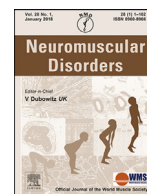




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## Expanding the muscle imaging spectrum in dysferlinopathy: description of an outlier population from the classical MRI pattern



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## ABSTRACT

Dysferlinopathy is a muscle disease characterized by a variable clinical presentation and is caused by mutations in the *DYSF* gene. The Jain Clinical Outcome Study for Dysferlinopathy (COS) followed the largest cohort of patients ( $n=187$ ) with genetically confirmed dysferlinopathy throughout a three-year natural history study, in which the patients underwent muscle function tests and muscle magnetic resonance imaging (MRI). We previously described the pattern of muscle pathology in this population and established a series of imaging criteria for diagnosis. In this paper, we describe the muscle imaging and clinical features of a subgroup of COS participants whose muscle imaging results did not completely meet the diagnostic criteria. We reviewed 184 T1-weighted (T1w) muscle MRI scans obtained at the baseline visit of the COS study, of which 106 were pelvic and lower limb only and 78 were whole-body scans. We identified 116 of the 184 patients (63%) who did not meet at least one of the established imaging criteria. The highest number found of unmet criteria was four per patient. We identified 24 patients (13%) who did not meet three or more of the nine established criteria and considered them as “outliers”. The most common unmet criterion (27.3% of cases) was the adductor magnus being equally or more affected than the adductor longus. We compared the genetic, demographic, clinical and muscle function data of the outlier patients with those who met the established criteria and observed that the outlier patients had an age of disease onset that was significantly older than the whole group (29.3 vs 20.5 years,  $p=0.0001$ ).

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This study expands the phenotypic muscle imaging spectrum of patients with dysferlinopathy and can help to guide the diagnostic process in patients with limb girdle weakness of unknown origin.

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## 1. Introduction

Dysferlinopathy is an autosomal recessive muscular dystrophy caused by mutations in the *DYSF* gene encoding the protein dysferlin, which is mainly located in the muscle fibre membrane. Patients with dysferlinopathy can present with variable muscle involvement, which can make it difficult to reach a diagnosis exclusively on clinical grounds, requiring complementary tests to be performed in order to distinguish dysferlinopathy from other forms of muscular dystrophies.

Muscle magnetic resonance imaging (MRI) has been demonstrated to be useful in guiding the genetic diagnosis of patients with neuromuscular diseases based on the findings identified on T1-weighted imaging and in Short Tau Inversion Recovery (STIR) imaging sequences [1–3]. The selective pattern of muscle involvement can differ from one disease to another. We described the pattern of muscle involvement in dysferlinopathy based on our review of the 184 muscle MRIs collected at the baseline visit of the COS study [4]. We found that dysferlinopathy is characterised by an early involvement of the posterior compartment of the thighs and legs along with fatty replacement of the paraspinal and subscapularis muscles. In the later stages of the disease, the proximal muscles of the arms, the periscapular muscles and the muscles of the anterior compartment of the thighs and legs are also involved. Finally, the finger flexor muscles of the forearm, abdominal muscles and gluteus major muscle are also affected [5]. This pattern was shown to be consistent, and led to the description of a series of 18 imaging criteria for the diagnosis of dysferlinopathy that were individually met on average by more than 92% of patients [6]. These criteria have also been reported for other dysferlinopathy cohorts, which supports their validity to suggest a diagnosis of dysferlinopathy in patients with undiagnosed muscle weakness [5,7–9]. However, we observed that several patients did not meet all criteria, suggesting that there were patients who, despite having a genetic diagnosis of dysferlinopathy, could present with a different pattern of muscle involvement on MRI. The aim of this study was to revisit the original cohort of 184 patients whose MRI was obtained at the baseline visit of the COS study, identify those patients who did not fully meet the MRI criteria, describe their imaging features, and identify whether there were differences in their demographic, genetic, clinical and disease progression features.

## 2. Materials and Methods

### 2.1. Muscle MRI

#### 2.1.1. Acquisition

One hundred and eighty-four patients from 15 sites (Newcastle, Barcelona, Seville, Munich, Berlin, Padua, Marseille, Paris, Saint Louis, Columbus, Charlotte, Washington DC, Stanford, Tokyo and Sydney) underwent a baseline muscle MRI scan, 78 of whom had whole-body and 106 had pelvic and lower-limb scans. The MRI protocol consisted of T1-weighted (T1w), Dixon, B1 map and T2-weighted sequences and can be found in the original publication [6].

#### 2.1.2. Semiquantitative assessment and review of pattern criteria

We reviewed the previously established 18 diagnostic criteria designed for the analysis of the T1w scans that were published in the original study (detailed in supplementary file 1). We selected those criteria that were met by less than 90% of the patients in the original publication. The selected criteria were the following: 1) the subscapularis muscle is the most affected of the shoulder girdle muscles, 2) the levator scapulae is the least affected shoulder girdle muscle, 3) the paraspinal muscles are equally or more severely involved than the abdominal muscles, 4) the gluteus minimus is equally or more severely involved than the gluteus medius and maximus, 5) the obturator externus is equally or more severely involved than the gluteus maximus, 6) the adductor magnus is equally or more severely involved than the adductor longus, 7) the rectus femoris is not spared when the vasti muscles are involved, and 8) the peroneus is equally or more severely involved than the tibialis anterior. Fig. 1 and Fig. 2 show T1w images of patients fitting the criteria described. As symmetry has been described as an imaging feature in patients with dysferlinopathy [5], we also reviewed this feature in the whole cohort and considered that a patient showed an asymmetric pattern when at least four muscles had two or more-point difference in the Mercuri score between the left and right side. Symmetry was considered the ninth imaging criteria. A neurologist with experience in MRI analysis (LL) reviewed the 184 baseline T1w MRI sequences, compared the Mercuri scores with that from the original study evaluation, and analysed whether each patient met the nine criteria selected. We calculated how frequently each criterion was not met by the patients in our series.

