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Tracking muscle wasting and disease activity in facioscapulohumeral muscular dystrophy by qualitative longitudinal imaging

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Abstract

Background Facioscapulohumeral muscular dystrophy (FSHD) is one of the most frequent late-onset muscular dystrophies, characterized by progressive fatty replacement and degeneration involving single muscles in an asynchronous manner. With clinical trials at the horizon in this disease, the knowledge of its natural history is of paramount importance to understand the impact of new therapies. The aim of this study was to assess disease progression in FSHD using qualitative muscle magnetic resonance imaging, with a focus on the evolution of hyperintense lesions identified on short-tau inversion recovery (STIR+) sequences, hypothesized to be markers of active muscle injury.

Methods One hundred genetically confirmed consecutive FSHD patients underwent lower limb muscle magnetic resonance imaging at baseline and after 365 ± 60 days in this prospective longitudinal study. T1 weighted (T1w) and STIR sequences were used to assess fatty replacement using a semiquantitative visual score and muscle oedema. The baseline and follow-up scans of each patient were also evaluated by unblinded direct comparison to detect the changes not captured by the scoring system.

Results Forty-nine patients showed progression on T1w sequences after 1 year, and 30 patients showed at least one new STIR+ lesion. Increased fat deposition at follow-up was observed in 13.9% STIR+ and in only 0.21% STIR- muscles at baseline ($P < 0.001$). Overall, 89.9% of the muscles that showed increased fatty replacement were STIR+ at baseline and 7.8% were STIR+ at 12 months. A higher number of STIR+ muscles at baseline was associated with radiological worsening (odds ratio 1.17, 95% confidence interval 1.06–1.30, P = 0.003).

Conclusions Our study confirms that STIR+ lesions represent prognostic biomarkers in FSHD and contributes to delineate its radiological natural history, providing useful information for clinical trial design. Given the peculiar muscle-by-muscle involvement in FSHD, MRI represents an invaluable tool to explore the modalities and rate of disease progression.

Keywords Muscle MRI; Muscle wasting; FSHD; Facioscapulohumeral muscular dystrophy; Biomarkers; STIR hyperintensity

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Introduction

Facioscapulohumeral muscular dystrophy (FSHD), the second most prevalent muscular dystrophy with onset in adulthood,[1] is a slowly progressing disorder characterized by a heterogeneous and often asymmetric muscle involvement, usually starting form facial and scapular fixator muscles and later spreading to upper arms, trunk, and lower limbs.[2] The clinical spectrum of the disease is wide, ranging from asymptomatic or minimally disabled patients to severe wheelchair

bound subjects.[3] In its most frequent form (FSHD1), it is inherited as an autosomal dominant trait associated with a contraction of the D4Z4 macrosatellite repeat array on the 4q chromosome which, together with a permissive haplotype distal to the last of these repeats, allows the inappropriate expression of the *DUX4* retrogene in adult skeletal muscle.[4]

Significant efforts have been made to develop therapies that could potentially counteract this disease, for example, preventing or slowing the loss of muscle mass, and promising approaches are emerging.[5,6] To evaluate the efficacy of such treatments, the research community is committed to identify biomarkers and develop outcome measures for clinical trial purposes.[7]

In the last years, the increasingly widespread use of standard and quantitative muscle magnetic resonance imaging (MRI) in FSHD patients suggested a non-linear model of disease progression that proceeds with a muscle-by-muscle type of involvement,[8] in which an early phase of muscle damage, identifiable by increased signal on short-tau inversion recovery (STIR) sequences accounting for oedema/inflammation, precedes fatty replacement of single muscles.[9–12] This would be consistent with the proposed model of disease pathophysiology, according to which bursts of inappropriate expression of the DUX4 retrogene, normally repressed in adult striated skeletal muscle, lead to a cascade of downstream events, not yet fully understood but potentially inclusive of an inflammatory-immune response,[13–16] that in the end generate skeletal muscle wasting.[17] In this context, STIR positive (STIR+) muscle lesions have been proposed as biomarkers of disease activity. Therefore, large studies to confirm that STIR+ lesions can be reliably correlated with phases of disease activity at single muscle level are primarily expected by the FSHD research community, but missing so far.[18]

