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ORIGINAL ARTICLE

# Developing a Natural History Progression Model for Duchenne Muscular Dystrophy Using the Six-Minute Walk Test

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**The 6-minute walk test (6MWT) is used as a clinical endpoint to evaluate drug efficacy in Duchenne Muscular Dystrophy (DMD) trials. A model was developed using digitized 6MWT data that estimated two slopes and two intercepts to characterize 6MWT improvement during development and 6MWT decline. Mean baseline 6MWT was 362 (±87) meters. The model predicted an improvement at a rate of 20 meters/year (95% confidence interval (CI) = 9.4–30) up until 10 years old (95% CI = 6.78–13.1), and then a decline at a rate of 85 meters/year (95% CI = 72–98). Interpatient slope variability for improvement and decline were similar at 21.9 percentage of coefficient of variation (%CV) and 23.3%CV, respectively. Model simulations using age demographics from a previous DMD natural history study could reasonably predict the trend in improvement and decline in the 6MWT. This model can be used to quantitate individual patient trajectories, identify prognostic factors for disease progression, and evaluate drug effect.**
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## Study Highlights

**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**

☑ The 6MWT is a clinical end point used to assess motor function in ambulatory subjects in Duchenne Muscular Dystrophy trials. The 6MWT is highly variable in part due to age-related changes making it a challenge to use this end point to demonstrate a drug effect.

**WHAT QUESTION DID THIS STUDY ADDRESS?**

☑ A disease progression population model was developed to characterize the extent of the age-related developmental improvements and disease-related decline in 6MWT performance.

**WHAT THIS STUDY ADDS TO OUR KNOWLEDGE**

☑ The model predicts that patients with DMD will improve in 6MWT performance up until a mean age of 10 years old, and then begin a rapid decline in performance. The base structural model provides a novel quantitative framework to characterize age-related changes in the 6MWT.

**HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?**

☑ The 6MWT disease progression model can be used to evaluate patient prognostic factors that contribute to disease progression in DMD, to design more effective trials for this rare disease and to evaluate a drug effect.

Duchenne Muscular Dystrophy (DMD) is a sex-linked genetic disease that affects 1 in 3,600 male births and is characterized by loss-of-function mutations in the dystrophin gene.[1] These mutations lead to mechanical muscle defects that eventually alter muscle function and accelerate mortality. Patients are typically diagnosed around 4 years old and are rendered nonambulatory in early to late teens and then succumb to the disease as young adults in their early 20s due to respiratory and cardiac complications.[1] Corticosteroids are the current standard of care (SOC) and offer some symptomatic benefit, but there is a need for more specific acting and potent drugs that can delay/prevent muscle function loss, improve the quality of life, and reduce mortality.[1,2]

The 6-minute walk test (6MWT) has been used as a measure of motor function in ambulatory patients with DMD in clinical trials[3–5] and in natural history studies.[6–9] The 6MWT was adapted and standardized for patients with DMD, as previously described.[10–12] Briefly, a flat indoor corridor is marked with cones 25-meters apart and the floor is taped at 1-meter increments. The subject is asked to continually walk around the cones for 6 minutes with a person following to assist with falls and to provide standard language of encouragement, while another person times the walk with a stopwatch. An early observation described when using the 6MWT and other motor function measures in patients with DMD was the age-dependence to motor function. Because patients with DMD are typically diagnosed during early childhood development, their motor function is continuing to improve (albeit less than healthy subjects). Eventually, developmental improvements plateau and disease-associated decline in motor function accelerates. The age-dependence to motor function was described in McDonald *et al.*[11] in 2010, in which it was reported that patients with DMD with baseline ages typically <7 years old, generally experienced improvements in motor function over the 1-year observation period, whereas patients with DMD with baseline ages >7 years old experienced motor function decline. Subsequent literature describing motor

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function progression in patients with DMD have used a baseline age stratification of 7 years to summarize motor function improvement and decline, but the age at which a patient with DMD begins to decline is likely to be highly variable.[6–8,13,14] In addition to the age-dependence to 6MWT variability, baseline motor function performance and steroid use have also been identified as sources of interpatient variability.[9] As a result of the 6MWT variability, it is challenging to design an effective trial that can detect a motor function difference between the study drug and placebo or SOC, and it often requires defining specific inclusion criteria and patient stratification.[15] Having a more objective disease progression model to characterize motor function trajectories in patients with DMD was important and prompted this work.

