

## **268th ENCM workshop - Genetic diagnosis, clinical classification, outcome measures, and biomarkers in Facioscapulohumeral Muscular Dystrophy (FSHD): relevance for clinical trials.**

September 30th – October 2nd 2022, Hoofddorp, The Netherlands

Federica Montagnese<sup>a</sup>, Katy de Valle<sup>b</sup>, Richard J.L.F. Lemmers<sup>c</sup>, Karlien Mul<sup>d</sup>, Julie Dumonceaux<sup>e</sup>, Nicol Voermans<sup>d</sup> on behalf of the 268th ENMC workshop participants<sup>#</sup>

<sup>a</sup>Friedrich-Baur-Institute, Department of Neurology, Ludwig-Maximilian University Munich, Germany

<sup>b</sup>The Royal Children's Hospital, Department of Neurology, Melbourne, Australia

<sup>c</sup>Leiden University Medical Center, Department Human Genetics, Leiden, The Netherlands

<sup>d</sup>Department of Neurology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, the Netherlands.

<sup>e</sup> NIHR Biomedical Research Centre, University College London, Great Ormond Street Institute of Child Health and Great Ormond Street Hospital NHS Trust, London WC1N 1EH, UK.

\*Corresponding author: Nicol Voermans, Email: [Nicol.Voermans@radboudumc.nl](mailto:Nicol.Voermans@radboudumc.nl), Department of Neurology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, the Netherlands.

<sup>#</sup>Listed at the end of the report

**Keywords:** Facioscapulohumeral, dystrophy, ENMC, clinical trials

### **1. Introduction**

The 268th ENMC International Workshop on “Genetic diagnosis, clinical classification, outcome measures, and biomarkers in Facioscapulohumeral Muscular Dystrophy (FSHD): relevance for clinical trials” took place on the 30th September - 2nd October 2022 as a hybrid meeting, with 23 participants on site in Hoofddorp (the Netherlands) and 8 connected remotely, from 10 different countries.

FSHD is the one of the most frequent muscular dystrophies with an estimate of 90.000 affected patients in Europe (1). Recent advancements in the understanding of FSHD pathogenesis have enabled progression toward clinical therapeutic trials. The scientific community is committed to reach clinical trial readiness; with this aim, two major consortia devoted to the acceleration of drug development and optimization of clinical trial design have been created (the FSHD European Trial Network - ETN, in Europe and the FSHD Clinical Trial Research Network - CTRN, coordinated from the United States and) (2-4).

Notably, FSHD is unique in its genetic mechanism (5) and very peculiar in the distribution and progression of muscle damage, compared to the other muscular dystrophies (6, 7). These aspects represent challenges for the development of effective drugs and for the success of clinical trials.

Genetic testing is the preferred tool to confirm a diagnosis of FSHD in a patient with suggestive clinical features. In the 2010 FSHD genetic diagnostic best practice guidelines, Southern blotting followed by FSHD-locus-specific hybridization emerged as the most suitable diagnostic method (8). The increased complexity in genetic result interpretation, due to newly identified genetic and epigenetic mechanisms coupled with the availability of new methods and technologies for genetic testing highlight the urgent need to update the FSHD diagnostic guidelines.

The broad clinical spectrum of FSHD with slow progression and asymmetrical muscular involvement poses major challenges for the selection and development of appropriate clinical outcome measures (COMs). Valid COMs are a strong determinant for the success or failure of clinical trials. For most of the available functional COMs and patients reported outcomes (PROs) there remains a paucity of validation studies to support their use in FSHD. To address this issue the FSHD-CTRN launched the ReSolve study with the aim of evaluating new (FSHD-Composite Outcome Measure (FSHD-COM), Reachable Workspace (RWS)) and existing (e.g., 6 Minute Walk Test (6MWT), Timed Up and Go (TUG)) COMs in FSHD (3). The European community is committed to join forces and expand the efforts of ReSolve by assessing the utility of other COM, by addressing neglected FSHD patient categories including paediatric, elderly or non-ambulatory patients and by implementing reliable home monitoring tools.

In addition to COMs, the identification of good biomarkers represents an important pillar for improved patient characterization and better evaluation of drug efficacy. The utility of muscle-MRI in FSHD was highlighted in a recent ENMC Workshop of the FSHD ETN, which defined the strengths and limitations of this tool for patients with diagnostic uncertainty, for patient stratification and for identification of disease progression or therapy response (9). Muscle biopsies also represent an important source of biomarkers. In particular, much attention has been drawn to the detection of DUX4 and PAX7 and the challenges related to their quantification (10). Blood biomarkers however are a more accessible alternative to an invasive muscle biopsy and some promising results have been demonstrated for inflammatory biomarkers such as IL-6 (11) and integral membrane protein SLC34A2 (12).

Four working groups (WG) created within the FSHD-ETN aim to address unmet needs in genetics (WG1), clinical outcome measures (WG2) and biomarkers (WG3) and on muscle imaging in FSHD

(WG4) (2). In preparation for this ENMC workshop, WG1, -2 and -3 defined their aims and future initiatives. This ENMC workshop provided the rare opportunity to bring together clinicians, patients, geneticists and basic science researchers, to share their knowledge and experience in FSHD to advance clinical trial readiness addressing the previously specified topics.

After a brief introduction and welcome from the ENMC representatives, *Nicol Voermans* introduced the workshop organizers, the working groups, the participants and illustrated the aims of the workshop (Table 1).

## **2. Session 1: optimize FSHD clinical care and monitoring throughout Europe (WG2)**

**Emma Matthews** opened this session discussing FSHD care from an adult neurologist perspective. An adult FSHD service should comprise a multidisciplinary care team (13). The team should include neuromuscular specialists, allied health and nursing clinicians (nurse, coordinator, physio and occupational therapists, orthotists, speech therapists, psychologists), clinical geneticists, pneumologists, orthopaedic surgeons and ophthalmologists. Given the importance and complexity of genetic diagnosis, a good collaboration between genetic laboratories, clinical geneticists and the neuromuscular team is essential. Availability of specialized staff to facilitate patient access to genetic testing, interpretation of results and advice on family planning is also beneficial. Information related to contraception, pregnancy and possible fertility treatments should be discussed early in the patient journey to allow the possibility to consider all options available.

Care should be tailored to the individual and the intervention personalized based on individual patient needs, which vary throughout the lifespan e.g., transition, or pregnancy. The administration of COMs, while time consuming, plays a vital component in clinical care. They do however need to be practical, informative, time efficient so as not to detract from other aspects of clinical care. More detailed COMs should be reserved for a research setting. Therapies play a vital role in the ongoing physical, environmental and psychosocial aspects of care. Physical aspects encompass the management of postural changes, orthotics, fatigue, pain, swallow and feeding, respiratory health and physical activity. Environmental considerations may include the risk of falls, home and community access, vocation, transportation and mobility. Psychosocial care supports diagnosis acceptance, implications for family members, intimacy, financial security, transition from paediatric to adult care and peer support. Ongoing challenges in the care of adults with FSHD include managing transition from paediatric to adult care, integration between clinicians and geneticists, psychological support, pain management, indications for scapular fixation and equity of access.

**Nicol Voermans** discussed FSHD care from a paediatric neurology perspective and presented the data of a systematic literature search on the clinical features of early onset FSHD (43 articles; 227 patients) performed in 2017. Infantile or early onset was estimated to occur in around 10% of all patients with FSHD. The mean age at reporting was 18.8 years, and 40% of patients were wheelchair-dependent at that age. Half of the patients had systemic features, including hearing loss (40%),

retinal abnormalities (37%) and developmental delay (8%). An inverse correlation between repeat size and disease severity, similar to adult-onset FSHD was described. De novo FSHD1 mutations were more prevalent than in adult-onset FSHD. However, a significant clinical heterogeneity was observed. Based on this review, early onset FSHD should be considered on the severe end of the FSHD disease spectrum, however there are also mildly affected children without systemic features (14).

After this review, a natural history study started in 32 children and confirmed that FSHD in childhood is more prevalent than previously known (1 in 100.000 children in the Netherlands), and the genotype resembles classic FSHD in adults. Main findings were facial weakness with normal or only mildly affected motor performance, decreased functional exercise capacity (6MWT), lumbar hyperlordosis, and increased echo intensity on muscle ultrasonography. The study concluded that FSHD in children mainly affects functional exercise capacity and quality of life (QoL)(15). The follow-up at two years showed a variable course. The most promising COMs to detect progression were the FSHD clinical score and muscle ultrasonography (16). Despite this disease progression, an improvement in functional capacity may still occur as the child grows up. Pain, fatigue, and a decreased QoL were common symptoms, which need to be addressed in the management of childhood FSHD. A qualitative study on social participation, communication and QoL by 15 in-depth interviews among children and adolescents is currently ongoing. Performance, fatigability and QoL are going to be assessed with validated endurance tests as has been used effectively in spinal muscular atrophy (SMA) (17). Based on these results, most children with FSHD will benefit from rehabilitation including energy management, physical training and psychological support. Furthermore, these topics remain important in transition to adult care.

**Elena Carraro** highlighted the importance of rehabilitation in FSHD patients. Rehabilitation is the process of enabling someone to live well with an impairment in the context of his or her environment; it requires a complex, individually tailored approach and should be based on the International Classification of Disability, Functioning and Health. Rehabilitation in FSHD should aim to maintain optimum health, to prevent or delay secondary complications, to maximize functional abilities and to improve or maintain QoL. Supporting patients to maximize their level of functioning requires the identification and monitoring of factors and activities that contribute to their well-being. Depending on the individual's age, signs, symptoms, and functional abilities, the plan may include assessment of balance and gait, posture, need for orthosis, management of pain and fatigue, recommendations about appropriate activities, and environmental modifications (4, 18, 19). Both in ambulant and in non-ambulant patients stretching (self-managed and rehabilitative stretching) of muscles and structures at risk of tightness should be performed not less than 4 to 6 times a week. Low-intensity aerobic exercise appears to be safe and potentially beneficial in FSHD (20) and should be encouraged, targeting the exercises based on weakness distribution to avoid falls or over-use damage (i.e. consider stationary bicycle instead of treadmill for patients with ambulatory difficulties).

The beneficial effect of strength training is still controversial, however, strengthening exercise was not noted to be harmful (21) and clinicians could propose personalized and safe programs using appropriate low/medium weights/resistance and taking into consideration the patients' physical

limitations. Eccentric exercise must be avoided, whereas concentric submaximal resistance exercise and moderate aerobic training are recommended (18). Balance training and reduction of falls are very important to consider; it is useful to consider the use of ankle foot orthoses for drop foot management, lumbar corset for lumbar hyperlordosis and customized manual or power wheelchair for fatigue management.

There are few data about Vibration therapy, FES training, Neuro or kinesio - taping application and robotic devices. There is also a lack of evidence about rehabilitation programs focusing on facial weakness, speech and swallowing.

**Katy de Valle's** presentation focused on the use of COM in clinical care. While COM instruments used in clinical care also require solid psychometric properties, they must contribute positively to anticipatory care with adequate utility to suit a real-world clinical environment and guide/support therapeutic interventions. A 2019 systematic review identified and graded the measurement property evidence of instruments used to measure physical functioning in individuals with FSHD. This review also generated a list of performance-based and self-reported outcome measures used in FSHD research to date (22). Currently, little is known about COMs use in FSHD clinical care settings.

A web-based survey aimed at identifying common instruments used to evaluate function and QoL in FSHD clinical care was designed to address this knowledge gap. This survey was distributed through established formal and informal neuromuscular and FSHD networks. Clinician respondents reported using a wide variety of outcome measure instruments to evaluate activity limitations and impairments of body structure and function. Inconsistent and infrequent evaluation of participation was reported in the clinical setting. The most frequently evaluated domains included muscle strength and falls reported by 93% and 88% of respondents respectively, followed by upper limb range of motion (80%) and timed motor function (78%). The most consistent COM included manual muscle strength using MRC scores, shoulder active range of motion and number of weekly falls reported by 78%, 59% and 56% of respondents. There was limited reported use of FSHD-specific disease severity measures including the FSHD-clinical score and FSHD-clinical severity scale in clinical service. This was a surprising finding given both these measures have excellent clinical utility, are freely available and have been used extensively to characterize disease severity in FSHD research (23, 24). Among the identified barriers to use of COMs across all domains except disease severity were lack of time and availability of therapists to administer instruments. Knowledge of- and familiarity with- disease severity instrument administration was the major reported barrier to utilizing these instruments in FSHD clinical care.

Achieving consistency in COM use in FSHD clinical care will aid identification and stratification of participants for clinical trials, help standardize clinical care monitoring and enhance anticipation of individual care needs.

