RESEARCH ARTICLE

LGMDD1 natural history and phenotypic spectrum: Implications for clinical trials

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ABSTRACT

Objective: To delineate the full phenotypic spectrum and characterize the natural history of limb girdle muscular dystrophy type D1 (LGMDD1). **Methods**: We extracted age at clinical events of interest contributing to LGMDD1 disease burden via a systematic literature and chart review. Manual muscle testing and quantitative dynamometry data were used to estimate annualized rates of change. We also conducted a cross-sectional observational study using previously validated patient-reported outcome assessments (ACTIVLIM, PROMIS-57) and a new LGMDD1 questionnaire. Some individuals underwent repeat ACTIVLIM and LGMDD1 questionnaire assessments at 1.5 and 2.5 years. **Results**: A total of 122 LGMDD1 patients were included from 14 different countries. We identified two new variants (p.E54K, p.V99A). In vitro assays and segregation support their pathogenicity. The mean onset age was 29.7 years. Genotype appears to impact onset age, weakness pattern, and median time to loss of ambulation (34 years). Dysphagia was the most frequent abnormality (51.4%). Deltoids, biceps, grip, iliopsoas, and hamstrings strength decreased by (0.5-1 lb/year). Cross-sectional ACTIVLIM and LGMDD1 questionnaire scores correlated with years from disease onset. Longitudinally, only the LGMDD1 questionnaire detected significant progression at both 1.5 and 2.5 years. Treatment trials would require 62 (1.5 years) or 30 (2.5 years) patients to detect a 70% reduction in the progression of the LGMDD1 questionnaire. **Interpretation**: This study is the largest description of LGMDD1 patients to date and highlights potential genotype-dependent differences that need to be verified prospectively. Future clinical trials will need to account for variability in these key phenotypic features when selecting outcome measures and enrolling patients.

Introduction

Limb-girdle muscular dystrophy D1 (LGMDD1) is an ultra-rare dominantly inherited neuromuscular disorder due to mutations in the HSP-40 co-chaperone, DNAJB6. Disease-causing mutations are primarily missense and are clustered within either the G/F or J domains of DNAJB6 (Fig. 1A). Although only recently genetically characterized in 2012, it is now one of the most commonly identified dominant LGMDs.[1] Disease onset ranges from early childhood to late adulthood. Individuals may present with a typical limb-girdle pattern of weakness, whereas others may have distal predominance. Many develop dysphagia, whereas respiratory and cardiac involvement is unclear. Promising therapeutic strategies are starting to emerge, accelerating the need for clinical trial readiness.[2,3] The preparatory steps for prospective natural history studies in other LGMDs, such as the clinical outcome study for dysferlinopathy, or sarcoglycanopathy genotype–phenotype studies, can inform our approach to LGMDD1.[4–6] To guide prospective natural history studies, and eventually, clinical trials, we sought to describe the phenotypic spectrum of LGMDD1 and identify factors that impact disease trajectory through a combination of systematic literature review, retrospective chart review, and a

cross-sectional observational study with a subset of longitudinal assessments (Fig. 1B).

Subjects/Materials and Methods

Standard protocol approvals, registrations, and consents

The work completed in this study, specifically, the chart review and questionnaire assessments, was approved by the Institutional Review Board of Washington University

St. Louis (IRB# 201903027). Written informed consent was obtained according to the Declaration of Helsinki from all participants or a parent/legal guardian. All clinical and genetic data were anonymized and entered into a secured database.

Systematic literature review

Starting in May 2019, a systematic literature review was initiated in PubMed to identify all published data on the timing for clinical events of interest that contribute to
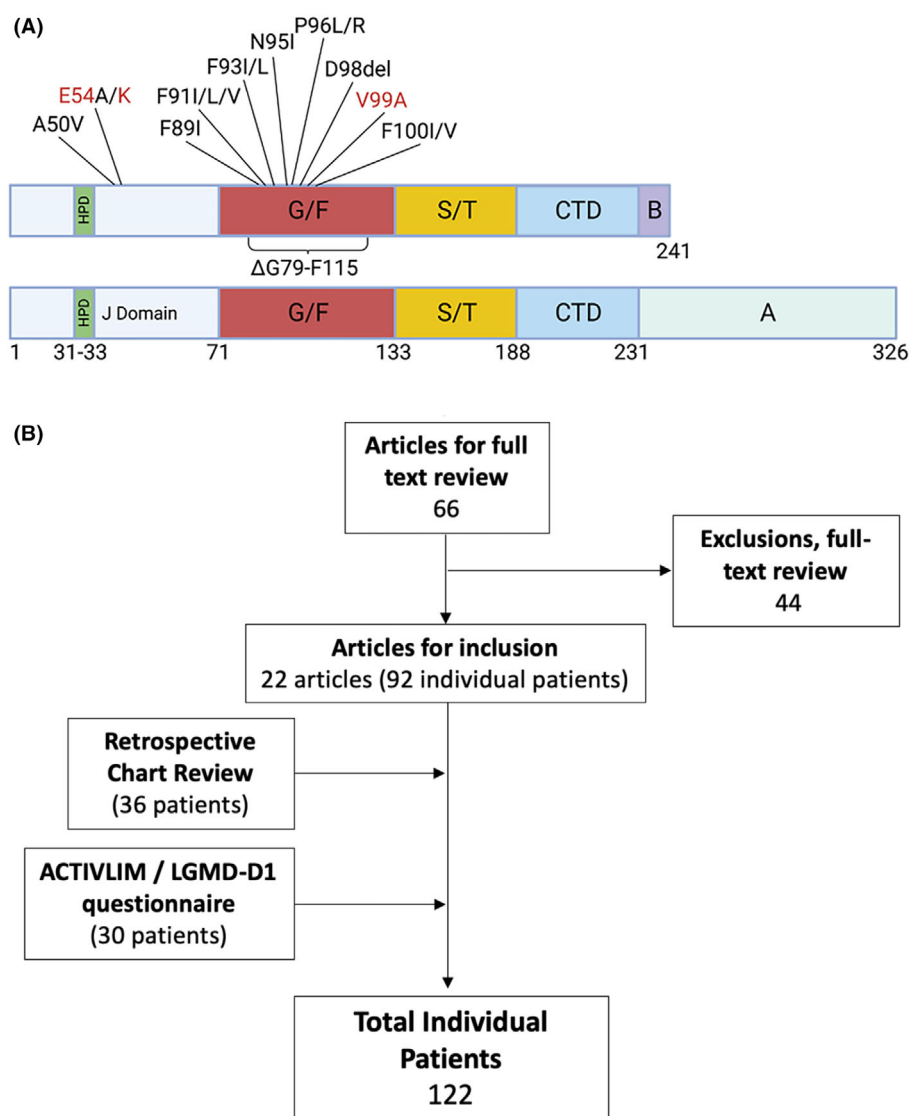
Figure 1. Summary of Study. (A) Mutation map of DNAJB6 A and B isoforms. Newly identified variants are noted in red. Key domains of the DNAJB6 protein are shown, with approximate amino acid numbers labeled below. HPD motif (contains histidine, proline, and aspartic acid). The G/F domain is rich in glycine and phenylalanine. S/T-rich domain. C terminal domain (CTD). Amino acids unique to DNAJB6b are shown by "B" and those unique to DNAJB6a are shown by "A". (B) PRISMA diagram illustrating the selection of eligible studies and source of clinical information for all patients included.

disease burden in LGMDD1 (supplemental 1). Clinical events of interest were defined by neuromuscular clinicians with expertise in LGMDD1 (ARF, CW). The literature review followed the 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[7] The search strategy included terms of interest for the study related to the Population, Intervention, Comparison, Outcome, Type of Study (PICOT) criteria (supplemental 1). Eligible studies contained individual-level data reporting the age at the occurrence of clinical events of interest. Articles were categorized as "included," "excluded," or "unsure" by two reviewers (SR, AF). Discrepancies were categorized as "unsure." The full text of articles meeting the inclusion criteria and those categorized as 'unsure' were then confirmed to meet inclusion criteria or excluded. The references of included studies were screened for relevant articles not captured by the search strategy. Values reported descriptively were excluded (i.e., "onset in 20 s" or "normal CK"). Data were gathered up until June 18, 2022.

