ARTICLE

MR biomarkers predict clinical function in Duchenne muscular dystrophy

Alison M. Barnard, DPT, PhD,* Rebecca J. Willcocks, PhD,* William T. Triplett, BS, Sean C. Forbes, PhD, Michael J. Daniels, ScD, Saptarshi Chakraborty, PhD, Donovan J. Lott, PT, PhD, Claudia R. Senesac, PT, PhD, Erika L. Finanger, MD, PhD, Ann T. Harrington, DPT, PhD, Gihan Tennekoon, MBBS, Harneet Arora, PT, PhD, Dah-Jyuu Wang, PhD, H. Lee Sweeney, PhD, William D. Rooney, PhD, Glenn A. Walter, PhD, and Krista Vandenborne, PT, PhD

Correspondence
Dr. Vandenborne
kvandenb@phhp.ufl.edu

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Abstract

Objective

To investigate the potential of lower extremity magnetic resonance (MR) biomarkers to serve as endpoints in clinical trials of therapeutics for Duchenne muscular dystrophy (DMD) by characterizing the longitudinal progression of MR biomarkers over 48 months and assessing their relationship to changes in ambulatory clinical function.

Methods

One hundred sixty participants with DMD were enrolled in this longitudinal, natural history study and underwent MR data acquisition of the lower extremity muscles to determine muscle fat fraction (FF) and MRI T_2 biomarkers of disease progression. In addition, 4 tests of ambulatory function were performed. Participants returned for follow-up data collection at 12, 24, 36, and 48 months.

Results

Longitudinal analysis of the MR biomarkers revealed that vastus lateralis FF, vastus lateralis MRI T_2, and biceps femoris long head MRI T_2 biomarkers were the fastest progressing biomarkers over time in this primarily ambulatory cohort. Biomarker values tended to demonstrate a nonlinear, sigmoidal trajectory over time. The lower extremity biomarkers predicted functional performance 12 and 24 months later, and the magnitude of change in an MR biomarker over time was related to the magnitude of change in function. Vastus lateralis FF, soleus FF, vastus lateralis MRI T_2, and biceps femoris long head MRI T_2 were the strongest predictors of future loss of function, including loss of ambulation.

Conclusions

This study supports the strong relationship between lower extremity MR biomarkers and measures of clinical function, as well as the ability of MR biomarkers, particularly those from proximal muscles, to predict future ambulatory function and important clinical milestones.

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*These authors contributed equally to this work.

From the Departments of Physical Therapy (A.M.B., R.J.W., W.T.T., S.C.F., D.J.L., C.R.S., H.A., K.V.), Statistics (M.J.D., S.C.), Pharmacology and Therapeutics (H.L.S.), and Physiology and Functional Genomics (G.A.W.), University of Florida, Gainesville; Departments of Pediatrics and Neurology (E.L.F., G.T., D.-J.W.) and Advanced Imaging Research Center (W.D.R.), Oregon Health & Science University, Portland; and Children's Hospital of Philadelphia (A.T.H.), PA.

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Glossary

BFLH = biceps femoris long head; **DMD** = Duchenne muscular dystrophy; **FF** = fat fraction; **GRA** = gracilis; **MG** = medial gastrocnemius; **MR** = magnetic resonance; **MRS** = MR spectroscopy; **PER** = peroneal group; **qMR** = quantitative MR; **ROC** = receiver operating characteristic; **6MWT** = 6-minute walk test; **SOL** = soleus; **STS** = supine-to-stand; **TA** = tibialis anterior; **TE** = echo time; **TP** = tibialis posterior; **TR** = repetition time; **VL** = vastus lateralis.

Duchenne muscular dystrophy (DMD) is a severe muscle degenerative disorder resulting in progressive skeletal and cardiac muscle weakness.[1–3] Currently, an unprecedented number of clinical trials are being initiated for this life-limiting disorder, and regulatory agencies have supported the development of biomarkers that can potentially be used as endpoints or surrogate outcomes.[4] The use of biomarker endpoints has the potential to accelerate approval of therapies that alter the natural history progression of DMD.[5]

Skeletal muscle MRI and magnetic resonance (MR) spectroscopy (MRS) measures are noninvasive biomarkers that are sensitive to pathologic changes in dystrophic muscles, and MR biomarkers have the potential to serve as clinical trial endpoints.[6,7] Muscle MRI transverse magnetization relaxation time constant (T_2) is altered in response to muscle sarcolemma disruption, inflammation, and fibrofatty infiltration, allowing it to be a global measure of muscle health.[8–11] Muscle fat fraction (FF) quantifies the level of fat infiltration and progresses from minimal levels of muscle fat to nearly complete fibrofatty replacement of muscle in individuals with DMD.[12,13]

Although a body of literature exists establishing quantitative MR (qMR) measures as high-quality biomarkers for DMD,[7,14–21] a high burden of proof is required to establish MR biomarkers as secondary endpoints or surrogate outcomes. The goal of this investigation was to use 48 months of qMR biomarker data from the multicenter ImagingDMD natural history study to characterize the longitudinal progression of lower extremity muscle MR biomarkers and to examine the relationship between MR biomarkers and function over time, as well as the ability of MR biomarkers to predict clinically meaningful sentinel events.

Methods

Standard protocol approvals, registrations, and patient consents

In September 2010, participants began enrolling in the longitudinal, natural history ImagingDMD study at 3 study sites (University of Florida, Oregon Health & Science University, and the Children's Hospital of Philadelphia). The study was approved by the Institutional Review Board at each site location and registered on ClinicalTrials.gov (NCT01484678). To enroll, participants were required to have a confirmed diagnosis of DMD, and they were excluded if they had contraindications to MRI, any comorbid muscle disorders, or cognitive or behavioral difficulties that precluded successful participation. Participants were initially required to walk at least 100 m and to be able to climb 4 stairs at the time of enrollment, but inclusion criteria were later expanded to include nonambulatory individuals. Before data collection, written informed consent to participate was obtained from the parent or guardian, while the participant provided written assent. Participants who were ≥18 years of age provided written informed consent themselves.

Study design

At the baseline visit, participants underwent an MRI and MRS examination of the lower leg and thigh, followed by clinical assessments of ambulatory function. Relevant medical history information such as fracture history and medication use was also collected. Participants returned annually (every 12 ± 2 months) for up to 7 years for follow-up MR, functional, and medical history data collection. A subset of participants had additional follow-up visits 3 and 6 months after baseline. These data have been previously reported, and only annual time points are included in this article to assess yearly changes.[18,21] Participants who missed a follow-up visit were allowed to continue their participation, and data were collected the following year. For ethical reasons, participants were not prohibited from enrolling in other natural history studies or clinical trials.

MR acquisition/analysis

MR data were collected on 3T MR systems (Philips Achieva [Best, the Netherlands]; Siemens Magnetom TIM Trio/Prisma^{fit} and Siemens Magnetom Verio [Munich, Germany]) to measure the MR biomarkers of interest: muscle MRI T_2 and FF determined by ^{1}H-MRS. Site-to-site reproducibility of the MR biomarkers on the different MR systems using site-specific coils has been previously established.[14] Trained MR operators performed all MR data acquisition using standardized manuals of operating procedures.