### **3. Session 1: FSHD Clinical Trial Readiness in Europe**

This session focused on the currently available COMs and PROs for use in clinical trials and discussed the steps needed to further develop digital COMs and implement existing FSHD registries.

**Federica Montagnese** presented the current panorama of motor COMs potentially suitable for clinical trials in patients with FSHD. Until a few years ago, the clinometric properties of several COMs in FSHD were found to be low to very low, given the lack of studies in this field (22). The ReSolve study was initiated with the aim to identify and validate the best motor COMs in FSHD. In particular, to determine the multi-site reliability and validity of FSHD-COM and assess its responsiveness in comparison to other COMs, such as Motor Function Measure (MFM32), 6MWT, 2MWT, TUG and RWS (3).

The strengths of using a composite score such as FSHD-COM include higher statistical efficiency (studies can be designed with fewer patients and for a shorter duration), and these scores are also useful if the choice of a unidimensional primary endpoint is not obvious. On the other hand, composite scores can make treatment seem more effective than it really is, especially if components of variable clinical importance are combined as in FSHD-COM (25). The first longitudinal results of the Resolve study presented at the last international research congress on FSHD (26), demonstrated FSHD-COM reliability in a multi-site study and validity when correlated with other measures of disease severity. The responsiveness was low, with significant changes likely to be detected at 18 months. Some alternative versions of the FSHD-COM are being validated for the paediatric population and for non-walkers. To include all FSHD subgroups, a new multi-site study (MOVE and MOVE+) has begun to identify and validate other COMs, without limitations of age (paediatric, adult, elderly), disability level (walkers and non-walkers) and genetic background (FSHD1 and 2). This study would better reflect the real-world FSHD population rather than selected FSHD patient's categories suitable for clinical trials as in ReSolve. Besides FSHD-COM, the MFM32 has ceiling and responsiveness limitations and 6MWT advantages in terms of reliability, validity and MDC95 in adults with FSHD.

Current knowledge suggests the most promising outcome measure for clinical trials is RWS technology, which evaluates the volume of reachable space. With data supporting validity, reliability, sensitivity to change and clinical meaningfulness of RWS, access to technical equipment and the complex data analysis are its major limitations (27). The relevance and usefulness of COMs developed to assess facial weakness (Iowa oral performance instrument, IOPI) remain open due to the lack of longitudinal data and debate related to progression of facial weakness in FSHD.

**Karlien Mul** highlighted the importance of patient reported outcome measures (PROs) for clinical trial readiness in FSHD. While physician-reported outcome measures are able to capture limitations on the levels of impairment (e.g. muscle strength) or activities (e.g. ability to handle buttons), the only way to gather information on the levels of social participation and QoL is to ask the patients. It is important in any clinical trial to realize that an improvement or decline in an impairment measure such as strength has no intrinsic meaning in the absence of correlation to quality of life.

Most PROs are questionnaires that provide ordinal-based measures. Ordinal scales allow a rank order but have unequal intervals between scores and provide nonlinear results that are unsuited for parametric statistical testing. Therefore, linear-weighted interval scales are preferred. Rasch analysis provides a mathematical model to transform ordinal-based scales into interval scales.

Another clinimetric aspect to consider is the transition from the now commonly used statistically significant differences in clinical trials, to more clinically relevant ‘minimally clinically important differences’ as the main statistical result. Next, the preference of FSHD specific scales over more generic scales was discussed, as daily and social tasks are not just dependent on having an illness but are rather disease specific. Three FSHD-specific PROs are the FSHD-Health Index (FSHD-HI) (28), the FSHD Rasch-built Overall Disability Scale (FSHD-RODS) (29) and the FSHD Facial Function Scale (FSHD-FFS) (30).

The FSHD-HI is an ordinal-based scale that intends to measure a patient's perception of the total disease burden through 116 questions covering 14 subscales (28). The FSHD-RODS measures activity and social participation limitations (29). It is an interval scale consisting of 32 items, which is available in five different languages. The FSHD-FFS assesses functional disabilities relating to facial weakness and consists of 25 items (30). For all three scales, studies are underway to assess their responsiveness and sensitivity to change. Finally, it is important to reach consensus on what PROs should optimally be used and strive for uniformity among clinicians and researchers on what COMs are used.

**Elisabetta Gazzero** presented an argument supporting the use of digital tools to address the use of real-world metrics for objective assessment of therapeutic effects on motor function in clinical trials. Current COMs, while useful, are susceptible to bias. They consist of self-reported questionnaires and observer-rated performance usually undertaken in an artificial hospital setting. Unlike digital tools, they are unable to detect changes in small intensity movements, overcome daily fluctuations in subjects’ conditions, test patient-relevant functioning and are mostly focused on lower limb motility. Although crucial for QoL and independence, available endpoints for upper limb motor profile remain limited, thereby constraining the recruitment of non-ambulant patients in clinical trials.

The stereo camera based RWS (27) adopted as primary endpoint in phase 3 FSHD clinical trial, visualizes and measures upper limb movements performed inside the working volume of the device. Measurement is restrained by a low efficiency in capturing free motion and limited by ceiling and floor effects. Similar bias is displayed by Microsoft-Kinect gaming interfaces associated with skeleton tracking algorithms. “Ubiquitous computing” via wearable or remote sensors embedded in everyday objects can smartly address this “knowledge gap” and support clinical research in acquiring longitudinal sensitive motion data collected in the home/community environment.

Proof of principle examples come from studies in Amyotrophic Lateral Sclerosis, Duchenne Muscular Dystrophy (DMD) and SMA (31). The stride velocity 95th centile (SV95C), measured at the ankle using the wearable-inertial magneto sensors (WMIS) Actimyo (Sysnav, France), received qualification from the European Medicines Agency (EMA) as an acceptable secondary endpoint in clinical trials of ambulant individuals with DMD (32). The same device was used for the first time to quantify home-based gait analysis, in a group of ten patients with FSHD. The representative values for stride speed and stride length were found to correlate with manual muscle testing scores (33, 34).

Research from our group focuses on the development of a new digital system aimed to provide real-world upper limb movement metrics and quantification of physical muscle activity using

accelerometers. This work aims to empower patients and to enhance patient-caregiver interaction. We tested our digital concept in a population of fifty individuals affected by different genetic diseases of which twelve (24%) were affected by FSHD. The feedback collected in this first community-building phase will drive subsequent validation.

**Giulia Ricci's** presentation emphasised the need to bridge the gap between clinical and registry data. Evidence from clinical practice supports the need for deep phenotyping of FSHD patients to promote more accurate diagnosis and treatment, to study the role of modifying factors and to develop appropriate COMs and biomarkers for trial readiness. All these needs and questions require the collection of a large amount of information. Therefore, along with a "Disease Registry" the collection of demographic data, should be directed toward a combination of genomic and clinical data.

Diseases Registries support the development of standards of care and research questions, by providing clinicians and scientists with information to learn more about the disease and representing a link between patients and the research community. Notably, deciding on the purpose of the registry is an important first step as it guides how the registry is designed. For example, all national registries that wish to participate in TREAT-NMD Global Registry Enquiries are required to collect a "mandatory dataset" for their specified disease; many registries take the opportunity to collect additional data, such as items relating to the disease natural history or QoL of registry participants.

In FSHD, clinical diagnosis and accurate recording of phenotypes and data regarding natural history are crucial aspects for genetic diagnosis and clinical management. One major problem is that results of clinical trials can be biased by the incorrect selection of patients or COMs interfering with interpretation of results. Therefore, the need to collect patient data in a harmonised manner across multiple countries has become increasingly apparent, especially when locating patients suitable for a particular trial or therapy poses a particular challenge. With this aim, the Italian Clinical Network for FSHD has defined the FSHD Comprehensive Clinical Evaluation Form (CCEF), a simple clinical tool applied during the neurological examination of patients (35). The CCEF describes clinical variability, beyond the degree of motor impairment, and classifies the phenotypic spectrum observed among patients through the identification of clinical categories sharing common features such as the muscle weakness distribution or presence of other additional signs or uncommon features. The categories outlined by the CCEF can assist diagnosis, genetic counselling, and natural history studies, and have useful potential in genotype-phenotype correlations studies.

**María Gómez-Rodulfo** a patient representative and member of FSHD-SPAIN and FSHD-EUROPE, emphasized the importance of including patient representatives in research progress meetings and highlighted the role patient representatives, embedded in FSHD associations, can play in the dissemination of information about upcoming clinical trials.

Maria participated in the Fulcrum Phase 1 clinical trial in 2019 and shared a 'patient's perspective' of what could be improved in the publicizing, recruitment and running of a clinical trial. Including: early publicizing of eligibility criteria and trial site locations to help determine feasibility for involvement; providing more information regarding trial expectations for example what happens in an MRI or muscle biopsy; giving participant's access to trial-based imaging, blood test and biopsy

results; mitigating participant burden by providing adequate financial support, careful site selection, scheduling appointments to limit participant disruption and providing access to wheeled mobility if required.

Post clinical trial participation could be enhanced by clear communication regarding ongoing access to the investigational product, potential involvement in subsequent phases of the trial and regular updates about how the follow-up phases of the trial have progressed. While involvement in clinical trials involve some level of risk and can invoke fear, involvement is often an extremely positive experience for affected individuals.

#### 4. Genetic testing modalities and harmonization

**Frederique Magdinier** presented the different genetic testing modalities for FSHD discussing their advantages and disadvantages. FSHD is genetically heterogeneous with at least two subtypes. In 95% of patients (FSHD1; MIM#158900), the disease is linked to the subtelomeric 4q35 locus (36, 37) and involves a reduction in the number of repeat units of the D4Z4 macrosatellite repeat (38). In the healthy population, the number of units is between 8 to more than 100 (39). FSHD1 patients carry a pathogenic contraction of the array (1-10 D4Z4 units) and the presence in cis of an FSHD-permissive haplotype (4qA)(39). Type 2 FSHD (FSHD2; MIM#158901, 5% of patients), is linked to mutations in chromatin modifiers (mostly the SMCHD1 gene (40) resulting in a loss of DNA methylation at D4Z4. FSHD2 also requires the presence of a permissive 4qA haplotype, which usually ranges here between 8-20 units. In both FSHD1 and FSHD2, a rough inverse correlation has been observed between the repeat size on the permissive allele and the severity.

In most laboratories worldwide, FSHD diagnosis is performed by Southern Blotting (SB) after digestion of DNA using *EcoRI* to determine the size of 4q and 10q arrays, linear (LGE) or pulsed-field (PFGE) gel electrophoresis and hybridization with the p13E-11 probe (D4S139 marker) that lies upstream of the first D4Z4 unit.(40)(41) Specific restriction enzyme of either 4q-derived (*Xapl/Apol*) (41) or 10q-derived (*BlnI*) (42) D4Z4 repeats are used in combination with *EcoRI* to discriminate between 4qTer and 10qTer-derived arrays. Determination of the haplotype (4qA, or non-permissive 4qB) requires an additional step of digestion using the *HindIII* restriction enzyme and hybridization of DNA blots with specific probes.

Over the past decade, two new diagnostic approaches have been developed. First, a molecular combing-based (MC) approach is a hybridization-based method on DNA fibers that provides a comprehensive analysis of the 4q and 10q alleles, the sizing of the D4Z4 arrays and determination of the haplotype in one go (42). More recently, Bionano Genomics has developed a novel diagnostic approach, based on the use of Single Genomic Optical Mapping (SMOM). For this approach, long fluorescently-tagged DNA molecules are stretched, imaged using fluorescence microscopy and assembled *in silico*. SMOM has been validated for sizing of the D4Z4 array on chromosomes 4 or 10 and determination of the haplotype (43, 44) that can be performed directly using the Bionano EnFocus™ FSHD 1.0 algorithm.

All four technologies have been found to be highly reliable for FSHD diagnosis with advantages and disadvantages for all of them. LGE and PFGE are cost effective but labor-intensive and non-

automated. LGE only visualizes <11 unit D4Z4 repeats, which can hamper the identification of somatic mosaicism and FSHD2. They also require additional steps for the determination of the haplotype. MC and SMOM provide a single step D4Z4 array sizing on both 4q and 10q alleles together with A- or B-haplotypes. However, these methodologies are more expensive and require dedicated equipment.

