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Workshop report

1st FSHD European Trial Network workshop: Working towards trial readiness across Europe

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1. Introduction

On April 23rd (Session 1–4) and May 7th 2021 (Session 5–7), the first meeting of the Facioscapulohumeral muscular dystrophy (FSHD) European Trial Network (ETN) took place. Forty-two participants from nine countries representing academic centres, FSHD patients' advocacy groups, and pharmaceutical companies were invited to participate in this virtual workshop on Zoom. Six themes were selected, and chairs of the sessions were encouraged to meet with the speakers of their session before the workshop.

FSHD is at the doorstep of clinical therapeutic trials. There is interest in FSHD from several pharmaceutical industries, and a few trials are ongoing in the USA and Europe. Guidelines for clinical trials, pharma regulation and participation, and health care provision in the different European countries differ in various subtle ways and would benefit from an overall strategy specifically catered to the European context.

The patient organization and the clinical and research networks in the USA (FSHD Society and FSHD Clinical Trial Research Network) are well established and collaborate successfully [1]. The level of organization in Europe lags behind, especially at the clinical level. In order to offer the FSHD patient community in Europe the best position in discussions with clinical and basic science researchers and with pharma, FSHD Europe initiated a virtual workshop with the following aims:

- Establish the foundation of European FSHD Trial Network;
- Harmonize criteria for clinical and genetic diagnosis, for registries and outcome measures;
- Create exchange of clinical experience and genetic reference material;
- Bring Europe on a par with the USA on trial-readiness in FSHD.

In addition, if compounds prove to have a positive effect, the European FSHD Trial Network will be able to act as a partner in the discussions with health care authorities and the regulator. This adds the following aims:

- Engage Pharma and EMA for a Europe wide collaboration;
- Harmonize treatment and care for all European FSHD patients.

Dr. Nicol Voermans and Prof. Pascal Laforêt were invited by FSHD Europe as chair and vice chair of the European Trial Network, and Maria Vriens-Munoz Bravo was appointed as secretary. Prof. George Padberg and Prof. Baziel van Engelen took on the role as advisors. Together, they invited experts from different European countries involved in FSHD research to form the core group of this network. The core group had three online meetings to compose the program of the workshop (January 15th; February 12th and March 5th).

Nicol Voermans (***the Netherlands***) opened the workshop with a short description of the initiative of FSHD Europe. Europe consists of 44 countries (27 are members of the European Union), with 24 official languages. It has 748 million inhabitants, representing 11% of the world population.

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Based on the assessed prevalence of 12 /100.000, Europe is estimated to have 90,000 FSHD patients [2]. This offers many possibilities for performing future trials, but at the same time poses large challenges for international multicentre phase 2 and / or phase 3 trials. The European FSHD Trial Network aims to unite the European experts in their endeavour to reach trial readiness. The experiences in other neuromuscular disorders such as spinal muscular atrophy and Pompe's disease offer important guidance on how to do this.

During these two days' workshop, the FSHD European Trial Network aimed to delineate the first steps to be taken. This included the foundation of the expert network, identification of knowledge gaps, constitution of working groups, delineation of a strategic plan, and coordination of tasks with the other organizations active in the field of trial readiness: FSHD Clinical Trial Research Network (CTRN) (USA), FSHD Society (USA), TreatNMD (UK), and the European Reference Network (ERN) EURO-NMD. The FSHD CTRN is currently testing the design of an international multicentre study and investigating the sensitivity of a number of clinical outcome measures. The FSHD Society together with the ERN EURO-NMD has started working on a revision of the international Standards of care for FSHD [3,4]. TREAT-NMD has recently initiated a FSHD Task Force, which will focus on Standards of care development, education, coordination of patient associations, and identifying & supporting developing countries.

Maria Vriens-Munoz Bravo (*the Netherlands, Spain*) introduced **FSHD Europe.** FSHD Europe was founded in 2010 and represents patient organizations across Europe. It has seven member organisations from six countries representing approximately 3000 people with FSHD. It has links with other patient organisations across Europe and is seeking to expand to other European countries.

