TOWARD AN OBJECTIVE MEASURE OF FUNCTIONAL DISABILITY IN DYSFERLINOPATHY

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ABSTRACT: *Introduction:* Understanding the natural history of dysferlinopathy is essential to design and quantify novel therapeutic protocols. Our aim in this study was to assess, clinically and functionally, a cohort of patients with dysferlinopathy, using validated scales. *Methods:* Thirty-one patients with genetically confirmed dysferlinopathy were assessed using the motor function measure (MFM), Modified Rankin Scale (MRS), Muscle Research Council (MRC) scale, serum creatine kinase (CK) assessment, baseline spirometry data, and echocardiographic and electrophysiologic studies. *Results:* MFM and MRC scores showed a significant negative correlation with disease duration and inverse correlation with MRS, but not with onset age, clinical phenotype, or CK levels. Percent forced vital capacity (%FVC) correlated negatively with disease duration and onset age. Eight known pathogenic mutations were identified recurrently, 4 of which accounted for 79% of the total. *Conclusions:* The results suggest that MFM is a reliable outcome measure that may be useful for longitudinal follow-up in dysferlinopathy. Recurrent mutations suggest a founder effect in the Chilean population.

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Additional Supporting Information may be found in the online version of this article.

Abbreviations: %FVC, percent of forced vital capacity; CFN, common fibular nerve; CK, muscle creatine phosphokinase; CMAP, compound muscle action potential; cMTS-ld, composite manual testing score distal lower limbs; cMTS-lp, composite manual testing score proximal lower limbs; cMTS-t, composite manual testing score for all muscle groups examined; cMTS-ud, composite manual testing score distal upper limbs; cMTS-up, composite manual testing score proximal upper limbs; cMTS, composite manual testing score; CNEMG, concentric needle electromyography; DHPLC, denaturing high-performance liquid chromatography; DMAT, distal myopathy with anterior tibial onset; *DYSF*, human dysferlin gene; EDB, extensor digitorum brevis; LGMD2B, limb girdle muscular dystrophy type 2B; MFM, motor function measure scale; MM, Miyoshi myopathy; MRC, Medical Research Council; MRS, Modified Rankin Scale; NCS, nerve conduction studies; NMJ, neuromuscular junction; RNS, repetitive nerve stimulation test; SFEMG, single-fiber electromyography

Key words: dysferlinopathy; *DYSF*; motor function measure; MRC score; recurrent mutations

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Primary dysferlinopathies define a group of muscular dystrophy phenotypes resulting from autosomal recessive mutations in the dysferlin gene (*DYSF*; OMIM # 603009).[1–3] Limb girdle muscular dystrophy type 2B (LGMD2B; OMIM # 253601), Miyoshi myopathy (MM; OMIM # 254130), and distal myopathy with onset in the tibialis anterior (DMAT; OMIM # 606768) are the 3 main clinical phenotypes associated with *DYSF* deleterious mutations.[1–4] However, a wide range of clinical phenotypes, including early subclinical to severe forms, may also be produced by similar mutations.[5–7]

Accordingly, a poor genotype-phenotype correlation has been reported commonly in most series of patients with dysferlinopathy,[2,5,8–10] and no evident "hot spot" has been identified in the *DYSF* gene, as detailed in the Universal Mutation Database for Dysferlin (www.umd.be/DYSF/).[11] Moreover, muscle MRI studies have established that muscle impairment is similar in all dysferlinopathy patients, regardless of the clinical phenotype.[12]

Although muscle strength scales have been applied to measure segmental distribution and progression of muscle impairment,[12,13] clinical evaluation of functional disability using validated scales in dysferlinopathy has not been reported. In this study, we aimed to assess the degree of functional disability produced by dysferlinopathy and to determine its correlation with disease duration using the motor function measure (MFM).[14] The MFM is a 32-item scoring system that provides a numerical measure of the motor capacity of a patient with neuromuscular impairment according to the following 3 functional dimensions: standing and transfers (D1); axial and proximal motor function (D2); and distal motor function (D3). We tested the discrimination validity of the MFM scale in patients with dysferlinopathies by correlation with the Modified

Rankin Scale (MRS, a measure of functional independence)[15] and with the Medical Research Council (MRC) muscle strength scale.[16]

Clinical assessment was completed with bi-dimensional Doppler echocardiography, baseline spirometry, and electrophysiologic testing.

In a previous study, we reported the first 2 Chilean cases of dysferlinopathy carrying novel mutations in *DYSF*.[17] We now report the clinical presentations, measures of motor disability, and genetic results in 31 patients belonging to 24 unrelated Chilean families. All families carry *DYSF* recurrent mutations of known pathogenic effect[11,18] and constitute the largest Latin-American cohort of patients with dysferlinopathy reported to date.

METHODS

Subjects. Thirty-three patients with dysferlinopathy were recruited continuously between 2009 and 2012 and assessed at the Department of Neurology and Neurosurgery of the Hospital Clínico Universidad de Chile (HCUCH), Santiago, Chile. Two patients were reported in detail in a previous study.[17] In the index cases, the preliminary diagnosis before genetic confirmation was based on either reduced or absent dysferlin by immunohistochemistry in at least 1 affected family member and in 2 patients by clinical findings only. Patients were syndromically defined as LGMD2B or MM at the first medical evaluation. Two patients, who harbor only 1 pathogenic mutation, were removed from the series, because muscle biopsies and/or blood samples were not available for dysferlin analysis, and thus the levels of dysferlin expression could not be assessed.

Detailed information was obtained for each patient to establish family history, including data on consanguineous marriages, age at onset, initial distribution of symptoms, pattern of muscle involvement, ambulatory status, disease progression, and serum creatine kinase (CK) levels. The clinical evaluation was completed with the MFM[14] scale, the MRS,[15] the Medical Research Council (MRC) muscle strength scale,[16] baseline spirometry, echocardiography, and standardized electrophysiologic studies. The ethics committee of HCUCH and the Chilean National Commission of Scientific Research and Technology (CONICYT) approved the informed consent used in this study.

