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The clinical outcome study for dysferlinopathy: An international multicenter study

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ABSTRACT

Objective: To describe the baseline clinical and functional characteristics of an international cohort of 193 patients with dysferlinopathy.

Methods: The Clinical Outcome Study for dysferlinopathy (COS) is an international multicenter study of this disease, evaluating patients with genetically confirmed dysferlinopathy over 3 years. We present a cross-sectional analysis of 193 patients derived from their baseline clinical and functional assessments.

Results: There is a high degree of variability in disease onset, pattern of weakness, and rate of progression. No factor, such as mutation class, protein expression, or age at onset, accounted for this variability. Among patients with clinical diagnoses of Miyoshi myopathy or limb-girdle muscular dystrophy, clinical presentation and examination was not strikingly different. Respiratory impairment and cardiac dysfunction were observed in a minority of patients. A substantial delay in diagnosis was previously common but has been steadily reducing, suggesting increasing awareness of dysferlinopathies.

Conclusions: These findings highlight crucial issues to be addressed for both optimizing clinical care and planning therapeutic trials in dysferlinopathy. This ongoing longitudinal study will provide an opportunity to further understand patterns and variability in disease progression and form the basis for trial design. ***Neurol Genet* 2016;2:e89; doi: 10.1212/NXG.0000000000000089**

GLOSSARY

a-NSAA = adapted North Star Ambulatory Assessment; **CK** = creatine kinase; **FVC** = forced vital capacity; **IB** = immunoblot; **IH** = immunohistochemistry; **LGMD** = limb-girdle muscular dystrophy; **LGMD2B** = limb-girdle muscular dystrophy type 2B; **ME** = monocyte expression; **MM** = Miyoshi myopathy; **MMT** = Manual Muscle Testing; **MRC** = Medical Research Council; **NSAA** = North Star Ambulatory Assessment; **OR** = odds ratio; **RFF** = rise from floor; **TUG** = Timed Up and Go.

Dysferlinopathy is a term for a group of rare muscular dystrophies with recessive mutations in the *DYSF* gene, which encodes the skeletal muscle protein dysferlin.[1,2] Two major phenotypes are Miyoshi myopathy (MM),[3] presenting with distal weakness and limb-girdle muscular dystrophy type 2B (LGMD2B),[4,5] affecting more proximal muscles. Other reported phenotypes

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include the more rapidly progressive distal myopathy with anterior tibial involvement,[6] proximodistal weakness, and a pseudometabolic presentation.[7,8]

Several studies have reviewed the phenotypes of dysferlinopathy demonstrating a high degree of variability in the initial pattern of weakness. Symptom onset in young adulthood, highly elevated serum creatine kinase (CK), and characteristic MRI pattern are generally consistent.[2,8–14] However, patients with atypical features are reported and the full spectrum of dysferlinopathy phenotypes and patterns of disease progression is yet to be fully described.[15–17]

As the neuromuscular field moves toward trial readiness, a clearer understanding of the natural history of these rare diseases is essential. This report describes baseline characteristics of participants in the Jain Foundation–funded clinical outcome study—a large cohort of patients with dysferlinopathy, enabling characterization of common and rarer phenotypic features. This work will form the baseline for longitudinal assessment aiming to define distinct disease trajectories and a robust set of outcome measures for clinical trials and to identify areas for improving clinical practice.

METHODS Inclusion criteria were ≥2 pathogenic mutations in *DYSF*, or 1 pathogenic mutation plus either absent dysferlin expression on immunoblot (IB)[18] or ≤20% dysferlin monocyte expression (ME).[19] Truncating mutations and splice-site mutations affecting the +1/−1 or 2 positions were deemed pathogenic. Pathogenicity of other splice-site mutations and missense mutations were defined according to the UMD Predictor (http://umd-predictor.eu).

Patients have 6 visits over 3 years (screening, baseline, 6 months, 1, 2, and 3 years). At each visit, a medical examination is conducted, and quality of life, exercise, and medical history data are collected via questionnaires. Blood is drawn for hematologic and biochemical assays. Patients can choose to provide DNA, RNA, serum, plasma, and skin biopsy for biobanking. Cardiac assessment by ECG and echocardiogram are performed at baseline and 3 years. MRI assessment (to be reported separately) includes lower limb T1W, T2, 3-point Dixon (lower limb), and magnetic resonance spectroscopy evaluation (3 sites) at baseline, 1, 2, and 3 years.

Physiotherapists, trained and assessed in investigator meetings, perform evaluations at each visit. They assess respiratory function (sitting forced vital capacity [FVC]), muscle strength (Manual Muscle Testing [MMT]), and functional status (adapted North Star Ambulatory Assessment [a-NSAA] in ambulant patients, timed tests [rise from floor (RFF), 10-m walk/run, 4 stair climb and descend, Timed Up and Go (TUG)], and 6-minute walk). Assessments were reviewed for consistency between screening and baseline by lead physiotherapists from Newcastle.

The a-NSAA is based on the validated NSAA, a 17-item scale with a maximum score of 34 used in Duchenne muscular dystrophy. This was adapted adding items relevant to ambulatory ability in dysferlinopathy creating a 22-item scale with a maximum score of 51 (table e-1 at Neurology.org/ng).

Using a-NSAA and ambulatory status, the cohort was stratified into mild (a-NSAA 40–51), moderate (a-NSAA 6–39), or severe (a-NSAA ≤5 or nonambulant) groups. Ambulation status was determined by the ability of patients to walk 10 m with shoes and usual walking aids or orthotics. Medical Research Council (MRC) power grades, timed tests, and respiratory status were reported according to this stratification.

For analysis, 5-point MRC power grades for MMT were converted to an 11-point scale (0, 1, 2, 3−, 3, 3+, 4−, 4, 4+, 5−, and 5).

