Deep phenotyping of an international series of patients with late-onset dysferlinopathy

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DEEP PHENOTYPING OF AN INTERNATIONAL SERIES OF PATIENTS WITH LATE ONSET DYSFERLINOPATHY

Running title: Late onset dysferlinopathy

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ABSTRACT

Background: To describe the clinical, pathological, and molecular characteristics of late onset (LO) dysferlinopathy patients.

Methods: Retrospective series of patients with LO dysferlinopathy, defined by an age at onset of symptoms ≥30 years, from neuromuscular centers in France and the International Clinical Outcome Study for dysferlinopathy (COS). Patients with early onset (EO) dysferlinopathy (<30 years) were randomly selected from the COS study as a control group**,** the North Star Assessment for Dysferlinopathy (NSAD) and Activity Limitation (ACTIVLIM) scores were used to assess functionality. Muscle biopsies obtained from 11 LO and 11 EO patients were revisited.

Results: Forty-eight patients with LO dysferlinopathy were included (28 females). Median age at onset of symptoms was 37 years (range=30-57) and most patients showed a limb-girdle (n=26) or distal (n=10) phenotype. However, compared to EO dysferlinopathy patients (n=48), LO patients more frequently showed atypical phenotypes (7 vs 1; p=0.014), including camptocormia, lower creatine kinase levels (2855 vs 4394 U/l; p=0.01) and higher NSAD (p=0.008) and ACTIVLIM scores (p=0.016). Loss of ambulation in LO patients tended to occur later (23±4.4 years after disease onset vs 16.3±6.8 years; p=0.064). Muscle biopsy of LO patients more frequently showed an atypical pattern (unspecific myopathic changes) as well as significantly less necrosis-regeneration and inflammation. Although LO patients more frequently showed missense variants (39.8% vs. 23.9%, p=0.021), no differences in dysferlin protein expression were found on Western blot.

Conclusions: LO dysferlinopathy patients show a higher frequency of atypical presentations, are less severely affected and show milder dystrophic changes in muscle biopsy.

INTRODUCTION

Dysferlinopathies are a group of muscle disorders caused by autosomal recessive variants in the *DYSF* gene (MIM # 603009)[1,2] encoding for dysferlin, a muscle-specific transmembrane protein involved in membrane repair, cell homeostasis, calcium-mediated signal translation, myogenesis, angiogenesis and microtubule dynamics.[2–6] The exact worldwide incidence and prevalence of dysferlinopathies are unknown, although dysferlinopathies have been estimated to account for up to 30% of recessive muscular dystrophies.[7]

Although dysferlin deficiency is usually associated with a recessive limb-girdle muscular dystrophy (LGMDR2) or a posterior compartment distal myopathy (Miyoshi's myopathy [MM]),[1,8] other, less frequent phenotypes have been described, including a proximo-distal, pseudometabolic and anterior compartment distal myopathy.[1,9,10] More than 450 different variants have been reported, with no clear genotype-phenotype correlation.[11,12] Consistent findings include symptom onset in young adulthood, slow progression, exceptional cardiorespiratory involvement, very high serum creatine kinase (CK) levels and a dystrophic appearance with possible inflammation and reduced or absent dysferlin protein expression in muscle biopsy.[8,13,14] Although the age at onset of symptoms is generally between 10 and 30 years,[8,10,15] late onset (LO) forms (≥ 30 years old) have been increasingly described,[16–19] contributing to considerable clinical heterogeneity.

To better characterize this subpopulation of patients, we analyzed clinical, pathological and molecular data of patients with LO dysferlinopathy (defined as patients with an age at onset of symptoms ≥ 30 years) followed at the neuromuscular reference centers in France

as well as those included in the International Clinical Outcome Study for dysferlinopathy (COS).

MATERIALS AND METHODS

Study design. Between January 2020 and March 2020, we retrospectively reassessed the clinical and paraclinical data of all patients with LO dysferlinopathy followed at the neuromuscular reference centers in France. Patients were selected based on the following inclusion criteria: 1) age at onset of symptoms $\geq$ 30 years; 2) absent or nearly absent dysferlin evidenced on muscle immunohistochemistry (IHC) and/or Western blot (WB) or a ≤20% expression of blood dysferlin in monocytes in either the patient or a family member with dysferlinopathy; 3) two pathogenic variants in the *DYSF* gene or one pathogenic variant and absent or nearly absent dysferlin on muscle biopsy WB.

Exclusion criteria were the following: 1) absence of symptoms (such as asymptomatic CK elevation); and 2) <2 *DYSF* variants and no information on WB dysferlin status.

We applied the same inclusion and exclusion criteria to select the patients from the JAIN Foundation's International COS study. Further information on the design of the COS study is available at ClinicalTrials.gov (NCT01676077).

As a control group, an equal number of patients with dysferlinopathy with an onset of symptoms before the age of 30 years were randomly selected from the COS study.

Clinical and paraclinical data. Detailed information from neuromuscular examinations (including manual muscle testing, scored using the Medical Research Council [MRC] scale), respiratory examinations (percentage of expected forced vital capacity [FVC] and indication of non-invasive ventilation [NIV]) and cardiac examinations (electrocardiogram

abnormalities and left ventricular ejection fraction [LVEF]) and demographic information (date of birth, sex, ethnic origin) was collected. Additional data such as CK level, muscle biopsy and molecular biology were reported. In terms of functional outcomes, the Gardner-Medwin and Walton (GMW) scale was available for the French series while the North Star Assessment for Dysferlinopathy (NSAD) and the self-reported activity limitation (ACTIVLIM) scores were available for patients from the COS study cohort.

