and participants were told to remove the device when showering or swimming. Our participants were instructed to wear the waterproof device on their dominant wrist continuously for a seven-day period, minimising our loss of data and perhaps capturing MVPA levels to a greater degree.

Placement of the activity monitor: There is much debate around the best placement of an accelerometer device for data collection. A large cohort study by the UK Biobank identified that a wrist worn device offers best compliance to wear time [55] for individuals, limiting the risk of missing data from removal of the device.

However, it is important to acknowledge that device placement may bias data collection with different populations, such as children, the elderly [55] and those with a disability, where non-ambulant individuals, or those using a wheelchair for part of the day may have varying levels of upper limb mobility. Colleagues in Newcastle upon Tyne, UK, assessed the usability of a tri-axial accelerometers (GENEActiv) during a lab based short walking tests in adults with Myotonic Dystrophy [21] and compared their results to healthy controls. They noted that

"accelerometer location impacts significantly when translating raw data into meaningful outputs" [21] and reported a reduction in their data reliability with increment of speed, when it is expected that upper limb force increases to accelerate over legs. It is unknown, but reasonable to assume that individuals with CMS may increase their upper limb movement to aid walking, with increasing levels of lower limb fatigue, leading to an inverse relationship between fatigue and PA levels detected. Caution should be given in our data analysis, and in the device placement for future studies, as although a wrist worn device improves wear compliance, it may limit the conclusions made.

PA levels in Congenital Myasthenic Syndrome: We have reported here the community based activity levels for a small cohort of individuals with CMS, however, the variety and heterogeneity of tools and methods used for PA analysis in the published research makes it difficult to compare results across the literature. [20] Furthermore, the small sample size and heterogeneity of this cohort further limits our ability to draw broad conclusions or repeat sub-analysis with different CMS genetic subtypes. There was no clear correlation between PA levels with either the MG-ADL or performance within the 6MWT, and so conclusions about PA habits of this population are limited.

A team of Dutch researchers explored self-reported fatigue levels and its association with physical activity in a population of over 700 Dutch individuals with myasthenia gravis (MG). [56] They identified that self-reported high levels of fatigue and low levels of physical activity were frequent, and strongly associated. However, it was unclear if individuals who engaging in higher levels of physical activity resulted in lower reported fatigue, or if lower levels of fatigue led to individuals engaging in more physical activity. Fatigue, exercise habits, lifestyle adjustments and/or sedentary careers chosen by a CMS population who experience daily muscle fatigability, could result in recorded low MVPA levels.

Myasthenia Gravis (MG) is significantly more common in the general population than CMS and thus there is a greater quantity of literature available to compare our cohort against. A study looking at "free living physical activity and sedentary behaviour in autoimmune myasthenia gravis" [57] whereby individuals wore a triaxial accelerometer at their waist, over a seven-day period, concluded that individuals with stable MG perform "less PA at lower intensities, and are more inactive than control individuals". Their results reflect similar findings to our data, where low vigorous PA than control subjects were observed. They also found a weak relationship between lower PA volumes and shorter distance walked in the 6MWT and felt

that there was a behavioural component to community PA levels, influenced by social, environmental, psychological, and other genetic factors.

Future analysis of longitudinal data, rather than across a cohort, as we have done, would allow us to better understand subtype variability and any changes observed with seasonality, medication, or other external factors, such as work and social habits. However, wider analysis such as this, is beyond the scope of this study and should be considered for future analysis at closure of the broader Natural History Study.

Myasthenia Gravis Activities of Daily Living questionnaire: The MG-ADL is a patient reported outcome measure widely used in the clinical care and research of individuals with myasthenia gravis (MG) an acute autoimmune condition, with often sudden symptom onset. [58–60] It is an 8-item questionnaire, whereby the clinician asks the individual questions about their perceived ability to carry out daily tasks. However, only two of the items focus on gross motor movement, and it is heavily weighted towards bulbar, respiratory, and ocular fatigue. The literature recognises a need to include more items for generalised weakness in the MG-ADL score.[59] Furthermore, individuals with CMS have by the very nature of the condition been living with their symptoms over a long period of time and have had time to adapt to their fatigable weakness, in contrast to individuals with MG who tend to experience short term weakness until effective treatment is achieved. Consideration must be given to what influence this may have on the response to questions, where individuals may use the arms of a chair to stand (item 6. impairment of ability to arise from a chair) because it is a learnt strategy to managing daily fatigue, rather than specifically needing to do this because of acute symptom onset.

