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265th ENMC International Workshop: Muscle imaging in Facioscapulohumeral Muscular Dystrophy (FSHD): relevance for clinical trials. 22–24 April 2022, Hoofddorp, The Netherlands

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1. Introduction

On 22–24 April 2022 the 265th ENMC International Workshop on "Muscle Imaging in Facioscapulohumeral Muscular Dystrophy (FSHD): relevance for clinical trials" took place as a hybrid meeting, with 21 participants on site in Hoofddorp (the Netherlands) and 5 connected remotely, from 11 different countries.

FSHD, one of the most frequent muscular dystrophies [1], has entered the era of clinical therapeutic trials. The scientific community is committed to achieve clinical trial readiness [2] and two major consortia, devoted to boost drug development, have been created: the FSHD Clinical Trial Research Network (CTRN), based in the USA, and more recently the FSHD European Trial Network (ETN) [3,4].

Muscle evaluation using magnetic resonance imaging (MRI) has lately been established as relevant to diagnose and monitor disease progression of different neuromuscular disorders [5]. In FSHD, evidence derived from MRI studies substantially contributed to a better understanding of this disease and its variable progression over time. However, a major need not yet addressed is the clear establishment of the importance of muscle imaging for the diagnosis and follow-up of FSHD patients, and the definition of its role in a clinical trial setting. This workshop provided a unique opportunity to gather the experts in the field, who shared their knowledge and experiences, and have dedicated time to focus and discuss on the usefulness and harmonization of the imaging techniques specifically in FSHD, since no previous meeting has been devoted to address these issues so far.

After a brief introduction and welcome from the ENMC representative and FSHD ETN chair *Nicol Voermans, Giorgio Tasca* provided an overview on disease pathophysiology and on the focus of the workshop. FSHD is unique in its genetic mechanism [6] and peculiar in the progression of muscle damage compared to the other muscular dystrophies, thus requiring dedicated efforts in the identification of imaging biomarkers and specific expertise. The underlying cause of the disease seems to be rooted in the inappropriate *DUX4* transcription in adult skeletal muscle, which is assumed to happen in random bursts in a minority of myonuclei [7], finally leading to muscle wasting through a multifaceted cascade of downstream events. This is supposed to take place, *in vivo*, predominantly in (parts of) the muscles that will display a

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T2-weighted short-tau inversion recovery (T2w-STIR) positive signal on MRI, which are the muscles where the *DUX4* signature, i.e. the dysregulation of DUX4 target gene transcripts, was found [8,9]. He also underlined that muscle imaging is not a "biomarker" by itself, but several different biomarkers can be obtained by MRI or other imaging modalities. The choice of the most appropriate biomarker for a specific trial would have to consider the supposed mechanism of action of the tested drug on what is known about disease pathophysiology.

Hence, the workshop aims were: (a) surveying the imaging facilities available in each center (Supplementary Table 1); (b) discussing about the best protocol/minimal set of images necessary for patients' diagnosis; (c) discussing the need to standardize MRI evaluation/protocols in multicentric trials; (d) discussing about the possible MRI outcomes to assess treatment efficacy (slowing fatty replacement, increasing the rate of disappearance of T2w-STIR positive/increased water T2 lesions or lowering the occurrence of new T2w-STIR positive/increased water T2 lesions, etc.); (e) discussing the role of imaging techniques other than qualitative/quantitative MRI, and in particular the use of muscle ultrasound in multicentric trials.

2. Qualitative MRI

Mauro Monforte opened the session discussing about the role of muscle MRI in the workup of suspect FSHD patients, considering on one hand the challenges in the interpretation of the genetic test given the estimated prevalence of contracted D4Z4 alleles in the general population [10], and on the other hand the clinical heterogeneity of the disease, which can manifest in incomplete or atypical phenotypes [11]. Qualitative MRI was able to identify specific susceptibility or resistance to damage of the different muscles in FSHD [12,13], as well as to define patterns with diagnostic value: the involvement of trapezius together with bilateral subscapularis sparing yielded a diagnostic accuracy of 0.89, with 0.90 sensitivity and 0.88 specificity, also in atypical patients [14]. Relevant for the understanding of disease mechanisms and progression, muscles with hyperintense lesions on T2w-STIR sequences showed higher likelihood of displaying inflammatory features on muscle pathology and increased expression of DUX4 targets [8,9,15,16], as well as a reduction of PAX7 targets [17]. Longitudinal MRI evaluations demonstrated that fat replacement occurs faster in T2w-STIR positive muscles than in T2w-STIR negative ones [18,19], and the higher the number of T2w-STIR positive muscles a patient displays at baseline, the higher the probability of radiological worsening at follow-up. Importantly, male patients had a significantly higher number of T2w-STIR positive muscles than females, but since sex was not an independent determinant of radiological worsening after one year in a longitudinal study, it is likely that T2w-STIR positive lesions in males are more frequently non-evolutive [19,20]. These results are valuable for targeted enrollment and prioritization in the context of a clinical trial, as the inclusion of patients in the active phase of the disease could increase the chances to detect the effect of the investigational drug. Decreasing the rate of appearance of new T2w-STIR positive lesions could also be considered as an outcome measure for specific drugs under development, although a population of approximately 350 patients studied with pelvic and lower limb MRI would be needed to perform a study aimed at halving this incidence (alpha 0.05, power 0.9). With regards to the imaging features of FSHD2, these are overlapping with the pattern described in FSHD1, since the same muscles are affected and spared, although with some possibly significant differences: the lower limb muscles are relatively more affected than the upper body ones in FSHD2 compared with FSHD1, and T2w-STIR positive muscles were more frequently detected [20–22].