MRC Scoring, MFM, and MRS. Two independent physicians (J.A.B. and C.C.) performed MRC muscle strength testing on all 31 patients as part of the routine physical evaluation on their first visit. No significant variability in the score was observed between examiners, and percentage of interobserver agreement was $93.56 \pm 6.54\%$ (mean $\pm$ SD). We examined the muscle groups that produce neck, elbow, wrist, hip, knee and ankle flexion and extension, arm abduction, and thigh abduction and adduction. To compare the MRC and MFM scores, and to compare proximal and distal degree of weakness between clinical phenotypes, a composite manual testing score (cMTS), adapted from Paradas *et al.*,[12] was constructed. The MRC grades were converted to a 6-point quantitative scale, and results from left and right sides were averaged for each muscle group. Five independent values were calculated, averaging the total score and 4 different body segments: (1) cMTS-up for proximal upper limb (including arm abduction and elbow flexion and extension); (2) cMTS-ud for distal upper limb (including wrist flexion and extension); (3) cMTS-lp for proximal lower limb (including hip extension; thigh flexion, adduction, and abduction; and knee flexion and extension); (4) cMTS-ld for distal lower limb (including ankle flexion and extension); and (5) total cMTS-t for all muscle groups examined and also including neck flexion and extension. Motor functional disability was determined using the MFM scale. The MRS (refer to Table S1 in the Supplementary Material available online) was used to investigate general functional abilities. The MFM ($n = 29$) was performed by the same examiners (C.C. and J.A.B.), and results were expressed as a percentage of the maximum possible score. Two patients (Dysf#19 and Dysf#20) did not perform the MFM (see Table S1). MRS ($n = 31$) was completed for all patients in the cohort. Comparisons between scores were made only for the 29 patients who had all 3 measurements completed.

Ancillary Testing. Serum CK activity was available for 28 patients. In the case of multiple measurements, the highest value was used for analysis. Patients underwent both baseline spirometry ($n = 22$) and bi-dimensional Doppler echocardiography ($n = 26$) to detect respiratory and/or cardiac involvement.

Electrophysiologic Assessment. The compound muscle action potential (CMAP) of the common fibular nerve (CFN) recorded from the extensor digitorum brevis (EDB) muscle was quantified in dysferlinopathy patients, correlated with disease duration, and compared with an age- and gender-matched healthy control group.

Because a novel role for dysferlin in the regulation of cholinergic synapse formation has been proposed in animal models *in vivo*,[19] thus implying possible neuromuscular junction (NMJ) dysfunction, repetitive nerve stimulation (RNS, $n = 17$) testing and single-fiber electromyography (SFEMG, $n = 9$) were performed, according to standardized protocols.[22] Nerve conduction studies (NCS) and concentric needle electromyography (CNEMG,

Table 1. Relative frequency and recurrence of *DYSF* pathogenic mutations found in Chilean patients.

Ranking	Exon	Nucleotide change	Amino acid change	Frequency (%)	Reference*
1	53	c.5979dupA	p.Glu1994Argfs*3	16 (25.8%)	*DYSF*_00054
2	27	c.2858dupT	p.Phe954Valfs*2	16 (25.8%)	*DYSF*_00214
3	26	c.2779delG	p.Ala927Leufs*21	9 (14.5%)	*DYSF*_00213
4	40	c.4390G>T	p.Glu1443*	8 (12.9%)	*DYSF*_00267
5	6	c.526C>T	p.Gln176*	5 (8.0%)	*DYSF*_00264
6	43	c.4756C>T	p.Arg1586*	2 (3.2%)	*DYSF*_00027
7	21	c.1948delC	p.Leu650Tyrfs*6	2 (3.2%)	*DYSF*_00317
8	13	c.1276G>A	p.Gly426Arg	2 (3.2%)	*DYSF*_00314

*The first 2 mutations accounted for 51.6% of the total, whereas mutations ranked 1–4 represented 79% of total, suggesting a founder effect. *Description according to Leiden Muscular Dystrophy Database records.*

$n = 27$) were performed according to standard protocols.[20–22]

Dysferlin Immunostaining. Dysferlin expression was determined by immunohistochemistry (IHC) on muscle biopsy tissue in 21 of 31 patients (Table S1). Skeletal muscle sections (10–12 μm) were immunostained with NCL-Hamlet anti-dysferlin monoclonal antibody (Novocastra, UK), as previously described.[17]

Dysferlin Gene Analysis. DNA was extracted from peripheral blood lymphocytes, as described previously.[17,19,23] Patients 1–14 were analyzed in Marseille (Département de Génétique Médicale, Hôpital d'Enfants de la Timone) using genomic denaturing high-performance liquid chromatography (DHPLC) with subsequent sequencing of abnormally eluted fragments.[18,23] Patients 15–44 and family members of the index patients in selected families were analyzed in Santiago (Programa de Genética Humana, ICBM, Facultad de Medicina, Universidad de Chile) by direct bidirectional sequencing of the 55 "canonical" exons (automated ABI Prism 3130 XL; Applied Biosystems, Waltham, Massachusetts). Primer sequences and the amplification conditions are available upon request. SeqMan Pro version 7.2.2 (DNASTAR Lasergene) software was used for sequence analysis. GenBank reference sequence NM_003494.3 was used to compare electropherogram results. Sequence variations are described at cDNA ("c") and protein ("p") levels, referring to the A in the ATG as position 1, in accordance with Human Genome Variation Society nomenclature (www.hgvs.org/mutnomen/) (Table 1).

In addition, the dysferlin alternative exons 1 of *DYSF-v1*, 5a, and 40a,[24,25] were analyzed for point mutations in Dysf#04 and Dysf#27, as described above.