Axial 2D multiecho spin-echo images were obtained in the lower leg and thigh to determine muscle MRI T_2 in the soleus (SOL), medial gastrocnemius (MG), tibialis anterior (TA), tibialis posterior (TP), peroneal group (PER), vastus lateralis (VL), biceps femoris long head (BFLH), and gracilis (GRA) muscles. For the 2D spin-echo images, a 6- to 8-slice stack was acquired (7-mm slice thickness, 3.5-mm slice gap, 0.75 × 1.5–mm in-plane resolution) at the belly of the calf and at midthigh. Repetition time (TR) was 3,000 milliseconds with 16 evenly spaced echo times (TEs) between 20 and 320 milliseconds. A monoexponential decay curve was fitted to the MR signal at 40-, 60-, 80-, and 100-millisecond TEs to determine muscle MRI T_2 on a pixel-by-pixel basis.[11] Trained analyzers selected 3 contiguous slices for analysis using predefined

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anatomic landmarks that ensured slice selection consistency between participants and from year to year. For the lower leg, the slices selected were the most distal slice in which the popliteus muscle was first visible and the 2 slices distal to it. In the thigh, the slices selected were the most proximal slice containing the biceps femoris short head and the 2 slices distal. Using custom software (Interactive Data Language; Harris Geospatial Solutions, Boulder, CO), analyzers from a single site (University of Florida) drew regions of interest just within the borders of the lower leg and thigh muscles in each slice, excluding large fascia, to determine muscle MRI T_2. The T_2 values of all pixels within the 3 analyzed slices were averaged to give mean muscle MRI T_2.

^{1}H-MRS was performed in the VL and SOL muscles to determine muscle FF. A stimulated echo acquisition mode sequence was used to obtain spectra from voxels placed within the belly of the SOL and VL muscles.[22] Voxels were made as large as possible while remaining completely within the muscle of interest. At follow-up visits, the voxel was visually matched to baseline location. Spectra were acquired with a TR of 3,000 milliseconds and TE of 108 milliseconds, and 16 acquisitions were averaged. Each summed spectrum was integrated to determine fat and water areas. The effects of T_1 and T_2 relaxation were corrected with previously published fat T_2, fat T_1, and water T_1 values, and water T_2 was determined from spectra acquired within the same voxel (TR 9,000 milliseconds, 8–16 TEs ranging from 11–243 milliseconds).[22] All spectrum analyses were automated with custom Interactive Data Language software.

A standardized quality control process was implemented to review all MRI and MRS. Images with excessive motion, signal inhomogeneity, or other obvious artifacts that would invalidate MRI T_2 values were excluded from analysis. Muscle regions of interest were reviewed to ensure compliance with analysis procedures. MRS data were reviewed for adequate signal-to-noise ratio, appropriate peak selection, suitable line width, and goodness of fit of the integrated regions and relaxation curves.

Functional outcomes

After the MR scans were completed, participants performed 4 tests of ambulatory function, including the 10-m walk/run, the 4-stair climb test (stair climb), the supine-to-stand (STS) test, and the 6-minute walk test (6MWT). All of these tests are recognized as reliable outcomes of functional ability in DMD and are used widely in natural history assessment and clinical trials.[23,24] For the 10-m walk/run, stair climb, and STS tests, 3 trials were performed, and the fastest time was recorded. If a participant could not complete the test within 45 seconds or without assistance, then the participant was considered to have lost the ability to perform the test. Loss of ambulation was defined as loss of the ability to perform the 10-m walk/run. The 6MWT was performed on a 25-m course, and the distance covered in 6 minutes was recorded. Detailed functional assessment methods from the ImagingDMD study have been previously published.[25]

Data analysis

All available MR and functional outcome data from annual visits were used for analysis unless the data had been deemed invalid after careful quality control review. Data from visits outside of the annual visit window were excluded, as were missing data. Data were analyzed with GraphPad Prism version 7.03 (La Jolla, CA) and R version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria). For data visualization purposes, functional test times were converted to velocities, with a velocity of 0 indicating an inability to perform the test. The 10-m walk/run velocity was computed by dividing 10 m by the test time to give a velocity in meters per second. Four-stair climb and STS velocities were computed by taking the reciprocal of the test time to give a velocity in tasks per second.

Functional test changes over 12 months were classified as improvement, stability, decline in performance, or loss of ability. For timed tests, improvement was defined as a change >-0.5 seconds; stability was defined as change between -0.5 and 0.5 seconds; and decline was defined as a change >0.5 seconds. For the 6MWT, 30 m has been estimated as the minimal clinically important difference. Therefore, for this analysis, improvement was defined as a >30-m increase, stability was defined as a change of 30 to -30 m, and decline was defined as a >30-m decrease.[26]

Receiver operating characteristic (ROC) curves were estimated to assess the ability of MR biomarkers, at year t, to predict future functional ability status at year $t + k$, where $k = 1$ or 2. Results are expressed as the C statistic (area under the curve). MR biomarker threshold values were determined from the point on the ROC curve that optimized the sum of sensitivity and specificity (Youden index). A discrete time hazard model was used to estimate the odds of loss of ambulation by year t given the biomarker at year $t - 1$, adjusting for age at study entry. For both ROC and odds ratio analyses, the nonparametric bootstrap (resampling participants with replacement) was used to characterize uncertainty given that multiple observations from the same participant were used.

Data availability

Anonymized data published within this article can be requested from the corresponding author by submitting a formal application. All data requests will be reviewed by the study executive committee.

Results

Cohort characteristics

One hundred sixty participants with DMD were enrolled in the ImagingDMD study. At the time of the analysis, a total of 566 participant visits were completed, with 79 participants completing 5 visits across 48 months (table 1). Consistent with the contemporary natural history of DMD, drug and supplement use (including corticosteroids, vitamin D, calcium, coenzyme

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Table 1 Participant characteristics and MR biomarker values

	Baseline (n = 160)	At 12 mo (n = 122)	At 24 mo (n = 110)	At 36 mo (n = 95)	At 48 mo (n = 79)
Demographics					
Age, y	8.6 (2.7)	9.3 (2.2)	10.2 (2.1)	11.4 (2.1)	12.3 (2.0)
Age, minimum/maximum, y	4.8/18.8	5.8/14.28	6.8/15.1	8.2/16.1	9.1/16.8
Age 25%–75%, y	6.4–10.6	7.3–11.0	8.5–11.8	9.7–13.1	10.7–13.9
Height, cm	120.9 (11.9)	123.3 (9.6)	126.0 (9.3)	129.4 (9.9)	131.9 (10.3)
Weight, kg	29.6 (12.0)	30.5 (9.9)	33.6 (11.0)	37.2 (12.1)	41.1 (13.2)
Corticosteroid use, n[a]	118/40/2	106/13/3	99/10/1	87/6/2	69/5/5
Functional ability, %					
Ambulatory	97	97	87	82	69
Able to climb 4 stairs	95	94	84	73	57
Able to rise from supine	88	79	70	50	39
MRS FF					
VL	0.20 (0.19)	0.24 (0.19)	0.31 (0.21)	0.37 (0.24)	0.42 (0.24)
SOL	0.11 (0.10)	0.12 (0.09)	0.16 (0.12)	0.20 (0.15)	0.24 (0.18)
MRI T_2					
VL	48.8 (10.3)	52.1 (11.5)	55.3 (12.2)	58.0 (13.3)	59.8 (12.4)
BFLH	51.2 (12.0)	54.9 (13.1)	59.3 (13.5)	62.8 (14.2)	64.6 (13.0)
GRA	39.8 (6.5)	40.6 (6.4)	42.1 (6.7)	42.8 (7.8)	43.9 (8.0)
SOL	43.4 (6.2)	44.1 (6.2)	46.1 (7.7)	48.3 (7.8)	49.1 (8.9)
MG	43.0 (6.7)	43.8 (7.6)	46.1 (9.2)	47.8 (9.2)	47.8 (8.9)
PER	43.5 (7.7)	44.6 (7.5)	47.0 (8.7)	49.3 (8.3)	49.6 (9.1)
TA	38.4 (6.1)	38.1 (4.9)	39.4 (5.5)	40.9 (6.5)	42.1 (7.3)
TP	37.3 (4.1)	37.2 (2.8)	37.9 (3.4)	38.5 (3.8)	39.5 (5.8)

Abbreviations: BFLH = biceps femoris long head; FF = fat fraction; GRA = gracilis; MG = medial gastrocnemius; MR = magnetic resonance; MRS = MR spectroscopy; PER = peroneal group; SOL = soleus; TA = tibialis anterior; TP = tibialis posterior; VL = vastus lateralis.
Values represent the mean (SD) unless otherwise denoted.
[a] For corticosteroid use, values represent the following statuses: on/off/unknown.