Recent longitudinal imaging studies indeed focused on quantitative evaluation of fatty replacement and its correlation with clinical measures of disease progression, providing only limited information on the rate of appearance and evolution of STIR+ lesions.[19–21]

To contribute to answer these relevant questions, we designed this longitudinal study using conventional muscle MRI with the following aims: (i) to determine the evolution of STIR+ lesions after 1 year time, in comparison with the evolution STIR- muscles; (ii) to assess the rate of appearance of new STIR+ lesions in the same time frame; (iii) to determine how often fatty replacement is preceded by a STIR+ lesion detectable 1 year earlier.

Patients and methods

Patients

All FSHD patients routinely followed up at the Fondazione Policlinico Universitario A. Gemelli IRCCS were asked to participate to this prospective longitudinal study that took place between January 2014 and December 2017. The target of enrolment was 100 consecutive patients. Among the enrolled patients, 74 were also involved in our previous large cross-sectional MRI study.[8] Inclusion and exclusion criteria were identical to that previous study and were designed to include only adult, genetically proven, symptomatic FSHD1 patients. Disease severity was evaluated with the clinical severity scale (CSS),[22] ranging from asymptomatic patients (score = 0) to patients who lost ambulation (score = 5). At the time of clinical examination, patients were asked to undergo a baseline (within 2 weeks) and a follow-up scan at 1 year distance (365 ± 60 days).

Magnetic resonance imaging protocol and evaluation

Lower limb muscle MRI scans were performed at a single centre using a 1.5T commercial scanner (Siemens Magnetom Espree, Erlangen, Germany). T1 weighted (T1w) and STIR sequences were acquired using published protocols.[8]

One neurologist expert in muscle imaging (M. M.) and one MD student trained in muscle imaging (M. B.) independently assessed all the scans. Baseline and follow-up images were first evaluated using a 5-point semiquantitative scale to assess the extent of fatty replacement in single muscles on T1w sequences[23] and using a binary score (i.e. yes or no) to judge the presence of hyperintensities on STIR sequences. A total of 35 muscles were assessed on each side throughout their length. Subsequently, to detect more subtle changes not identified by the former assessment, the observers performed a direct visual comparison between the baseline and the follow-up scan of each patient on the same computer monitor. After careful alignment, using bony landmarks (femoral head and tibial plateau) as reference, the observers classified every muscle into one of the 16 categories summarizing all the possible combinations of evolution on T1w and STIR sequences (*Table* 1). Radiological worsening in single muscles was defined as an increase in fatty replacement or evident decrease in size and/or an appearance of a new hyperintensity on STIR sequences, as defined in the categories 4–6, 8–12, 14, and 15 of *Table* 1. Time needed to perform the scoring of one scan was 25 min; time needed to perform one comparison was 35 min on average.

Statistical analysis

Data are expressed as mean ± standard deviation unless otherwise specified and were analyzed using IBM SPSS Statistics ver. 22. All *P*-values <0.05 were considered statistically significant. Shapiro–Wilk test was used to assess data distribution, and each time, the appropriate statistic was chosen

Table 1 Classification system used to identify the differences between baseline and follow-up MRI scans and results of the comparisons

Category	Comparison baseline ⇨ follow-up MRI	Number of muscles	%
0	T1-/STIR- ⇨ T1-/ STIR-	3618	51.7
2	T1+/ STIR- ⇨ T1+/ STIR-	2501	35.7
3	T1+/ STIR+ ⇨ T1+/ STIR+	620	8.86
15	T1+/ STIR+ ⇨ T1++/ STIR+	87	1.24
1	T1-/STIR+ ⇨ T1-/ STIR+	74	1.06
4	T1-/ STIR- ⇨ T1-/ STIR+	27	0.39
14	T1+/ STIR+ ⇨ T1++/ STIR-	15	0.21
10	T1+/ STIR- ⇨ T1+/ STIR+	14	0.20
13	T1+/ STIR+ ⇨ T1+/ STIR-	11	0.16
8	T1-/ STIR+ ⇨ T1+/ STIR+	10	0.14
12	T1+/ STIR- ⇨ T1++/ STIR+	8	0.11
7	T1-/ STIR+ ⇨ T1-/ STIR-	7	0.10
9	T1-/ STIR+ ⇨ T1+/ STIR-	3	0.04
11	T1+/ STIR- ⇨ T1++/ STIR-	3	0.04
5	T1-/ STIR- ⇨ T1+/ STIR+	2	0.03
6	T1-/ STIR- ⇨ T1+/ STIR-	0	0.00