We have compared our assessment with the original analysis of the series and have seen no major discrepancy between Mercuri scores.

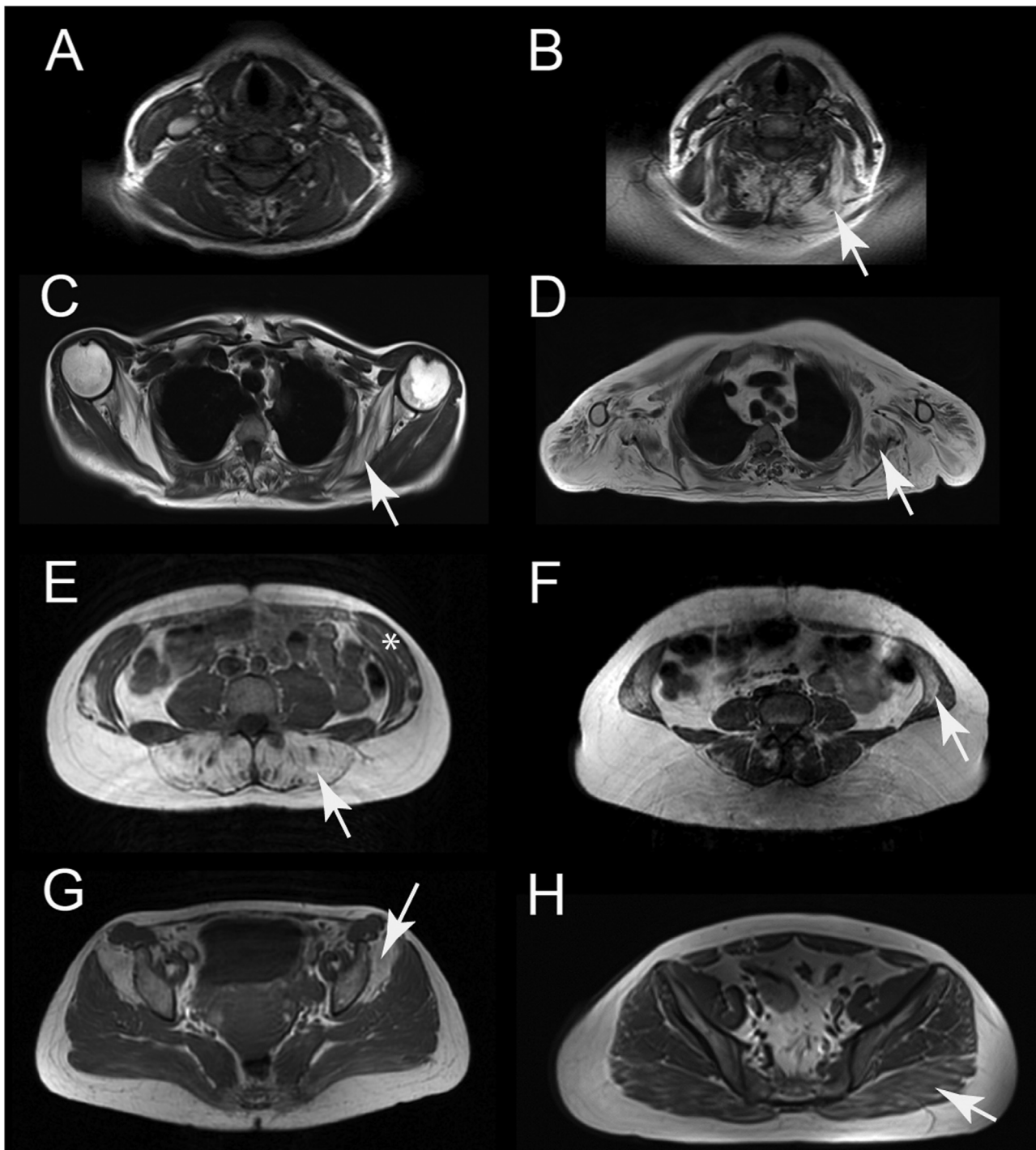
#### 2.1.3. Defining outliers from the common MRI pattern

We defined those patients who did not meet 3 or more of the 9 investigated criteria as ‘outliers’. As we identified many patients with asymmetries in one or two muscles (only 51.6% had zero asymmetric muscles), we defined the criterion of ‘asymmetry’ as having at least four muscles showing differences of two or more points on the Mercuri score. As dysferlinopathy has been consistently defined as a symmetrical disease by many reports [5,7–9], we also considered as outliers those patients not fulfilling just two criteria if one of them was asymmetry.

### 2.2. Imaging and clinical data collected

We collected the following imaging data from all of the patients: the type of MRI performed at baseline (lower limbs including pelvis, lower limbs without pelvis, and whole-body images); whether the patient met/did not meet each pattern criterion; when significant left-right asymmetry was observed in any muscle (2 or more points on the Mercuri score); the number and name of the asymmetric muscles; the number of criteria not met by each patient.