Obtaining data for a rare disease is often a challenge and utilizing literature data can provide an initial starting point. Here, we describe the use of digitized, longitudinal data from the literature from a natural history study and from the placebo arm of a randomized trial to develop a linear mixed-effect population model. This model captured the age-dependence to the developmental and disease-induced changes in the 6MWT and provided a way to quantitate both the population mean as well as individual patient trajectories. Simulations with the model using different age demographics and trial durations reported in the literature could reasonably describe observed changes in the 6MWT. This base structural model of the 6MWT disease progression will be used to identify intrinsic and extrinsic patient factors that may contribute to motor function heterogeneity and loss of ambulation for more effective trial design.

## METHODS

### Data sources

Longitudinal 6MWT data from two publications were digitized using plot digitizer (http://plotdigitizer.sourceforge.net).[6,8] McDonald *et al.*[8] (2013) described 6MWT data from the placebo arm of a randomized, controlled clinical trial across multiple clinical sites and the data is presented in **Figure 4** of their publication. In total, 53 subjects were digitized with two data points per subject (one at baseline and one at 48 weeks) for a total of 106 observations. The Goemans *et al.*[6] (2013) publication described 6MWT data from a natural history study conducted by the Leuven Neuromuscular Reference Center and the data is presented in **Figure 1** of their publication. Multiple 6MWT data points for each subject at baseline and throughout the 2-year study were digitized for a total of 35 subjects and 122 observations. The datasets were combined for a total 88 subjects and 228 6MWT observations (**Figure 1**). All of the subjects from the Goemans *et al.*[6] publication were on a stable, daily steroid regimen with 90% of the subjects treated with deflazacort. The McDonald *et al.*[8] publication had a more heterogeneous population with 70% of the subjects on steroids and details on the type of steroid or regimen were not provided.

### Data analysis platform

The Phoenix nonlinear mixed effect version 6.4 (NLME; https://www.certara.com/software/pkpd-modeling-and-simulation/phoenix-nlme/) was used for model estimation and simulation. Diagnostic graphics, exploratory analysis, and postprocessing of the simulation data were performed in Phoenix. Age demographics for the simulations were generated in R version 3.2.3, R Studio version 0.99.491.

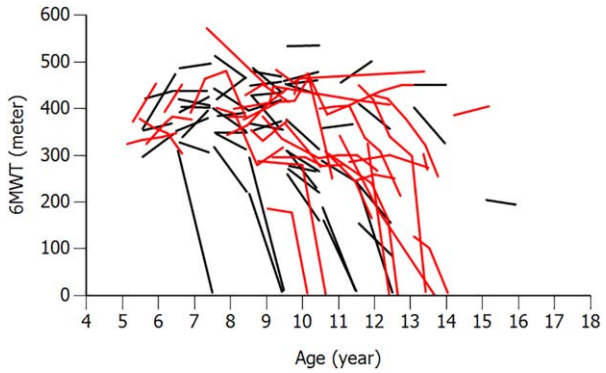


**Figure 1** Digitized data from two separate publications showing individual 6-minute walk test (6MWT) trajectories on the y-axis and the subject age on the x-axis. The red lines indicate data from the Goemans *et al.*[6] 2013 publication and the black lines indicate data from the McDonald *et al.*[8] 2013 publication. Details of data digitization can be found in the Methods section.

### Model development

The following six models were evaluated with the goal to describe the full progression of the 6MWT, including early age (growth/improvement) and late age (decline). For models 1 and 2, the subject age refers to the baseline subject age. Subjects that could no longer perform the test were censored and the likelihood-based M3 method was used during estimation.[16,17] The below the quantification limit of 50 meters was used because all digitized data were above 50 meters. A total of 13 of 228 data points (6%) were censored, representing 13 of 88 subjects (15%) that lost ambulation. The Quasi-Random Parametric Expectation Maximization (QRPEM) algorithm can handle the M3 method for below the quantification limit data and was used for all models.[18] Between-subject variability (BSV) was evaluated on all fixed terms in the models initially and removed stepwise starting with the lowest BSV estimates and/or high shrinkage on the estimates and retained in the model if there was an improvement in the goodness of fit (GOF) plots and Akaike information criterion (AIC)/Bayesian information criteria (BIC).

*Model 1: Linear with categorical age covariate.* Baseline age was defined as a categorical covariate (1 or 0) on slope and intercept (subject age $\leq$7 years, isLE7 (less than equal to 7) $==$ 1, else "0") to characterize the upward trajectories for subjects with a baseline age $\leq$7 years and the downward trajectory for subjects with a baseline age $>$7 years. Interpatient variability and residual error modeled as additive.

$$6\text{MWT} = \text{Intercept} + (\text{isLE7} == 1) * \theta_1 + (\text{Slope} + (\text{isLE7} == 1) * \theta_2 + \eta\text{Slope}) * \text{Age},$$

where $\theta_1$ and $\theta_2$ are estimated covariate effects.

