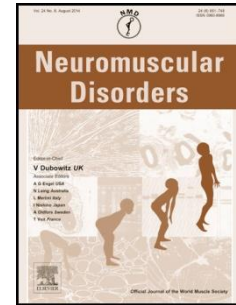


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CONFIDENTIAL

Categorizing natural history trajectories of ambulatory function measured by the 6-minute walk distance in patients with Duchenne muscular dystrophy

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Highlights

- The paper shows that DMD boys could be grouped into classes sharing trajectories.
- Using analysis accounting for trajectory classes, the variation was strongly reduced.
- Reducing unexplained variation could help to improve DMD clinical trial design.

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ABSTRACT

(Word Count: 250)

Background: High variability in patients' changes in 6 minute walk distance (6MWD) over time has complicated clinical trials of treatment efficacy in Duchenne muscular dystrophy (DMD). We assessed whether boys with DMD could be grouped into classes that shared similar ambulatory function trajectories as measured by 6MWD.

Methods: Ambulatory boys aged 5 years or older with genetically confirmed DMD who were enrolled in a natural history study at 11 care centers throughout Italy were included. For each boy, standardized assessments of 6MWD were available at annual intervals spanning 3 years. Trajectories of 6MWD vs. age and trajectories of 6MWD vs. time from enrollment were examined using latent class analysis.

Results: A total of 96 boys were included. At enrollment, the mean age was 8.3 years (mean 6MWD: 374 meters). After accounting for age, baseline 6MWD, and steroid use, four latent trajectory classes were identified as explaining 3-year 6MWD outcomes significantly better than a single average trajectory. Patient trajectories of 6MWD change from enrollment were categorized as having fast decline (n=25), moderate decline (n=19), stable function (n=37), and improving function (n=15) during the 3-year follow-up. After accounting for trajectory classes, the standard deviation of variation in 6MWD was reduced by approximately 40%.

Conclusions: The natural history of ambulatory function in DMD may be comprised of distinct trajectory classes. The extent to which trajectories are associated with novel and established prognostic factors warrants further study. Reducing unexplained variation in patient outcomes could help to further improve DMD clinical trial design and analysis.

Keywords: Duchenne muscular dystrophy, ambulatory function, natural history, latent class trajectory analysis, variability

1. INTRODUCTION

Approximately one in every 3,500 male births is affected by Duchenne muscular dystrophy (DMD), an X-linked disease arising from mutations to the dystrophin gene [1]. Early signs generally appear at 2 to 3 years of age, and include frequent falls and developmental delay [2]. Ambulatory function may improve in younger patients due to age-related growth and development. However, disease progression eventually reverses these gains with progressive impairment of motor function and loss of ambulation by between 9 and 14 years on average, depending on the steroid regimen used [3, 4] and subsequent progressive involvement of upper limb function [5, 6].

Changes in ambulatory ability measured by the 6-minute walk distance (6MWD) test have been characterized in several longitudinal natural history studies [7-10], and this test has been used as the primary functional outcome measure for clinical trials of the first three disease modifying DMD treatments under investigation (ataluren, drisapersen and eteplirsen). At the time of reporting initial results from these trials, challenges were apparent in using 6MWD to measure treatment effects [11, 12]. In particular, rates of change in 6MWD showed significantly greater cross-patient variability than expected, resulting in limited statistical power to measure treatment effects with available sample sizes.

Variability in the rate of progression of 6MWD is known to be influenced by age, baseline 6MWD, and steroid use [2]. Longitudinal studies of 6MWD over 12, 24 and 36 months have identified a number of possible cut-off points that help to identify different profiles of progression, such as baseline 6MWD \geq 350 meters and age \geq 7 years [7, 13, 14]. On average, boys below the age of 7 have shown annual improvements on the 6MWD while boys above the age of 7 have shown annual declines [7]. However, individual boys may show improving or declining function on either side of this age threshold.

In addition to variability in rates of progression from boy to boy, shorter-term, measurement-related variability has been characterized across repeated 6MWD

assessments within individual boys. Although 6MWD is effort based, and can be influenced by motivation, test-retest reliability is high, with correlations exceeding 0.9. The variability in 6MWD that has frustrated clinical trials is primarily due to high inter-patient variability in rates of change, rather than the smaller-scale, intra-patient measurement noise. For this reason, there remains a clear need for better characterizing the variability in 6MWD rates of change.

To objectively characterize the natural history of 6MWD in DMD, the present study sought to classify patients based on their trajectories of ambulatory function over time. Classification of patients based on disease trajectories has been used to understand natural histories in a number of diseases, including cardiovascular diseases and mental health conditions [15, 16], and has been used to identify associations between patient outcomes and biomarkers [17] and to help interpret treatment effects in randomized trials [18-21]. We applied latent trajectory analysis to study the natural history of ambulatory function in DMD, as measured by 6MWD, and explored whether trajectory classes could help to further explain variation in disease progression after accounting for age, steroid use and baseline 6MWD.

2. METHODS

2.1 Patient Selection and Outcomes Measurement

The patients and outcomes included in this study have been previously described as part of an ongoing natural history study of DMD [9, 10, 22]. Included boys were enrolled at 11 tertiary neuromuscular care centers in Italy between January 2008 and June 2010. Inclusion required boys to have genetically confirmed DMD, to be aged 5 years or older, to be able to walk independently for at least 75 meters, and to be free of moderate or severe learning difficulties or behavioral problems. All boys fulfilling these criteria at the study centers were enrolled. The present study sample includes 96 boys with 3 years of annual 6MWD assessments conducted at the same study center at intervals of 12 ± 3 months. 6MWD was assessed at enrollment and annually according to the American

Thoracic Society guidelines [23] with the modification of having two examiners (one recording time and distances and one staying close to the patient for safety issues) [24]. The last follow-up visit in the study sample was performed in August 2013. Steroid use prior to and during follow-up was into three categories: no steroids, intermittent steroids, and daily steroids.