STIR, STIR sequences; T1, T1w sequences; -, unaffected; +, affected; ++, worsening of an already affected muscle. An evident change in the size of the muscle (hypotrophy) was also considered as worsening. White background: muscle showing no changes from baseline to follow-up MRI; red background: muscle displaying changes (i.e. radiological worsening); and green background: muscle with STIR+ lesions only at baseline without progression on T1w sequences.

accordingly. T1 score was calculated as the sum of the scores of involvement of the individual muscles in each patient, using the 5-point scale for fatty replacement. Independent sample *t*-test was used to evaluate differences regarding age, EcoRI fragment length, CSS, baseline T1 score, and number of STIR + muscles. Paired *t*-test was used to ascertain differences in T1 score and number of STIR+ muscles between baseline and follow-up scans; χ^2 test was used to assess differences between the number of STIR+ and STIR- muscles with increased fatty replacement at follow-up. Cohen's κ was used to determine if there was agreement between the two raters on the classification system adopted for this project. A logistic regression was performed to ascertain the effects of age, gender, EcoRI fragment length, CSS, T1 score, and number of STIR+ muscles at baseline on the likelihood that the patients displayed radiological progression in at least one muscle.

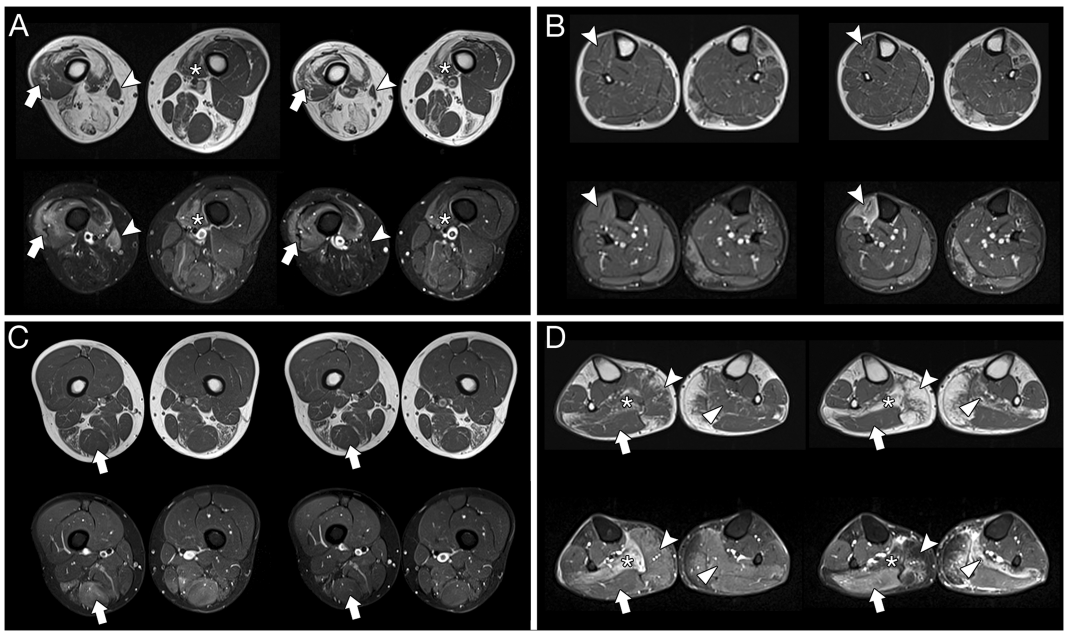
Results

Patients and baseline results

Baseline MRI scans were acquired in the period January 2014–December 2016. Forty-nine men and 51 women were enrolled. Mean age at baseline was 44.45 ± 13.99 years (range 18–71), mean EcoRI fragment length was 24.18 ± 5.62 kb (range 10–38), and median CSS was 3.0 (interquartile range 1.8, range 0.5–4.5).

