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Longitudinal motor function in proximal versus distal *DMD* pathogenic variants

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Abstract

Introduction/Aims: There is considerable heterogenicity in clinical outcomes in Duchenne Muscular Dystrophy (DMD). The aim of current study was to assess whether dystrophin gene *(DMD*) pathogenic variant location influences upper extremity and lower extremity motor function outcomes in a large prospective cohort.

Methods: We used longitudinal timed and quantitative motor function measurements obtained from 154 boys with DMD over a 10-year period by the Cooperative International Neuromuscular Research Group Duchenne Natural History Study (CINRG-DNHS) to understand how the trajectories of motor function differ based on proximal versus distal *DMD* pathogenic variants. Proximal variants were defined as located proximal to 5' *DMD* intron 44, and distal variants as those including nucleotides 3' *DMD* including intron 44. Distal *DMD* variants are predicted to alter the expression of short dystrophin isoforms (Dp140, Dp116, and Dp71). We compared various upper extremity and lower extremity motor function in these two groups, after adjusting for total lifetime corticosteroid use.

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Author contributions

Name	Location	Contribution
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Results: The time to loss-of-ambulation and timed motor function measurements of both upper and lower limbs over a ten-year period were comparable between boys with proximal (n = 53) and distal *DMD* pathogenic variants (n = 101). Age had a significant effect on several motor function outcomes. Boys younger than 7 years of age (n = 49) showed gain in function whereas boys 7 years and older (n = 71) declined, regardless of dystrophin pathogenic variant location.

Discussion: The longitudinal decline in upper and lower motor functions is independent of proximal versus distal *DMD* pathogenic variants.

Introduction

Duchenne Muscular Dystrophy (DMD) is a chromosome X-linked genetic disease caused by mutations in the dystrophin gene (*DMD*) encoding dystrophin protein (1, 2). *DMD* consists of 79 exons and encodes full-length dystrophin (dp427) and shorter dystrophin isoforms (dp260, dp140, dp116, and dp71) (3). Proximal *DMD* pathogenic variants (proximal to 5' *DMD* intron 44) affect the expression of full-length dystrophin (dp427) and dp260 isoform, whereas distal DMD pathogenic variants (mutations in 3' *DMD* including intron 44) are predicted to affect the expression of shorter dystrophin isoforms (dp140, dp116, dp71).

An enduring challenge even in a monogenic disease such as DMD is disease heterogeneity, and variability in clinical outcomes is evident across multiple organs (4–16). We and others have shown that both *DMD* genotype and genetic modifiers affect motor, respiratory, and cognitive outcomes in DMD. Bello et al. using the Cooperative International Neuromuscular Research Group Duchenne Natural History Study (CINRG-DNHS) cohort showed that boys with a non-sense mutation in *DMD* lost ambulation at a median age of 11.1 years, compared to those boys with deletions amenable to *DMD* exon 44 skipping, who lost ambulation at a median age of 14.8 years. Likewise, genetic modifiers such as *SPP1*, *LTBP4* and *CD40* affect the age of loss-of-ambulation in DMD; these modifiers have been validated in multiple, independent cohorts of DMD (9–13).

Recently, boys with distal *DMD* pathogenic variants were shown to have lower pulmonary function measures compared to those with proximal *DMD* pathogenic variants (17). Similarly, cognitive burden in DMD vary depending between boys with proximal and distal *DMD* pathogenic variants (14–16, 18–19). Boys with distal pathogenic variants have higher incidence of speech delay, neurodevelopmental disorders like autism, attention-hyperactivity, and poorer performance on neuropsychological assessment.

While skeletal muscle pathology is largely ascribable to lack of full-length Dp427 dystrophin, the role of shorter isoforms in muscle is under-studied and possibly under-recognized; furthermore, the interactions between the central nervous system (where the importance of short dystrophin isoforms is well-established) and muscle are manifold and complex. Whether distal *DMD* pathogenic variants affect motor outcomes or influence the trajectory of functional decline is not known and forms the scientific rationale for our present work.

The drug pipeline in DMD is rich and newer agents have recently been approved for clinical use. In this context, showing drug efficacy in clinical trials will draw upon knowledge

from natural history studies of DMD that is particularly informative of pathophysiological mechanisms. Knowledge gained from natural history studies not only help understand disease heterogeneity, they are also helpful for prognostication during clinical care, optimal stratification of subjects, and in improving clinical trial design in DMD. The latter is important owing to the resource-intense nature of clinical trials in rare diseases such as DMD. With these long-term goals in mind, in the present study, we evaluated the longitudinal trajectory in upper and lower motor strength in proximal and distal *DMD* pathogenic variants using a large, prospective, well-characterized, international cohort of DMD subjects. We hypothesize that subjects with distal *DMD* pathogenic variants show decline in motor function earlier compared to those with proximal *DMD* pathogenic variants.