*Model 2: Linear with if/else conditional statement.* Age at maximum fixed to 7 years (Phoenix code "?" = if; ":" = else). Interpatient variability modeled as exponential (in contrast to model 1, which was modeled as additive) and residual error as additive.

$$6\text{MWT} = ((\text{Age} <= 7)?(\text{Intercept} + \text{Slope} * \exp(\eta\text{Slope}) * \text{Age}) : (\text{Intercept2} + \text{Slope2} * \exp(\eta\text{Slope2}) * \text{Age}).$$

*Model 3: Quadratic.* Interpatient variability modeled as exponential and residual error as additive.

$$6\text{MWT} = \text{Alpha} + \text{Beta} * \exp(\eta\text{Beta}) * \text{Age} + \text{Gamma} * \text{Age}^2.$$

*Model 4: Linear with simultaneous estimation.* Interpatient variability modeled as exponential and residual error as additive.

$$6\text{MWT}(1) = \text{Intercept} + \text{Slope} * \exp(\eta\text{Slope}) * \text{Age}.$$

$$6\text{MWT}(2) = \text{Intercept2} + \text{Slope2} * \exp(\eta\text{Slope2}) * \text{Age}.$$

6MWT = min (6MWT(1),6MWT(2)); min = minimizing function in Phoenix NLME.

For each individual, the age at maximum response ($\text{Age}_{max}$) is the x value ($\text{Age}_{max}$) at which the two lines intersect (6MWT(1) = 6MWT(2)).

$$\text{Age}_{max} = (\text{Intercept2} - \text{Intercept})/(\text{Slope} * \exp(\eta\text{Slope}) - \text{Slope2}) * \exp(\eta\text{Slope2}).$$

Simultaneous estimation using the "min" function in Phoenix NLME means that both linear equations are used in a simultaneous estimation step to minimize the response and objective function, as opposed to estimating the slope/intercept for each linear model separately. For each value of x (Age), the response (6MWT) is determined using the slope and intercept for the first line (6MWT(1)) and the second line (6MWT (2)) simultaneously. The linear fit that results in the minimal response and objective function for a given x value uses that linear model to estimate the parameters. The Age at maximum ($\text{Age}_{max}$) response is determined after estimation by calculating the age when the response from the first line and second line are equal. By incorporating the eta ($\eta$) parameters from the slope estimation, Bayesian estimates were used to calculate the $\text{Age}_{max}$ for each subject. It is important to note that model 4 is not differentiable at the point of intersection of the two lines where 6MWT(1) = 6MWT(2), which is an assumption of mixed effect approaches that use linear approximation methods (i.e., first order conditional estimation). However, model 4 did converge, and gave reasonable goodness of fit plots and parameter estimates with the QRPEM algorithm, suggesting that the mixed effect approach was successfully executed, despite lack of differentiability when 6MWT(1) = 6MWT(2). Although differentiability is required for methods, such as first order conditional estimation, expectation maximization methods (i.e., QRPEM, Monte Carlo Parametric Expectation Maximization, and stochastic approximation expectation maximization) do not seem to have this requirement, but rather use finite sampling strategies of the probability density function to arrive at an approximate likelihood.[18] However, to further support the use of the minimum function in Phoenix NLME, if linearization approximation methods are used, the data was refit using a differentiable approximation to model 4 (see **Supplementary Model Code**) and the parameter estimates were found to be comparable to the original form of model 4 (**Supplementary Table S2**).

Model 4 was selected based on GOF plots, AIC/BIC and the ability to estimate the age at maximum 6MWT performance (**Supplementary Table S1**).

The following additional models (Bateman Function and Indirect Response Model) were also evaluated, but parameters could be not estimated accurately due to high percentage of relative standard error (%RSE) or confidence intervals (CIs) that included zero with these models.

*Model 5: Bateman function.* The 6MWT = A*k/(k-k2)* (exp(-k2*Age)-exp(-k*Age)).

A is a coefficient and k and k2 are rate constants. Interpatient variability was modeled as exponential and evaluated on k and k2. A residual additive error model was used.

*Model 6: Indirect response model.* The derivative (E = Kin* (1 + $E_{max}$ *Age/(Age + EC50))-E*Kout*(1+slope*Age)).

A saturation limited input and linear output direct response model.

The E is the 6MWT response. Kin is the rate constant describing the input, $E_{max}$ is the maximum response, and EC50 is the age at the midpoint of the maximum response. Kout is the rate constant describing the output and the slope describes the linear change in the output.

Interpatient variability was modeled as exponential and evaluated on EC50 and slope. A residual additive error model was used.

### Model evaluation

The final model (model 4) was evaluated using 100 bootstraps (despite the small dataset) to obtain CIs of the parameter estimates to evaluate model robustness. A visual prediction check using 2,000 replicates was also performed to compare model predictions with the observed data to evaluate model misspecification.