Our data identified variability across the cohort, with RAPSYN and Slow-Channel sub types reporting lowest levels of impairment, and DOK7, COLQ and AChR deficiency reporting some of the highest. There was greatest range in MG-ADL scores amongst individuals with DOK7 subtype, which is also reflected in the wide range of total distance achieved in the 6MWT by individuals with DOK7. However, a low MG-ADL score did not directly correlate with a low 6MWT walking distance.

There was very limited correlation between the total and sub scores (items for upper and lower limb activity) of the MG-ADL and the observed PA levels in this cohort. It is equally important to acknowledge that observed PA in the community reflects the activities, habits, and lifestyle choices of an individual and not necessarily their actual abilities. For although it may be true that an individual with lower levels of myasthenic fatigue may have higher activity

levels, it is equally true that individuals may still require high activity levels despite fatigue (e.g. a mother with young children) or may have low levels of fatigue but choose a more sedentary lifestyle (e.g. someone without children, working from home in a desk-based role). A combination of clinic-based assessments and observed community PA levels should be considered when counselling individuals about exercise and rehabilitation.

6.3 Gait analysis

6MWT & distance walked: Walking ability is linked with better health outcomes [61] and is a useful objective measure in the management of neuromuscular conditions to plot disease progression and response to treatment [62,63]. Our results identified a wide range of total distance walked, in a cohort of 40 individuals with CMS, with DOK7, GFPT1 and AChR deficiency recording some of the lowest distances (25m, 50m and 100m respectively) over a 6MWT.

Five participants with COLQ, were all able to walk a minimum of 180m, and yet reported some of the highest MG-ADL scores in this study. This is likely to reflect the ocular, bulbar and respiratory disabilities of this cohort,[8] again reflecting the caution required with using the MG-ADL as a predictive tool for physical ability in the CMS cohort. Overall participants with RAPSYN achieved some of the largest walking distance (mean = 711m) in the 6MWT, in contrast only 50% of DOK7 participants walking greater than 400m.

Normal 6MWT values: Several papers have explored normative reference values for 6MWT distance in different populations, but limited consensus was identified to support a normal distribution value that our cohort could be compared against. A systematic review [64] in 2016, identified that “total walking distance in six minutes for healthy children and adolescents can vary up to 159m”. They also cited variability in how the tests were performed (e.g., variable length of walking course), the height of the child and relative leg length impact.

A study of 26 older healthy adults [65] explored whether the method of instruction in the 6MWT (“walk as far as you can” vs “walk as fast as you can”) can impact the rate of perceived exertion of the person performing the test. They concluded that the method of instruction made no differences in walking distance in health community-dwelling populations. For this study we adopted the phrase “I want to see how far you are able to walk in six minutes”.

We must also consider the influence of other factors on our results, such as completing the assessment in a hospital (albeit quiet) corridor compared with a lab-based assessment. The bias that having a trained clinician who understands CMS performing the test, might encourage greater participant engagement, with some reportedly walking further in this test that they would normally attempt in the community setting, and the scheduling of this test within the context of the wider study (e.g., being the last item, they perform at the end of the study visit). It is also unclear if individuals who showed minimal muscle fatigue during the 6MWT, would have experienced signs of muscle fatigue if encouraged to walk beyond the six-minute threshold. Capturing those unable to effectively pace themselves over a longer period. And to consider any delayed fatigue that could be experienced by individuals pushing themselves in the assessment, only to spend an increased amount of time the following day at rest, biasing the levels of sedentary PA data captured in this study.

Unpicking the 6MWT in CMS: Beyond the total distance walked, we were able to identify trends in variability of walking, between laps and times. Difference in distance walked and lap time across the 6MWT proved to correlated best with total distance walked. Individuals with the greatest difference in both distance and lap time, walked below the mean total distance for this CMS cohort. Those with a small difference, be it an increase or reduction in speed, appeared to be able to pace themselves more effectively and walk further than the mean distance for this CMS cohort. Individuals who walked the shortest distance were unable to either maintain a steady pace or continue to walk beyond a certain point of task failure. The difference in recorded values from beginning to end of the test, might prove to be the most effective way of identifying myasthenic fatigue as individuals are unable to compensate.