We collected clinical data such as demographic features including gender, ethnicity, age at onset and symptom duration at baseline MRI, as well as data on exercise levels during adolescence,

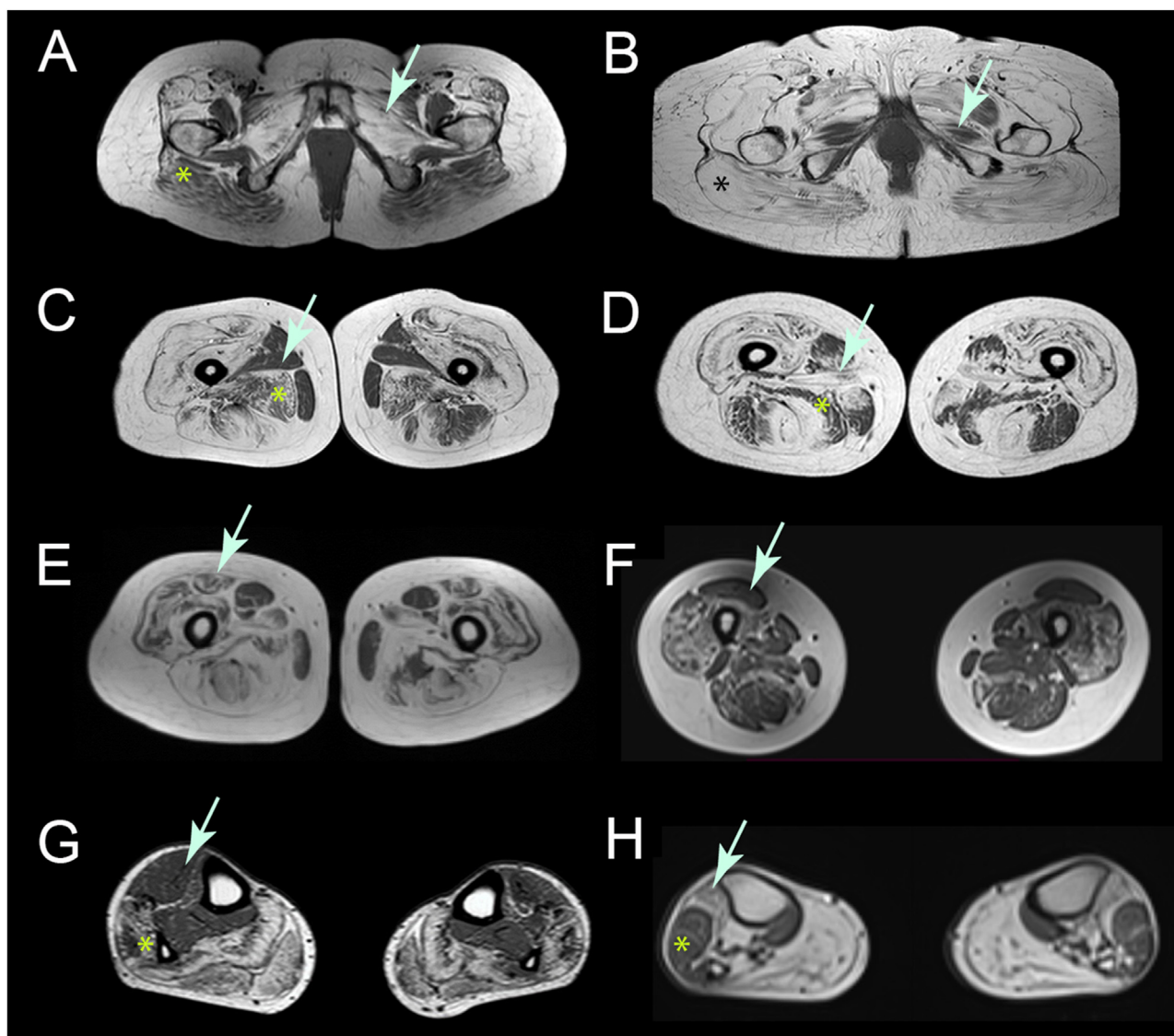


**Fig. 1.** Dysferlin MRI pattern: common findings and exception to the rules (1).

Panel of images of patients with muscular dystrophy produced by mutation in the dysferlin gene showing the characteristic features and examples of the exceptions to the rules. A) Cervical muscles are commonly non affected in this disease. B) Example of a patient with involvement of the cervical muscles (arrow). C) Subscapularis tends to be the most affected periscapular muscle (arrow). D) Example of a patient with severe involvement of all periscapular muscles, while subscapularis is less affected (arrow). E) Paraspinal muscles (arrow) are more severely affected than abdominal muscles (asterisk). F) Paraspinal muscles (arrow) are more severely affected than the paraspinal ones. G) Gluteus minor (arrow) is more affected than the gluteus maximus in patients. H) Example of a patient who has higher fat replacement in the gluteus maximus (arrow) than in the gluteus minor.

classified by metabolic equivalents (METs), as previously described in the same cohort (0: no physical activity; 1: mild activity; 2: moderate activity; 3: vigorous activity) [10]. The following data were also collected: muscle involvement at clinical presentation, isolated hyperCKemia, genetic data including the number of pathogenic/likely pathogenic variants, type of mutation (nonsense,

missense, frameshift or splice site) and homozygosity versus compound heterozygosity. For each patient we analysed whether dysferlin was residually expressed or absent based on the data provided by skeletal muscle western blot (WB). If WB was not available, protein expression was collected based on monocyte dysferlin expression studies and/or muscle immunohistochemistry



**Fig. 2.** Dysferlin MRI pattern: common findings and exception to the rules (II).

A) Obturator externus (arrow) is equally or more severely involved than the gluteus maximus in dysferlinopathy patients (asterisk). B) Example of a patient with a more severe involvement of the gluteus maximus (asterisk) than the obturator externus (arrow). C) Adductor magnus (asterisk) is equally or more severely involved than the adductor longus (arrow). D) Example of a patient with a more severe involvement of the adductor magnus (asterisk) than the adductor longus (arrow). E) Rectus femoris (arrow) is affected if vasti muscles are severely involved. F) Example of a patient with a not affected rectus femoris (arrow) despite severe fat replacement of vasti muscles. G) The peroneus (asterisk) is equally or more involved than the tibialis anterior (arrow). H) Example of a patient with peronei muscles non affected (asterisk) despite severe fat replacement of tibialis anterior (arrow).