**Nienke van der Stoep and Sarah Burton-Jones** discussed the challenges of genetic testing, sharing their experience on how to deal with false positive and false negative results, the overlap between FSHD1, FSHD2, patient samples displaying 8-10 units and when to continue to use follow up verification and complementary studies such as 4qA/4qB haplotype and methylation analysis. Some of the technical and logistic challenges related to the use of Southern blot include: the need of large amounts of DNA in comparison to next generation sequencing (NGS) techniques; the need of fresh EDTA blood for good quality DNA; the challenge of delivering fast results especially important for prenatal testing; the resolution of larger repeats 7-10 which is not very accurate and can vary across laboratories. Therefore, the reports should always include both repeat sizes in kb and number of D4Z4 units.

Southern blot technique using LGE has some detection limitations in case of somatic mosaicism and for complex D4Z4 rearrangements, like translocations between chromosomes 4 and 10 and for FSHD alleles where the p13E-11 probe is deleted. For these situations, the use of PFGE-based Southern blot analysis or the newer technologies offer a real advantage. In cases where the FSHD1 diagnosis cannot be confirmed with the sole clinical assessment and the 4q35 D4Z4 repeat size, such an extended analysis might be helpful. In particular, the 4qA/4qB haplotype should be determined if: 1) the patient has a 4q35 D4Z4 allele with 8 to 10 D4Z4 repeat units; 2) the patient has more than one 4q35 D4Z4 allele in the pathogenic repeat size range; 3) the tested individual has 4q35 D4Z4 allele in the pathogenic range but the symptoms are atypical for FSHD (or absent in case of a presymptomatic family member or control); 4) the identified short 4q35 D4Z4 fragment does not segregate with the phenotype in the family and 5) in a prenatal diagnosis, the suspected FSHD allele in one of the parents is from a *de novo* contraction. SSLP analysis can assist in cases to elucidate the genotype, in case that complex rearrangements are suspected (like p13E-11 probe deletions, or translocations between chromosomes 4 and 10). However, this approach remains a risk calculation and is, without further complete D4Z4 repeat information, no final result. Finally, FSHD2 testing should be performed: in patients with FSHD phenotype and where no FSHD1 allele is identified; in patients with confirmed positive family history for FSHD2; if the inheritance pattern is not clearly dominant in the pedigree or the short fragment does not segregate with disease symptoms in pedigree; and when a high clinical severity is found in combination with an 8-10 unit D4Z4 repeat size.

**Richard Lemmers and Sarah Burton-Jones** discussed the key aspects of FSHD2 which has a digenic inheritance pattern, combining a permissive 4qA allele with loss of function mutation in a gene (SMCHD1, DNMT3B, ...) encoding a protein involved in DNA methylation or heterochromatin structure on D4Z4. They presented diagnostic strategies, comparing methylation and sequencing, discussing how to proceed with variants of unknown significance (VUS) in SMCHD1 and DNMT3B.

In most FSHD2 patients, heterozygous mutations or deletions are found in the SMCHD1 gene and sometimes in DNMT3B or LRIF1 (45-47), which cause hypomethylation at the D4Z4 repeat. For some patients the epigenetic causative gene has not yet been identified. Because different chromatin modifier genes are involved in FSHD2 and because the defect is sometimes difficult to identify when this is a variant of uncertain (or unknown) significance, a far intronic variant or a partial or complete deletion of the involved gene, it is highly recommended to do D4Z4 methylation analysis besides sequence analysis.

All FSHD2 patients carry a 4qA chromosome, with a D4Z4 repeat array mostly ranging between 8-20 units, while in control this ranges between 9-100 U. In general, the shorter the repeat array, the more severe the phenotype in both FSHD1 and FSHD2. As FSHD2 requires hypomethylation and a permissive 4qA chromosome, FSHD2 patient's family members who inherited the same SMCHD1 mutation but with only 4qB chromosomes will not develop FSHD. Yet, these unaffected SMCHD1 mutation carriers have a significant risk of transmitting the disease to their offspring. Therefore, it is important that these families become aware of this risk and are examined for potential carriership.

FSHD2 genetic analysis (Fig. 1) is required for FSHD2 patient's family members, or following a negative FSHD1 genetic analysis, but also in case a D4Z4 repeat array with 8-10 U is identified on a 4qA allele (as this size is found in about 10% of FSHD2 patients). If the D4Z4 methylation level is below the usual FSHD2 threshold, FSHD2 is confirmed in combination with a clinical diagnosis of FSHD. Subsequent steps can be the identification of the causative variant in an FSHD2 gene and determination of the D4Z4 haplotype and repeat array size. FSHD2 can be excluded if the methylation is not below the threshold. Patients who are genetically not confirmed as FSHD1 or FSHD2 (but clinically diagnosed with definitive FSHD), should be marked as 'clinical FSHD, genetic cause unknown'. In the discussion that followed this presentation, the importance of setting and sharing the thresholds for methylation in FSHD1 and FSHD2 has been highlighted.

**Emiliano Giardina and Nicol Voermans:** shared their experience with prenatal and preimplantation testing. Genetic test is a medical analysis addressed to identify variations in chromosomes, genes or proteins, whose results can confirm or rule out a suspected genetic condition, or can assess the individual risk to develop or transmit a disease (48). In the case of FSHD, genetic testing can be applied to confirm a suspicion or a clinical diagnosis of FSHD and provide the basis for assessing the risk of transmission of a genetic variation and the recurrence risk of disease (4, 49). On this subject, pre-test genetic counselling is crucial, and it is strongly recommended for FSHD patients considering pregnancy, who may benefit from Pre-implantation Genetic Testing (PGT) and Prenatal Diagnosis (PND).

However, both tests present some limitations. The big amount of DNA (>500 ng) required for D4Z4 sizing (for all available technologies) hampers its application for PGT. Indirect testing is possible, but it can be complicated by the telomeric location of the *D4Z4 array*, which makes the selection of appropriate genetic markers difficult and there is an increased risk of recombination. The general success rate of PGT is 25-30% per embryo transfer and it has 5% risk of misdiagnosis for FSHD. Therefore, PND is always offered to confirm PGT and exclude possible recombination events (49, 50). Currently, PGT is not suited for sporadic FSHD cases and somatic mosaicism. Prenatal genetic

testing in FSHD is performed on chorionic villus samples and/or cultured cells, by direct analysis (i.e. *D4Z4* array sizing). If possible, indirect analyses (STRs and/or SNPs analysis) are also recommended to exclude contamination by maternal DNA (51). Moreover, pregnancy of FSHD patients may worsen the disease course and symptoms severity (approximately 12-24% of cases).

The results of the genetic test have always to be interpreted considering the reduced penetrance and inter/intra-familial clinical variability (age of onset, progression rate of muscle weakness) of FSHD as well as the unpredictable severity of disease and genotype-phenotype correlation (50, 51). Regardless of the results, post-test genetic counselling should be performed in all cases, and it is fundamental to discuss all the implications of the genetic test for the patients and their families.

**Victoria Williams:** presented the efforts of the European Molecular Genetics Quality Network (EMQN) in order to implement the offer of FSHD diagnostics all over the world. EMQN is a non-profit global quality assurance provider in genomics, who helps laboratories to provide accurate and reliable test results by promoting professional quality in diagnostic genomic testing and appropriate analytical and interpretative performance. EMQN has experts and volunteers all over the world and develop interlaboratory comparison guidelines on planning, operation, evaluation and reporting. Since 2021, six EMQN associated laboratories have been performing the FSHD1 genetic analysis and 4 laboratories the FSHD2 genetic analysis. All laboratories require 20-40 micrograms of DNA and perform enzymatic digestion and southern blot. The assessment of the quality of the results across different laboratories showed that no major errors were detected, as all labs reached the correct diagnosis, however she showed slight heterogeneous results (+/- unit) as regards repeat size across labs. As regards FSHD2, all labs used NGS to analyze SMCHD1 gene and obtained the same result, with no critical errors, even though some labs did not provide the classification of pathogenicity or the heterozygous status.

The post presentation discussion highlighted the importance of integrating clinical data to enable improved interpretation of the genetic results.

**Emiliano Giardina** discussed the importance of including minimal clinical patient information in physician referrals also highlighting problems with genetic counselling and clinical variability. FSHD heterogeneity can complicate the diagnosis and the genotype-phenotype correlation, delaying diagnosis, clinical care and follow-up of patients and families. The clinical diagnosis of FSHD can be challenging. Dedicated measures (such as the FSHD clinical score and the CCEF score) have been developed with the purpose of addressing the phenotypic heterogeneity and variable severity of FSHD and to standardize the clinical evaluation of patients (52). Moreover, the molecular diagnosis of FSHD can be complicated by technical issues related to non-standardized methods (such as Southern blot analysis), which are labour- and time-intensive and require large amounts of DNA (49).

The adoption of a standardized protocol for molecular diagnosis as well as the availability of reference centres for clinical diagnosis are therefore highly desirable for enhancing the management of patients and families with FSHD. The medical, psychological, familial and reproductive implications of the disease-related testing highlight the importance of genetic counselling in FSHD (49, 53). Genetic counselling requires collection of patient data such as clinical

and instrumental evaluation and laboratory results thereby providing a bridge between neurologists and the genetic laboratory. In addition, genetic counselling also provides a family history evaluation that is crucial to highlight undiagnosed cases, intra-familial variability and phenotype peculiarities possibly linked to the genotype. Importantly, genetic counselling should be conducted with the purpose of assessing the risk of recurrence, understanding the patients' expectations and enabling them to make informed reproductive choices. In this context, a multidisciplinary approach including a neurological evaluation of patients based on standardized clinical methods and the concomitant genetic counselling could improve the accuracy of FSHD diagnosis and genotype-phenotype correlation, reduce unnecessary testing and improve the management of patients and families with FSHD.

**Sarah Burton-Jones and Richard Lemmers:** proposed the minimal requirements for the genetic confirmation of FSHD1 and FSHD2. Genetic confirmation of FSHD1 or FSHD2 may involve multiple testing steps, depending upon the diagnostic methods employed and the individual genotype. The patient's clinical features and family history are key to the interpretation of genetic test results, and details must be provided to the diagnostic laboratory. A universal form is proposed, accessible online, to capture this information. Alternatively, the laboratory may accept a descriptive clinic letter or email. Testing for FSHD starts with determining the size of the smallest 4q35 D4Z4 repeat allele in the patient.

All current methods involve measurement uncertainty equivalent to  $\pm 1$  D4Z4 repeat unit (U). Results of optical genome mapping (OGM) or molecular combing analysis will include 4qA/B haplotype (42, 44). Using Southern blot, this involves an additional test (39). If the FSHD patient has 1-7 U on a 4q35 allele, this is consistent with a diagnosis of FSHD1. If analysis shows 8-10 U, segregation studies and confirmation of haplotype4q A may support a diagnosis of FSHD1. In addition to these investigations, testing for FSHD2 is recommended for 8-10 U patients with severe/early-onset FSHD, and in other cases if appropriate. Where the smallest 4q35 D4Z4 allele is  $>10U$ , FSHD2 testing is indicated. Testing for a hybrid D4Z4 repeat array or deletion of the p13E-11 sequence targeted by the Southern blot probe may also be undertaken if not already excluded. SSLP analysis (54) may be performed to confirm the presence of a compatible 4qA haplotype, particularly alongside LGE-based Southern blotting.

FSHD2 associates with hypomethylation of the D4Z4 repeat regions on chromosomes 4 and 10, in the presence of a permissive 4q35-A allele of 8-20U (rarely  $>20$  U). Different DNA methylation analysis methods target the *DUX4* promoter on chromosomes 4 and 10, or the 3'UTR on chromosome 4 (55). Therefore, the designated hypomethylation threshold may vary between laboratories. Detection of a pathogenic *SMCHD1* variant supports an FSHD2 diagnosis and enables segregation analysis. *DNMT3B* and *LRIF1* may also be analysed where available. If D4Z4 hypomethylation is severe, identifying a causative gene variant is not crucial for a diagnosis of FSHD2. When D4Z4 methylation is mildly reduced with no pathogenic variant identified, the diagnosis will remain uncertain. The laboratory report should specify the applied methods and their limitations. Risks to offspring should be stated. In case of clinical or pedigree anomaly, further testing may be undertaken to investigate potential complexity. It is acknowledged that a group of

FSHD patients remains without a genetic cause identified, whose diagnosis rests on clinical presentation only.