Q10, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, and others) was common in this cohort. Overall, participants reported taking corticosteroids at 87% of visits. Of those taking steroids, 74% took deflazacort, and 26% took prednisone/prednisolone. A number of participants also took conditionally approved drugs (11.9% ataluren, 9.3% eteplirsen) or were enrolled in ongoing clinical trials at some point during the course of their study participation (7.5%).

At baseline, the cohort was young (mean age 8.6 years, n = 55 age ≤6 years) and highly functional with the exception of 5 individuals recruited later in the study who were nonambulatory at baseline. By 48 months, the mean age of the cohort was 12.3 years, and >30% of the cohort was nonambulatory. Mean MR biomarker values for the cohort are reported for each 12-month interval in table 1, and illustrative lower extremity MRIs and corresponding functional results acquired in 12-month intervals are shown in figure 1.

MR biomarker trajectories

All available MR data from the ImagingDMD cohort were binned by participant age to examine the group mean progression in MRI T_2 and MRS FF of the lower extremity muscles. Of the 8 muscles analyzed for MRI T_2, values in the BFLH and VL were highest for each age bin and increased most quickly with age (figure 2A). PER, SOL, and MG MRI T_2 values increased at intermediate rates, whereas GRA, TA, and TP MRI T_2 values increased very slowly with increasing age. MRS FF values for the VL and SOL demonstrated an age-related increase similar to that of MRI T_2, with VL FF increasing approximately twice as quickly as SOL FF (figure 2B). In the most affected individuals, MRI T_2 of the BFLH

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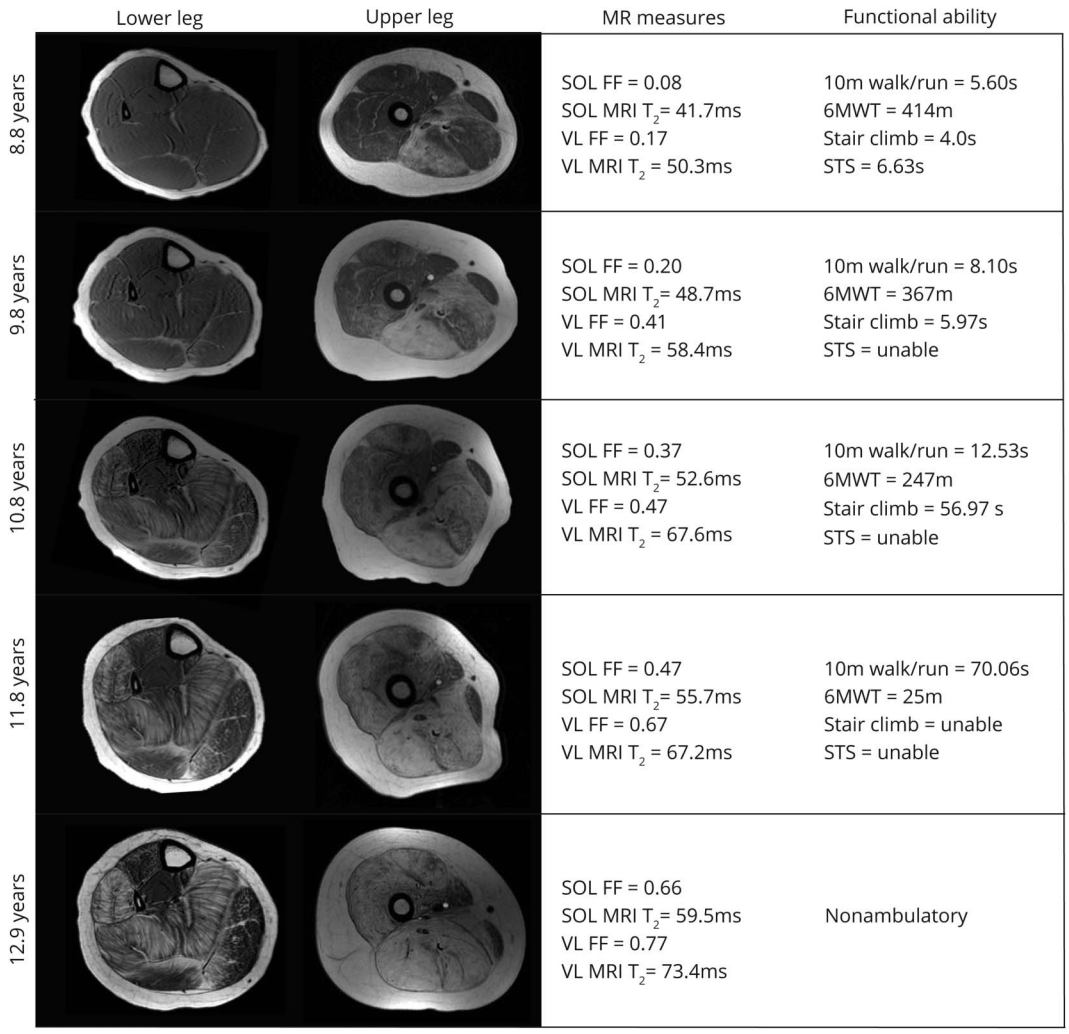
Figure 1 MRIs, MR biomarker values, and functional status for a participant across 48 months



This figure represents the natural history of a single individual's disease progression from 8.8 years (baseline) to 12.9 years (48 months) of age. The lower leg and thigh images are axial T_1-weighted images that demonstrate the progressive fatty infiltration of the musculature. Magnetic resonance (MR) spectroscopy fat fraction (FF) and MRI T_2 values with the corresponding functional test results are listed for each year. 6MWT =6-minute walk test; STS = supine-to-stand; VL = vastus lateralis.

and VL reached values as high as 80 to 85 milliseconds, and VL FF reached values near 0.85.

Individual longitudinal trajectories of MR biomarkers from 2 key muscles, the VL and SOL, were examined as a function of age (figure 2, C–F). A high degree of between-participant heterogeneity in FF and MRI T_2 biomarker progression was observed, with some individuals displaying early and rapid increases and others appearing relatively stable, even after 10 years of age. The average rate of progression over 12 months appeared to be dependent on baseline MR biomarker values. Individuals with very low FF or MRI T_2 values (VL FF <0.10, VL MRI T_2 <45 milliseconds) tended to have small increases in MR values over the next 12 months (VL FF change <0.05, MRI T_2 change <3 milliseconds). The largest increases were noted in individuals with a VL FF between 0.10 and 0.50 (mean annual change 0.10) or a VL MRI T_2 between 45 and 65 milliseconds (mean annual change of 5.8 milliseconds).

Longitudinal relationship between MR biomarkers and function

The VL is a key lower extremity extensor muscle, and MR biomarkers of the VL have demonstrated a strong relationship to functional ability. Therefore, the trajectories of VL FF and MRI T_2 were examined in relation to performance on each of the ambulatory functional tests. Longitudinally, there was a consistent decline in 6MWT distance and 10-m walk/run velocity with increasing VL FF and increasing VL MRI T_2 (figure 3). A VL FF of 0.40 and a VL MRI T_2 of 65 milliseconds appear to be approximate lower thresholds for loss of ambulation. Similarly, 4-stair climb and STS velocities decreased as VL FF and VL T_2 increased.