Muscle biopsy. Muscle biopsies obtained at the time of diagnosis, and processed according to standard methods,[20] were reanalyzed. The results of WB and/or IHC with anti-dysferlin monoclonal antibodies (NCL-hamlet) and anti-calpain 3 antibodies (Calp3d/2C4, and Calp3c/12A2) were reassessed. All patients gave informed consent for research at the time of the muscle biopsy.

The muscle biopsies of the French patients with LO dysferlinopathy (n=11) were revisited by an expert neuropathologist (TE) and later compared with the previous biopsy report. The following parameters were evaluated and scored as indicated: 1) presence of nuclear internalizations, necrosis and regeneration, muscle fiber size disproportion, intermyofibrillar disorganization, perimysial and perivascular inflammation (scored 0-3: none, rare, moderate, abundant); 2) connective and adipose tissue quantity (scored 0 to 4: none, slight, moderate, major, end stage); 3) muscle fiber type distribution (normal, type 1 or type 2 predominance) and the presence of cytochrome oxidase-negative (COX-negative) fibers, ragged red fibers or rimmed vacuoles. The findings were compared with muscle biopsies from 11 early onset (EO) patients, randomly selected but matched for sex

and a similar time interval from disease onset to biopsy. WBs were available for review in 9 LO patients to search for a lower molecular weight or truncated dysferlin.

Blood dysferlin in monocytes. Immunolabeling of dysferlin on peripheral blood monocytes was performed according to a previously described protocol.[21]

Molecular biology. Genomic DNA was extracted from the patient's whole blood using standard procedures. Exons and flanking intronic sequences of the *DYSF* gene were amplified by PCR using primers and sequenced using quantitative fluorescent PCR analysis. RNA extraction from muscle and RT-PCR analysis were also performed when deemed necessary.

Statistical analysis. Data analysis was carried out using SPSS 26 (IBM). Mean and median values, standard deviation and range of quantitative variables and absolute or relative frequencies of categorical variables were reported. Association was studied through the Student's t test (continuous dependent variables), Chi-squared test (categorical dependent variables), one-way ANOVA (dependent variables for more than two groups) and Kruskal-Wallis test (ordinary dependent variables for more than two groups). Normality was verified through the Shapiro-Wilk test. The Pearson correlation coefficient test was used to identify significant associations between the continuous variables. Regression analyses were performed to search for predictive variables: logistic regression for loss of ambulation (LoA) and linear regression for age at LoA. Kaplan-Meier curves with the log-rank test were used to examine time to LoA. Statistical significance was established at $p \leq 0.05$. For statistical purposes, muscle biopsy categories for each variable were regrouped in none or mild versus moderate, marked or end stage (for nuclear

internalization, necrosis, regeneration, fiber size, intermyofibrillar structure, connective and adipose tissue) or in yes/no (for perimysial and perivascular inflammation).

Ethics approval. In accordance with French legislation, the study was approved by the local Ethics Committee and by the French data protection authority (CNIL, *Commission nationale de l'informatique et des libertés*). The COS study was also approved by ethics review boards in each country, and was registered at ClinicalTrials.gov (NCT01676077).

RESULTS

Forty-eight LO dysferlinopathy patients fulfilled the inclusion and no exclusion criteria. The neuromuscular reference centers across France (Amiens, Angers, Bordeaux, Lausanne, Marseille, Nantes, Rouen, Saint-Nazaire, Saint-Etienne, Strasbourg) contributed 21 patients while 27 were from the international COS study (total n=48) (Table 1). The median age at examination was 54.5 years (range=35-86); 28 patients were females (58.3%) and the majority of patients were Caucasians (83.3%). Fifteen patients had at least one affected sibling, and differences in age at onset could vary widely between siblings. In total, we found 58 different variants, with no particular hotspot. Forty-five individuals had two class IV or V ACMG variants and we report four novel variants. Supplementary material 1 shows a list of all variants including the novel ones, as well as symptom onset for each sib-pair. Muscle biopsy was performed for diagnostic purposes in 39 patients, and WB for dysferlin was available for 29. In 19 patients dysferlin was completely absent, while 10 patients had traces of dysferlin expression.

Symptom onset and clinical presentation at diagnosis. The median age at onset of symptoms was 37 years (range=30-57) and the mean diagnostic delay was 7.7±7.7 years.

Isolated lower limb weakness was the most frequently reported initial symptom (n=30). In 50% of patients, weakness was initially symmetrical. The most common clinical diagnoses were LGMD (n=26) and MM (n=10). In five patients, the proximal or distal onset of symptoms could not be delineated and therefore they were classified as “proximo-distal”. Seven patients presented with atypical phenotypes; five had a pseudometabolic presentation, with pain and exercise intolerance, and two patients presented with isolated camptocormia and evidence of axial musculature involvement on muscle MRI (Figure 1). Furthermore, 2 more patients initially classified as pseudometabolic later developed an almost isolated camptocormia. Patients with an atypical phenotype showed a later onset of symptoms (mean 47.6±9.8 years). Globally, an initial misdiagnosis of polymyositis was reported in 9 patients.