Methods

Standard protocol approvals, registrations, and patient consents

The study was fully compliant with the Principles of Good Clinical Practice according to the International Conference on Harmonization and the Declaration of Helsinki (2000). Each local Institutional Review Board or Ethics Committee approved the study. Written parental consent and assent from children older than 12 years were obtained prior to study enrollment. The study design and characteristics of the CINRG-DNHS cohort have been previously published (20). The CINRG-DNHS cohort is registered with Clinical Trials.gov, number NCT00468832.

Subject characteristics and subgroups

The inclusion criteria for CINRG-DNHS have been previously published (21). Briefly, study subjects were required to meet at least one of the diagnostic criteria. These criteria included (i) out-of-frame deletion or duplication in *DMD* using multiplex ligation-dependent probe amplification, (ii) complete *DMD* sequencing predicting an out-of-frame pathogenic variant in *DMD,* (iii) absent dystrophin immunofluorescence and/or absent dystrophin on immunoblot, (iv) an X-linked pedigree with an older male sibling with out-of-frame pathogenic variant in *DMD* or a muscle biopsy demonstrating absent dystrophin. The CINRG-DNHS had two cohorts of subjects, ages 2 to 28 years recruited between 2006 to 2009 (parent CINRG-DNHS) (n = 340), and ages 4 to <8 years) recruited between 2012 to 2016 (the younger cohort) (n = 100).

We used loss-of-ambulation (LoA) data from 212 subjects belonging to the parent CINRG-DNHS (22). Longitudinal upper extremity and lower extremity motor function were analyzed from both cohorts on whom *DMD* pathogenic variants data was available. Based on *DMD* pathogenic variant location, we divided the subjects into those with “proximal” pathogenic variants, situated entirely 5’ of *DMD* intron 44, predicting normal expression of Dp140 (Dp140+), and those with “distal” pathogenic variants as those including nucleotides 3’ *DMD* including intron 44, predicting potential disruption of Dp140 (Dp140−).

Clinical endpoints assessed during the CINRG-DNHS

All subjects underwent several timed functional tests, quantitative muscle strength assessments, and standardized goniometry techniques by trained clinical evaluators during each study visit as previously described (21). Briefly, the times tests included (i) 6MWT, (ii) time to walk/run 10 meters, and (iii) time to supine-to-stand among others. Isometric strength of elbow flexors and extensors, and knee flexors and extensors using the CINRG Quantitative Measurement System (CQMS) were performed in all participants, regardless of their mobility as previously described (21). Age at LoA was defined as patient-reported and evaluator-verified (where possible) age at full-time wheelchair use, as previously described (22).

Statistical analyses

Statistical tests appropriate for each demographic characteristic were performed comparing those with a proximal versus distal pathogenic variants at the first study visit. These methods included Fisher's exact tests and one-way analysis-of-variance (ANOVA). The Kaplan-Meier method was used to calculate the median time to LoA. The Cox regression model was used to model *DMD* pathogenic variant location as the predictor, and corticosteroid use (defined as having at least 1 year of use before LoA versus no treatment or less than 1 year while ambulatory) as a binary co-variate. Analysis of each motor function over time was performed using mixed-effect linear models where the outcome was the dependent variable and age, total lifetime corticosteroid exposure, and *DMD* pathogenic variant location were the independent variables. Each model included a random effect for participant to account for repeated measurements. The quantitative muscle strength values were square-root transformed for analysis. Grip strength showed a curvilinear relationship, and therefore a quadratic term was included in each model. Lastly, mixed-effect linear models were again used to assess functional outcomes stratified by those <7 years versus those age 7 or greater at the time of assessment. Power calculations for loss-of-ambulation analyses were performed with the "powerSurvEpi" package in R v.3.5.3. Statistical significance was defined at a significance level of 0.05.

Results

Time-to-event analyses of LoA

Of the 212 participants from whom LoA data were analyzed, 66 participants (31%) had proximal *DMD* pathogenic variants and 146 participants (69%) has distal *DMD* pathogenic variants. As detailed in Table 1, Cox regression analysis with use of corticosteroids as a co-variate showed no significant statistical difference, although median age at LoA in subjects with distal *DMD* pathogenic variants was 0.5 years earlier (p = not significant; Figure 1).