Retrospective chart review

We reviewed genetically confirmed LGMDD1 patient charts from the Washington University Neuromuscular Clinic and patients from other institutions that enrolled in our Muscle Disease Phenotyping study. We extracted the same clinical event data as done for the systematic literature review. Manual muscle testing (MMT) and quantitative muscle testing data were also collected. Medical research council (MRC) scores were converted to a 11-point scale as previously described.[4] Right and left sides were averaged for each muscle. A combined score was generated for the proximal arm (deltoid, biceps, triceps), proximal leg (iliopsoas, quadriceps, hamstrings), distal arm (wrist extension, first dorsal interosseous, abductor pollicis brevis), and distal leg (tibialis anterior, gastrocnemius, extensor hallucis longus).

Questionnaires

Patients completed three different patient-reported outcome (PRO) questionnaires: Activity limitations (ACTIV-LIM), NIH Patient-Reported Outcomes Measurement Information System (PROMIS)-57, and a customized LGMDD1 questionnaire (supplemental 2). A subset of patients underwent repeated assessments with the ACTIV-LIM and LGMDD1 questionnaire at 1.5 and 2.5 years. The ACTIVLIM includes 22 questions on daily activities to be rated as impossible (0), difficult (1), or easy (2). It has been validated in patients with neuromuscular diseases.[8–10] The PROMIS-57 includes 57 questions covering seven domains: physical function, social functioning, etc,

and has been validated in several LGMD studies.[11–13] The total raw PROMIS score for each participant is converted to standardized T-score with a mean = 50 (US general population average) and standard deviation (SD) of 10. The customized LGMDD1 questionnaire was collaboratively developed by neuromuscular physicians with LGMDD1 patient input. It aimed to both retrospectively and prospectively catalog the age at onset for key disease events (i.e., running, climbing stairs), as well as the age at significant difficulty and loss of ability. Age at first use of various assistive equipment was also assessed (i.e., cane, wheelchair). For prospective LGMDD1 questionnaire assessments, participants updated their prior questionnaire, noting any changes in activity or assistive device use that occurred in the prior year. Similar to the ACTIV-LIM, participants answered activity questions as if assistive equipment and human aids were unavailable. Points are given for each activity participants had any difficulty, significant difficulty, or loss of ability, as well as for use of assistive equipment. Scores increase with the progression of disability, up to a maximum of 42 points.

Data synthesis and analysis

Individuals with data from multiple sources were counted as only one patient. For conflicting values between different sources (i.e., age of onset), the earliest reported age was used. Loss of ambulation (LOA) was defined as wheelchair-dependent or non-ambulant. If the ambulatory status was not mentioned at an assessment, it was assumed it had not changed. For other clinical events of interest, it was assumed they were not assessed unless specifically noted. Cardiac abnormalities included: palpitations, syncope, congestive heart failure, cardiomyopathy, conduction abnormalities on electrocardiogram (ECG), and structural abnormalities or ejection fraction (EF) of <50% on echocardiogram. Respiratory abnormalities included dyspnea, orthopnea, need for ventilatory support, or FVC <80%. For dysphagia, abnormalities included choking, nasal regurgitation, food getting stuck, difficulty swallowing, or abnormalities noted on formal swallow evaluations. For individuals with multiple CK assessments, the highest level was recorded, whereas for multiple FVC assessments, the lowest was recorded.

Data were analyzed using two-sided tests with a significance of $p < 0.05$. Group comparisons were evaluated using Fisher exact tests for categorical variables and T-test or one-way ANOVA for continuous variables. The Tukey method was applied for multiple comparisons. Kaplan–Meier curves were used to estimate the median years from onset to the first occurrence of disease events and log-rank tests were used to determine significance. Cross-sectional annual disease progression was estimated using

linear regression. Longitudinal annualized disease progression was estimated using the linear mixed-effect modeling (LMEM) restricted-maximum likelihood method with random effects on intercept and slope. Differences between longitudinal PRO assessments were evaluated using a mixed effects model with repeated measures, restricted maximum likelihood, and Šídák's multiple-comparison test. Longitudinal PRO assessment change from baseline score was similarly evaluated using a mixed effects model but with Dunnett's multiple-comparison test. Correlations were determined by Pearson correlation coefficient. Sample size estimates for mean differences used typical assumptions (independent-sample T-test, 80% power, alpha = 0.05, two-sided). All analyses were conducted using graphpad prism v9 except LMEM and power calculations (SPSS v28).

Plasmid constructs

Human DNAJB6b constructs were cloned using site-directed mutagenesis, digested with HindIII/XhoI, and ligated into pcDNA3.1 containing GFP. Mutations were generated with the QuikChange Mutagenesis Kit (Agilent Technologies; 200517). The creation of human TDP-43 fused to mCherry was described previously (33).

TDP-43 aggregation assay

The TDP-43 aggregation assay has been described previously.[14] U2OS cells were co-transfected with wild-type or mutant GFP-DNAJB6b constructs and mCherry-TDP-43. 24 hours later, cells were heat-shocked (42°C) for 1 h, then fixed. The proportion of cells containing mCherry-TDP-43 aggregates was determined by fluorescence microscopy. For each condition at least 300 cells were counted by a blinded assessor, and the experiment was repeated three times.

Results

Cohort description

From the 22 total studies that met inclusion criteria, 92 patients were identified across 14 different countries. Retrospective chart data were reviewed for 36 patients, and 30 patients completed questionnaires, 23 of which underwent repeat assessments for up to 2.5 years (Fig. 1B). In total 122 distinct patients with 17 different dominantly inherited DNAJB6 mutations were included in this study (Fig. 1A). Two new variants (c.160G > A p.E54K; c.296 T > C p.V99A) were identified during the course of the study (Fig. 1A).

General characteristics of the cohort are presented in Table 1. The mean age of onset was 29.7 years with a wide range (4–69). Most individuals were male (60%) and had proximal predominant weakness (73%). Certain mutations were associated with either proximal or distal weakness patterns (Fig. 2A). All patients with F89, F91, and F93 mutations (*n* = 80) had proximal weakness, whereas 83.3% of patients with P96 mutations had distal weakness. Genotype, but not sex or weakness pattern, significantly impacted the age of onset (Fig. 2B–D). Focusing on the most prevalent mutations, F89I, F91I/L, and P96R/L all had a significantly earlier disease onset than F93I/L (Fig. 2D). Due to large differences in age of onset, all clinical event data were normalized by calculating years from disease onset. Kaplan–Meier survival analysis found a median of 34 years from disease onset to LOA (Fig. 2E). Genotype, but not sex, weakness pattern, or age of onset, appears to impact time to LOA (Fig. 2E–H). P96L/R (20y) and F91I/L (29y) both decline significantly faster than F89I (44y) and F93I/L (35y) (Fig. 2H).