Heart Rate: There was no correlation between an increase in heart rate (HR) and distance walked in 6MWT, which limits the use of HR as an indicator of effort in this population. Due to the fatigable nature of this condition, it is possible that those who achieved short distances in the 6MWT are likely to be more severely affected by muscle fatigability and may either work harder (elevating their HR) to achieve their final distance or may stop walking before we detect a significant HR elevation. Conversely those walking further may be less severely affected by CMS muscle fatigue, having a higher general fitness level and limited HR elevation. This suggests all participants put in equal amount of effort, with no greater effort for those achieving a longer distance.

Walking time and velocity: Walking time and velocity varied across the cohort, with walking velocity ranging from 0.4m/s to 2.0m/s. Subtypes such as AChR deficiency and DOK7 had significant outliers and reflects those participants who paused walking in the test, slowing their overall walking speed across the 6WMT. These pauses reflect a higher disease burden in these CMS subtypes.

A deeper gait analysis: Gait speed is commonly reported as an outcome in mobility research, but it offers little information about gait quality. Gait speed is determined by step length and step frequency (cadence), [66] and changes in gait can result from a change in either variable. This relationship between step length and cadence is termed the walk ratio (WR) and this ratio may give us greater insight into the compensations seen in individuals with CMS as muscle fatigability increases. WRs in healthy adults do "not change between preferred and fast walking speed conditions, with individuals optimising energy expenditure and stability" [66] and offers a way of comparing individuals who walk at different speeds. Normal walk ratios are around 0.65 cm/steps/min [66–68], with lower WR resulting from increased cadence with reduced step length, perhaps indicative of cautious gait, or poor balance.[69] A study in patients with Multiple Sclerosis found a reduction in WR by 20%.[68] Surprisingly, in this CMS cohort, higher ratios occurred as a result of lowering cadence and not a change in step length.

Gait analysis alongside the 6MWT allowed a first look at how this cohort of individuals might compensate for their muscle fatigability to avoid task failure. There was a weak correlation between mean stride length and cadence, against total distance walked. However, in the first of its kind in-depth look at walk ratios (WR) for individuals with CMS, this study identifies variability of gait during the 6MWT, driven by a reduction in cadence (steps/minute), which points towards an inability to take the same frequency of steps as muscle fatigability increases, but with maintenance of a stable stride length.

Understanding walk ratios in CMS: The speed of human walking is "determined by the product of step-length and step-rate. At a given speed, therefore, one can walk with infinite combinations of step-length and step-rate." [70] This relationship is commonly known as walk ratio (WR) and remains relatively stable when individual's pace their own gait. Where there is reported variability is at extreme walking speeds; either very fast (transitioning into a run) or very slow, [70,71] and when transitioning between walking patterns. We anticipated that a change in WR might be evident at the point of muscle fatigability in individuals with CMS, capturing the point at which they are unable to compensate to maintain a stable WR.

WR can vary when walking on uneven surfaces, or distractions within the environment. [66] This was minimised in our test where the 6MWT was completed indoors, on a clear and relatively quiet hospital corridor, with little distraction, and instructions to walk between two clearly marked points. This allows greater confidence that any WR variability is due to change in the individual, most likely fatigue resulting from deficit at the neuromuscular junction.

AChR, DOK7 and CHAT recorded the smallest walking distances overall in the 6MWT and saw some of the biggest changes in WR. This could be a result of muscle fatigability, with individuals unable to compensate and maintain a stable walk ratio, or due to distribution of muscle weakness between subtypes, and/or behavioural variation.

Myasthenic muscle fatigue and considerations:

Muscle fatigue has been described across the literature in many different forms, and is referred to as a motor deficit, or a decrease in maximal force or power. [72,73] It can develop soon after sustained physical activity and is not necessarily at the point of task failure. However, the primary outcome of many assessments designed to capture myasthenic muscle fatigue, is at the point of task failure (e.g., the point at which an individual can no longer hold their arms out, an item in the quantitative myasthenia gravis (QMG) score), which fails to capture the nuances of muscle fatigability throughout the given task.

By using gait analysis during the 6MWT, we have been able to identify changes in gait ahead of task fatigue, which could be signs of muscle fatigability, driven by a deficit at the neuromuscular junction (NMJ). However, it remains clear that muscle fatigability in CMS is complex and multifaceted, with both a NMJ deficient, potential impact of deconditioning and mental endurance, and we cannot assume that all fatigue seen in this data reflects NMJ muscle fatigue.

A systematic review of fatigue in patients with MG [74] identified two categories of fatigue; 1) peripheral fatigue; a direct result of muscle fatigability from a disorder of the neuromuscular junction, and 2) central fatigue; a “lack of energy and feelings of tiredness” not related to muscle weakness. The 6MWT aims to capture NMJ or peripheral fatigue and every effort was made to reduce bias and limit other external factors, although it is impossible to fully separate them, and some “central fatigue” will certainly bias our results to some degree.

