Assessing Dysferlinopathy Patients Over Three Years With a New Motor Scale

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Objective: Dysferlinopathy is a muscular dystrophy with a highly variable clinical presentation and currently unpredictable progression. This variability and unpredictability presents difficulties for prognostication and clinical trial design. The Jain Clinical Outcomes Study of Dysferlinopathy aims to establish the validity of the North Star Assessment for Limb Girdle Type Muscular Dystrophies (NSAD) scale and identify factors that influence the rate of disease progression using NSAD.
Methods: We collected a longitudinal series of functional assessments from 187 patients with dysferlinopathy over 3 years. Rasch analysis was used to develop the NSAD, a motor performance scale suitable for ambulant and non-ambulant patients. Generalized estimating equations were used to evaluate the impact of patient factors on outcome trajectories.
Results: The NSAD detected significant change in clinical progression over 1 year. The steepest functional decline occurred during the first 10 years after symptom onset, with more rapid decline noted in patients who developed symptoms at a younger age ($p = 0.04$). The most rapidly deteriorating group over the study was patients 3 to 8 years post symptom onset at baseline.
Interpretation: The NSAD is the first validated limb girdle specific scale of motor performance, suitable for use in clinical practice and clinical trials. Longitudinal analysis showed it may be possible to identify patient factors associated with greater functional decline both across the disease course and in the short-term for clinical trial preparation. Through further work and validation in this cohort, we anticipate that a disease model incorporating functional performance will allow for more accurate prognosis for patients with dysferlinopathy.

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Abbreviations

6MWT	6 minute walk test
10MWT	10 meter walk/run test
a-NSAA	adapted North Star Ambulatory Assessment
ClinRO	clinician reported outcome measure
COS	Clinical Outcomes Study of dysferlinopathy
DIF	differential item functioning
GEE	generalized estimating equations
ICC	intraclass correlation coefficient
LGMD	limb girdle muscular dystrophy
MFM-20	Motor Function Measure-20
MM	Miyoshi myopathy
MMD1	Miyoshi myopathy dystrophy 1
NSAA	North Star Ambulatory Assessment
NSAD	North Star Assessment for limb girdle type muscular dystrophies
RFF	timed Rise From Floor test
TUG	Timed Up and Go test

Dysferlinopathy is a rare, autosomal recessive, inherited form of muscular dystrophy caused by mutations in the *DYSF* gene, which encodes the skeletal muscle protein dysferlin.[1,2] The most common clinical diagnoses associated with dysferlinopathy are limb girdle muscular dystrophy R2 dysferlin related (formerly LGMD 2B) and Miyoshi myopathy (MM or Miyoshi myopathy dystrophy 1 [MMD1]).[3–5] Although onset typically occurs during young adulthood, clinical presentation is inconsistent, with a wide range of age at onset, patterns of muscle weakness, and severity, despite a shared loss of dysferlin protein expression.[1,6–8] Likewise, disease progression is variable; loss of ambulation occurs 5 to 35 years after onset, whereas a minority of patients remain mildly affected for decades.[9,10] A number of factors that may influence the clinical phenotype and progression of dysferlinopathy have been proposed, including exercise and the specific mutation,[6,11,12] although no clear pattern of decline or phenotype–genotype relationship has been established.

The variable progression of dysferlinopathy presents numerous challenges for clinical management and identifying patient groups that could be targeted for clinical trials. The lack of disease specific, validated clinician reported outcome measures (ClinRO) of motor performance specific to the LGMD population has hampered interpretation when monitoring progression. Clinically meaningful, validated outcomes are essential to accurately evaluate change[13,14] and response to therapeutic interventions.[15] Existing dysferlinopathy research suggests the level of decline varies according to the current functional state and is not stable across short time periods.[16] Showing efficacy in clinical trials is particularly difficult in slowly progressing and heterogeneous diseases.[17,18] Thus, to appropriately power an interventional clinical trial aiming to slow or stabilize progression in a rare disease like dysferlinopathy, the natural history of the disease must be well-characterized using robust and meaningful outcomes, with a characterized population likely to progress over the course of a defined clinical trial.

The Jain International Clinical Outcome Study (COS) of Dysferlinopathy was established to address the lack of comprehensive natural history data for dysferlinopathy and to identify and, if necessary, develop appropriate outcome measures for monitoring disease progression. Using data from 3 years of follow-up, the aims of this paper are: part 1) describe the development of the North Star Assessment for Limb Girdle Type Muscular Dystrophies (NSAD; formally the North Star Assessment

for Dysferlinopathy),[19] part 2) report on its ability to detect change over a 1 year period, and part 3) describe longitudinal disease progression to inform clinical prognostication and identify patients best suited for inclusion in clinical trials.

Methods

The Jain COS of dysferlinopathy is a multicenter, international study of patients with a confirmed diagnosis of dysferlinopathy. Detailed study methods have been published previously.[9,16] To be included in the Jain COS of dysferlinopathy, patients were required to have 2 predicted pathogenic mutations in *DYSF*, or 1 predicted pathogenic mutation plus either absent dysferlin expression on immunoblot or < 20% dysferlin monocyte expression.[20] Each participating site received local ethics approval and written informed consent was obtained for all patients. The study was registered at ClinicalTrials.gov (NCT01676077).