Of the assessed clinical events of interest, dysphagia was the most common, occurring in 51.4% of patients, with a median disease duration of 30 years to onset (Fig. 3A). Respiratory (38.3%) and cardiac (29.7%) abnormalities were less frequent and occurred later with median disease durations of 42 and 46 years respectively (Fig. 3B,C). Genotype, but not sex or weakness pattern significantly impacted disease duration to both cardiac and respiratory abnormalities (Fig. 3A–I). FVC and CK did not correlate with years from disease onset. (Fig. 3J,K).

We collected MMT values from 23 patient charts. Rates of strength change were estimated using LMEM analysis. Fit lines illustrate distal muscle groups progress faster in patients with distal predominant weakness. However, proximal muscle groups appear to have a more similar rate of change regardless of weakness pattern (Fig. 4A–D).

Quantitative dynamometry data were available for eight patients with proximal predominant weakness (Fig. 4E,F). Hamstrings, iliopsoas, and deltoids (proximal muscles)

Table 1. Key characteristics of LGMDD1 patients.

Overview of data	
Characteristic	Value
Total individuals	122
Number of different mutations	17
Mean age of onset, *n* (SD)	29.7, 120 (15.3)
Female, *n* (%)	49 (40%)
Male, *n* (%)	73 (60%)
Proximal predominant weakness, *n* (%)	88 (73%)
Distal predominant weakness, *n* (%)	33 (27%)
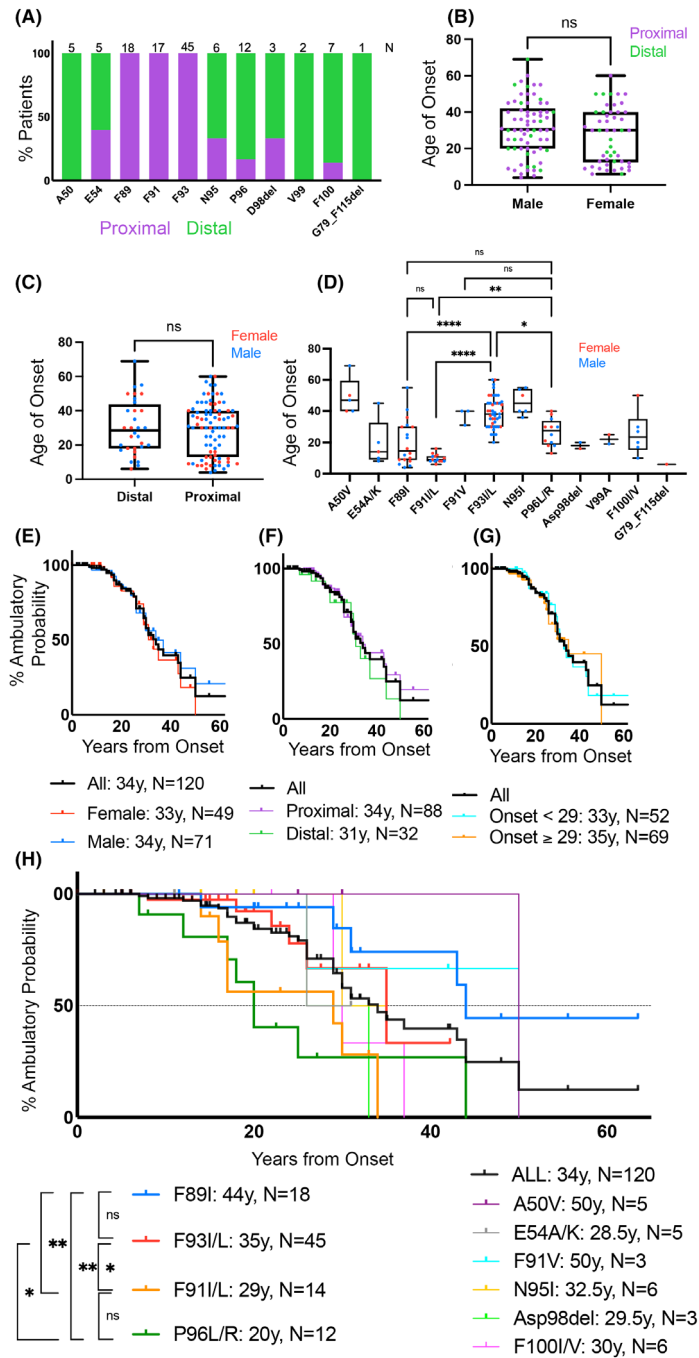
Figure 2. Weakness pattern, age of onset, time to loss of ambulation. (A) Bar graph demonstrating % of patients with either distal or proximal weakness for each genotype. There is a significant interaction between genotype and weakness pattern ($x^2 = 92.142$, $P < 0.001$). (B–D). Boxplots of age of disease onset comparing male/female (B), weakness pattern (C), and genotype (D). Age of onset ranged from 4 to 69 years and was significantly different among genotypes (one-way ANOVA $P < 0.0001$). There were also significant differences in age of onset between some of the most commonly encountered mutations; specifically, F93I/L had a later age of onset compared with F89I, F91I/L, and P96R/L. P96R/L had a later disease onset compared with F91I/L (one-way ANOVA with post-hoc Tukey test, $*P < 0.05$, $**P < 0.01$, $****P < 0.0001$). Dots represent individual patients, the middle horizontal line represents the median age of onset, the whiskers represent the range, and the upper and lower borders of the box represent the first (25%) and third quartiles (75%). (E-H): Kaplan–Meier analysis of years from disease onset to LOA. Values listed next to each group: years to LOA, number of subjects (N). (E) There were no