6.4 Relationship between gait analysis and physical activity levels

There is a weak correlation between the observed community-based PA levels of this cohort and their total distance walked in the 6MWT. We cannot therefore reliably use a 6MWT to predict community activity levels.

We cannot also rely in the MG-ADL as a robust patient reported outcome measure (PROM) for community activity levels for individuals with CMS, as this questionnaire presents a bias towards ocular, bulbar and respiratory function, with limited depth of gross motor function.

6.5 Limitations of the study

Participants were recruited to this study through a national NHS clinic and patient information days. Details of the study were made publicly available on both a national study database platform and UK patient charity for myasthenia. However, due to the rarity of the condition, and single centre study design, this led to a small sample size and limits the strength of our analysis and broader themes than can be concluded to the wider population.

The heterogeneity of CMS further limits our ability draw strong themes, with some subtypes reported as one participant, and the most common subtypes (AChR deficiency and DOK7) having only 12 participants each. There is also the risk that travel burden and a post COVID environment might bias recruitment to milder sub types.

Further population bias might be considered as individuals willing to participate in research are likely to be more motivated and adhere to guidance given. They are more likely to be aware of their condition and hold good habits for better health and fitness and it is reasonable to assume that some might change their habits during the observed week, despite guidance to continue with normal activity.

This study evaluates data collected as part of a wider natural history study, with several physically demanding assessment outcomes, the prioritisation and order of assessments might negatively impact on the distance achieved in the 6MWT and risk bias of individuals self-pacing. A learning response to the 6MWT has been reported in the literature, [37] although attempts to minimised this by capturing the first visit where the 6MWT and PA data was available, this might be a limiting factor in future longitudinal analysis. We have not reported any comorbidities that this cohort may have in addition to CMS, which could further impact our analysis and conclusions drawn. Typical comorbidities of this population include scoliosis,

spinal surgery, respiratory impairment, and joint contractures, which may impact on the activity levels and outcomes of the 6MWT in this population.

At this time suitable data parameters for community PA analysis in a disabled cohort and the small CMS data size available, limit our ability to draw any strong conclusions about predicting activity levels in the community, based on results from the 6MWT. Further longitudinal analysis will prove beneficial in understanding activity variability and unpicking the multifaceted approach required for fatigue management in this population.

General Discussion

The 40 participants included in this study had a range of CMS subtypes, including the most common (AChR deficiency, DOK7 and RAPSYN). Overall, there was a weak relationship between distance walked on the 6MWT and community PA outcomes.

There are several limitations to the application of accelerometery for capturing community based physical activity in CMS, including robust data parameters, device placement and application to wheelchair users. The heterogeneity and rarity of this disease, limits our current ability to establish more robust data parameters.

Gait analysis alongside the 6MWT allowed a first look at how this cohort of individuals compensate for their muscle fatigability to avoid task failure and identified that variability of gait during the 6MWT, is driven by a reduction in cadence (steps/minute), which points towards an inability to take the same frequency of steps as muscle fatigability increases, but maintenance of a stable stride length.

Difference in distance walked and lap time across the 6MWT correlates best with total distance walked and might prove to be the most effective way of identifying muscle fatigability in CMS.

An instrumented 6MWT allows us to describe with greater detail the walking fatigue in this population, which may prove useful in clinical and research data collection and help with condition management, however, it would be prudent to review whether there was variability within an individual's activity over time, and comparison of longitudinal data from repeated assessments should be considered.

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Appendix A – HRA approval

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

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10 August 2021

Dear Professor Palace

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**A Natural History Study of Congenital Myasthenic Syndromes, to establish reliable outcome measures suitable for clinical and research assessment**
IRAS project ID:	**289835**
Protocol number:	**1/090421**
REC reference:	**21/LO/0480**
Sponsor	**Oxford University Hospitals**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report

(including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The standard conditions document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **289835**. Please quote this on all correspondence.

