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# Miyoshi myopathy and limb girdle muscular dystrophy R2 are the same disease

Ursula Moore[a], Heather Gordish[b,c], Jordi Diaz-Manera[d,e], Meredith K. James[a], Anna G. Mayhew[a], Michela Guglieri[a], Roberto Fernandez-Torron[a], Laura E. Rufibach[f], Jia Feng[b], Andrew M. Blamire[g], Pierre G. Carlier[h], Simone Spuler[i], John W. Day[j], Kristi J. Jones[k], Diana X. Bharucha-Goebel[l,m], Emmanuelle Salort-Campana[n], Alan Pestronk[o], Maggie C. Walter[p], Carmen Paradas[q], Tanya Stojkovic[r], Madoka Mori-Yoshimura[s], Elena Bravver[t,1], Elena Pegoraro[u], Linda Pax Lowes[v], Jerry R. Mendell[v], Kate Bushby[a], Volker Straub[a,*], on behalf of The Jain COS Consortium

[a] *The John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Institute, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Central Parkway, Newcastle upon Tyne, United Kingdom*

[b] *Center for Translational Science, Division of Biostatistics and Study Methodology, Children's National Health System, Washington, DC, United States*

[c] *Pediatrics, Epidemiology and Biostatistics, George Washington University, Washington, DC, United States*

[d] *Centro de Investigación Biomédica en Red en Enfermedades Raras (CIBERER), Barcelona, Spain*

[e] *Neuromuscular Disorders Unit, Neurology Department, Hospital de la Santa Creu i Sant Pau*

[f] *The Jain Foundation, Seattle, Washington DC, United States*

[g] *Magnetic Resonance Centre, Translational and Clinical Research Institute, Newcastle University, United Kingdom*

[h] *AIM & CEA NMR Laboratory, Institute of Myology, Pitié-Salpêtrière University Hospital, 47-83 Paris, France*

[i] *Charite Muscle Research Unit, Experimental and Clinical Research Center, a Joint Cooperation of the Charité Medical Faculty and the Max Delbrück Center for Molecular Medicine, Berlin, Germany*

[j] *Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA, United States*

[k] *The Children's Hospital at Westmead, and The University of Sydney, Australia*

[l] *Department of Neurology Children's National Health System, Washington, DC, United States*

[m] *National Institutes of Health (NINDS), Bethesda, MD, United States*

[n] *Service des maladies neuromusculaire et de la SLA, Hôpital de La Timone, Marseille, France*

[o] *Department of Neurology Washington University School of Medicine, St. Louis, MO, United States*

[p] *Friedrich-Baur-Institute, Department of Neurology, Ludwig-Maximilians-University of Munich, Germany*

[q] *Neuromuscular Unit, Department of Neurology, Hospital U. Virgen del Rocío/Instituto de Biomedicina de Sevilla, Sevilla, Spain*

[r] *Centre de référence des maladies neuromusculaires, Institut de Myologie, AP-HP, Sorbonne Université, Hôpital Pitié-Salpêtrière, Paris, France*

[s] *Department of Neurology, National Center Hospital, National Center of Neurology and Psychiatry Tokyo, Japan*

[t] *Neuroscience Institute, Carolinas Neuromuscular/ALS-MDA Center, Carolinas HealthCare System, Charlotte, NC, United States*

[u] *Department of Neuroscience, University of Padova, Italy*

[v] *The Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH United States*

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**Abstract**

This study aims to determine clinically relevant phenotypic differences between the two most common phenotypic classifications in dysferlinopathy, limb girdle muscular dystrophy R2 (LGMDR2) and Miyoshi myopathy (MMD1). LGMDR2 and MMD1 are reported to involve different muscles, with LGMDR2 showing predominant limb girdle weakness and MMD1 showing predominant distal lower limb weakness. We used heatmaps, regression analysis and principle component analysis of functional and Magnetic Resonance Imaging data

* Corresponding author.
*E-mail addresses:* Volker.straub@newcastle.ac.uk, volker.straub@ncl.ac.uk (V. Straub).

[1] Deceased

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to perform a cross-sectional review of the pattern of muscle involvement in 168 patients from the Jain Foundation's international Clinical Outcomes Study for Dysferlinopathy. We demonstrated that there is no clinically relevant difference in proximal vs distal involvement between diagnosis. There is a continuum of distal involvement at any given degree of proximal involvement and patients do not fall into discrete distally or proximally affected groups. There appeared to be geographical preference for a particular diagnosis, with MMD1 being more common in Japan and LGMDR2 in Europe and the USA. We conclude that the dysferlinopathies do not form two distinct phenotypic groups and therefore should not be split into separate cohorts of LGMDR2 and MM for the purposes of clinical management, enrolment in clinical trials or access to subsequent treatments.


*Keywords:* [16] Clinical neurology examination; [21] Clinical trials methodology; [54] Cohort study; [176] All neuromuscular disease; [185] Muscle disease.

## 1. Introduction

Dysferlinopathy is an autosomal recessively inherited form of muscular dystrophy, which predominantly affects skeletal muscle, resulting in progressive weakness and muscle wasting.

Several clinical phenotypes are associated with mutations in the *DYSF* gene, most commonly limb girdle muscular dystrophy R2 (LGMDR2) and Miyoshi myopathy type 1 (MMD1). LGMDR2 describes a phenotype with predominant proximal lower and upper limb weakness at presentation [1], and was previously called LGMD2B before recent consensus review of LGMD nomenclature [2]. MMD1 describes a phenotype with predominantly distal lower limb weakness at presentation [3].

The *DYSF* gene was identified as the candidate gene simultaneously in patients with predominantly proximal [4] and predominantly distal disease [5]. Patients with the same DYSF mutations, including full siblings, have been described with clinically different patterns of weakness and ascribed different phenotypic diagnoses [4–6]. However, doubt about the presence of two truly distinct diagnosis was raised when pattern of muscle involvement on lower limb MRI was shown not to differ between LGMDR2 and MMD1 [7].

Dysferlinopathy is becoming a focus for clinical trials [8]. Multiple clinical diagnoses creates difficulties for trial design and therapy licencing. Pharmaceutical companies want to develop therapies for as many patients as possible, which is particularly important in a rare disease like dysferlinopathy. Regulators want to know if results from a trial involving LGMDR2 patients are applicable to patients with MMD1.

Here we compare the demographic, MRI, functional and genetic differences between 168 genetically confirmed dysferlinopathy patients with a clinical diagnosis of LGMDR2 or MMD1 to determine if they are distinct clinical phenotypes.

## 2. Methods

We analysed data from 168 patients with genetically confirmed dysferlinopathy enrolled in the Jain Foundation's 3-year longitudinal Clinical Outcomes Study for Dysferlinopathy (COS). This study received ethical approval in all participating countries. Participants attended 15 international sites for six evaluations (screening, baseline, 6 months, 12 months, 24 months, 36 months) between November 2012 and March 2018. Visits involved medical review, functional assessments and T1 weighted MRI of the lower limb muscles. The study protocol, patient demographics and genetic information, baseline results and a review of functional progression have been previously published [9,10].

Individual centres identified patients with dysferlinopathy from clinic lists to invite for screening. At study enrolment, 209 patients were screened and 197 met the inclusion criteria. Diagnostic inclusion criteria were the presence of a) two (predicted) pathogenic mutations in the DYSF gene, b) one (predicted) pathogenic mutation and absent dysferlin on muscle immunoblot or c), one (predicted) pathogenic mutation and dysferlin protein level $\leq$20% of normal level determined by blood monocyte testing. Patients also needed to be $\geq$ 10 years of age, able to perform study assessments, attend appointments and provide informed consent. Exclusion criteria were the presence of significant co-morbidity (in the opinion of the consenting clinician) or anticipated medical intervention which might interfere with ability to attend the assessments. Genetic and protein expression information are shown in supplementary Tables 1 and 2 and in Fig. 1.

Of these 197, all 10 patients from one site have been excluded from further analysis due to incomplete longitudinal data. One further patient became ineligible, after further genetic analysis identified a collagen VI related myopathy.

Patients' current clinical diagnosis was reported by the assessing clinician at screening either from reference to clinical notes (sites seeing local patients) or from patient self-report of the diagnosis given by their usual clinician (out of area patients). Of the 186 patients, 18 did not report a diagnosis of MMD1 or LGMDR2 – but rather proximodistal dysferlinopathy, asymptomatic hyperCKaemia, pseudometabolic dysferlinopathy or paravertebral muscular dystrophy. They were therefore excluded from this analysis, leaving 168 patients who had a baseline assessment. Of these, five further patients dropped out before year 2 and three missed the visit window, giving 160 (95%) with a 36-month assessment.