significant differences between males and females (hazard ratio [HR]: 1.27, 95% confidence interval [CI]: 0.66–2.42; $P = 0.46$), (F) proximal versus distal weakness (HR: 0.76, 95% CI: 0.37–1.53; $P = 0.40$), or (G) early versus late onset (HR: 0.95, 95% CI: 0.49–1.81; $P = 0.86$). (H) Genotype does significantly impact years to LOA (log-rank test (Mantel-cox) $x^2 = 23.96$, $P = 0.004$). Significant differences between the most commonly encountered mutations are noted.
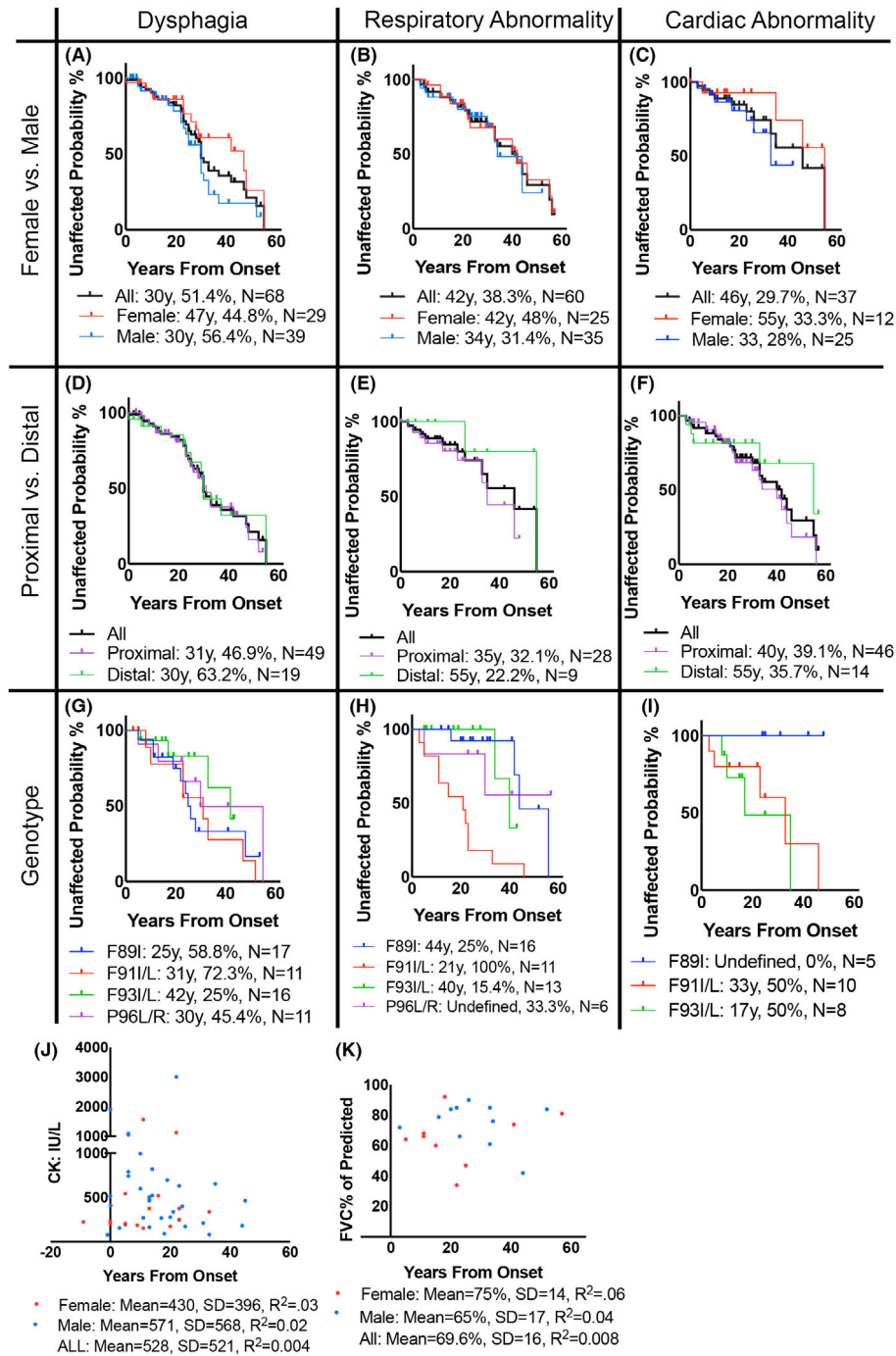
Figure 3. Kaplan–Meier analysis of years from disease onset to clinical events. Values listed next to each group: median years to event, frequency of abnormality, number of subjects assessed (N). (A) There was no significant difference between males vs. females for dysphagia (HR 0.57, 95% CI 0.29 = 1.09, $P = 0.07$), (B) respiratory abnormality (HR 0.91, 95%CI: 0.4–2.04, $P = 0.8$), or (C) cardiac abnormality (HR 1.99, 95% CI 0.61–6.51, $P = 0.19$). (D) There was no significant difference between individuals with proximal vs distal weakness for dysphagia (HR 0.86, 95% CI 0.44–1.68, $P = 0.64$), (E) respiratory abnormality (HR 0.58, 95% CI 0.25–1.4, $P = 0.26$), or (F) cardiac abnormality (HR 0.37, 95% CI 0.11–1.26, $P = 0.14$). (G). There was no significant difference between individuals with different mutations for dysphagia (Log-rank test (Mantel-cox) $?^2 = 2.418$, $P = 0.49$). (H). There were significant differences between genotypes for respiratory abnormality ($?^2 = 22.49$, $P = 0.0002$), and I. cardiac abnormality ($?^2 = 8.71$, $P = 0.034$). For G-I, only mutations with >5 individuals assessed were included in graphs. (J). CK values versus years from disease onset show no significant correlation. (K) FVC% versus years from disease onset shows no significant correlation.
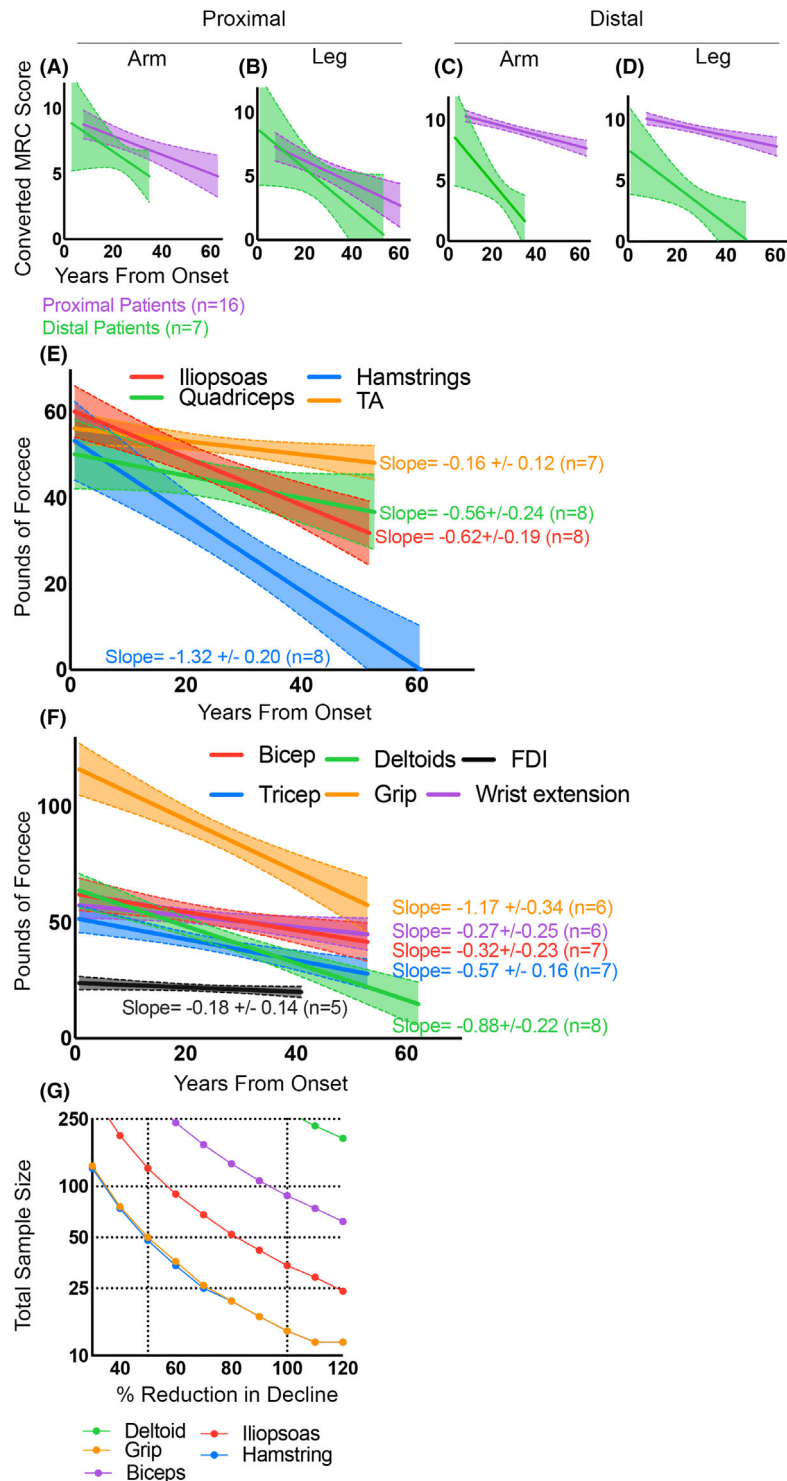
Figure 4. Longitudinal strength testing. (A-D) Manual muscle testing MRC scores converted to 11-point scale. Fit lines from LMEM modeling analysis are shown with 95% CI boundaries. The average of several muscles was taken to generate a combined score for proximal arm (A) proximal leg (B) distal arm (C) distal leg (D). (E) Quantitative strength testing of leg muscles, and arm muscles (F). Fit lines from LMEM analysis are shown with 95% CI boundaries. Slopes (annualized linear decline) and standard errors are listed for individual muscles. (G) Sample size estimates for the detection of reduced decline of quantitative dynamometry progression in a parallel group (1:1) 1-year interventional trial. MRC = medical research council; TA = tibialis anterior; FDI = first dorsal interosseous.