Data Analysis. Statistical tests were carried out using IBM SPSS Statistics 21 (IBM Corp., 2012). A 2-tailed *t*-test or Mann-Whitney *U*-test was used for comparative analysis between phenotype groups and for proximal and distal scores of manual muscular testing. Spearman correlations were used to describe associations between quantitative variables.

RESULTS

Clinical Assessment. At onset, 22 patients (13 of 15 men and 9 of 16 women) had a typical distal phenotype and were classified as MM; the other 9 patients had a proximal phenotype and were classified as LGMD2B (see Table S1 online). Mean age at onset across all patients was 20.4 (range 10–33) years. The age of onset for MM patients was 20 ± 5.2 years (mean ± SD), whereas the onset of LGMD2B was 21.4 ± 7.2 years (non-significant). Furthermore, lower limb weakness and atrophy, mainly in calf and thighs, was the first symptom manifested in all patients (Fig. 1A, left 2 images), resulting in difficulties in standing on tiptoes, climbing stairs, and running. In 5 women, atrophy of the posterior compartment of the legs and thighs was not evident after several years of disease (Fig. 1A, right 2 images). Symptoms invariably progressed to the upper limbs and affected the anterior compartment of the arms and shoulders within the first 5 years (Fig. 1B). Forearm and hand involvement was clinically evident after ≥10 years of disease, with relative sparing of the hand and finger extensors. In a patient with 48 years of disease, upper limb movements were almost restricted to finger extension, and there was severe atrophy of the deltoids (Fig. 1B) and first dorsal interossei (not shown). The mean time to require walking support was 15.4 ± 7.1 years, and the mean time to wheelchair use was 19.7 ± 7.0 years.

The mean serum CK level was increased in all patients, ranging from 591 to 25,000 IU/L ($10{,}140 \pm 6{,}393$ IU/L) with an average increase of 54 (± 36) times over normal. No significant differences on CK levels were found between phenotypes.

Dysferlin IHC labeling was absent on muscle biopsy in 23 of 24 patients and markedly reduced in 1 patient (see Table S1 online).

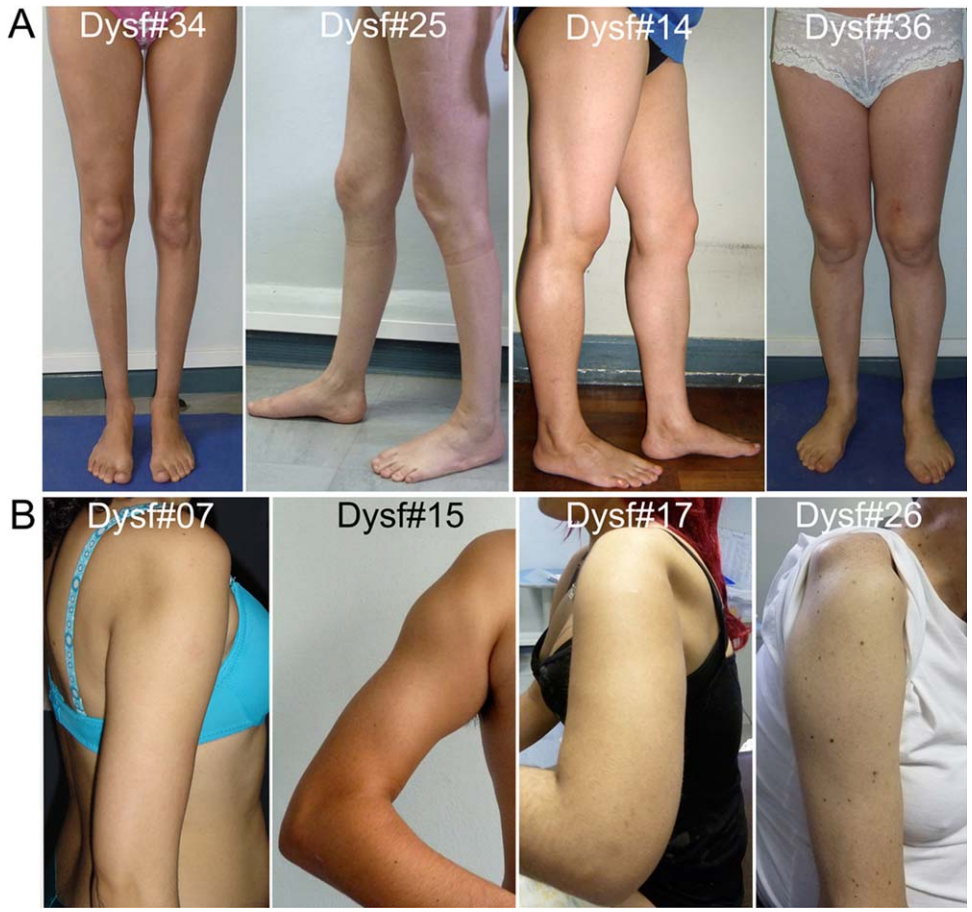

FIGURE 1. Clinical features of lower **(A)** and upper **(B)** limbs. **(A)** From left to right: the first 2 pictures are women with the MM phenotype (Dysf#34 and Dysf#25); the third and fourth pictures are LGMD2B women (Dysf#14 and Dysf#36) who lack atrophy of the lower limbs despite functional involvement. **(B)**. The "bulge" sign of the deltoid and "*the boule du biceps*" were observed in 38% (12 of 31) of the patients (as seen in Dysf#07 and Dysf#15). Atrophy was marked and produced a hollow of the affected muscle/area in 13% (4 of 31) of patients at the posterior deltoid (as seen in Dysf#07 and Dysf#17). Patient Dysf#26, with a disease course of 48 years, had complete atrophy of the deltoid.