Only anonymous, de-identified data were analyzed in the present study. Data collection was approved by the Ethical Committees of all 11 of the participating centers. As the assessments were already part of the clinical routine in all centers, with the approval of the Ethics Committees, verbal consent to record the anonymized data in a database was obtained by the parents for the boys under age. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

2.2 Statistical analysis

The goal of this study was to characterize variation in 6MWD trajectories. A first step in characterizing variation is to measure its magnitude. The magnitude of variation in 6MWD outcomes has been widely reported in DMD, with standard deviations for annual change ranging from 80-100 meters. A next step, taken in this study, is to ask whether such a variable population might be better understood as a mixture of several distinct groups. Suppose, as a hypothetical example, that annual rates of change in 6MWD exhibited a bimodal distribution across patients with one peak around 50 meters of decline and another peak around 100 meters of decline. This distribution would suggest that the population can be treated as a composite of two groups, and that appropriately classifying patients (e.g., based on a cutoff somewhere between 50 and 100 meters of decline) would yield subgroups worthy of further characterization. In this hypothetical example the classification can be based on a single number (annual rate of decline).

Changes in ambulatory function in DMD, especially over the 3-year time period addressed in the current study, are complex, and can include periods of both improvement and decline. They cannot be well characterized by a single number. For this reason, the present study employed a statistical method, latent class trajectory

analysis, which extends the intuition applied in the hypothetical example above to settings in which each individual is characterized by multiple measurements over time. Considering all measurements, the method asks whether there are distinct peaks in the distribution across patients, i.e. clusters of patients that share similar trajectories.

latent class trajectory analysis was also used to model annual 6MWD outcomes up to and including the first visit reflecting a loss of ambulation (defined as a 6MWD of zero meters). These analyses assessed whether the observed trajectories of 6MWD versus age were adequately described by a single underlying mean trajectory or, alternatively, by a mixture of two or more underlying mean trajectories, with each mean trajectory corresponding to a latent class [25-27]. Within each latent class, mean 6MWD was modeled as a quadratic function of age, thus allowing for periods of increasing and decreasing 6MWD. Variation in 6MWD around the mean trajectory for each individual was modeled using a subject-specific random intercept and independent Gaussian errors at each visit. Models with one, two, three, etc., latent trajectory classes were fit to the 6MWD data using maximum likelihood, and were compared in terms of their unexplained variation in 6MWD. Sensitivity analyses were conducted with 6MWD modeled as a more flexible function of age (i.e. including cubic terms) and with autoregressive error models.

Because adding latent trajectory classes to the model for 6MWD outcomes would necessarily improve the fit to the data, thereby reducing unexplained variation, Akaike's Information Criterion (AIC) and the Bayesian Information Criterion (BIC) were used for model selection [28, 29]. These measures help optimize the tradeoff between a model's goodness of fit and its complexity, as measured by the number of model parameters governing the mean trajectory in each latent class. The number of latent classes that minimizes the AIC and BIC provides a description of the data that is parsimonious and better fitting than models with fewer numbers of latent classes. After selecting the appropriate number of classes using AIC and BIC, each patient was assigned to the class that best matched their individual trajectory. This assignment was made based on posterior probabilities of class membership, which account for the likelihood that an

individual trajectory would occur in each class and the relative sizes of each class. Patients were assigned to the class for which they had the maximum posterior probability of membership, i.e., that class to which they were most likely to belong based on the fitted model. Confidence in these assignments was assessed by averaging the assignment probabilities across the patients within each class.

Because clinical trials of treatments for DMD typically measure changes in function from the time of enrollment, additional latent class trajectory analyses were conducted from this perspective. In particular, within each latent class, mean changes in 6MWD from the time of enrollment were modeled as a linear function of age at enrollment, 6MWD at enrollment, steroid use, and post-baseline visit time as a categorical variable (with levels of 1, 2 and 3 years). Variation in change in 6MWD around the mean was modeled using Gaussian errors with a common variance. Models with subject-specific random effects and autoregressive errors were also explored. AIC and BIC were used to select the number of latent classes, and, based on the selected model, patients were assigned and classified as described above. For each group, patients were characterized in terms of age, 6MWD at enrollment, and steroid use. These analyses were repeated while including all 6MWD assessments equal to zero meters following loss of ambulation (as opposed to terminating the included data following the first visit with loss of ambulation, as in the primary analyses). All analyses were implemented using the *lcmm* package in R [27].

3. RESULTS

3.1 *Patient characteristics*

Among the 96 boys included in the present study, age at enrollment ranged from 5 to 17 years with a mean age of 8.3 years and a standard deviation (SD) of 2.1 years. Sixty eight boys (71%) were aged 7 years or older. Mean 6MWD at enrollment was 373.7 meters (SD 82.4). Nearly 65% had a 6MWD greater than or equal to 350 meters. Of the 96 patients in the study, 92 (96%) were on a corticosteroid regimen: 42 received daily steroids and 50 received intermittent steroids. Four patients were classified as

having no steroids. A total of 24 boys lost ambulation during the study period: 3 by the end of the first year, 16 by the end of the second year and 24 by the end of the third year.

3.2 Classification of 6MWD trajectories vs. age

Individual patient trajectories of 6MWD vs. age exhibited wide variation (Figure 1). When a single mean trajectory of 6MWD vs. age was fit to these data, the residual, unexplained variation in 6MWD had a standard deviation (SD) of 71.5 meters. A latent class model with four classes was identified as the best fitting model, based on reduced AIC and BIC. This best fitting four-class model reduced the residual variation in 6MWD to an SD of 43.5 meters.