Robert-Yves Carlier discussed the different protocols used for qualitative MRI acquisitions. The use of whole-body MRI examinations has increased in neuromuscular disorders as all the recently installed scanners have the possibility to perform whole-body MRI. The most commonly used technique employs a phased array network of coils with stack-by-stack consecutive acquisitions, combined together offline. One manufacturer offers a continuously moving table, like in computed tomography spiral acquisitions, which is a time-saving option, but only few sequences are customized for this type of table, and the system is very expensive. The network of coils classically includes head and neck coils, posterior coils inserted in the table, anterior body surface coils and lower-limb coils. Variations are possible depending on the center's equipment. Upper limbs should be positioned just next to or above the trunk to be able to analyze arm and forearm muscles due to field inhomogeneities close to the edge of the magnet bore. Qualitative and quantitative sequences are complementary, and the same type of equipment and positioning can be used for both. Classical qualitative examination relies on the consecutive acquisition of T1-weighted images, to detect fatty replacement and inform on muscle shape and size, and T2-weighted with fat saturation images in order to detect an increase in water content. A spin-echo T2-weighted sequence with fat and water separation (T2-Dixon) could be used to obtain in a single acquisition visually interpretable images comparable to T1-weighted and fat-saturated T2-weighted images respectively, and a complete, continuous acquisition could take less than 25 min. Contiguous axial slices with sufficient spatial resolution avoid a lack of detection between slices. T2-Dixon, even if with an inferior performance compared with 3-point gradient echo Dixon, also allows the quantification of muscle fat content, preserving the possibility of a quick visual evaluation of fatty replacement and edema-like changes at single muscle level.

Sabrina Sacconi stressed the importance of dealing with heterogeneity in the study of FSHD, which has to be taken into account in analyzing possible differences in the response to therapies and appearance of side effects. Several MRI-derived biomarkers could be used according to the purpose of the study (they can be diagnostic or prognostic for instance) and should be correlated to clinical measures. Qualitative MRI could be also implemented to target a muscle biopsy if tissue biomarkers are required in the trial, and thorough efforts should be made to standardize the baseline and repeated samplings. She then presented the panorama of the natural history studies based on the France National Registry, such as the "Clinical Trial Readiness Network FSHD France", which is a prospective, multicenter, 24-month natural history study (NCT04038138) on 100 FSHD patients involving three centers (Nice University Hospital, Myology Institute Paris, Lille University Hospital), aimed at developing outcome measures for adult ambulatory type 1 FSHD patients. In this study, clinical data collection is coupled with 3T WB-MRI acquisitions and biological sample collection (DNA, RNA, serum) to investigate the correlation between functional scores, paraclinical data derived from MRI, and serum biomarkers specifically focused on inflammation. The MRI protocol used in this study was developed by AMRA Medical (Linköping, Sweden), and consists of a standardized, quantitative MR acquisition using Dixon imaging with semiautomatic skeletal muscle segmentation. It quantifies the 3-dimensional muscle volume and fat fraction to measure the extent of skeletal muscle tissue replaced by fat in FSHD patients. The variability characteristic of the disease will be addressed leveraging the use of artificial intelligence and specifically designed complex algorithms.

Roberto Fernandez-Torrón presented data from the San Sebastian (Spain) cohort. This center has performed the molecular diagnosis collecting samples from all the country for the last 20 years, accounting for $n = 2009$ FSHD1 and $n = 72$ FSHD2 patients diagnosed so far. Fernandez-Torrón presented the clinical and molecular data from FSHD1 patients currently followed ($n = 50$), as well as the muscle imaging qualitative data in 17 patients evaluated by Mercuri scale [23]. None of the FSHD1 patients had a normal muscle MRI. The pattern of fat replacement in FSHD1 was consistent with what was previously reported [12,13]. Half of the patients had at least one T2w-STIR positive muscle. He also showed the clinical, molecular and muscle imaging data from the local FSHD2 cohort ($n = 14$), which was partially included in the international collaboration published by Giacomucci et al. [21]. Eleven FSHD2 patients were scanned in San Sebastian (8 applying a whole-body protocol), 8 females and 3 males, and all the patients had a pathogenic variant in *SMCHD1*. The mean age at muscle imaging was 53 years (range 33–83 years) with 7.7 years from onset to scan (1–19 years). Nine out of 11 patients had abnormal imaging and 4 had at least one T2w-STIR positive muscle.

3. Quantitative MRI

John Vissing opened the session on quantitative MRI showing that the paraspinals are among the most affected muscles in FSHD, and that muscle fat fractions correlate closely with clinical parameters such as the FSHD clinical score, muscle strength and the 6-min walking test (6MWT) [24]. This suggests that quantitative MRI could be appropriate for clinical staging in FSHD [25]. In contrast to the severely affected paraspinal muscles, the psoas muscle is well preserved in FSHD, unlike patients with limb girdle muscular dystrophy type 2I/R9 and Bethlem myopathy [26]. An MRI follow-up study in LGMD2I/R9 showed significant progression in fat replacement of muscle on quantitative MRI after one year, although functional muscle tests were unchanged [27]. After 6 years fat replacement of muscle had further progressed and at that time functional muscle tests declined as well. These data indicate that MRI is a good candidate as a surrogate marker for progression in follow-up and clinical trials [25]. In a 1-year follow-up study in 45 patients with FSHD, all muscles studied showed an increase in fat fraction, on average 3.6% [28]. Muscles with an intermediate fat fraction of 40–60% progressed the most and were more frequently hyperintense on T2w-STIR sequences compared to muscles with low or end-stage levels of fat fraction [18]. At the same time, functional muscle tests such as the 6MWT, timed up and go and 5 times sit to stand tests did not change. To map the temporal evolution of T2w-STIR positive muscles, 10 patients were sequentially MR-scanned for 2.7 years. The study showed that muscles with long T2 relaxation times progressed much more in fat fraction than muscles with short T2 relaxation time [29], and muscles tended to keep being edematous/inflamed until they reached full fat replacement. Whether edematous muscle changes have an active role or represent an epiphenomenon in FSHD disease progression remains to be determined, since these radiological abnormalities are found in other muscular dystrophies and neuromuscular disorders in general and are not specific for FSHD.