Participants were evaluated 6 times over the course of the study: screen, baseline, 6, 12, 24, and 36 months between November 2012 and March 2018. This multipart study includes data on: part 1 = 330 assessments from 154 patients, using available data from a data cut at 20 months after the first patient enrolled. Part 2 = 309 assessments from years 2 and 3 and, part 3 = 187 patients at 14 sites over 3 years (one original site was excluded due to the level of missing data). Three-year follow-up was high with 163 participants (87.2%) completing all study visits.

Demographic Measures

Self-reported race and patient sex was recorded, and age at each visit was calculated from the patient's year of birth. Symptom duration was calculated as the difference in years between patient-reported symptom onset (defined as the onset of muscle weakness) and age at each visit. Patient-reported clinical diagnosis, as given by diagnosing clinician, including LGMD2B and MM or other was recorded. Maximum exercise level prior to disease onset was classified as none, low, moderate, or high.[21]

Outcome Measures

A wide range of measures, including function and muscle strength, were performed in order to establish their usefulness in assessing disease progression over time.[9] For part 1, two scales of motor performance, the adapted-North Star Ambulatory Assessment (a-NSAA)[22] and Motor Function Measure-20 (MFM-20),[23] were evaluated with Rasch analysis at year 1, which evolved into the NSAD used for parts 2 and 3. For part 3, the most sensitive outcome measures identified during the first year of the study were selected[16] alongside the newly developed NSAD assessment introduced at year 2. To evaluate the NSAD across all 3 years, scores prior to year 2 were calculated based on a-NSAA and MFM-20 items captured at previous study visits using a defined algorithm; new items were imputed based on item difficulty hierarchies. Outcome measures selected showed consistently significant changes over both 6 and 12 months with relatively high standardized response means, including the 10 meter walk/run (10MWT) and the ACTIVLIM patient report of daily function questionnaire. The ACTIVLIM is a validated patient-reported outcome measure of functional ability based on perceived difficulty in performing specific activities of daily living.[24,25] Although the 6 minute Walk Test (6MWT) was previously found to be less sensitive in the dysferlinopathy population,[16] it was included for comparison. Timed function tests were converted to velocity measures (meters or task per second), and 0 m/s or 0 task/s was assigned when the patient was unable to complete the test due to disease progression. To appraise usefulness as inclusion criteria, the Timed Up and Go (TUG) and Rise from Floor (RFF) tests were evaluated as predictors of decline to determine whether they could better profile baseline function.

Statistical Analysis

Part 1: Development of NSAD Using a-NSAA and MFM-20 Collected at Year 1 - Psychometric Evaluation Using Rasch Analysis. A Rasch analysis examines the extent to which the observed data (physiotherapists' ratings of subject performance on items on the 2 scales) "fit" with predictions of those ratings from the Rasch model (which defines how a set of items should perform to generate reliable and valid measurements).[26] The difference between observed and expected scores indicates the degree to which a valid measurement is achieved.[26,27] Rasch analysis has been used in the development and validation of scales of motor performance in neuromuscular disorders, including the North Star Ambulatory Assessment (NSAA) and MFM.[13,28] Rasch analysis examined 7 tests for reliable and valid measurement and compared the items of the 2 scales and their targeting ability on the Jain COS of dysferlinopathy population. Available data from patients with between 1 and 4 assessments each over the first year period were included in this analysis. Data were entered into Rasch Unidimensional Measurement Model[26] RUMM2030 software.[29]

Part 2: Detecting Change Over 1 Year Using the NSAD. Following part 1 analysis, a proposed amalgamation and rescoring of items from the a-NSAA and MFM-20 was presented. The new scale, the NSAD, was

introduced at year 2 visits. The NSAD assessments at years 2 and 3 visits were examined using the Rasch analysis, as described above, and the Wilcoxon signed rank for longitudinal change with SPSS 24. Inter-rater reliability was assessed using intraclass correlation coefficients (ICCs).

Part 3: Longitudinal Disease Progression Over 3 Years. For part 3, the primary outcome was disease progression over time, both since symptom onset in years, and time in months from baseline to subsequent study visits. To account for correlation between repeated measures of individual patients, generalized estimating equations (GEEs) with sandwich variance estimation were used to evaluate change in functional measures over time. An exchangeable covariance structure was assumed for all models based on quasi information criteria measures suggesting best fit. For direct assessment of demographic and clinical factors, a 2-tailed $p < 0.05$ was considered significant. Nonlinear associations were considered in symptom duration models by including a quadratic time term in models to evaluate any plateau effects. All models evaluating progression controlled for age at baseline. For all demographic and clinical factors considered, a factor by time term was included in models to assess any differences in trajectories; interaction terms significant at $p \leq 0.20$ were noted. To better visualize potential trajectory differences, continuous measures with notable interactions were categorized either through commonly used age groupings or study-derived quartiles for graphing. Predicted values above or below the possible values for a given measure were bounded at the upper and lower limits for plotting. All analyses were completed using SAS version 9.4.

Results

Characteristics of the study patients included in the longitudinal analysis are presented in Table 1. Of the study subjects, 53.5% were women and most patients identified as non-Hispanic white (71.1%). Age at baseline ranged from 11 to 86 years and symptom duration at baseline from 1 to 51 years. Most of the patients were diagnosed as LGMDR2/2B, and three-quarters of the participants were ambulant at baseline. Mean follow-up time was 35.0 ± 5.8 months.