Manual Muscle Testing and Muscle Functional Assessment. The results of the composite manual testing score are summarized in Tables S1 and S2 (online). Composite muscle manual testing total score (cMTS-t) at first examination was not statistically different between MM and LGMD2B patients (MM: 3.53 ± 0.7; LGMD2B: 3.81 ± 0.4), but it had a significant negative Spearman correlation with disease duration ($r = -0.76$, $P < 0.001$). Scores for distal and proximal lower limbs showed significant differences when all patients were compared together, regardless of their clinical phenotype (cMTS-lp: 3.21 ± 0.7; cMTS-ld: 1.9 ± 1.4; $P < 0.001$), but there was no difference for upper limb data (cMTS-up: 4.11 ± 0.7; cMTS-ud: 4.38 ± 0.7). Comparison of distal lower limb cMTS scores between phenotypes were statistically different (MM cMTS-ld: 1.5 ± 1.1; LGMD2B cMTS-ld: 2.9 ± 1.5; $P = 0.02$), but scores for proximal lower limb (cMTS-lp), proximal upper (cMTS-up), and distal upper (cMTS-ud) limbs showed no statistical differences (see Table S2 online).

Patients who presented initially with a distal MM phenotype had significantly lower scores in the distal lower limbs (cMTS-lp: 3.16 ± 0.8; cMTS-ld: 1.5 ± 1.1; $P < 0.0001$), but not in the upper limbs (cMTS-up: 4.06 ± 0.8; cMTS-ud: 4.3 ± 0.8; $P = 0.25$). In the 9 patients who presented with the LGMD2B phenotype, no significant difference between proximal and distal cMTS scores in the upper (cMTS-up: 4.2 ± 0.7; cMTS-ud: 4.6 ± 0.4) or lower (cMTS-ld: 2.9 ± 1.5; cMTS-lp: 3.3 ± 0.5) limbs was observed (Fig. 2, and Table S1 online).

The mean disease duration at time of MFM examination was 10.8 (range 3–48) years. On the MFM scale, patients had major impairment on standing and transfers (D1), with a mean score across all patients of $41.82 \pm 23.2\%$. Axial/proximal (D2) and distal motor function (D3) were clearly less affected (mean $87.53 \pm 18.5\%$ and $92.94 \pm 10.1\%$, respectively). A significant negative Spearman correlation ($r = -0.76$, $P < 0.001$) was observed between disease duration and total MFM score, and especially with D1 score ($r = -0.79$,

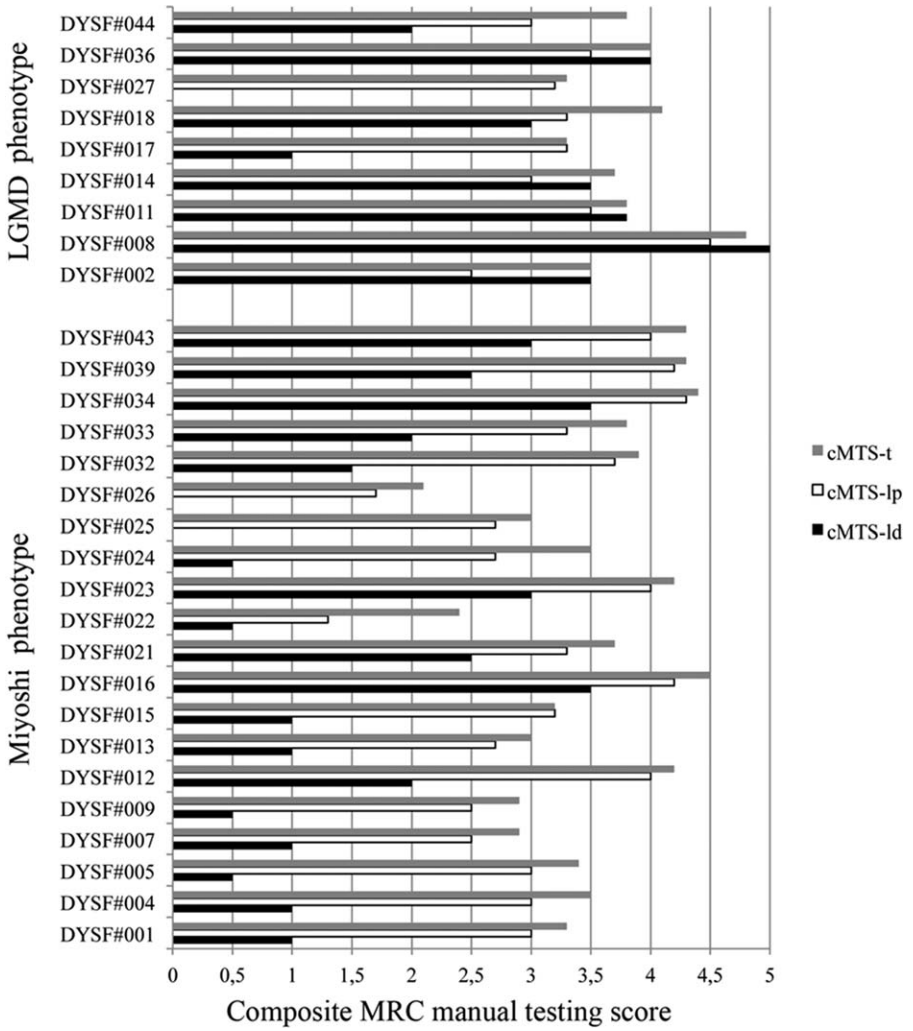

FIGURE 2. Composite manual testing scores (cMTS) for total and lower limbs according to phenotype. Total score (cMTS-t, grey bars) and scores for proximal (cMTS-lp, white bars) and distal (cMTS-ld, black bars) lower limbs are presented. Limb girdle muscular dystrophy type 2B (LGMD2B) patients are in the upper part; Miyoshi distal myopathy phenotype (MM) patients are in the lower part of the graph (in the same order as in Table S1 online). Total scoring (cMTS-t) was not statistically different between groups (Mann–Whitney *U*-test, $P = 0.36$). Black bars, representing distal lower limb score, are consistently shorter in the MM group (Mann–Whitney *U*-test, $P < 0.0001$) than in the LGMD2B group (Mann–Whitney *U*-test, $P = 1.0$). See also Table S2 (online).