Statistical analysis was performed using Prism software (GraphPad Software Ltd., La Jolla, CA). Demographics were collected for ethnicity, sex, age, ambulatory status, years symptomatic, and mutation details. Median values and ranges were calculated for the number of years symptomatic, age at symptom onset, age at diagnosis, and MMT analysis. Mean values (SD and ranges) were calculated for serum CK, serum creatinine, and serum urea. Percent predicted FVC and timed tests (10-m run, TUG, RFF, stair ascend, descend, and 6-minute walk test) are stratified by disease severity. MMT median values were also stratified by disease severity and analyzed for symmetry between right and left, anterior and posterior, and upper and lower limb muscle groups using the Wilcoxon signed-rank test, considering a p value of less than 0.05 statistically significant.

Standard protocol approvals, registrations, and patient consents. All study participants provided informed consent. The study was approved by ethical review boards in each country.

RESULTS **Study demographics.** Included were 193 patients from 15 sites (Newcastle, Barcelona, Seville, Munich, Berlin, Padova, Marseille, Paris, Saint Louis, Columbus, Charlotte, Washington, DC, Stanford, Tokyo, and Sydney) representing 8 countries (United Kingdom, Spain, Germany, Italy, France, the United States, Japan, and Australia). Participants' ethnicities were white (71%), Asian (17%), black (3%), Hispanic (6%), and other (3%). Participants were 52% female and 48% male. Ages range from 12 to 88 years (mean age 40 years). Participants were 75% ambulant (36% male/39% female) and 25% nonambulant (13% male/12% female). At assessment, the majority reported symptoms for 25 years or less (77%). Median symptom duration was 17 years (range 3–52 years).

Genetic and protein expression findings. In total, 175 different mutations were observed (table e-2), 112 only in a single individual; 49.2% of mutations were truncating: 32.8% frameshift and 16.4% nonsense. The remainder were missense (32.8%), splice-site (17.5%), or in-frame duplication (0.6%).

Mutations were widely distributed throughout the gene. Table 1 shows the most frequently observed mutations. Two previously reported founder mutations were identified: c.2779delG,[20] in 3 individuals

Table 1 Frequent mutations

No. of occurrences	Mutation	Protein effect	Class
14	c.5979dupA	p.Glu1994ArgfsX3	Frameshift
7	c.3444_3445delinsAA	p.Tyr1148X	Nonsense
6	c.3112C>T	p.Arg1038X	Nonsense
6	c.855+1delG	Intronic	Splicing
6	c.4756C>T	p.Arg1586X	Nonsense
5	c.757C>T	p.Arg253Trp	Missense
5	c.6124C>T	p.Arg2042Cys	Missense
5	c.5698_5699delAG	p.Ser1900GlnfsX14	Frameshift
5	c.1392dupA	p.Asp465ArgfsX9	Frameshift
5	c.2643+1G>A	Intronic	Splicing
5	c.2997G>T	p.Trp999Cys	Missense

Mutations observed on 5 or more instances in the Clinical Outcome Study for dysferlinopathy (COS) cohort. Reference sequence NM_003494.3.

with Hispanic ethnicity and c.5713C>T,[21] observed in 4 individuals of diverse ethnicities.

Eighty-four percent of participants had 2 pathogenic mutations in *DYSF*. Thirteen percent had a single heterozygous mutation and absent or reduced (i.e., <20%) dysferlin expression on IB or ME. Three percent had >2 pathogenic mutations.

Dysferlin ME, IB, or immunohistochemistry (IH) data were available for 153 (79%) patients. Of these, 68% had absent and 30% had reduced dysferlin expression. Symptom onset age did not vary according to dysferlin expression levels. Normal dysferlin expression was observed in 3 individuals who presented with moderate or severe disease.

Of the 40 patients (21%) with no protein expression data, 28 had 2 clearly deleterious mutations (frameshift, splice-site, or nonsense), 8 had 1 clearly deleterious mutation and 1 missense mutation that was predicted to be pathogenic by the UMD predictor, and 4 patients had 2 missense mutations predicted to be pathogenic. There was no relationship between genotype (homozygosity for missense, splicing, or truncating mutations) and protein expression, age at onset, or disease severity.

Symptom onset and diagnosis. Self-reported age at "first muscle symptoms" ranged from 3 to 60 years (median 19 years) (figure 1). Most patients had symptoms preceding diagnosis; however, 24% were diagnosed after an incidental finding of elevated CK and 13% after diagnosis in a relative.

Initial symptoms varied, with some patients reporting multiple symptoms. Most commonly reported was lower limb weakness (72%); this was proximal (15%), distal (32%), or both (25%). Upper limb weakness was less common (7%). Others described muscle wasting (27%—predominantly distal lower limbs), pain, stiffness, or cramps (13%), or pseudohypertrophy (6%—predominantly distal lower limbs). Seventeen percent described onset following trauma or illness, but the majority described symptom onset over months.

Prior to symptom onset, 80% reported frequent participation in sports; daily (13%) or several times a week (42%). Forty-four percent reported "average" sporting ability, with 19% competing at the regional or national level.

The median age at confirmed diagnosis was 25 years (range 3–62 years) (figure 1). Mean time from symptom onset to diagnosis in the 1970s was 20.5 years (SD 10.7), falling to 3.1 years (SD 2.6) with onset in 2000s. Patient-reported clinical diagnoses were LGMD2B (60%), MM (30%), proximodistal dysferlinopathy (6%), hyperCKemia (3%), and "other" including paravertebral muscular dystrophy or pseudometabolic dysferlinopathy (2%). Clinical diagnosis varied by research site but not by patient ethnicity or age at symptom onset. MM was the most common diagnosis in Japan: odds ratio (OR) 7.01 (2.10–23.46) and LGMD2B in England: OR 6.12 (2.28–16.25).