(IH). Moreover, we also collected data on muscle function, including muscle strength assessed by manual muscle testing (MMT) and muscle function using the North Start Ambulatory Assessment for patients with Dysferlinopathy (NSAD) at baseline and 3 years follow up that was available in the COS study.

### 2.3. Statistical analysis

The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test the distribution of continuous variables. In our cases, none of the variables followed normality ( $p < 0.05$ ) and therefore non-parametric tests were used for the analysis of these variables. Comparative analysis of age at symptom onset and symptom duration between the two groups was performed using the Mann-Whitney  $U$  test. To compare categorical variables (gender, outlier yes/no, asymmetry yes/no, levels of protein expression reduced/absent) and ordinal variables (ethnicity and level of

exercise) between outliers and non-outliers, we used the Chi-square test. All tests were bilateral and a  $p$ -value ( $\alpha$ ) of 0.05 or lower was considered significant. The software package SPSS V21 was used for all analyses.

## 3. Results

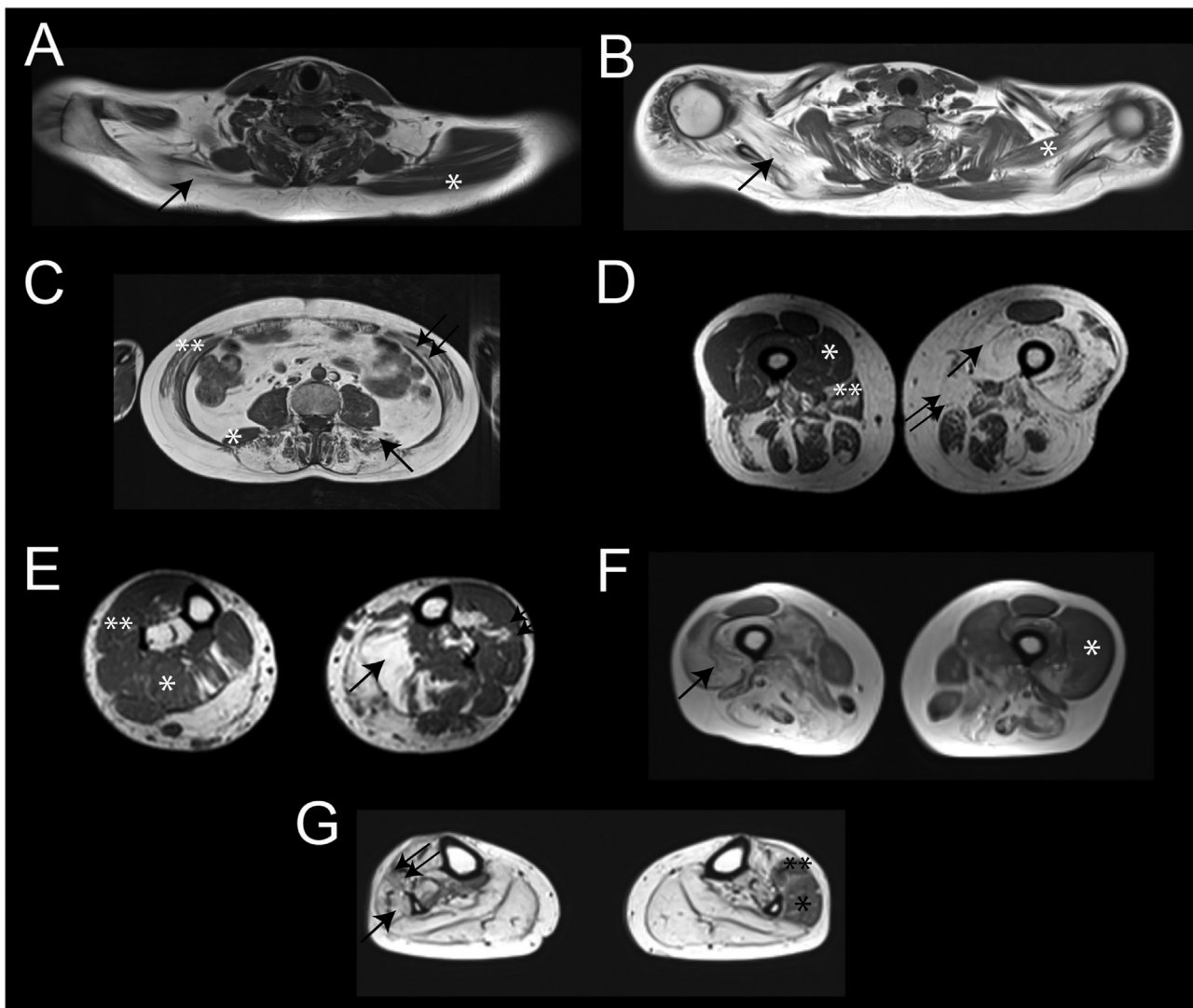
### 3.1. Criteria selected for the analysis

We selected the criteria that were met by a lower number of patients in the original publication, that is, those criteria fulfilled by 67 to 95% of patients. The selected criteria can be found in [Table 1](#). As significant asymmetries were found in only a minority of the patients studied both in the original paper and in other reports, we also selected symmetry as a criterion to be reviewed in all patients in this cohort.