## 5. Biomarkers in FSHD: opportunities and challenges

**Julie Dumonceaux** introduced the session on biomarkers in FSHD. A biomarker is a measurable indicator of a biological state or condition. There are different types of biomarkers according to their applications (56). Diagnostic biomarkers are used to detect or confirm the presence of a disease or to identify individuals with a subtype of the disease. Prognostic biomarkers are used to identify the likelihood of clinical events, disease recurrence, or disease progression in diseased patients. Monitoring biomarkers is used to repeatedly check disease status for medical products and assess patient response to therapeutic interventions. In this session, we mainly focused on therapeutic biomarkers, which ideally meet several criteria. A good biomarker should be quantitative, non-invasive, applicable across the entire range of severity of affected patients, sensitive to change, reliable and clinically meaningful. Most of the current and past clinical trials for FSHD involve nonspecific approaches targeting different levels of FSHD pathophysiology to increase muscle mass and strength, reduce oxidative stress or modulate the immune response (2). The development of new drugs specifically targeting the primary cause of the disease, namely the DUX4 transcription factor, highlighted the need to develop new biomarkers. During this session, the potential biomarkers were presented, and their limitations, validation and harmonization discussed.

**Lorenzo Guizzaro** discussed the requirements a biomarker should have from the perspective of the EMA. The development of medicines for a complex disease with heterogeneous progression such as FSHD should leverage on the use of biomarkers. Such use encompasses at least two domains: the selection and characterization of the patients to study and the demonstration of a treatment effect. Beyond diagnostic confirmation, prognostic biomarkers can aid to either find populations to study separately (with the possibility – depending on the status of knowledge - to extrapolate findings to the wider population) or to optimize assignment and analysis in the same trial.

Depending on the mode of action of a candidate medicine, selection might also be based on expected predictive biomarkers. Biomarkers would also be helpful for early detection of a treatment effect. A good understanding of the mode of action of candidate medicines would allow pharmacodynamics biomarkers to select promising candidates to go into late phase clinical trials. Biomarkers could also in principle be used to establish efficacy, but this requires a very precise understanding of the causal relationship between the biomarker, the pathological process leading to clinically meaningful changes, and the medicine. A correlation between a biomarker and the clinical status in observational studies – while important to deepen our understanding – is not in itself sufficient to validate a surrogate biomarker. The EMA encourages developers of biomarkers (as well as of COM tools) to engage in the Qualification process (57).

**Robert Bloch** presented the advantages and limitations of using SLC34A2 as a biomarker in FSHD. He and his team have developed a xenograft model of FSHD in which they generate mature FSHD

muscle tissue in the hindlimbs of immunodeficient mice (58). These xenografts contain mature skeletal muscle tissue, as indicated by the presence of striations and the near absence of embryonic myosin heavy chain. They are also innervated. Crucially, they reproduce key features of FSHD: (i) They express DUX4 and several of its downstream gene products at much higher levels than xenografts made from unaffected muscle cells; (ii) They show the same level of hypomethylation as the biopsies from which the cells that generate the graft were originally obtained; (iii) They express one of the gene products, SLC34A2 as both mRNA and protein, at ~10-fold higher levels in FSHD grafts than in controls, and at ~10-fold higher levels in biopsies of FSHD muscles than in healthy controls (12). Based on the latter observation, they have been studying SLC34A2 protein as a possible biomarker for FSHD. SLC34A2 is a Na<sup>+</sup>-P<sub>i</sub> cotransporter that is normally expressed in epithelial tissues but not in muscle. However, it is expressed at readily detectable levels in immunoblots of FSHD muscle. The amounts of SLC34A2 detected in immunoblots of FSHD myotubes are at least 20-fold higher than in control myotubes, and they decrease ~2-fold when myotubes are exposed to any of 3 different inhibitors of p38 MAPkinase, ralemetinib, pamapimod and losmapimod, the drug currently in Fulcrum's clinical trials. The protein can also be detected at higher levels in immunoblots of the engrafted FSHD muscles than in control grafts, as well as in the serum of mice carrying FSHD grafts. Consistent with this result, we find ~30% higher levels of SLC34A2 protein in the sera from FSHD patients compared to controls. In recent experiments, using an antibody to the exposed extracellular domain of SLC34A2, tagged with IR-647 and injected into FSHD xenografts IM, they observed a subset of human fibres that were labelled *in situ*. If these results stand up to further scrutiny, they open the prospect of tracking disease progression and the efficacy of different experimental therapies for FSHD in the same individuals over time, without the need for muscle biopsies.

In the discussion that followed the presentation, two important aspects were highlighted, firstly this marker is influenced by nutrition (vitamin D) therefore it needs to be assessed under controlled conditions (e.g. fasting). Secondly, about the functional relevance of this biomarker, it has been suggested that more studies be undertaken in order to better correlate the SLC34A2 levels and their variation with changes in muscle function and disease severity.

**Sabrina Sacconi** shared her group's results on IL-6 as an interesting biomarker for FSHD. Inflammatory pathway activation induced by inappropriate DUX4 expression seems to play a role in the early stages of disease progression. Indeed, muscle inflammation plays a central role in FSHD pathophysiology, with subsequent muscle atrophy and fibrofatty degeneration. Several studies on skeletal muscle biopsies and human primary myoblasts have highlighted a link between the cytotoxicity of DUX4 and the deregulation of adaptive and innate immunity (59, 60). The common proposed model is that the expression of DUX4 and its target genes, which are usually expressed in an immune-privileged environment, trigger an immune response (59, 60). Nevertheless, the exact pathway of muscle inflammation needs to be further clarified to better understand its contribution in FSHD pathophysiology. Her group has retrospectively analysed serum cytokines in a large cohort of 100 adult patients with FSHD1 (51 males and 49 females) to identify potential biomarkers of disease activity. The results showed that among the 20 cytokines tested, 10 displayed a significantly different expression level between patients and healthy controls sex and age matched. FSHD1 patients displayed an overall higher level of inflammatory cytokines (GM-CSF, IL-6, IL-7, IL-8, IL-

12p40, IL-15, IL-16, TNF $\alpha$  and VEGF) and a reduced level of anti-inflammatory cytokines (IL-10) (11). However, IL-6 was the only one with concentrations strongly correlating with several well-established clinical severity and functional scores (Manual Muscle Testing sum score, Brooke score, Vignos score, and Clinical Severity Score) in the overall FSHD1 population in both male and female subsets. Further, IL-6 levels were higher in the FSHD1 population compared to healthy controls. In vivo experiments using an FSHD-like mouse model (the ACTA1-MCM; FLExD mouse model) showed that IL-6 levels were elevated in serum and muscle compared to control mice and increased with the disease severity and DUX4 expression, suggesting that IL-6 levels were linked to DUX4 expression in skeletal muscles, and that circulating, and muscle resident cells could contribute to IL-6 production (11, 61, 62). Based on these results, serum IL-6 levels show promise as a serum biomarker of FSHD activity in FSHD patients. Furthermore, these results highlight the potential use of IL-6 levels as a suitable tool for phenotypic stratification and a candidate target for therapy in FSHD. Further studies will be a crucial matter of research for therapeutic development since anti-IL-6 receptor monoclonal antibodies have already been approved for the treatment of rheumatoid arthritis and some other IL-6-related pathologies.

**Enrico Bugiardini** focused his presentation on the detections of circulating biomarkers, which have the advantage of being non-invasive, can be repeated over time, can be assessed in previously stored samples and these markers are usually cheap to collect and can provide a general view of disease burden. He presented a review of available evidence and main studies on circulating biomarkers in FSHD. The main circulating biomarkers assessed are related to microRNA (miRNA) and proteomics studies. Few studies evaluated miRNAs in FSHD (63-66) and there is limited information available. Interestingly miR-206, a muscle-derived miRNA with an important role in skeletal muscle differentiation was found elevated in two of these .

Proteomics studies have rapidly increased in the last few years using a variety of approaches ranging from Mass Spectrometry to innovative affinity-based methods (64, 67-70). Of interest, S100A8, a subunit of calprotectin, was identified in two of them. S100A8 is also an established biomarker in rheumatic diseases and modulates the inflammatory response. Further studies are needed to confirm the value of S100A8 as an FSHD biomarker. Overall, the current review of literature on circulating biomarkers in FSHD shows that there is still limited information available and a scarcity of clinical/radiological data in most studies performed. Most candidate biomarkers are also not DUX4-specific. It is paramount to advance biomarker research to extend studies on current candidate biomarkers (i.e. miR-206, S100A8, IL6, SLC34A2), combine shotgun and affinity-based approaches, re-test innovative affinity-based approaches with broader and/or tailored (DUX4-related) panels and explore the use of metabolomics.

**Alexandra Belayew** discussed the challenges and opportunities of DUX4 and PAX7 as FSHD biomarkers. DUX4 mRNA detection in FSHD muscle cells is a major challenge both for technical and specificity reasons. The first successful detection methods for DUX4 RNA and protein are detailed in Dixit et al 2007 (71). DUX4 expression only occurs at random for very short times (bursts) in very few myonuclei. Its sequence is very GC rich and similar to RNAs expressed from hundreds of 3.3-kb elements scattered in the human genome, raising issues in the selection of DUX4-specific primers

for RT, PCR, or in situ hybridization. The most similar genes are DUX4C located 42kb centromeric from D4Z4, and LEUTX and DUXA which are induced by DUX4, and keep activating most of DUX4 target genes after DUX4 extinction (72). Because of these issues, most research groups have turned to the detection of DUX4 “footprint genes” i.e. genes that are either direct targets or activated in the transcription deregulation cascade initiated by DUX4. Several groups have thus defined DUX4 signatures, increased in FSHD muscle biopsies and including genes expressed either early (Banerji et al and Choi et al - 212 genes), (73, 74) or late after DUX4 expression (Yao et al 2014 (75), 114 genes; Banerji et al 2017 (73) - Geng et al 2012 (59), 165 genes).

The single PAX7 homeodomain is similar to those of DUX4, and PAX7 can rescue DUX4-induced cytotoxicity in mice (76). A PAX7 signature of 311 up- and 290 down-regulated genes was defined (73) and found decreased in FSHD muscle biopsies. DUX4 and PAX7 can bind similar DNA elements and compete on reporter target gene activation. Several publications have discussed the relative interests of either signature to distinguish FSHD from control samples, association with FSHD severity and disease progression over 1 year (10). DUX4 interferes with PAX7 at the protein but not RNA level (Zammit group: unpublished data). This extends the concept of an FSHD biomarker from activation of DUX4 “footprint genes” to inhibition of PAX7 target genes.

As a potential new FSHD muscle-blood biomarker, DUX4 and its target genes were detected in lymphoblasts and PBMC (10). Detailed analysis of PAX7 target genes correlated to severity (Ricci and Lamperti score, disease duration) yielded 64 up- and 9 down-regulated genes which constitute a “muscle and blood biomarker”, decreasing with increasing FSHD severity and easily assessed in PBMC (77).

**Peter Jones:** presented the experiences of his group in developing *in vitro* and *in vivo* models for FSHD and discussed their usefulness. Many model organisms have DUX4 orthologues, however they do not have useful DUX4 homologs and no natural animal models of FSHD exist or can be created. Fortunately, several DUX4-based transgenic mouse models have been developed that are applicable for FSHD therapeutic development and preclinical testing. The most widely used model is the FLExDUX4/MCM model that contains a floxed and inverted human DUX4 transgene under control of the Rosa26 promoter (61, 62). When mated to the ACTA1-mER-cre-mER mouse line containing a tamoxifen inducible cre, mosaic DUX4 expression can be induced in skeletal muscles. In addition, there is low leaky mosaic expression of DUX4 in skeletal muscles in the absence of tamoxifen. These mouse models show progressive FSHD-like histopathology and muscle weakness with severity dependent upon amount of tamoxifen used. Although there are two other DUX4 transgenic mouse models available (78, 79), almost all academic labs and biotech companies around the world use this double transgenic mouse model for testing therapeutics. In addition, FSHD-like minipigs with the same double transgenic design as the mouse model have recently been generated and are currently undergoing characterization for use as large animal models of FSHD. These should start to become available to the FSHD community in 2023.

**Julie Dumonceaux:** discussed the importance of harmonization of assessment methods for biomarkers and functional tests in mice and proposed the development of specific standard operating procedures (SOPs). Lessons learned from discontinued translational research in

neuromuscular diseases have shown that lack of robust data to support the validity of a measure is one of the reasons for termination (80). In the FSHD landscape, several articles describing the development of therapeutic approaches using DUX4 mouse models have been published. Only a few of them evaluated functional outcomes (79, 81-84). Two mouse models were used: the ACTA1-MCM/Flex DUX4 and the TIC-DUX4, both expressing DUX4 after tamoxifen injection. Major differences in protocols used for functional outcome measures (exhaustion test, force test) or to measure the expression of DUX4 and DUX4-network genes (nested PCR, qPCR, PCR, different oligonucleotides, different PCR programs etc.) were highlighted.