Part 1: Development of the North Star Assessment for Limb Girdle Type Muscular Dystrophies

One hundred fifty-eight assessments were available for the a-NSAA and 172 assessments included in the MFM-20 Rasch analysis. Overall, the a-NSAA and MFM-20 performed well against the battery of psychometric tests but were not without issue (Table 2).

TABLE 1. Characteristics of the Study Sample (N = 187)

Demographics	No.	%
Gender		
Male	87	46.5
Female	100	53.5
Race/ethnicity		
White	133	71.1
Asian	32	17.1
Hispanic	12	6.4
Other	10	5.4
Age at baseline, mean (SD), min/max	38.5 (13.0)	11, 86
Clinical factors		
Age at symptom onset, mean (SD), min/max	21.8 (8.6)	0, 60
Symptom duration at baseline, mean (SD), min/max	17.1 (10.5)	1, 51
Clinical diagnosis		
LGMD2B/R2	116	62.0
Miyoshi myopathy	54	28.9
Other	17	9.1
Ambulant at baseline		
Ambulatory	140	74.8
Nonambulatory	47	25.1
Maximum exercise level during teen years		
None	46	25.4
Low	17	9.4
Moderate	62	34.3
High	56	30.9
Study follow-up		
Number of visits, mean (SD), min/max	4.8 (0.5)	2, 5
Follow-up time in months, mean (SD), min/max	35.0 (5.8)	6, 44

Adapted North Star Ambulatory Assessment. The a-NSAA item fit was excellent with good coverage of disease severity (ie, very few items measured the same level of ability).

There was minimal floor effect, but some ceiling effect. Three of 22 items had fit residuals outside the recommended range, and one item misfit with a significant v^2 probability. Unidimensionality was acceptable (t test 4.2%, binomial test lower 95% confidence interval [CI] proportion, < 0.01). Reliability was supported by a high PSI (0.96), similar to Cronbach's alpha. Six of 22 items displayed disordered item response (scoring) thresholds. Ten pairs of bilateral items (including stand on one leg, hopping, and climbing on and off box step) had residuals that were highly correlated (> 0.40), implying that a response to one influenced the response to the other. When left-sided items were removed, the PSI remained high (0.95), suggesting the dependency did not artificially inflate reliability. There was no uniform or non-uniform differential item functioning (DIF) for gender, indicating that gender did not influence performance on this scale.

MFM-20. Rasch targeting identified redundant items (too easy for the population) and a ceiling effect, with a lack of items measuring stronger ambulant individuals. Eight of the 20 items had fit residuals outside the recommended range, and 4 items had a significant v^2 probability. Thirteen of 20 items had disordered scoring thresholds. Reliability was supported by a high PSI (0.94). Unidimensionality was not achieved (t test 14.5%, binomial test lower 95% CI proportion, > 0.05). One item expressed uniform DIF (ankle dorsiflexion), indicating that the ability to dorsiflex the ankle may be influenced by gender.

With data for ambulant patients collected on both scales, it was possible to examine the interaction between the scales. The a-NSAA measures stronger patients more effectively with some items from the MFM-20 contributing to better measurement of weaker and nonambulant individuals. Ordered response categories (thresholds) are paramount for accurate scale performance, as a higher score on individual items must represent a higher level of overall ability. The a-NSAA had 73% ordered thresholds. The MFM-20 had only 35% of items with ordered thresholds, with 4 scoring categories being too complex in 65% of items.

Following this review, 29 items were retained for the NSAD (all 22 items from the a-NSAA and 7 items from the MFM-20) with a range from 0 to 54 (the higher the score the better the ability). The NSAD rescored the disordered items, removed redundant and duplicate items (available from the authors), and was re-ordered to improve efficiency for patients and prevent unnecessary fatigue. The NSAD replaced the a-NSAA and MFM-20 in years 2 and 3 of the Jain COS of dysferlinopathy.

Part 2: Ability of the NSAD to Detect Change in 1 Year

We examined 309 NSAD assessments, 149 male patients and 160 female patients from year 2 and year 3 visits. The NSAD performed well on Rasch analysis (see Table 2). All but one of the items (Gets to sitting) clearly fit the construct with appropriate fit residual locations. Four items at year 2, and 5 items at year 3, misfit with significant chi square values of $p < 0.05$. Reliability was demonstrated by a high PSI of 0.97. Unidimensionality was acceptable (binomial test lower 95% CI proportion, < 0.05). Although a ceiling effect still existed for the strongest and potentially asymptomatic subjects, the motor performance of both ambulant and nonambulant subjects was targeted successfully by the items of the NSAD (Fig 1).

In the absence of a second independent rare disease population on whom to perform traditional scale validation, Rasch analysis was repeated on 5 randomly generated cohorts of 80% of the discovery population (see Table 2). The NSAD performed well in all of these analyses, confirming the scale is a fit for purpose instrument in this population.

The NSAD as a whole detected a statistically significant deterioration in the population over 1 year, with a mean change score of −1.73 points (Wilcoxon signed rank-sum test $p < 0.0001$, 95% CI = –2.33 to −1.14; Table 3). Inter-rater reliability was established with an ICC of 0.99.