Baseline MR biomarker values were predictive of change in function over the next 12 months, and the estimated probabilities of functional improvement, stability, decline, or loss of ability by VL FF are plotted in figure 4. The probability of

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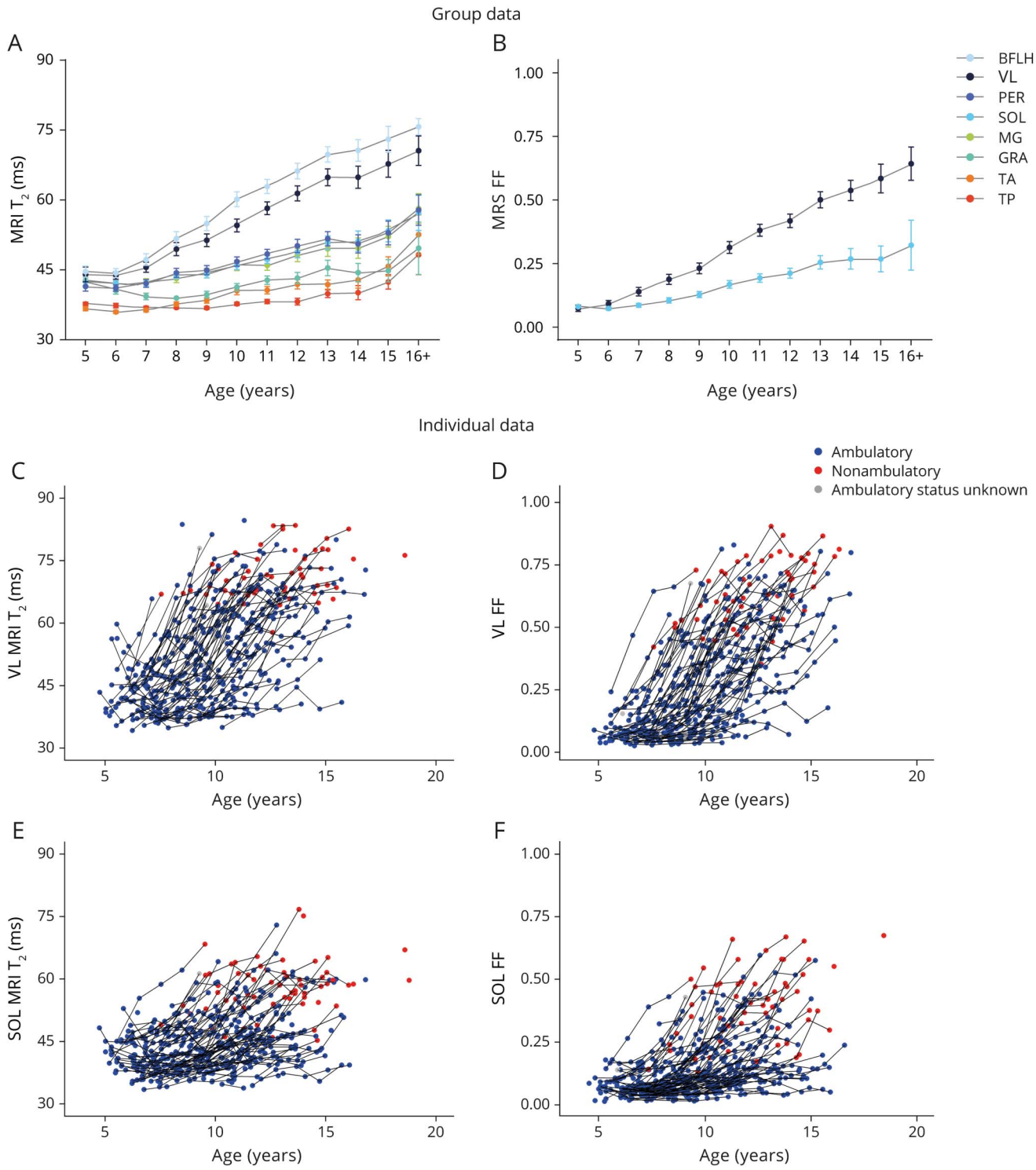
Figure 2 Binned and individual participant MR biomarker trajectories



(A and B) With binning of all available data points for each age, the progression of MRI T_2 and magnetic resonance (MR) spectroscopy (MRS) fat fraction (FF) with increasing age reveals that vastus lateralis (VL) FF, VL MRI T_2, and biceps femoris long head (BFLH) MRI T_2 are most elevated within each age group. (C–F) The trajectories of MR biomarkers from each individual participant demonstrate heterogeneity in disease progression among participants, even participants of similar ages. GRA = gracilis; MG = medial gastrocnemius; PER = peroneal group; SOL = soleus; TA = tibialis anterior; TP = tibialis posterior.

experiencing functional stability or improvement over 12 months was >50% in individuals with very low VL FFs (<0.1), while the probability of declining or losing function was highest above an FF of 0.40.

The magnitude of 12-month change in the MR biomarkers was also associated with the likelihood of functional improvement, stability, decline, or loss of ability. More than half of individuals with negligible or small changes in VL FF (change ≤0.02)

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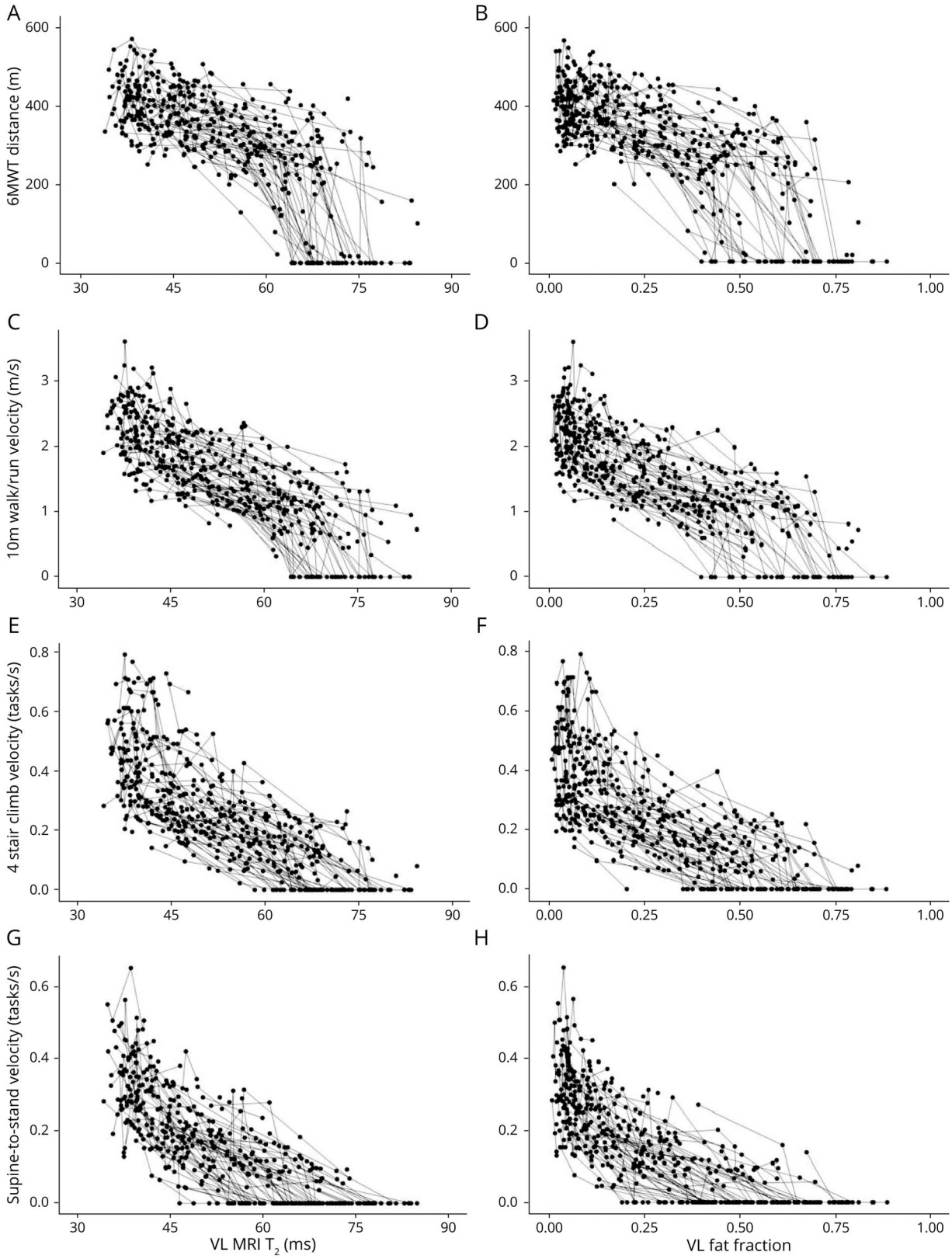
Figure 3 Longitudinal relationship between VL MR biomarkers and ambulatory function