were some of the muscles that progress most rapidly (0.5–1 lb per year). Grip strength (distal muscles) also changed at slightly over 1 lb per year. Using average annualized progression estimates for the most affected muscles, we calculated preliminary sample sizes required to detect different treatment effects in a clinical trial. For example, in a 1-year placebo-controlled study (1:1 randomization), a total of 48 patients would be required to detect a 50% reduction in hamstring strength progression (80% power). Similarly, 50 patients would be needed for a study looking at grip strength, and 128 for iliopsoas (Fig. 4G).

Cross-sectional annualized disease progression based on ACTIVLIM patient measure scores, correlated with years from disease onset and showed an average progression of −0.11 points per year (patient measure, not raw score) (Fig. 5A). Longitudinal annualized disease progression as estimated by LMEM analysis was similar, at −0.16 points per year (Fig. 5B). However, there was prominent variability between individuals. There were no notable differences in rates of progression between genotypes (Fig. 5B).

For the PROMIS-57, cross-sectional but not longitudinal data were available. Only the physical function domain T score was significantly reduced in LGMDD1 patients compared with the general population ($T = 36.07$, SD = 11.13). Disease duration significantly correlated with T scores for the social roles, physical function, and fatigue domains (Fig. 5C). ACTIVLIM scores correlated with T scores for social roles ($r = 0.71$ $P = 1.67$ e-4) and physical function domains ($r = 0.84$, $P = 4.07$ e-7).

In parallel with other questionnaires, participants also completed a customized LGMDD1 questionnaire collaboratively developed by neuromuscle specialists and LGMDD1 patients. Patients entered their age at which assistive equipment was first used as well as when functional activities first became difficult, significantly difficult, or impossible. This allowed for retrospective characterization of key disease events at their first assessment (Fig. 5D). For repeat, prospective assessments, individuals updated their prior questionnaire as new disease events occurred. Similar to the ACTIVLIM, participants answered questions as if technical and human help were unavailable. Scores correlated with ACTIVLIM ($r = −0.952$, $P < 0.0001$) and disease duration ($r = 0.66$, $P < 0.0001$). Cross-sectional annualized change (+0.66 points/year, SE 0.05) was similar to longitudinal annualized change (+0.73 points/year, SE 0.09) (Fig. 5E,F) Although there was still interindividual variability, the LGMDD1 questionnaire lacked spurious improvements in scores and was able to capture functional progression in this cohort of patients (Fig. 5F–H). We used the average change from baseline score at 1.5 and 2.5 years to estimate preliminary sample sizes required to detect different

treatment effects in a placebo-controlled clinical trial (1:1 randomization, 80% power) (Fig. 5I). To detect a 100% reduction in worsening of LGMDD1 questionnaire scores (i.e., no progression of disease), 32 patients would be needed for a 1.5-year trial, and 16 total patients for a 2.5-year trial. There were no significant differences between patients with F89I or F93L mutations in their average change in LGMDD1 questionnaire scores at both 1.5 and 2.5 years (Fig. 5J).

Case descriptions

During the course of the study, we identified two previously unreported variants. Here we describe their cases and provide in vitro data asserting each variant's pathogenicity.

Family 1

Patient II:1 was a 47-year-old Caucasian male of Swedish ancestry who at age 7 was told he had an awkward gait. In high school, he was unable to jump vertically and ran without lifting his heels. Later his hands were involved causing difficulty with buttons, and at age 52, he developed dysphagia. His father had a similar gait pattern, and his daughter (III:2) also became affected (Fig. 6A). Examination at age 47 noted atrophy of intrinsic hand and foot muscles, distal forearms, and distal legs (both compartments). He could stand from sitting without difficulty, but could not stand on his toes or heels. There was no scapular winging. Strength assessment was notable for symmetric distal predominant weakness affecting legs more than arms (Table 2). CK was 474 IU/L. Nerve conduction studies were normal, and electromyography demonstrated myopathic units with fibrillations and positive sharp waves predominantly in distal musculature. A quadriceps biopsy was notable for rimmed vacuoles and eosinophilic aggregates (Fig. 6C). Electron microscopy demonstrated vacuoles containing electron-dense myeloid debris and tubulo-filamentous-like inclusions (images and grids were no longer available). He carried a clinical diagnosis of Welander's distal myopathy but never underwent genetic testing and died at age 59 due to melanoma.