**Table 1**  
Criteria selected for dysferlinopathy pattern.

Criteria	% of cases not fulfilling the criteria
1) The subscapularis muscle is the most affected in the upper limb girdle	16.7
2) Levator scapulae is the less affected muscle in the upper limb girdle	1.3
3) Paraspinal muscles are equally or more involved than abdominal muscles	3.4
4) Gluteus minimus is equally or more severely involved than gluteus medius and maximus	3.3
5) Obturator externus is equally or more severely involved than the gluteus maximus	16.2
6) Adductor magnus is equally or more severely involved than the adductor longus	27.3
7) Rectus femoris is not spared when the vasti muscles are involved	19.1
8) Peroneus is equally or more involved than the tibialis anterior	13.6
9) Symmetric involvement between left and right side	22.3

Table 1: Muscle MRI diagnostic criteria selected for this analysis and % of cases not fulfilling each of the criteria in the whole cohort.

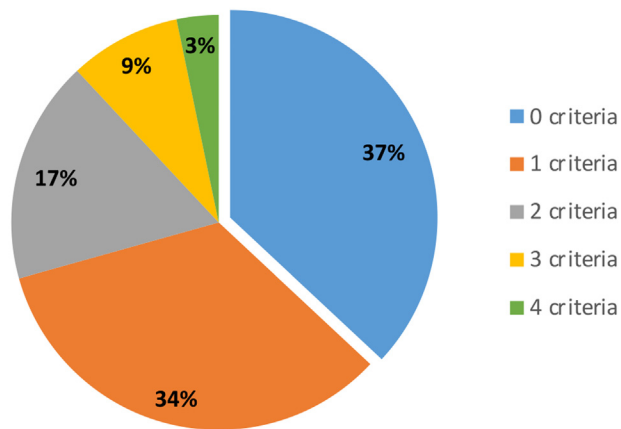


**Fig. 3.** Examples of dysferlin patients with asymmetric changes in muscle MRI. Asterisks show the preserved muscle while arrows show the more affected contralateral muscle. A) MRI showing asymmetric involvement of the trapezius; B) MRI showing asymmetric involvement of the subscapularis muscle. C) MRI showing asymmetric involvement of the quadratus lumborus (single arrow/asterisk) and transversus abdominis (double arrow/asterisk); D and F) MRIs showing asymmetric involvement of the quadriceps (single arrow/asterisk) and sartorius (double arrow/asterisk); E) Patient showing asymmetric involvement of the soleus (single arrow/asterisk) and extensor digitorum (double arrow/asterisk). G) Patient showing asymmetric involvement of the peronei (single arrow/asterisk) and the extensor digitorum (double arrow/asterisk).

MRI scans showing exceptions to each criterion are shown in Fig. 1 and Fig. 2 and examples of patients with asymmetries are shown in Fig. 3. We identified 63% of the patients (116/184) with at least one discordant criterion; most of these patients showed discordance in only 1 criterion (33.7%, 62/184), followed by patients discordant for 2 criteria (17.4%, 32/184), 3 criteria (8.7%, 16/184) or 4 criteria (3.2%, 6/184) (see Fig. 4).

In the upper limbs, the criterion that had the most exceptions to the rule was the subscapularis being the most severely affected periscapular muscle (13/78 patients). In these 13 cases, we observed that other periscapular muscles, such as the supraspinatus and infraspinatus, were more affected than the subscapularis. On the other hand, the most commonly met criterion was the levator scapulae being the least affected muscle

## Discordant criteria (n=184)



**Fig. 4.** Distribution of patients not fulfilling MRI proposed diagnostic criteria. Pie graph showing the percentage of patients with not discordant criteria or with 1 to 4 discordant criteria.

in the scapular girdle, with only 1/78 patients not meeting this criterion. In the lower limbs, the criterion with the most exceptions (50/183) was the adductor magnus being more affected than adductor longus, either uni- or bilaterally, while the criterion with the fewest exceptions was the gluteus minimus being more affected than the gluteus medius or maximus (5/151). The percentage of patients not fulfilling each criterion are shown in Fig. 5.

Based on the total number of discordant criteria found in each patient, 24 outliers were identified (13% of the 184 patients). Of these, 21 patients were selected for having three or more discordant criteria and only three patients were selected for having two discordant criteria when one of the criteria was severe asymmetric involvement. Four out of the five patients not meeting the criteria “gluteus minimus more affected than gluteus maximus” were outliers with three or more discordant criteria (all of them in the lower limbs). Similarly, the only patient that did not meet the elevator scapulae criterion, and two out of three patients not meeting the paraspinal muscles criteria (different patients than those discussed above), were also outliers with three discordant criteria. This fact could suggest that not meeting the

gluteus minimus, paraspinal muscles and elevator scapulae criteria (see Table 1) can predict significant discordance to more pattern criteria in the lower limbs and may reasonably predict outlier patients.

### 3.2. Demographic characteristics

Of the 24 outlier patients, 12 were female and 12 were male, while in the non-outlier group there were 79 females and 81 males. Regarding ethnicity, 20 out of the 24 outliers were white Caucasian (83.3%), while there were only 1 Hispanic (4.2%) and 3 Asian patients (12.5%). The distribution of sex and ethnicity between the two groups was compared using the Chi-square test and we did not find statistically significant differences ( $p = 0.97$  for gender and  $p = 0.67$  for ethnicity).