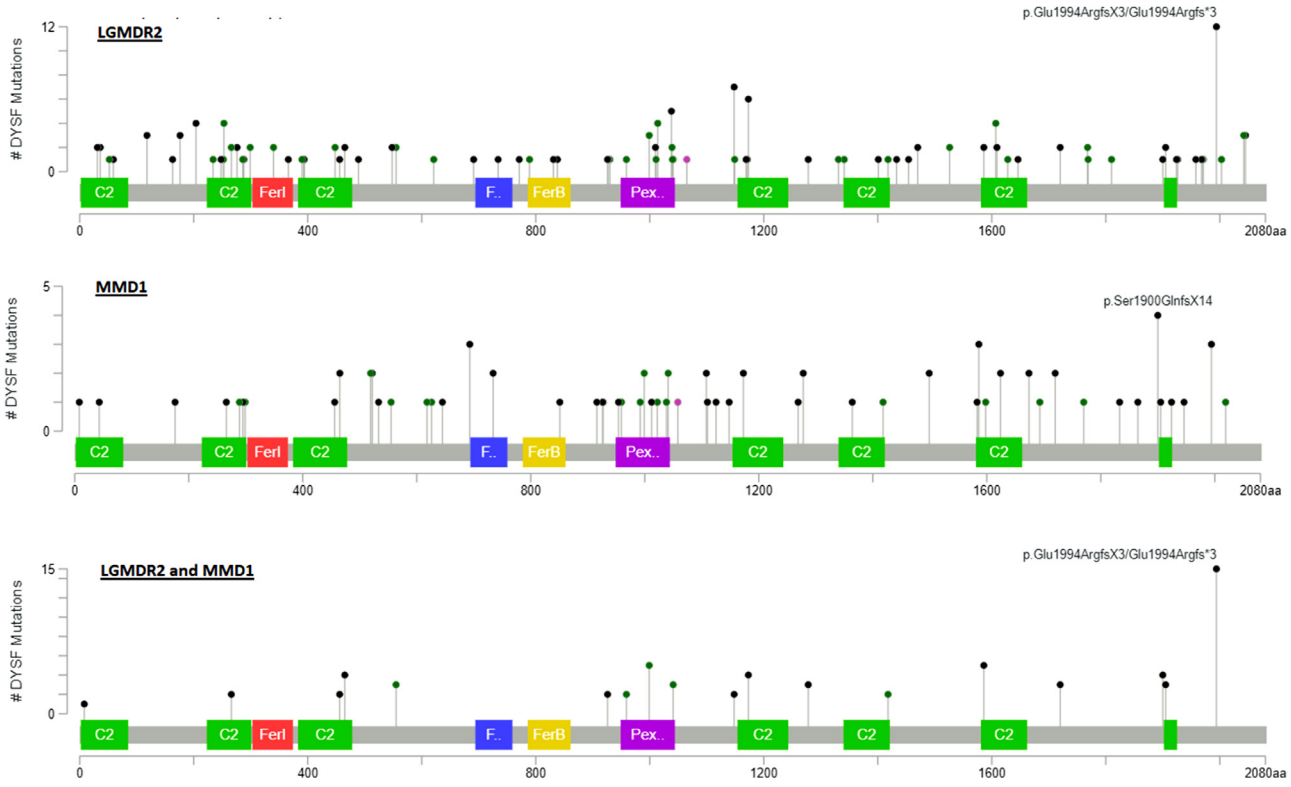


Fig. 1. Lollipop chart showing the location of pathogenic mutations in dysferlin exons. The chart separates mutations in LGMDR2 patients (top row), MMD1 patients (second row) and then shows those mutations that occur in both groups of patients (bottom row). The height of the bar corresponds to mutation frequency. Black circles denote truncating mutations and green circles denote missense mutations. This figure was generated using the open source 'mutation mapper' at https://www.cbioportal.org/mutation_mapper.

We used Wilcoxon rank sum testing to compare mean age of onset of muscle weakness (patient reported) and duration of symptoms and Chi-squared testing for comparing gender, previous steroid use, teenage exercise level and specific sports performed between groups.

We categorised teenage exercise levels into high and low, based on the metabolic equivalents and frequency of the exercise reported by patients, as previously described [11]. High levels of exercise describe 'moderate activity multiple times per week or vigorous activity at least weekly', with low levels as less than this.

To compare the pattern of muscle involvement between LGMDR2 and MMD1, we used assessments that graded functional ability or pathology in various muscle groups; North Star assessment for limb girdle type muscular dystrophies (NSAD), manual muscle testing (MMT) scores and Mercuri scores from T1 MRI. NSAD scores were taken from the year 2 study visit. MRI and MMT results were from the baseline assessment as this visit had the most complete assessments. Semiquantitative assessment of MRIs were performed by a blinded neurologist (RF-T) and radiologist (JL), who independently evaluated axial T1-weighted sequences with the Lamminen-Mercuri visual scale, as previously reported [12]. Patients with missing data were excluded from the analysis of the scale for which they did not have data. When the scales were combined, only patients with data from all of the scales were included in the comparison. Numbers of patients with complete data for each assessment are detailed below.

### 2.1. NSAD score

The NSAD score is a dysferlinopathy specific functional scale of motor performance, developed using RASCH analysis, which demonstrates measurable change over one year in Dysferlinopathy [13]. The scale comprises 29 tasks involving both upper and lower limbs, testing proximal and distal muscle function. Patients can score 0, 1 or 2 in each domain of the scale, corresponding to 'unable to perform task', 'modified method, but achieves task independent of physical assistance from another' and 'able to perform task'. Score declines as functional abilities are lost. As the scale was developed using the first assessments in the natural history study, direct measurements using the new scale were not performed until the year 2 visit. The NSAD scores used in this analysis are therefore from the year 2 visit.

To compare the order in which functional abilities were impacted, we ordered NSAD items by the cohorts mean score for each item. Items with the lowest score were those that fewest patients could complete. Thus, the order demonstrated the average sequence in which the ability to perform an item were lost. All item scores of each patient with LGMDR2 were compared to those with MMD1 using a heatmap (Fig. 2a). Mean item scores for LGMDR2 and MMD1 patients were

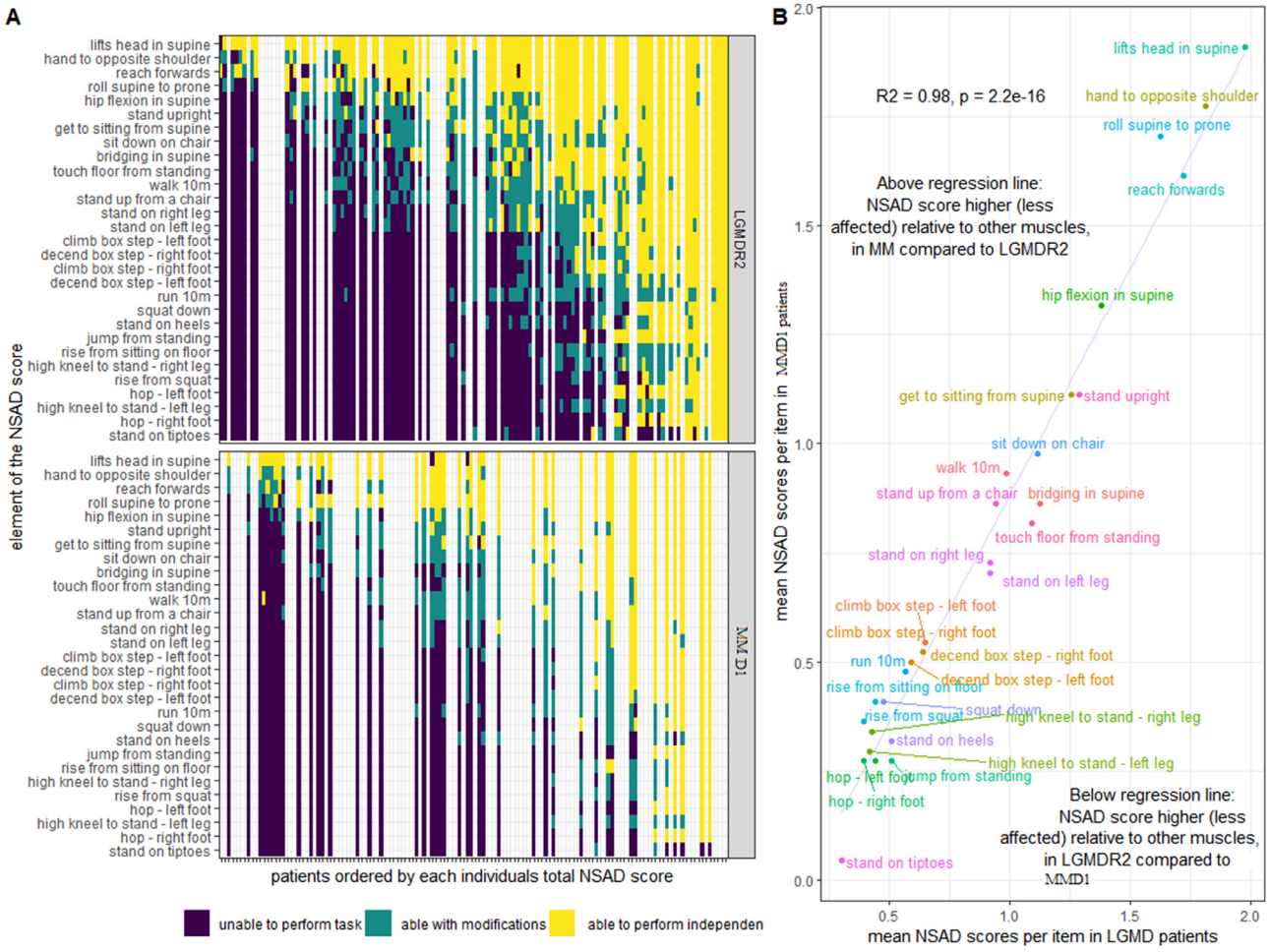


Fig. 2. Functional ability on the NSAD score compared by clinical diagnosis. A: Heatmap of individual patients NSAD scores, split by clinical diagnosis of LGMDR2 or MMD1 ($n = 130$). B: Scatter plot with linear regression line of the mean score on each component of the NSAD assessment for patients with a clinical diagnosis of LGMDR2 compared to patients with a clinical diagnosis of MMD1 ($n = 130$).

compared using linear regression (Fig. 2b). Points above the regression line represent preserved ability to complete these items, relative to other items, in MMD1 compared to LGMDR2. The standardised residual for each item was calculated using the R Function "rstandard()".