Part 3: Disease Progression

Disease Duration (Time From Symptom Onset). For all outcomes assessed, a significant decline in function was seen across disease duration ($p < 0.0001$), with nonlinear trajectories noted (Table SS1). Plots of the NSAD, 10MWT, and 6MWT across symptom duration suggested disease progression for approximately 30 years, at which point decline reaches the measurement floor or levels off (data not shown). A milder plateau effect was noted for the ACTIVLIM.

Results of GEE models suggested that age at onset may play a role in disease progression (Table SS1). Later age at onset was associated with better function according to the NSAD adjusting for disease duration (β = 0.26 points for each year later, 95% CI = 0.06–0.45, $p = 0.03$), and patients with earlier age at onset showed faster progression than those with later onset ($p = 0.04$). Graphs suggested the steepest declines occurred during the first 10 years post onset regardless of age at symptom onset, with earlier onset patients experiencing continued linear decline compared with later onset patients who began to plateau (Fig 2A). Additionally, adjusted for disease

TABLE 2. Summary Rasch Results for Part 1 - a-NSAA and MFM-20 and Part 2 - NSAD

	Item Fit Mean (SD)	Person Fit Mean (SD)	Item-trait interaction chi squared value (DF)	Reliability PSI with extremes	Item Fit Ordered thresholds	Number of items with good fit*	Dependency (number of pairs)	Unidimensionality and DIF (by gender)
Part 1-a-NSAA and MFM-20 analysis								
a-NSAA n = 158 assessments 10 extremes	−0.95 (1.41)	−0.12 (0.42)	116 (44)	0.96	16/22 (73%)	19/22[a] (86%) 1 < p 0.01[b]	10 pairs	Acceptable. No DIF for gender
MFM-20 N = 172 assessments 10 extremes	−0.85 (2.17)	−0.25 (0.65)	524 (40)	0.94	7/20 (35%)	12/20[a] (60%) 4 < *p* 0.01[b]	1 pair	Not acceptable. DIF present on 1 item (ankle dorsiflexion)
Part 2- NSAD								
NSAD Year 2 N = 153 assessments 7 extremes	−0.27 (1.08)	−0.26 (0.57)	243.5 (116)	0.97	28/29 (97%)	28/29[a] 4 < 0.01[b]	14 pairs	Acceptable (< 0.05) and no DIF for gender
NSAD Year 3 N = 156 assessments 6 extremes	−0.51 (1.34)	−0.34 (0.61)	350.4 (116)	0.97	28/29 (97%)	26/29[a] 5 < 0.01[b]	19 pairs	Acceptable (< 0.05) and no DIF for gender
NSAD internal validation on discovery cohort								
Random cohort 1 N = 122 4 extremes	−0.25 (1.06)	−0.25 (0.60)	267.4 (116)	0.97	27/29 (93%)	29/29[a] 3 < 0.05[b]	12 pairs PSI 0.97 with one of pair removed	Acceptable (< 0.05) and no DIF for gender
Random cohort 2 N = 122 4 extremes	−0.28 (0.97)	−0.27 (0.57)	212.12 (116)	0.97	28/29 (97%)	29/29[a] 3 < 0.05[b]	13 pairs PSI 0.97 with one of pair removed	Acceptable (< 0.05) and no DIF for gender
Random cohort 3 N = 122 5 extremes	−0.25 (1.01)	−0.24 (0.59)	275.22 (116)	0.97	28/29 (97%)	28/29[a] 3 < 0.05[b]	12 pairs PSI 0.97 with one of pair removed	Acceptable (< 0.05) and no DIF for gender
Random cohort 4 N = 122 2 extremes	−0.56 (1.22)	−0.37 (0.72)	344.39 (116)	0.97	28/29 (97%)	26/29[a] 3 < 0.05[b]	14 pairs PSI 0.96 with one of pair removed	Acceptable (< 0.05) and no DIF for gender
Random cohort 5 N = 122 2 extremes	−0.50 (1.18)	−0.30 (0.54)	289.57 (116)	0.97	28/29 (97%)	27/29[a] 4 < 0.05[b]	13 pairs PSI 0.96 with one of pair removed	Acceptable (< 0.05) and no DIF for gender

*Fit: Defined as fit residual inside the recommended range (−2.50 to 2.50)[a] and nonsignificant chi squared probability ($p < 0.01$)[b].
a-NSAA = adapted North Star Ambulatory Assessment; DIF = differential item functioning; MFM-20 = Motor Function Measure-20; NSAD = North Star Assessment for Limb Girdle Type Muscular Dystrophies.

duration, Asian patients performed worse on the 10MWT (β = −0.53 m/s, 95% CI = –0.89 to −0.16, $p = 0.0005$) and 6MWT (β = −0.25, 95% CI = –0.42 to −0.08, $p = 0.004$) than White patients, on average, across the disease course. Slight differences in 10MWT trajectories by race were also noted ($p = 0.11$), with Asian and White patients appearing to decline more quickly than Hispanic and other patients (Fig 2B). Similarly, on average, women had higher NSAD scores (β = 4.60 points, 95% CI = 0.90–8.31, $p = 0.02$) and slightly better 6MWT velocities (β = 0.12 m/s, 95% CI = –0.02 to 0.25, $p = 0.08$) than male patients, controlling for disease