With recent advances in the development of drugs targeting DUX4, harmonization in DUX4 measurement is crucial to compare results from different laboratories as it allows definition of universal reference values. It can also be of interest to measure DUX4 in patient muscle biopsies. Harmonization through the implementation of standard operating procedures (SOP) and guidelines will facilitate the identification of relevant biomarkers of therapeutic efficacy and shorten timelines for drug development. In this perspective, it was proposed and discussed to form an international effort to establish SOPs for DUX4 detection and guidelines about minimum information for publication of work including animal models for FSHD.

**Karlien Mul:** highlighted the role and collection of muscle biopsies as an important biomarker source. The way muscle biopsies are collected, stored, shared may affect the results of research on these specimens. First, different methods of collecting muscle biopsies were discussed. Importantly, because muscle biopsies are performed on only a few muscles of the human body, knowledge obtained comes from limited upper and lower extremity muscles and results may also differ from biopsies taken from muscles commonly affected or spared in FSHD.

Muscle can be collected through open biopsies, which are more invasive and cosmetically less attractive but provide the surgeon with a clear view of the muscle. Needle muscle biopsies, which are minimally invasive and can yield more than one smaller sized specimen but are limited by the accuracy of needle location in the muscle. Especially in a disease like FSHD where pathology in the muscle can be focal and differ between different regions within one muscle, it is relevant to have information on where in the muscle the biopsy was taken. Therefore, there is increasing interest in imaging guided muscle biopsies.

One technique is to perform an 'imaging informed' muscle biopsy, where muscle MRI (or ultrasound) is obtained before performing the biopsy procedure and a fiducial or grid is placed on the skin to localize the biopsy site. Another option is an 'imaging guided' biopsy where the site of the biopsy is verified during the biopsy procedure. In the case of MRI-guided biopsies, the biopsy is performed within the MRI scanner (85). Although these procedures enable repeated biopsies in the same area of the muscle, it is unclear how a previous biopsy in the same area affects future findings. Regarding blood samples, variability in the circumstances under which the sample was taken is often not recorded. This includes, but is not limited to, time of day, exercise before sampling, diet, recent infections, etc. Additionally, the amount of clinical information that is available per sample varies greatly and ideally a standardized set in minimal clinical information (yet to be determined) should be available.

Storage and sharing of samples has improved with the introduction of biobanks in many centres. These biobanks often offer standardized collection, storage and management of biomaterials, but

just as importantly provide support regarding IT, legal-ethical aspects and data safety. The use of biobanks enables researchers and other parties such as pharmaceutical companies to request samples from other groups.

## 6. Integration

**Emma Weatherley** presented the goals and actions of FSHD Global, a research foundation advocating for clinical trial readiness in Australia to ensure that investments into medical research translate into future treatments for the disease. This roadmap aims to improve diagnostics, capture fully characterized patient data, build medical infrastructure that connects across the country, and to capture and promote the patient voice at every stage.

In Australia, there are many barriers to diagnosis for patients, including: i) Limited disease awareness among the medical profession, ii) Lack of patient motivation to seek a formal diagnosis, iii) Remote and rural patient populations with limited access to specialist services, or genetic testing, iv) Lack of funded diagnostic testing resulting in significant out of pocket costs for patients, v), Common misdiagnosis, or medical professionals offering only a clinical diagnosis vi) Limited ability to test for FSHD type 2.

FSHD Global seeks to uplift diagnostics to excellent standards by implementing current gold standard diagnostics including Bionano Optical Genome Mapping and considering new and emerging technology to improve the diagnostic patient journey, decrease time required to confirm a clinical diagnosis and reduce barriers to diagnostics including AI analysis of MRI scans and saliva testing.

Patient data will be captured and recorded in a fully characterized disease registry including genetic test results, epigenetic information, patient surveys, MRI scans and AI analysis of MRI scans. This multi-dimensional, fully characterized patient registry provides baseline and natural history information. The creation of this National FSHD Diagnostic and Medical Network supports connectivity of clinicians and institutions across the country.

By improving diagnostics and removing barriers to diagnosis for patients, capturing current patient data and including innovative information in our disease registry such as methylation and AI analysis, we encourage the development of a documented, well understood patient population with current data available to attract pharmaceutical interest to host clinical trials in Australia.

**Giorgio Tasca** presented the summary results of a parallel ENMC meeting held in April 2022, which was dedicated to muscle imaging in FSHD and its relevance for clinical trials (9). Participants in this workshop agreed on the diagnostic usefulness of MRI especially in the context of; i. cases with a borderline number of D4Z4 repeats or discrepancy between clinical severity and D4Z4 repeat number, ii. suspected FSHD2, iii. atypical or incomplete phenotypes, and iv. The role MRI can play in patient stratification for clinical trials (86, 87). Ultrasound, whose expertise is currently restricted to specific centres, emerged as a potentially interesting technique that, if standardized, could help in providing additional information to MRI.

Given the heterogeneity and unpredictability of FSHD, researchers agreed that imaging protocols should aim to achieve whole-body coverage, as well as to include specific sequences to assess and quantify disease activity. The idea to tailor the choice of the specific imaging biomarker(s), based on the action of the investigated drug also emerged. To increase validity and obtain additional insights on disease mechanism and progression, participants reached consensus on; the need to perform a global analysis of the quantitative MRI data published in the different natural history studies, and on the benefits of acquiring and analysing placebo arm imaging data from past trials (including those whose results were not published). Steps towards the implementation of new advanced imaging protocols, which could provide more complete information in a shorter acquisition time, should be pursued. The ENMC and CTRN imaging working groups have adopted a coordinated approach to maximize efforts in the standardization of protocols for imaging data collection and analysis.

**Enrico Bugiardini** joined the TREAT-NMD FSHD-taskforce as a representative of the FSHD-ETN. The taskforce was created in 2021 to advance diagnosis, care and therapies for patients with FSHD. The general aims are to create a bridge between academic-scientific networks and patient associations and representatives and facilitate trial readiness. To reach their objectives, the FSHD taskforce is planning to 1) support educational programs/training to harmonize protocols and procedures; 2) promote awareness of existing networks, trials, points of diagnosis and care amongst patients; 3) create FSHD Taskforce Working Groups, with a specific focus on improving specific areas agreed by the Taskforce; 4) maintain independence from any biased stakeholder interest 5) direct courses of action, which reflect the best interests of the FSHD patient cohorts. The first actions were to set up the task force and review current FSHD working groups. During the workshop, a summary of the landscape review was presented with details of ongoing FSHD working groups and their key priorities. Key priorities listed were i) registries, ii) biomarkers, iii) standards of care, iv) imaging, v) trial readiness, vi) outcome measures. While there is not a single network involved in all the activities, there are several FSHD working groups that currently provide adequate coverage of the key priorities. In this context, the TREAT-NMD task force has the role of acting as a liaison between the different organizations/networks facilitating collaboration, integration and ongoing identification of unmet needs.

**Karlien Mul's** presentation highlighted to the group the roles of the different networks and where collaboration opportunities exist. The CTRN is a consortium of academic research centres in the US and Europe with expertise in FSHD clinical research and/or conducting neuromuscular clinical trials. Their major goals are to create a common research infrastructure and help centres implement clinical studies on FSHD. This includes, but is not limited to, standardization of data collection and standard operating procedures, training of personnel, consults for protocol development and organization of focus groups. The CTRN has two ongoing natural history studies: ReSolve and MOVE (3). The ReSolve study follows over 250 FSHD patients for a two-year period to validate new COMs and refine trial planning strategies. The MOVE study follows approximately 450 FSHD patients for at least three years, using a shorter 'clinic-based' protocol with less strict inclusion criteria compared to ReSolve including patients of all ages and functional abilities. The ETN started as a patient initiative by FSHD Europe with the goal to optimally prepare European countries for clinical trials in

FSHD. They aim to harmonize clinical and genetic diagnostics, but also treatment and care. The overarching aim is the accessibility of trials and eventually drugs to all European countries. Four working groups with experts from across Europe work on genetic testing, COMs, biomarkers, and muscle imaging (2).

The overlap in members between the two networks provides a solid basis for collaboration and ensures that both networks are kept up to date regarding each other's activities. Working groups on muscle imaging from both networks have already initiated a joint meeting. In the future, new collaborative projects could be facilitated using these networks.

Multi-site clinical trials are necessary to achieve greater participation and avoid recruitment saturation at well-established trial sites. To expand networks and include investigational sites with geographical diversity, minimal requirements for a clinical trial study site should be established.

**Piraye Oflazer**, from Turkey, discussed the ideal features a site entering a clinical trial should have. She highlighted the minimal requirements needed and proposed some solutions to facilitate the participation of centres into clinical trials.

She proposed as clinical trial site requirements: 1) Neuromuscular clinic, 2) Adequate patient population 3)

Access to patients' organizations, registries, databases, 4) Access to genetic testing, 5) Access to muscle biopsy, MRI, biomarkers collection and storage, 6) Clinical trial unit, 7) Structural and human resources for outcome assessments, 8) Institutional data protection and a reliable reporting system, 9) Established systems such as Clinical Research Organization (CRO), Institutional Review Board (IRB), Independent National Ethics Committee (IEC).

She suggested as possible actions to overcome barriers: 1) Neuromuscular training, 2) Increased awareness and improved diagnosis, 3) Networking with existing organizations, 4) Partnerships with genetic testing labs in neighboring countries, 5) Collection at site, then couriered to neighboring site for further processing and storage, 6) GCP training, Training of investigators and study nurses, 7) Adoption of easy to implement outcome measures minimizing technical sophistication.

## **7. The road ahead: session with FSHD Europe, FSHD Society, FSHD CTRN, Pharmaceutical companies**

**Yann Pereon** described to the group some lessons learned from recent pharmaceutical based clinical trials in FSHD. A clinical trial led by Fulcrum Therapeutics has been undertaken over the past three years to assess the efficacy and safety of losmapimod in treating adults with FSHD. The primary endpoint selected for this trial, which was not met, was the change in DUX4-driven gene expression, included as an experimental biomarker. Some secondary endpoints however were positive, including decreased muscle fat infiltration (MRI), improved RWS volumes and improved Patient Global Impression of Change. Similar issues have been encountered in clinical trials in other neuromuscular diseases, including DMD, raising the critical question of endpoints and outcome assessment selection in these sorts of clinical trials.

First and foremost, the primary aim of therapeutic treatment should always be to improve the QoL of individuals affected by disease, rather than affecting the level of any RNA or protein expression.

Secondly, careful consideration should be paid to the potential difficulties in reaching endpoint significance when the assessed drug is provided in a slowly progressive disease, such as FSHD, and when the therapeutic benefit for the patient is not immediate and dramatic. Finally, precise knowledge of the natural course of the disease, including the natural course of the biomarkers selected for a clinical trial is critical when designing a clinical trial, selecting outcome measures, and setting participant stratification criteria. All these lessons should be considered in the future when designing new clinical trials in FSHD.

With clinical trials for patients with FSHD under development, it was thought important to ensure that trials were designed to reflect what is important to patients, measuring the impact the condition, and any treatments may have on their day-to-day lives. Patient representative, **Sheila Hawkins**, shared preliminary results of a European Patient Survey. Undertaken with support from pharmaceutical companies, FSHD Europe commissioned the John Walton Muscular Dystrophy Research Centre at Newcastle University to design and administer a survey of patients across Europe to collect:

- Demographic data to show the spread and incidence of FSHD
- Disease impact on individual mobility and functioning to date
- Factors influencing participation in clinical trials
- Patient perceptions of what a ‘good’ treatment outcome means

The survey was administered through an online questionnaire available in six European languages. The survey remained open for three weeks in April 2022 and was completed by 1147 patients across and beyond Europe. The survey showed a wide spread of respondents across the participating countries, an even spread between male and female respondents, and a spread of ages. The highest number of respondents reported onset of symptoms in teenage years, with an average of eight years to get a diagnosis. All respondents felt that their condition had deteriorated in the last three years, and no one reported seeing any improvement in their condition. The identified priorities for patients are to prevent further deterioration and loss of function, reduce fatigue and pain and gain muscle strength and mobility. The survey showed that while patients were keen to take part in clinical trials, recruitment and retention could be maximized by patient-centered clinical trial design focused on overall trial organization and accessibility. The results of this survey have not yet been published although information about publication should be available in 2023.

The FSHD Society (USA) was represented by their chief science officer **Jamshid Arjomand**, who outlined their efforts at advancing clinical trial readiness. Following the first Industry Collaborative Workshop for Therapy Development in FSHD that took place in March 2019, participating pharmaceutical companies helped identify a series of hurdles that could hinder the advancement of all clinical programs in FSHD. To address these gaps, the FSHD Society established the Therapeutic Accelerator, consisting of a series of initiatives being deployed in a collaborative manner with all stakeholders. Advances for selected initiatives relevant to the workshop topics were presented.