### 2.2. MMT score

The MMT score is a clinical measurement of strength widely used in general clinical practice. We used an 11 point conversion of the 5 point MRC scale (0, 1, 2, 3−, 3, 3+, 4−, 4, 4+, 5−, and 5) [9]. We ordered each movement by the mean MMT scores achieved by the cohort, as done for NSAD scores, and compared scores of each individual patient using a heatmap (Fig. 3a). Mean muscle MMT scores for LGMDR2 and MMD1 patients were compared using linear regression (Fig. 3b).

### 2.3. Mercuri score

Repeating the methods above, a heatmap was created for Mercuri MRI scores (Fig. 4a) and linear regression performed comparing the mean Mercuri score for each muscle between the two diagnoses (Fig. 4b). Mercuri scoring of the MRIs was performed manually with visual inspection of the muscle image [12].

### 2.4. Distal to proximal involvement ratio

To assess for subgroups of distally or proximally affected patients irrespective of clinical diagnosis, we compared proximal and distal MMT and Mercuri scores. The mean MMT score of distal muscles was plotted against the mean MMT score of proximal muscles for each individual to create a distal: proximal scatter plot (Fig. 3c). This method was repeated for Mercuri MRI scores (Fig. 4c). All muscle groups listed in the heat maps were used. 'Proximal' muscles were those above, or working across, the knee or elbow and 'distal' muscles were below the knee or elbow, working across the ankle or wrist.

As mean values were not normally distributed, the median MMT ratio was calculated for LGMDR2 and for MMD1 and compared using a Wilcoxon signed rank test. The Spearman's correlation coefficient between distal and proximal muscle

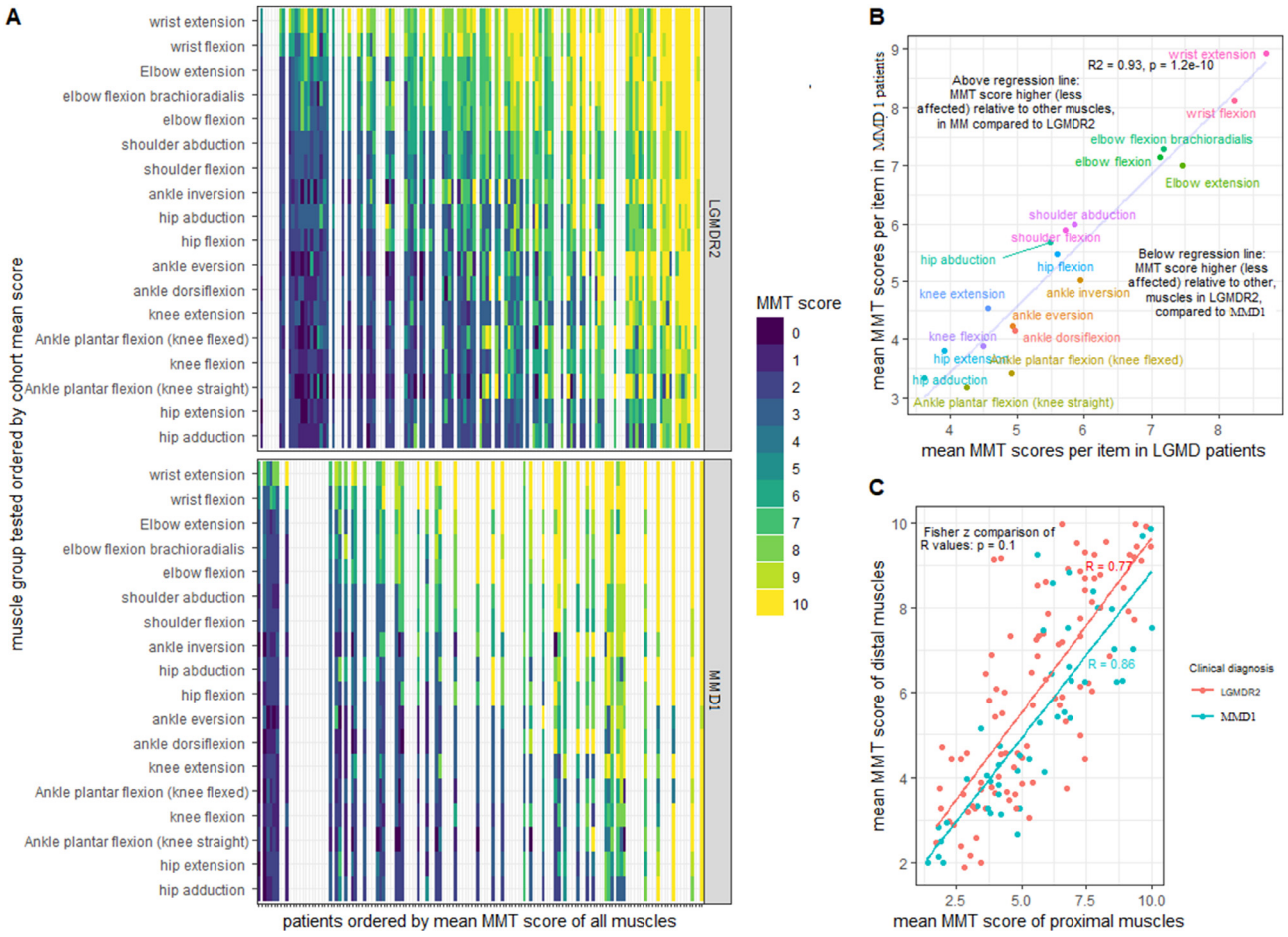


Fig. 3. Pattern of weakness assessed by the MMT score compared by clinical diagnosis. A: Heatmap of individual patients MMT scores, split by clinical diagnosis of LGMDR2 or MMD1, ($n = 143$). B: Scatter plot with linear regression line of the mean score for each movement tested by MMT for patients with a clinical diagnosis of LGMDR2 compared to patients with a clinical diagnosis of MMD1 ($n = 143$). C: Scatter plot with linear regression line of mean distal MMT score against mean proximal MMT score for each individual patient ($n = 143$).

groups for LGMDR2 and for MMD1 was compared using z-scores for a two tailed test, after a Fisher z transformation. Power calculation demonstrated 87% power to detect a difference in median distal:proximal MMT score ratio of $>0.2$.

To assess for site specific differences in muscle involvement, the mean MMT and MRI Mercuri score distal: proximal ratios were compared between patients from Tokyo (high number of MMD1 patients) and Newcastle (high number of LGMDR2 patients) using a scatter plot and Spearman's correlation (Fig. 6a and b) in the same way as for comparing clinical diagnosis.

Post-hoc power calculations, to assess the ability to detect differences between MMD1 and LGMD groups, were performed using an online tool [14].

### 2.5. Principle component analysis

A principal component analysis (PCA) was carried out using the R package ggbiplot [15]. This was completed for NSAD scores, MMT scores and MRI both individually and with all three assessments combined. PCA is a statistical analysis and data visualisation tool for comparing multiple variables [16]. It takes all of the variables for each individual and positions that individual on a 2D chart of principal components, in a way that maximises the variation between individuals. This means that each variable becomes a vector along which individuals are positioned. In this way, groups of individuals with distinct characteristics are separated – for example if MMD1 patients had a weaker soleus muscle relative to the rectus femoris than LGMDR2 patients, then MMD1 and LGMDR2 would form two distinct groups on the PCA, without the programmer needing to know which variable would pick out the differences.

### 2.6. Data availability statement

Anonymised aggregate data will be provided on reasonable request. Requests should be made to the clinical outcomes study steering committee by contacting the corresponding author.