All the patients except one, whose CSS was 0.5, showed lower limb involvement on MRI at baseline. Mean number of muscles affected on T1w sequences was 32.56 ± 19.59 (range 0–68) per patient. Mean T1 score was 71.9 ± 50.79 (range 0–183). Eighty-four patients had at least one STIR+ muscle. Mean number of STIR+ muscles per patient was

Figure 1 Different types of progression at 1 year in single muscles (baseline on the left-hand side and follow-up on the right of each panel). (A) Patient 41: worsening on T1w sequences with persistence of STIR hyperintensity in vastus lateralis (arrow) and medialis (asterisk); in the same patient, the STIR + sartorius (arrowhead) becomes atrophic and STIR- at follow-up; (B) Patient 35: a new hyperintense lesion in STIR sequences accompanied by volume loss is evident in the tibialis anterior (arrowhead); (C) Patient 65: normalization of STIR signal without apparent changes on T1w images in the semitendinosus (arrow); (D) Patient 63: right soleus (asterisk, STIR+ at baseline), left soleus (triangle), and right gastrocnemius medialis (arrowhead, STIR+ at follow-up scan) becoming almost completely replaced by fat tissue after 1 year; appearance of a new STIR+ muscle (gastrocnemius lateralis, arrow) with normal signal in T1w sequences. STIR, short-tau inversion recovery.



8.27 ± 6.77 (range 0–33), and the total number of STIR+ muscles was 827 (11.8% of the total).

We found no differences comparing male and female groups with respect to age, CSS, and T1 score while a statistically significant difference in EcoRI fragment length (men 25.98 ± 5.8 kb, women 22.45 ± 4.9 kb, P = 0.001) and number of STIR+ muscles (men 10.39 ± 6.85, women 6.24 ± 6.09, P = 0.002) was observed. A summary of clinical, genetic, and radiological features is reported in Supporting Information, *Table* S1.

Follow-up scans

The observers agreed in classifying muscles in one of the 16 categories in 93% of cases, and in the remaining 7%, consensus was reached by consultation. There was substantial agreement between the two raters' judgements, with κ = 0.861 (95% confidence interval 0.841–0.878, $P < 0.001$).

Mean T1 score at follow-up was higher than baseline (72.28 ± 50.92, range 0–184, $P < 0.001$). Twenty-three patients showed increased T1 score at follow-up, while no patient showed a decrease. The direct comparison of the scans allowed to detect an increase in fat deposition in at least one muscle in 26 additional patients. Overall, 49 patients (25 men and 24 women) showed detectable changes on T1w sequences after 1 year.

The majority of the muscles (*n* = 6813, 97.3% of the total, categories 0–3, *Table* 1) did not show changes at follow-up; an increase of at least one point in the semiquantitative score was found in 0.53% of the muscles (*n* = 37), while the direct comparison showed an increase in fat content in 1.83% (*n* = 128; categories 5, 6, 8, 9, 11, 12, 14, and 15, *Table* 1). The muscles that more frequently showed progression on T1w images were the gracilis (*n* = 14), gastrocnemius medialis (*n* = 14), and tibialis anterior (*n* = 10) (Supporting Information, *Figure S1). This progression consisted in muscle hypotrophy rather than increase of fatty replacement in a minority of cases and only for some muscles that were STIR+ at baseline: tibialis anterior (4 out of 8 cases), gracilis (5 out of 12 cases), and sartorius (3 out of 8 cases). Examples of different types of progression are shown in Figure 1. The complete scoring data set is available as Supporting Information, Table S2.*

No significant difference was found in the number of STIR+ muscles at follow-up (8.45 ± 6.76, range 0–33) compared with baseline evaluation. A new STIR+ lesion was displayed by 0.73% of the muscles at follow-up (*n* = 51, 25 with a score 0, 21 with a score 1–2, 5 with a score 3, and none with a score 4).