(A and B) For the 6-minute walk test (6MWT), distances declined in a linear manner as vastus lateralis (VL) MRI T_2 and VL fat fraction (FF) increased. Once participants were walking only ≈200 m, VL MRI T_2 was >65 millisecond, or VL FF was >0.4, loss of the ability to perform the 6MWT became likely. (C–H) Functional test times were expressed as velocities to allow visualization of loss of ability. As the velocity of functional task performance decreased, there was an associated increase in magnetic resonance (MR) biomarker values. Supine-to-stand velocity tended to decrease more rapidly and at lower VL MR biomarker values than stair climb or 10-m walk/run velocities.

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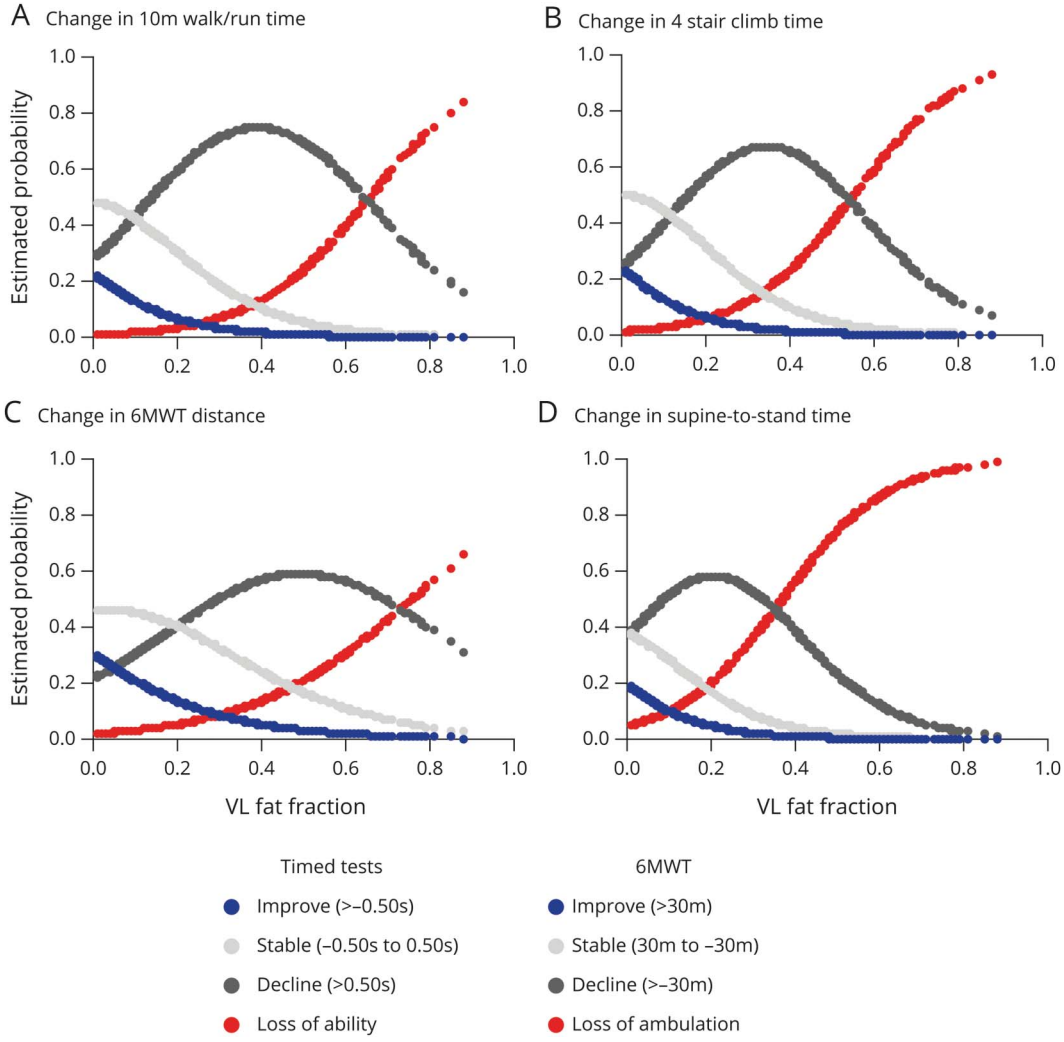
Figure 4 Baseline VL FF and the probability of functional test change



(A–D) The probability of functional test improvement, stability, decline, or loss over 12 months was estimated with ordinal logistic regression. At baseline fat fractions (FFs) <0.10, the probability of improvement or stability is highest. At vastus lateralis (VL) FFs of 0.20 to 0.40, decline in functional tests is highly likely with a smaller chance of loss of ability. The exception is the supine-to-stand test, for which the probability of loss of ability becomes more likely than decline at ≈0.36. 6MWT = 6-minute walk test

either remained stable or had improved functional test performance over 12 months. Conversely, nearly 90% of individuals with increases in VL FF > 0.15 declined in function or lost function. From the entire cohort, the individuals with the fastest and slowest rates of VL FF progression were identified, and their functional ability was assessed. These individuals with extremely fast or slow disease progression by MR biomarkers were also outliers by functional outcomes.

MR biomarkers predict loss of function

A Kaplan-Meier curve for loss of functional skills as a function of VL FF illustrates the range of values over which loss of function is most likely (figure 5A). VL FFs for individuals who lost and did not lose functional abilities over the subsequent 24 months were significantly different. Participants with a baseline VL FF <0.2 were likely to retain the ability to ambulate, climb stairs, and rise from the floor over the following 12 and 24 months (figure 5, B–D). In contrast, individuals with a baseline VL FF >0.3 were more likely to lose functional ability over 24 months, with >50% of these individuals losing the ability to ambulate.

ROC curves of the relationship between baseline lower extremity MR biomarkers and loss of ambulation at 12 months were significant for all MR biomarkers (table 2). VL FF, VL T_2, and SOL T_2 had the largest C statistic (area under the curve) when predicting loss of ambulation and loss of stair climbing. These same VL biomarkers, as well as BFLH T_2, also had the largest C statistic for loss of STS. A baseline VL FF of 0.39 and a VL MRI T_2 of 59.6 milliseconds produced the highest sum of sensitivity and specificity for loss of ambulation in the following 12 months.