Patient III:2 is the daughter of II:1. At age 15, she reported no symptoms but was examined by a neuromuscular specialist due to her father having a distal predominant vacuolar myopathy. She had wasting of her thenar eminence and extensor digitorum brevis bilaterally. Strength testing was normal except for distal upper extremity muscles (Table 2). Sensation and reflexes were normal. During high school and college, she played competitive sports (volleyball, basketball, and track). At 30, she noticed trouble with squats due to tight heel cords. At 35 she had dysphagia (solids and liquids), as well as
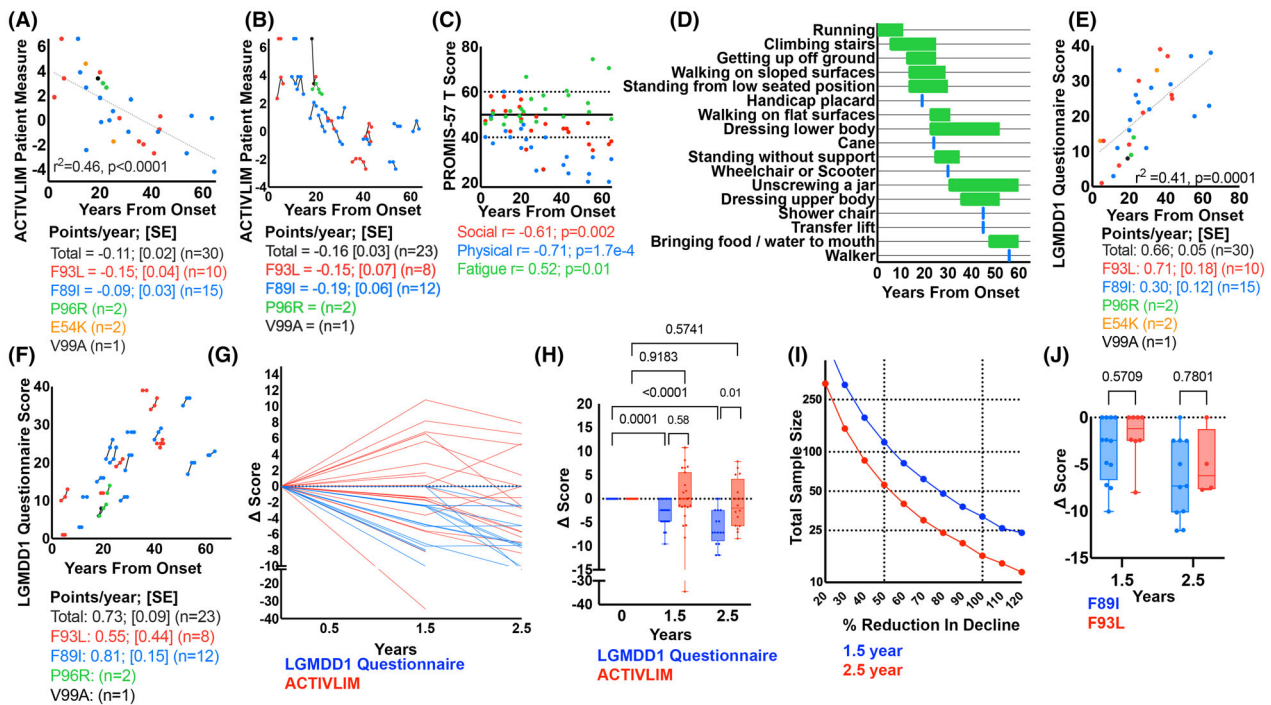
Figure 5. Patient-reported outcome assessments. (A) ACTIVLIM cross-sectional analysis. Slopes and their standard error from linear regression are noted for each genotype. The dotted line is the regression analysis for all data points. (B) Longitudinal analysis of ACTIVLIM. Longitudinal annualized disease progression was assessed via LMEM analysis with restricted maximum likelihood and random effects on slope and intercept. (C) PROMIS-57 scores versus years from onset. Scores from domains that correlated with years from onset are shown. Population T score mean is the solid line (50), and 1 standard deviation is dotted line (40, 60). (D) LGMDD1 questionnaire retrospective analysis. Kaplan–Meier curves were used to determine median disease duration to onset vs. loss of certain functional abilities as well as time to first use of various assistive equipment. Bars represent median years to onset (left side) and to loss of ability (right side). For assistive equipment, blue lines mark the median time to first use. (E) LGMDD1 questionnaire score vs. years from onset. (F) Longitudinal analysis of LGMDD1 questionnaire score. Longitudinal annualized disease progression was assessed via LMEM analysis with restricted maximum likelihood and random effects for slope and intercept. (G) Comparison of ACTIVLIM and LGMDD1 questionnaire scores. To facilitate comparison, scores from both questionnaires were converted to a 100-point scale, and LGMDD1 questionnaire scores were inverted such that scores decreased with progression. Change from baseline score was calculated for each patient. Repeat assessments occurred at 1.5 and 2.5 years. Lines represent individual participants. (H) LGMDD1 questionnaire scores were significantly different compared with ACTIVLIM at 2.5 years but not 1.5 years (mixed-effects model, repeated measures, restricted maximum likelihood, with Šídák's multiple-comparison test). Removal of a single outlier within the data set (identified via ROUT method, Q = 1%) followed by re-analysis, resulted in statistical significance at 1.5 years ($P = 0.004$). LGMDD1 questionnaire scores were significantly different compared with base-line at both 1.5 and 2.5 years, whereas ACTIVLIM scores were not (mixed-effect model, repeated measures, restricted maximum likelihood, with Dunnett's multiple-comparison test). (I) Sample size estimates for the detection of reduced decline of LGMDD1 questionnaire scores for a parallel group (1:1) 1.5- or 2.5-year interventional trial and were based on the average and standard deviation of delta score as determined in H. Sample size estimates for the ACTIVLIM were too high and are not shown. (J) Comparison of LGMDD1 questionnaire delta score for F89I and F93L geno-types. There were no significant differences at 1.5 or 2.5 years (mixed-effect model, repeated measures, restricted maximum likelihood, with Šídák's multiple-comparison test).

difficulty with hand dexterity. CK was 81 IU/L at age 41. Electrodiagnostics demonstrated a non-irritable myopathy in distal muscles at age 42. Whole genome sequencing with segregation analysis via Variantyx at age 43 identified an unreported variant in *DNAJB6* (c.296 T > C, p.V99A) that was not present in her unaffected mother or brother. DNA was unavailable from her affected, deceased father. This amino acid is within the G/F domain of DNAJB6 where many other mutations are known to cause LGMDD1 to reside (Fig. 1B) and is conserved primarily within mammals with a phastCons score of 1 and phyloP score of 4.388. Using in silico analysis tools, the variant is predicted to be disease-causing in Mutation Taster, dele-terious in SIFT with a score of 0, and probably damaging in polyphen with a score of 0.985.

Family 2

Patient II:2 is a 45-year-old male of Spanish and Nicara-guan descent. Weakness started at age 10 with the
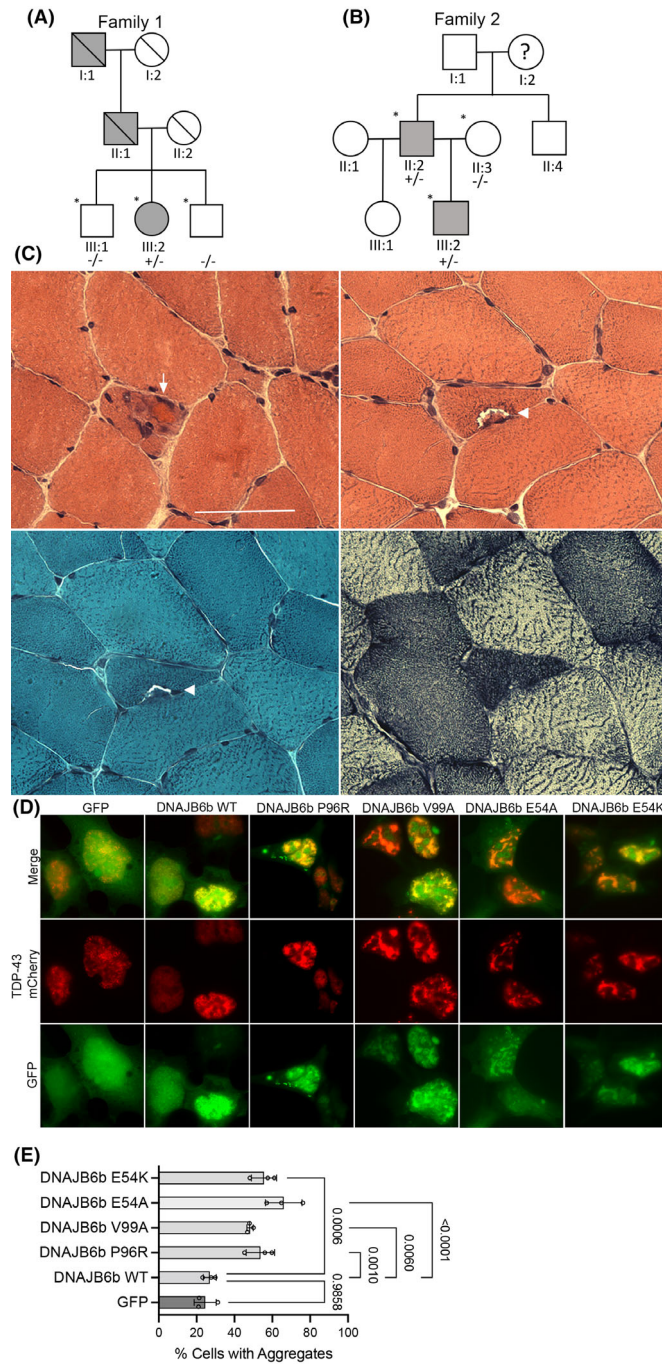
Figure 6. Case presentations and in vitro DNAJB6b variant testing. (A) Family tree 1 and (B) Family tree 2. Clinically affected individuals are shaded in gray. There is no clinical knowledge of I:2 in family 2. Individuals for whom DNA was available are marked with *. The genetically tested family members are indicated as negative (−/−) or heterozygous (+/−) for the DNAJB6 variants. (C) Muscle biopsy from family 1, II:1. Top left panel illustrating abnormal fiber with large eosinophilic aggregate. Scale bar = 50 μM. Other panels are serial sections demonstrating a fiber with vacuolar changes. In clockwise order starting in the top left: hematoxylin and eosin, congo red, NADH, gomori trichrome. NADH stain illustrates some linearization of internal architecture. (D) Representative images of U2OS cells co-transfected with GFP or GFP-tagged DNAJB6b, and mCherry-TDP-43, then subjected to 1-hour heat shock. (E) Quantitation of % of cells with aggregated nuclear TDP-43 following heat shock. All DNAJB6b variants (P96R, V99A, E54A, E54K) increased nuclear TDP-43 aggregation compared with WT DNAJB6b after heat shock (one-way ANOVA with Dunnett's multiple-comparison test). Bars show the percentage of cells with TDP-43 accumulations (mean ± SD) in three replicates.