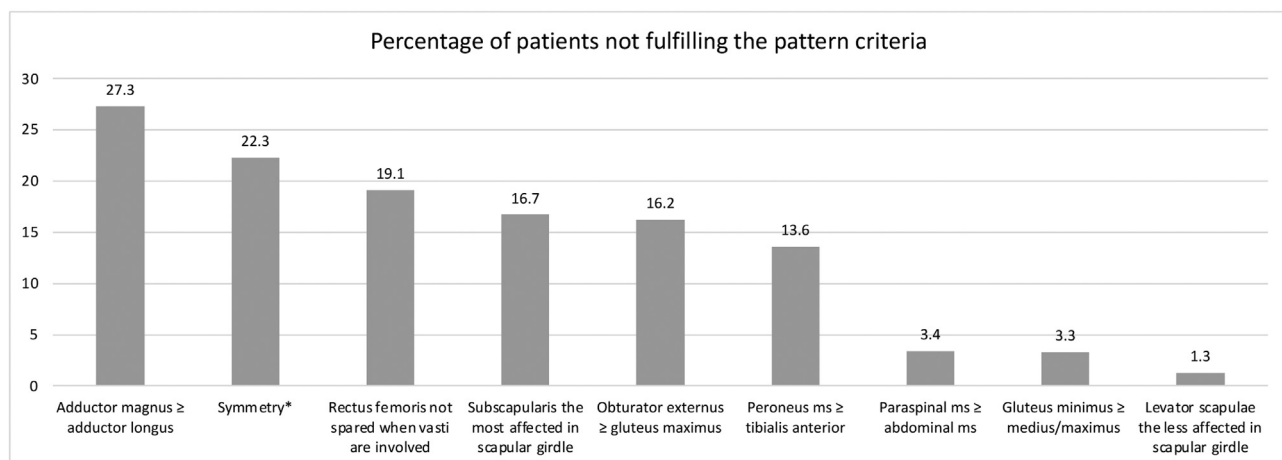
### 3.3. Phenotype

Of the 24 patients, 17 had a clinical presentation as limb-girdle muscular weakness (LGMDR2: 70.83%), 5 had Miyoshi myopathy (MM: 20.83%) and 2 had asymptomatic hyperCKemia (8.33%). Comparing the clinical phenotype (LGMDR2, MM or hyperCKemia), there were no differences in distribution between the two groups ( $p = 0.39$ ).

### 3.4. Age of onset and muscle function data

There were no data on the age of symptom onset or disease duration in 5 of the 160 non-outlier patients. In the outlier group, mean age at symptom onset was 29.3 years (SD = 10.8 years) with a range of 12-86 years of age, while the mean was 20.5 years in the non-outlier group (SD = 7.5y, range 8-67y). This difference was statistically significant using the Mann-Whitney U test ( $p = 0.0001$ ). There were no differences regarding symptom duration across the two groups ( $p = 0.73$ ). The mean disease duration for all patients was 16.7 years (SD = 9.9 years) with a range of 1-51 years.

We also compared muscle function between the two groups of patients. Outlier patients had statistically significant better results on the MMT assessment of muscle strength compared to non-outliers in the muscle strength assessed using MMT between outlier and non-outliers (outliers median value 7.50 [IQR 6.13-7.99] vs non-outliers median value 4.97 [IQR 3.50-7.15], Mann Whitney U test, 0.009). However, we did not identify significant



**Fig. 5.** Percentage of patients not fulfilling each MRI proposed diagnostic criteria. Bar graph showing the percentage of patients not filling each of the proposed criteria analyzed in this paper. Ms: muscle.

asymmetries in muscle strength in outlier patients. Moreover, there were significant differences between outliers and non-outliers in NSAD at baseline (outlier median value 35 [IQR 25.75–45.75] vs non-outliers median value 24 [IQR 16.25–43], Mann Whitney U test, 0.03) and at 3 years follow-up (outlier median value 26.5 [IQR 19.75–38.75] vs non-outliers median value 12 [IQR 4–27], Mann Whitney U test, 0.003). However, there were no differences in the progression rate during the follow-up between both groups (median decline in NSAD outliers  $-2.5$  [IQR  $-10.75$  –  $-0.5$ ] vs median decline in NSAD non-outliers  $-4$  [IQR  $-8.50$  –  $-2$ ], Mann Whitney U test 0.7).

### 3.5. Exercise levels prior to disease onset

Exercise data was not available for 12 out of the 160 non-outlier patients; however, we had data on exercise levels for all of the outliers. The four ordinal categories of exercise levels prior to disease onset (0 to 3, from absent to intense) were compared between the two groups using the Chi-square test, without finding statistically significant differences ( $p = 0.23$ ).

### 3.6. Genetic mutations

Of the 24 outlier patients, 6 had homozygous mutations (3 missense, 2 frameshift and 1 nonsense) and 18 had compound heterozygous mutations (no identified predominance between missense, nonsense, splice site or frameshift mutations). All the heterozygous outlier patients had two pathogenic or likely pathogenic DYSF variants except for one who had a pathogenic variant and a second variant of unknown significance (VUS). This patient had absent levels of dysferlin in WB analysis of muscle biopsy, compatible with the diagnosis of dysferlinopathy. In the non-outlier group, there were only six patients with only one known DYSF variant but otherwise a compatible diagnosis of dysferlinopathy according to low/absent levels of dysferlin in muscle biopsies' IH, WB and/or monocyte expression. We did not identify a specific genotype–phenotype correlation neither presence of more uncertain variants in the outlier group.

### 3.7. Protein expression

Regarding the levels of dysferlin expression, WB performed on muscle biopsies was taken as the gold standard to assess levels of protein expression (absent versus reduced). In the patients without available muscle WB, data concerning monocyte WB was analysed if this was performed [11]. If not, data from the muscle IH was collected. The comparison between the levels of protein expression with muscle WB in the two groups did not show significant differences ( $n = 84$ ,  $p = 0.82$ ), and neither did the comparison regarding protein expression based on IH ( $n = 92$ ,  $p = 0.52$ ). To try to maximise the sample size, we included the monocyte dysferlin expression or IH if the WB was not available, following this hierarchical order to categorise patients as expressing or not expressing dysferlin (muscle WB, monocyte WB and then muscle IH). We did not identify any differences between the groups, and the same percentage of outliers was identified in absent versus reduced protein expression groups (14% of outliers per group,  $p = 0.94$ , Chi-square).