Yours sincerely,
Rebecca Evans
Approval Specialist

Email: approvals@hra.nhs.uk HCRW.approvals@wales.nhs.uk

Copy to: *Mrs Shahista Hussain*

Appendix B – Participant Consent Form – Adults & YP 16+

Oxford University Hospitals NHS
NHS Foundation Trust
The Oxford Congenital Myasthenic Syndrome Service
Level 3, Neurosciences
West Wing
John Radcliffe Hospital
Oxford
OX3 9DU

Participant Identification Number: ☐

CONSENT FORM – for adults and young persons above 16 years of age

Title of Study: A Natural History Study of Congenital Myasthenic Syndromes (CMS)

Name of the Chief and Principal Investigators: Professor J Palace and H Ramjattan

If you agree, please initial box

1. I confirm that I have read and understood the information sheet for the above study (version 1.2) dated 05/12/2021. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2. I understand that my participation is voluntary and that I am free to withdraw my consent at any time without giving any reason, without my medical care or legal rights being affected now or in the future.	
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the Sponsor, from regulatory authorities and from the OUH NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
4. I understand that my GP or local clinical team will be informed of any relevant clinical information	
5. I agree to take part in this study.	

6. Optional: I agree for my anonymised data to be used in future research, here or abroad, which has ethics approval and may be commercial.	Yes	No
7. Optional: I agree to video recording being taken as part of this study, which will be used in research reports and publications.	Yes	No
8. Optional: I agree to completing a self-assessment diary at home, between study visits.	Yes	No
9. Optional: I agree to be contacted about ethically approved research studies for which I may be suitable. I understand that agreeing to be contacted does not oblige me to participate in any further studies.	Yes	No
10. Optional: I agree to wearing an activity monitor as part of this study. I understand that this data will be collected and anonymised for data analysis with a third party outside of OUH NHS FT.	Yes	No

Name of Participant	*Date*	*Signature*
Name of Person taking Consent	*Date*	*Signature*

**1 copy for participant; 1 copy for medical notes; 1 (original) to be kept in site file.*

Appendix C – Participant Consent Form – Parent & Guardian

Oxford University Hospitals NHS
NHS Foundation Trust

The Oxford Congenital Myasthenic Syndrome Service
Level 3, Neurosciences
West Wing
John Radcliffe Hospital
Oxford
OX3 9DU

Participant Identification Number: ☐

CONSENT FORM – for parents or guardians of children and young persons under 16 years of age

Title of Study: A Natural History Study of Congenital Myasthenic Syndromes (CMS)

Name of the Chief and Principal Investigators: Professor J Palace and H Ramjattan

If you agree, please initial box

1. I confirm that I have read and understood the parent information sheet for the above study (version 1.2) dated 05/12/2021. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2. I understand that me and my child's participation is voluntary and that my child and I are free to withdraw consent at any time without giving any reason, without my child's medical care or legal rights being affected now or in the future.	
3. I understand that relevant sections of my child's medical notes and data collected during the study may be looked at by individuals from the Sponsor, from regulatory authorities and from the OUH NHS Trust, where it is relevant to my child taking part in this research. I give permission for these individuals to have access to my child's records.	
4. I understand that my child's GP or local clinical team will be informed of any relevant clinical information	
5. I agree to my child taking part in this study.	

6. Optional: I agree for my child's anonymised data to be used in future research, here or abroad, which has ethics approval and may be commercial.	Yes	No
7. Optional: I agree to video recording being taken of my child as part of this study, which will be used in research reports and publications.	Yes	No
8. Optional: I agree to my child completing a self-assessment diary at home, between study visits.	Yes	No
9. Optional: I agree to be contacted about ethically approved research studies for which my child may be suitable. I understand that agreeing to be contacted does not oblige me or my child to participate in any further studies.	Yes	No
10. Optional: I agree to my child wearing an activity monitor as part of this study. I understand that this data will be collected and anonymised for data analysis with a third party outside of OUH NHS FT.	Yes	No

_____	_____	_____
Name of Participant/ Parent	*Date*	*Signature*
_____	_____	_____
Name of Person taking Consent	*Date*	*Signature*

**1 copy for participant; 1 copy for medical notes; 1 (original) to be kept in site file.*

Appendix D – Participant Assent Form – C&YP age 11-15

Oxford University Hospitals NHS
NHS Foundation Trust

The Oxford Congenital Myasthenic Syndrome Service
Level 3, Neurosciences
West Wing
John Radcliffe Hospital
Oxford
OX3 9DU

Participant Identification Number: ☐

ASSENT FORM – children and young persons 11-15 years of age

Title of Study: A Natural History Study of Congenital Myasthenic Syndromes (CMS)

Name of the Chief and Principal Investigators: Professor J Palace and H Ramjattan