Follow-up clinical findings. Median age at last examination was 54.5 years (range=35-86) with a mean disease duration of 16.7±10 years. Gastrocnemius atrophy was the most frequent finding (n=30) and was far more frequent than pseudohypertrophy (n=7). Additional frequent features were Achilles tendon contractures (n=14). Six patients had lost ambulation after 23±4.4 years, at a mean age of 59.8±7.5 years. Eight patients developed restrictive respiratory insufficiency, and four patients needed nocturnal NIV for obstructive sleep apnea syndrome. Patients needing NIV were generally older. Two patients had ischemic cardiomyopathy with LVEF < 50% (74 and 76 years, respectively). Nine patients had ECG abnormalities, with atrial fibrillation being the most frequent one (n=5, median age: 74 years, range=54-76), as well as grade I auriculo-ventricular block

(n=1), atrial extrasystoles (n=1), complete right bundle branch block (n=1) and sinus bradycardia (n=1).

Further phenotyping data were available for the French series (n=21). Five patients presented camptocormia, with involuntary trunk anterior flexion when standing or walking, ameliorated in the recumbent or supine position. Scapular winging was present in only 3 patients. In terms of function and disability, at a mean disease duration of 16.7±10 years, 8 patients were able to walk unaided, 7 needed unilateral support, 3 needed bilateral support and 3 were full-time wheelchair users. Median GMW scale was 4 (cannot climb stairs), ranging from 1 (unable to run) to 9 (unable to sit unsupported).

Comparison with patients with early onset dysferlinopathy. Forty-eight patients with dysferlinopathy from the COS study with an onset of disease before the age of 30 years were randomly selected as a control group (Table 2). Median age at disease onset was 18 years (range=0-28) compared to 37 years (range=30-57) for LO patients. The two groups did not differ for sex (p=0.306). The atypical phenotypes (pseudometabolic or camptocormia) were significantly more common in LO patients (p=0.014) and CK levels were lower in LO patients (p=0.01). After 15 years of disease duration, only 6 LO patients had lost ambulation compared to 13 EO patients (p=0.081), which also tended to occur later in the disease course for LO patients (23±4.4 years vs 16.3±6.8 years; p=0.064). In a sub analysis of patients from the COS study where the NSAD and ACTIVLIM scores were available, LO patients were less severely affected, both on the NSAD (p=0.008) and the ACTIVLIM functional scores (p=0.016). However, progression at 3-year follow-up was

similar in both groups, based on NSAD (p=0.45) and ACTIVLIM (p=0.556) functional scores (Figure 2).

With regard to protein expression on muscle, the proportion of patients with completely absent vs nearly absent dysferlin on WB did not differ between EO and LO patients (p=0.353), even after subdividing patients into very LO (≥40 years), LO (30-39 years) and EO subgroups (p=0.78).

Finally, LO patients more frequently showed missense variants and inframe deletions (41.9% vs. 26.1%; p=0.021) than loss of function variants compared to EO patients. Furthermore, homozygous variants of any type were more frequent in EO patients (18 vs 7; p=0.011). Pathogenic variants in LO patients were spread across all domains of the protein with the exception ferlin B and I and C2E domains, and no particular hotspot was observed (Figure 3).

Muscle biopsy findings in late vs early onset patients. Muscle biopsies from 11 LO dysferlinopathy patients could be revisited in detail and compared with those from 11 randomly selected EO patients. Biopsies were mainly from the deltoid muscle and the groups were matched for sex and time from disease onset to biopsy. However, patients in the LO group, as expected by the above mentioned clinical findings, were less severe on GMW scale and their CK levels were lower.

Two main muscle phenotypes were found: a classic dystrophic pattern with frequent necrosis, regeneration, increased connective and adipose tissue; and a mild myopathic pattern with frequent internalized nuclei, rare necrosis or regeneration, and without increased connective or adipose tissue (Figure 4). The dystrophic pattern predominated in

the EO patients (10/11), while in the LO group a mild myopathic pattern was quite frequent (5/11), but the difference between the groups did not reach statistical significance (p=0.056). The mild myopathic pattern was almost exclusively present in the atypical (axial or pseudometabolic) presentations (5/6). LO patients showed less perimysial inflammation (p=0.003) and necrosis (p=0.008), and although there was less proliferation of connective and adipose tissue in LO patients, the difference was not significant (p=0.392 and p=0.375) (Supplementary table 1). The intermyofibrillar structure (non-specific changes) was similarly altered in both groups, and COX-negative and rimmed vacuoles were infrequent. There was no evidence of a truncated dysferlin on WB in LO patients.

DISCUSSION

In our series of 48 patients with LO dysferlin-related muscle disorders, when compared to EO dysferlinopathy patients, we demonstrated 1) an increased prevalence of atypical phenotypes, including camptocormia and exercise intolerance along with the classical LGMD and Miyoshi distal phenotype, 2) lower severity on functional scales and a trend towards later loss of ambulation, and 3) milder muscle destruction with less dystrophic muscle changes and inflammation.