$P < 0.001$), but also for D2 ($r = -0.47$, $P < 0.009$) and D3 ($r = -0.52$, $P < 0.003$) scores (Fig. 3). Significant Spearman correlations were observed between total MFM and D1 scores and total cMTS-t score ($r = 0.83$, $P < 0.0001$ and $r = 0.80$, $P < 0.0001$, respectively).

The MRS score increased with disease duration ($r = 0.80$, $P < 0.001$), in agreement with MFM and cMTS score results (see Table S1 online). Total MFM and D1 scores showed significant negative Spearman correlations with MRS ($r = -0.74$, $P < 0.001$ and $r = -0.79$, $P < 0.001$, respectively). After 10 years of disease, all patients consistently scored at least 4 on the MRS, thus reflecting an inability to walk independently. Differences between MM and LGMD2B patients on the scores for global MFM scale and subscores were not significant.

Respiratory and Cardiac Assessment. Patients reported neither cardiologic nor respiratory symptoms. A significant Spearman correlation was seen between baseline %FVC and disease duration ($r = -0.58$, $P = 0.004$), but values were within the normal range in 18 patients tested (Fig. 4, and Table S4 available online). Age at onset also had a significant correlation with %FVC ($r = 0.47$, $P = 0.03$). An asymptomatic restrictive defect was observed in 3 patients after 10, 18, and 48 years of disease, respectively. Spirometry in patient Dysf#26 after 35 years of disease showed an FVC of 72%, and was 61% after 48 years of disease, thus providing evidence of progressive respiratory decline. Echocardiogram values were within the normal range in 24 of 25 patients tested, and no correlation was observed between ejection fraction (EF) and disease duration (see Table S1 online). Significant

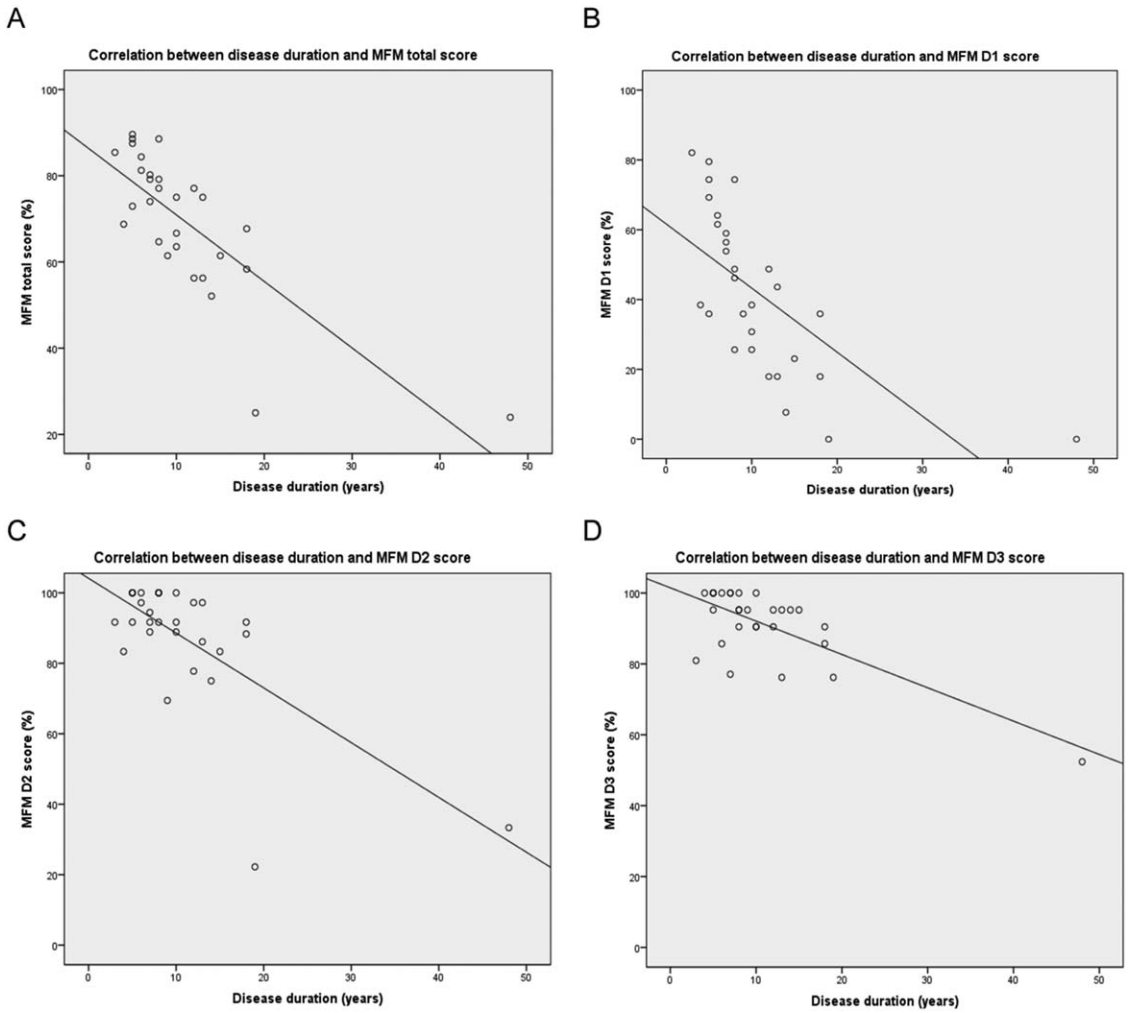

FIGURE 3. Spearman correlation data for MFM scores and subscores vs. disease duration. Scatterplot representation of Spearman correlations between disease duration vs. total MFM score ($r = –0.76$, $P < 0.001$) **(A)**, D1 MFM score ($r = –0.79$, $P < 0.001$) **(B)**, D2 MFM score D2 ($r = –0.47$, $P < 0.009$) **(C)**, and D3 MFM score ($r = –0.52$, $P < 0.003$) **(D)** in 29 of the 31 patients of the cohort. D1 score is more representative of disease progression than the other MFM scores.

differences were not found between phenotypes on baseline spirometry and echocardiography.