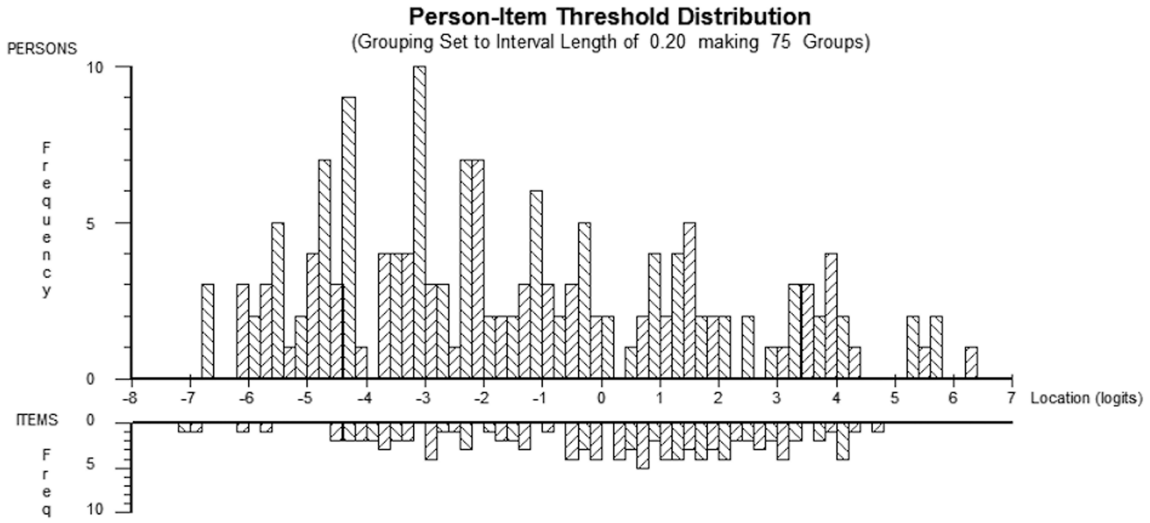

FIGURE 1: Person–item targeting of North Star Assessment for Limb Girdle Type Muscular Dystrophies at year 2 and 3 visits, people are the top row and items are the bottom row. The strongest patients and the hardest items are on the right, and the weakest patients and the easiest items are on the left. Good coverage of ability of both ambulant and nonambulant. Ceiling exists for asymptomatic subjects.

TABLE 3. Means and Change Scores for the NSAD over 12 Months, Years 2 to 3

	Baseline NSAD	Baseline	12 month changes
Total scores Whole cohort	Mean (ranges) [95% CI] *p* value	21.6 (0 to 54) (n = 140)	−1.73 (−15.0 to 8.00) [−2.33 to −1.14] < 0.0001 (n = 123)
Total scores Ambulant	Mean (ranges) [95% CI] *p* value	28.86 (7 to 54) (n = 97)	−2.18 (−15 to 8) [−0.296, −1.41] < 0.0001 (n = 87)
Total scores Nonambulant	Mean (ranges) [95% CI] *p* value	5.73 (0 to 15) (n = 43)	−0.64 (−5 to 5) [−1.36 to 0.08] 0.0613 (n = 36)

The patients whose visits fell outside the 12-month visit window for years 3 and 2 were excluded from the analysis (n = 13).
CI = confidence interval; NSAD = North Star Assessment for Limb Girdle Type Muscular Dystrophies.

duration. Some evidence that men progressed faster than women was noted for the NSAD ($p = 0.17$; Fig 2C) and 10MWT ($p = 0.19$), although differences in trajectories were not significant. Although absolute scores did not differ based on teenage exercise levels, assessment of disease trajectories suggested that patients with high exercise levels during the teenage years progressed slightly faster than those in the none and moderate exercise groups (Fig 2D; $p = 0.08$).

Three-Year Progression. All outcome measures showed significant decline during the 3-year follow-up period ($p < 0.0001$; Table S1). Beta estimates (Table S1) for follow-up time represented average monthly declines. For all outcomes, older age at onset and faster TUG and RFF velocities at baseline were associated with better function, whereas longer symptom duration was associated with worse function, controlling for patient age ($p < 0.001$). Compared with White patients, patients who identified as Asian or "other" had lower 10MWT and 6MWT velocities ($p < 0.05$).

Age at baseline and symptom duration at baseline were significantly associated with disease progression during the study period, primarily NSAD and 10MWT decline. The greatest decline during the study period was seen in patients who had been symptomatic for 3 to