The aim of FSHD Europe is to act as a catalyst, working with all stakeholders to promote joint work around FSHD that will enable the faster development of treatments, and make them accessible to people with FSHD. The stakeholders are researchers, scientists and clinicians, statutory and regulatory bodies such as the European Medicine Agency, pharmaceutical companies, and people with FSHD and their families. Current projects include the foundation of this network and the Patient Pharma project, which will define what patients see as important outcomes from clinical trials and how they could be measured. The recently performed investigation by SMA Europe serves as an excellent example [5].

The members are: Amis FSH (France), AFM Télethon (France), FSHD-Diagnosegruppe in der DGM e. V. (Germany), FSHD Spain (Spain), FSHD Italia (Italy), Spierziekten Nederland (The Netherlands), FSH-MD Support Group UK (United Kingdom). The scientific advisers are George Padberg and Julie Demonceaux. FSHD Europe is a member of Eurordis: https://www.eurordis.org/The voice of rare disease patients, Treat NMD: https://treat-nmd.org/Neuromuscular Network, and FSHD Alliance.

2. Lessons learnt from other trials and network

This session was chaired by Alexandre Mejat and Isabelle Desguerre.

Susana Quijano-Roy (France), scientific coordinator of the SMA Register France presented the challenge of launching a National SMA Registry. The large spectrum of age, severity and progression and the multi-systemic complications constitute important issues to launch a registry. Moreover, there is an important burden for patients and clinicians and making a registry feasible requires professional management. In fact, a SMA registry had been launched previously in France, but only a few paediatric centres were able to participate and it was not continued after 2013 (UMD-SMA).

This situation changed in 2017 when new therapies in SMA appeared. This triggered a new impulse, and clinicians, stake-holders patients and pharma converged in a common aim. Progressive steps were taken in a consensual pathway in order to ensure launching and maintenance of the database. The French neuromuscular federation (FILNEMUS) first estimated the population followed in the reference centers. FILNEMUS and AFM-Téléthon next facilitated meetings of a multidisciplinary working group devoted to define the items, choose the adequate sponsor and nominate the scientific coordinator. An institutional sponsor was preferred, to ensure academic independence. A protocol with clear objectives (epidemiological, clinical therapeutic response and tolerance, becoming a backbone for research satellite projects) was defined. The selected items were reviewed, taking into account the pre-existing international registries (SMARTCARE, iSMAc), international recommendations (TREAT-NMD) and preference of the French clinical community.

The current registry was launched in January 2020 in a retrospective version and later with prospective data collection. The operative team was structured around the scientific coordinator and the methodologist. Other important key-persons were a part-time curator, a full-time project manager and a well know clinician in the neuromuscular network charged of heading the steering committee. An independent body (scientific counsel) is in course of constitution.

In March 2020, COVID pandemics perturbed the planning dramatically, which required a timeline readjustment. The operative team then developed a dynamic management, projecting progressive phases with some flexibility and re-negotiating with pharma the time deliveries but preserving the fixed objectives. Nevertheless, the target number of inclusions (400 patients) was reach in the first trimester of 2021. Additional funding from new pharmaceutical companies directly interested in the development of the registry made it possible to spread an "army" of formed technicians devoted to the collection of data in real time in each center.

While the first year was mainly devoted to opening centers and including patients, the following steps currently ongoing are directed to improve the quality of data and implement patients' and carers' questionnaires about daily life and

autonomy. While waiting to have a functional database, the pediatric neuromuscular community collected data on their own in order to get results in real-time of the different therapies available : nusinersen (Audic, 2020) and gene therapy (under submission).

Simon Khosla from SMA Europe (***Switzerland***) presented the **SMA Europe campaigns** to improve the quality of life of people living with SMA, to bring effective therapies to patients in a timely and sustainable way and to encourage optimal patient care.

SMA Europe is an umbrella organization which includes 25 SMA patient and research organisation from 23 countries in Europe. Their clinical trial readiness strategy is to strengthen the relationship with clinical centres in Europe through workshops, info sessions and targeted expertise. They aim to intensify the connection between new centres and industry and to increase best practice sharing between centres to improve the quality and processes of clinical trials. In addition, they intend to extend the SMA Europe clinical trial working group team with medical expertise and process knowledge, and enforce the engagement with industry, centres and healthcare professionals.