To better catalogue the natural history of the disease, the FSHD Society has launched an integrated analysis of the longitudinal clinical study “Clinical Trial Readiness to Solve Barriers to Drug Development in FSHD” (ReSolve). This initiative, carried out in collaboration with the FSHD CTRN,

leverages powerful machine learning and artificial intelligence offered through BullfrogAI. In addition, the FSHD Society established a collaboration with the Food and Drug Administration's Critical Path Institute's Rare Disease Cures Accelerator – Data and Analytics Platform (RDCA-DAP) to incorporate the placebo arm of several prior clinical trials to help guide the design of future clinical studies. These include University of Rochester's albuterol, Wyeth's MYO-029, aTyr's ATYR1940 and Acceleron's ACE-083 trials.

TestFSHD, a genetic testing initiative co-sponsored by several industry partners, was designed to overcome the barriers in genetic testing in the United States and fulfil the eligibility inclusion criteria requirements for enrolment into clinical trials. The fully sponsored pilot program provided comprehensive genetic counselling, medical referrals, as well as optical genome mapping for FSHD1, and SMCHDI1 whole exome sequencing for FSHD2 testing for up to 150 eligible patients.

Finally, an overview of the FSHD Society's circulating biomarker initiative was presented. The primary objective of this initiative was to develop an assay to report on DUX4 activity by measuring DUX4-regulated proteins in circulating blood. The two approaches to date build on existing published data and use commercially available assays. For the first attempt, DUX4-regulated genes were screened for the presence of a secretory signal and cross-referenced with available assays offered by Olink. Only two proteins-of-interest (POIs) matched these criteria (ALPP and CRHBP). In vitro pilot studies using inducible-DUX4 myoblasts or patient myoblasts only confirmed the detection of ALPP from the supernatant of the cultures, while patient sera failed to distinguish patients from controls. These results are available on MedRxiv. Currently, patient and control samples are being queried using quantitative mass spectrometry for the presence of DUX4-regulated protein peptides previously identified by Jagannathan et al. (87), with results still pending.

## **8. Discussion and workshop deliverables**

This ENMC workshop has seen the participation of many important stakeholders working together to improve trial readiness: patients and patients' organizations (FSHD-Europe, FSHD-Society and FSHD Global), neuromuscular clinicians, geneticists, basic researchers, representatives of the TREAT-NMD network, the FSHD-CTRN and EMA, thus allowing a fruitful discussion and the identification of common goals to work on. Many topics emerged during the different workshop sessions and the last day of the workshop was therefore dedicated to the identification of tasks and actions that should be put in place to improve care and trial readiness in FSHD.

As regards the care of FSHD patients, the workshop participants agreed on the importance of a multidisciplinary team, involving a close collaboration between neuromuscular specialists, clinical geneticists and therapists. The detailed clinical characterization of patients is essential for patient management, classification, for the interpretation of the genetic results, genetic diagnosis and family counselling.

COMs represent useful tools for the standardized collection of clinical features but need to be selected to match the clinical setting of use. For patient care, they need to be informative, with practical and time efficient utility so as not to detract from clinical care. For clinical trial purposes, the need to be reliable, valid, meaningful and sensitive to change to better depict therapeutic responses. Several COMs used in FSHD were discussed during the workshop and a survey distributed

to workshop participants to guide the discussion and reach consensus on COMs most suited for clinical practice and clinical trials for the adult FSHD population. Only one out of the 12 neuromuscular clinicians who participated in the workshop worked with children therefore, no consensus on best practice or COMs could be reached for the pediatric FSHD population. The need to create a dedicated working group for pediatric FSHD patients was discussed.

The RWS, 6MWT, quantitative muscle testing and FSHD-COM were considered feasible for the clinical trial setting. Manual muscle testing (MMT), range of motion and the Brook scale could be adopted in clinical practice but were deemed unsuitable for clinical trials and the 10m walk run test, TUG, handgrip and FSHD clinical severity scales were considered COMs feasible in both clinical settings (Fig. 2). To achieve greater survey engagement and address additional questions regarding COM for the non-ambulant and pediatric population and to evaluate pain and fatigue we plan to extend this survey and open discussion with members of the WG2 who were unable to attend the ENMC meeting. The results will guide the development of a European clinical evaluation form that should be used in all FSHD-centres and that will be harmonized with other existing evaluation forms (e.g. CCEF) and TREAT-NMD registry core data set (88). Finally, the field of home monitoring tools should be largely implemented, this should be feasible and well received in the FSHD population.

An optimized clinical evaluation and genetic test form is also one of the goals of WG1. Clinical features play a key role in the interpretation of genetic results and adopting the same genetic test form, available online, among different laboratories will improve collaboration and data sharing.

The advent of new genetic testing modalities such as molecular combing (MC) and Single Genomic Optical Mapping (SMOM) in addition to the well-known and largely used Southern blot poses new opportunities and challenges for FSHD diagnosis. Genetic confirmation may involve multiple testing steps, depending upon the diagnostic methods employed and the individual genotype. All available technologies are reliable for FSHD diagnosis with advantages and disadvantages for all of them. Southern blot (LGE and PFGE) is cost effective but labour intensive and non-automated. MC and SMOM provide an “all-in-one” approach with D4Z4 array sizing on both 4q and 10q alleles and A- or B-haplotypes assessment in one-step. However, these methodologies are more expensive, require dedicated equipment and do not provide information on 4q-10q interchromosomal exchanges that are frequent. A diagnostic flowchart for FSHD1 and FSHD2 has been proposed and discussed (Fig. 1). A consensus was reached on the definition of FSHD2 as a disease with a FSHD clinical phenotype with reduced methylation of the 4qA allele with or without a pathogenic variant in SMCHD1, DNMT3B or LRIF1 genes.

The best practice guidelines on genetic diagnosis of FSHD will be updated, considering the advantages and indications of using newer technologies and updated recommendations for prenatal and preimplantation testing. Pre-test genetic counselling should be offered to all FSHD patients considering pregnancy, who may benefit from Pre-implantation Genetic Testing (PGT) and Prenatal Diagnosis (PND). The PGT is performed by indirect analysis and has an estimated success rate of 25-30% per embryo transfer and a 5% risk of misdiagnosis for FSHD, therefore PND is always offered to confirm PGT and exclude possible recombination events (49, 50). Furthermore, PGT is not suited for sporadic FSHD cases and somatic mosaicism. PND is performed on chorionic villus samples and/or cultured cells, by direct analysis (i.e. D4Z4 sizing). If possible, indirect analyses (STRs and/or SNPs analysis) are also recommended to exclude contamination by maternal DNA (50). Regardless

of the results, post-test genetic counselling should be performed in all cases to discuss all the implications of the results for patients and their families.

In order to better address the challenges related to the FSHD genetic confirmation, periodic virtual meetings will take place to discuss difficult cases and allow training of less experienced genetic centres. WG1 will draw up an updated list of genetic centres in Europe and neighbouring countries encompassing contact details, genetic testing modalities and information whether genetic counselling and prenatal testing is performed. The list will then be made available on the webpage of FSHD Europe.

Another important unmet need for clinical trial readiness in FSHD is the identification of good therapeutic biomarkers, which ideally should be quantitative, non-invasive, applicable across the entire range of disease severity, sensitive to change, reliable and clinically meaningful. Promising data were shown for DUX4 (DUX4 signature), PAX7 (PAX7 signature), SLC34A2, IL-6, several micro-RNAs (as miR206), proteomic studies (e.g. S100A8). In the discussion emerged however how critical standard assessment conditions are for nearly all these biomarkers and therefore the need for further validation and harmonization of laboratory procedures. A recent example of an unsuccessful assessment of DUX4 occurred in the Fulcrum Phase 2 trial where inconsistent and inefficacious DUX4 measurements may have been contributing factors to not meeting the primary endpoint, a major issue being the selection of muscle biopsy sites. The WG 3 will therefore start by producing standard operating procedures (SOPs) for DUX4 detection. Similarly, large differences in the reporting of studies performed on animal models, thus hindering interpretation, repeatability and comparison of the results need to be addressed. Guidelines regarding minimum information for publication of work including animal models for FSHD will therefore be published. Further gaps and hurdles for the development of biomarkers in FSHD will be assessed with a questionnaire to be distributed among researchers.

The next important topic that has been discussed is the need to improve FSHD patient access to clinical trials and best medical care in non-EU, non-US sites. The patient representatives highlighted the importance of early communication (e.g. sites involved in a clinical trial, inclusion criteria) and enhanced patient support for trial participation considering study site accessibility and geographical distribution of study centres involved as well as financial aspects including compatibility with working life.

In order to attract pharmaceutical companies to conduct clinical trials in non-EU/non-US sites and thus improve patients' access to trials, the clinical and diagnostic pathway need to be further improved. Therefore, actions aiming at increasing disease awareness, reaching remote and/or rural patient populations, improving access to genetic testing (including FSHD2), reducing the economic burden of diagnostic testing and defining minimal requirements for a clinical trial study centre need to be supported and implemented as testified by FSHD Global and summarized by Piraye Oflazer.

Finally, various possibilities of collaboration between FSHD Europe, FSHD Society and FSHD Global were discussed, including translation of the extensive information on the website of FSHD Society into various languages (for the FSHD Alliance Website) and reaching out to countries not yet actively involved.

The FSHD Society is collaborating with FSHD Europe and the ETN to organize the FSHD International Research Conferences, including the organization of a patient conference at the same time (FSHD Connect and FSHD Alliance).

The different networks (TREAT-NMD, ETN, CTRN) aim to collaborate as much as possible to coordinate their efforts and reach emerging unmet needs. The members of the working groups agreed on the need to have regular joint meetings in the future to keep on the work for clinical trial readiness in FSHD.

## Funding

This work was supported by the FSHD Society, FSHD Global Research Foundation and European Neuromuscular Centre (ENMC).

## Workshop participants

- Giorgio Tasca (Unità Operativa Complessa di Neurologia, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy)
- Maria Gomez-Rodulfo, patient representative (Spain)
- Nicol Voermans (Department of Neurology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, the Netherlands)
- Sabrina Sacconi (Université Cote d'Azur (UCA), Peripheral Nervous System, Muscle and ALS Department, Pasteur 2 Hospital, Nice; Université Cote d'Azur, Inserm, CNRS, Institute for Research on Cancer and Aging of Nice (IRCAN), Nice, France)
- Federica Montagnese (Friedrich-Baur Institute, Neurology Department, Ludwig-Maximilians University Munich, Germany)
- Katy de Valle (The Royal Children's Hospital and Murdoch Children's Research Institute, Melbourne, Australia)
- Richard Lemmers (Department of Human Genetics) Leiden University Medical Center, Leiden, The Netherlands
- Julie Dumonceaux, National Institute for Health and Care Research Great Ormond Street Hospital Biomedical Research Centre/University College London Great Ormond Street Institute of Child Health, London, UK
- Pilar Camaño, Clinical Laboratory Geneticist, Biodonostia, San Sebastian, Spain
- Emiliano Giardina Genomic Medicine Laboratory-UILDm, Santa Lucia Foundation IRCCS, Rome, Italy; Department of Biomedicine and Prevention, Tor Vergata University, Rome, Italy.
- Nienke van der Stoep, Clinical Laboratory Geneticist, Department of Clinical genetics, Leiden University Medical Center, Leiden, The Netherlands
- Sarah Burton-Jones, Clinical Laboratory Geneticist, NORTH BRISTOL NHS TRUST, Bristol, UK
- Frederique Magdinier, Aix Marseille Univ, INSERM, Marseille Medical Genetics, MMG, Marseille, France
- Valérie Race, Clinical Laboratory Geneticist, Human Genetics, UZ Leuven, Leuven, Belgium
- Sheila Hawkins. Patient Representative, FSHD Europe
- Alexandre Méjat, AFM-Telethon, France
- Piraye Oflazer: Dept of Neurology, Koç University Medical Faculty, Koç University Hospital Muscle Center, İstanbul, Turkey
- Lorenzo Guizzaro, European Medicines Agency. Amsterdam, The Netherlands.
- Jamshid Arjomand, Chief Science Officer, FSHD Society, USA
- Yann Péréon, Reference Centre for Neuromuscular Diseases, Filnemus, Euro-NMD, University Hospital, CHU Nantes, Nantes, France
- Giulia Ricci, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy.
- Enrico Bugiardini, Department of Neuromuscular Disorders, UCL Queen Square Institute of Neurology, University College London, London, United Kingdom

- Alexandra Belayew, Professor emerita, Dept Medicine and Pharmacy, University of Mons, Mons, Belgium

### **Acknowledgements**

The workshop and early-career programme are made possible thanks to the financial support of the European Neuromuscular Centre (ENMC) and its Full Partners: Association Française contre les Myopathies (France), Deutsche Gesellschaft für Muskelkranke (Germany), Muscular Dystrophy Campaign (UK), Muskelsvindfonden (Denmark), Prinses Beatrix Spierfonds (The Netherlands), Schweizerische Stiftung für die Erforschung der Muskelkrankheiten (Switzerland), Spierziekten Nederland (The Netherlands), Telethon Foundation (Italy). In addition, we would like to thank the Associated Partners: Finnish Neuromuscular Association (Finland), Österreichische Muskelforschung (Austria), SMA Europe and World Duchenne Organisation, and the members of the ENMC Company Forum: Amicus Therapeutics, Astellas, Biogen, Ionis Pharmaceuticals, Lupin Neuroscience, Novartis, PerkinElmer, Roche, Sanofi, and Sarepta.