Electrophysiologic Assessment. CNEMG and NCS were performed in 27 of 31 patients. All patients had normal nerve conduction. Myopathic findings were observed in at least 1 muscle in 26 of 27 patients, with the exception being patient Dysf#08. The CMAP of the CFN recorded from the EDB correlated with marked hypertrophy of the muscle, contrasting with the severe atrophy of the distal legs (see Fig. S1 online). RNS and SFEMG were within the normal range for all patients tested. None of the patients assessed showed any neurogenic features. There were no electrophysiologic differences between MM and LGMD2B phenotypes.

Genetic Analysis. Genetic findings are summarized in Table 1 and Table S3 (online). We identified 8 different disease-causing mutations in 31 patients from 24 unrelated families. None were novel and have been described previously as frameshift (c.1948delC, c.2858dupT, c.2779delG, and c.5979dupA), nonsense (c.526C>T, c.4390G>T, and c.4756C>T), and missense (c.1276G>A). The previously reported pathogenic mutations affecting exons 53 (c.5979dupA), 27 (c.2858dupT), and 26 (c.2779delG)[23,26] were detected in 41 of 62 alleles (Table 1). Also, the non-pathogenic mutation c.3624C>G on exon 33 was detected in families 16, 19, and 43 (data not shown).

Genotype and Clinical Correlation. No major differences were observed between mutation type with respect to disease onset and duration, MRC cMTS, MFM and MRS scores, CK level, baseline spirometry, or echocardiographic findings.

DISCUSSION

We identified and extensively studied the phenotype of 31 Chilean patients with dysferlinopathy. At least 1 disease-causing mutation was identified

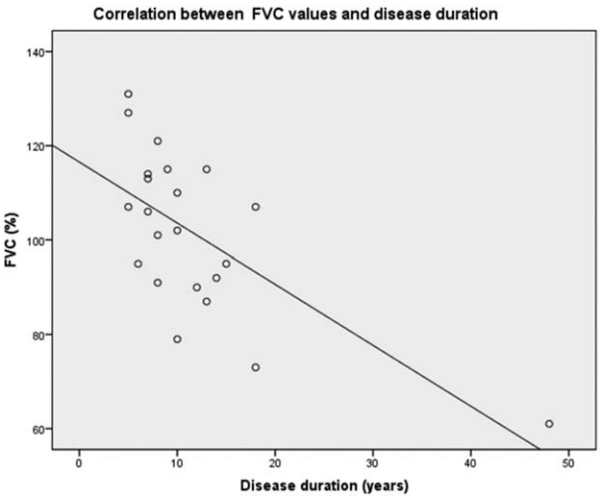

FIGURE 4. Spearman correlation data for respiratory function vs. disease duration. Scatterplot represents baseline spirometry (%FVC) vs. disease duration, showing the Spearman correlation of the parameters. A significant decrease in %FVC was observed ($r = -0.58$, $P = 0.004$). See Table S4 (online) for a list of patients included in the correlation.

in all patients. None of these mutations were novel, and their pathogenicity had been established previously.[3,18,23,26–28] This is consistent with our immunohistochemistry data, as dysferlin was markedly decreased (Dysf#27) or absent in all patients assessed. As in previous studies, the mutation type in our cohort was not useful for predicting phenotypic differences or disease severity,[2,5,8–11,13] and thus no genotype-phenotype correlation was found. Accordingly, we observed a Miyoshi phenotype in 22 of our patients regardless of mutation type, the presence of both phenotypes in 3 families (families 7, 11, and 36), and different mutations associated with same phenotype. This suggests that other genetic and/or environmental factors may influence the clinical heterogeneity (i.e., MM or LGMD2B) observed at the onset of the disease, as has been recently proposed.[29] Moreover, no significant differences between MM and LGMD2B phenotypes were observed in disease duration, mean serum CK level, ejection fraction, or baseline spirometry. Global muscle strength measured with composite MRC manual testing (cMTS-t), total MFM score and subscore, and MRS scale all correlated similarly with disease duration in both phenotypes.

Despite the significant proximal-distal difference found in lower and upper limbs of MM patients who presented with weaker distal lower and proximal upper limbs (cMTS-ld and cMTS-up), this difference was not evident on the functional MFM scale. Although no significant segmental differences were found in upper and lower limbs of LGMD2B patients, such a result should be verified, as it may be due to the low number of patients in the group ($n = 9$). Taken together, these results show that there were no significant differences between dysferlinopathy phenotypes and suggest that the differences observed in muscle strength distribution, although measurable and statistically significant for patients with MM, did not affect MFM and MRS functional scores.

We obtained a relatively high score (92.94%) on the MFM distal function (D3) subscale, indicating a low level of impairment. This may be explained by the fact that the distal motor function score on MFM applies mainly to upper limbs that are less or exclusively affected in late stages of the disease. This is in agreement with the score observed with cMTS for upper limbs. The inclusion of tiptoe stand and walking as additional sub-items of the MFM scale may help reflect the early involvement of the posterior compartment of the legs in MM patients, but it seems not to be relevant to the overall functional performance. On the other hand, the D1 MFM dimension as a measure of standing position and transfers showed the greatest degree of impairment and correlated with years of disease duration and total muscle strength (cMTS-t) score. This suggests that functional impairment in dysferlinopathy is mainly determined by the D1 dimension in MFM, and that the D1 subscore is reliable. Therefore, its validation as an outcome measure for longitudinal follow-up should be tested.