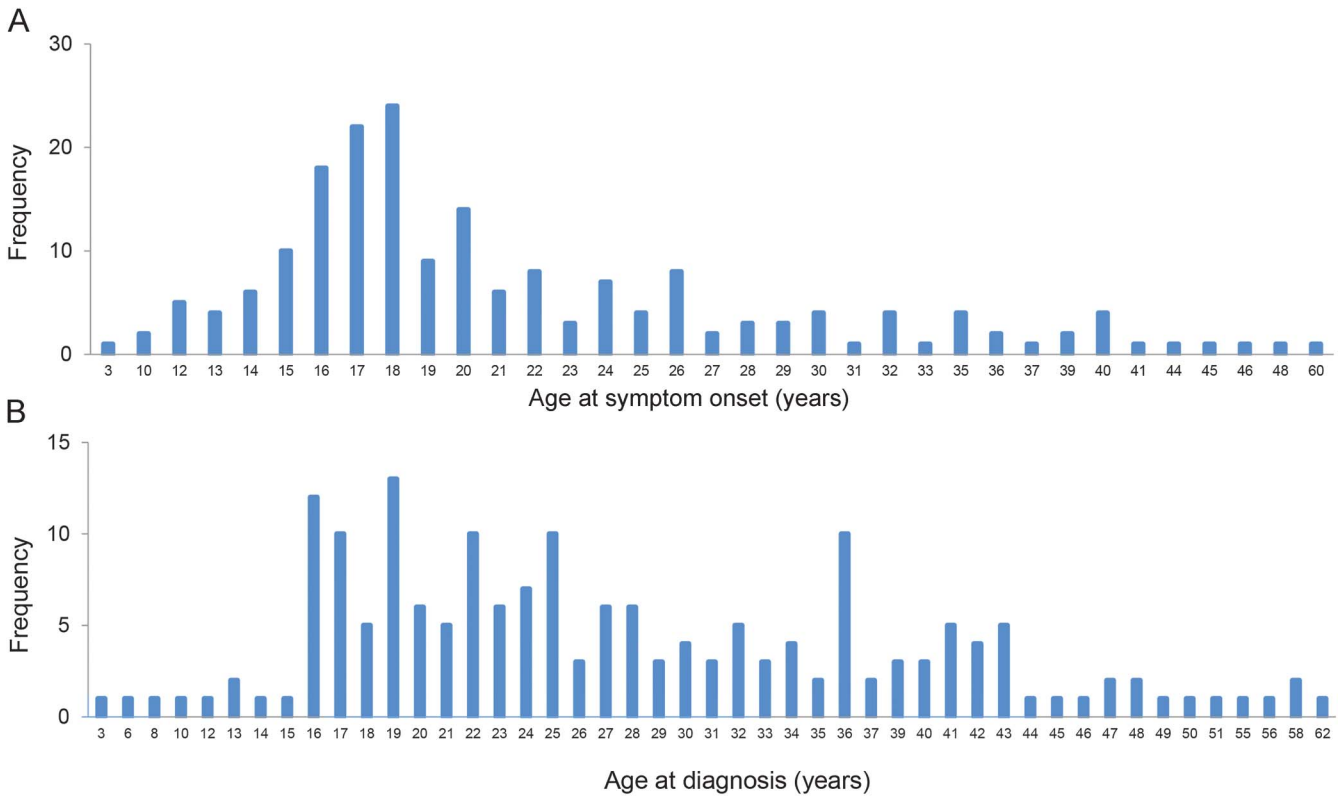
An initial diagnosis of polymyositis was reported by 16%, and 25% reported previous corticosteroid use. There were geographical differences in prior steroid use, with none in Australia and >60% in Germany, possibly because of a previous clinical trial.[22]

The longer the duration of symptoms, the greater proportion of patients with severe disease (figure 2). However, 2 patients with symptoms for over 30 years remain mildly affected. Four patients, aged 16 to 30, reported no muscle symptoms.

Physical examination. Thirty-six percent of patients had joint contractures, commonly affecting ankles, knees, and elbows. Muscle wasting was observed in 80% of patients most commonly in distal lower limbs (71%). Pseudohypertrophy was noted in 11%; usually in distal lower limbs but sometimes proximal lower limbs, upper arms, shoulders, or neck. Additional features observed include scoliosis (8%), rigid spine (7%), tremor (5%), facial weakness (3%), tongue fasciculations (3%), dysarthria (0.5%), or myotonia (0.5%).

Clinical investigations. Mean serum CK at baseline was 4,562 IU/L (SD 3,937; range 209–23,124 IU/L) with values falling with increasing age and disease duration. Serum creatinine was abnormally low in 70% of patients (mean 36.7 μmol/L; range 11–145 μmol/L, SD 18), likely reflecting reduced muscle mass. Mean serum urea was within normal range (mean 6.2 mmol/L; range 1.1–23.9 mmol/L and SD 3.4 mmol/L). Elevated alanine aminotransferase (91%) and aspartate

Figure 1 Age of patients at symptom onset and diagnosis



The mean time from onset to diagnosis was 6 years.

transaminase (93%) levels were seen in most patients, consistent with muscular dystrophy. Elevated alkaline phosphatase (6%) and total bilirubin (9%) was less common.

Baseline MMT. The characteristics of the median MMT values are summarized in figure 3. Strength was better preserved in upper limbs than lower limbs: median MMT scores 8/10 vs 3/10, respectively ($p \leq 0.0001$) There was no asymmetry between right and left in any muscle group. Analysis of MMT scores in posterior vs anterior muscle groups in the lower limbs demonstrated a difference in hip muscles with hip flexion stronger than extension (hip flexion score 6/10, extension 3/10 [$p < 0.0001$, Wilcoxon signed-rank test]).