If you agree, please initial box

1. I confirm that I have read and understood the information sheet for the above study (version 1.1) dated 04/07/2021. I have had the opportunity to consider the information, ask questions and have had these answered so that I understand.	
2. I understand that being a part of this study is my choice and is voluntary. I understand that I can stop being in this study and withdraw my consent at any time without giving any reason.	
3. I understand that relevant people outside of the study team make look at data collected on me during the study and I give permission for these individuals to have access to my records.	
4. I understand that my GP or local clinical team will be informed of any relevant clinical information	

5. I agree to take part in this study.		
6. Optional: I agree for my anonymised (not identifiable) data to be used in future research, here or abroad, which has ethics approval.	Yes	No
7. Optional: I agree to video recording being taken as part of this study, which will be used in research reports and publications.	Yes	No
8. Optional: I agree to completing a self-assessment diary at home, between study visits.	Yes	No
9. Optional: I agree to wearing an activity monitor as part of this study.	Yes	No

Name of Participant	*Date*	*Signature*
Name of Person taking Consent	*Date*	*Signature*

**1 copy for participant; 1 copy for medical notes; 1 (original) to be kept in site file.*

Appendix E – Activity Monitor Information Sheet

Oxford University Hospitals
NHS Foundation Trust
The Oxford Congenital Myasthenic Syndrome Service
Level 3, Neurosciences
West Wing
John Radcliffe Hospital
Oxford
OX3 9DU

Activity Monitor Information Sheet - for adults and young persons above 16 years of age

Title of Study: A Natural History Study of Congenital Myasthenic Syndromes (CMS)

Name of the Chief and Principal Investigators: Professor J Palace and H Ramjattan

About your activity monitor

This document will tell you how and when to wear your activity monitor, and how to return it to us afterwards. Please note the wearing of the monitor is optional. If you are happy to wear it, it is particularly important for our research that you wear the activity monitor all the time for the full
week.

Why am I being asked to wear this device?

The sensor is the size of a watch and will be worn on the wrist to monitor physical activity. It records activity level continuously, such as movement and how many steps you take over a 7-day period, but it does not track the type of activity.

It is useful for the study team to see how active you are outside of clinic, so that we can better understand how your myasthenia affects you throughout a normal day. You will not be judged on how active you are, and you will be asked to do your normal activities whilst wearing it.

It records the number of hours you sleep indirectly in the absence of movement for longer periods at night. The sensor does not record your location (no GPS data).

How do I get the device?

If you are happy to wear an activity monitor for one week, then we will give you the device at your clinic visit.

You will be asked to remove the monitor after 7 days and return it to us in the pre-paid envelope given to you with the device. You will be provided with a new device at each

subsequent study visit and instructed how to wear and return the device to the study team.

How do I wear the device?

Please wear the activity monitor on your least affected (or dominant) wrist for 7 nights and days. You can simply ignore it and engage in your normal activities throughout the day. Please try not to do anything different to normal in this week. You can continue to wear it in the shower or when swimming.

You will not have to charge the device within that time.

What data is the device is collecting?

The collected data is initially stored on the device and will be extracted by us once you give the activity monitor back to the research team.

How do I return the device?

After a week, you can return the activity monitor and the rest of the pack via the prepaid envelope given to you at your study visit.

What if I have a problem with the device?

The responsibility for the devices lies with the research team. If you discover any problems or discomfort with the device, you can take it off without any worry.

Please then contact the study team Principal Investigator on the details below and we will instruct you on what to do next.

If the device is lost or stolen, please contact the study team for a replacement, at no cost to you.

Study Team contact details:

Hayley Ramjattan, Physiotherapist / Principal Investigator
Tel: 01865 231986
Email: hayley.ramjattan@ouh.nhs.uk

COVID-SAFE

Your activity monitor has been thoroughly washed and disinfected before being given to you. They will be washed and disinfected after it is returned to the research team.

Appendix F – Six-minute walking test (6MWT)