The MRS was introduced as a reference, as this scale has been widely used and validated.[15] It measures independence rather than performance of specific tasks, incorporating physical adaptations to the neurologic deficits. In dysferlinopathy, MRS scoring was based exclusively on motor disability. It showed a consistent correlation with MFM score and disease duration, thus proving to be a clinically meaningful measure of level of functional impairment associated with disease duration (i.e., loss of independent ambulation at 10 years of symptomatic disease). In summary, the MFM scale, particularly the D1 MFM dimension, as well as MRS and cMTS scores, were consistently reliable indicators of the level of functional disability and muscle strength in dysferlinopathy. Such scores also correlate with years of disease, and therefore it will be worth validating them as tools for longitudinal follow-up and for monitoring therapeutic interventions. Our study was limited to a single time evaluation, so findings should be interpreted with caution. Currently, repeated application of these scales and correlation of the findings with MRI on this cohort of patients are being done.

The clinical and genetic overlap between MM and LGMD2B observed in our cohort provides further evidence that dysferlinopathy is sufficiently homogeneous clinically, thus prompting us to

combine both phenotypes in further dysferlinopathy research, as has been suggested previously.[12]

Baseline spirometry and echocardiography were within normal ranges in most patients despite disease duration or severity, as noted elsewhere.[9,10,13] However, we found a significant correlation between disease duration and %FVC that may represent increased muscle mass and power loss throughout the disease course, instead of being a specific dysferlinopathy sign. Further functional respiratory analyses are required to redefine the relevance of respiratory management in dysferlinopathy—for example, measuring %FVC differences between the sitting and lying positions.[10] According to this evidence, periodic evaluation of respiratory function would be advisable in patients with dysferlinopathy after the first decade of symptoms in order to avoid respiratory complications.

Because electrophysiologic testing is still used widely in our country as a diagnostic tool for myopathic patients, we sought to define the electrophysiologic profiles of patients in the cohort. Sparing of the EDB in distal myopathies, including dysferlinopathy, is an important finding in the evaluation of myopathic distal weakness.[30] However, to date, no objective measure of this finding exists. NCS and CNEMG did not show specific alterations. Instead, they showed consistent electrophysiologic findings, including sparing of craniofacial and distal upper limb muscles, normal nerve conduction velocity with CMAP normality of the common fibular nerve, and normal neuromuscular transmission. This correlates with previous observations of EDB hypertrophy in distal myopathies[30] and rules out NMJ involvement in dysferlinopathy, contrary to what has been implied in animal models.[19]

To date, this series of Chilean patients with dysferlinopathy is the largest cohort to characterize the landscape of dysferlinopathy in Latin America.[17,31–35] In general terms, no major differences were observed in this cohort as compared with previous reports in France,[5] Spain,[8] the UK,[9] Japan,[10] The Netherlands,[13] and USA.[36] The high frequency of mutations affecting exons 53 and 27 (c.5979dupA and c.2858dupT, respectively) found in Chilean patients (51.6% of cases) suggests a founder effect that may be attributable to European and/or Asian immigrations in the 17th century.[37]

Dysferlinopathy was not recognized clinically in Chile until 2009.[17] Our results not only demonstrate its existence in Chile, but also suggest that the prevalence of this form of muscular dystrophy is higher than previously estimated.[18,38]

A reliable description of the prevailing dysferlinopathy phenotypes and the different forms of the disease would contribute to patient identification and understanding of disease pathophysiology worldwide. This observational study, based on data collected from the largest cohort of dysferlinopathy patients in Latin America, provides further evidence of the potential usefulness of functional scales for longitudinal follow-up and therapeutic trial monitoring of these patients.

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REFERENCES

1. Bashir R, Britton S, Strachan T, Keers S, Vafiadaki E, Lako M, et *al.* A gene related to *Caenorhabditis elegans* spermatogenesis factor fer-1 is mutated in limb-girdle muscular dystrophy type 2B. Nat Genet 1998; 20:37–42.
2. Liu J, Aoki M, Illa I, Wu C, Fardeau M, Angelini C. et al. Dysferlin, a novel skeletal muscle gene, is mutated in Miyoshi myopathy and limb girdle muscular dystrophy. Nat Genet 1998;20:31–36.
3. Aoki M, Liu J, Richard I, Bashir R, Britton S, Keers SM, et *al.* Genomic organization of the dysferlin gene and novel mutations in Miyoshi myopathy. Neurology 2001;57:271–278.
4. Illa I, Serrano-Munuera C, Gallardo E, Lasa A, Rojas-García R, Palmer J, et *al.* Distal anterior compartment myopathy: a dysferlin mutation causing a new muscular dystrophy phenotype. Ann Neurol 2001;49:130–134.
5. Nguyen K, Bassez G, Krahn M, Bernard R, Laforêt P, Labelle V, et *al.* Phenotypic study in 40 patients with dysferlin gene mutations: high frequency of atypical phenotypes. Arch Neurol 2007;64:1176–1182.
6. Illa I, De Luna N, Domínguez-Perles R, Rojas-García R, Paradas C, Palmer J, et *al.* Symptomatic dysferlin gene mutation carriers: characterization of two cases. Neurology 2007;;68:1284–1289.
7. Paradas C, González-Quereda L, De Luna N, Gallardo E, García-Consuegra I, Gómez H, et *al.* A new phenotype of dysferlinopathy with congenital onset. Neuromuscul Disord 2009;19:21–25.
8. Vilchez JJ, Gallano P, Gallardo E, Lasa A, Rojas-García R, Freixas A, et *al.* Identification of a novel founder mutation in the DYSF gene causing clinical variability in the Spanish population. Arch Neurol 2005;62:1256–1259.
9. Klinge L, Aboumousa A, Eagle M, Hudson J, Sarkozy A, Vita G, et *al.* New aspects on patients affected by dysferlin deficient muscular dystrophy. J Neurol Neurosurg Psychiatry 2010;81:946–953.
10. Takahashi T, Aoki M, Suzuki N, Tateyama M, Yaginuma C, Sato H, et *al.* Clinical features and a mutation with late onset of limb girdle muscular dystrophy 2B. J Neurol Neurosurg Psychiatry 2013;84: 433–440.
11. Blandin G, Beroud C, Labelle V, Nguyen K, Wein N, Hamroun D, et *al.* UMD-DYSF, a novel locus specific database for the compilation and interactive analysis of mutations in the dysferlin gene. Hum Mutat 2012;33:E2317–2331.
12. Paradas C, Llauger J, Diaz-Manera J, Rojas-García R, De Luna N, Iturriaga C, et *al.* Redefining dysferlinopathy phenotypes based on clinical findings and muscle imaging studies. Neurology 2010;75: 316–323.
13. Linssen WH, de Voogt WG, Krahn M, Bernard R, Levy N, Wokke JH, et *al.* Long-term follow-up study on patients with Miyoshi phenotype of distal muscular dystrophy. Eur J Neurol 2013;20:968–974.
14. Bérard C, Payan C, Hodgkinson I, Fermanian J, The MFM Collaborative Study Group. A motor function measure for neuromuscular diseases. Construction and validation study. Neuromuscul Disord 2005; 15:463–470.
15. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. Stroke 1988;19:604–607.
16. Medical Research Council. Aids to the examination of the peripheral nervous system. Memorandum No. 45. London: Her Majesty's Stationery Office; 1981.
17. Bevilacqua JA, Krahn M, Pedraza L, Gejman R, Gonzalez S, Lévy N. Dysferlinopathy in Chile evidence of two novel mutations in the first reported cases. Genet Test Mol Biomarkers 2009;13:105–188.
18. Krahn M, Béroud C, Labelle V, Nguyen K, Bernard R, Bassez G, et *al.* Analysis of the DYSF mutational spectrum in a large cohort of patients. Hum Mutat 2009;30:E345–375.