In the present study, the two classic phenotypes, LGMD and MM, were the most common within LO forms of dysferlinopathy. However, 2 patients presented with an isolated camptocormia, and 2 other patients developed an almost isolated camptocormia years after the initial onset of exercise intolerance and myalgia. Dysferlin-related muscle disorders are known to involve paraspinal muscles, although clinical manifestation of an

axial deficit is infrequent.[10,22] In line with this, two case reports have shown severe fatty degeneration of the lumbar erector spinae muscle on imaging and associated dysferlin deficiency without clinical evidence of camptocormia.[23,24]

Camptocormia may be present in extrapyramidal disorders, spine degenerative joint disease and neuromuscular diseases. Neuromuscular diseases account for 35% to 64% of all cases, although 40-50% of these neuromuscular cases are of undetermined origin.[25–27] Reported neuromuscular causes of camptocormia encompass amyotrophic lateral sclerosis, fascioscapulohumeral muscular dystrophy, inflammatory myopathies including inclusion body myositis, myotonic dystrophy and myasthenia gravis.[25] In line with our findings, in a series of 276 patients with camptocormia, two were classified as dysferlinopathy.[25] Therefore, clinical awareness of this subgroup of LO patients is critical to increase the diagnostic accuracy and direct the clinician towards the immunohistochemical study of sarcolemma proteins.

Disease progression in dysferlinopathy, although usually slow, is quite heterogeneous.[8,28] We show that LO patients have a slower disease progression. However, it is crucial to flag that differences in progression between the two groups were found retrospectively after a mean disease duration of 15 years, and not in the 3-year follow-up of the COS study. Therefore, stratification of the population by age at onset in clinical trials seems relevant in order to avoid analyzing populations with different underlying long-term progression curves.[29]

The fact that CK levels were significantly lower in LO patients, even though they were less severely affected, supports the hypothesis that muscle destruction is milder in LO patients

and led us to revisit in detail the muscle biopsies. Indeed, LO patients showed lower degrees of necrosis and regeneration, as well as of inflammation. Previous evidence suggests that decreased myotube fusion in dysferlinopathy could be attributed to intrinsic inflammatory activation,[30] although inhibition of inflammation with celastrol failed to improve muscle function in dysferlin-deficient mice.[31] The difference in inflammation levels and disease activity would need to be taken into account in future therapeutic choices and drug combinations.

Unlike other muscular dystrophies such as dystrophinopathies and sarcoglycanopathies,[32,33] the proportion of patients in the present study with nearly absent dysferlin protein expression in muscle did not differ between EO and LO dysferlinopathy patients, nor did it correlate with a less dystrophic muscle pattern. In line with this, previous reports failed to find a correlation between dysferlin expression and clinical disease severity or progression.[10] Furthermore, the clinical and anatomopathological differences observed cannot be fully explained by the type of variant. We found a wide variety of variants among the LO patients, without a mutational hotspot. In the Japanese population, the p.Trp999Cys variant has been reported to be associated with a later onset and lower CK values,[34] and, more recently, a hotspot of six missense variants was reported in the inner dysfF domain, also in Japanese patients.[35] Although we found variants targeting the dysfF domain (14/48 or 26.2%), we were not able to confirm this finding in our primarily Caucasian series as only two patients carried the p.Trp999Cys variant. We found almost double the frequency of missense variants in LO vs EO patients, which has also been recently reported,[36] and would support a milder

protein disruption of this variant type. On the other hand, the intrafamilial difference in age at onset in our study varied from 2 to 28 years, making it difficult to explain this difference by a monogenic change. Furthermore, homozygotes predominated in the EO group, and therefore other genetic and epigenetic actors could play a role in modulating the disease onset and severity. In line with this, an increased expression of annexin A1 and A2 protein present in muscle biopsies from patients with dysferlinopathy and other muscular dystrophies has been reported.[37] Progressive accumulation of annexin A2 (AnxA2) in myofiber causes fibroadipogenic progenitors (FAP) to differentiate into adipocytes in dysferlin-related LGMD.[38] Furthermore, AnxA2 facilitates muscle inflammation in dysferlinopathy and regenerating muscle.[39] We have shown that, on muscle biopsy, LO patients present less inflammation and necrosis, and a trend towards lower levels of adipose tissue infiltration, compared to EO patients. Furthermore, a truncated dysferlin protein was not observed on muscle WB in LO patients. Other disease gene modifiers should be further explored with the potential to become alternative approaches to therapeutic dysferlin overexpression.

Our study has some limitations. First, to be able to analyze 48 LO dysferlinopathy patients, an international effort was required, and thus patients were ascertained from two different series. Consequently, there was some heterogeneity in data collection and subsequently all variables may not have been recorded in both series, which may prevent a more in-depth clinical description. Nevertheless, both LO series were homogenous for the most relevant clinical and paraclinical findings, such as disease onset, CK level findings and dysferlin expression on muscle biopsy. In patients presenting with camptocormia,

muscle biopsies were not taken from the paraspinal muscles. Muscle pathology may vary from one muscle group to another; however, paraspinal muscles are rarely sampled clinically, and normative data have not been published.[40]

In summary, when compared to EO dysferlinopathy patients, patients with LO dysferlinopathy showed a higher frequency of atypical presentations, including isolated camptocormia or exercise intolerance. They also showed a slower progression of the disease over a long period. Furthermore, they presented milder dystrophic changes and less inflammation in muscle biopsy. These findings cannot be fully explained by dysferlin protein expression and do not seem to be correlated with specific dysferlin gene variants. These important findings should be taken into account to reduce study population heterogeneity and improve therapeutic targeting in clinical trials.