When patients were assigned to the four classes based on maximum posterior probabilities, there were 8 patients assigned to class 1, 22 assigned to class 2, 22 assigned to class 3, and 44 assigned to class 4 (Figure 2). Average trajectories in the 4 classes overlapped substantially at ages younger than 7 years, but the separated along increasingly distinct trajectories with increasing age. Among trajectories with observed loss of ambulation, the first experience of 6MWD=0 meters occurred at ages 8.9-10, 10.3-12.4, 13-14.4 and after 15 years, respectively, for patients in classes 1-4 (Figure 2). Age at loss of ambulation was not well represented in the data for this last class; only one patient was observed to lose ambulation in his late teens (at age 19 years), but was much older than all other patients in the sample. (Repeating the analyses after excluding this patient did not substantially change the results.) Steroid use was present in all classes. However, all 4 of the patients without steroid use were assigned to class 1. Steroids were used intermittently for 1 (12.5%), 9 (40.9%), 13 (59.1%) and 27 (61.4%) of the patients assigned to classes one through four, respectively, with the remaining patients receiving daily steroids. The assignment to classes based on posterior probabilities could, on average, be made with high confidence; the average posterior probability of being in the assigned class was over 80% (Table A2).

3.3 Classification of 6MWD trajectories vs. time from enrollment

A second latent class analysis studied 6MWD trajectories as a function of time from enrollment. By the end of the 3-year study period, average 6MWD had decreased by 104.2 meters from the time of enrollment, and a total of 24 patients had lost ambulation. Individual patient trajectories varied substantially around the mean trajectory (Figure 3). A model with a single mean trajectory for change in 6MWD, which was allowed to depend on baseline age, 6MWD, and steroid use, had a residual standard deviation of 95.4 meters across all post-baseline visits. Alternative single-class models including indicators for baseline age ≥ 7 years and baseline 6MWD ≥ 350 meters did not substantially improve the model fit, nor did models including an interaction between baseline 6MWD and visit number (residual SD exceeded 90 meters in all cases). However, the addition of latent classes improved model fit. Models with two and three latent classes reduced the unexplained variation to 71 and 62 meters, respectively. The model with four latent classes was identified as providing the most parsimonious description of changes in 6MWD, based on minimization of BIC and AIC, and after adjustment for baseline age, 6MWD, and steroid use. This model had a residual standard deviation of 55.6 meters.

The four trajectory classes in the selected model exhibited the following patterns (Figure 4). In the first class, labeled as “fast decline,” 6MWD declined at an average rate of approximately 117 meters per year. In the second class, labeled as “moderate decline,” 6MWD declined at approximately 50 meters per year. The third class has relatively “stable” mean 6MWD over the 3-year study period, with an average decline of approximately 10 meters after 3 years. In the final class, labeled “improvement,” mean 6MWD increased by almost 100 meters during the study period.

When patients were assigned to these classes based on maximum posterior probabilities there were 25 patients classified as having “fast decline,” 19 patients classified as “moderate decline,” 37 classified as “stable” and 15 classified as having “improvement” (Table 1). The average posterior probability of being in the assigned class was over 80% for each class (Supplementary Table A3).

Average baseline characteristics differed among patients assigned to these 4 trajectory classes (Table 1). Patients assigned to the “improvement” class were the youngest, with a mean age of 6.5 years and less than one third older than 7 years at enrollment. In contrast, over 90% of the patients assigned to the “fast decline” class were older than 7 years. Patients with 6MWD \geq 350 meters at enrollment were present in all classes, representing the minority in the “fast decline” class and the large majority in the “improvement” class.

Assignments to classes based on both change in 6MWD vs. age and change in 6MWD vs. time from enrolment provided complementary descriptions of natural history, and are both illustrated in Supplementary Figure A1.

As a sensitivity analysis, model selection was repeated for an analysis of all visits, including recurrent visits following loss of ambulation (at which 6MWD=0 meters). This analysis assessed the extent to which unexplained variation may be reduced by latent trajectory analysis in intent-to-treat analyses that included all follow-up assessments. In this sensitivity analysis, the single class model adjusting for baseline age, baseline 6MWD, and steroid use had a residual standard deviation of 102 meters. Adding up to 4 latent classes to this model decreased both the BIC and AIC, and reduced the residual standard deviation to 56 meters.

4. DISCUSSION

In this retrospective analysis of DMD natural history data, classes of boys who shared similar trajectories of ambulatory function were detected. Accounting for these trajectory classes explained significant variation in 6MWD, even after accounting for age, steroid use and baseline 6MWD. Baseline 6MWD and age exhibited expected associations with subsequent rates of changes in 6MWD, with faster declining groups having lower average 6MWD, higher average age and a higher proportion aged 7 years or older, compared to groups with stable or improving trajectories.

The degree to which trajectory classes reduced unexplained variation in 6MWD has important implications for DMD clinical trial design and analysis. Smaller sample

sizes can shorten the time and reduce the costs required for enrollment and completion of a trial. Reduced variation in outcomes translates into increased power and smaller sample size requirements. For example, to detect a 30 meter treatment effect on change in 6MWD, a proposed minimal important difference [30], with 80% power, approximately 110 boys would be required with $SD=56$, and more than 310 boys would be required with $SD=95$. If a study design reduced variation to 75 meters, approximately 50% of the way between 95 and 56 meters, a sample size of 200 would be required. Enabling trials to achieve adequate power with smaller numbers of patients would help expand the diversity of treatments that could be investigated across the DMD community at the same time.