Characteristics of participants in the longitudinal data

Table 2 summarizes the characteristics of the 154 participants who were included in the longitudinal data analyses. There were 53 boys (34%) with proximal *DMD* pathogenic variants and 101 boys (66%) with distal *DMD* pathogenic variants. The mean age and

ambulatory status of subjects in the two *DMD* subgroups were comparable. The mean follow-up period was 4.6 years (SD 2.9 years, range 0 to 8.2 years) in those with proximal *DMD* pathogenic variants, and 3.6 years (SD 2.8 years, range 0 to 9.9 years) in those with distal *DMD* pathogenic variants. The lifetime use of corticosteroids was comparable between the two subgroups (data not shown).

The longitudinal follow-up intervals for subjects with proximal and distal *DMD* pathogenic variants are listed in Supplemental Table 1. Table 3 summarizes corticosteroid use at first study visit in this cohort.

Timed and quantitative measurements of upper and lower extremities strength based on *DMD* pathogenic variant location

Functional motor outcomes adjusted for total lifetime corticosteroid use were comparable between proximal and distal *DMD* pathogenic variants (Table 4). Age had a significant effect on several of the functional motor outcomes.

We performed additional evaluation of how age affects 6MWT over a 3-year period. Boys 7 years of age and older, over a 3-year period, declined more rapidly, than boys younger than 7 years of age, regardless of *DMD* pathogenic variant location (Table 5).

Discussion

Our longitudinal data provides a historical account of the trajectory of motor function decline in DMD. Our hypothesis that DMD subjects with distal *DMD* pathogenic variants would show decline in motor function earlier that those with proximal *DMD* pathogenic variants, was not informed by our data. Furthermore, the longitudinal decline in functional motor outcomes did not differ between those with proximal and distal *DMD* pathogenic variants. The observed trend towards earlier LoA with distal *DMD* pathogenic variants is similar to those reported previously (19). In a Dutch study focusing on central nervous system (CNS) phenotypes, participants with pathogenic variants in 5’ region of *DMD* lost ambulation at 11 years (SD 2.6) compared to those with pathogenic variants in the 3’ domain, who lost ambulation at 9.9 years (SD 1.3) (19). We acknowledge that the number of individuals in each category was small, and outliers in either group could skew accurate interpretation of the results.

This potential direction of effect is the same as observed in association with the respiratory phenotype, and may be explained by a worsening of motor or respiratory performance (both mediated by voluntary striated muscles) secondary to some form of CNS involvement, of which there is ample evidence in association with pathogenic variants disrupting Dp140 (5). We propose a viewpoint, supported by literature that individuals with distal *DMD* pathogenic variants, because of poor executive function, are probably unable to accurately forecast future consequences, or strictly adhere to medical recommendations, or medical treatment. To cite, individuals with DMD who had higher intellectual functioning survived longer (23). Likewise, those with worse intellectual function had poorer long-term outcomes in skeletal, respiratory and cardiac health (24). Altogether, these findings open new perspectives on the tissue-specificity of dystrophin isoforms in mediating end-organ damage.

The discordance between genotype-phenotype associations observed in CNS, muscle, and respiratory phenotypes cannot be fully explained in the light of currently available data, and warrants an expansion of "natural history" and observational studies. If we assume that the Hazard Ratio of 0.85 for carrying a proximal *DMD* pathogenic variant (i.e. 15% per-year reduction of the risk of losing ambulation) is an accurate estimate, and considering that proximal pathogenic variants are observed in 30% of the DMD population, our power calculation estimates a sample size of approximately 1300 non-ambulatory DMD participants to identify this effect with 80% power ($1\text{-}\beta = 0.8$) and a type-I error ($\alpha = 0.05$). This large sample size may be reached by future, larger multi-center natural history studies, and/or meta-analyses of existing datasets.

Clinically, it is well known that some skeletal muscles are disproportionately affected compared to others in DMD. Further, timed motor function tests is a read-out of strength of muscle groups, rather than an individual muscle. We therefore reason that a perfect correlation between all timed motor function tests is not biologically plausible. Therefore, it is not incongruous that age was not statistically significant predictor of *all* timed motor function tests.