### Model application

The final model (model 4) was used to simulate the 6MWT vs. age using age demographics and subject number from the original publications used for digitization and from an additional publication[7] (**Table 1**). Age demographics were generated using a random normal distribution in R using the mean and SD listed in the publications. Trial durations of 1 year (McDonald *et al.*[8] and Mazzone *et al.*[7]) and 2 years (Goemans *et al.*[6]) were simulated and the 6MWT change from baseline was compared to the observed data in the publications. For each simulation, the subject number reported in the published paper was used with 100 trial replicates. The 6MWT change from baseline (CFB) was calculated at 6-week intervals.

**Table 1** A summary of two literature references used to build the model and one reference used to evaluate the model predictions.

| Publication | Study type | Steroid use | Subject number reported | Subject number digitized (≤7) | Mean age in years (SD, range) | Trial duration |
|---|---|---|---|---|---|---|
| McDonald *et al*.[8,a] 2013 | Placebo controlled trial | 70% of subjects | 57 | 53 (13)[b] | 8.3 (2.3, 5–15) | 48 weeks |
| Goemans *et al*.[6,a] 2013 | Natural history study | 100% of subjects | 65 | 35 (6)[c] | 9.5 (2.3, 5.1–15.3) | 2 years |
| Mazzone *et al*.[7] 2011 | Natural history study | 52% of subjects | 106 | N/A | 8.3 (2.3, 4.1–17) | 1 year |

N/A, not applicable.
[a]Digitized data to build the model.
[b]Two data points per subject.
[c]Two to six data points per subject.

$$\text{6MWT change from baseline (t=i)} = \text{6MWT (t=i)} - \text{6MWT (t=0)},$$

where i= time in year.

## RESULTS

### Digitized data

Data was digitized from two publications (McDonald *et al*.[8] and Goemans *et al*.[6]), which included figures of individual subject performance on the 6MWT (meters) vs. age (years) (**Figure 1**). These publications were selected due to the longitudinal nature of the data at the subject level. In addition, in these more recent publications, nearly all of the subjects were on SOC steroid treatment (**Table 1**), which is known to impact 6MWT performance and delay loss of ambulation by 3 years.[19]

The use of digitized data was important for disease progression modeling of this rare disease and provided a path forward in the absence of actual data, but it came with some limitations. Although individual subject trajectories can be digitized, subject-specific intrinsic (i.e., dystrophin genotype and race) and extrinsic factors (i.e., steroid use) are not available. In addition, the accuracy of information in the publication cannot be confirmed. Data from two separate publications were combined to avoid drawing conclusions from one specific study. The McDonald *et al*.[8] article included longitudinal data from the placebo arm of a randomized controlled, multisite clinical trial conducted internationally. The Goemans *et al*.[6] article included longitudinal data from a natural history study conducted at a single center (Leuven Neuromuscular Reference Center in Belgium).

In total, 88 subjects were digitized for a combined total of 228, 6MWT observations (**Figure 1**). The majority of subjects came from the McDonald *et al*.[8] publication (60%) with two observations per subject and the remaining from the Goemans *et al*.[6] publication with two to six observations per subject for a total of 122 observations from Goemans *et al*.[6] and 106 observations from McDonald *et al*.[8]; (**Table 1**).

### Model selection

Six structural models were evaluated, as described in the Methods section. It was important to have a model that could capture in an unbiased way the maximum in 6MWT performance. The statistical performance of each model is presented in **Supplementary Table S1**. Our first attempt to characterize the data (model 1) implemented a categorical covariate effect on the slope and intercept such that subjects that had a baseline age of $\leq$7 (category = 1), and had slopes and intercepts that were modified by the estimated fixed covariate effect. This effectively allowed for a model that could characterize the upward and downward trajectories, but required fixing the categorical cut of the baseline age to 7 years old. Model 2 was similar to model 1, but instead of using a covariate effect to modulate the slope and intercept to capture the upward and downward trajectory, an if/then statement was used in the model using the 7-year-old age cutoff, allowing for two estimated slopes and intercepts. Models 1 and 2 had improved statistical fits based on AIC/BIC criteria, but required defining *a priori* the maximum at which decline would occur. In addition, these models had parameter estimates that could not be accurately determined, as evidenced by CIs that included zero. Both models 3 and 4 allowed for determination of the maximum in 6MWT performance thereby circumventing the bias. However, model 4 was selected over model 3 given lower AIC/BIC values and the fact that model 3 was found to be unstable and dependent on the initial estimates. Two additional models: model 5 (Bateman Function) and model 6 (Indirect Response Model) were also evaluated, but statistically were not improved fits over model 4 (**Supplementary Table S1**), included parameter estimates with high %RSE and CIs that included zero (data not shown).