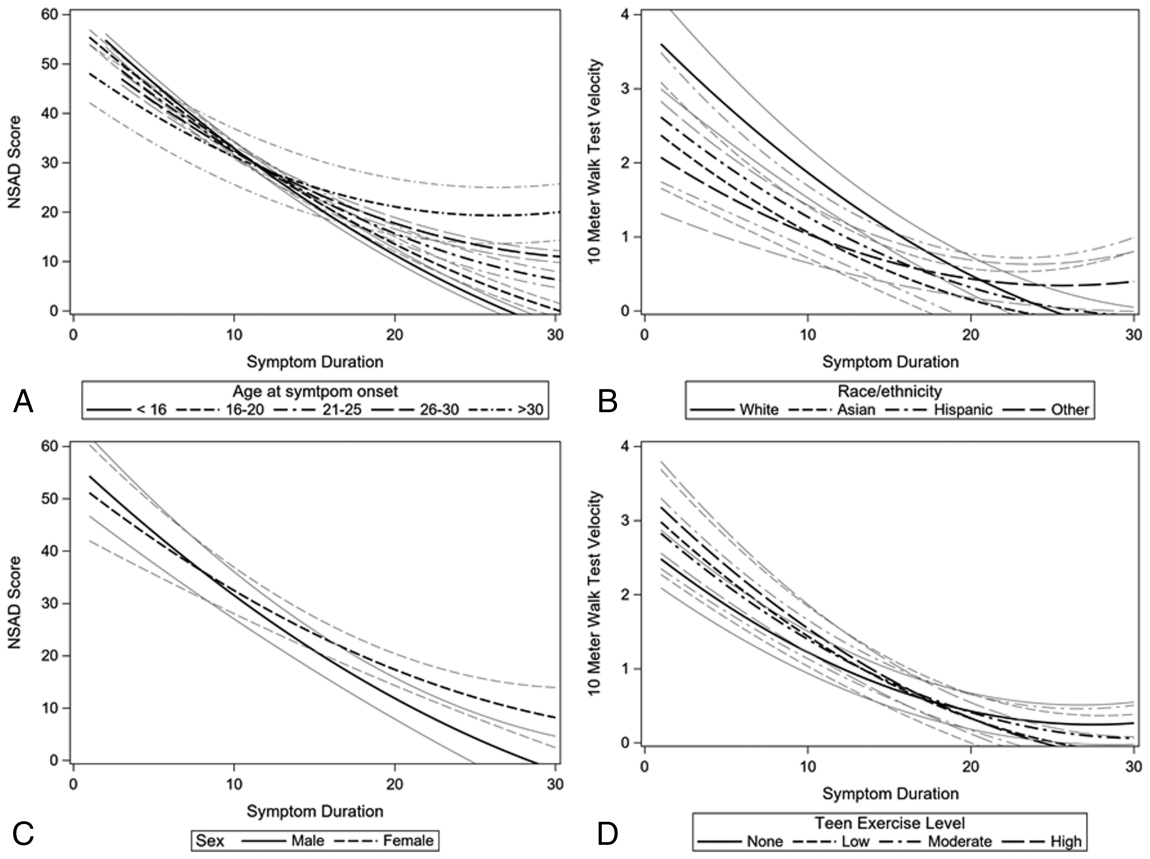

FIGURE 2: Outcome trajectories across symptom duration by patient characteristic. Fitted regression lines with 95% confidence intervals from generalized estimating equation modeling for (A) North Star Assessment for Limb Girdle Type Muscular Dystrophies (NSAD) score by age at symptom onset, (B) 10 meter walk/run test (10MWT) by race/ethnicity, (C) NSAD score by sex, and (D) 10MWT by teen exercise level.

8 years (Fig 3A, B). Likewise, patients with earlier age at symptom onset tended to show a greater decline during the study period than those with later onset (p = 0.06).

Lower TUG velocity at baseline was associated with greater decline in self-reported function on the ACTIVLIM during the follow-up period (p = 0.03), with some evidence of greater progression on timed function tests (p = 0.08). Likewise, patients with lower RFF velocity showed larger declines on the ACTIVLIM (Fig 3C; p = 0.001) and 6MWT (Fig 3D' p = 0.003), with some evidence of steeper progression on the 10MWT (p = 0.08). Quartile estimates suggested little decline over the 3-year study period for patients with high TUG velocity > 0.13 t/s (~ 7.7 seconds) or RFF velocity > 0.21 t/s (~ 4.7 seconds).

Sample Size Estimation for Clinical Trial Readiness. Outcome measures associated with decline over a short time period identified in 3-year GEE models were selected to characterize possible clinical trial inclusion criteria. Sample size is highly dependent on estimated treatment effect and variability around the estimate, whereby larger effects and decreased variability necessitate smaller samples. Sample size was estimated using PASS 15 software (Table 4). Mean decline and standard deviation for each 6 and 12-month period (± 2 months) were calculated. Sample sizes for a hypothetical clinical trial targeting halting of progression with the NSAD as the outcome measure ranged from 14 using the strictest inclusion criteria to 32 based on symptom duration at baseline alone for a 1-year trial and 32 to 52 for a 6-month trial based on the same criteria.