The odds of losing ambulation within 12 months were determined with a discrete time hazard model. An increase in

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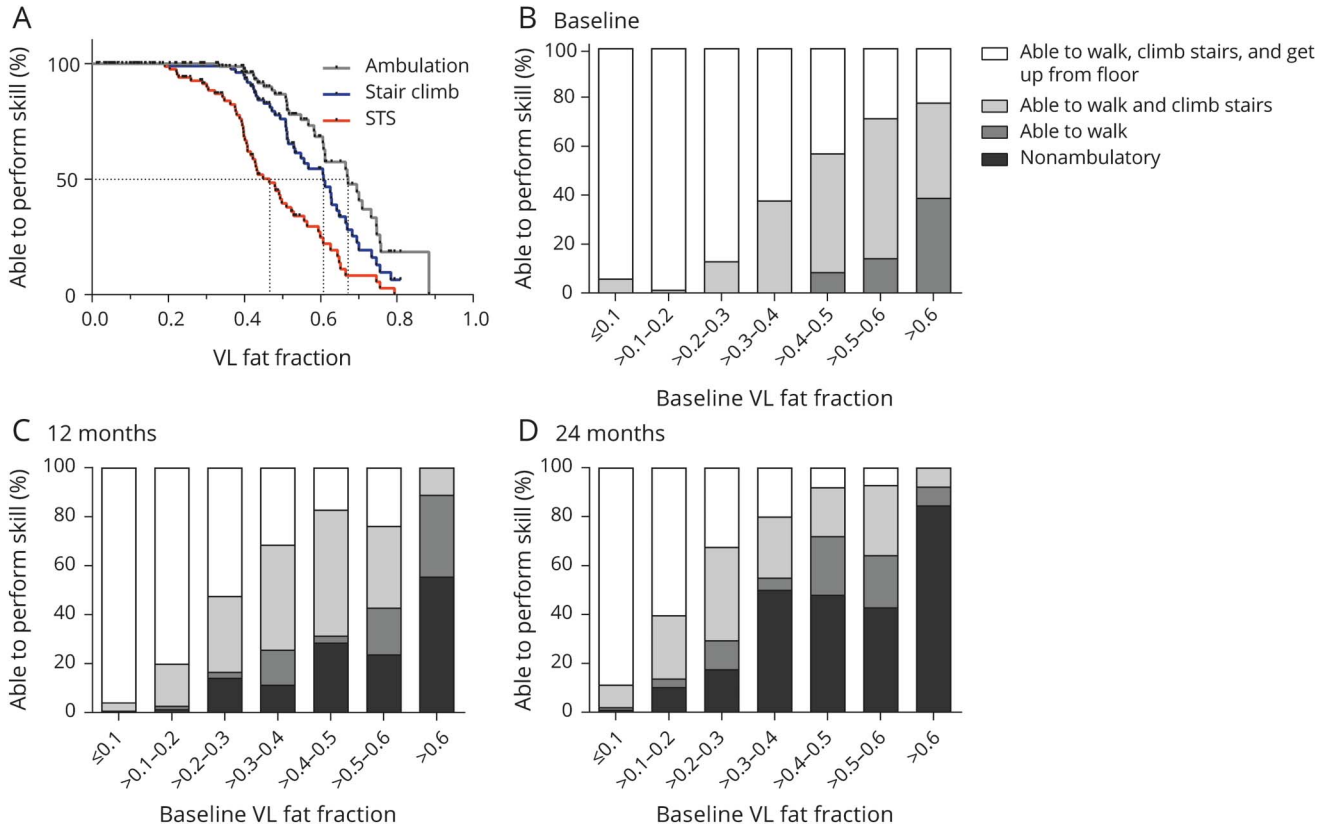
Figure 5 Loss of functional ability vs magnetic resonance biomarkers



(A) A Kaplan-Meier plot for loss of 3 functional skills (supine-to-stand [STS], stair climb, and ambulation) in relation to vastus lateralis (VL) fat fraction (FF). (B–D) There is a strong relationship between baseline VL FF and the loss of functional skills over 24 months (white = ambulatory, can climb stairs, and can perform STS; light gray = ambulatory and can climb stairs but cannot perform STS; dark gray = ambulatory, unable to climb stairs or perform STS; black = non-ambulatory). Very few individuals with VL FFs ≤0.20 lost functional skills over 12 months, and only a small proportion lost abilities over 24 months. Individuals who were ambulatory at baseline with VL FFs >0.30 were the most likely to lose ambulation within 24 months. (Note that all participants included in this analysis were ambulatory at baseline.)

several MR biomarker values was significantly associated with an increased odds of loss of ambulation (table 2). Specifically, a 1-SD increase in VL FF produced the highest odds of loss of ambulation over the next 12 months with a 10.8-fold increase ($p < 0.0001$), while a 1-SD increase in VL MRI T_2 increased the odds of loss of ambulation by 4.4-fold ($p < 0.0001$).

Discussion

A large body of literature supports the potential use of qMR biomarkers of skeletal muscle health to track disease progression in DMD, setting the stage for its future use as an endpoint in clinical trials. This study used the well-characterized ImagingDMD cohort to comprehensively assess the trajectory of qMR biomarkers in the lower extremity muscles and their relationship to functional outcomes. The 48-month multicenter data presented here represent analyses of 2 different lower extremity MR biomarkers (muscle MRI T_2 and MRS FF), paired with clinical measures of ambulatory function, in a large, contemporary cohort. The results of this study describe the trajectory of MRI T_2 and MRS FF in DMD over a range of ages and disease severities in multiple lower extremity muscles. In addition, we demonstrate the relationship between qMR biomarkers and decline in ambulatory function, including the ability of qMR biomarkers to predict future ambulatory function and loss of function.

Most longitudinal lower extremity MR biomarker studies examining disease progression in DMD have been limited to small samples sizes and short durations, with all prior studies outside of the ImagingDMD cohort having <30 participants.[7,15–17,27] The present investigation represents the largest MR natural history study to date, with 160 participants enrolled and 79 participants completing 48 month follow-up visits. Having a large cohort is powerful because it has allowed evaluation of disease progression across a wide range of ages and functional levels. In addition, our large cohort reveals the high level of interindividual disease variability. For example, boys 7 to 12 years of age, who are commonly targeted for inclusion in clinical trials, have VL FF values ranging from <0.05 to >0.75. This wide spectrum of disease severity highlights the challenges associated with age-based trial inclusion criteria. Using MR biomarker-based inclusion criteria alone or in conjunction with function-based criteria may help homogenize clinical trial cohorts.

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Table 2 Predicting loss of ambulation by MR biomarker

	ROC analysis at 12 mo		Odds ratio prediction at 12 mo		
Predictor	**C statistic (SE)**	**Threshold (SE)**	**Odds ratio**	***p* Value**	**1 SD**
MRS FF					
VL	0.88 (0.02)	0.39 (0.05)	10.8	<0.0001[a]	0.20
SOL	0.86 (0.03)	0.14 (0.02)	3.9	<0.0001[a]	0.12
MRI T_2					
VL	0.91 (0.02)	59.6 (2.1)	4.4	<0.0001[a]	11.8 ms
BFLH	0.87 (0.03)	68.0 (2.1)	3.8	<0.0001[a]	13.6 ms
GRA	0.79 (0.04)	40.8 (1.9)	1.0	0.767	6.5 ms
SOL	0.88 (0.03)	47.0 (1.2)	1.2	0.084	6.8 ms
MG	0.87 (0.03)	46.7 (1.9)	1.4	0.004[a]	8.1 ms
PER	0.86 (0.03)	45.5 (2.3)	1.4	0.007[a]	8.1 ms
TA	0.82 (0.04)	40.1 (0.7)	1.0	0.786	5.2 ms
TP	0.81 (0.03)	37.9 (1.0)	0.9	0.015[a]	3.0 ms

Abbreviations: BFLH = biceps femoris long head; FF = fat fraction; GRA = gracilis; MG = medial gastrocnemius; MR = magnetic resonance; MRS = MR spectroscopy; PER = peroneal group; ROC = receiver operating characteristic curve; SE = standard error; SOL = soleus; TA = tibialis anterior; TP = tibialis posterior; VL = vastus lateralis.
All MR biomarkers were significantly predictive of loss of ambulation over 12 and 24 months, with baseline VL FF, VL T_2, BFLH T_2, and SOL T_2 having the largest C statistics (areas under the curve). Thresholds represent MR biomarker values that have the highest sum of sensitivity and specificity for predicting loss of ambulation over the given time period. The SE was calculated for each C statistic and threshold with the bootstrap. A discrete time hazard model was used to determine the odds of loss of ambulation over the following 12 months given a 1-SD change in an MR biomarker. Increases in VL FF and VL MRI T_2 were the strongest predictors of loss of ambulation.
[a] The odds ratio was statistically significant.