Randomized trials of drug effects could reduce outcome variation through enrichment of particular patient trajectories, or by stratification or adjustment for baseline characteristics that predict outcome trajectories. In addition to reducing variation in outcomes, clinical trial design may seek to enrich for patients whose trajectories of ambulatory function are thought to be most modifiable by a particular study drug's mechanism of action. All of these considerations apply to both randomized trials and study designs that include comparisons to natural history controls, and are particularly relevant to many of the ongoing or planned clinical trials using exon skipping that target small numbers of boys with specific subgroups of DMD mutations. The initial clinical trials for exon skipping interventions are in the largest sub-populations, and as the population sizes decrease, the limited numbers of patients available to participate in these studies may become prohibitive for placebo-controlled study designs. It will therefore be important to identify and qualify appropriate controls for prospective clinical study designs, and for potential comparison to a control arm drawn from natural history studies matched on characteristics that predict outcome trajectories. These results provide a proof of concept, indicating that latent class trajectory analysis can contribute to the characterization of heterogeneous ambulatory outcomes in DMD. The approach may also help characterize variation in rates of change of non-ambulatory (e.g., pulmonary or cardiac) outcomes in DMD, or surrogate markers (e.g., imaging of skeletal muscle).

To the best of our knowledge, this is the first mathematically-driven quantification of rates of DMD disease progression using latent class methodology. However, observable differences between groups of patients across a range of outcome measures have previously been reported.

Translation of the trajectory classes identified in this study into statistical tools for clinical trial design and analyses could be further explored following two approaches. The first approach is to identify baseline predictors of outcome trajectories. Beyond baseline age, 6MWD, and steroid regimen, potential predictors include other functional assessments (e.g., time to climb stairs, time to rise from supine and other components of the North Start Ambulatory Assessment), height, weight, direct measures of muscle strength, age at steroid initiation and dystrophin genotype. Other possible predictors include genetic modifiers [31, 32], MRI measures of muscle composition [33, 34], or other biomarkers currently being investigated. The maximum extent to which combinations of currently available measures can predict ambulatory outcomes in DMD is an important topic for future research. An accurate prediction model for trajectories of ambulatory function would enable improved enrichment, stratification, baseline adjustment, and matching in clinical trials with randomized or natural history controls.

The second approach for utilizing the trajectories identified in the present study is the direct application of latent class trajectory analysis in the study of drug effects in DMD. Such analyses have been used to explore drug effects in a number of other therapeutic areas, and, in addition to reducing unexplained variation, can identify potential treatment effect heterogeneity across patients with different underlying trajectories [18-21]. Care must be taken in the interpretation of these analyses because the latent classes are identified using post-baseline or post-randomization outcomes. In addition, given the sample sizes available for drug trials in DMD, it will be important to develop ways of incorporating knowledge gained from analyses of natural history data into the analyses of drug effects.

The present study has important limitations. Although the sample size and duration of follow-up is large for a DMD study, the number of patients limited our ability

to evaluate models with more than 4 latent trajectory classes, more complex shapes for mean trajectories within classes, more flexible models for correlation over time, and non-normal error distributions. With more participants, it may be possible to further describe the trajectories and explain variation. It is possible that analyses of larger sample sizes would identify more latent classes than the four identified in the present study. Furthermore, the reproducibility of these results should be explored, and new studies are in progress to assess the reproducibility of trajectory classes in separate DMD natural history databases.

5. CONCLUSIONS

The results of this preliminary study, designed as a proof of concept, demonstrate that it is possible to reduce the high variability of 3-year change in 6MWD in DMD patients by approximately 40% over and above the 10-15% reduction provided by baseline age, 6MWD and steroid use. If these results are reproducible, latent class trajectory analysis may enrich understanding of natural history in DMD and complement other approaches to improving and accelerating clinical trials of important therapeutic candidates.

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Specific author contributions:

SJW and JES co-developed the concept for this study. All authors participated in the design of the study and contributed to the manuscript development. Statistical analyses were conducted by JES, ES and JS and were interpreted in collaboration with all co-authors. EM and JES contributed equally to this research. All the authors vouch for the accuracy and completeness of the data reported and the adherence of the study to the protocol and all the authors made the decision to submit the manuscript for publication.

Members of cTAP contributed to the conceptualization and interpretation of this research, and to review of the publication. Contributing members include: Giles Campion, MD Biomarin; Carl Morris, PhD and Michael Binks, MD, Pfizer; Robert Spiegel, MD, Allen Reha, PhD, PTC Therapeutics; Ed Kaye, MD, Sarepta Therapeutics; Serene Josiah, Ph.D., Shire; Lawrence Charnas, MD, PhD, advisor to Parent Project Muscular Dystrophy; and Michael Kelly, PhD, Cure Duchenne. This manuscript was approved for submission by the cTAP Joint Steering Committee.