AUTHORS CONTRIBUTIONS

Gorka Fernández-Eulate: neurologic evaluation of patients, French patients' data retrieval, statistical analysis, writing of manuscript. Giorgia Querin: statistical analysis and revision of the manuscript. Ursula Moore: COS patients' data retrieval. Anthony Behin, Marion Masingue, Guillaume Bassez, Pascal Laforêt: neurologic evaluation of patients. Sarah Leonard-Louis: neurologic evaluation of patients and muscle biopsy interpretation. Thierry Maisonobe: muscle biopsy interpretation. Philippe-Edouard Merle, Marco Spinazzi, Guilhem Solé, Thierry Kuntzer, Anne-Laure Millet, Emanuelle Salort-Campana, Shahram Attarian, Yann Pereon, Leonard Feasson, Julie Graveleau, Aleksandra Nadaj-Pakleza: neurologic evaluation of patients and French patients' data retrieval. France Leturcq: genetic data and Western blot interpretation and data retrieval. Svetlana Gorokhova:

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Liu J, Aoki M, Illa I, et al. Dysferlin, a novel skeletal muscle gene, is mutated in Miyoshi myopathy and limb girdle muscular dystrophy. *Nat Genet*. 1998;20(1):31-36. doi:10.1038/1682
2. Bashir R, Britton S, Strachan T, 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(1):37-42. doi:10.1038/1689
3. Britton S, Freeman T, Vafiadaki E, et al. The third human FER-1-like protein is highly similar to dysferlin. *Genomics*. 2000;68(3):313-321. doi:10.1006/geno.2000.6290
4. Huang Y, Laval SH, Remoortere A, et al. AHNAK a novel component of the dysferlin protein complex, redistributes to the cytoplasm with dysferlin during skeletal muscle regeneration. *FASEB J*. 2007;21(3):732-742. doi:10.1096/fj.06-6628com
5. Cárdenas AM, González-Jamett AM, Cea LA, Bevilacqua JA, Caviedes P. Dysferlin function in skeletal muscle: Possible pathological mechanisms and therapeutical targets in dysferlinopathies. *Exp Neurol*. 2016;283(Pt A):246-254. doi:10.1016/j.expneurol.2016.06.026
6. Bulankina A V., Thoms S. Functions of Vertebrate Ferlins. *Cells*. 2020;9(3):534. doi:10.3390/cells9030534
7. Urtizberea J, Bassez G, Leturcq F, Nguyen K, Krahn M, Levy N. Dysferlinopathies. *Neurol India*. 2008;56(3):289-297. doi:10.4103/0028-3886.43447
8. Harris E, Bladen CL, Mayhew A, et al. The clinical outcome study for dysferlinopathy an international multicenter study. *Neurol Genet*. 2016;2(4).

doi:10.1212/NXG.0000000000000089

9. Illa I, Serrano-Munuera C, Gallardo E, et al. Distal anterior compartment myopathy: A dysferlin mutation causing a new muscular dystrophy phenotype. *Ann Neurol*. 2001;49(1):130-134. doi:10.1002/1531-8249(200101)49:1<130::AID-ANA22>3.0.CO;2-0

10. Nguyen K, Bassez G, Krahn M, et al. Phenotypic study in 40 patients with dysferlin gene mutations: High frequency of atypical phenotypes. *Arch Neurol*. 2007;64(8):1176-1182. doi:10.1001/archneur.64.8.1176

11. Aoki M, Liu J, Richard I, et al. Genomic organization of the dysferlin gene and novel mutations in Miyoshi myopathy. *Neurology*. 2001;57(2):271-278. doi:10.1212/WNL.57.2.271

12. Krahn M, Béroud C, Labelle V, et al. Analysis of the *DYSF* mutational spectrum in a large cohort of patients. *Hum Mutat*. 2009;30(2):E345-E375. doi:10.1002/humu.20910

13. Fanin M, Angelini C. Progress and challenges in diagnosis of dysferlinopathy. *Muscle and Nerve*. 2016;54(5):821-835. doi:10.1002/mus.25367

14. Angelini C, Grisold W, Nigro V. Diagnosis by protein analysis of dysferlinopathy in two patients mistaken as polymyositis. *Acta Myol*. 2011;30(DECEMBER):185-187. https://pubmed.ncbi.nlm.nih.gov/22616201/. Accessed August 22, 2020.