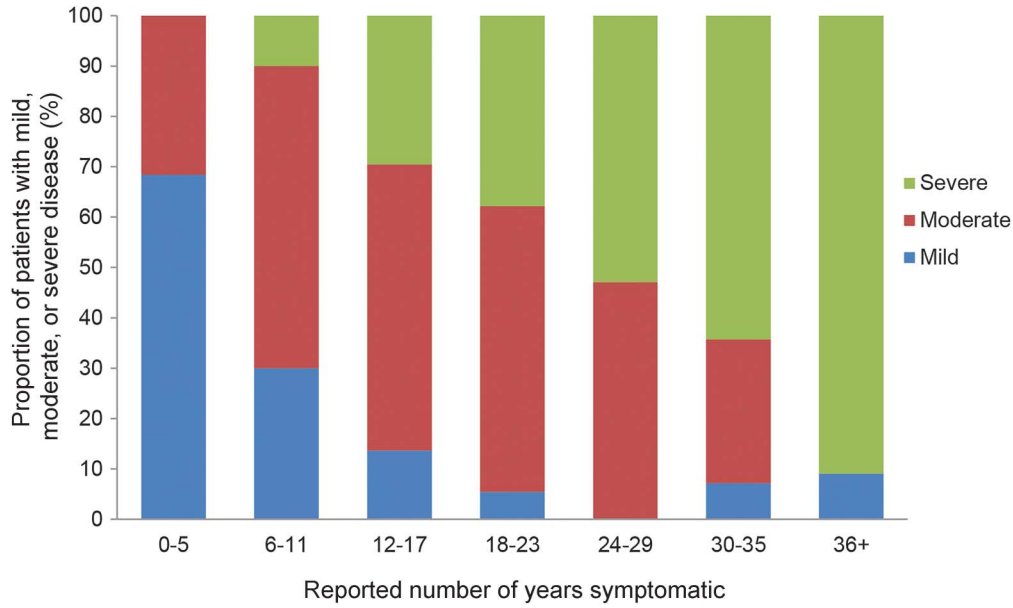
MMT was evaluated depending on disease severity scores. In the mild cohort, median MRC power was ≥4 in all muscle groups, with lower limbs generally weaker than upper limbs. Elbow and wrist flexion and extension, knee extension, and ankle inversion, eversion, and dorsiflexion were typically of normal power. In the moderate cohort, on average, lower limbs were weaker in all muscle groups than upper limbs with median MRC scores of ≤4 and ≥4, respectively. Hip adduction was weakest (median MRC 2.5), and wrist flexion and extension were strongest (median MRC 5−). In the severe cohort, lower limb proximal and distal muscle groups were similarly affected (median MRC 1 or 2, with the exception of hip abduction MRC 3−). Ankle dorsiflexion, eversion, and plantar flexion were weakest (median MRC grade 1). In upper limbs, there was proximal weakness (median MRC 3−) with distal strength preservation (wrist flexion/extension median MRC 4−/4+).

When baseline timed tests were stratified by disease severity (table 2), there was overlap between groups, indicating the variability of physical ability within the cohort.

Cardiac findings. Impaired left ventricular function, defined as ejection fraction <55%, was detected by study-related echocardiography in 7 patients (aged 29–69) and will be further evaluated by cardiac MRI. To date, 3 of these are completed—2 are normal and 1 confirms cardiomyopathy (patient aged 51). One additional patient had cardiomyopathy diagnosed prior to the study, at age 46 years. Of the 2 patients with cardiomyopathy, one had reduced FVC at 67% predicted and used nocturnal ventilation.

Respiratory findings. Increased disease severity was associated with lower FVC (table 2). Nocturnal noninvasive ventilation was used by 4 patients, all of whom reported a diagnosis of sleep apnea, and 3 of whom had a body mass index of >30. Disease

Figure 2 Patient stratification by the reported duration of symptoms and disease severity at the time of assessment



The percentage of patients within each severity category is given. Severity is defined as mild if the adapted North Star Ambulatory Assessment score is 40–51, moderate: 6–39, severe: 5 or less or nonambulatory. Symptomatic patients for whom sufficient data were available to assign severity were included (n = 182). Numbers of patients within each category are as follows: mild n = 34, moderate n = 89, severe n = 59.

severity in these 4 individuals ranged from mild to severe disease with predicted FVC between 50% and 82%.

Previous diagnosis. Clinical features were evaluated by preexisting clinical diagnosis. All patients with a clinical diagnosis of hyperCKemia fell into the mild category. Patients diagnosed as LGMD2B or MM were seen in mild, moderate, and severe groups. Median MMT values were similar between LGMD2B and MM groups, with proximal and distal lower limb weakness and predominantly proximal upper limb weakness. Ankle inversion was better preserved in LGMD2B patients (median MRC 4− vs median MRC 2 in MM). Patients diagnosed with proximodistal dysferlinopathy were more severely affected, with weakness extending to distal upper limbs (wrist extension median MRC 2, wrist flexion median MRC 3), and none had mild disease. Mean symptom duration was 17 years for the whole cohort. Apart from hyperCKemia, with median 5 years, this did not differ according to clinical diagnosis.