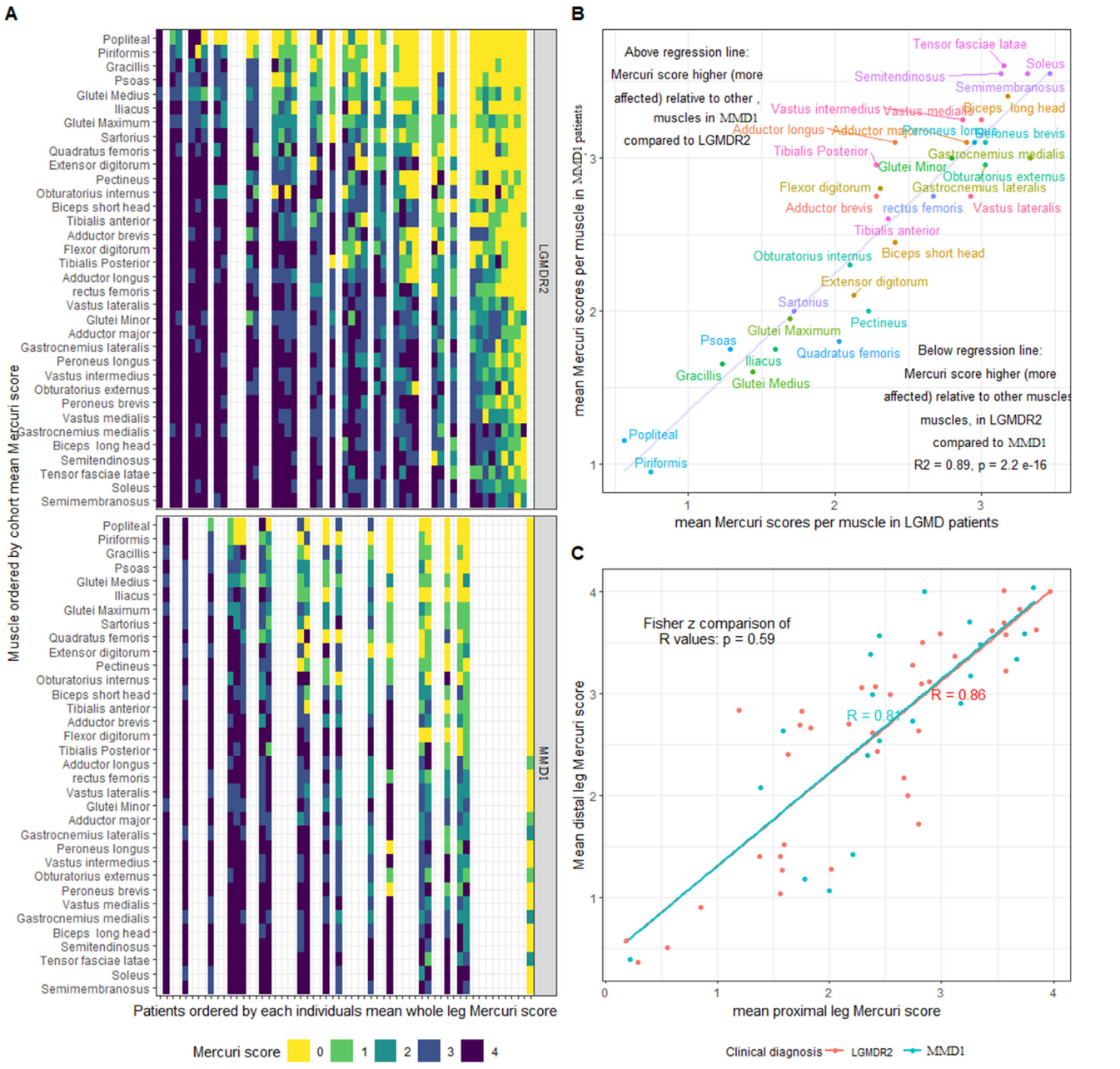


Fig. 4. Pattern of weakness assessed by Mercuri scoring of leg T1W MRI compared by clinical diagnosis. A: Heatmap of individual patients Mercuri MRI scores, split by clinical diagnosis of LGMDR2 or MMD1 ($n = 59$). B: Scatter plot with linear regression line of mean Mercuri score for each leg muscle for patients with a clinical diagnosis of LGMDR2 compared to patients with a clinical diagnosis of MMD1 ($n = 59$). C: Scatter plot with linear regression line of Mean Mercuri grading for each distal muscle against mean Mercuri grading for each proximal muscle ($n = 59$).

## 3. Results

### 3.1. Demographics

At baseline, the cohort consisted of 114 patients with a clinical diagnosis of LGMDR2 and 54 patients with MMD1 (Table 1). Age at symptom onset and at assessment did not differ between clinical diagnoses ($p > 0.05$). Median symptom duration was higher in MMD1 patients than in LGMDR2 patients ($p = 0.048$). Gender, proportion of non-ambulant patients, previous steroid treatment and teenage exercise intensity did not differ between clinical diagnoses ($p > 0.05$).

### 3.2. Genetics

LGMDR2 and MMD1 occurred in patients with the same genotype, with 12 patients showing the same pair of mutations as a patient with the other clinical diagnosis (supplementary Table 1 and 2). Discordant phenotypes for the same genotype did not segregate based on gender, ethnicity, location or teenage exercise level. There were 12 pairs of siblings, who all shared their sibling's diagnosis.

Patients with both LGMDR2 and MMD1 displayed a range of missense, non-sense and splice site mutations throughout

Table 1
Demographic information.

| Clinical diagnosis | LGMDR2 | MMD1 |
|---|---|---|
| Number of participants | 114 | 54 |
| Median age symptom onset (range) | 19 yrs (0–48) | 18 yrs (12–46) |
| Median age at assessment (range) | 36.5 yrs (11–86) | 37 yrs (19–62) |
| Median symptom duration*(range) | 14 yrs (1–51) | 20 yrs (4–45) |
| Male: female | 45:69 | 30:24 |
| Ambulant | 86 (75%) | 37(69%) |
| Previous steroid treatment | 38 (33%) | 26 (48%) |
| active teenage lifestyle (cat 2/3) | 74 (65%) | 35 (65%) |
| *Ethnicity* | | |
| White (%) | 79 (69%) | 38 (70%) |
| Asian (%) | 21 (18%) | 11 (20%) |
| Black (%) | 3 (3%) | 0 (0%) |
| Hispanic (%) | 9 (8%) | 2 (4%) |
| Mixed race (%) | 2 (2%) | 0 (0%) |
| Other (%) | 0 (0%) | 3 (6%) |
| *Patient location* | | |
| Europe (%) | 65 (57%) | 27 (50%) |
| Spain (Barcelona, Seville) | 9 (5, 4) | 6 (0, 6) |
| Italy (Padova) | 7 | 8 |
| Germany (Berlin, Munich) | 11 (4, 7) | 2 (0, 2) |
| France (Paris) | 4 | 6 |
| UK (Newcastle) | 34 | 5 |
| USA (Charlotte, Columbus, Stanford, St Louis, Washington DC) (%) | 41 (6, 21, 10, 4, 0) (36%) | 17 (6, 2, 2, 2, 5) (31%) |
| Australia (Sydney) (%) | 4 (4%) | 1 (2%) |
| Japan** (Tokyo) (%) | 4 (4%) | 9 (17%) |

No difference in age symptom onset or age at assessment on Wilcoxon rank sum test.

* Symptom duration differs between groups ($p=0.048$ on Wilcoxon rank sum test).Gender, previous steroid treatment and teenage activity category not significantly different on Chi squared testing.

** Using Chi squared test patients were more likely to have a MMD1 diagnosis in Japan than in Europe ($p=0.01$) or US sites ($p=0.02$).

the length of the *DYSF* gene. Neither group displayed a mutational hotspot (Fig. 1).

### 3.3. NSAD sequence

NSAD scores were available for 130 patients at visit 5 (86 LGMDR2 and 44 MMD1). Scores were generally lower for the MMD1 patients (Fig. 2b), suggesting a weaker cohort. The sequence in which functional abilities were lost was variable between patients but similar between LGMDR2 and MMD1 (Fig. 2a and 1b). The most common ability to be lost was standing on tiptoes for both clinical diagnoses (Fig. 2b). In general, distal functions appeared below the regression line, suggesting they were more impaired in MMD1 than LGMDR2 patients for a given overall score, although standardised residuals for all items were small (<1.5).

### 3.4. MMT sequence

Complete MMT scores were available for 143 patients at baseline (visit 2) (95 LGMDR2 and 48 MMD1. Scores were generally lower for the MMD1 patients (Fig. 3b). The sequence in which MMT scores deteriorated was variable between patients (Fig. 3a) but similar between LGMDR2 and MMD1 (Fig. 3b). Distal lower limb movements appeared slightly more affected in MMD1, (below the regression line in Fig. 3b), particularly ankle plantar flexion (knee flexed), which showed the largest residual. Proximal lower limb movements were involved slightly more prominently in LGMDR2, with hip extension having the largest residual. Upper limb movements were generally less affected in both groups of patients and did not appear to separate by diagnosis. Overall, while the ratio of mean distal: proximal involvement did vary between patients (Fig. 3c) it did not vary by diagnosis, with no significant difference between median distal: proximal ratios (LGMDR2 = 1.06, MMD1 = 0.97, $p=0.1$) and no difference between regression coefficient ($p=0.10$) (Fig. 3c). Sample size was adequate to detect a true difference in ratio of greater than 0.18. These ratios did not highlight discrete groups of distally or proximally affected patients, irrespective of diagnosis (Fig. 3c).

### 3.5. MRI sequence

A complete set of Mercuri scores for thigh and lower leg muscles (whole leg) was available for 59 patients (39 LGMD and 20 MMD1) and for lower leg muscles for 131 patients (84 LGMDR2 and 47 MMD1) at their baseline visit. Mercuri scores across all muscles were generally lower for the MMD1 patients (Fig. 4b). Heatmaps and regression showed a variable pattern of muscle involvement between patients (whole leg – Fig. 4a, lower leg – Fig. 5), but the sequence of severity of involvement was similar between LGMDR2 and MMD1 (whole leg - Fig. 4b). The largest standardised residuals were seen for the gastrocnemius medialis muscle, being more prominently involved in LGMDR2 than MMD1, and for the