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Potential competing interests:

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REFERENCES

- [1] Hoffman EP, Brown RH, Jr., Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell* 1987;51:919-28.
- [2] Emery AEH, Muntoni F, Quinlivan R, Duchenne muscular dystrophy. 4th ed. Oxford monographs on medical genetics. 2015, Oxford: Oxford University Press. ix, 308 p.
- [3] Ricotti V, Ridout DA, Scott E, et al. Long-term benefits and adverse effects of intermittent versus daily glucocorticoids in boys with Duchenne muscular dystrophy. *J Neurol Neurosurg Psychiatry* 2013;84:698-705.
- [4] Pane M, Fanelli L, Mazzone ES, et al. Benefits of glucocorticoids in non-ambulant boys/men with Duchenne muscular dystrophy: A multicentric longitudinal study using the Performance of Upper Limb test. *Neuromuscul Disord* 2015;25:749-53.
- [5] Mazzone E, Bianco F, Main M, et al. Six minute walk test in type III spinal muscular atrophy: a 12month longitudinal study. *Neuromuscul Disord* 2013;23:624-8.
- [6] Seferian AM, Moraux A, Annoussamy M, et al. Upper limb strength and function changes during a one-year follow-up in non-ambulant patients with Duchenne Muscular Dystrophy: an observational multicenter trial. *PLoS One* 2015;10:e0113999.
- [7] McDonald CM, Henricson EK, Abresch RT, et al. THE 6-minute walk test and other endpoints in Duchenne muscular dystrophy: Longitudinal natural history observations over 48 weeks from a multicenter study. *Muscle Nerve* 2013;48:343-56.
- [8] Goemans N, van den Hauwe M, Wilson R, van Impe A, Klingels K, Buyse G. Ambulatory capacity and disease progression as measured by the 6-minute-walk-distance in Duchenne muscular dystrophy subjects on daily corticosteroids. *Neuromuscul Disord* 2013;23:618-23.
- [9] Pane M, Mazzone ES, Sormani MP, et al. 6 minute walk test in Duchenne MD patients with different mutations: 12 month changes. *PLoS One* 2014;9:e83400.
- [10] Pane M, Mazzone ES, Sivo S, et al. Long term natural history data in ambulant boys with Duchenne muscular dystrophy: 36-month changes. *PLoS One* 2014;9:e108205.
- [11] Voit T, Topaloglu H, Straub V, et al. Safety and efficacy of drisapersen for the treatment of Duchenne muscular dystrophy (DEMAND II): an exploratory, randomised, placebo-controlled phase 2 study. *Lancet Neurol* 2014;13:987-96.
- [12] Bushby K, Finkel R, Wong B, et al. Ataluren treatment of patients with nonsense mutation dystrophinopathy. *Muscle Nerve* 2014;50:477-87.
- [13] McDonald CM, Henricson EK, Han JJ, et al. The 6-minute walk test as a new outcome measure in Duchenne muscular dystrophy. *Muscle Nerve* 2010;41:500-10.
- [14] Mazzone E, Vasco G, Sormani MP, et al. Functional changes in Duchenne muscular dystrophy: a 12-month longitudinal cohort study. *Neurology* 2011;77:250-6.
- [15] McLaughlin KA, King K. Developmental trajectories of anxiety and depression in early adolescence. *J Abnorm Child Psychol* 2015;43:311-23.
- [16] Tielemans SM, Geleijnse JM, Menotti A, et al. Ten-year blood pressure trajectories, cardiovascular mortality, and life years lost in 2 extinction cohorts: the Minnesota Business and Professional Men Study and the Zutphen Study. *J Am Heart Assoc* 2015;4:e001378.

- [17] Pietrzak RH, Lim YY, Ames D, et al. Trajectories of memory decline in preclinical Alzheimer's disease: results from the Australian Imaging, Biomarkers and Lifestyle Flagship Study of ageing. *Neurobiol Aging* 2015;36:1231-8.
- [18] Leoutsakos JM, Bandeen-Roche K, Garrett-Mayer E, Zandi PP. Incorporating scientific knowledge into phenotype development: penalized latent class regression. *Stat Med* 2011;30:784-98.
- [19] Shiyko MP, Li Y, Rindskopf D. Poisson Growth Mixture Modeling of Intensive Longitudinal Data: An Application to Smoking Cessation Behavior. *Struct Equ Modeling* 2012;19:65-85.
- [20] Muthen B, Brown HC. Estimating drug effects in the presence of placebo response: causal inference using growth mixture modeling. *Stat Med* 2009;28:3363-85.
- [21] Hunter AM, Muthen BO, Cook IA, Leuchter AF. Antidepressant response trajectories and quantitative electroencephalography (QEEG) biomarkers in major depressive disorder. *J Psychiatr Res* 2010;44:90-8.
- [22] Mazzone ES, Pane M, Sormani MP, et al. 24 month longitudinal data in ambulant boys with Duchenne muscular dystrophy. *PLoS One* 2013;8:e52512.
- [23] Laboratories ATSCoPSfCPF. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 2002;166:111-7.
- [24] McDonald CM, Widman LM, Walsh DD, Walsh SA, Abresch RT. Use of step activity monitoring for continuous physical activity assessment in boys with Duchenne muscular dystrophy. *Arch Phys Med Rehabil* 2005;86:802-8.
- [25] Muthen B, Shedden K. Finite mixture modeling with mixture outcomes using the EM algorithm. *Biometrics* 1999;55:463-9.
- [26] Muthen B, Muthen LK. Integrating person-centered and variable-centered analyses: growth mixture modeling with latent trajectory classes. *Alcohol Clin Exp Res* 2000;24:882-91.
- [27] Proust-Lima C, Dartigues JF, Jacqmin-Gadda H. Joint modeling of repeated multivariate cognitive measures and competing risks of dementia and death: a latent process and latent class approach. *Stat Med* 2015.
- [28] Akaike H. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 1974;19.
- [29] Schwarz G. Estimating the dimension of a model. *The Annals of Statistics* 1978;6:461-464.
- [30] McDonald CM, Henricson EK, Abresch RT, et al. The 6-minute walk test and other clinical endpoints in duchenne muscular dystrophy: Reliability, concurrent validity, and minimal clinically important differences from a multicenter study. *Muscle Nerve* 2013;48:343-56.
- [31] van den Bergen JC, Hiller M, Bohringer S, et al. Validation of genetic modifiers for Duchenne muscular dystrophy: a multicentre study assessing SPP1 and LTBP4 variants. *J Neurol Neurosurg Psychiatry* 2015;86:1060-5.
- [32] Bello L, Kesari A, Gordish-Dressman H, et al. Genetic modifiers of ambulation in the Cooperative International Neuromuscular Research Group Duchenne Natural History Study. *Ann Neurol* 2015;77:684-96.