Our study has some limitations. The duration of follow-up of individuals with proximal versus distal pathogenic variants were different, and we are therefore taking a nuanced interpretation of our data. Undoubtedly, longitudinal follow-up more equally representing both groups will be highly beneficial. Second, we analyzed *DMD* pathogenic variant location as a binary variable. This approach makes it more difficult to identify clinically significant pathogenic variant effects in some individuals. Third, for the time to loss of ambulation analysis, we used steroid exposure during ambulation as a covariate defining those who had less than one year of steroid use while ambulatory versus those who had 1 year or greater exposure while ambulatory. This exposure dichotomy is a commonly used method; it does treat participants with many years exposure with those having only 365 days of exposure. Also, LoA data was available in 212 participants compared to longitudinal timed function tests in 154 participants, which may affect data analysis. Last, and a much-needed consideration for future research studies, is to improve the participation of non-white and Hispanic populations to improve research equity.

One of the widely used clinical outcome endpoints in DMD is 6MWT. Earlier studies reported that 6MWT declined annually, and that the trajectory of decline was markedly different in boys younger and older than age 7 years. Boys older than age 7 years showed progressive decline in 6MWT (25–27). These earlier works have shown that age 7 and above appears to be an "inflexion" point and slopes of deterioration in 6MWT, NSAA are evident (25–27). This evidence let us to perform this specific analysis. Further, we wanted to take advantage of the depth of the CINRG data set given that many clinical trials in DMD are focusing on boys of ages 4 to less than 8 years. In concordance with earlier publications, we found that as a cohort, over a three-year period, boys 7 years and older declined more rapidly than boys younger than 7 years. Further, in our cohort, the decline in 6MWT was regardless of *DMD* pathogenic variant location. The decline in 6MWT also depends on mutation type as reported by Brogna, who showed that individuals with pathogenic variants amendable to exon 44 skipping had better 6MWT at baseline and 36 months, compared

to those with pathogenic variants amendable to exon 53 skipping (28). These results, also supported by findings from the CINRG-DNHS (7) and other cohorts (29, 30), suggest that accounting for *DMD* pathogenic variant type, as well as longer follow-up, may be necessary to fully understand drug efficacy in clinical trials.

In summary, our study along with earlier published literature demonstrates that well-designed natural history studies in rare disease like DMD provides critical insights for clinical trial design.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations

DMD	Dystrophin gene
DMD	Duchenne Muscular Dystrophy
CINRG	Cooperative International Neuromuscular Research Group
DNHS	Duchenne Natural History Study
6MWT	6-minute Walk Test
LoA	Loss-of-ambulation
CQMS	CINRG Quantitative Measurement System
ANOVA	Analysis-of-variance
CNS	Central nervous system

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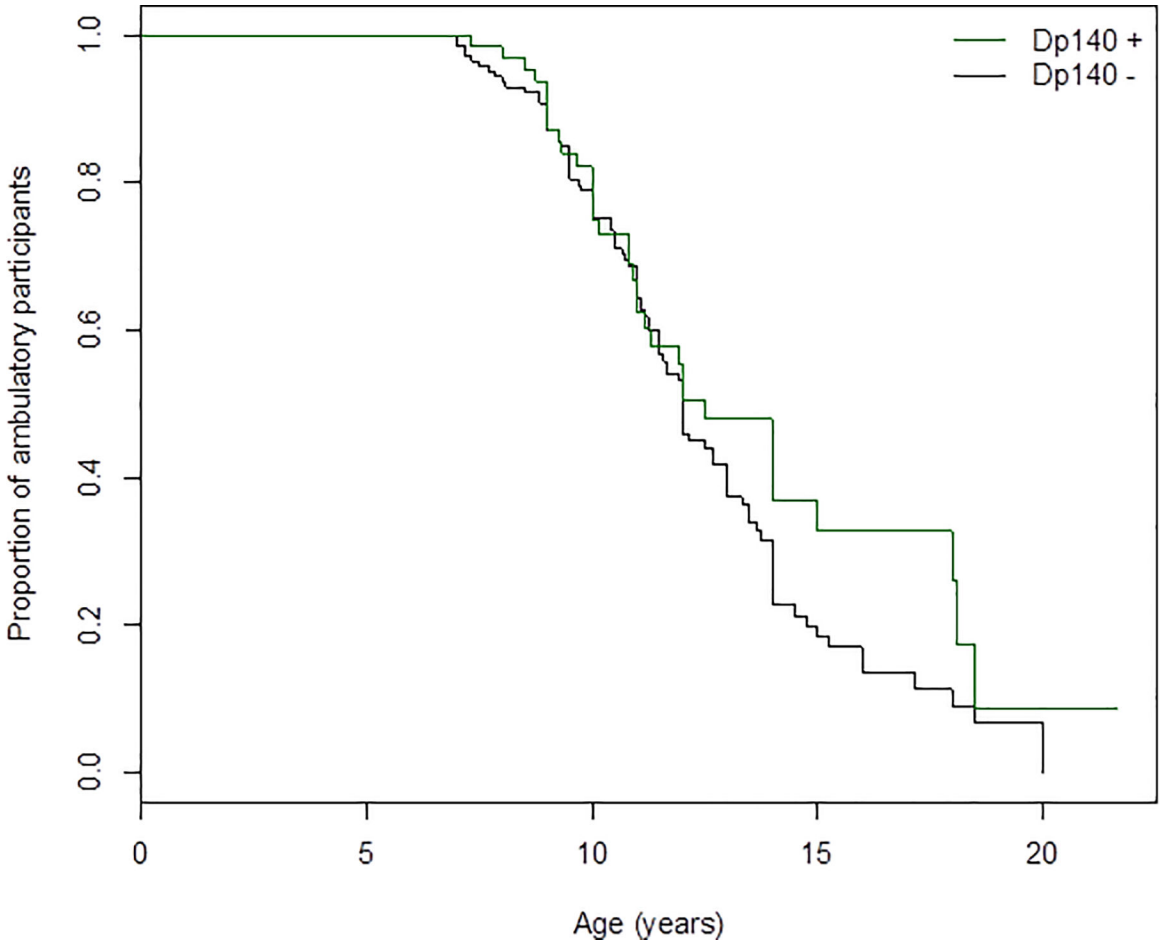