The Italian experience on longitudinal quantitative MRI in FSHD patients was presented by *Anna Pichiecchio*. Thirty-five FSHD subjects with at least one T2w-STIR positive muscle in the lower limbs were scanned at baseline (T0), 6 months, 12 months (T12) and 24 months. Advanced MRI techniques (6-point Dixon and multi-spin-echo T2) were used for the detection of fat-fraction and water T2 on a 3T scanner [30], with dynamic MRI scans performed at the end of the protocol [31]. The study is still ongoing, and data from the longitudinal evaluation of 25 patients at T0-T12 were shown (8 females, 17 males, age = 45.1 ± 9.5 years). Global MR disease burden had a negative correlation at T12 with 6MWT ($p < 0.05$) and a positive correlation with the clinical severity scale (Ricci score [32]) ($p < 0.05$). Mean fat fraction change at T12 in all muscles averaged on all subjects was around 2%. Intermediate fat fraction (20–40%) values at baseline predicted the highest fat fraction change at 12 months and a higher global water T2 muscle value also correlated with a higher fat fraction change at T12.

Francesco Santini discussed the use of phase-contrast MRI during muscle contraction using dynamic scans, performed on a 2D parasagittal slice across the quadriceps, in synchronization with neuromuscular electrical stimulation [33]. The intensity of the stimulation was chosen in order to elicit a visible twitch in the muscle with no force output. Maximum muscle strain, a parameter that can be derived from phase-contrast MRI scans, as well as buildup and release rates, were calculated for each patient. According to the changes in maximum strain occurring between the first and second time point, patients could be divided into two categories: those who showed an increased strain, similarly to healthy volunteers, and those who had a decrease in strain, who were generally more severely affected.

Pierre Carlier presented on the possibility to perform a whole-body water T2 measurement. Muscle water T2 reflects water mobility in muscle tissue. It is a non-specific but rather sensitive biomarker of ongoing damage in diseased muscle. T2w-STIR imaging is the qualitative equivalent of water T2 maps. In FSHD, T2w-STIR imaging has identified that hyperintensities can develop rapidly in muscles in different regions of the body. These muscles with active damage are progressively and sometimes rapidly destroyed and replaced by fat. Whole-body water T2 maps would help to quantify the severity of the active muscle damage and might improve the predictive values of hyperintensities seen in T2w-STIR imaging, and therefore the question whether whole-body muscle water T2 measurement is feasible or not was raised. As always in nuclear magnetic resonance, the answer is determined by the constraints that are imposed. In a clinical setting, acquisitions should ideally not take more than 30 min, should be combined with water-fat imaging, and have a millimetric in-plane resolution ideally with no interslice gap, which should in any case be smaller than twice the slice thickness. To achieve this, several options could be considered, ranging from the most available to the most sophisticated ones (Fig. 1):

- The very standard multi-slice multi spin echo sequence (MESE) generates series of images that can be processed to obtain fat fraction maps and water T2 maps. The scapular and pelvic girdles, the thighs and the legs can be scanned in approximately 30 min. The processing has to be performed offline, but several freeware solutions are now available.
- T2w turbo spin echo Dixon sequences, which are also standard on modern scanners and are automatically processed. They provide quantitative information although T2w fat fraction maps overestimate fat fraction and the T2w water images remain qualitative. T2w hyperintensities have to be further quantified by a separate MESE acquisition centered on them. If this quantitative step is added, there is no real gain in acquisition time compared to the first option.
- MESE can be accelerated by the use of parallel imaging, multislice simultaneous excitation and compressed sensing. These techniques can be combined resulting in acceleration factors of 4 to 8. It can even be speeded up when artificial intelligence-based denoising is added.
- Magnetic Resonance Fingerprinting is a very different way to quantify multiple MR variables at once, by varying the acquisitions parameters pseudo-randomly and generating signal profiles that are specific of a particular combination of MR

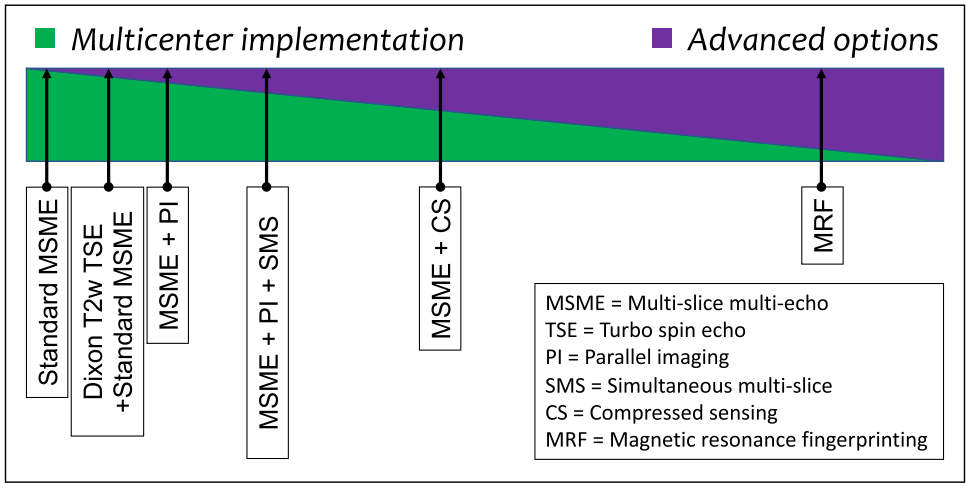

Fig. 1. Tradeoffs between multicenter implementation and technical resources available at the different centers for the different protocols aimed at measuring whole-body fat content and water dynamics in short timeframes.

variables. Profiles allowing the determination of fat fraction, water T2 and T1 maps can be acquired in 14–16 s for one slice.