19. Krajacic P, Pistilli EE, Tanis JE, Khurana TS, Lamitina ST. FER-1/Dysferlin promotes cholinergic signaling at the neuromuscular junction in *C. elegans* and mice. Biol Open 2013;2:1245–1252.
20. Kane NM, Oware A. Nerve conduction and electromyography studies. J Neurol 2012;259:1502–1508.
21. Daube JR, Rubin DI. Needle electromyography. Muscle Nerve 2009; 39:244–270.
22. Stalberg E, Sanders DB. Jitter recordings with concentric needle electrodes. Muscle Nerve 2009;40:331–339.
23. Nguyen K, Bassez G, Bernard R, Krahn M, Labelle V, Figarella-Branger D, et *al*. Dysferlin mutations in LGMD2B, Miyoshi myopathy, and atypical dysferlinopathies. Hum Mutat 2005;26:165.
24. Pramono ZA, Tan CL, Seah IA, See JS, Kam SY, Lai PS, et *al*. Identification and characterisation of human dysferlin transcript variants: implications for dysferlin mutational screening and isoforms. Hum Genet 2009;125:413–420.
25. Krahn M, Labelle V, Borges A, Bartoli M, Lévy N. Exclusion of mutations in the dysferlin alternative exons 1 of DYSF-v1, 5a, and 40a in a cohort of 26 patients. Genet Test Mol Biomarkers 2010;14: 153–154.
26. Cagliani R, Fortunato F, Giorda R, Rodolico C, Bonaglia MC, Sironi M, et *al*. Molecular analysis of LGMD-2B and MM patients: identification of novel DYSF mutations and possible founder effect in the Italian population. Neuromuscul Disord 2003;13:788–795.
27. Therrien C, Dodig D, Karpati G, Sinnreich M. Mutation impact on dysferlin inferred from database analysis and computer-based structural predictions. J Neurol Sci 2006;250:71–78.
28. Liewluck T, Pongpakdee S, Witoonpanich R, Sangruchi T, Pho-Iam T, Limwongse C, et *al*. Novel DYSF mutations in Thai patients with distal myopathy. Clin Neurol Neurosurg 2009;111: 613–618.
29. Gallardo E, Ankala A, Núñez-Álvarez Y, Hegde M, Diaz-Manera J, Luna ND, et *al*. Genetic and epigenetic determinants of low dysferlin expression in monocytes. Hum Mutat 2014;35:990–997.
30. Udd B. Distal muscular dystrophies. Handb Clin Neurol 2011;101: 239–262.
31. Werneck L, Marrone CD, Scola RH. Distal myopathies: clinical, laboratory, electromyographic, histologic-histochemical analysis of 8 cases. Arq Neuropsiquiatr 1993;51:475–486.
32. Soares CN, de Freitas MR, Nascimento OJ, da Silva LF, de Freitas AR, Werneck LC. Myopathy of distal lower limbs: the clinical variant of Miyoshi. Arq Neuropsiquiatr 2003;61:946–949.
33. Rosas-Vargas H, Gómez-Díaz B, Ruano-Calderón L, Fernández-Valverde F, Roque-Ramírez B, et *al*. Dysferlin homozygous mutation G1418D causes limb-girdle type 2B in a Mexican family. Genet Test 2007;11:391–396.
34. Vernengo L, Oliveira J, Krahn M, Vieira E, Santos R, Carrasco L, et *al*. Novel ancestral Dysferlin splicing mutation which migrated from the Iberian peninsula to South America. Neuromuscul Disord 2011; 21:328–337.
35. Gómez-Díaz B, Rosas-Vargas H, Roque-Ramírez B, Meza-Espinoza P, Ruano-Calderón LA, Fernández-Valverde F, et *al*. Immunodetection analysis of muscular dystrophies in Mexico. Muscle Nerve 2012;45: 338–345.
36. Rosales XQ, Gastier-Foster JM, Lewis S, Vinod M, Thrush DL, Astbury C, et *al*. Novel diagnostic features of dysferlinopathies. Muscle Nerve 2010;42:14–21.
37. Cruz-Coke R. Ethnic origin and evolution of the Chilean population. Rev Med Chil 1976;104:365–368.
38. Barthélémy F, Wein N, Krahn M, Lévy N, Bartoli M. Translational research and therapeutic perspectives in dysferlinopathies. Mol Med 2011;17:875–882.