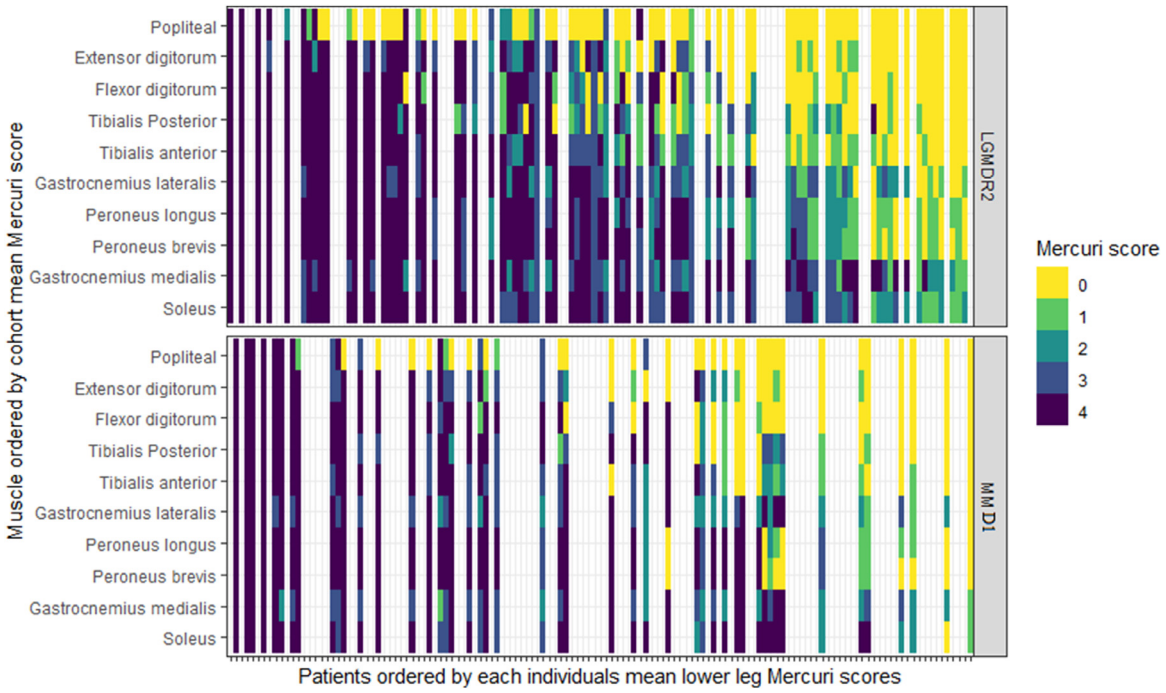


Fig. 5. Heatmap of the lower leg T1W MRI Mercuri scores ($n = 131$) by clinical diagnosis.

adductor longus muscle, being more prominently involved in MMD1 than in LGMDR2. The ratio of mean distal: proximal involvement did vary between patients (Fig. 4c), but not by diagnosis, with no difference between median distal: proximal ratio (LGMDR2 = 1.08, MMD1 = 1.04, $p = 0.97$) and no difference between regression coefficient ($p = 0.59$) (Fig. 4c). Sample size was adequate to detect a true difference in ratio of greater than 0.37. These ratios did not highlight discrete groups of distally or proximally affected patients, irrespective of diagnosis (Fig. 4c).

### 3.6. Site to site variation

A greater proportion of Japanese patients had a diagnosis of MMD1 (64%) than Europeans (30%, true difference on chi-squared $p = 0.01$) or Americans (32%, $p = 0.02$). In comparing patients from Tokyo (high proportion of MMD1) and Newcastle (high proportion of LGMDR2), there was no difference in regression coefficients for distal: proximal MMT ($p = 0.87$) and Mercuri MRI ($p = 0.56$) scores (Fig. 6a and b). In Newcastle, all patients with an MMD1 diagnosis showed relative distal weakness on MMT distal: proximal ratio, while the pattern was more varied in LGMDR2 patients (Fig. 6a). In Tokyo, all patients with an LGMDR2 diagnosis showed relative proximal weakness on MMT distal: proximal ratio, while the pattern was more varied in MMD1 patients (Fig. 6a). On MRI, patients distal: proximal ratio of Mercuri scores did not group by diagnosis at either site (Figure b). PCA analysis of NSAD score, MMT score and MRI did not split Tokyo and Newcastle patients into distinct groups (Fig. 7b)

### 3.7. Principal component analysis

LGMDR2 and MMD1 patients did not separate into distinct groups based on PCA analysis of NSAD score, MMT score or MRI analysis when each scoring systems was assessed individually (data not shown) or when combined (Fig. 7a).

## 4. Discussion

We have demonstrated that MMT scores, the NSAD and the pattern of muscle involvement on MRI were similar between the two most common clinical diagnoses in dysferlinopathy - MMD1 and LGMDR2.

Distal involvement was common to both diagnoses, even in patients with otherwise mild functional impairment, with difficulty standing on tiptoes being the most commonly affected function in both LGMDR2 and MMD1. Although MMD1 patients showed a slightly more distal phenotype, with ankle plantar flexion (knee flexed) and some distal NSAD functions being more prominently involved than in LGMDR2, these differences were not statistically significant and there was a high degree of overlap in pattern of weakness between both diagnoses. Upper limb involvement, as measured by MMT scores and functional elements, was less common in both diagnosis and distal upper limb weakness was not more common in either group.

In addition to the overlap between groups, some patients with an MMD1 diagnosis displayed an LMGDR2 phenotype, with striking preservation of distal strength but marked proximal weakness and vice versa for LGMDR2 patients

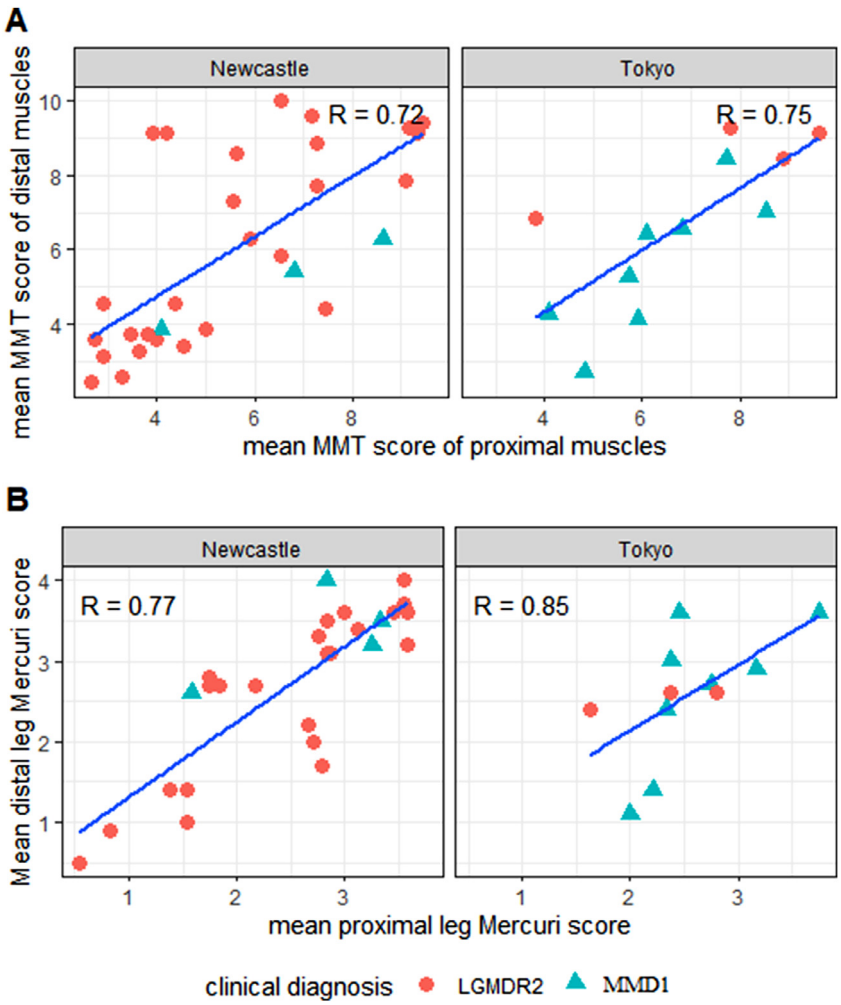


Fig. 6. Comparison of distal vs proximal weakness in Newcastle and Tokyo. A: Mean distal MMT score against mean proximal MMT score for each individual patient in Newcastle ($n=30$) and Tokyo ($n=12$). There was no significant difference between Spearman's R coefficient ($p=0.87$). B: Mean distal Mercuri score against mean proximal Mercuri score for each individual patient in Newcastle ($n=26$) and Tokyo ($n=11$). There was no significant difference between Spearman's R coefficient ($p=0.56$).

displaying an MMD1 phenotype. This suggests either that phenotypes were variable and have changed since the time of diagnosis, or that factors other than pattern of weakness influenced the diagnosis.

One potential factor influencing diagnosis is a sites' previous experience. A clinical diagnosis of MMD1 was more common in Japan while a diagnosis of LGMDR2 was more common in Europe and the United States; however, we found no purely ethnic variation in diagnosis prevalence, no distinct patterns of weakness on PCA and no variation in the degree of distal muscle involvement between sites. Only those Tokyo patients with markedly greater proximal than distal weakness had an LGMDR2 diagnosis, while patients in Newcastle only had an MMD1 diagnosis if they were particularly weak distally. We speculate that this may be explained by a geographical preference for diagnosis. Japanese sites may be more likely to diagnose MMD1 as the default, unless presentation is strikingly different, due to site experience, as MMD1 was first described in a Japanese cohort [3]. LGMDR2 may be the default diagnosis in the West, having been first described in a European cohort [1]. However, we did not assess what the pattern of weakness was at initial presentation. It remains possible that a true difference in pattern of presentation exists, but this study was not close enough to the time of diagnosis, and that MMD1 and LGMDR2 were both described in the geographic locations where each presentation was more common.