In a multicentric clinical trial, the diversity of the imaging platforms would need to use the most standard options, whilst the introduction of more advanced sequences will be possible in some sites only. Programming these sequences in a vendor-independent environment will facilitate the dissemination of more sophisticated and faster acquisition schemes. Unfortunately, the burden of ever-increasing regulations poses restraints that complicate the task.

Hermien Kan shared the experience on identifying muscle MRI biomarkers in another disorder, Becker Muscular Dystrophy (BMD), which is characterized by slow progression and high variability between patients. The methods used in data analysis could be of value for other slowly progressive muscle diseases like FSHD, especially the choice for a specific muscle or muscle group as the most sensitive to change, and which parameters can predict a change in fat fraction over time. At baseline, BMD patients had increased fat fractions compared to healthy controls, and increased standard deviation of the water T2 relaxation time (stdT2) and phosphodiester ATP ratio (PDE/ATP) in fat replaced muscles. In the longitudinal analysis, a stepwise approach was performed looking for a high sensitivity to detect change indicated by a standardized response mean above 0.8 and a correlation to functional tests of at least 0.8 [34]. All remaining parameters were then ranked based on reproducibility. They included fat fraction of the three center slices and the whole muscle of 19 individual muscles and 6 muscle groups, and contractile cross sectional area. In their cohort of BMD patients, 9 of the fat fraction measures, but none of the contractile cross sectional area parameters, had a sufficiently high sensitivity to detect change. Analysis of the three center slices of the whole thigh was the optimal parameter, closely followed by analysis of the whole thigh/whole muscle. A similar analysis could be done in FSHD. Given the slow and heterogenous disease progression in BMD, chances of observing clinically relevant endpoints during a typical clinical trial duration are low. Parameters that can predict disease progression could allow stratification of patients in whom measurable disease progression is expected. The fat fraction in many muscle diseases seems to follow a sigmoidal trajectory with age, where the rate of disease progression is not constant over time, but highest around the middle region of the fat fraction range. As such, baseline fat fraction is a good predictor for the rate of fat fraction increase over time. A mixed model analysis to show that the fat fraction increase over time in BMD follows a sigmoid was used, and determined that baseline fat fraction was the strongest predictor of the change in fat fraction over two years [35]. Including the stdT2 and PDE/ATP did not improve the prediction.

Sanne Vincenten presented the results of a longitudinal FSHD study in the Netherlands, aimed at determining the relationship between changes on quantitative MRI and changes in clinical outcomes. All the enrolled patients were assessed twice: at baseline [20] and at 5-year follow up. 2pt-Dixon and turbo inversion recovery magnitude (TIRM) MR sequences, equivalent of T2w-STIR, were acquired, and muscle fat fraction and T2w-STIR positivity of 19 leg muscles were determined bilaterally. The MRI compound score was defined as the mean fat fraction of all muscles combined. Clinical outcome measures included the Ricci score [32], FSHD clinical score [36] and Motor Function Measure (MFM) [37]. One-hundred and five FSHD patients were included (41% male, mean age 54±14 years, median Ricci score 7 (range 0–10)). The median change over 5 years in the MRI compound score was 2.0% (range −2.5–10.5; $p < 0.001$). Median change over 5 years in all clinical outcome measures was small but significant, with z-scores of 5–7.2 ($p < 0.001$). Change in MRI compound score correlated moderately ($\rho = 0.3$, $p < 0.001$) with the change in FSHD clinical score and MFM, subscore D1. The largest median increase in MRI compound score was seen in subgroups of patients with an MRI compound score between 20 and 40% (4.9%), or with two or more T2w-STIR positive muscles (3.9%), or FSHD clinical score 5–10 (3.3%). In conclusion, this 5-year study showed significant changes in the MRI compound score and clinical outcome measures, and a significant correlation between them. In addition, the results showed which subgroups of patients are most prone to radiological progression. This knowledge further establishes MRI as a tool to derive prognostic biomarkers in FSHD, as well as efficacy biomarkers in upcoming clinical trials.

Doris Leung, at the Kennedy Krieger Institute and the Johns Hopkins Medical Center in the USA, has collaborated on several studies to examine quantitative imaging biomarkers in muscular dystrophy. A cross-sectional observational study of multi-voxel ^{1}H-MR spectroscopy has shown that the trimethylamine/creatine ratio is reduced in muscles of patients with FSHD compared to healthy volunteers, even when the muscles are not fatty replaced on qualitative imaging. The trimethylamine/creatine ratio was also significantly associated with muscle strength measured by dynamometry [38]. Their research team has also developed a deep learning algorithm that uses texture analysis to identify tissue-

specific signatures for muscle, fat, and fatty replaced muscle [39]. Fat fractions of the thigh calculated using this technique strongly correlated to measures of lean body mass and whole-body fat derived from dual energy X-ray absorptiometry (DEXA) scans in limb-girdle muscular dystrophy 2I/R9 [40]. The fat fractions were also significantly correlated with strength and timed function testing. This technique is currently being used to analyze whole-body MRI data from a longitudinal cohort of 30 subjects with FSHD who were scanned at 5 time points over 21 months. Imaging studies in FSHD are also being performed by investigators at the Seattle Wellstone Center, and their work has demonstrated that higher levels of *DUX4* expression are found in T2w-STIR hyperintense muscles from patients with FSHD [41]. Follow-up imaging over 1 year has shown that fatty replacement increases most in T2w-STIR hyperintense muscles with intermediate levels of fat replacement at baseline [42]; however, increasing fatty replacement can also be observed in muscles that are not T2w-STIR hyperintense at baseline. Investigators from both these United States centers are currently collaborating with the FSHD-CTRN to conduct a clinical trial preparedness study of muscle MRI and imaging-guided biopsy in FSHD. This study aims to enroll 200 subjects across multiple sites to characterize imaging correlates to muscle function and pathophysiology in FSHD.