Table 2. Patient strength assessments.

Family	ID	Age	D	B	T	Gr	WE	FE	FDI	APB	IP	HAd	Q	H	TA	G
1	II:1	47	5/5	5/5	4+/4+	4/4		3+/3+	3+/3+		5/5	5/5	5/5	5/5	2/2	2/2
	III:2	15	5/5	5/5	5/5	5/5	5/4+	4/4	5−/5−	4/4	5/5	5/5	5/5	5/5	5/5	5/5
		44	5/5	5/5	5/5	4/4	5/5	4/4	4/4	4/4	5/5		5/5	5/5	4/4	
2	II:2	35	3+/3+	3+/3+	3+/3+				3/3	3/3	3/3	3+/3+	3/3	4−/4−	3/3	3/3
		45	3+/3+	3/3	2/2		1/1		1/1	1/1	0/0	0/0	0/0	0/0	0/0	0/0

MRC scores are reported as left/right. D, deltoid; B, bicep; T, triceps; Gr, grip; WE, wrist extension; FE, finger extension; FDI, first dorsal interosseous; APB, abductor pollicis brevis; IP, iliopsoas; Had = hip adduction; Q, quadriceps; H, hamstrings; TA, tibialis anterior; G, gastric. No strength assessments are listed for family 2 III:2 as their examination was descriptive, without MRC scores provided.

inability to run fast. By age 20 he had difficulty climbing stairs. At 30 he noticed difficulty gripping objects, and by age 35 he required a wheelchair. His 9-year-old son was similarly affected. His father was unaffected. He had not been in contact with his mother since age 6 (Fig. 6B). Examination at age 35 showed diffuse weakness with slight distal predominance involving legs more so than arms (Table 2). Re-exam at age 45 illustrated disease progression with more clear distal predominance in the upper extremities. A quadriceps biopsy at age 25 demonstrated myopathic changes with regions of prominent fibrofatty replacement of muscle with occasional necrotic fibers and rimmed vacuoles. Biopsy slides and tissue were discarded by the performing laboratory 10 years after it was performed. Genetic testing (Invitae comprehensive muscular dystrophy panel) in 2021 identified a previously unreported variant in *DNAJB6* (c.160G > A p.E54K). This amino acid is within the J domain of DNAJB6 where another cluster of disease-causing mutations reside.[15] A disease-causing mutation resides at this exact residue (p.E54A).[15] This amino acid is conserved primarily within mammals with a phastCons score of 1 and phyloP score of 5.392. The variant is predicted to be disease-causing in Mutation Taster, deleterious in SIFT with a score of 0, and probably damaging in polyphen with a score of 0.975.

Patient III:2 is a 9-year-old boy, (son of II:2). His mother first suspected weakness at age 6 that progressed to cause falls and difficulty climbing stairs by age 8. Examination at age 9 was notable for ankle dorsiflexion weakness, slow running, and difficulty getting up off the ground. CK was 80 (cutoff was 160 IU/L). Genetic testing (Invitae comprehensive neuromuscular panel) identified the same *DNAJB6* variant (c.160G > A, p.E54K) identified in his father.

In-vitro functional studies

LGMDD1-mutant DNAJB6b increases the abundance of TDP-43 positive nuclear accumulations following heat shock. We co-expressed mCherry-tagged TDP-43 with GFP-tagged DNAJB6b-WT or LGMDD1-mutant DNAJB6b. We included G/F domain (P96R) and J-domain (E54A) mutations for comparison. Both new variants (E54K, V99A) increased the proportion of cells with nuclear TDP-43 accumulations following heat shock (Fig. 6D,E).

Discussion

To initiate clinical trial readiness efforts for LGMDD1, we used multiple study types including systematic literature review, retrospective chart review, and prospective PRO assessments. From this, we generated detailed phenotype and natural history information, as well as preliminary sample size calculations. This study is the largest description of LGMDD1 patients to date and provides quantitative data on longitudinal progression.

We identified genotype as a likely source for phenotypic variability in LGMDD1 patients. Genotype appears to impact several aspects with implications for clinical trials: weakness pattern, age of onset, and possibly rate of disease progression to LOA. Differences in weakness pattern (Fig. 2A) likely affect muscle-specific rates of progression (Fig. 4A–D), and therefore, the responsiveness of certain strength-based assessments. Ideally, future studies will identify outcome measures that are sensitive to change regardless of weakness pattern. If not feasible, future trials may need to perform subgroup analyses based on weakness patterns, or specify either proximal or distal weakness in the inclusion/exclusion criteria.

Age of onset was highly variable among individuals with different mutations and was not predictive of the rate of disease progression to LOA (Fig. 2G). Future studies ideally will not subgroup or stratify participants based on age, but instead based on years from disease onset or using functional outcome measures.

The rate of disease progression to LOA may also be impacted by mutation, with F89I and F93I/L progressing more slowly than F91I/L and P96R/L. However, due to the small sample size and retrospective nature of data, genotype's impact on the rate of disease progression will

need to be verified in larger prospective studies. This again raises an issue of subgroup analysis vs. limiting inclusion and exclusion criteria to certain genotypes. While these strategies may reduce variability in a trial, any benefit is likely offset by reduced power in this ultra-rare disease. Prospective natural history studies should aim to identify outcome measures capable of capturing a similar rate of progression in all genotypes to avoid limiting enrollment criteria in future trials.