A potential weakness in this study is the wide range of age and disease severity of patients when they were assessed. MMD1 patients had generally had symptoms for longer, were slightly weaker than the LGMDR2 patients and may have presented later initially if they only had mild calf weakness. In addition, because the NSAD assessment was developed and validated during COS, the most complete scores were from the year 2 (5th) visit, adding a further 2 years to each individual's disease progression at the point of assessment. The variation between individuals was greatest in less affected patients, so it is possible that a larger cohort of minimally affected patients would demonstrate true phenotypic differences. However, in this cohort, even amongst

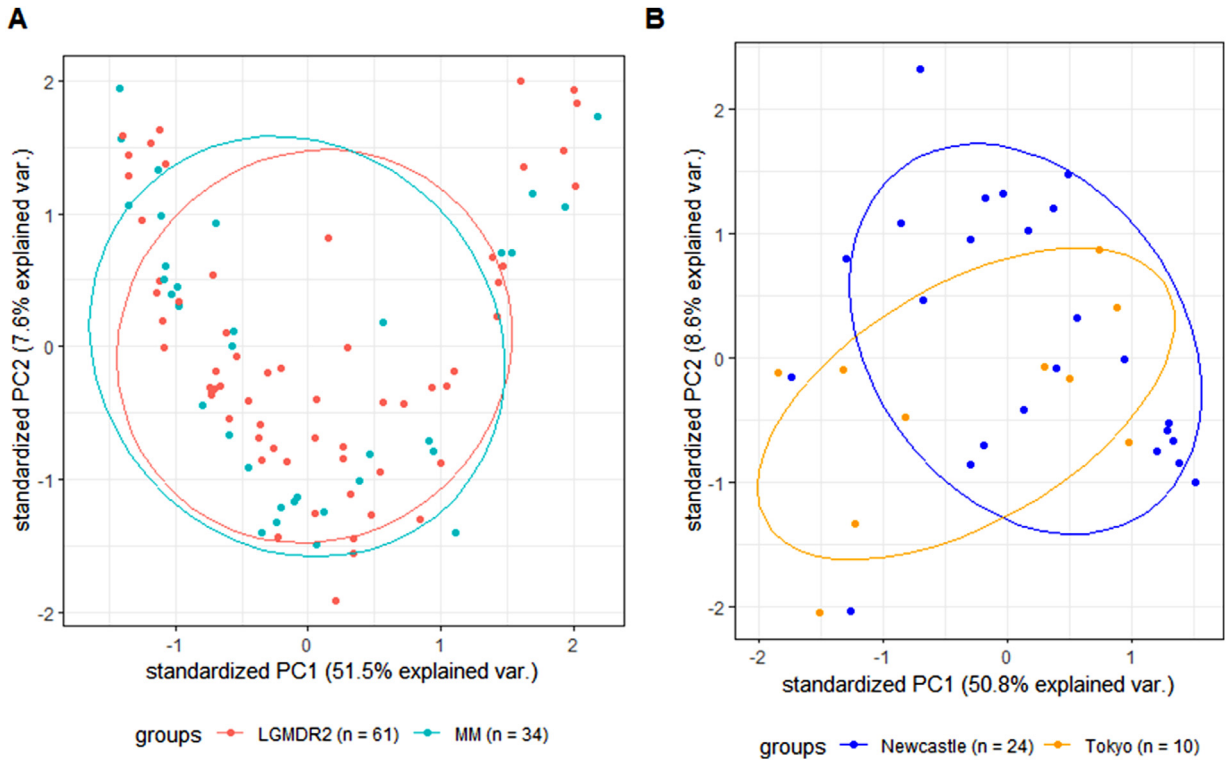


Fig. 7. Principal component analysis of assessments between a. diagnosis and b. study site. A: Principle component analysis (PCA) of NSAD, MMT scores and lower leg Mercuri MRI score showing no difference in weighting of principle components between diagnoses. B: Principle component analysis (PCA) of NSAD, MMT scores and lower leg Mercuri MRI score showing no difference in weighting of principle components between Newcastle and Tokyo.

the mildly affected patients, the pattern of muscle involvement varied and overlapped between diagnostic groups. Even if there were a difference early in the disease course, it seems that the clinical diagnoses given at presentation does not continue to best describe the phenotype of a dysferlinopathy patient several years into their disease.

Although not forming distinct groups, a wide range in pattern of muscle involvement is evident and suggests there may be multiple, rather than a single, genetic or environmental disease modifying factors [17]. The variation is not explained by the underlying dysferlin mutations, as patients in this (and previous) studies with the same dysferlin genotype have different clinical diagnoses and patterns of weakness [5,6]. Although siblings in this cohort shared the same clinical diagnosis, previous studies have demonstrated otherwise [6]. Ethnicity also does not seem to influence diagnostic prevalence. We also reviewed exercise intensity and type, which are known to vary culturally, [18] but did not identify any association with clinical diagnosis. This suggests that pattern of weakness is not related to demographic factors, such that these findings should be generalizable to the wider population of patients with dysferlinopathy.

The patient population with dysferlinopathy demonstrate variation in rates of disease progression and the degree of distal:proximal muscle involvement, particularly early in the disease. Developing interventional therapies for such a heterogenous cohort is complex and there are incentives to form smaller, more homogeneous cohorts of patients. However, for several reasons, we argue that a patient's clinical diagnosis should not be the element used to create cohorts for clinical trials:

(1) Diagnostic criteria for each diagnosis are not clearly defined allowing individual and regional variation in ascribing diagnosis.
(2) There is significant phenotypic overlap between clinical diagnostic groups even in early stages.
(3) Validated, dysferlinopathy specific, outcome measures (the NSAD) are available that incorporate both distal and proximal muscle functions to allow progression to be monitored across the spectrum of disease.
(4) Outcome measures including the NSAD, 10 m walk and timed up and go show no difference in rate of progression over three years between patients with MMD1 and LGMDR2 [19].

These points suggest that combining the clinical diagnoses of MMD1 or LGMDR2 into a common unified group of 'dysferlinopathies', is important for clinical trials in order to prevent potential detrimental artificial distinctions and the risk oftreatments being developed with and for one specific group and not the other, when both could benefit.

Mutations in the *DYSF* gene are associated with multiple different clinical diagnoses, including LGMDR2 and MMD1. We have shown that pattern of weakness did not separate patients into distinct clinical entities, with significant functional overlap between and within diagnostic groups. Initial clinical diagnosis was not a reliable predictor of future

pattern of weakness, functional ability or rate of deterioration. Therefore, for the purposes of monitoring and the evaluation of therapeutic interventions, we recommend that patients with dysferlinopathy be considered as a single cohort, rather than being separated based on initial clinical diagnosis.

## Declaration of Competing Interest

None.

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## Statistical analysis

Statistics conducted by Dr Ursula Moore, academic affiliation 1.

Statistical approach advised by Dr Heather Gordish Dressman, academic affiliation 2 and 3.

## Study funding

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## Study sponsorship and author disclosures

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As detailed below individuals disclosure interests outside the scope of this work and the grant from the Jain Foundation. As a natural history study, there are no products that are being tested in this study. Therefore in the view of the corresponding author and consortium lead there are no conflicts of interest relevant to this work.

Ursula Moore reports the grant from the Jain Foundation

Heather Gordish reports the grant from the Jain Foundation

Jordi Díaz-Manera reports no disclosures

Meredith K. James reports the grant from the Jain Foundation

Anna G. Mayhew reports no disclosures

Michela Guglieri reports the grant from the Jain Foundation

Roberto Ferenandez Torron reports no disclosures

Laura E. Rufibach works for the Jain Foundation

Jia Feng reports no disclosures

Andrew M. Blamire reports the grant from the Jain Foundation

Pierre G. Carlier MD reports no disclosures

Simone Spuler reports no disclosures

John W. Day reports the grant from the Jain Foundation, personal fees from Biogen, Ionis, Avexis, Roche, Sarepta, Sanofi, Genzyme, Scholar Rock, Pfizer plus patents from Athena Diagnostics.

Kristi J. Jones reports no disclosures

Diana X. Bharucha-Goebel reports membership of the Gene Therapy Network (Avexis)

Emmanuelle Salort-Campana reports no disclosures

Alan Pestronk reports the grant from the Jain Foundation

Sabine Krause reports no disclosures

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Carmen Paradas reports no disclosures

Tanya Stojkovic reports no disclosures

Madoka Mori-Yoshimura reports no disclosures

Elena Bravver deceased

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Linda Pax Lowes reports no disclosures

Jerry R. Mendell reports no disclosures

Kate Bushby reports no disclosures

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2021.01.009.