Thirty patients (18 men and 12 women, mean age 40.47 ± 15.6, median CSS 3.0 interquartile range 2.0, mean EcoRI fragment length 24.5 ± 6.53 kb, mean T1 score at baseline

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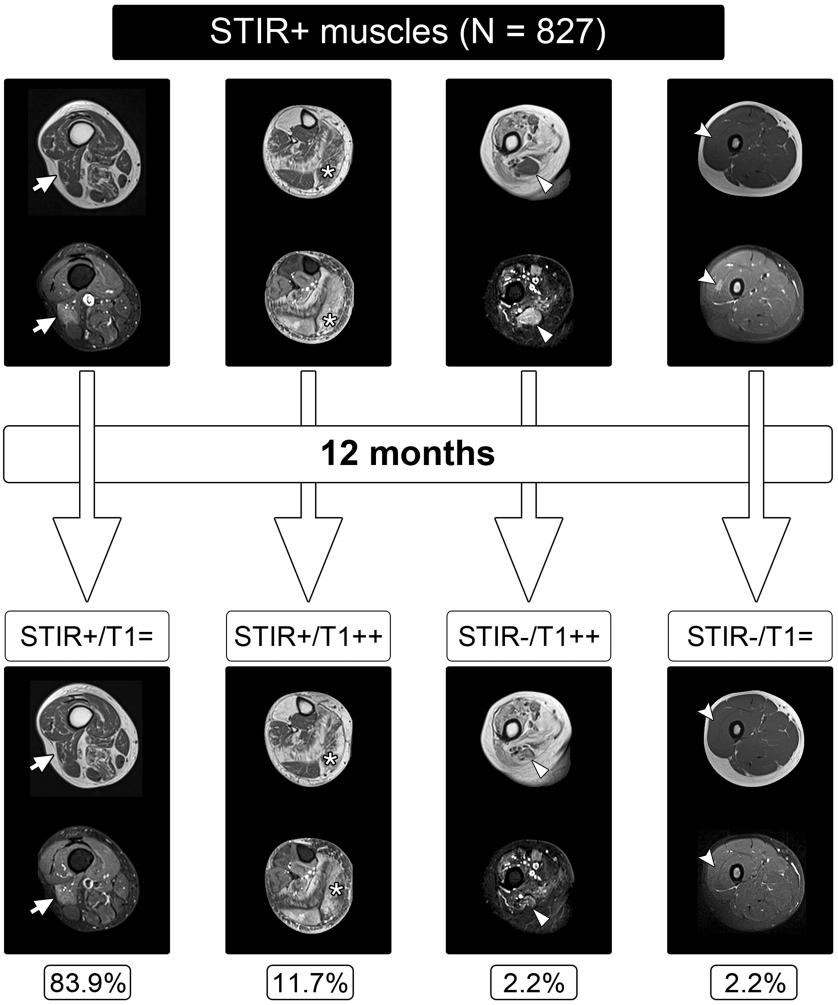
Figure 2 Evolution of STIR+ muscles at baseline. Patients (left to right) 80, 40, 9, 5, and 24. T1, T1w sequences; -, unaffected; +, affected; ++, worsening; =, no changes. STIR, short-tau inversion recovery.



69.2 ± 55.54) showed at least one muscle with a new STIR+ lesion.

Evolution of STIR+ muscles

Out of the 128 muscles that showed progression on T1w images, 115 (89.9%) were STIR+ at baseline and 10 (7.8%) were STIR+ at follow-up.

The majority of the STIR+ muscles at baseline (n = 694, 83.9%) showed no changes either on T1w or on STIR sequences at follow-up while 13.9% (n = 115) worsened on T1w sequences, thus displaying increased fatty replacement. On the contrary, only 0.21% of the STIR- muscles at baseline (n = 13) showed progression on T1w images at follow-up. In 2.2% of the STIR+ muscles (n = 18, corresponding to 11 patients, 6 men and 5 women), a normalization of STIR signal without detectable changes on T1w sequences was observed (*Figure* 2). The difference between STIR+ and STIR- muscles in the progression to fatty replacement at follow-up was statistically significant ($P < 0.001$). Higher values of T1 score (94.94 ± 41.58 vs. 55.77 ± 48.57, $P < 0.001$) and CSS (median 3.5 interquartile range 1.0 vs. median 3.0 interquartile range 2.0, $P < 0.001$) were found in patients (n = 48, 25 men and 23 women) who had at least one STIR+ muscle that progressed on T1w sequences, compared with patients (n = 35, 21 men and 14 women) who had only STIR+ muscles that did not show increase in fatty replacement.