### **Disclaimer**

The views expressed in this article are the personal views of the authors and may not be understood or quoted as being made on behalf of or reflecting the position of the agencies or organisations with which the authors are affiliated.

### **Figure's Legends:**

Figure 1: Minimal criteria for genetic confirmation of FSHD

Figure 2: Results of the ENMC survey on outcome measures in FSHD. On the left the ones considered suitable only for clinical trials, on the right the ones that might find use only in clinical practice, in the middle the ones that might be used in both clinical settings.

## References:

1. Deenen JC, Arnts H, van der Maarel SM, Padberg GW, Verschuuren JJ, Bakker E, et al. Population-based incidence and prevalence of facioscapulohumeral dystrophy. *Neurology*. 2014;83(12):1056-9.
2. Voermans NC, Vriens-Munoz Bravo M, Padberg GW, Laforet P, group FETNws, van Alfen N, et al. 1st FSHD European Trial Network workshop: Working towards trial readiness across Europe. *Neuromuscul Disord*. 2021;31(9):907-18.
3. LoRusso S, Johnson NE, McDermott MP, Eichinger K, Butterfield RJ, Carraro E, et al. Clinical trial readiness to solve barriers to drug development in FSHD (ReSolve): protocol of a large, international, multi-center prospective study. *BMC Neurol*. 2019;19(1):224.
4. Tawil R, Kissel JT, Heatwole C, Pandya S, Gronseth G, Benatar M, et al. Evidence-based guideline summary: Evaluation, diagnosis, and management of facioscapulohumeral muscular dystrophy: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology and the Practice Issues Review Panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. *Neurology*. 2015;85(4):357-64.
5. Lemmers RJ, van der Vliet PJ, Klooster R, Sacconi S, Camano P, Dauwerse JG, et al. A unifying genetic model for facioscapulohumeral muscular dystrophy. *Science*. 2010;329(5999):1650-3.
6. Janssen BH, Pillen S, Voet NB, Heerschap A, van Engelen BG, van Alfen N. Quantitative muscle ultrasound versus quantitative magnetic resonance imaging in facioscapulohumeral dystrophy. *Muscle Nerve*. 2014;50(6):968-75.
7. Friedman SD, Poliachik SL, Otto RK, Carter GT, Budech CB, Bird TD, et al. Longitudinal features of STIR bright signal in FSHD. *Muscle Nerve*. 2014;49(2):257-60.
8. Lemmers RJ, O'Shea S, Padberg GW, Lunt PW, van der Maarel SM. Best practice guidelines on genetic diagnostics of Facioscapulohumeral muscular dystrophy: workshop 9th June 2010, LUMC, Leiden, The Netherlands. *Neuromuscul Disord*. 2012;22(5):463-70.
9. Monforte M, Attarian S, Vissing J, Diaz-Manera J, Tasca G, th Ewp. 265th ENMC international workshop: Muscle imaging in Facioscapulohumeral Muscular Dystrophy (FSHD): Relevance for clinical trials. 22-24 April 2022, Hoofddorp, The Netherlands. *Neuromuscul Disord*. 2022.
10. Banerji CRS, Zammit PS. Pathomechanisms and biomarkers in facioscapulohumeral muscular dystrophy: roles of DUX4 and PAX7. *EMBO Mol Med*. 2021;13(8):e13695.
11. Gros M, Nunes AM, Daoudlarian D, Pini J, Martinuzzi E, Barbosa S, et al. Identification of Serum Interleukin 6 Levels as a Disease Severity Biomarker in Facioscapulohumeral Muscular Dystrophy. *J Neuromuscul Dis*. 2022;9(1):83-93.
12. Mueller AL, O'Neill A, Jones TI, Llach A, Rojas LA, Sakellariou P, et al. Muscle xenografts reproduce key molecular features of facioscapulohumeral muscular dystrophy. *Exp Neurol*. 2019;320:113011.
13. Paganoni S, Nicholson K, Leigh F, Swoboda K, Chad D, Drake K, et al. Developing multidisciplinary clinics for neuromuscular care and research. *Muscle Nerve*. 2017;56(5):848-58.
14. Goselink RJM, Voermans NC, Okkersen K, Brouwer OF, Padberg GW, Nikolic A, et al. Early onset facioscapulohumeral dystrophy - a systematic review using individual patient data. *Neuromuscul Disord*. 2017;27(12):1077-83.
15. Goselink RJM, Schreuder THA, van Alfen N, de Groot IJM, Jansen M, Lemmers R, et al. Facioscapulohumeral Dystrophy in Childhood: A Nationwide Natural History Study. *Ann Neurol*. 2018;84(5):627-37.

16. Dijkstra JN, Goselink RJM, van Alfen N, de Groot IJM, Pelsma M, van der Stoep N, et al. Natural History of Facioscapulohumeral Dystrophy in Children: A 2-Year Follow-up. *Neurology*. 2021;97(21):e2103-e13.
17. Bartels B, de Groot JF, Habets LE, Wadman RI, Asselman FL, Nieuwenhuis EES, et al. Correlates of Fatigability in Patients With Spinal Muscular Atrophy. *Neurology*. 2021;96(6):e845-e52.
18. Lombardo ME, Carraro E, Sancricca C, Armando M, Catteruccia M, Mazzone E, et al. Management of motor rehabilitation in individuals with muscular dystrophies. 1(st) Consensus Conference report from UILDM - Italian Muscular Dystrophy Association (Rome, January 25-26, 2019). *Acta Myol*. 2021;40(2):72-87.
19. Tawil R, van der Maarel S, Padberg GW, van Engelen BG. 171st ENMC international workshop: Standards of care and management of facioscapulohumeral muscular dystrophy. *Neuromuscul Disord*. 2010;20(7):471-5.
20. Olsen DB, Orngreen MC, Vissing J. Aerobic training improves exercise performance in facioscapulohumeral muscular dystrophy. *Neurology*. 2005;64(6):1064-6.
21. van der Kooi EL, Vogels OJ, van Asseldonk RJ, Lindeman E, Hendriks JC, Wohlgemuth M, et al. Strength training and albuterol in facioscapulohumeral muscular dystrophy. *Neurology*. 2004;63(4):702-8.
22. de Valle K, McGinley JL, Woodcock I, Ryan MM, Dobson F. Measurement properties and utility of performance-based outcome measures of physical functioning in individuals with facioscapulohumeral dystrophy - A systematic review and evidence synthesis. *Neuromuscul Disord*. 2019;29(11):881-94.
23. Aristizabal JF, Smit RM. Orthodontic treatment in a patient with unilateral open-bite and Becker muscular dystrophy. A 5-year follow-up. *Dental Press J Orthod*. 2014;19(6):37-45.
24. Ricci E, Galluzzi G, Deidda G, Cacurri S, Colantoni L, Merico B, et al. Progress in the molecular diagnosis of facioscapulohumeral muscular dystrophy and correlation between the number of KpnI repeats at the 4q35 locus and clinical phenotype. *Ann Neurol*. 1999;45(6):751-7.
25. McKenna SP, Heaney A. Composite outcome measurement in clinical research: the triumph of illusion over reality? *J Med Econ*. 2020;23(10):1196-204.
26. Leung DG, Kinoshita J, Arjomand J, Dumonceaux J, committee FIP, co c. Meeting report: The 2022 FSHD International Research Congress. *Neuromuscul Disord*. 2022;33(2):196-8.
27. Hatch MN, Kurillo G, Chan V, Han JJ. Motion sensor-acquired reachable workspace correlates with patient-reported upper extremity activities of daily living (ADL) function in facioscapulohumeral dystrophy. *Muscle Nerve*. 2021;63(2):250-7.
28. Hamel J, Johnson N, Tawil R, Martens WB, Dilek N, McDermott MP, et al. Patient-Reported Symptoms in Facioscapulohumeral Muscular Dystrophy (PRISM-FSHD). *Neurology*. 2019;93(12):e1180-e92.
29. Mul K, Hamadeh T, Horlings CGC, Tawil R, Statland JM, Sacconi S, et al. The facioscapulohumeral muscular dystrophy Rasch-built overall disability scale (FSHD-RODS). *Eur J Neurol*. 2021;28(7):2339-48.
30. Mul K, Wijayanto F, Loonen TGJ, Groot P, Vincenten SCC, Knuijt S, et al. Development and validation of the patient-reported "Facial Function Scale" for facioscapulohumeral muscular dystrophy. *Disabil Rehabil*. 2022:1-6.
31. Youn BY, Ko Y, Moon S, Lee J, Ko SG, Kim JY. Digital Biomarkers for Neuromuscular Disorders: A Systematic Scoping Review. *Diagnostics (Basel)*. 2021;11(7).
32. Servais L, Camino E, Clement A, McDonald CM, Lukawy J, Lowes LP, et al. First Regulatory Qualification of a Novel Digital Endpoint in Duchenne Muscular Dystrophy: A Multi-Stakeholder

Perspective on the Impact for Patients and for Drug Development in Neuromuscular Diseases. *Digit Biomark*. 2021;5(2):183-90.

33. Gidaro T, Gasnier E, Annoussamy M, Vissing J, Attarian S, Mozaffar T, et al. Home-based gait analysis as an exploratory endpoint during a multicenter phase 1 trial in limb girdle muscular dystrophy type R2 and facioscapulohumeral muscular dystrophy. *Muscle Nerve*. 2022;65(2):237-42.
34. Statland JM, Karanevich A, Bruetsch A, Huisinga J. A pilot study of the responsiveness of wireless motion analysis in facioscapulohumeral muscular dystrophy. *Muscle Nerve*. 2019;60(5):590-4.
35. Ricci G, Ruggiero L, Vercelli L, Sera F, Nikolic A, Govi M, et al. A novel clinical tool to classify facioscapulohumeral muscular dystrophy phenotypes. *J Neurol*. 2016;263(6):1204-14.
36. Wijmenga C, van Deutekom JC, Hewitt JE, Padberg GW, van Ommen GJ, Hofker MH, et al. Pulsed-field gel electrophoresis of the D4F104S1 locus reveals the size and the parental origin of the facioscapulohumeral muscular dystrophy (FSHD)-associated deletions. *Genomics*. 1994;19(1):21-6.
37. Sarfarazi M, Wijmenga C, Upadhyaya M, Weiffenbach B, Hyser C, Mathews K, et al. Regional mapping of facioscapulohumeral muscular dystrophy gene on 4q35: combined analysis of an international consortium. *Am J Hum Genet*. 1992;51(2):396-403.
38. van Deutekom JC, Wijmenga C, van Tienhoven EA, Gruter AM, Hewitt JE, Padberg GW, et al. FSHD associated DNA rearrangements are due to deletions of integral copies of a 3.2 kb tandemly repeated unit. *Hum Mol Genet*. 1993;2(12):2037-42.
39. Lemmers RJ, de Kievit P, Sandkuijl L, Padberg GW, van Ommen GJ, Frants RR, et al. Facioscapulohumeral muscular dystrophy is uniquely associated with one of the two variants of the 4q subtelomere. *Nat Genet*. 2002;32(2):235-6.
40. Lemmers RJL, de Kievit P, van Geel M, van der Wielen MJ, Bakker E, Padberg GW, et al. Complete allele information in the diagnosis of facioscapulohumeral muscular dystrophy by triple DNA analysis. *Ann Neurol*. 2001;50(6):816-9.
41. Deidda G, Cacurri S, Piazzo N, Felicetti L. Direct detection of 4q35 rearrangements implicated in facioscapulohumeral muscular dystrophy (FSHD). *J Med Genet*. 1996;33(5):361-5.
42. Nguyen K, Puppo F, Roche S, Gaillard MC, Chaix C, Lagarde A, et al. Molecular combing reveals complex 4q35 rearrangements in Facioscapulohumeral dystrophy. *Hum Mutat*. 2017;38(10):1432-41.
43. Dai Y, Li P, Wang Z, Liang F, Yang F, Fang L, et al. Single-molecule optical mapping enables quantitative measurement of D4Z4 repeats in facioscapulohumeral muscular dystrophy (FSHD). *J Med Genet*. 2020;57(2):109-20.
44. Stence AA, Thomason JG, Pruessner JA, Sompallae RR, Snow AN, Ma D, et al. Validation of Optical Genome Mapping for the Molecular Diagnosis of Facioscapulohumeral Muscular Dystrophy. *J Mol Diagn*. 2021;23(11):1506-14.
45. Lemmers RJ, Tawil R, Petek LM, Balog J, Block GJ, Santen GW, et al. Digenic inheritance of an SMCHD1 mutation and an FSHD-permissive D4Z4 allele causes facioscapulohumeral muscular dystrophy type 2. *Nat Genet*. 2012;44(12):1370-4.
46. van den Boogaard ML, Lemmers R, Balog J, Wohlgemuth M, Auranen M, Mitsuhashi S, et al. Mutations in DNMT3B Modify Epigenetic Repression of the D4Z4 Repeat and the Penetrance of Facioscapulohumeral Dystrophy. *Am J Hum Genet*. 2016;98(5):1020-9.
47. Hamanaka K, Sikrova D, Mitsuhashi S, Masuda H, Sekiguchi Y, Sugiyama A, et al. Homozygous nonsense variant in LRIF1 associated with facioscapulohumeral muscular dystrophy. *Neurology*. 2020;94(23):e2441-e7.