## Appendix 1. Authors

| Name | Location | Contribution |
|---|---|---|
| Ursula Moore | *The John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Institute, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Central Parkway, Newcastle upon Tyne, UK* | conception and design of the study, acquisition and analysis of data, preparation of the manuscript |
| Heather Gordish | *Centre for translational science, division of biostatistics and study methodology, children's national health system, washington, dc, usa,* | Review and design of statistical analysis for the study, drafting |
| Jordi Diaz-Manera | *Neuromuscular Disorders Unit, Neurology Department, Hospital de la Santa Creu i Sant Pau,* | Conception and design of the study, acquisition and analysis of data, drafting |
| Meredith K. James | *The John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Institute, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Central Parkway, Newcastle upon Tyne, UK* | Conception and design of the study, acquisition and analysis of data, drafting |
| Anna G. Mayhew | *The John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Institute, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Central Parkway, Newcastle upon Tyne, UK* | Conception and design of the study, acquisition and analysis of data, drafting |
| Michela Gulgieri | *The John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Institute, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Central Parkway, Newcastle upon Tyne, UK* | Acquisition and analysis of data, drafting |
| Roberto Fernandez Torron | *The John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Institute, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Central Parkway, Newcastle upon Tyne, UK* | Acquisition and analysis of data |
| Laura E. Rufibach | *6 The Jain Foundation, Seattle, Washington, USA* | Conception and design of the study, acquisition and analysis of data, drafting |
| Jia Feng | *Centre for translational science, division of biostatistics and study methodology, children's national health system, washington, dc, usa,* | Acquisition and analysis of data |
| Andrew M. Blamire | *Magnetic Resonance Centre, Translational and Clinical Research Institute, Newcastle University, UK* | Conception and design of the study, drafting |
| Pierre G. Carlier | *AIM & CEA NMR Laboratory, Institute of Myology, Pitié-Salpêtrière University Hospital, 47–83, Paris, France* | Conception and design of the study, drafting |
| Simone Spuler | *Charite Muscle Research Unit, Experimental and Clinical Research Centre, a joint cooperation of the Charité Medical Faculty and the Max Delbrück Centre for Molecular Medicine, Berlin, Germany* | Acquisition and analysis of data, drafting |
| John W. Day | *Department of Neurology and Neurological Sciences, Stanford University School of Medicine; Stanford, CA, USA* | Acquisition and analysis of data, drafting |
| Kristi J. Jones | *The Children's Hospital at Westmead, and The University of Sydney, Australia.* | Acquisition and analysis of data, drafting |
| Diana X. Bharucha-Goebel | *Department of Neurology Children's National Health System, Washington, DC, USA* | Acquisition and analysis of data, drafting |
| Emmanuelle Salort-Campana | *Service des maladies neuromusculaire et de la SLA, Hôpital de La Timone, Marseille, France* | Acquisition and analysis of data, drafting |
| Alan Pestronk | *Department of Neurology Washington University School of Medicine, St. Louis, MO, USA* | Acquisition and analysis of data, drafting |
| Maggie C. Walter | *Friedrich-Baur-Institute, Dept. of Neurology, Ludwig-Maximilians-University of Munich, Germany* | Acquisition and analysis of data, drafting |
| Carmen Paradas | *Neuromuscular Unit, Department of Neurology, Hospital U. Virgen del Rocío/Instituto de Biomedicina de Sevilla, Sevilla, Spain* | Acquisition and analysis of data, drafting |
| Tanya Stojkovic | *Centre de référence des maladies neuromusculaires, Institut de Myologie, AP-HP, Sorbonne Université, Hôpital Pitié-Salpêtrière, Paris, France* | Acquisition and analysis of data, drafting |
| Madoka Mori-Yoshimura | *Department of Neurology, National Centre Hospital, National Centre of Neurology and Psychiatry Tokyo, Japan* | Acquisition and analysis of data, drafting |
| Elena Bravver | *Neuroscience Institute, Carolinas Neuromuscular/ALS-MDA Centre, Carolinas HealthCare System, Charlotte, NC, USA.* | Acquisition and analysis of data, drafting |
| Elena Pegoraro | *Department of Neuroscience, University of Padova, Italy* | Acquisition and analysis of data, drafting |
| Linda Pax Lowes | *The Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, Ohio USA* | Acquisition and analysis of data, drafting |
| Jerry R. Mendell | *The Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, Ohio USA* | Acquisition and analysis of data, drafting |
| Kate Bushby | *The John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Institute, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Central Parkway, Newcastle upon Tyne, UK* | Study design, acquisition and analysis of data, drafting |
| Volker Straub | *The John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Institute, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Central Parkway, Newcastle upon Tyne, UK* | Conception and design of the study, acquisition and analysis of data, drafting, final sign off, corresponding author |

## Appendix 2 Coinvestigators - The Jain COS Consortium

We acknowledge the work of the following members of the Consortium who have contributed to the collection of the data, but do not qualify for authorship on this paper.

| Name | Location | Role | Contribution |
|---|---|---|---|
| Marni Jacobs | Children's National Medical Centre Washington DC, USA | Study Statistician | Oversight of data management Data Management and Training |
| Adrienne Arrieta MS | Children's National Medical Centre Washington DC, | Lead Data Management Expert | Data Base development and training |
| Esther Hwang | Jain Foundation, Seattle, USA- | Patient Advocate | Development of study questionnaires Recruitment, |
| Elaine Lee PhD | Jain Foundation, Seattle, USA-, | Patient Advocate | Development of study questionnaires Recruitment |
| Isabel Illa MD | Hospital de la Santa Creu i Sant Pau/ CIBERER, Barcelona, Spain | Director of the Neuromuscular Unit | Study Design |
| Eduard Gallardo MD | Hospital de la Santa Creu i Sant Pau/ CIBERER, Barcelona, Spain | Consultant Scientist | Confirmation of Eligibility using monocyte assay |
| Izaskun Belmonte Jimeno PT | Servei de Medicina F ísica i Rehabilitació, Hospital de la Santa Creu i Sant Pau | Physiotherapist | Acquisition of physiotherapy data |
| Elena Montiel- Morillo | Servei de Medicina F ísica i Rehabilitació, Hospital de la Santa Creu i Sant Pau | Physiotherapist | Acquisition of physiotherapy data |
| Irene Pedrosa-Hernandez | Servei de Medicina F ísica i Rehabilitació, Hospital de la Santa Creu i Sant Pau | Physiotherapist | Acquisition of physiotherapy data |
| Jaume LLauger Rossello | Radiology department, Universitat Autònoma de Barcelona, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, | Radiologist | Collection and Analysis of MRI data |
| Bruce Harwick | Department of Radiology, CMC Mercy Charlotte, Carolinas Healthcare System Neurosciences Institute, Charlotte, NC, USA | NMR Technologist | MRI Data Acquistion |
| Jackie Sykes RN, BSN | Carolinas HealthCare System, Charlotte, NC, | Study Coordinator | Study Coordination and Data Management |
| Susan Sparks | Carolinas HealthCare System, Charlotte, NC, | Neurologist | Recruiting PI and PI for first three visits. |
| Scott Holsten | Carolinas HealthCare System, Charlotte, NC, | Physiotherapist | Acquisition of physiotherapy data |
| Lindsay Alfano | Nationwide Children's Hospital, Columbus, OH, | Physiotherapist | Acquisition of physiotherapy data |
| Brent Yetter, MS | Nationwide Children's Hospital, Columbus, OH, | Study Coordinator | Study Coordination and Data Management |
| Mark Smith MS, DABMP RT(MR) | Department of Radiology, Nationwide Children's Hospital, Columbus, OH, USA, | MRI Technologist | MRI Data Acquisition |
| Bernard Lapeyssonie PT | Neuromuscular and ALS Centre, La Timone Hospital, Aix-Marseille Université, Marseille, | Physiotherapist | Acquisition of physiotherapy data |
| Bruno Vandevelde | Neuromuscular and ALS Centre, La Timone Hospital, Aix-Marseille Université, Marseille, | Physiotherapist | Acquisition of physiotherapy data |
| David Bendahan PhD | Centre de Résonance, MagnétiqueBiologique et Médicale, UMR CNRS 7339, Marseille, France, | MRI Lead | MRI Technologist |
| Yann Le Fur PhD | Aix-Marseille Université, Marseille, France, | MRI Technologist | MRI Data Acquisition |
| Attarian Shahram, MD, PhD | Neuromuscular and ALS Centre, La Timone Hospital, Aix-Marseille Université, Marseille, | Study Doctor | Medical History, Medical and Physical Examination |
| Testot-Ferry Albane, CRA | Neuromuscular and ALS Centre, La Timone Hospital, Aix-Marseille Université, Marseille, | Study Coordinator | Study Coordination and Data Management |
| Eva M. Coppenrath MD, | Department of Clinical Radiology, Ludwig-Maximilians-University Munich, Germany, | Radiologist | MRI Data Acquisition |
| Simone Thiele | Ludwig-Maximilians-University Munich, Germany, | Physiotherapist | Acquisition of physiotherapy data |
| Sabine Krause | Ludwig-Maximilians-University Munich, Germany, | Study Doctor | Medical History, Medical and Physical Examination |
| Olivia Schreiber- Katz | Ludwig-Maximilians-University Munich, Germany, | Study Doctor | Medical History, Medical and Physical Examination |
| Elizabeth Harris MD | The John Walton Muscular Dystrophy Research Centre, Newcastle upon Tyne, UK | Study Doctor | Medical History, Medical and Physical Examination |
| Michela Guglieri MD | The John Walton Muscular Dystrophy Research Centre, Newcastle upon Tyne, | Study Doctor | Medical History, Medical and Physical Examination |
| Teresinha Evangelista MD | The John Walton Muscular Dystrophy Research Centre, Newcastle upon Tyne, | Study Doctor | Medical History, Medical and Physical Examination |