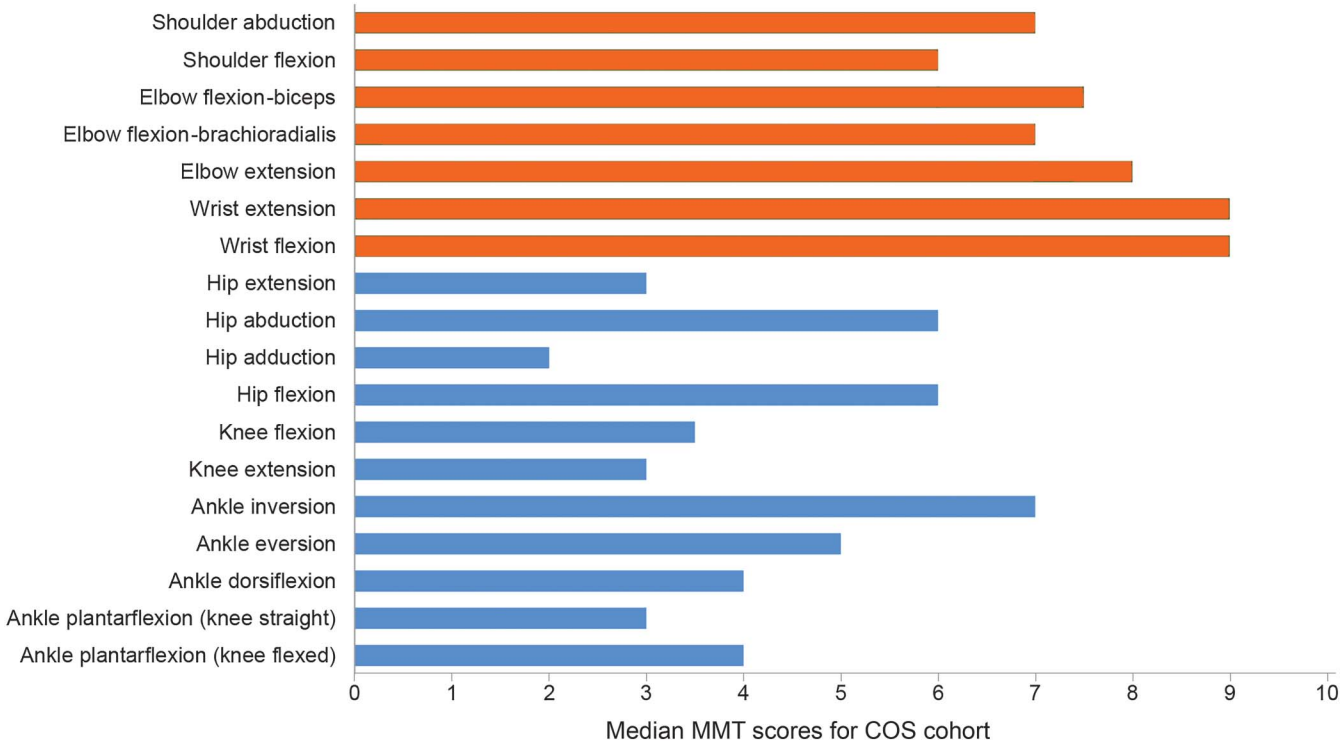
DISCUSSION We report the initial findings of an international observational study of patients with genetically confirmed dysferlinopathy. This cross-sectional analysis of a large and geographically diverse cohort of patients highlights both typical features and disease course, and outlying characteristics. This will form the basis for future longitudinal analysis of clinical outcomes, cardiac and respiratory evaluations, and muscle MRI data (the latter being reported separately).

Inclusion criteria for this study aimed to replicate the strict diagnostic criteria required for clinical trials: all patients have 2 mutations or a heterozygous mutation with additional evidence of absent or disease-range dysferlin protein expression by ME or IB.

Genetic data from this cohort support the high degree of genetic heterogeneity reported previously.[2,7,23] One-third of patients have nonsense mutations, indicating that nonsense read-through therapies, now licensed in Europe for use in Duchenne muscular dystrophy, may be a potential therapy for some patients with dysferlinopathy.

A high percentage of the mutations were missense mutations. Although usually associated with absent or reduced dysferlin expression (table e-2), further analysis is needed to determine the mechanism by which these missense mutations lead to disease. Some investigations link missense mutations to protein instability causing reduced dysferlin levels.[24] Others have demonstrated that missense mutations can lead to normal protein expression levels, but abnormal protein localization, which results in clinical disease.[25] Assays for the functionality of dysferlin protein with various missense mutations are currently being investigated by the Jain Foundation and may help to refine the diagnostic process in the future.

Figure 3 Comparison of median manual muscle test scores in the upper and lower limbs



Data were available for 189 study participants. The 5-point Medical Research Council power grade was converted to an 11-point scale (0, 1, 2, 3−, 3, 3+, 4−, 4, 4+, 5−, and 5). Observed Manual Muscle Testing scores ranged from 0 or 1 to 10 for each movement assessed, with the exception of wrist extension for which the lowest observed score was 2. Overall, the most severely affected muscle groups were hip adduction, extension, knee flexion and extension, and ankle plantar flexion, dorsiflexion, and eversion. The least severely affected muscle groups were wrist flexion and extension. Red indicates the upper limb muscles and blue indicates the lower limb muscles. COS = Clinical Outcome Study.

Most patients included in this study have absent or reduced dysferlin on IH, IB, or ME. Absence of dysferlin was more commonly noted than reduction, irrespective of the severity of the clinical phenotype or mutation type. We identified 3 patients with 2 *DYSF* missense mutations in whom dysferlin protein levels were normal. The typical diagnostic procedure for dysferlinopathy diagnosis has been to identify absent or reduced dysferlin protein levels and then sequence the dysferlin gene. Therefore, patients with pathogenic *DYSF* mutations and normal dysferlin protein levels are rarely identified.[26] As genetic testing becomes more prevalent as a first-line investigation, patients with normal dysferlin levels may be increasingly recognized and caution will be required before generalizing results from this study to that population.

More than 2 dysferlin mutations were found in 3% of cases. However, aside from one novel mutation (c.6056G>A), all of these missense mutations have previously been associated with reduced dysferlin expression when in the homozygous state or in combination with one other mutation,[7,25–29] which supports their pathogenicity.

We identified that time from symptom onset to diagnosis has reduced. As our data indicate that 30% of patients are moderately affected within 5 years, earlier diagnosis is likely to reduce unnecessary testing or potentially detrimental steroid treatment.[22] As therapies become available, any delay becomes more costly because the window of opportunity to treat may be missed. We hope improved awareness and delineation of the dysferlinopathy phenotype will continue to improve time to diagnosis.