Discussion

Part 1 of this study evaluated the suitability of the a-NSAA and MFM-20 scales to measure motor performance in dysferlinopathy. The subsequent new scale, the NSAD, addressed the measurement issues within these 2 scales, re-ordered items to improve efficiency and reduce patient fatigue, and made the scale applicable for both ambulant and nonambulant patients. Analysis in part 2 confirmed that the NSAD could detect change in clinical progression of individuals with dysferlinopathy over 1 year and demonstrated excellent inter-rater reliability. This further supports the reported results for the first year of the Jain

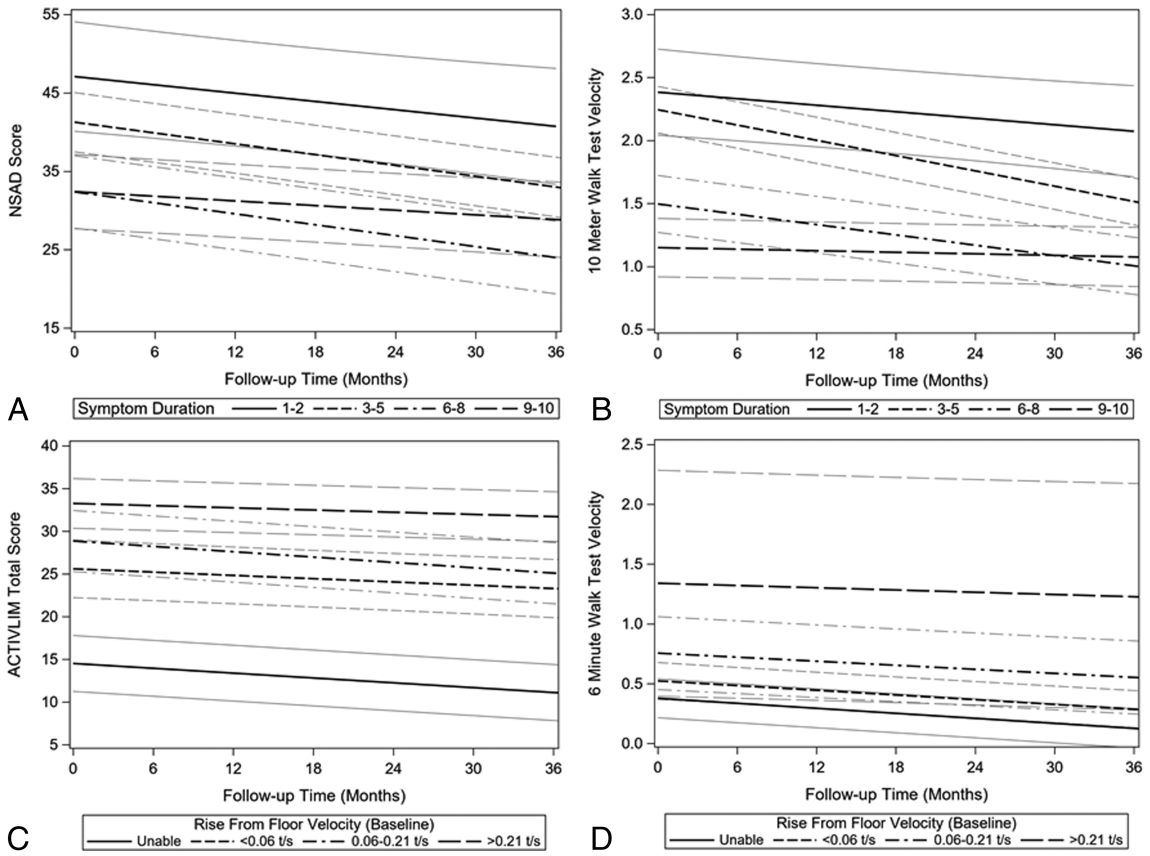

FIGURE 3: Functional outcome trajectories during the study period by patient characteristic. Fitted regression lines with 95% confidence intervals from generalized estimating equation modeling for (A) North Star Assessment for Limb Girdle Type Muscular Dystrophies (NSAD) score among symptom duration ≤ 10 years, (B) 10MWT among symptom duration ≤ 10 years, (C) ACTIVLIM by Rise from Floor velocity quartile, and (D) 6 minute walk test by Rise from Floor velocity quartile.

COS of dysferlinopathy, where the a-NSAA was suggested as a possible primary outcome for ambulant patients.[16]

The NSAD tests the abilities of anterior and posterior muscles of the leg and calf, which positions the assessment well to measure change in motor function across other LGMDs and conditions involving the pelvic girdle, such as Becker muscular dystrophy. The NSAD is currently being utilized in longitudinal natural history studies of LGMD types, including LGMDR1 Calpain3 related, LGMDR3 alpha-sarcoglycan related, and LGMDR4 beta-sarcoglycan related.[30,31] In these populations, the NSAD was highly correlated to the 100 meter walk/run and RFF tests, and differentiated rates of functional decline.[30,31] It is also being utilized as an end point to demonstrate improvement in motor function in the ongoing phase I gene therapy trial for LGMD R4.[15] Further work is being undertaken to examine its relationship to patient-reported outcome measures and validation in other diseases.

Results from part 3 suggested disease progression primarily occurs during the first 30 years after symptom onset, at which point progression levels off. Patients with an earlier disease onset may represent a more severe phenotype. Patients earlier on in their disease, particularly those 3 to 8 years from symptom onset, showed the steepest rate of progression, particularly in the NSAD and 10MWT over 3 years of follow-up. This finding is supported by a small study of 18 patients with dysferlinopathy where patients progressed from normal function to difficulty standing at an average of 8 years after disease onset.[11] Baseline TUG and RFF tests provided additional information, suggesting more noticeable declines in other outcome measures among patients with velocity measures below the highest quartile.