(*continued on next page*)

| Name | Location | Role | Contribution |
|---|---|---|---|
| Alex Murphy MD | The John Walton Muscular Dystrophy Research Centre, Newcastle upon Tyne, | Study Doctor | Medical History, Medical and Physical Examination |
| Michelle Eagle PhD | The John Walton Muscular Dystrophy Research Centre, Newcastle upon Tyne, | Physiotherapist | Study Design, Acquisition of physiotherapy data |
| Heather Hilsden | The John Walton Muscular Dystrophy Research Centre, Newcastle upon Tyne, | Project Manager | Coordination of whole study |
| Robert Muni Lofra | The John Walton Muscular Dystrophy Research Centre, Newcastle upon Tyne, | Physiotherapist | Acquisition of physiotherapy data |
| Dionne Moat BSc | The John Walton Muscular Dystrophy Research Centre, Newcastle upon Tyne, | Physiotherapist | Acquisition of physiotherapy data |
| Jassi Amritpal Singh Sodhi BSc | The John Walton Muscular Dystrophy Research Centre, Newcastle upon Tyne, | Physiotherapist | Acquisition of physiotherapy data |
| Helen Sutherland | The John Walton Muscular Dystrophy Research Centre, Newcastle upon Tyne, | Clinical Trial and Data Manager | Study Coordination and Data Management |
| Tim Hodgson MClinRES, | Magnetic Resonance Centre, Newcastle University, UK, | Radiologist | MRI Data Acquisition |
| Fiona E. Smith | Magnetic Resonance Centre, Newcastle University, UK, | NMR Scientist | MRI analysis and Training |
| Ian Wilson | Magnetic Resonance Centre, Newcastle University, UK, | NMR Scientist | MRI analysis |
| Dorothy Wallace BSc, | Magnetic Resonance Centre, Institute for Cellular Medicine, Newcastle University, UK, | Radiologist | MRI Data Acquisition |
| Louise Ward DCR, | Magnetic Resonance Centre, Institute for Cellular Medicine, Newcastle University, UK | Radiologist | MRI Data Acquisition |
| Debra Galley | Magnetic Resonance Centre, Newcastle University, | Radiology Assistant | MRI Data Acquisition |
| Chiara Calore | University of Padova, Padova, Italy, | Study Doctor | Medical History, Medical and Physical Examination |
| Claudio Semplicini | University of Padova, | Sub PI | Acquisition of physiotherapy data Medical History, Medical and Physical Examination |
| Luca Bello | University of Padova, Padova, Italy, | Sub PI | Acquisition of physiotherapy data Medical History, Medical and Physical Examination |
| Roberto Stramare MD | Radiology Unit, Department of Medicine, University of Padova, Padova, Italy, | Radiologist | MRI Data Acquisition |
| Alessandro Rampado MRT | Radiology Unit, Department of Medicine, University of Padova, Padova, Italy, | Radiologist | MRI Data Acquisition |
| Aurelie Canal | Institut de Myologie, Paris, | Physiotherapist | Acquisition of physiotherapy data |
| Jean Yves Hogrel | Institut de Myologie, Paris, | Research Scientist | Supervision of Physiotherapy and Study Coordination |
| Suna Turk MSc | AIM & CEA NMR Laboratory, Institute of Myology, Pitié-Salpêtrière University Hospital, 47–83, Paris, France, | Radiologist | MRI Data Acquisition |
| Harmen Reyngoudt | AIM & CEA NMR Laboratory, Institute of Myology, Pitié-Salpêtrière University Hospital, 47–83, Paris, France, | NMR Scientist | MRI Training and analysis |
| Ericky Caldas | AIM & CEA NMR Laboratory, Institute of Myology, Pitié-Salpêtrière University Hospital, 47–83, Paris, France | NMR Scientist | MRI Training and analysis |
| Cyrille Theis | Institut de Myologie, Paris | Radiologist | MRI Data Acquisition |
| Oumar Diabaté | Institut de Myologie, Paris, | Study Coordinator | Study Coordination and Data Management |
| Matthew Harms | Washington University, St. Louis, MO, USA, Co PI | Co PI, Study Doctor | Recruitment, Medical History, Medical and Physical Examination |
| Julaine M. Florence | Washington University, St Louis, MO, USA, | Physiotherapist | Acquisition of physiotherapy data |
| Catherine Siener | Washington University, St Louis, MO, USA, | Physiotherapist | Acquisition of physiotherapy data |
| Linda Schimmoeller | Washington University, St. Louis, MO, USA, | Study Coordinator | Study Coordination and Data Management |
| Glenn Foster rtr (mr) | Centre for clinical imaging research ccir, washington university, st louis, mo, usa, | MRI Technologist | MRI Data Acquisition |
| Pilar Carbonell MD | Hospital U. Virgen del Rocío/Instituto de Biomedicina de Sevilla Seville, | Study Doctor | Medical History, Medical and Physical Examination |
| Macarena Cabrera MD | Hospital U. Virgen del Rocío/Instituto de Biomedicina de Sevilla, Seville, | Study Doctor | Medical History, Medical and Physical Examination |
| Juan Bosco Mendez | Hospital U. Virgen del Rocío/Instituto de Biomedicina de Sevilla, Seville, | Rehabilitation Doctor | Acquisition of physiotherapy data |

(*continued on next page*)

| Name | Location | Role | Contribution |
|---|---|---|---|
| Nieves Sanchez-Aguilera Praxedes | Hospital U. Virgen del Rocío/Instituto de Biomedicina de Sevilla, Seville, | Physiotherapist | Acquisition of physiotherapy data |
| Yolanda Morgado MD | Hospital U. Virgen de Valme /Instituto de Biomedicina de Sevilla, Seville, | Study Doctor | Medical History, Medical and Physical Examination |
| Susana Rico Gala MD | Department of Radiology, Hospital U. Virgen de Valme, Sevilla, Spain, | Radiologist | MRI Data Acquisition |
| Jennifer Perez | Stanford University School of Medicine, Stanford, | Study Coordinator | Study Coordination and Data Management |
| Anne Marie Sawyer FSMRT, | Lucas Centre for Imaging, Stanford University School of Medicine; Stanford, CA, USA | MRI Lead | MRI data acquisition |
| Carolina Tesi-Rocha | Stanford University School of Medicine; Stanford, CA, USA | Sub- I Study Doctor | Medical History, Medical and Physical Examination |
| Tina Duong | Stanford University School of Medicine; Stanford, CA, USA | Physiotherapist | Training of physiotherapists and Acquisition of physiotherapy data |
| Richard Gee | Stanford University School of Medicine; Stanford, CA, USA | Physiotherapist | Acquisition of physiotherapy data |
| Nigel F Clarke MD | Institute for Neuroscience and Muscle Research, Sydney r | Original PI. *Deceased.* | Recruitment and Study Design |
| Kristy Rose | Institute for Neuroscience and Muscle Research, Sydney | Physiotherapist | Training of physiotherapists and Acquisition of physiotherapy data |
| Sarah Sandaradura MD | Institute for Neuroscience and Muscle Research, Sydney | Study Doctor | Acquisition of Medical History, medical and physical assessments |
| Roula Ghaoui MD | Institute for Neuroscience and Muscle Research, Sydney | Study Doctor | Acquisition of Medical History, medical and physical assessments |
| Kayla Cornett | Institute for Neuroscience and Muscle Research, Sydney | Physiotherapist | Acquisition of physiotherapy data |
| Claire Miller PT | Institute for Neuroscience and Muscle Research, Sydney | Physiotherapist | Acquisition of physiotherapy data |
| Sheryl Foster MHlthSc | Department of Radiology, Westmead Hospital; Faculty of Health Sciences, The University of Sydney, Australia | Radiologist | MRI data acquistion |
| Anthony Peduto MBBS | Department of Radiology, Westmead Hospital; Faculty of Health Sciences, The University of Sydney, Australia, | Radiologist | MRI data acquistion |
| Noriko Sato MD PhD | Department of Radiology, National Centre Hospital, National Centre of Neurology and Psychiatry, Tokyo, Japan | Radiologist | MRI data acquistion |
| Takeshi Tamaru MRT | Department of Radiology, National Centre Hospital, National Centre of Neurology and Psychiatry, Tokyo, Japan, | Radiologist | MRI data acquistion |
| Shin'ich Takeda | National Centre of Neurology and Psychiatry, Tokyo, | Director | Supervision of Site Investigators |
| Ai Ashida PT | National Centre of Neurology and Psychiatry, Tokyo, | Physiotherapist | Acquisition of physiotherapy data |
| Chikako Sakamoto PT | National Centre of Neurology and Psychiatry, Tokyo, | Physiotherapist | Acquisition of physiotherapy data |
| Tatayuki Tateishi | National Centre of Neurology and Psychiatry, Tokyo | Physiotherapist | Acquisition of physiotherapy data |
| Hiroyuki Yajima | National Centre of Neurology and Psychiatry, Tokyo, | Physiotherapist | Acquisition of physiotherapy data |
| Takahiro Nakayama MD PhD | Yokohama Rosai Hospital, Yokohama, | Study Advisor | Study Coordinator |
| Kazuhiko Segawa MD PhD | National Centre of Neurology and Psychiatry, Tokyo, | Study Doctor | Acquisition of Medical History, medical and physical assessments |
| Makiko Endo | National Centre of Neurology and Psychiatry, Tokyo, | Study Coordinator | Study Coordination and Data Management |
| Meganne E Leach, MSN, APRN | Children's National Health System, Washington, | Research Nurse | Acquisition of Medical History, medical and physical assessments |
| Nora Brody, PT, DPT | Children's National Health System, Washington, | Physiotherapist | Acquisition of physiotherapy data |
| Brittney DeWolf | Children's National Health System, Washington, | Physiotherapist | Acquisition of physiotherapy data |
| Allyn Toles | Children's National Health System, Washington, | Study Coordinator | Study Coordination and Data Management |
| Stanley T. Fricke PhD | Department of Diagnostic Imaging and Radiology, Children's National Health System, Washington DC, USA, | Radiologist | MRI data acquistion |
| Hansel J. Otero MD | Department of Diagnostic Imaging and Radiology, Children's National Health System, Washington DC, USA, | Radiologist | MRI data acquistion |
| Ulrike Grieben MD | Charite, Berlin, | Study Doctor | Acquisition of Medical History, medical and physical assessments |
| Elke Marron | Physio Plus, Berlin, | Physiotherapist | Acquisition of physiotherapy data |
| Juliana Prugel | Physio Plus, Berlin, | Physiotherapist | Acquisition of physiotherapy data |

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