Dysferlinopathy is often assigned a particular clinical phenotype, most commonly MM or LGMD. The pattern of weakness between patients given these 2 diagnoses did not differ in our study. A clinical diagnosis of proximodistal dysferlinopathy was associated with more severe disease, and this appears unrelated to symptom duration. The 3% patients labeled as hyperCKemia had symptoms for a shorter duration. Longitudinal study will clarify whether this is a presentation of early disease or a distinct phenotype. We noted a number of occasionally reported features, such as tremor or dysarthria, the significance of which is unclear. Above-average sporting ability before symptom onset has been reported previously in dysferlinopathy[10] and is supported here with 19% of our cohort participating in sport at the regional or national level. The basis for this remains unknown.

Table 2 Respiratory function and timed tests by disease severity

	Stratification of disease severity by a-NSAA and ambulation status		
	Mild	Moderate	Severe
% predicted FVC	98 (CI 94–102)	91 (CI 88–94)	81 (CI 76–86)
No. (%) of patients with FVC <80% predicted	3 (8)	16 (18)	25 (43)
No. (%) of patients with FVC <50% predicted	0 (0)	1 (1.1)	5 (8.6)
Timed 10-m walk/run	4.5 s (range 2.2–9 s) (100%)	11.4 s (range 4.8–25.8 s) (96%)	18.11 s (range 9.6–26.8 s) (14%)
Timed Up and Go	6.7 s (range 3.8–10 s) (100%)	13.2 s (range 3.8–35.8 s) (82%)	31.9 s (range 28.8–36.2 s) (5%)
Rise from floor	3.8 s (range 0.9–12.4 s) (100%)	10.2 s (range 2.9–29.3 s) (57%)	Not applicable[a] (0%)
Time to ascend 4 stairs	2.7 s (range 1.1–5.1 s) (97%)	8.4 s (range 2.2–40 s) (83%)	23.1 s (range 6.7–35.2 s) (5%)
Time to descend 4 stairs	2.3 s (range 1.1–4.4 s) (97%)	6.27 s (range 1.3–26.5 s) (83%)	31 s (range 6.6–77 s) (5%)
6 min walk/run	495 m (range 304–656 m) (100%)	299 m (range 72–515 m) (92%)	138 m (range 9–295 m) (20%)

Abbreviations: a-NSAA = adapted North Star Ambulatory Assessment; CI = confidence interval; FVC = forced vital capacity.
This table displays respiratory and timed test data according to disease severity at the time of assessment. Mean predicted FVC according to the height and weight at baseline assessment. Overall, 24% of patients had FVC <80% predicted and 3.2% had FVC <50% predicted. Mean duration of symptoms in the 6 patients with FVC <50% predicted was 23 years (range 12–33 years). Mean values are provided for timed tests, and the percentage of patients who completed each test is given in brackets.
[a] No patients in the severe category were able to complete the rise from floor test.

We stratified patients by a-NSAA score and ambulation status into mild, moderate, and severely affected. This baseline analysis has demonstrated that weakness predominantly affects lower limbs in both proximal and distal muscle groups, regardless of disease severity. Increasing proximal upper limb weakness is connected with more severe disease. An increasing proportion of patients are more severely affected with increasing symptom duration. The rate of disease progression is variable, with >30% of patients mildly or moderately affected ≥30 years from symptom onset, while a similar proportion are severely affected after ≤17 years of symptoms (figure 2). The cause of this variability is not known, but differing presentations within a single family or common genotypes suggest the presence of disease-modifying factors.[30,31] Given this highly variable severity, pattern of weakness and rate of progression in dysferlinopathy, we anticipate that longitudinal data will help to elucidate potentially distinct disease trajectories.

We observed that 6 patients with moderate or severe disease had an FVC <50%, supporting the need for respiratory function monitoring in moderate or severe disease.[32] Four patients used nocturnal ventilation and reported sleep apnea. This may be coincidental as all have FVC ≥50% and 3 patients have body mass index >30. Two patients were identified with cardiomyopathy. Echocardiogram analysis for left ventricular dysfunction will be explored further in this study. Cardiac abnormalities have previously been reported in dysferlinopathy, but whether these are a consequence of dysferlinopathy or an alternative etiology is not established.[9,32–35] Low serum creatinine seen in 70% of patients is relevant for renal function monitoring, as creatinine-dependent methods will be uninformative.[36]

This analysis has identified a number of findings pertinent to the clinical care and planning of trials for patients with dysferlinopathy. Diagnosis is frequently delayed. Detailed analysis of muscle strength and function across different clinical diagnoses suggests that distinctions in pattern of weakness between MM, LGMD2B, and other phenotypes are limited. Emerging longitudinal data will allow us to assess whether progression of weakness is also similar, allowing patients with different clinical diagnoses to be considered as a whole in planning clinical studies. A small proportion of patients have respiratory dysfunction and cardiac abnormalities. Although the general phenotype is of a slowly progressive disease manifesting in young adulthood, there are patients with disease onset at extremes of age and divergent rates of progression. The etiology of this variability is unclear but important to understand for clinical trials and developing validated outcome measures. As longitudinal data on this cohort emerge, we anticipate being able to contribute to the trial readiness of this patient group.