The long-term, longitudinal trajectories of MRS FF and MRI T_2 allow a better understanding of the progression of qMR biomarkers from the different lower extremity muscles over time and in relation to one another. BFLH and VL MR biomarkers increase most quickly over time, whereas PER, MG, and SOL biomarkers all increase at similar intermediate rates. GRA, TA, and TP biomarkers progress most slowly. In selecting a muscle to investigate as a clinical trial endpoint, both the amount of muscle available to target therapeutically and the expected progression of the biomarker in the study population over the trial duration should be considered in order to maximize the detection of treatment effects. MR biomarker trajectory analysis reveals that the expected changes over time are nonlinear. One study found age-related differences in disease progression over 12 months, noting that individuals <7 years old had an annual increase of 3.2% in thigh FF, while those >7 years of age had annual increases of 9.1%.[7] This study showed that annual changes in FF and MRI T_2 are largest in individuals with baseline FF levels between 0.10 and 0.50. The annual changes are smaller in early disease progression, with low FF levels, and in advanced disease progression, when the muscle is largely replaced by fat.

In addition to their sensitivity to change over time, it is important that MR biomarkers for DMD are related to current and future functional ability. A strong cross-sectional correlative relationship has previously been demonstrated in the ImagingDMD cohort and in other cohorts,[19,28,29] but the longitudinal relationship between qMR biomarkers and clinical function has not been explored comprehensively. In study planning, it is critical to understand the natural history of MR biomarkers, the functional ability of the target cohort, and the relationship between these variables. The natural history data presented here demonstrate that MR biomarkers and ambulatory function are strongly related over time. The longitudinal relationships also shed light on the muscular changes underlying decline in and loss of function in DMD. Our data suggest that individuals in whom VL FF progresses rapidly relative to their peers also experience rapid functional progression, indicating a global link between the rate of progression of MR biomarkers and the rate of functional progression.

The current work shows that MR biomarkers can predict functional decline over the following year, which has implications for both care and clinical trial planning. Individuals with a low VL FF (<0.2) are likely to experience stability or improvement in functional performance over the coming 12 months, while individuals with a VL FF between 0.2 and 0.4 are most likely to experience declines in, but not loss of, walking and running performance/ability over the following 12 months. Likewise, individuals who had small increases in VL FF over 12 months (≤0.02) were the most likely to remain functionally stable, indicating that stability in an MR biomarker is associated with stability in ambulatory function.

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We used several different analyses to demonstrate the link between MR biomarker progression and loss of functional ability, particularly loss of ambulation. In this cohort, at a VL FF greater than ≈0.4, 25% of individuals lost ambulation over the following 12 months, and nearly 50% lost ambulation over the following 24 months, indicating that this is a population who should be encouraged to prepare for the transition to full-time wheelchair use. Large changes (>0.15) in VL FF over 12 months were associated with a higher percentage of participants losing functional abilities, and an increase of 0.20 in VL FF was associated with a >10 times increase in the odds of loss of ambulation. Across muscles, the quickly progressing VL and BFLH muscle biomarkers were the strongest predictors of loss of functional ability. SOL MR biomarkers were also good predictors of loss of ambulation and loss of stair climbing, but in agreement with conclusions from another observational study SOL biomarkers were not strongly related to loss of supine to stand, likely because of the limited role of the SOL in this functional movement.[27]

There was a range of VL and BFLH biomarker values over which loss of ambulation occurred. Some of this variability may be accounted for by differential involvement of other key muscles of ambulation such as the gluteal and calf muscles. In addition, we speculate that individuals with slower disease progression may have more time to develop compensatory movement strategies to maintain function and display loss of abilities at higher levels of overall pathology.

The extensive characterization of MR biomarkers longitudinally and their predictive relationship to clinical function presented here strengthen the case for the use of lower extremity MR biomarkers of disease progression in clinical trials of therapeutics for DMD. The design of clinical trials, including the choice of appropriate endpoints, is challenging in DMD because of the disease heterogeneity, the small cohort sizes, and the motivation dependence of clinical outcome measures. MR biomarkers can be used either as secondary endpoints to bolster functional outcome findings or as surrogate endpoints for clinical outcomes to conduct shorter or smaller trials with sufficient power to detect proof of drug efficacy or inefficacy. This study demonstrates the predictive ability of MR biomarkers, implying that a therapeutic agent that slows the increase in VL FF is also likely to delay declines in ambulatory function and loss of ambulation, an important disease milestone in DMD.

There are limitations and points of consideration to acknowledge for this large natural history study. First, most analyses of lower extremity MR biomarkers are for ambulatory individuals. Although many individuals in this study lost ambulation and MR biomarkers predicted loss of ambulation, there was not a large enough cohort to comprehensively assess the sensitivity and progression of MR biomarkers after loss of ambulation. Recent small studies have explored MR biomarkers of the upper extremity, particularly in nonambulatory individuals.[30–32] Upper extremity MR biomarker data collection is now ongoing in the ImagingDMD cohort, and these data, in conjunction with data from other studies, will be useful to help determine the most appropriate biomarkers for nonambulatory individuals. Another consideration for this study is that the data are natural history data rather than data from a placebo-controlled trial. Thus, differences in clinical care and medication add potential variability to the data. Although the findings presented here and in prior studies provide strong evidence for the ability of MR biomarkers to serve as secondary endpoints or surrogate outcomes for clinical trials, the thoughtful and standardized inclusion of MR biomarkers in trials of therapeutics demonstrating clinical efficacy can provide definitive evidence.

With numerous clinical trials underway to evaluate a variety of therapeutic approaches to treat DMD, clinical trial design and the choice of endpoints are areas of intense interest and investigation. The relationship to current and future function presented here supports the use of MR biomarkers as endpoints in trials, and the longitudinal lower extremity muscle MR biomarker data will assist in clinical trial planning. The goals of using MR biomarker endpoints in trials are to noninvasively evaluate muscle health, to limit the need for muscle biopsies when possible, to bolster findings from other efficacy endpoints, and to reduce trial length or size by using MR measures as surrogate outcomes. Most important, the hope is that inclusion of muscle MR biomarkers will accelerate development and approval of disease-modifying therapeutics for DMD.

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Appendix Authors

Name	Location	Role	Contribution
Alison M. Barnard, DPT, PhD	University of Florida, Gainesville	Author	Data acquisition, data analysis and interpretation, drafted and edited the manuscript for intellectual content, approved final version of submitted manuscript
Rebecca J. Willcocks, PhD	University of Florida, Gainesville	Author	Designed and conceptualized study, data acquisition, data analysis and interpretation, drafted and edited the manuscript for intellectual content, approved final version of submitted manuscript
Michael J. Daniels, ScD	University of Florida, Gainesville	Author	Designed and conceptualized study, data analysis and interpretation, statistical analysis, edited the manuscript for intellectual content, approved final version of submitted manuscript
Saptarshi Chakraborty, PhD	University of Florida, Gainesville	Author	Data analysis and interpretation, statistical analysis, approved final version of submitted manuscript
William T. Triplett, BS	University of Florida, Gainesville	Author	Designed and conceptualized study, data acquisition, data analysis and interpretation, approved final version of submitted manuscript
Sean C. Forbes, PhD	University of Florida, Gainesville	Author	Designed and conceptualized study, data acquisition, data analysis and interpretation, approved final version of submitted manuscript
Donovan J. Lott, PT, PhD	University of Florida, Gainesville	Author	Designed and conceptualized study, data acquisition, data analysis and interpretation, approved final version of submitted manuscript
Claudia R. Senesac, PT, PhD	University of Florida, Gainesville	Author	Designed and conceptualized study, data acquisition, edited the manuscript for intellectual content, approved final version of submitted manuscript
Ann T. Harrington, DPT, PhD	Children's Hospital of Philadelphia, PA	Author	Designed and conceptualized study, data acquisition, data analysis, approved final version of submitted manuscript
Gihan Tennekoon, MBBS	Children's Hospital of Philadelphia, PA	Author	Data acquisition, data interpretation, approved final version of submitted manuscript
Harneet Arora, PT, PhD	University of Florida, Gainesville	Author	Data acquisition, data analysis and interpretation, approved final version of submitted manuscript
Dah-Jyuu Wang, PhD	Children's Hospital of Philadelphia, PA	Author	Designed and conceptualized study, data acquisition, data analysis and interpretation, approved final version of submitted manuscript