The population fit (**Figure 2a**) and the individual fits (**Figure 2b**) for model 4 show the expected upward trajectory at younger ages and downward trajectories at older ages. Model 4 was the only model of the four tested that accurately captured the steepness of the decline trajectory (data not shown). Representative individual fits from model 4 illustrate the similarity in slopes across selected individuals in the decline phase (**Supplementary Figure 1S**). All of these subjects were $>$10 years old at baseline. The fits are reasonable for this base, structural model and there is expectation for further model improvement when patient covariates are included.

The GOF plots for model 4 are summarized in (**Figure 3a–d**). By including an intersubject variability term on the upward and downward slopes, the individual (**Figure 3b**) predictions were improved relative to the population predictions (**Figure 3a**). Adding the intersubject variability term on both slopes also allowed for a way to determine individual performance trajectories from the model. There was no apparent trend between the conditionally weighted residuals and age (**Figure 3c**). The model adequately described the 6MWT progression over time (**Figure 3d**).

Parameter estimates from the final selected model and the bootstrap results ($N$ = 100) are comparable, indicating the model parameters are not sensitive to the input dataset

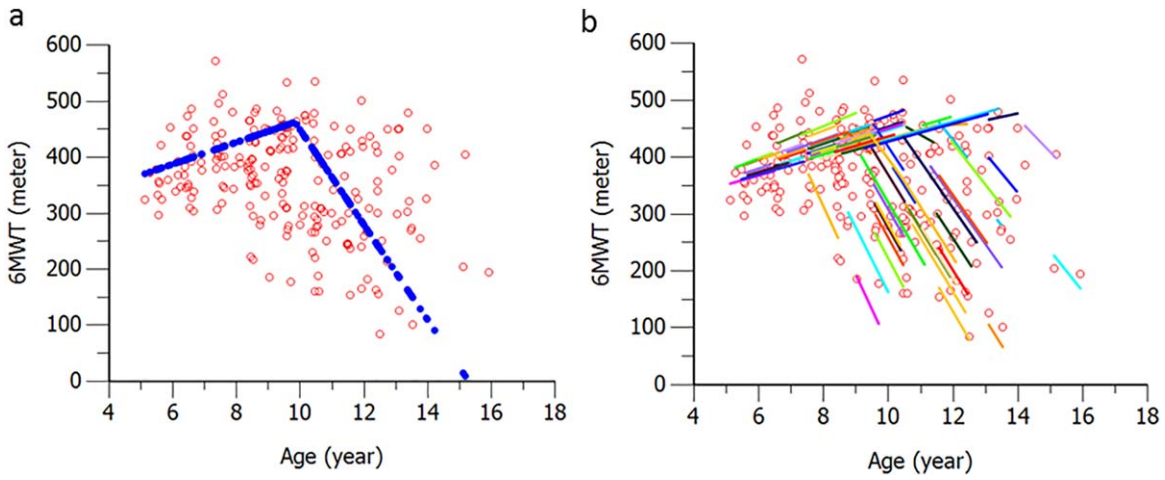


**Figure 2** Population (**a**) and individual (**b**) fits of the data to model 4. The digitized data is indicated with red circles and the population prediction in blue circles on the left plot **a** and individual predictions in multicolors for each individual on the right plot **b**. 6MWT, 6-minute walk test.

(**Table 2**). The mean slope for improvement was 20 meters/year and for decline it was 85 meters/year. The mean age of maximum performance across individual subjects was predicted to be 10.0 years (95% CI = 6.78–13.1).

### Model evaluation

A visual prediction check was performed (**Figure 4 and Supplementary Figure 3S** with increased bin number) to evaluate model performance in describing the central tendency of the data and the distribution around the population average. The model described the central tendency and the 5th and 95th percentiles of the data reasonably well with the exception of ages >12 years old. For this age range, the model predicted a slightly lower median 6MWT response compared with the observed data. This may be the consequence of having limited data over this age range, in which patients with DMD generally lose the ability to walk. It will be important to evaluate this model with a larger dataset covering more subjects >12 years old and to evaluate the impact of covariates that may impact the decline.

### Model application

The model was used to simulate 100 datasets, each dataset containing the age demographics, subject number, and trial duration reported in the publications used to build the model (McDonald *et al.*[8] and Goemans *et al.*[6]) and a third set of demographics from a dataset not used to build the model (**Table 1**). Summary statistics were generated for each simulation replicate. The mean simulated 6MWT change from baseline was plotted vs. trial duration at 6-week intervals for subjects ≤7 and >7 years old at baseline for the McDonald *et al.*[8] and Goemans *et al.*[6] predictions (**Supplementary Figure S2a,b**). The model predicted the overall mean trajectories reasonably well for subjects ≤7, which showed a 6MWT improvement for both datasets consistent with the publications (**Supplementary Figure S2a,b**). For the Goemans *et al.*[6] predictions, which extended out to 2 years, the ≤7-year-old age group began to show a loss in 6MWT performance around 1.5 years, which is also consistent with the publication.