AUTHOR CONTRIBUTIONS

Elizabeth Harris and Catherine L. Bladen contributed to data analysis, drafting, statistics, and writing. Anna Mayhew contributed to data analysis, drafting, and writing. Meredith James contributed to data analysis. Karen Bettinson and Ursula Moore contributed to data analysis and drafting. Fiona E. Smith contributed to drafting. Laura Rufibach and Avital Cnaan contributed to study design and revising for intellectual content. Diana X. Bharucha-Goebel, Andrew M. Blamire, Elena Bravver, and Pierre G. Carlier contributed to study design. John W. Day and Jordi Díaz-Manera contributed to study design and revising for intellectual

content. Michelle Eagle, Ulrike Grieben, Matthew Harms, Kristi J. Jones, and Hanns Lochmüller contributed to study design. Jerry R. Mendell contributed to study design and revising for intellectual content. Madoka Mori-Yoshimura and Carmen Paradas contributed to study design. Elena Pegoraro and Alan Pestronk contributed to study design and revising for intellectual content. Emmanuelle Salort-Campana, Olivia Schreiber-Katz, Claudio Semplicini, Simone Spuler, and Tanya Stojkovic contributed to study design. Volker Straub contributed to study design and revising for intellectual content. Shin'ich Takeda, Carolina Tesi, and M.C. Walter contributed to study design. Kate Bushby contributed to study design, drafting, revising for intellectual content.

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DISCLOSURE

Elizabeth Harris and Catherine L. Bladen report no disclosures. Anna Mayhew has served on the scientific advisory board of BioMarin; has been a consultant for BioMarin, Pfizer Inc., Sarepta Therapeutics, PTC Therapeutics, Summit Therapeutics, Eli Lilly, and Amicus; and has been involved with clinical procedures/imaging studies for the John Walton MD Centre. Meredith James has been a consultant for BioMarin, Pfizer Inc., Sarapeta Therapeutics, PTC Therapeutics, Summit Therapeutics, Eli Lilly, FibroGen, and Amicus Therapeutics. Karen Bettinson, Ursula Moore, and Fiona E. Smith report no disclosures. Laura Rufibach has served on the scientific advisory board of the Neuromuscular Disease Foundation; has received travel funding from Neuromuscular Disease Foundation; and has received research support from/been an employee of the Jain Foundation. Avital Cnaan has served on scientific advisory boards for NIH/NIDDK and the FDA; and has received research support from the Department of Defense, NIH/National Institute of Neurological Disorders and Stroke, the Department of Education, NIH/NCATS, NIH/NIAMS, NIH/NICHD, PCORI, the Jain Foundation, and the Foundation to Eradicate Duchenne. Diana X. Bharucha-Goebel has received research support from T32 AR 56993-4. Andrew M. Blamire has received research support from the European Commission, EPSRC, the UK Academy of Medical Sciences, Arthritis Research UK, and the Alzheimer's Society UK. Elena Bravver reports no disclosures. Pierre G. Carlier has served on the scientific advisory board for the EU FP7 BIOIMAGE project; has served on the editorial board of the *Journal of Neuromuscular Diseases*; has been a consultant for ProSensa; and has received research support from EU FP7 SCOPE NMD, EU FP7 BIOIMAGE, EU FP7 SKIP NMD, and France Life Imaging. John W. Day has served on a scientific advisory board funded by NIH, PPMD, and Marathon Pharmaceuticals; has received the following gifts: (1) Nonprofit for myotonic dystrophy cognitive function in adolescents, from family benefactor (2) Nonprofit for myotonic dystrophy genotype-phenotype correlations, from family benefactor; has received travel funding/speaker honoraria from Cytokinetics Inc., Biogen Inc., Roche Inc., Isis Pharmaceuticals, the Spinal Muscular Atrophy Foundation, Parent's Project Muscular Dystrophy, Myotonic Dystrophy Foundation, the American Association of Pediatrics, PPMD, and the Carrel-Krusen Organization; holds patents for Myotonic Dystrophy type 2 genetic testing (licensed to Athena Diagnostics) and Spinocerebellar Ataxia type 5 genetic testing (licensed to Athena Diagnostics); has been an employee of Stanford University; has been a consultant for Isis Pharmaceuticals, Biogen Inc., Cytokinetics, Sarepta Therapeutics, and PTC Therapeutics; has received research support from Genzyme Corporation, Isis Pharmaceuticals, Sarepta Pharmaceuticals, Cytokinetics Inc., BioMarin Pharma, NIH/National Institute of Neurological Disorders and Stroke, the Muscular Dystrophy Association, the Myotonic Dystrophy Foundation, and the Spinal Muscular Atrophy Foundation; and has received royalty payments from Athena Diagnostics. 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Hanns Lochmüller has served on the scientific advisory boards of German Duchenne parents project, IRDiRC Interdisciplinary Scientific Committee, German Muscular Dystrophy Network, Myotubular Trust Patient Registry, Action Duchenne Patient Registry, and German Patient Registries on DMD and SMA; has received travel funding/speaker honoraria from PTC Therapeutics Inc. and Ultragenyx Pharmaceuticals Inc.; has served on the editorial boards of the *Journal of Neuromuscular Diseases* and the *Journal of Neurology*; has been a consultant for Roche Pharmaceuticals, ASD Therapeutics Partners LLC, IOS Press, Alexion Pharmaceuticals Inc., Ultragenyx Pharmaceuticals Inc., and Fondazione Cariplo; and has received research support from Marigold Foundation Ltd., Ultragenyx Pharmaceuticals Inc., PTC Therapeutics Inc., Eli Lilly and Co., Action Benni & Co., GlaxoSmithKline, Trophos SA, the European Commission, the Medical Research Council, NIHR, Action Duchenne, Association Francaise Contre les Myopathies, the British Heart Foundation, Muscular Dytrophy UK, the National Cancer Institute, Spinal Muscular Atrophy Support UK, Wellcome Trust, Jennifer Trust, and Duchenne Parent Project. Jerry R. Mendell has been a consultant for AveXis Therapeutics and Serapta Therapeutics; and has received research support from AveXis Therapeutics, Serapta Therapeutics, the Nationwide Children's Hosptial Foundation, and the MDA Clinical Research Network. Madoka Mori-Yoshimura reports no disclosures. Carmen Paradas holds a patent for Computerized image analysis method for the diagnosis of neuromuscular diseases; and has received research support from the Andalusia Government (Consejeria de Salud, Spain). Elena Pegoraro has received travel funding from Genzyme; and has received research support from the University of Padova and the Italian Telethon (Italian Ministry of Health). Alan Pestronk has served on the scientific advisory board of the Myositis Association; has received travel funding from the Myositis Association; holds patents for TS-HDS antibody 7,175,989, GALOP antibody 6,121,004, GM1 ganglioside antibody 6,077,681, and Sulfatide antibody 6,020,140; has served on the speaker's bureaus of Athena and the Myositis Association; has received research support from Genzyme, Insmed, Knopp, Ultragenyx, Ionis, Sanofi, Cytokinetics, GlaxoSmithKline, Biogen, CSL Behring, BioMarin, NIH, the Washington University Neuromuscular Research Fund, the CINRG Children's Hospital Washington, DC, and the Muscular Dystrophy Association; holds stock in Johnson & Johnson; has received license fee payments from Athena; and has received royalty payments for GALOP antibody 6,121,004, GM1 ganglioside antibody 6,077,681, and Sulfatide antibody 6,020,140. Emmanuelle Salort-Campana reports no disclosures. 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Volker Straub has served on the scientific advisory boards of Pfizer, Italfarmaco, Audentes Therapeutics, Bristol-Myer Squibb, Summit Therapeutics, Tivorsan, and the Nationwide Children's Hospital (Ohio); has received travel funding/speaker honoraria from Sanofi/Genzyme; has served on the editorial boards of *Neuromuscular Disorders*, the *Journal of Neuromuscular Diseases*, and *PLOS Currents Muscular Dystrophy*; has been a consultant for Sanofi/Genzyme; and has received research support from Sanofi/Genzyme, BioMarin, Ionis Pharmaceuticals, Sarepta Therapeutics, Ultragenyx, the European Commission, the UK Medical Research Council, Newcastle University, the Parent Project Muscular Dystrophy, Association Francaise Contre les Myopathies, the LGMD2I Research Fund, Wellcome Trust, the Sylvia Aitken Charitable Trust, Muscular Dystrophy UK, and Action Medical Research. Shin'ichi Takeda has served on the scientific advisory boards of the Myology Institute in Paris and the National Center for Child Health and Development (Japan); has received travel funding/speaker honoraria from the Japanese Society of Neurology, the Pharmaceutical and Medical