### 3.8. Asymmetry

Of the 184 patients, 41 had asymmetric involvement (22.3%), while 21 of the 24 outliers had asymmetric involvement (87.5% of the outlier group). We compared asymmetric patients, including the outliers and non-outliers, to non-asymmetric patients, adjusted for every other variable. We identified significantly more outliers

among the asymmetric group (21 out of 41 asymmetric, compared to 3 in 143 symmetric patients,  $p = 0.0001$ , Chi Square test), which is expected but reinforces the fact that asymmetry in MRI findings is possible in dysferlinopathy and could be independently assessed as it tends to predict outlier patients more than other pattern criteria. We also identified a significantly different age at symptom onset between the two groups, which was higher on the asymmetric group (28.2 vs 20.6 years with  $p = 0.001$ , Mann-Whitney U test). We did not identify any other differences in demographic or clinical data between these patients with and without an asymmetric involvement.

## 4. Discussion

Several studies have described the common muscle MRI pattern of selective fatty replacement observed in dysferlinopathy patients [7,9]. A predominant and early involvement of posterior thigh and lower-leg muscles was reported as the main imaging feature of patients in the COS study, as well as in other, smaller cohorts of patients in Chile, China and Russia. The prevalence of these findings across the different cohorts varies between 40% and 80% [5–7,12]. Other muscles typically affected in the early stages are the subscapularis muscle, the thigh adductors, the gluteus minimus, the external and internal obliques, the lumbar and thoracic erector spinae, the biceps brachialis and the flexor digitorum profundus in the forearm. In later stages of the disease, fatty transformation affects the anterior compartments of the leg and thigh, the posterior compartments of arms and forearms, trunk and other paravertebral muscles. Fat replacement in muscle MRI correlates positively with disease duration and negatively with motor functional scales [4,5,12].

The muscles which are relatively spared throughout the disease course in the pelvic girdle and lower limbs are the piriformis, gracilis, sartorius and popliteus. In the scapular girdle and upper limbs, the levator scapulae and the posterior compartments of the forearms are the only muscles which are relatively spared throughout the disease course. Additionally, craniofacial muscles are not usually affected.

Despite several imaging studies in patients with dysferlinopathy having been published, only a few of them included upper-limb images [9,12]. The Jain COS MRI study is still the largest cohort of dysferlinopathy patients imaged so far, acquiring either lower-limb or whole-body MRI in 184 patients [6]. The large number of patients included in that study enabled a precise description of the muscles that were affected and established diagnostic criteria that described common MRI features, which aimed to help clinicians in the identification of potential dysferlinopathy patients using MRI. Although these criteria were met by a large majority of patients, there were several patients who did not meet all the criteria, suggesting that the pattern of muscle involvement in dysferlinopathy could be more heterogeneous than expected. A recent study with a Russian cohort of 25 patients showed a high variability in MRI patterns among the thigh muscles, which was greater than in the pelvic and leg muscles [12]. The study described, apart from the most prevalent posterior-dominant pattern, less frequent patterns with anterior-dominant and diffuse distribution; the latter was described in patients that were not at the end stage of the disease. Here we have revisited nine of the pattern criteria described previously and identified patients who did not follow these criteria, the so-called “outlier patients”, and described their pattern of muscle involvement and their demographic and clinical data [13]. We would like to highlight that 71% of patients met all the criteria or all except one, and only a minority (3%) did not meet a maximum of four criteria, demonstrating that the already established MRI pattern criteria are still valid and very reliable for a correct diagnosis indeed.

We identified 24 outlier patients out of 184 who did not meet the classical imaging criteria. After the least reliable criterion, which is the adductor magnus more affected than the adductor longus in thighs (27.3% of exceptions), symmetric involvement was the most frequently unmet criterion in 22.3% of the patients. This frequency is similar to that described in other cohorts: for example, a recent Chinese study described 18.3% of a total of 60 patients having asymmetric involvement in muscles of the thigh [14]. We have also observed that other criteria were commonly not met, such as the subscapularis being the most affected muscle in the scapular girdle in 16.7% of the patients. As found in 7 out of 9 patients, our data support the fact that not meeting the gluteus minimus, paraspinal muscles or elevator scapulae criteria can predict more discordance in other criteria and help predict outlier patients.

We also identified a greater proportion of outliers among the asymmetric patients (52.5% versus 13% in the whole cohort), suggesting that asymmetry can also predict belonging to the outlier group when used independently of the other imaging criteria.