The model predicted and observed data at 1 year are presented for all three datasets (**Table 3**). Overall, the model can reasonably predict the mean change from baseline in 6MWT at 1 year for the combined age groups and the age stratified groups, even for the Mazzone *et al.*[7] dataset, which was not used to build the model. There is a slight trend for the model to underpredict the decline for subjects ≥7 years old at baseline, but the predicted mean data are maintained within the SD of the observed data (**Table 3**). It was noted that for the Mazzone *et al.*[7] dataset, the model predicted an improvement in 6MWT for the ≤7 years old, but the observed data had a decline in performance. This may be due to the fact that some of the subjects may not have been on a stable steroid regimen and the model was built from subjects primarily on steroids (**Table 1**). It will be important to determine if the inclusion of specific patient factors, such as steroid use, can improve the model prediction in the future.

## DISCUSSION

Understanding disease progression for rare diseases, such as DMD, is important for effective trial design. Clinical trials usually include a small number of patients and placebo-controlled randomized trials are difficult to recruit and conduct making it even more challenging to determine if the drug is efficacious. Having a quantitative model that can describe changes in the 6WMT over the course of the disease and that can incorporate patient-specific factors that influence the 6MWT trajectory for each subject would benefit future trials in DMD and perhaps circumvent the historical challenges in using the 6MWT end point.[15,20] A disease progression model can be used to supplement placebo data and will likely be more sensitive in picking up a drug effect, especially with an end point like the 6MWT that exhibits nonlinear progression over time.