15. Katz JS, Rando TA, Barohn RJ, et al. Late-onset distal muscular dystrophy affecting the posterior calves. *Muscle and Nerve*. 2003;28(4):443-448. doi:10.1002/mus.10458

16. Griger Z, Nagy-Vincze M, Bodoki L, Gherardi RK, Dankó K, Hortobágyi T. Late onset dysferlinopathy mimicking treatment resistant polymyositis. *Jt Bone Spine*. 2016;83(3):355-356. doi:10.1016/j.jbspin.2015.03.017

17. Li F, Yin G, Xie Q, Shi G. Late-Onset Dysferlinopathy Presented as "Liver Enzyme" Abnormalities. *JCR J Clin Rheumatol*. 2014;20(5):275-277. doi:10.1097/RHU.0000000000000126

18. Klinge L, Dean AF, Kress W, et al. Late onset in dysferlinopathy widens the clinical spectrum. *Neuromuscul Disord*. 2008;18(4):288-290. doi:10.1016/j.nmd.2008.01.004

19. Suzuki N, Aoki M, Takahashi T, et al. Novel dysferlin mutations and characteristic muscle atrophy in late-onset miyoshi myopathy. *Muscle and Nerve*. 2004;29(5):721-723. doi:10.1002/mus.20025

20. Udd B, Stenzel W, Oldfors A, et al. 1st ENMC European meeting: The EURO-NMD pathology working group Recommended Standards for Muscle Pathology Amsterdam, The Netherlands, 7 December 2018. In: *Neuromuscular Disorders*. Vol 29. Elsevier Ltd; 2019:483-485. doi:10.1016/j.nmd.2019.03.002

21. Gallardo E, de Luna N, Diaz-Manera J, et al. Comparison of dysferlin expression in human skeletal muscle with that in monocytes for the diagnosis of dysferlin myopathy. *PLoS One*. 2011;6(12). doi:10.1371/journal.pone.0029061

22. Diaz-Manera J, Fernandez-Torron R, Llauger J, et al. Muscle MRI in patients with dysferlinopathy: Pattern recognition and implications for clinical trials. *J Neurol Neurosurg Psychiatry*. 2018;89(10):1071-1081. doi:10.1136/jnnp-2017-317488

23. Seror P, Krahn M, Laforet P, Leturcq F, Maisonobe T. Complete fatty degeneration of lumbar erector spinae muscles caused by a primary dysferlinopathy. *Muscle and Nerve*. 2008;37(3):410-414. doi:10.1002/mus.20910

24. Nagashima T, Chuma T, Mano Y, et al. Dysferlinopathy associated with rigid spine syndrome. *Neuropathology*. 2004;24(4):341-346. doi:10.1111/j.1440-1789.2004.00573.x

25. Ali F, Matsumoto JY, Hassan A. Camptocormia: Etiology, diagnosis, and treatment response. *Neurol Clin Pract*. 2018;8(3):240-248. doi:10.1212/CPJ.0000000000000453

26. Ghosh PS, Milone M. Camptocormia as presenting manifestation of a spectrum of myopathic disorders. *Muscle and Nerve*. 2015;52(6):1008-1012. doi:10.1002/mus.24689

27. Laroche M, Cintas P. Bent spine syndrome (camptocormia): A retrospective study of 63 patients. *Jt Bone Spine*. 2010;77(6):593-596. doi:10.1016/j.jbspin.2010.05.012

28. Moore U, Jacobs M, James MK, et al. Assessment of disease progression in dysferlinopathy: A 1-year cohort study. *Neurology*. 2019;92(5):E461-E474. doi:10.1212/WNL.0000000000006858

29. Mahjneh I, Marconi G, Bushby K, Anderson LVB, Tolvanen-Mahjneh H, Somer H. Dysferlinopathy (LGMD2B): A 23-year follow-up study of 10 patients homozygous for the same frameshifting dysferlin mutations. *Neuromuscul Disord*. 2001;11(1):20-26. doi:10.1016/S0960-8966(00)00157-7

30. Cohen T V., Cohen JE, Partridge TA. Myogenesis in dysferlin-deficient myoblasts is

inhibited by an intrinsic inflammatory response. *Neuromuscul Disord*. 2012;22(7):648-658. doi:10.1016/j.nmd.2012.03.002

31. Dillingham BC, Benny Klimek ME, Gernapudi R, et al. Inhibition of inflammation with celastrol fails to improve muscle function in dysferlin-deficient A/J mice. *J Neurol Sci*. 2015;356(1-2):157-162. doi:10.1016/j.jns.2015.06.042

32. Hoffman EP, Fischbeck KH, Brown RH, et al. Characterization of Dystrophin in Muscle-Biopsy Specimens from Patients with Duchenne's or Becker's Muscular Dystrophy. *N Engl J Med*. 1988;318(21):1363-1368. doi:10.1056/NEJM198805263182104

33. Alonso-Pérez J, González-Quereda L, Bello L, et al. New genotype-phenotype correlations in a large European cohort of patients with sarcoglycanopathy. *Brain*. 2020. doi:10.1093/brain/awaa228

34. Takahashi T, Aoki M, Suzuki N, et al. Clinical features and a mutation with late onset of limb girdle muscular dystrophy 2B. *J Neurol Neurosurg Psychiatry*. 2013;84(4):433-439. doi:10.1136/jnnp-2011-301339

35. Izumi R, Takahashi T, Suzuki N, et al. The genetic profile of dysferlinopathy in a cohort of 209 cases: Genotype–phenotype relationship and a hotspot on the inner DysF domain. *Hum Mutat*. 2020. doi:10.1002/humu.24036

36. Park HJ, Hong Y Bin, Hong J, et al. Null variants in DYSF result in earlier symptom onset . *Clin Genet*. November 2020. doi:10.1111/cge.13887