Device Regulatory Science Society of Japan, the International Collaboration Forum of Human Gene Therapy for Genetic Disease, the Japan Health Sciences Foundation, Jichi Medical University, MSD K.K., the Japan Society of Human Genetics, Chugai Pharmaceutical Co. Ltd., and Eisai Co. Ltd.; has served on the editorial boards of the *Journal of Neuromuscular Diseases*, the *American Journal of Pathology*, and *Neuromuscular Disorders*; holds patents for Antisense nucleic acid—sequence for exon 53 skip, Antisense nucleic acid—sequence for exon 44 skip, Antisense nucleic acid—sequence for exon 51 skip, and Antisense nucleic acid—sequence for block skip; has received publishing royalties from Springer; has been a consultant for Ono Pharmaceutical Co. Ltd., Chugai Pharmaceutical Co. Ltd., Taiho Pharma, Daiichisankyo Co. Ltd., and Takeda Pharmaceutical Co. Ltd.; and has received research support from Taiho Pharma, GlaxoSmithKline K.K., Nippon Shinyaku Co. Ltd., Takara Bio Inc., JCR Pharmaceuticals Co. Ltd., the Japan Agency for Medical Research and Development (AMED), AMED, the Ministry of Education, Sports, Science and Technology (MEXT), the National Center of Neurology and Psychiatry (NCNP), NCNP, the National Cerebral and Cardiovascular Center, and the National Center for Child Health and Development. Carolina Tesi Rocha has served on the scientific advisory boards of Sarepta and Marathon; and has been a consultant for Advance Medical. M.C. Walter has served on the scientific advisory boards of Novartis Pharma, the Steering Committee for a Prospective Observational Study in Sporadic Inclusion Body Myositis (sIBM), PTC Therapeutics, Roche Pharma, Grünenthal Pharma, and AveXis; has received travel funding/speaker honoraria from the EMG Seminar (Vienna), Novartis Pharma, and Biogen Pharma; has been a consultant for Guidepoint Global, GLG Consult, Olson Research, and Novartis; and has received research support from GlaxoSmithKline, Trophos AG (now Roche Pharma AG), Griofols, Novartis, the Federal Ministry of Education and Research (Germany), the Jain Foundation, Deutsche Gesellschaft für Muskelkranke, Association contre les Myopathies (AFM), and Friedrich-Baur-GmbH. Kate Bushby has served on the scientific advisory boards of Acceleron, AVI Biopharma, GlaxoSmithKline, Genzyme, Prosensa, PTC, Santhera, ELIX-ER, and BioMarin Pharmaceuticals; has served on the editorial board of *Neuromuscular Disorders*, has received publishing royalties from Cambridge University Press; has been an employee of Newcastle University; has been a consultant for Debiopharm, Lilly Pharmaceuticals, Summit Corporation, Insight Research Group, Galapagos SASU, Shire Human Genetics Therapies Inc., Amsterdam Molecular Therapeutics, European Neuromuscular Centre, Bristol-Meyers Squibb Company, and Solid Ventures LLC; has received research support from PTC, AVI, Pfizer Global Research and Development, Medical Research Council UK, The European Union, NIH, NHS England, the US Department of Defense, Muscular Dystrophy Campaign, Association Francaise contre les myopathies, INC Research, Duchenne Children's Trust, British Heart Foundation, Duchenne Parent Support, Wellcome Trust, Muscular Dystrophy Group of GB, and Parent Project Muscular Dystrophy. Go to Neurology.org/ng for full disclosure forms.

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