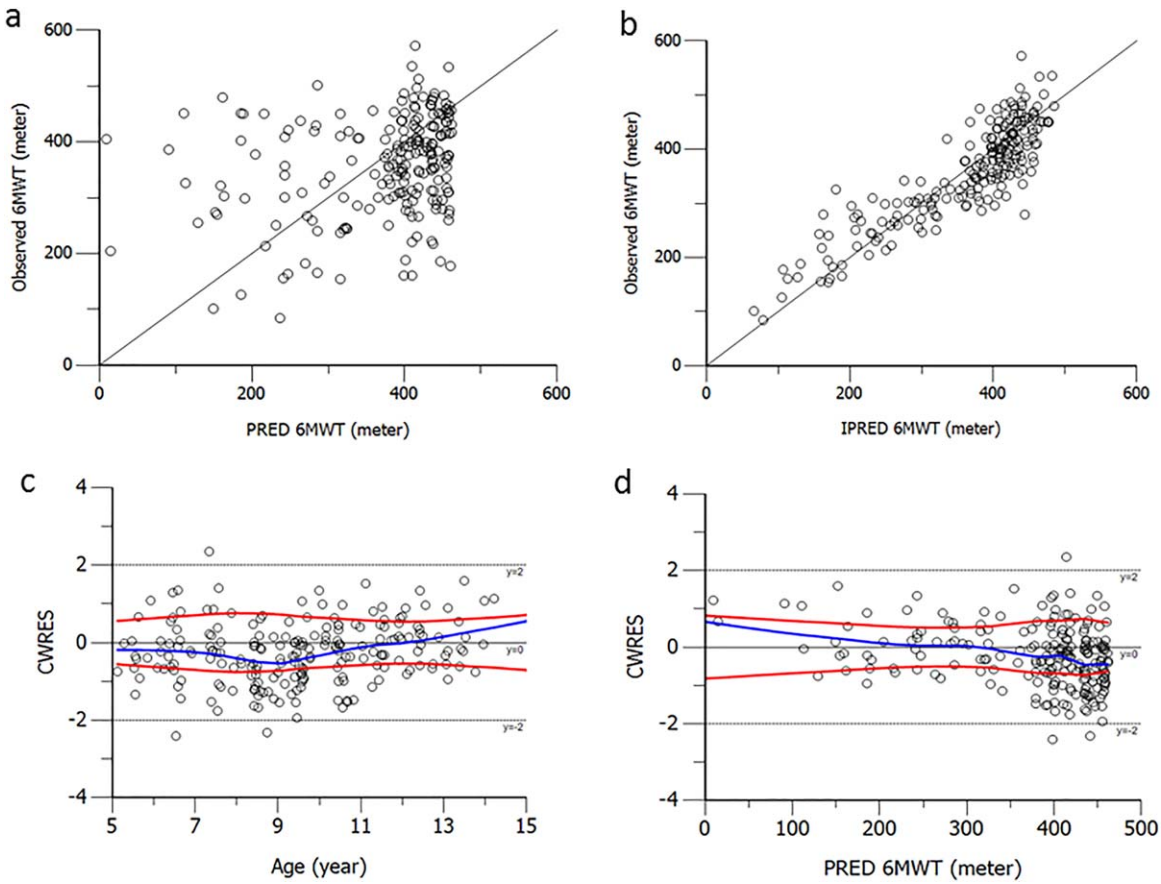


**Figure 3** Goodness of fit plots are presented for model 4. Observed data vs. population predicted (PRED) (**a**) and individual predicted (IPRED) (**b**). The line of unity is indicated. Conditional weighted residuals (CWRES) are plotted vs. age (**c**) and predicted 6-minute walk test (6MWT) (**d**). The blue curve is the loess curve for the residuals. The top red curve is the loess curve for the absolute values of the residuals and the bottom red is a reflection of the top curve; these show the trend in the magnitude of the residuals.

There have been several natural history studies evaluating the 6MWT in DMD and linear regression models have been used to characterize the increase and decrease in 6MWT performance separately to help identify sources of variability.[6,8,11–13,21–23] It was found that the 6MWT can have SDs ranging from 94–157 meters with corresponding percentage of coefficient of variations (%CVs) ranging from 26–51% in the decline phase when comparing the end point across subjects.[8] Using modeling approaches that can explain the sources of variability is important. Mercuri *et al.*[24] recently described an approach to explain 6MWT variability using latent class trajectory analysis by classifying subjects into four classes: fast decline; moderate decline; stable function; or improved function; and within each class estimating the trajectories using a quadratic model. This approach was able to reduce unexplained residual variability from 72 meters when fitting the mean trajectories to 44 meters when stratifying into class trajectories.[24]

Here, we described a series of structural models that were used in an attempt to identify a structural model that could

**Table 2** Parameter estimates and bootstrap results from model 4

| Parameter, units | Symbol | Estimate | %RSE | 95% CI | BSV %CV | Estimate bootstrap[a] | %RSE bootstrap | 95% CI bootstrap |
|---|---|---|---|---|---|---|---|---|
| Developmental intercept, m | Intercept | 270 | 13.9 | 197–344 | N/A | 284 | 20.6 | 228–404 |
| Developmental slope, m/y | Slope | 19.6 | 26.5 | 9.4–29.8 | 22 | 17.5 | 36.2 | 5.6–23.8 |
| Disease-induced intercept, m | Intercept2 | 1,298 | 5.5 | 1,158–1,437 | N/A | 1,594 | 135 | 786–3,121 |
| Disease-induced slope, m/y | Slope2 | −84.9 | 7.6 | −97.6 to −72.2 | 23 | −94.1 | 87 | −226 to −40.7 |
| SD, m[b] | stdev | 56.9 | 4.2 | 52.2–61.6 | N/A | 59.6 | 70.5 | 43.8–72.5 |
| Age at maximum, y[c] | $Age_{max}$ | 10.0 | 16.1 | 6.78–13.1 | N/A | N/A | N/A | N/A |

BSV, between-subject variability; CI, confidence interval; CV, coefficient of variation; m/y, meter per year; m, meter; N/A, not applicable, RSE, relative standard error.
[a]100 bootstraps.
[b]Additive error model.
[c]Calculated secondary parameter.

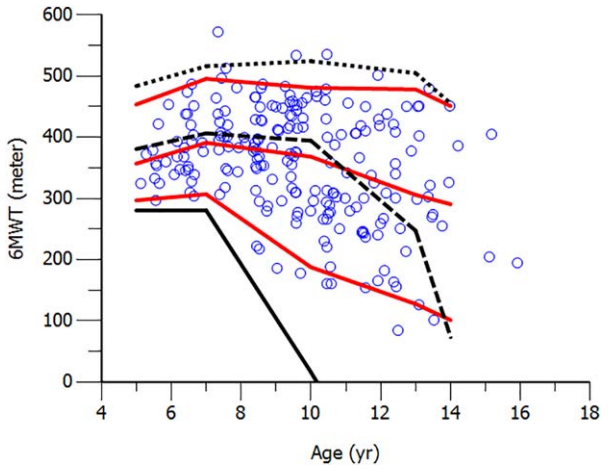


**Figure 4** A visual prediction check using 2,000 replicates. The observed data are indicated with blue open circles. The observed quantiles are in red and the predicted quantiles are in black. The dotted line represents the 95th percentile, the dashed line the 50th percentile, and the solid line the 5th percentile. 6MWT, 6-minute walk test.

capture the upward and downward trajectories of the 6MWT in an unbiased way. Each of the models had benefits and limitations, but it was found that model 4 in both the nondifferentiable form (at the point of intersection of the two lines) and in the differentiable approximation to this form could adequately describe the current dataset in an unbiased way to predict the age at which 6MWT performance begins to decline. A population modeling approach that can estimate individual trajectories adds value and provides a basis for future analysis to identify patient covariates that can impact subject trajectories.