Determinants of radiological worsening

Radiological worsening in at least one muscle, as previously defined, occurred in 59 patients (31 men and 28 women). The logistic regression model built to ascertain the effects of age, gender, EcoRI fragment length, CSS, T1 score, and

number of STIR+ muscles at baseline on the likelihood of displaying radiological worsening in at least one muscle was statistically significant, $\chi^2(6) = 29.89$, $P < 0.001$. The model explained 34.8% (Nagelkerke R^2) of the variance and correctly classified 76% of cases. Among all these variables, only an increase in the number of STIR+ muscles was associated with a higher likelihood of radiological worsening at follow-up (odds ratio 1.17, 95% confidence interval 1.06–1.30, P = 0.003).

Discussion

Several cross-sectional imaging studies reported the most frequent patterns of involvement in FSHD,[8,24,25] but only scarce longitudinal data are present in the literature, mostly focused on quantitative evaluation of fatty replacement and its correlation with clinical measures of disease progression with no or limited information on the rate of appearance and evolution of STIR+ lesions.[19–21] In this study, we performed a longitudinal follow-up of a large cohort of FSHD patients by lower limb muscle MRI. Lower limb MRI was chosen because more widely available and for the higher likelihood of finding STIR+ lesions compared with upper girdle imaging.[8] In a heterogeneous and slowly progressive disease such as FSHD, conventional MRI examination was able to detect signs of progression in terms of fatty replacement in almost half of the studied patients after 1 year. The direct comparison of the baseline and follow-up scans identified an increase of fat accumulation with higher sensitivity than the 5-point semiquantitative evaluation. Other limited natural history data on FSHD are available: a clinical study that followed patients over 36 months showed a slight decline in a mean voluntary isometric contraction test after 12 months and of an averaged composite manual muscle testing score after 18 months, but no predictors of disease progression were identified in that study.[26] Quantitative MRI demonstrated that fat fraction progressed in different muscles on average by 3.6% per year,[19] without differential evaluation of STIR+ and STIR- muscles. Clinical tools to more accurately identify disease progression are currently under development; muscle imaging depicts different and complementary aspects of disease progression, and the integration of the two assessments could be the best way to define reliable, sensitive, and meaningful outcome measures.

The results provided with the present study are relevant for the upcoming clinical trials in FSHD for different reasons: (i) enrolling patients with STIR+ muscles, thus more prone to show disease progression in a short time frame, would maximize the chances to detect a meaningful effect of an investigational treatment; (ii) slowing down the evolution of STIR+ lesions towards fatty replacement, increasing the rate of disappearance of STIR+ lesions, or lowering the occurrence of new STIR hyperintensities could be considered proof of efficacy of the treatment.

We indeed found that STIR+ muscles progress faster towards fatty replacement: after 1 year, almost 14% of the STIR+ muscles showed signs of increased fat amount, while only 0.2% of STIR- muscles demonstrated such an evolution. The evidence that a higher number of STIR+ muscles at baseline predicts worsening at follow-up further supports the link between STIR+ lesions and disease progression. Similarly, we found that the increase in fatty replacement at single muscle level is almost invariably preceded by a phase characterized by the presence of STIR+ lesions detectable 12 months before. Our results thus confirm that STIR hyperintense lesions are prognostic biomarkers as they can identify an active phase of the disease with faster progression towards muscle destruction. We cannot rule out the possibility that even all the evolutions towards fat replacement are preceded by a phase of STIR positivity, which would be virtually impossible to prove. This is however further suggested by the fact that most of the STIR- muscles at baseline that increase in fat content show STIR positivity at follow-up.