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- [33] Willcocks RJ, Arpan IA, Forbes SC, et al. Longitudinal measurements of MRI-T2 in boys with Duchenne muscular dystrophy: effects of age and disease progression. *Neuromuscul Disord* 2014;24:393-401.
- [34] Forbes SC, Willcocks RJ, Triplett WT, et al. Magnetic resonance imaging and spectroscopy assessment of lower extremity skeletal muscles in boys with Duchenne muscular dystrophy: a multicenter cross sectional study. *PLoS One* 2014;9:e106435.

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Figure 1. Individual patient trajectories of 6MWD versus age

Each point represents a patient's 6MWD, with lines connecting annual assessments from the same individual patient.

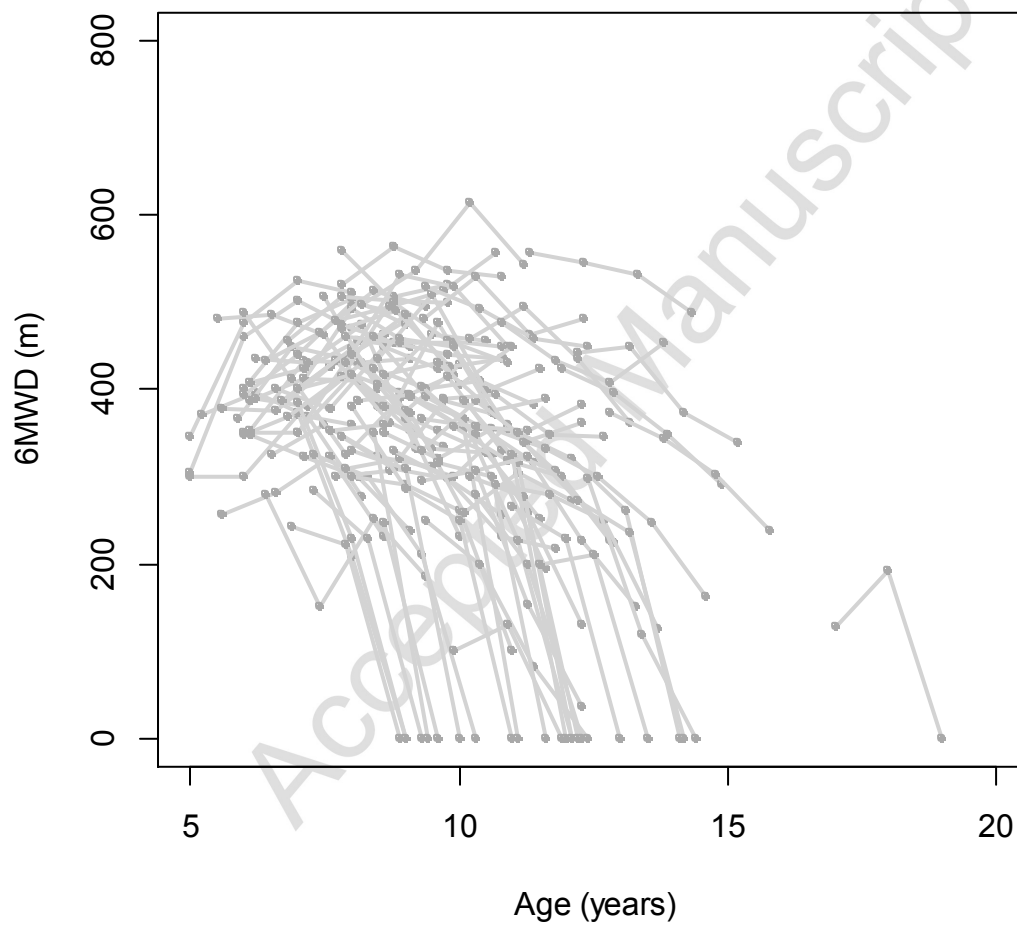


Figure 2. Predicted and observed trajectories of 6MWD vs. age

Solid black lines represent the predicted mean 6MWD trajectory in each class; gray lines represent the individual patient trajectories assigned to each class.