Testing Guidelines

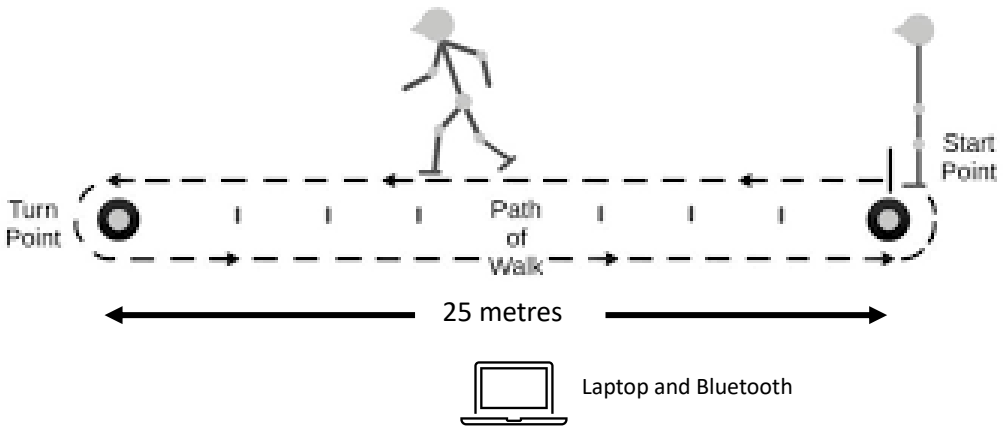
- A 10-minute rest period should always be given prior to the start of the test.
- A wheelchair should always be used to transport the participant to the test area.
- Participants should wear comfortable clothing and appropriate shoes for walking (i.e., trainers, etc).
- No orthotic devices are allowed other than insoles (extending below the ankle joint only).
- Walking aids (e.g., canes, walkers, rollators) should not be used.
- No support may be given by an assistant unless the participant needs help to rise from a fall or to sit down.
- Participants may not touch the wall while walking unless leaning on the wall to rest.

Course Set up

The test should be performed indoors, along a flat, straight, enclosed, and seldom travelled corridor at least 6 feet (approximately 2 meters) wide with a hard surface. The test area will be marked with a 25-meter tape line. The tape line should be placed in the middle of the corridor. Arrows indicating the counterclockwise direction and path of movement should be placed in a half-circle at the ends of the course. A tape should be placed as a starting line to the right of the first cone. Note that due to the possibility of patient falls, the course should be within easy access of appropriate medical assistance.

Testing Directions

- Set the stopwatch and count ticker to zero.
- Ask the patient to stand with his/her toes at the starting line, immediately adjacent to axis of the "home" cone.
- The following information should be communicated to the patient in a way they will understand.
  "You will be walking back and forth around these cones without crossing the line in the middle. You will walk around the cone in a half circle without slowing down. Then you will go back the other way. Remember that the object of this test is to walk as far as you can in six minutes without running."
- One "lap" is the distance from one cone to the other (i.e. 25 meters)
- When the patient is ready, say *"Ready, set, go!"*, and start the stopwatch.
- Every time the patient reaches the each end of the course, mark the worksheet to record the time at each 25 meters completed. Record the time as the participant crosses the mid-way point of the cones.
- A marker should be added for every 1 minute completed during the test.



Appendix G - Physical Activity sub analysis with adult (Eslinger) and Paediatric (Phillips) data parameters

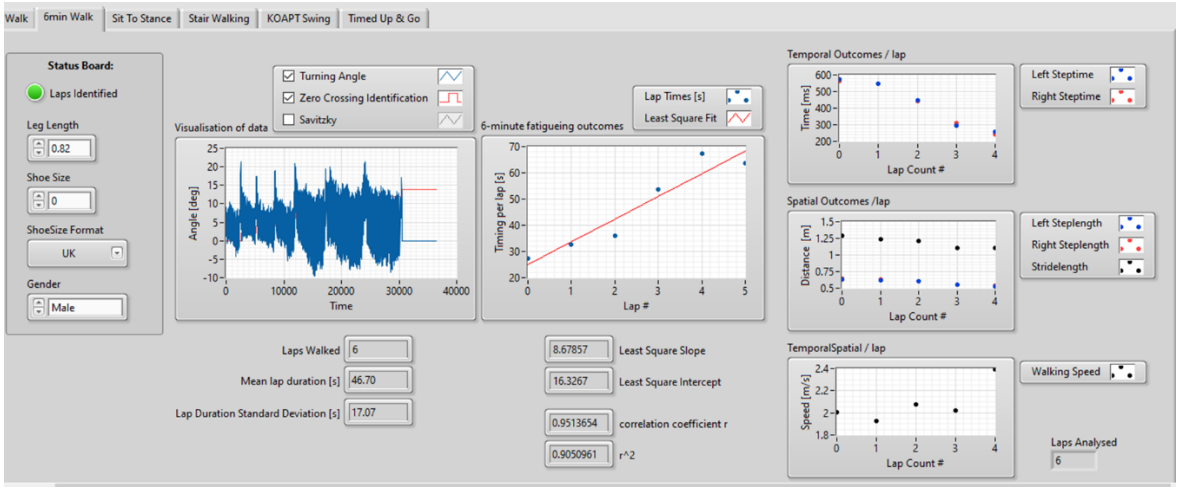
Genetic subtype	Age [years]	Height [cm]	Gender	Sedentary [%activity/week]		Light [%activity/week]		Moderate [%activity/week]		Vigorous [%activity/week]		MVPA [%activity/week]	
				Adult*	Paeds**	Adult*	Paeds**	Adult*	Paeds**	Adult*	Paeds**	Adult*	Paeds**
RAPSYN	11	141	Female	80.3	79.3	2.3	17.9	16.3	2.3	1.1	0.5	17.4	2.8
DOK7	11	132.5	Male	79.9	78.5	2.6	18.7	16.5	2.5	0.9	0.3	17.4	2.8
RAPSYN	15	182	Male	87.1	86.4	1.4	10.8	10.9	2.5	0.7	0.3	11.5	2.8
AChR def	16	155	Female	73	68.2	8.4	31.1	18.5	0.6	0.1	0	18.6	0.6
COLQ	10	146	Female	88.7	87.9	1.4	10	9.3	1.9	0.6	0.1	9.9	2.1
DOK7	10	139.5	Female	81.7	80.2	2.5	16.7	14.5	2.5	1.3	0.5	15.8	3.1
AChR def	13	169	Male	81.6	77.6	4.1	19.8	13.3	2.1	1.1	0.5	14.4	2.6
Mean				81.8	79.7	**3.2**	**17.9**	**14.2**	**2.1**	0.8	0.3	15.0	2.4