37. Cagliani R, Magri F, Toscano A, et al. Mutation finding in patients with dysferlin deficiency and role of the dysferlin interacting proteins annexin A1 and A2 in

muscular dystrophies. *Hum Mutat*. 2005;26(3):283. doi:10.1002/humu.9364

38. Hogarth MW, Defour A, Lazarski C, et al. Fibroadipogenic progenitors are responsible for muscle loss in limb girdle muscular dystrophy 2B. *Nat Commun*. 2019;10(1). doi:10.1038/s41467-019-10438-z

39. Defour A, Medikayala S, Van der Meulen JH, et al. Annexin A2 links poor myofiber repair with inflammation and adipogenic replacement of the injured muscle. *Hum Mol Genet*. 2017;26(11):1979-1991. doi:10.1093/hmg/ddx065

40. Witting N, Andersen LK, Vissing J. Axial myopathy: An overlooked feature of muscle diseases. *Brain*. 2016;139(1):13-22. doi:10.1093/brain/awv332

FIGURE LEGENDS

FIGURE 1

Axial involvement on MRI of patients with camptocormia.

Two patients with very late onset dysferlinopathy (onset after 50 years of age) presenting with isolated camptocormia. **A)** Patient A's muscle MRI: complete paraspinal muscle degeneration is observed, as well as nearly complete degeneration of both gastrocnemius medialis and right soleus muscle. Some proximal anterior and posterior bilateral thigh fat replacement is observed. **B)** Patient B's muscle MRI: complete paraspinal muscle degeneration is observed as well as mild bilateral gastrocnemius muscle fat replacement. Ps, paraspinal; GAm, gastrocnemius medialis; So, soleus; VI, vastus intermedius; VM, vastus medialis; AM, adductor magnus; SM, semimembranosus.

FIGURE 2

Progression rate in patients with dysferlinopathy.

A) Creatine kinase (CK) levels in EO (red) vs LO (blue) patients. **B**) Correlation of age at loss of ambulation from onset and age at onset of symptoms in EO (red) vs LO (blue) patients. **C)** Time to loss of ambulation from disease onset in EO (green) vs LO (orange) patients.

EO, early onset; LO, late onset.

FIGURE 3

Genetic findings in patients with dysferlinopathy.

Total number of variants: 93 in late onset patients, 92 in early onset patients.

Previously reported functions of specific dysferlin domains as well as interactions sites with other muscular protein are represented.

*Binding sites to interacting proteins.

Domains: Fer, Ferlin domain; NdysfF, dysferlin domain N-terminal region; IdysfF, inner dysferlin domain; CdysfF, dysferlin domain C-terminal region; TM, transmembrane domain.

FIGURE 4

Muscle biopsy findings in patients with dysferlinopathy.

EO patients with dysferlinopathy: 1) Classic dysferlin-related dystrophic pattern in a patient with an early onset (<20 years) LGMD: a) perimysial and perivascular Inflammation as well as necrosis on x10 H&E; b) perivascular inflammation and necrosis on x20 H&E; and c) connective tissue infiltration as well as necrosis on x20 H&E. **2)** A dystrophic pattern in a second EO patient with a Miyoshi myopathy: a) necrosis and connective tissue

infiltration on x20 H&E and b) GT, as well as, interestingly, c) some rods on x40 GT. **LO patients with dysferlinopathy: 3)** Mild myopathic pattern in a patient with very late onset (>40 years) pseudometabolic phenotype subsequently developing a camptocormia: a) mild nuclear internalization, sometimes centralized on x20 H&E and b) x20 GT; c) mild intermyofibrillar alteration on x20 DPNH; d) no COX-negative fibers; e) absent dysferlin on IHC; and f) present dystrophin as control. **4)** A second mild myopathic pattern in a patient with very late onset camptocormia: a) moderate nuclear centralization on x20 H&E; and b-c) some necrotic fibers on x20 H&E. **5)** A dystrophic pattern in a patient with a very late onset proximo-distal phenotype: a) fiber disproportion and some necrosis, including a tunneled fiber on x20 H&E, b) necrosis on x20 GT, and c) no intermyofibrillar alteration on x20 DPNH. **6)** WB of 14 patients with LO-dysferlinopathy. No lower molecular weight or truncated dysferlin is observed, but some patients may show a secondary calpain-3 deficiency. H&E, hematoxylin-eosin; GT, Gomori's trichrome; DPNH, dinitrophenylhydrazine; COX, cytochrome oxidase; WB, Western blot; C, control.

TABLES

TABLE 1 Demographic characteristics of patients with late onset dysferlinopathy.