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Appendix *(continued)*

Name	Location	Role	Contribution
Erika L. Finanger, MD, PhD	Oregon Health & Science University, Portland	Author	Designed and conceptualized study, data acquisition, approved final version of submitted manuscript
William D. Rooney, PhD	Oregon Health & Science University, Portland	Author	Designed and conceptualized study, data acquisition, data analysis and interpretation, approved final version of submitted manuscript
H. Lee Sweeney, PhD	University of Florida, Gainesville	Author	Designed and conceptualized study, approved final version of submitted manuscript
Glenn A. Walter, PhD	University of Florida, Gainesville	Author	Designed and conceptualized study, data acquisition, data analysis and interpretation, edited the manuscript for intellectual content, approved final version of submitted manuscript
Krista Vandenborne, PT, PhD	University of Florida, Gainesville	Author	Designed and conceptualized study, data acquisition, data analysis and interpretation, edited the manuscript for intellectual content, approved final version of submitted manuscript

References

1. Hoffman EP, Brown RH Jr, Kunkel LM.Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 1987;51:919–928.
2. Petrof BJ, Shrager JB, Stedman HH, Kelly AM, Sweeney HL. Dystrophin protects the sarcolemma from stresses developed during muscle contraction. Proc Natl Acad Sci USA 1993;90:3710–3714.
3. Peverelli L, Testolin S, Villa L, et al. Histologic muscular history in steroid-treated and untreated patients with Duchenne dystrophy. Neurology 2015;85:1886–1893.
4. Duchenne Muscular Dystrophy and Related Dystrophinopathies: Developing Drugs for Treatment—Guidance for Industry. Silver Spring; US Food and Drug Administration; 2018.
5. Fleming TR. Surrogate endpoints and FDA's accelerated approval process. Health Aff 2005;24:67–78.
6. Carlier PG, Marty B, Scheidegger O, et al. Skeletal muscle quantitative nuclear magnetic resonance imaging and spectroscopy as an outcome measure for clinical trials. J Neuromuscul Dis 2016;3:1–28.
7. Bonati U, Hafner P, Schädelin S, et al. Quantitative muscle MRI: a powerful surrogate outcome measure in Duchenne muscular dystrophy. Neuromuscul Disord 2015;25:679–685.
8. Maillard SM, Jones R, Owens C, et al. Quantitative assessment of MRI T2 relaxation time of thigh muscles in juvenile dermatomyositis. Rheumatology (Oxford) 2004;43:603–608.
9. Mathur S, Vohra RS, Germain SA, et al. Changes in muscle T2 and tissue damage after downhill running in mdx mice. Muscle Nerve 2011;43:878–886.
10. Kim HK, Serai S, Lindquist D, et al. Quantitative skeletal muscle MRI: part 2, MR spectroscopy and T2 relaxation time mapping–comparison between boys with Duchenne muscular dystrophy and healthy boys. Am J Roentgenol 2015;205:W216–W223.
11. Arpan I, Forbes SC, Lott DJ, et al. T2 mapping provides multiple approaches for the characterization of muscle involvement in neuromuscular diseases: a cross-sectional study of lower leg muscles in 5–15-year-old boys with Duchenne muscular dystrophy. NMR Biomed 2013;26:320–328.
12. 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.
13. Burakiewicz J, Sinclair CDJ, Fischer D, Walter GA, Kan HE, Hollingsworth KG. Quantifying fat replacement of muscle by quantitative MRI in muscular dystrophy. J Neurol 2017;264:2053–2067.
14. Forbes SC, Walter GA, Rooney WD, et al. Skeletal muscles of ambulant children with Duchenne muscular dystrophy: validation of multicenter study of evaluation with MR imaging and MR spectroscopy. Radiology 2013;269:198–207.
15. Hooijmans MT, Doorenweerd N, Baligand C, et al. Spatially localized phosphorous metabolism of skeletal muscle in Duchenne muscular dystrophy patients: 24–month follow-up. PLoS One 2017;12:e0182086.
16. 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.
17. Mankodi A, Bishop CA, Auh S, Newbould RD, Fischbeck KH, Janiczek RL. Quantifying disease activity in fatty-infiltrated skeletal muscle by IDEAL-CPMG in Duchenne muscular dystrophy. Neuromuscul Disord 2016;26:650–658.
18. Willcocks RJ, Rooney WD, Triplett WT, et al. Multicenter prospective longitudinal study of magnetic resonance biomarkers in a large Duchenne muscular dystrophy cohort. Ann Neurol 2016;79:535–547.
19. Barnard AM, Willcocks RJ, Finanger EL, et al. Skeletal muscle magnetic resonance biomarkers correlate with function and sentinel events in Duchenne muscular dystrophy. PLoS One 2018;13:e0194283.
20. Kim HK, Laor T, Horn PS, et al. T2 mapping in Duchenne muscular dystrophy: distribution of disease activity and correlation with clinical assessments 1. Radiology 2010;255:899–908.
21. Arpan I, Willcocks RJ, Forbes SC, et al. Examination of effects of corticosteroids on skeletal muscles of boys with DMD using MRI and MRS. Neurology 2014;83:974–980.
22. Triplett WT, Baligand C, Forbes SC, et al. Chemical shift-based MRI to measure fat fractions in dystrophic skeletal muscle: MR measurements of fat fraction in dystrophic muscles. Magn Reson Med 2014;72:8–19.
23. Mendell JR, Goemans N, Lowes LP, et al. Longitudinal effect of eteplirsen versus historical control on ambulation in Duchenne muscular dystrophy: eteplirsen in DMD. Ann Neurol 2016;79:257–271.
24. 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;48:343–356.
25. Arora H, Willcocks RJ, Lott DJ, et al. Longitudinal timed function tests in Duchenne muscular dystrophy: ImagingDMD cohort natural history. Muscle Nerve 2018;58:631–638.
26. 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;48:357–368.
27. Godi C, Ambrosi A, Nicastro F, et al. Longitudinal MRI quantification of muscle degeneration in Duchenne muscular dystrophy. Ann Clin Transl Neurol 2016;3:607–622.
28. Gaeta M, Messina S, Mileto A, et al. Muscle fat-fraction and mapping in Duchenne muscular dystrophy: evaluation of disease distribution and correlation with clinical assessments: preliminary experience. Skeletal Radiol 2012;41:955–961.
29. Fischmann A, Hafner P, Gloor M, et al. Quantitative MRI and loss of free ambulation in Duchenne muscular dystrophy. J Neurol 2013;260:969–974.
30. Hogrel JY, Wary C, Moraux A, et al. Longitudinal functional and NMR assessment of upper limbs in Duchenne muscular dystrophy. Neurology 2016;86:1022–1030.
31. Willcocks RJ, Triplett WT, Forbes SC, et al. Magnetic resonance imaging of the proximal upper extremity musculature in boys with Duchenne muscular dystrophy. J Neurol 2017;264:64–71.
32. Wary C, Azzabou N, Giraudeau C, et al. Quantitative NMRI and NMRS identify augmented disease progression after loss of ambulation in forearms of boys with Duchenne muscular dystrophy. NMR Biomed 2015;28:1150–1162.

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