The link between STIR+ lesions and disease progression had already been suggested by smaller studies: Friedman *et al*. found that STIR+ muscles evolved to fatty replacement in 3 out of 9 patients followed by MRI of the calves over 2 years, while no evolution was seen in STIR- muscles.[11] At variance, one recent study by the same group on a cohort of 15 patients followed for 1 year found that only 4 out of 7 muscles that progressed on T1w sequences were STIR+ at baseline,[27] partly questioning this proposed link. However, we might hypothesize that the small number of patients examined, the heterogeneity of the disease, or even the different MRI protocols and subjective evaluation of STIR images could account for this discrepancy with our and previous studies.[10]

Here, we also confirmed earlier findings that women display STIR+ muscles less frequently than men,[8,28] although without a corresponding significant decrease in radiological worsening over 1 year, thus suggesting that STIR+ lesions are somehow more long lasting in men. This is in keeping with the recent evidences of a proposed protective effect of oestrogens by antagonizing DUX4 activity and at least partially preventing the activation of the cascade of pathogenic events downstream DUX4 transcription,[29] while according to recent clinical study, differences in endogen oestrogen exposure during life did not seem to have a clinically relevant modifying effect on disease severity in female patients.[30] It also suggests that other sex-related factors might play a role in the development or further prevention of DUX4-mediated muscle damage as well.

It is known that STIR+ muscles are characterized by an inflammatory component, whose role is not yet fully understood. The fact that the majority of STIR hyperintensities are rather long lasting (83.9% remained unchanged at 1 year) and that some of them may even normalize on MRI

without macroscopic consequences on T1w images is intriguing. The implementation of quantitative MRI techniques and pathology studies might clarify whether this evolution corresponds to an accumulation of fat that is under the sensitivity threshold of standard MRI or whether it corresponds to deposition of fibrotic tissue, whose assessment is a current limitation of all MRI techniques, but can be for instance visualized by muscle ultrasound.[31] The molecular link between DUX4 activation and STIR positivity has been evaluated by a recent study on MRI-targeted muscle biopsies, finding that abnormal STIR signal is a reliable predictor of the presence of DUX4 target expression.[32] Further studies aimed at clarifying if inflammation represents a protective attempt against the DUX4-driven deleterious effects or a direct detrimental component of the DUX4 cascade are needed to understand more of the disease and for therapy development.

A limitation of the present study is that we did not measure the difference in longitudinal fatty replacement between STIR+ and STIR- muscles with quantitative techniques for fat or water content. It is known that semiquantitative imaging scores globally correlate with the actual fat fraction, but a wide range of fat fractions can exist within the same visual grade, so quantitative imaging is definitely more precise in measuring time differences in single muscles.[33] Furthermore, advanced MRI techniques allow to measure the water T2, that is more accurate to muscle oedema changes compared with STIR imaging.[34,35] Our study is however complementary to quantitative ones, providing different information by allowing an extensive and fast evaluation of all the muscles in the lower limbs along all their length. It also helps to inform longitudinal quantitative MRI studies to find suitable targets and regions of interest to be effectively monitored (or avoided) in a 1 year time frame.

In summary, in a slowly progressive disease such as FSHD, where clinical assessment alone may fail to detect modifications of the natural history of the disease induced by treatments in a relatively short time frame, having sensitive prognostic biomarkers is extremely useful for clinical trial design, including correct patient stratification and evaluation of treatment efficacy. Our work sheds light on the radiological natural history of FSHD and further supports the role of this technique in defining predictors of disease progression.

Acknowledgement

We are gratefully to thank the FSHD Italia Onlus Association and all the patients that participated to this study.

Online supplementary material

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

Figure S1. Progression on T1w images and appearance of new STIR+ lesions in single muscles
Table S1. Summary of clinical, genetic and radiological features
Table S2. Complete scoring dataset according to the classification into different categories

Conflict of interest

Pursuant to the terms of a Master Academic Services Agreement with the Catholic University of the Sacred Heart, M.M. and E.R. provided central reading services for MRI scans generated in aTyr's clinical trials of Resolaris (ATYR1940). E.R. was the site principal investigator for some of these trials. F.L., P.O., M.R.B., A.P., and G.T. have nothing to report.

Ethical standards

The authors certify that they comply with the ethical guidelines for authorship and publishing of the Journal of Cachexia, Sarcopenia and Muscle.[36] This study was approved by the Ethics Committee of the 'Fondazione Policlinico A. Gemelli IRCCS' and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All the involved subjects gave their written informed consent prior to the inclusion in the study.

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