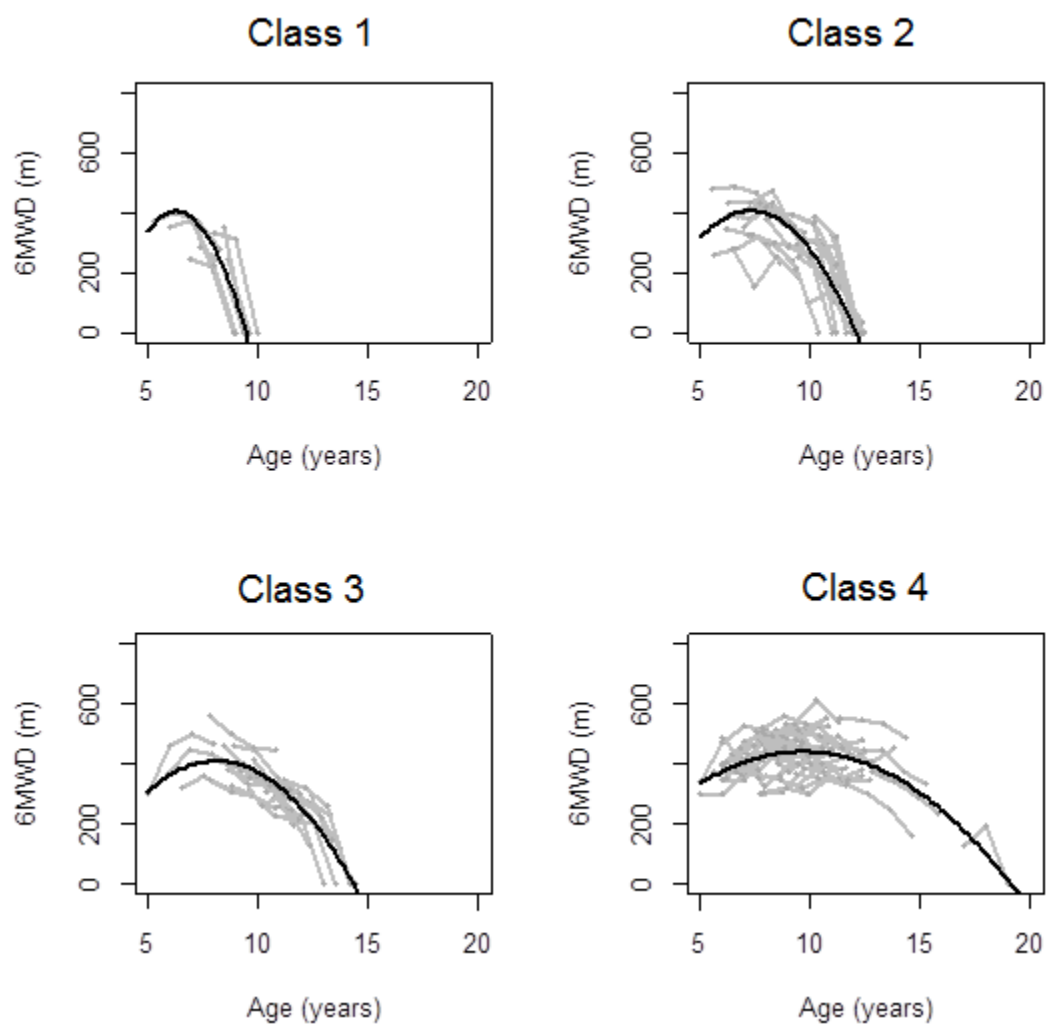


Figure 3. Changes in 6MWD vs. time from enrollment

The solid black line represents the mean change in 6MWD from enrollment at each of the 3 follow-up years; error bars represent \pm one standard deviation. Gray lines represent individual patient trajectories. Solid black points represent visits with loss of ambulation (defined as 6MWD = 0 meters).

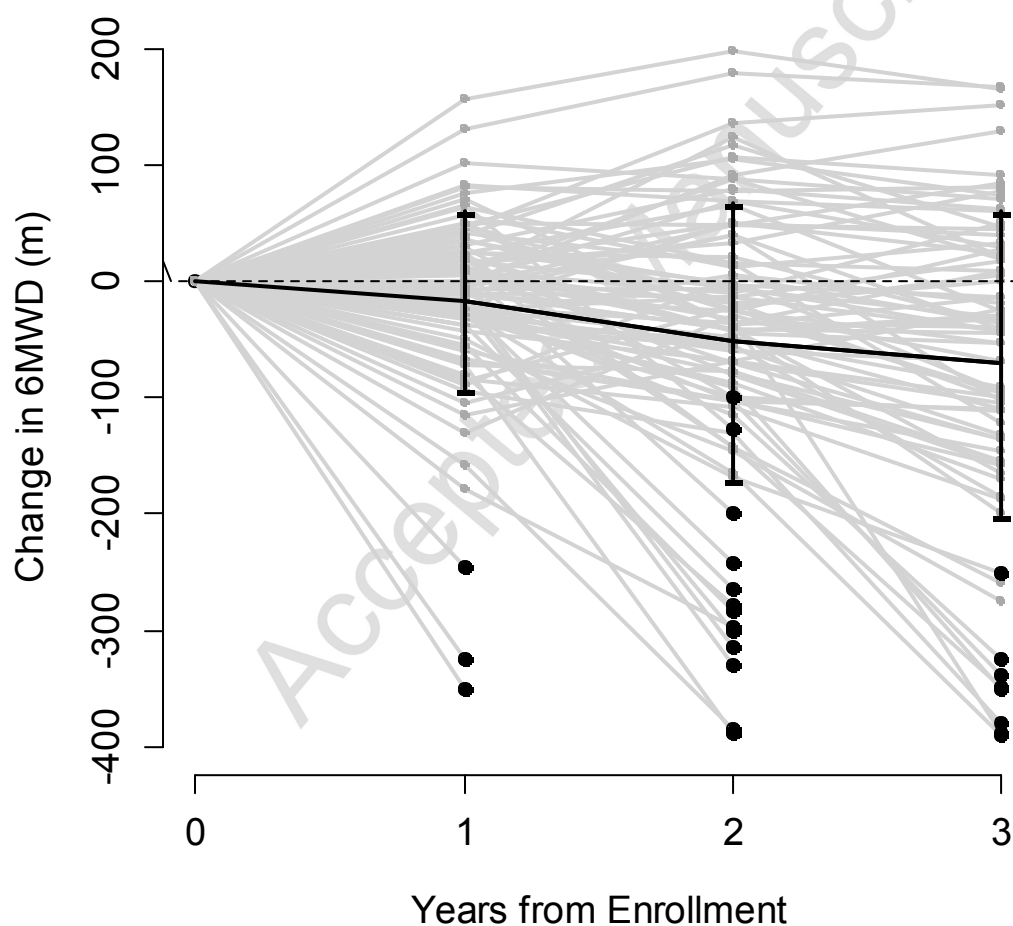


Figure 4. Predicted and observed trajectories of 6MWD versus time from enrollment

Solid colored lines represent the predicted mean 6MWD trajectory in each class; gray lines represent the individual patient trajectories assigned to each class. Solid black points represent visits with loss of ambulation (defined as 6MWD = 0 meters).