Other myopathies characterized by marked asymmetry, such as inclusion body myositis (IBM), facioscapulohumeral dystrophy (FSHD), LGMD produced by mutations in the ANO5 gene or carriers of mutations in the dystrophin gene should be taken into consideration in the differential diagnosis of an asymmetric pattern involvement, making muscle biopsy and molecular studies essential to guide the final diagnosis. Reasons for such asymmetries in myopathies are still unknown. In the case of FSHD, some studies performed by Tasca et al., based on different stages of muscle involvement considering two MRI patterns(15), showed different transcriptional patterns in the STIR+ muscles similar to that seen in inflammatory myopathies, with distinct upregulation of selective inflammation and angiogenesis genes [16]. Interestingly, they used four dysferlinopathy samples as controls, which had a broad gene expression distribution, but intermediate between STIR+ FSHD patients and healthy controls. In the case of IBM, it is thought that asymmetric involvement could be due to inflammation progressing on a different pace between muscles [17]. In view of these findings, it is tempting to hypothesise that differences in the degree of inflammation or gene expression among muscles could explain marked asymmetry in some dysferlinopathy patients, either driven by epigenetic changes such as DNA methylation or by the existence of variants in other genes that could modulate the asymmetry of muscle involvement. However, we do not have enough material to confirm this hypothesis as muscle biopsies comparing affected and spared muscles from asymmetric patients were not available and the diagnosis was established sometimes using different approaches. In some cases, diagnosis was obtained using sanger sequencing of the DYSF gene after phenotypic and muscle biopsy data were reviewed, while in others the diagnosis was obtained through NGS after running a limb-girdle muscular dystrophy panel or an exome. Some of them have variants of unknown significance (VUS) in DYSF other than the two variants classified as pathogenic or likely pathogenic. However, there was never recorded if any other variants in other genes were present, and a new genetic analysis would be necessary to identify them. To date, there has not been any research on dysferlin's modifier genes that we are aware of, and this topic should be worth exploring in the future.

When comparing disease characteristics among outlier patients and non-outliers, we found a significant later age at symptom onset in the outlier group as well as in the patients with asymmetries. We did not find any significant difference regarding ethnicity, gender, phenotype, symptom duration and prior exercise levels between outliers and non-outliers. However, we did find difference in muscle strength measured using MMT and muscle

function measured by NSAD between the two groups either at baseline and three years after, although the progression rate was not significantly different between both groups. These results support the fact that later disease onset in dysferlinopathy patients is associated with an atypical MRI pattern, and that in these cases muscle function would be relatively preserved when first MRI is obtained. Despite this different MRI pattern and muscle function, it does not have influence on disease progression, probably because a widespread fat replacement takes place across all muscles over time.

We did not find any difference in the genotype among outliers and non-outliers, and we hypothesised that the later age of symptom onset could be better explained by a difference in dysferlin expression in muscle fibres or blood monocytes – for instance, that reduced dysferlin in outliers produced a later onset in contrast to more absent dysferlin in non-outliers – but this did not seem to be the case. A possible explanation of this falsely similar protein expression found between groups could be the existence of different levels of dysferlin expression among muscles, leading to inaccurate levels measured in just one biopsy. Comparing the protein expression solely in monocytes would amend this bias but the latter technique was performed in a low proportion of patients. A second hypothesis could be a difference in dysferlin isoform among groups not reflected in the available quantitative tests. Thus, we cannot currently explain the difference in disease onset observed in our cohort based on pathophysiology. However, we think that our results will improve practice with regard to clinical suspicion of this heterogeneous disease, even in the presence of atypical MRI patterns.

This study has several limitations, including that MRI assessment focused exclusively on T1-weighted images. In the COS study, STIR sequences were not collected; instead, T2 maps were collected to provide quantitative data on free water in the muscles but not assessed in this study [1]. We are aware that STIR+ or water T2+ images may correlate histologically with muscle inflammation or oedema in many patients with dysferlinopathy, mainly in the early stages of the disease, which tends to decrease with disease progression [2]. In fact, there is a negative correlation between fatty infiltration and oedema scores. Previously reported data show that among thigh and leg, oedema occurs more frequently in the anterior-medial compartments where fat infiltration is seen in later stages, suggesting that oedema precedes fatty replacement throughout the disease course [5,18,19]. This fact has been described in other hereditary myopathies such as FSHD, with a histopathological correlation and changes in gene expression compared to STIR- muscles [15,16]. For that reason, STIR images seem to anticipate which muscles will undergo fatty replacement in later stages, so it could be stated that we are here analysing muscle involvement at a later period within the same pathological process of muscle wasting. Despite this, as other rare phenotypes have been described with pronounced muscle oedema and mild fatty infiltration, known as pseudo-myositis or pseudo-metabolic (prevalence of 2% in 193 European patients [4,12], assessing muscle oedema in our patients would likewise have been an interesting outcome and could be useful in a future to select the proper time to start treating patients. Another potential limitation of this work is that MRIs were scored by one reviewer only on this occasion, although the scores were compared with the original ones published in 2018 that were obtained by two independent reviewers not observing significant discrepancies between these two analyses.

## 5. Conclusions

Using the large Jain COS for Dysferlinopathy cohort and the previously described typical muscle MRI pattern for



dysferlinopathy, we assessed how many patients met nine previously described imaging criteria in T1w images of 184 baseline MRI scans. We identified that most of the patients met the established criteria, suggesting that they are useful for predicting the disease when clinical suspicion is present. However, we categorised as “outliers” 13% of them that did not meet three or more imaging criteria, or two criteria when one was severe asymmetry. Up to 87.5% of the outliers had asymmetric muscle involvement. We identified another three criteria that can probably predict outlier imaging findings when used independently, thus helping to simplify the diagnosis in the revision of MRI criteria. We analysed the outliers’ baseline characteristics compared to non-outliers and found that they have a significant later age at onset and better muscle function at baseline, but without difference in progression rates.

In conclusion, the present study has widely explored the use of the imaging diagnostic criteria in dysferlinopathy and expands the characterisation of this heterogeneous disease, providing robust data which may be especially useful for diagnostic approach in centers where muscle MRI is more available than genetic testing, or in cases where just one mutation in DYSF gene is identified in NGS studies.

### Declarations of Competing Interest

Authors have no conflict of interest related to the content of this paper.

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### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.nmd.2023.02.007](https://doi.org/10.1016/j.nmd.2023.02.007).

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