Model 4 was able to describe the developmental increase and disease-induced decline in the 6MWT simultaneously. It is important to emphasize that the model has implicitly accounted for the steroid impact on the 6WMT because all of the subjects in the Goemans *et al.*[6] study and well over the majority (70%) of subjects in the McDonald *et al.*[8] study were on steroids. The model predicted a mean 6MWT improvement of 20 meters per year for patients with DMD in the development stage and is consistent with previous publications that stratified performance using an age threshold of 7 years old.[7,9] Pane *et al.*[9] reported a mean increase of 18.5 meters per year for subjects <7 years old that were on a steroid regimen and Mazzone *et al.*[7] reported an improvement of 18.8 meters per year for this age group. As a comparison, healthy boys over the 5–8 year age range had a mean improvement of approximate 40 meters per year.[25] Healthy boys go on to show a more gradual improvement in 6MWT performance from 8–12 years that begins to plateau, whereas boys with DMD begin to decline.[25] The model predicted a mean decline of 85 meters per year that on average was predicted to start at 10 years old. This mean age of decline is higher than the previously reported 7–8 year range and is hypothesized to be due to the fact that the 6MWT data used to build the model came primarily from subjects who were on stable steroid use. Bello *et al.*[19] have shown that a stable steroid regimen can delay loss of ambulation by 3 years. The mean rate of decline per year predicted by the model is within the reported literature range from −23 to −115 meters per year with an admittedly high observed variability.[6–9,11,22]

An important model qualification was to determine how accurately the model can predict observed data. The model reasonably predicted the change from baseline in 6MWT for the literature data used to build the model and a third dataset, which was not used to build the model (**Table 3**). There was a trend for the predicted mean estimates to underpredict slightly the mean magnitude of decline of the observed data for all three studies, but the mean estimates were within the SD of the observed data. Incorporating additional sources of variability in the future may help to improve model predictability.

A population model was developed using digitized data to accelerate our understanding of 6MWT disease progression in DMD. The model could reasonably predict observed data and can be used for future disease progression modeling to identify patient-specific factors that may explain additional sources of 6MWT variability. The current limitation of the model is its ability to account for all of the observed variability in the 6MWT, because observed SDs were still higher than the model predicted. In addition, because digitized data were used, it will be important to confirm model acceptability and predictability using a larger dataset to screen for patient covariates, such as dystrophin genotype or lung function that have potential to influence 6MWT trajectories. Importantly, the model can be used to evaluate a drug effect by determining the extent to which treated subjects have improvements in the developmental or decline slope compared to placebo. A drug therapy that

**Table 3** Model predicted 6MWT change from baseline at 1 year obtained from simulations and compared to the observed data published in the literature

| Mean (SD) change from baseline 6MWT at 1 year | All age groups | ≤7 years old | >7 years old |
|---|---|---|---|
| McDonald *et al.*[8] predicted | −20.4 (33.4) $n=57$ | 13.3 (9.5) $n=23$ | −43.2 (22.6) $n=34$ |
| McDonald *et al.*[8] observed[a] | −44.1 (88.0) $n=55$ | 34.1 (53.9) $n=6$ | −58.9 (81.9) $n=33$ |
| Goemans *et al.*[6] predicted | −36.6 (34.4) $n=65$ | 12.0 (11.5) $n=9$ | −44.4 (30.2) $n=56$ |
| Goemans *et al.*[6] observed[b] | −42.9 (89.9) $n=25$ | 8.6 (84.2) $n=3$ | −50.0 (90.2) $n=22$ |
| Mazzone *et al.*[7] predicted | −23.1 (27.3) $n=106$ | 11.0 (10.2) $n=28$ | −35.4 (20.2) $n=78$ |
| Mazzone *et al.*[7] observed | −25.8 (74.3) $n=106$ | −7.8 (63.9) $n=35$ | −42.3 (73.9) $n=71$ |

The data are summarized across all age groups and stratified by 7 years old.
6MWT, 6-minute walk test.
[a]Age categories defined as <7 years and ≥7 years in the publication.
[b]Age categories defined as below and above 7.5 years in the publication.

is anticipated to improve muscle function might be expected to improve the developmental slope and to delay the age of decline, which could be evaluated using this model.

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**Conflict of Interest.** All authors were employees of Bristol-Myers Squibb at the time of this work.

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