Physical Activity sub analysis with adult (Eslinger) and Paediatric (Phillips) data parameters. Adult (Eslinger 2011 data cut for adults)* [31] *Paeds** (Phillips 2012 data points for children age 10-16)*

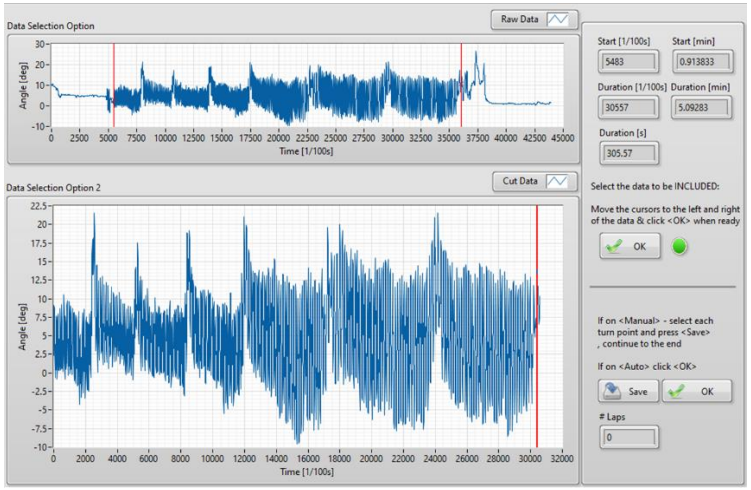
Appendix H – Myasthenia Gravis Activities of Daily Living (MG-ADL) score

Rate	0	1	2	3	Score (0,1,2 or 3)
1. Talking	Normal	Intermittent slurring or nasal speech	Constant slurring or nasal speech, but can be understood	Difficult to understand speech	
2. Chewing	Normal	Fatigue with solid food	Fatigue with soft food	Gastric tube	
3. Swallowing	Normal	Rare episode of choking	Frequent choking necessitating changes in diet	Gastric tube	
4. Breathing	Normal	Shortness of breath with exercise	Shortness of breath at rest	Ventilator dependence	
5. Impairment of ability to brush teeth or comb hair	None	Extra effort, but no rest periods needed	Rest periods needed	Cannot do one of these functions	
6. Impairment of ability to arise from a chair	None	Mild, sometimes uses arms	Moderate, always uses arms	Severe, requires assistance	
7. Double vision	None	Occurs, but not daily	Daily, but not constant	Constant	
8. Eyelid droop	None	Occurs, but not daily	Daily, but not constant	Constant	
				MG-ADL score total (items 1-8)	=

Appendix I – Gait Analysis 6MWT data interpretation



Screenshot capturing the LPMS data and gait analysis software view, with example of changes in cadence, lap time and step asymmetry.



Screenshot capturing the LPMS data and gait analysis software view, with example of increasing lap time and fatigue after 100m.