We previously estimated 46 moderately affected ambulant patients would be needed to show a halting of disease progression over 1 year on the a-NSAA.[16] Limiting inclusion criteria to patients 3 to 8 years from onset, with the NSAD as the outcome, could reduce this number to 32 and with timed function test criteria to 14. However, the necessary sample size must be balanced against the available population that meets the inclusion criteria. In the present study, only 40 patients fell into the 3 to

TABLE 4. Sample Size Estimates for a 6-Month or 1-Year Placebo-Controlled Clinical Trial with Variable Patient Inclusion Criteria

Outcome measure	Average change (SD)[a]	Target treatment effect				
		50% Reduction in progression	75% Reduction in progression	Halting of progression	20% Improvement	50% Improvement
Disease duration 3–8 yr						
6 mo						
NSAD	−1.53 (1.93)	202	92	52	38	26
ACTIVLIM	−0.74 (1.61)	598	268	152	106	70
10MWT	−0.14 (0.20)	260	116	68	48	32
6MWT	−0.04 (0.07)	388	174	100	70	46
1 yr						
NSAD	−2.81 (2.68)	116	54	32	22	16
ACTIVLIM	−1.31 (2.03)	310	138	78	56	36
10MWT	−0.20 (0.18)	104	48	28	20	14
6MWT	−0.09 (0.09)	128	58	34	24	18
Disease duration 3–8 yr and *either* TUG velocity < 0.13 *or* Rise from Floor velocity < 0.21						
6 mo						
NSAD	−1.90 (1.91)	130	60	34	26	18
ACTIVLIM	−1.33 (1.39)	140	64	38	26	18
10MWT	−0.15 (0.21)	250	112	64	46	30
6MWT	−0.04 (0.06)	286	128	74	52	34
1 yr						
NSAD	−2.78 (2.66)	118	54	32	24	16
ACTIVLIM	−2.14 (1.75)	86	40	24	18	12
10MWT	−0.20 (0.13)	56	26	16	12	10
6MWT	−0.11 (0.08)	70	32	20	14	10
Disease duration 3–8 yr and TUG velocity < 0.13 *and* Rise from Floor velocity < 0.21						
6 mo						
NSAD	−2.20 (2.14)	122	56	32	24	16
ACTIVLIM	−1.39 (1.64)	178	80	46	34	22
10MWT	−0.07 (0.08)	166	76	44	32	22
6MWT	−0.03 (0.07)	684	306	174	122	78
1 yr						
NSAD	−3.07 (1.79)	46	22	14	10	8
ACTIVLIM	−1.80 (1.25)	64	30	18	14	10
10MWT	−0.16 (0.10)	52	24	16	12	8
6MWT	−0.12 (0.10)	90	42	24	18	12

[a]Calculations are based on an observed change in outcome score in untreated patients, representing the expected control effect. Numbers quoted represent the total sample size based on equally sized treatment and control groups.

6MWT = 6 minute walk test; 10MWT = 10 meter walk/run test; NSAD = North Star Assessment for Limb Girdle Type Muscular Dystrophies; TUG = Timed Up and Go test.

8 years post-onset criteria across 14 sites, whereas just 10 patients met the most stringent criteria, highlighting the difficulty in meeting specific inclusion criteria for clinical trials. In addition, caution should be taken when considering years from symptom onset in inclusion criteria as onset of symptoms is often subjective, especially in retrospect. Although the 6MWT and ACTIVLIM remained sensitive measures of decline for more severely affected patients, timed function tests based on walking, including the 6MWT, are unsuitable once a patient loses ambulation, and are thus limited in terms of clinical and research utility.

Although we think the development of the LGMD-specific NSAD motor performance measure provides a valuable assessment tool previously lacking in dysferlinopathy, a limitation in the present investigation was the change to the scale mid-study. To address this issue, a detailed conversion plan was developed by expert physiotherapists; average decline per year estimated by GEE models (−1.7 points/year) was similar to mean annual changes of directly collected NSAD scores from year 2 to year 3 (−1.8).

The present study further supports results reported previously for the first year of study follow-up.[16] Annual change scores estimated from GEE models controlling for age were similar to median change scores reported previously for the outcome measures considered. The use of GEE modeling here allowed us to directly assess differences in rates of disease progression, which may have both clinical and trial implications.

A major strength of the present study was the availability of a large, diverse, multinational cohort, with characterized long-term follow-up that permitted assessment of longitudinal progression and trends previously unavailable. However, despite this large sample, certain patient groups remained small given the rarity of the disease; for example, only 12 patients identified as Hispanic and 5 as Black across all sites. Thus, findings involving race should be interpreted with caution. We aim to address this in future studies by expanding to additional sites.

The present investigation provided a broad estimate of functional trajectories across disease duration and aimed to identify patient groups and outcome measures for efficiently powered clinical trials. Future studies will explore additional factors, including genetic and biomarkers, as well as examine the role of the upper limb. Continued follow-up of the present population will also permit a more detailed understanding of long-term performance trajectories and functional loss. The NSAD is the first LGMD specific scale of motor performance. It allows measurements of patients in both the ambulant and non-ambulant stages of disease, providing a continuous scale of functional ability throughout the course of the disease, making it suitable for clinic, natural history studies, and interventional clinical trials.

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Author Contributions

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Potential Conflicts of Interest

The authors declared no conflict of interest.

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