Figure 1.

Kaplan-Meier plot of age of loss-of-ambulation based on the proximal *DMD* pathogenic variants (proximal to 5' *DMD* intron 44) versus distal *DMD* pathogenic variants (as those including nucleotides 3' *DMD* including intron 44) from the parent CINRG-DNHS cohort (n = 212).

Table 1.

Parameters for time-to-event analyses of loss-of-ambulation (LoA) from the parent CINRG-DNHS (n = 212).

Cox regression factor	Level of factor	N	%	LoA events (n, %)	Median age (years) at LoA (95% CI)	HR (95% CI)	p-value
***DMD* pathogenic variant alocation**	Proximal to 5' *DMD* intron 44	66	31%	34 (52%)	12.5 (11.2 – 18.1)	0.85 (0.57 – 1.27)	ns
	Distal as those including nucleotides 3' *DMD* including intron 44	146	69%	98 (67%)	12.0 (11.5 – 13.0)	1 *	-
Corticosteroid treatment	Untreated or treated for less than one year	55	26%	48 (87%)	9.7 (9.0 – 11.0)	1 *	-
	Treated at least for 1 year while ambulatory	157	74%	84 (54%)	13.5 (12.5 – 14.0)	0.24 (0.16 – 0.35)	***<0.0001***
Total		212	100%	132 (100%)	12.0 (11.5 – 13.0)	-	-

LoA: loss of ambulation; CI: confidence interval; HR: Hazard Ratio.

* A HR of 1 is given for factor levels that are taken as reference in the Cox regression model.

Table 2.

Demographic and anthropometric characteristics of the longitudinal cohort at first study visit.

Characteristic	Proximal *DMD* pathogenic variants			Distal *DMD* pathogenic variants			P-value
	N (%)	Mean ± SD	Median (IQR, Range)	N (%)	Mean ± SD N (%)	Median (IQR, Range)	
Age (years)	53	6.5 ± 1.4	6.2 (2.3, 4.8)	101	6.6 ± 1.9	6.5 (2.1, 16.3)	0.72
Calculated height (cm) *	53	116.0 ± 9.6	114.9 (14.2, 38.9)	100	117.4 ± 10.8	117.8 (13.7, 79.9)	0.41
Weight (kg)	53	22.3 ± 5.7	21.8 (6.8, 27.5)	101	22.3 ± 7.4	20.7 (6.6, 52.9)	0.98
Ambulatory							0.99
Yes	52 (98.1%)			100 (99.0%)			
No	1 (1.9%)			1 (1.0%)			
Ethnicity							0.17
Hispanic	1 (1.9%)			8 (7.9%)			
Non-Hispanic	52 (98.1%)			93 (92.1%)			
Race							0.048
White	39 (73.6%)			71 (70.3%)			
Black	0 (0%)			1 (1%)			
Pacific Islander	2 (3.8%)			0 (0%)			
Asian	6 (11.3%)			24 (23.8%)			
Other	4 (7.6%)			2 (2.0%)			
Unknown	2 (3.8%)			3 (3.0%)			

* Height calculated from ulna length using Gould equation.