4. Experience from FSHD trials using MRI

Mauro Monforte, Linda Heskamp and *Shahram Attarian* discussed the clinical trial pipeline of the ATYR1940 drug (Resolaris™). This double-blinded, phase 1b/2a study evaluated the safety, tolerability, pharmacokinetics, immunogenicity, and biological activity of multiple ascending doses of intravenous ATYR1940 in adults with FSHD [43]. Exploratory outcome measures included the quality of life INQoL questionnaire, manual muscle testing, and targeted quantitative MRI of lower extremity muscles. Weekly doses of 0.3 ($n = 4$), 1.0 ($n = 8$), and 3.0 ($n = 8$) mg/kg were tested over 4, 4, and 12 weeks, respectively. Patients ($n = 20$) were randomized 3:1 (ATYR1940: placebo) across all dose groups and followed for 4 and 12 weeks after the last study drug dose.

Potentially eligible patients underwent a screening MRI. This scan was assessed by a central reader to locate T2w-STIR positive muscles with minimal or intermediate fatty replacement. The targeted MRI was focused on one of the T2w-STIR positive muscles. The protocol included a 3pt-Dixon sequence for quantification of fat replacement and muscle size, and a MESE sequence and ^{1}H MR spectroscopy sequence for the quantification of water T2 as a measure of the inflammatory response. This was the first drug trial in FSHD using MRI for screening of patients and using quantitative MRI as the main outcome measure. Lessons learned were reviewed, with particular regard to the difficulties of training sites and getting reproducible positioning of image stacks between time points. Furthermore, potential ways to correct for misalignment afterwards were discussed, e.g. minimizing slice gaps and analysing only overlapping slices.

Jordi Díaz-Manera, Doris Leung and *Sabrina Sacconi* discussed their experience in the ReDUX4 trial, a randomized placebo-controlled double-blinded phase 2b study, sponsored by Fulcrum Therapeutics, investigating losmapimod for the treatment of FSHD. The study lasted 48 weeks and enrolled 80 patients with 1:1 randomization ratio [44]. The primary endpoint was a change in DUX4-driven gene expression in needle muscle biopsies. Among the secondary endpoints were changes in different WB musculoskeletal MRI indexes, measured using the AMRA Medical protocol. Although the primary endpoint failed, treated participants showed a reduced increase of muscle fat infiltration (MFI, i.e. fat fraction calculated within voxels with less than 50% fat content) in muscles with intermediate fat fraction (less than 50%) at follow-up [45–47]. Participants discussed the rationale behind the threshold for MFI computation, the possibilities to reanalyze the data to determine the validity of the threshold, the selection of muscles analyzed, the difficulties related to COVID19 pandemic and the possible efforts to increase the accuracy of sampling for MRI-targeted biopsies.

Olof Dahlqvist Leinhard, representative of AMRA Medical, presented a commercial pipeline for the analysis of whole-body quantitative MRI, implemented in multi-center clinical trials to assess all major muscles affected in FSHD and adequately describe the disease heterogeneity. FSHD is a slowly and heterogeneously progressing disease affecting muscles all over the body, making it difficult to detect treatment effects in short clinical trials by using functional tests. Biomarkers based on quantitative MRI have proven sensitive to detect disease progression in FSHD but are mostly assessed only in a small portion of the muscles or only in a region of the body. This approach has limitations to describe the heterogeneity of the disease within individual muscles as well as the distribution of affected muscles throughout the body. Whole-body quantification of different features of the muscle composition such as lean muscle volume, muscle fat fraction, and MFI can provide a comprehensive view of muscle disease processes and separate the different components of disease progression. By combining measurements from multiple muscles into composite scores, a holistic representation of an individual's muscles can be obtained [46]. By further utilizing the knowledge that muscles with different degrees of fat replacement have different rates of progression, the composites can be enriched with muscles showing a high progression rate increasing the sensitivity to detect both disease progression and treatment response [45]. Combining and analyzing muscles not by anatomical location, but rather by the disease state of the muscle, also opens the possibility to monitor patients at a wide range of disease stages where different regions of the body are affected differently. This framework also allows for constructing composite scores by combining muscles involved in a specific task for correlation with functional outcomes [45].

5. Correlation with functional outcomes and other techniques

Jordi Díaz-Manera reviewed the published data on the correlation between muscle MRI findings and muscle function tests. Several publications demonstrate that the amount of fat present in the muscles correlates with muscle function studied either with muscle strength measurements, timed tests or functional scales in different neuromuscular diseases, including Duchenne Muscular Dystrophy (DMD), limb-girdle muscular dystrophies and FSHD [24,48,49]. Short-term longitudinal studies suggest that quantitative MRI detects changes in the muscle structure, mainly increase in the amount of fat, before patients experience any clinical decline [50,51]. There is recent evidence from long-term follow-up studies showing that changes in muscle fat content correlate with changes in muscle function [52–54]. Muscle fat fraction has been shown also to predict changes in functional performance and clinical milestones in DMD patients [55–57]. There is less evidence published on the correlation between water T2 and muscle function tests. The scarce data published shows that there is no correlation between water T2 and muscle function tests at a given time point. However, preliminary data in dysferlinopathy suggests that water T2 might predict changes in muscle function over time, although this needs to be confirmed in further studies [58].