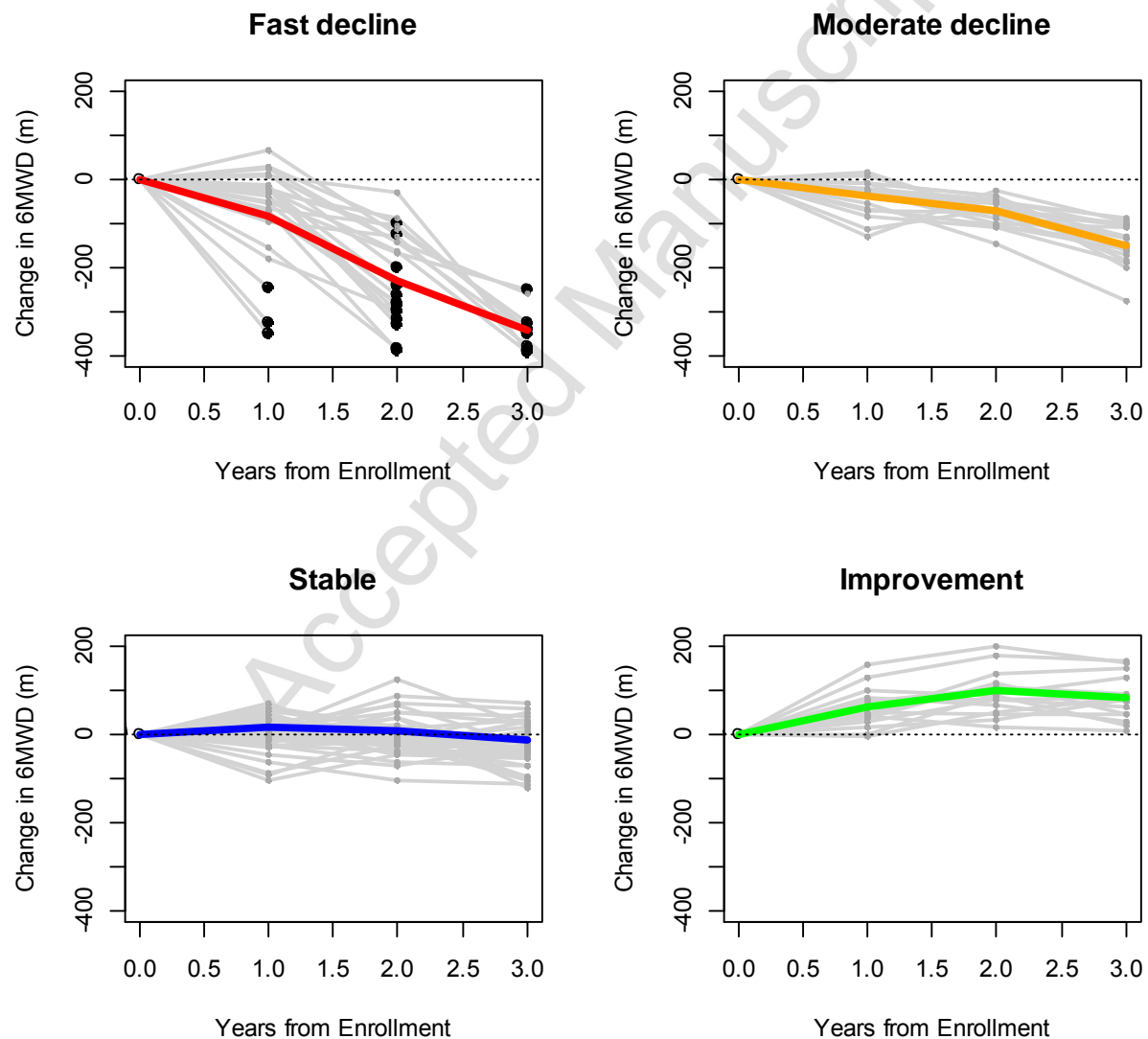


Table 1. Patient characteristics stratified by trajectory class (based on changes in 6MWD from enrollment)

	“Fast decline”	“Moderate decline”	“Stable”	“Improvement”
Characteristics	N=25	N=19	N=37	N=15
Age at enrollment, years				
Mean ± SD	9.5 ± 2.3	9.4 ± 1.8	7.4 ± 1.6	6.5 ± 1.0
Median (range)	9.4 (6.0,17.0)	9.1 (6.1, 12.8)	7.7 (5.0, 11.3)	6.1 (5.0, 8.2)
Age ≥ 7 years, n (%)	23 (92.0)	17 (89.4)	24 (64.9)	4 (26.7)
6MWD at baseline, meters				
Mean (SD)	300.1 ± 76.2	369.7 ± 56.0	412.7 ± 72.4	405.0 ± 62.3
6MWD ≥ 350 meters, n (%)	7 (28.0)	11 (57.9)	31 (83.8)	13 (86.7)
Age ≥ 7 years & 6MWD ≥ 350 meters, n (%)				
	7 (28.0)	11 (57.9)	21 (56.8)	4 (26.7)
Steroid use, n (%)				
No steroids	3 (12.0)	0 (0.0)	1 (2.7)	0 (0.0)
Intermittent	10 (40.0)	10 (52.6)	21 (56.8)	9 (60.0)
Daily	12 (48.0)	9 (47.4)	15 (40.5)	6 (40.0)

Caption: SD= standard deviation.

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