Table 3:

Descriptive statistics of corticosteroid use at first study visit

Characteristic	Subjects with proximal *DMD* pathogenic variants			Subjects with distal *DMD* pathogenic variants			P-value
	N	Mean ± SD N (%)	Median (IQR, Range)	N	Mean ± SD N (%)	Median (IQR< Range)	
Total lifetime corticosteroid use (days)	53	355 ± 423	208 (563, 1579)	101	266 ± 364	82 (468, 1665)	0.30
Current corticosteroid user							0.86
No		19 (35.9%)			39 (38.6%)		
Yes		34 (64.2%)			62 (61.4%)		
Corticosteroid currently used *							0.96
None		19 (35.9%)			39 (39.0%)		
Prednisone		15 (28.3%)			25 (25.0%)		
Deflazacort		11 (20.8%)			22 (22.0%)		
Prednisolone		8 (15.1%)			14 (14.0%)		

* One participant in the distal pathogenic variant group is a current corticosteroid user, but the specific drug is unknown

Table 4:

Assessment of functional outcomes over time with adjustment for total lifetime corticosteroid use

Outcome	N (Observations) N (Subjects)	DMD pathogenic variant location	DP140 isoform coefficient (P-value)	Age coefficient (p-value)
10 m run/walk velocity (m/sec)	1061 154	*Proximal*	0.132 (p=0.31)	−0.103 (p=0.008)
		Distal	---	
Standing from supine velocity (rise/sec)	985 154	*Proximal*	0.004 (p=0.85)	−0.029 (p<0.001)
		Distal	---	
Climbing velocity (task/sec)	1039 154	*Proximal*	0.016 (p=0.59)	−0.014 (p=0.13)
		Distal	---	
6MWT distance traveled (m)	377 93	*Proximal*	−6.8 (p=0.78)	−0.48 (p=0.95)
		Distal	---	
Grip strength (lbs.) **	1009 145	*Proximal*	0.01 (p=0.90)	0.217 (p<0.001)
		Distal	---	
Elbow extensor strength (lbs.) *	893 141	*Proximal*	−0.02 (p=0.70)	−0.110 (p<0.001)
		Distal	---	
Elbow flexor strength (lbs.) *	901 141	*Proximal*	−0.04 (p=0.59)	−0.053 (p=0.09)
		Distal	---	
Knee extensor strength (lbs.) *	907 142	*Proximal*	−0.30 (p=0.60)	−0.297 (p<0.001)
		Distal	---	
Knee flexor strength (lbs.) *	895 140	*Proximal*	0.07 (p=0.49)	0.0235 (p=0.52)
		Distal	---	

* Strength outcomes were square-root transformed for analysis, and transformed values are presented here.

** This relationship is curvilinear, therefore the coefficient for each DP140 group estimates the instantaneous rate of change in grip strength when all other predictors are zero, rather than the average rate of change over all ages.

Table 5.

Analysis of 3-year change in 6-minute walk test in subjects with proximal and distal *DMD* pathogenic variants.

Cohort	Age*	N (obs.) N (indiv.)	Baseline 6MWT (Mean ± SE)**	6MWT at 3 years (Mean ± SE)**	Calculated 3 year change in 6MWT***
All	< 7 years	189 49	353 ± 15	439 ± 31	85.7
	≥ 7 years	188 71	393 ± 15	337 ± 17	−55.2
Proximal *DMD* pathogenic variants	< 7 years	45 12	324 ± 38	341 ± 52	16.8
	≥ 7 years	47 22	395 ± 29	331 ± 34	−64.4
Distal *DMD* pathogenic variants	< 7 years	144 37	363 ± 16	454 ± 13	91.0
	≥ 7 years	141 49	391 ± 17	339 ± 19	−52.2

*Note an individual can be in both the <7 and ≥ 7 years sub-cohorts at different times based on age. A participant can contribute assessments while younger than 7 in one model and contribute assessments while 7 or older to the other model.

**Mean and SE are predicted values from longitudinal model.

***Change over three years is based on the rate of change (coefficient) for the model.