Segmentation of individual muscles or muscle compartments in MR images is the necessary preliminary step before quantitative

measurements, and it has been largely acknowledged that manual segmentation is a time-consuming task prone to interoperator variability. Because of this, various semiautomatic or fully automatic methods have been developed. *David Bendahan*'s presentation assessed the potential of various segmentation methods. Results clearly illustrate that fatty replacement is a limiting factor for segmentation processes based on identification of muscle borders. Active contour methods combined to clustering analysis can provide a very effective segmentation of the whole muscle compartment in each image [59]. In case of severe fatty replacement, this method has to be combined with a manual double-check. Multi-atlas-based methods can be used for the segmentation of individual muscles with a mean volume error of a small percentage (3%). However, the efficiency varies widely among the different muscles and the individual volume error can reach up to 20%. Fatty replacement is also a limiting factor for this kind of method. With the aim of following up changes in a given subject, a single-atlas-based method in which the dataset recorded at baseline can be used as an atlas for the segmentation of datasets recorded at later times has been developed [60]. The semi-supervised method is definitely more robust to fatty replacement. It is based on the manual segmentation of a limited number of slices (10 to 15%) and registration and propagation processes are used for an automatic segmentation of the remaining slices [61]. Interestingly, this method can be used not only for the propagation of segmentation masks within a given dataset but also among multiple datasets recorded over time.

Francesco Santini reported on the use of deep learning, a data-driven artificial intelligence method which has been recently widely used for a variety of problems, including the segmentation of medical images. While multiple algorithms (models) exist, they all require a considerable amount of data, which must be representative of the range of physiological and pathological appearances of the organs that need to be segmented. The failure to include enough patient data in the training of the segmentation algorithms can prevent the generalization of the developed methods. A few studies have dealt with the segmentation of muscle MR images, focusing either on the detection of intramuscular adipose tissue [62], the segmentation of muscle groups [63], or of single muscles [64]. To allow the inclusion of data from multiple institutions, the free software Dafne (https://dafne.network/) uses federated learning, where the model improvement is performed at the users' side, and the improved models are subsequently integrated centrally.

Teresa Gerhalter presented on advanced MR methods, such as MR spectroscopy and X-nuclei imaging. These techniques can provide information on metabolic derangements that might occur before morphological changes are detectable, and only few studies investigated their potential to track metabolic changes in patients with FSHD. A ^{1}H-MRS study reported reduced Trimethylamine/Creatine ratio in normal appearing muscles of the hamstring. However, the underlying biological mechanism of this change remains unknown [38]. ^{31}P-spectroscopy revealed changes in the phosphorus metabolic indices in fat replaced muscles, but not in normal appearing ones, in FSHD patients [65]. The progressive phase in FSHD was characterized by distal to proximal fat replacement and altered energy metabolism in intermediately and highly fat replaced muscles [66]. Overall, disturbances in the phosphorus metabolism have been only observed in more advanced disease stages. In DMD, ^{23}Na-MRI offered sensitive markers such as the total sodium concentration, which was systematically elevated even in spared muscles [67]. In FSHD, disease activity as reflected by water T2 and sodium indices was systematically higher in moderately fatty replaced muscles [68]. However, some muscles without any increase in fat fraction showed also increased disease activity. The sodium measures increased even in some T2w-STIR negative muscles, highlighting the potential of ^{23}Na-MRI to detect early pathological changes not detected by qualitative imaging.

Aurea Martins-Bach's presentation focused on fibrosis, a pathological feature in FSHD. Methods capable of assessing skeletal muscle fibrosis specifically, quantitatively and non-invasively are still lacking. However, there are indications that MR imaging and ultrasound can be sensitive to skeletal muscle fibrosis. In a mouse model, different imaging modalities were altered in fibrotic muscles and abnormalities correlated with collagen fraction from histology. These parameters included extracellular volume estimated from plasma and muscle T1 maps before and after injection of a gadolinium contrast agent, texture features from high-resolution T2*-weighted images, water-T2, and viscoelastic index from ultrasound shear wave elastography [69]. However, when fibrosis co-exists with inflammation, degeneration and fatty replacement, like in FSHD, it is challenging to disentangle how each pathological process contributes to changes in MRI and ultrasound measurements. In these complex scenarios, multiple MRI and spectroscopy modalities, like water-T2, ultra-short time-to-echo (UTE), X-nuclei imaging, and magnetization transfer, can be still sensitive to fibrosis [70]. Nevertheless, these methods lack specificity and are also affected by the concomitant pathological processes like inflammation and fatty replacement. Contrast agents with high affinity to collagen allow a more specific assessment of fibrosis [71], but their use has been limited to preclinical studies due to safety concerns. When analyzed together with MRI, ultrasound images could potentially allow the visualization of skeletal muscle fibrosis, but only in the absence of fatty replacement [72]. Finally, multispectral optoacoustic tomography appears to be a sensitive and specific technique to detect skeletal muscle fibrosis, but its use is still limited to superficial muscles [73].