Although most individuals in this study were male (60%), we did not identify any significant phenotypic differences between males and females. Future studies could perform intrafamilial comparisons between male and female patients to limit any confounding impact from genotype.

We used two validated PRO assessments (ACTIVLIM, PROMIS-57) and our customized questionnaire in this LGMDD1 cohort. Although ACTIVLIM scores correlated well with disease duration (Fig. 5A,B), they were highly variable between longitudinal assessments, with no significant change from baseline at both 1.5- and 2.5-year repeat assessments. ACTIVLIM scores actually increased for certain individuals at some timepoints. While these score increases may reflect noise, one cannot rule out that LGMDD1 does not significantly worsen within this time period. For the PROMIS-57, only cross-sectional data were available. Scores from a subset of domains (social roles/activities, physical function, fatigue) correlated with disease duration. If included in future studies, questions could be limited to these domains to minimize questionnaire fatigue.

The LGMDD1 questionnaire was initially designed to retrospectively catalog age at key disease events to help inform when leg vs. arm outcome measures might be most helpful in a trial. For example, the window for capturing functional decline in the legs ranges from 0 to ~30 years after disease onset, whereas the functional decline in the arms starts later and extends beyond 50 years from disease onset (Fig. 5D). This questionnaire asked similar functional questions to the ACTIVLIM but differed in that participants prospectively updated their prior questionnaire (unblinded) by entering their age at which new disease events occurred. The structure of this questionnaire makes it less capable of detecting functional improvement and likely impacts recall bias by providing participants with their prior answers. We did not observe the same score improvements as seen with the ACTIVLIM (Fig. 5B,F), and it captured a significant decline at both 1.5 and 2.5-year assessments (Fig. 5H). Although LGMDD1 questionnaire scores correlated well with ACTIVLIM ($r = -0.952$, $P < 0.0001$) and disease duration ($r = 0.66$, $P < 0.0001$), it is important to acknowledge the ACTIVLIM is a validated tool and the

LGMDD1 questionnaire is not. These discrepant results for longitudinal analysis should therefore prompt caution in the use and interpretation of the custom LGMDD1 questionnaire. LGMDs do invariably decline over time, and any improvement is likely transient or subjective. While having treatments for LGMDs that result in functional improvement would be ideal, trials thus far have repeatedly illustrated therapeutics are much more likely to slow progression. It is, therefore, important to have tools that are more sensitive to detecting slowing of decline. Although scores for the two most common mutations in our longitudinal study (F89I, F93L) progressed at similar rates on this questionnaire (Fig. 5J), additional testing will need to include more patients with different genotypes as well as individuals with distal predominant weakness. Preliminary sample size estimates are close to feasible for this slowly progressive ultra-rare disease. However, these initial calculations should be viewed with caution given the LGMDD1 questionnaire has not been validated, and these estimates were generated from a small number of participants. Future changes to this questionnaire could expand its ability to detect functional improvement. Although this questionnaire was designed with input specifically from individuals with LGMDD1, it may be useful in other neuromuscular disorders.

Quantitative dynamometry provided detailed data illustrating muscle-specific rates of progression in patients with proximal weakness for up to 60 years after disease onset. Consistent with prior descriptions of disease, some of the most affected muscles were hamstrings, iliopsoas, biceps, and deltoids.[16–18] Grip strength also progressed relatively fast in this small group of individuals. While this may represent a later onset of distal weakness in proximal predominant patients, grip strength is known to decline in healthy individuals with increasing age.[19] We chose not to normalize strength measurements (% change from baseline) as it skews data for patients with severe weakness. For example, someone with 2lbs of strength at baseline and 1 lb on repeat assessment has had a 50% change from baseline. Quantitative dynamometry was, therefore, presented as raw strength data (lbs/year) to more accurately capture the overall clinical spectrum. However, this approach is also problematic as it biases rates of progression towards stronger muscle groups with higher baseline values (grip). Similar to the preliminary sample size estimates for the LGMDD1 questionnaire, those for quantitative dynamometry should also be viewed with caution and require validation with a larger prospective study.

MMT data were available for both distal and proximal predominant patients. Variability between physicians and their use of the MRC scale (i.e., some do not use 5- and

3-) makes this an imprecise tool for quantifying declining strength. Despite these limitations and the small sample sizes, it appears proximal muscles progress at a similar rate in both distal and proximal predominant patients. This will need to be clarified in prospective trials with quantitative dynamometry. If true, it suggests testing proximal muscles could be an effective outcome measure regardless of weakness pattern. The distal vs. proximal predominant weakness seen in LGMDD1 is somewhat reminiscent of the clinical spectrum seen in dysferlinopathy patients. It is possible with more in-depth phenotyping, we will similarly find LGMDD1 is not two discrete groups of distal and proximal patients but more of a continuum of distal involvement for a given degree of proximal weakness.

Of the clinical events assessed, dysphagia was the most frequent (51.4%), followed by respiratory (38.3%), and cardiac (29.7%). Although commonly present, dysphagia did not seem to progress to severe levels necessitating enteral feeding. Cardiac and respiratory abnormalities were typically mild (FVC slightly below 80% or minor EKG abnormality). These frequencies may be inaccurate due to the retrospective nature of this study. Dysphagia, cardiac, and respiratory complications are disease events that prompt medical attention but maybe missed on questioning. Disease event frequency may be falsely inflated by the low number of individuals who reported undergoing assessments. Patients may preferentially remember abnormal assessments. In addition, clinic notes from chart review and studies within the systematic literature review tended to only report pertinent positives. We did not control for other confounders such as smoking and other cardiovascular risk factors.

We identified two new variants: c.296 T > C, p.V99A and c.160G > A, p.E54K that highlight the extremes of phenotypic variability in LGMDD1. The V99A variant was associated with a milder distal predominant vacuolar myopathy. In vitro data support the pathogenicity of this mutation. Unfortunately, the variant was confirmed in only one affected individual. The proband's affected father died before mutations in *DNAJB6* were known to cause a myopathy. p.E54K was associated with a more severe phenotype with childhood onset, diffuse weakness with slight distal predominance, and vacuolar changes on biopsy. Genetic changes were identified in two affected individuals, and in vitro data were also supportive of pathogenicity. The prominent clinical differences between these two new variants exemplify the wide range of LGMDD1 disease severities illustrated in this study, as well as the significant impact of genotype.

There are many limitations to this study including but not limited to retrospective data, the small longitudinal cohort size, and the limited number of individuals with distal predominant weakness. Additionally, the mixed nature of data from PRO assessments (prospective) and strength testing (retrospective), does not allow for correlation analysis. Despite these shortcomings, this study provides a starting point to guide detailed longitudinal analysis and future trials. The observation of genotype impacting weakness pattern and rates of disease progression suggest that both future natural history studies and treatment trials will need to identify and include outcome measures capable of capturing decline across the phenotypic spectrum. The relatively high numbers in our preliminary sample size estimates highlight the need for future trials to be adequately powered to accommodate the slow but variable rate of progression in individuals with LGMDD1.

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Author Contributions

Conception and design of the study: ARF, CCW. Acquisition and analysis of data: ARF, SR, SP, MS, RB. Drafting a significant portion of the manuscript and preparing the figures: ARF.

Conflicts of Interest

The authors report no competing interests.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplemental 1.: Systematic literature review summary table.
Supplemental 2.: Customized LGMDD1 questionnaire.