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.

ABBREVIATIONS

| Abbreviation | Description |
|--------------|-------------|
| 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.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 change13,14 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),

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

Methods
The Jain COS of dysferlinopathy is a multicenter, inter-
national study of patients with a confirmed diagnosis of
dysferlinopathy. Detailed study methods have been pub-
lished 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 multi-
part 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 useful-
ness 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 out-
come measures identified during the first year of the study
were selected16 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 previ-
ous 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 standard-
ized 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 activi-
ties 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 compari-
sion. Timed function tests were converted to velocity mea-
sures (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 val-
ification of scales of motor performance in neuromuscular
disorders, including the North Star Ambulatory Assess-
ment (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 Measure-
ment Model26 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 LGMD2B/R2, 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 ($p < 0.0001, 95\% CI = -2.33$ 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 ($\beta = 0.26$ 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...
duration, Asian patients performed worse on the 10MWT ($\beta = -0.53$ m/s, 95% CI = -0.89 to -0.16, $p = 0.0005$) and 6MWT ($\beta = -0.25$, 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 ($\beta = 4.60$ points, 95% CI = 0.90–8.31, $p = 0.02$) and slightly better 6MWT velocities ($\beta = 0.12$ m/s, 95% CI = -0.02 to 0.25, $p = 0.08$) than male patients, controlling for disease

| TABLE 2. Summary Rasch Results for Part 1 - a-NSAA and MFM-20 and Part 2 - NSAD |
|---------------------------------|-----------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Item Fit | Person Fit | Item-trait interaction chi squared value | Reliability | Item Fit | Number of items with good fit | Dependency | Unidimensionality and DIF (by gender) |
| Mean (SD) | Mean (SD) | PS with extremes | Ordered thresholds | | |  |
| 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 (86%) | 10 | 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 (60%) | 1 | 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* | 4 | 0.01b | 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* | 5 | 0.01b | 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* | 3 | 0.05b | 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* | 3 | 0.05b | 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* | 3 | 0.05b | 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* | 3 | 0.05b | 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* | 4 | 0.05b | Acceptable (≤ 0.05) and no DIF for gender |

*Fit: Defined as fit residual inside the recommended range (−2.50 to 2.50) and nonsignificant chi squared probability (p < 0.01). 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. 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
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 ($\sim 7.7$ seconds) or RFF velocity $> 0.21$ t/s ($\sim 4.7$ 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 ($\pm$ 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...
COS of dysferlinopathy, where the a-NSAA was suggested as a possible primary outcome for ambulant patients.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{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 \(\leq 10\) years, (B) 10MWT among symptom duration \(\leq 10\) years, (C) ACTIVLIM by Rise from Floor velocity quartile, and (D) 6 minute walk test by Rise from Floor velocity quartile.}
\end{figure}
| Outcome measure | Target treatment effect | 50% Reduction in progression | 75% Reduction in progression | Halting of progression | 20% Improvement | 50% Improvement |
|-----------------|-------------------------|------------------------------|------------------------------|------------------------|----------------|----------------|
|                 |                        | Average change (SD)\(^a\)    |                              |                        |                |                |
|                 |                         | 6 m o                        | 1 y r                        | Disease duration 3–8 yr | 6 m o          | 1 y r          |
| 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             |
|                 |                        | 6 m o                        | 1 y r                        | Disease duration 3–8 yr and either TUG velocity < 0.13 or Rise from Floor velocity < 0.21 | 6 m o          | 1 y r          |
| 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             |
|                 |                        | 6 m o                        | 1 y r                        | Disease duration 3–8 yr and TUG velocity < 0.13 and Rise from Floor velocity < 0.21 | 6 m o          | 1 y r          |
| 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             |
|                 |                        | 6 m o                        | 1 y r                        | Disease duration 3–8 yr | 6 m o          | 1 y r          |
| 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             |
|                 |                        | 6 m o                        | 1 y r                        | Disease duration 3–8 yr and TUG velocity < 0.13 and Rise from Floor velocity < 0.21 | 6 m o          | 1 y r          |
| 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             |
|                 |                        | 6 m o                        | 1 y r                        | Disease duration 3–8 yr | 6 m o          | 1 y r          |
| 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. 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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Potential Conflicts of Interest
The authors declared no conflict of interest.

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