6. Muscle ultrasound

Nens van Alfen opened the session on muscle ultrasound, a technique that has been used for 40 years, with a significant development over the past 10 years. It has been extensively tested and validated in children and adults as a screening tool for neuromuscular disorders and as a potential technique to derive disease-related biomarkers. Muscle ultrasound allows scanning superficial muscle layers in any body region, and the analysis could benefit from the integration of several techniques, such as grayscale analysis, texture and pattern recognition, and elastography. With standardized measurements, visual analysis using Heckmatt grading has a sensitivity of around 75–80% for detecting intramuscular pathologic alterations, while quantitative grayscale analysis is even more robust with around 90% sensitivity and 85% predictive values [74]. In FSHD different cohort studies, including a longitudinal 1-year prospective follow-up study, have found that ultrasound is a responsive to disease-induced changes [75]. Ultrasound derived measurement correlated well with cross-sectional clinical scales (r values around 0.8–0.9). On follow-up, in this monocentric cohort of 22 patients, 41% of the muscles showed an increased mean gray level score after one year, while clinical measures did not significantly change. Longitudinal studies of larger cohorts are currently ongoing. In addition, in one study ultrasound was able to detect abnormalities in a number of muscles (7%) with "nearly" normal MRI signal, while MRI was better at quantifying end-stage degenerated muscles which looked normal on ultrasound [72]. Based on these results, muscle ultrasound has been proposed as an ideal companion tool for MRI in clinical trials, finding abnormalities apparently not detected by MRI, with the potential to reveal muscle fibrosis and the ability for

frequent repeated scanning that can be performed bedside or in a wheelchair if needed. Challenges lie in cross-center standardization of scanning protocols and quantification, and deep learning is being explored as a possible solution for that.

Sanne Vincenten presented a large cohort study combining muscle ultrasound with clinical outcome measures. Ultrasound images of truncal, upper and lower extremity and facial muscles were acquired bilaterally at specific reference points. The echogenicity z-score and muscle thickness z-scores of each muscle image were calculated and the Heckmatt score was visually determined. Clinical outcome measures included the Ricci score [32], FSHD clinical score [36], MFM [37] and a 4-point facial weakness score [76]. The ultrasound compound score was defined as the mean fat fraction of all muscles combined. One-hundred and fifteen FSHD patients (52% male, mean age 52±14 years, median Ricci score 6 (range 0–10)) were enrolled. An abnormal z-score (≥2) was found in 38% of all muscles and 94% of all patients had one or more abnormal muscles. The trapezius muscle was affected most frequently (78% of the cohort), while the rectus abdominis was the least affected (19%). The highest agreement between Heckmatt and echogenicity z-scores was found in the biceps brachii, trapezius, rectus femoris and vastus lateralis (96–100%). Both the compound z-score and Heckmatt score strongly correlated with the clinical outcome measures used (ρ=0.8, $p < 0.05$). Analysis of echogenicity z-scores in the facial muscles showed that the muscles of facial expression were most frequently affected (15–22% of the cohort), as opposed to muscles of mastication and swallowing (0–3%). Heckmatt assessment of facial muscles was highly variable both inter- and intra-rater. The conclusions of the study were that quantitative muscle ultrasound can be used to derive biomarkers in FSHD with some caveats: consider the muscles with the highest agreement between Heckmatt and z-scores, re-assess all muscle images visually to prevent misinterpretation and use it preferably in early disease stages of FSHD.

Kristen M. Meiburger further discussed the role of artificial intelligence methods, in particular deep learning, for medical image processing. Deep learning aims to mimic the network of neurons in the brain and consists of neural networks with many layers with an end-to-end learning, showing nonlinear hierarchical representations. A few important features about deep learning methods regard how they are “data-hungry”, needing hundreds and thousands of images with a ground truth label, and their explainability, which is especially critical when directly classifying medical images. Deep learning techniques can be extremely helpful when analyzing ultrasound images in three main aspects: image segmentation, image classification, and image generation and clinician training. Deep learning methods have shown better performances than those based on traditional image-processing methods for muscle segmentation in ultrasound images [77,78] and can assist clinicians by providing an automatic segmentation of the cross-sectional area for the computation of the gray scale mean [79]. Texture features computed on ultrasound musculoskeletal images have shown to be informative for pathology differentiation [80] and the implementation of deep learning methods for FSHD staging and classification merging ultrasound and MRI data along with clinical parameters is a field that needs investigation and will be the focus of future collaborations. Finally, deep learning methods can be helpful for image generation. The ability to synthesize musculoskeletal ultrasound images from semantic labels with controllable echogenicity can be useful for both automated algorithms by augmenting datasets and filling the gap in underrepresented classes, as well as for clinicians by providing a tool for assessing how a certain muscle with a different echogenicity level may look.

7. Discussion and workshop deliverables

At the end of each session, and during the last session of the workshop, all the participants discussed several topics that emerged from the presentations. The participants agreed on the added value of muscle MRI in many different contexts for FSHD patients.

First, the patient representatives put forward the importance for them of having an MRI scan to better understand and visualize the degree and distribution of muscle involvement caused by their disease. They defined a scan time less than one hour as acceptable, with longer periods possible only after interruption of the examination to allow for a short break and change of position, and a scan time of less than 30 min as ideal. The scan would also set a baseline for the evaluation of disease burden, being useful for subsequent comparisons, and could help patient management by more accurately tailoring rehabilitation treatments and the use of orthoses.

The use of qualitative MRI, with dedicated protocols including at least the scapular girdle, but if possible covering also the lower limbs, was considered particularly helpful for diagnostic purposes in the following cases: (a) patients with fragment length in the upper abnormal range (7–10 D4Z4 repeat units) or discrepancy between clinical severity and number of D4Z4 repeats; (b) patients diagnosed with suspect FSHD2; (c) patients with atypical or incomplete phenotypes (i.e., patients with a myopathy presenting some FSHD features in association with other uncommon characteristics or patients not presenting a simultaneous involvement of facial and scapular girdle muscles); (d) patients with no family history; (e) to address and correctly interpret FSHD genetic testing in patients that are still undiagnosed even after massive parallel sequencing. The use of muscle MRI as a diagnostic support for FSHD in the above-mentioned cases should be considered in the future revisions of the clinical guidelines on this disease [81].