Patients (n)	**Total (48)**	French series (21)	International cohort (27)
Demography			
Sex (female)	**28 (58.3)**	14 (66.7)	14 (51.9)
Affected sibling	**16 (33.3)**	8 (38.1)	8 (29.6)
Ethnicity			
Caucasian	**40 (83.3)**	19 (90.5)	21 (77.8)
Asian	**4 (8.3)**	0	4 (14.8)
African	**3 (6.3)**	2 (9.5)	1 (3.7)
Hispanic	**1 (2.1)**	0	1 (3.7)
Onset of disease			
Age at disease onset (years)	**37 (30-57)**	39 (30-57)	35 (30-48)
Onset group			
Late	**29 (60.4)**	11 (52.4)	18 (66.7)
Very late	**19 (39.6)**	10 (47.6)	9 (33.3)
Symptoms at onset			
Weakness	**30 (62.5)**	14 (66.7)	16 (59.3)
Pain	**4 (8.3)**	2 (9.5)	2 (7.4)
Exercise intolerance	**1 (2.1)**	1 (4.8)	0
Combination	**13 (27.1)**	4 (19)	9 (33.3)
Phenotype			
LGMD	**26 (54.2)**	5 (23.8)	21 (77.8)
Miyoshi myopathy	**10 (20.1)**	6 (28.6)	4 (14.8)
Proximo-distal	**4 (8.33)**	4 (19)	0
Pseudometabolic	**5 (10.4)**	4 (19)	1 (3.7)
Camptocormia	**2 (4.2)**	2 (9.5)	0
UK	**1 (2.1)**	0	1 (3.7)
Polymyositis misdiagnosis	**9 (18.8)**	6 (28.6)	3 (11.1)
Weakness symmetry	**24 (50)**	14 (66.7)	10 (37)
Follow-up			
Age at diagnosis (years)	**43 (26-65)**	51.5 (33-65)	40 (26-56)
Diagnostic delay (years)	**7.7 (±7.7)**	10.8 (±8.2)	4.9 (±6)
Age at examination (years)	**54.5 (35-86)**	58 (41-76)	52 (35-86)
Time since onset (years)	**16.7 (±10)**	17.6 (±10.6)	16 (±9.6)
Loss of ambulation	**6**	3	3
Age at loss of ambulation (years)	**55 (55-72)**	63.5 (55-72)	55 (55-62)

Baseline CK level (mean U/l)	**2984 (±2182)**	2846 (±2057)	3056 (±2282)
Cardio-respiratory			
Cardio			
LVEF < 50%	**2 (4.2)**	2 (9.5)	0
ECG abnormalities	**9 (18.8)**	5 (23.8)	4 (14.8)
Respiratory			
FVC (%)	**87.4 (±22.1)**	93.3 (±25.5)	84 (±19.7)
FVC <70%	**8 (16.7)**	3 (14.3)	5 (18.5)
NIV	**4 (8.3)**	3 (14.3)	1 (3.7)
Age at NIV (years)	**54 (47-70)**	62 (54-70)	47 (47-47)
Biopsy	**39 (81.3)**	17 (81)	22 (81.5)
IHC available	**32**	13	19
Reduced	**7 (21.9)**	2 (15.4)	5 (26.3)
Absent	**25 (78.1)**	11 (84.6)	14 (73.7)
WB available	**29**	15	14
Reduced	**10 (34.5)**	4 (26.7)	6 (42.9)
Absent	**19 (65.5)**	11 (73.3)	8 (57.1)

For categorical variables: absolute number of patients with the percentage in parentheses. For continuous variables: age at disease onset, age at diagnosis, age at examination, age at loss of ambulation and age at NIV shown as: median (range); diagnostic delay, time since onset and baseline CK shown as mean (± standard deviation). LO, late onset; EO, early onset; LGMD, limb girdle muscle dystrophy; UK, unknown; CK, creatine kinase; LVEF, left ventricular ejection fraction; ECG, electrocardiogram; FVC, forced vital capacity; NIV, non-invasive ventilation; IHC, immunohistochemistry; WB, Western blot.

TABLE 2 Clinical characteristics of patients with late onset versus early onset dysferlinopathy.

Patients (n)	**LO (48)**	**EO (48)**	**p**
Age at disease onset (years)	27 (30-57)	18 (0-28)	
Sex (female)	23 (47.9)	28 (58.3)	0.306
Protein expression			
IHC (absent)	17/25 (68)	25/32 (78.1)	0.249
WB (absent)	21/28 (75)	19/29 (65)	0.353
Polymyositis	9 (18.8)	9 (18.8)	0.96
Phenotype			0.076
LGMD	26 (54.2)	24 (50)	
Miyoshi myopathy	10 (20.8)	17 (35.4)	
Proximo-distal	4 (8.3)	5 (10.4)	
Pseudometabolic or axial	8 (16.7)	1 (2.1)	0.014
Isolated CK	0	1 (2.1)	
Disease duration (years)	15.5 (1-45)	15 (2-46)	0.584
LoA	6 (12.5)	13 (27.1)	0.081
Time to LoA from onset of symptoms (years)	16.3 (±6.8)	23 (±4.4)	0.064
Baseline CK level (U/l)	2855 (±2020)	4394 (±3192)	0.01
NSAD score	19	30	
Baseline	35 (14-53)	22 (4-51)	0.008
At 3 years	33 (6-54)	18 (2-44)	0.005
Difference	3 (-11-14)	4 (-4-27)	0.45
ACTIVLIM score	21	42	
Baseline	28 (10-36)	26 (2-35)	0.016
At 3 years	26 (7-36)	22.5 (0-36)	0.074
Difference	3 (-6-10)	2 (-7-17)	0.57

For categorical variables: absolute number of patients with the percentage in parentheses. For continuous variables: age at disease onset, disease duration, NSAD and ACTIVLIM scores shown as median (range); time to LoA from onset of symptoms and CK levels shown as mean (± standard deviation). LO, late onset; EO, early onset; IHC, immunohistochemistry; WB, Western blot; LGMD, limb girdle muscle dystrophy; CK, creatine kinase; LoA, loss of ambulation; NSAD, North Star Assessment for Dysferlinopathy; ACTIVLIM: measure of activity limitation.