48. Franceschini N, Frick A, Kopp JB. Genetic Testing in Clinical Settings. *Am J Kidney Dis*. 2018;72(4):569-81.
49. Zampatti S, Colantoni L, Strafella C, Galota RM, Caputo V, Campoli G, et al. Facioscapulohumeral muscular dystrophy (FSHD) molecular diagnosis: from traditional technology to the NGS era. *Neurogenetics*. 2019;20(2):57-64.
50. Vincenten SCC, Van Der Stoep N, Paulussen ADC, Mul K, Badrising UA, Kriek M, et al. Facioscapulohumeral muscular dystrophy-Reproductive counseling, pregnancy, and delivery in a complex multigenetic disease. *Clin Genet*. 2022;101(2):149-60.
51. Wohlgemuth M, Lemmers RJ, Jonker M, van der Kooi E, Horlings CG, van Engelen BG, et al. A family-based study into penetrance in facioscapulohumeral muscular dystrophy type 1. *Neurology*. 2018;91(5):E444-E54.
52. Ghasemi M, Emerson CP, Jr., Hayward LJ. Outcome Measures in Facioscapulohumeral Muscular Dystrophy Clinical Trials. *Cells*. 2022;11(4).
53. Ruggiero L, Mele F, Manganelli F, Bruzzese D, Ricci G, Vercelli L, et al. Phenotypic Variability Among Patients With D4Z4 Reduced Allele Facioscapulohumeral Muscular Dystrophy. *JAMA Netw Open*. 2020;3(5):e204040.
54. Lemmers RJ, van der Vliet PJ, van der Gaag KJ, Zuniga S, Frants RR, de Knijff P, et al. Worldwide population analysis of the 4q and 10q subtelomeres identifies only four discrete interchromosomal sequence transfers in human evolution. *Am J Hum Genet*. 2010;86(3):364-77.
55. Lemmers RJ, Goeman JJ, van der Vliet PJ, van Nieuwenhuizen MP, Balog J, Vos-Versteeg M, et al. Inter-individual differences in CpG methylation at D4Z4 correlate with clinical variability in FSHD1 and FSHD2. *Hum Mol Genet*. 2015;24(3):659-69.
56. BEST (Biomarkers, EndpointS, and other Tools) Resource. Silver Spring (MD) Bethesda (MD)2016.
57. Manolis E, Vamvakas S, Isaac M. New pathway for qualification of novel methodologies in the European Medicines Agency. *Proteomics Clin Appl*. 2011;5(5-6):248-55.
58. Sakellariou P, O'Neill A, Mueller AL, Stadler G, Wright WE, Roche JA, et al. Neuromuscular electrical stimulation promotes development in mice of mature human muscle from immortalized human myoblasts. *Skelet Muscle*. 2016;6:4.
59. Geng LN, Yao Z, Snider L, Fong AP, Cech JN, Young JM, et al. DUX4 activates germline genes, retroelements, and immune mediators: implications for facioscapulohumeral dystrophy. *Dev Cell*. 2012;22(1):38-51.
60. Snider L, Geng LN, Lemmers RJ, Kyba M, Ware CB, Nelson AM, et al. Facioscapulohumeral dystrophy: incomplete suppression of a retrotransposed gene. *PLoS Genet*. 2010;6(10):e1001181.
61. Jones T, Jones PL. A cre-inducible DUX4 transgenic mouse model for investigating facioscapulohumeral muscular dystrophy. *PLoS One*. 2018;13(2):e0192657.
62. Jones TI, Chew GL, Barraza-Flores P, Schreier S, Ramirez M, Wuebbles RD, et al. Transgenic mice expressing tunable levels of DUX4 develop characteristic facioscapulohumeral muscular dystrophy-like pathophysiology ranging in severity. *Skelet Muscle*. 2020;10(1):8.
63. Nunes AM, Ramirez M, Jones TI, Jones PL. Identification of candidate miRNA biomarkers for facioscapulohumeral muscular dystrophy using DUX4-based mouse models. *Dis Model Mech*. 2021;14(8).
64. Heier CR, Zhang A, Nguyen NY, Tully CB, Panigrahi A, Gordish-Dressman H, et al. Multi-Omics Identifies Circulating miRNA and Protein Biomarkers for Facioscapulohumeral Dystrophy. *J Pers Med*. 2020;10(4).
65. Koutsoulidou A, Koutalios D, Georgiou K, Kakouri AC, Oulas A, Tomazou M, et al. Serum miRNAs as biomarkers for the rare types of muscular dystrophy. *Neuromuscul Disord*. 2022;32(4):332-46.

66. Matsuzaka Y, Kishi S, Aoki Y, Komaki H, Oya Y, Takeda S, et al. Three novel serum biomarkers, miR-1, miR-133a, and miR-206 for Limb-girdle muscular dystrophy, Facioscapulohumeral muscular dystrophy, and Becker muscular dystrophy. *Environ Health Prev Med.* 2014;19(6):452-8.
67. Corasolla Carregari V, Monforte M, Di Maio G, Pieroni L, Urbani A, Ricci E, et al. Proteomics of Muscle Microdialysates Identifies Potential Circulating Biomarkers in Facioscapulohumeral Muscular Dystrophy. *Int J Mol Sci.* 2020;22(1).
68. Petek LM, Rickard AM, Budech C, Poliachik SL, Shaw D, Ferguson MR, et al. A cross sectional study of two independent cohorts identifies serum biomarkers for facioscapulohumeral muscular dystrophy (FSHD). *Neuromuscul Disord.* 2016;26(7):405-13.
69. Statland J, Donlin-Smith CM, Tapscott SJ, van der Maarel S, Tawil R. Multiplex Screen of Serum Biomarkers in Facioscapulohumeral Muscular Dystrophy. *J Neuromuscul Dis.* 2014;1(2):181-90.
70. Wong CJ, Wang L, Holers VM, Frazer-Abel A, van der Maarel SM, Tawil R, et al. Elevated plasma complement components in facioscapulohumeral dystrophy. *Hum Mol Genet.* 2022;31(11):1821-9.
71. Dixit M, Anseau E, Tassin A, Winokur S, Shi R, Qian H, et al. DUX4, a candidate gene of facioscapulohumeral muscular dystrophy, encodes a transcriptional activator of PITX1. *Proc Natl Acad Sci U S A.* 2007;104(46):18157-62.
72. Chau J, Kong X, Viet Nguyen N, Williams K, Ball M, Tawil R, et al. Relationship of DUX4 and target gene expression in FSHD myocytes. *Hum Mutat.* 2021;42(4):421-33.
73. Banerji CRS, Panamarova M, Hebaishi H, White RB, Relaix F, Severini S, et al. PAX7 target genes are globally repressed in facioscapulohumeral muscular dystrophy skeletal muscle. *Nat Commun.* 2017;8(1):2152.
74. Choi SH, Bosnakovski D, Strasser JM, Toso EA, Walters MA, Kyba M. Transcriptional Inhibitors Identified in a 160,000-Compound Small-Molecule DUX4 Viability Screen. *J Biomol Screen.* 2016;21(7):680-8.
75. Yao Z, Snider L, Balog J, Lemmers RJ, Van Der Maarel SM, Tawil R, et al. DUX4-induced gene expression is the major molecular signature in FSHD skeletal muscle. *Hum Mol Genet.* 2014;23(20):5342-52.
76. Bosnakovski D, Xu Z, Gang EJ, Galindo CL, Liu M, Simsek T, et al. An isogenetic myoblast expression screen identifies DUX4-mediated FSHD-associated molecular pathologies. *EMBO J.* 2008;27(20):2766-79.
77. Banerji CRS. *Brain Communications.* 2023 in press.
78. Bosnakovski D, Chan SSK, Recht OO, Hartweck LM, Gustafson CJ, Athman LL, et al. Muscle pathology from stochastic low level DUX4 expression in an FSHD mouse model. *Nat Commun.* 2017;8(1):550.
79. Giesige CR, Wallace LM, Heller KN, Eidahl JO, Saad NY, Fowler AM, et al. AAV-mediated follistatin gene therapy improves functional outcomes in the TIC-DUX4 mouse model of FSHD. *JCI Insight.* 2018;3(22).
80. Mayhew AG, Nelson L, Guglieri M, Willis T. Editorial: Lessons Learned from Translational Research in Neuromuscular Diseases: Impact on Study Design, Outcome Measures and Managing Expectation. *Front Genet.* 2022;13:840074.
81. Lu-Nguyen N, Dickson G, Malerba A, Popplewell L. Long-Term Systemic Treatment of a Mouse Model Displaying Chronic FSHD-like Pathology with Antisense Therapeutics That Inhibit DUX4 Expression. *Biomedicines.* 2022;10(7).

82. Bouwman LF, den Hamer B, van den Heuvel A, Franken M, Jackson M, Dwyer CA, et al. Systemic delivery of a DUX4-targeting antisense oligonucleotide to treat facioscapulohumeral muscular dystrophy. *Mol Ther Nucleic Acids*. 2021;26:813-27.
83. Lu-Nguyen N, Malerba A, Antoni Pineda M, Dickson G, Popplewell L. Improving Molecular and Histopathology in Diaphragm Muscle of the Double Transgenic ACTA1-MCM/FLEXDUX4 Mouse Model of FSHD with Systemic Antisense Therapy. *Hum Gene Ther*. 2022;33(17-18):923-35.
84. Lu-Nguyen N, Malerba A, Herath S, Dickson G, Popplewell L. Systemic antisense therapeutics inhibiting DUX4 expression ameliorates FSHD-like pathology in an FSHD mouse model. *Hum Mol Genet*. 2021;30(15):1398-412.
85. Lassche S, Janssen BH, T IJ, Futterer JJ, Voermans NC, Heerschap A, et al. MRI-Guided Biopsy as a Tool for Diagnosis and Research of Muscle Disorders. *J Neuromuscul Dis*. 2018;5(3):315-9.
86. Loonen TGJ, Horlings CGC, Vincenten SCC, Beurskens CHG, Knuijt S, Padberg G, et al. Characterizing the face in facioscapulohumeral muscular dystrophy. *J Neurol*. 2021;268(4):1342-50.
87. Giacomucci G, Monforte M, Diaz-Manera J, Mul K, Fernandez Torron R, Maggi L, et al. Deep phenotyping of facioscapulohumeral muscular dystrophy type 2 by magnetic resonance imaging. *Eur J Neurol*. 2020;27(12):2604-15.
88. Mul K, Kinoshita J, Dawkins H, van Engelen B, Tupler R, Consortium F. 225th ENMC international workshop:: A global FSHD registry framework, 18-20 November 2016, Heemskerk, The Netherlands. *Neuromuscul Disord*. 2017;27(8):782-90.