MRI has a role in patient stratification identifying active versus non-active disease through the presence and/or extent of T2w-STIR positive lesions. The differences between FSHD1 and FSHD2 patients, which should also be taken into account in the analysis of the results of natural history and interventional trials, were considered to belong to the variability of the whole disease spectrum.

Notably, all the longitudinal quantitative MRI studies, despite the different protocols used, were concordant on the yearly increase of fat fraction and on the fastest progression of T2w-STIR positive muscles and of the muscles with intermediate fat fraction at baseline. The group agreed that a structured meta-analysis would be worth to confirm the robustness of published and unpublished data that emerged from the presentations, and that the availability of the placebo-arm quantitative MRI findings from the already concluded interventional trials could strengthen this evidence. Researchers, patients’ associations and other stakeholders should move together in the direction of requesting to share data even if obtained from sponsored studies, as well as to publish or warrant access to negative trial results, which are informative and valuable to avoid duplication or replicating mistakes in science [82].

Since FSHD is a multifocal disorder progressing asynchronously in the different muscles, an optimized protocol for tracking disease progression in a reasonably short timeframe should allow fat and water quantitative imaging with a whole-body coverage. Dedicated scanning protocols would be a suboptimal but still valuable or indicated option in selected trials and for specific research questions, since particular biomarkers could be more informative than others according to the putative action of the drug tested (Table 1). Therefore, the need for the development of new

Table 1
Expected modification of muscle function and of different MRI-derived biomarkers based on the putative drug action, according to the current knowledge on FSHD pathophysiology.

Drug mechanism	Muscle function	Muscle size	Muscle fat fraction (composite)	Muscle fat fraction of T2w-STIR+ lesions	Muscle fat «Infiltration»	water T2 (composite)	water T2 signal of T2w-STIR+ lesions	Rate of T2w-STIR+ appearance
Inhibition of DUX4 expression	Reduced decrease/ stabilization	Reduced decrease/ stabilization	Reduced increase/ stabilization	Most likely none, or reduced increase	Reduced increase/ stabilization	Decrease	None	Decrease
Inhibition of DUX4 transcriptional activity	Reduced decrease/ stabilization	Reduced decrease/ stabilization	Reduced increase/ stabilization	Reduced increase/ stabilization	Reduced increase/ stabilization	Decrease	Decrease	Decrease
Anabolic drugs	Reduced decrease/ stabilization/ increase	Reduced decrease/ stabilization/ increase	Reduced increase/ stabilization/ reduction	Most likely none	Reduced increase/ stabilization	None	None	None
Anti-inflammatory drugs	Reduced decrease/ stabilization	Reduced decrease/ stabilization	Reduced increase/ stabilization	Reduced increase/ stabilization (?)	None, or reduced increase/ stabilization	Decrease (?)	Decrease	Decrease (?)

protocols and open pipelines for analysis/processing tools to gain further insight into FSHD pathophysiology and disease progression was underlined, and collaboration between neurologists and neuromuscular specialists, neuro- and musculoskeletal radiologists, physics, engineers and patients will be crucial to this aim. The time required for the examination, the number of centers potentially involved in a multicentric trial and their specific expertise are all factors with impact on the technical choices. The possibility to implement 2pt gradient echo Dixon with optimized MESE sequences for water T2 quantification in the lower limbs with contiguous slices was discussed. Regarding the segmentation process, the use of region of interests encompassing muscle compartments instead of single muscles could be sufficiently sensitive to change for most purposes [83], streamlining the data analysis toolchain.

Ultrasound might have a complementary role to MRI, thanks to an interesting potential sensitivity, despite low specificity, for muscle fibrosis, and the very good patient compliance for the technique. The group agreed that current drawbacks are the possibility to have standardized assessments limited to specific muscles/muscle sections, the software and hardware dependency of the results, and the relatively long training required for the operators, which presently limit its applicability in a multicentric setting. However, novel image analysis and processing tools for ultrasound alone or in combination with MRI data are being explored, with promising applications in the assessment of disease progression and therapeutic interventions.

Finally, the two consortia, FSHD CTRN and ETN, will coordinate the efforts to reach common aims, and the members of the respective imaging working groups agreed on the need to have regular joint meetings in the future.

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Declaration of Competing Interest

- Shahram Attarian reports no disclosures related to the present work;
- David Bendahan reports no disclosures related to the present work;
- Pierre Carlier reports no disclosures related to the present work;
- Robert-Yves Carlier reports no disclosures related to the present work;
- Olof Dahlqvist Leinhard is a shareholder and employer of AMRA Medical AB; provided consulting activity for Fulcrum Therapeutics;
- Jordi Diaz-Manera reports no disclosures related to the present work;
- Roberto Fernandez-Torron reports no disclosures related to the present work;
- Teresa Gerhalter reports no disclosures related to the present work;
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- Aurea Martins-Bach reports no disclosures related to the present work;
- Kristen Meiburger reports no disclosures related to the present work;
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- Sabrina Sacconi provided consulting activity for Fulcrum, Dyne therapeutics and Roche; received a grant from Roche; collaborates with AMRA Medical;
- Francesco Santini have received honorary as a consultant for Hoffmann - La Roche, paid to his Institution;
- Giorgio Tasca has acted as consultant on advisory boards and participates as steering committee member on a study in FSHD with Roche;
- Nens van Alfen performs editorial services for Wiley Inc., payment goes to their employer;
- Sanne Vincenten reports no disclosures related to the present work;
- John Vissing has acted as consultant on advisory boards and participates as PI on studies in FSHD with Fulcrum Therapeutics and Roche;
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Supplementary materials

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