SMA Europe has recently conducted two surveys on trial readiness in 2018–2019, sent to 182 centres in Europe. The response rate was 44% (24 countries). The key results were: (1) Many centres were willing to start clinical trials; (2) Many centres need training (best practice sharing, documentation and a better understanding of outcome measures); (3) Three types of centres were identified: Level 1 (well established and SMA experienced centres), Level 2 (centres striving to become competent), and Level 3 (centres with no SMA competency and do not apply Standards of Care). The SMA Europe clinical trial working group then established three main areas of training activities: best practice educational workshops culminating in a curriculum, educational materials and a knowledge database, and the expansion of the landscape survey. The first CT best practice workshop was held at the second SMA Scientific Congress in Paris Evry in Feb 2020 and was a great success. The third SMA Europe Scientific Congress will be held in Barcelona in February 2022 where over 1000 worldwide participants are expected.

Rabi Tawil *(USA)* presented a **brief history of the FSHD CTRN**. The FSHD CTRN was developed over the past 6 years to assure trial readiness and prepare a network of sites for upcoming clinical trials with FSHD. Initially started with 4 sites in the United states, it has now expanded to 12 US sites and 6 European sites [6].

The CTRN consortium agreement insures that every site has equal representation in the network and oversight of the CTRN is led by an external advisory committee composed of advocacy groups, industry as well as individuals with FSHD. Additionally, working groups on Imaging, Biomarker development and Clinical outcome measures were established also involving academia, advocacy and industry. This is critical, as consensus needs to be reached for establishing the key functional, motor tasks and patient reported outcomes needed in FSHD, establishing validated biomarkers and standardizing MRI in FSHD. One of the other important aspects of the CTRN is the development of uniformly training of physical therapist and other clinical evaluators to assure the collection of high quality, consistent and reliable data. The CTRN has attracted a number of pharmaceutical companies that have funded protocols to be run by the FSHD CTRN.

3. Genetics, participants and registries

This session was chaired by Teresinha Evangelista and Pascal Laforêt.

Silvère van der Maarel *(the Netherlands)* presented a summary on **the standardization of molecular analysis for diagnosis of FSHD**. In ∼95% of cases, FSHD is caused by a contraction of the D4Z4 macrosatellite repeat on chromosome 4q (FSHD type 1: FSHD1) [7]. In unaffected individuals the D4Z4 repeat varies between 8 and 100 units while in FSHD1 individuals the repeat size is between 1 and 10 units. D4Z4 contractions are associated with local chromatin relaxation in somatic cells, marked by D4Z4 hypomethylation, and expression of DUX4 from the repeat in skeletal muscle [8,9].

DUX4 is an early embryonic and germline transcription factor and its misexpression in skeletal muscle disturbs muscle homeostasis leading to muscle cell death [10]. The remaining FSHD type 2 (FSHD2; ∼5%) cases are caused by mutations in D4Z4 chromatin factors that contribute to DUX4 silencing in skeletal muscle, most notably the Structural Maintenance of Chromosomes Hinge Domain 1 (SMCHD1) gene, combined with a D4Z4 repeat of 10–20 units [11–13]. In both situations, D4Z4 chromatin relaxation needs to occur on a 4qA genetic background, which encodes for a somatic polyadenylation signal for DUX4 that is necessary for its stable production in skeletal muscle (Fig. 1).

FSHD1 diagnosis is accurate and traditionally performed by serial hybridizations of Southern blots to size the D4Z4 repeat and to establish the 4qA background [14]. Recently, newer technologies provide equally well performing alternatives to Southern blotting including molecular combing and single molecule optical mapping [15,16]. FSHD2 diagnosis requires sizing and haplotyping of the D4Z4 repeats combined with mutation analysis of the FSHD2 genes and D4Z4 methylation analysis. FSHD1 and FSHD2 are not separate disease entities but form a continuum with affected individuals having an FSHD1 and FSHD2 mutation [17].

For registries it is recommended to document the D4Z4 repeat size in repeat units including the genetic background (4qA) irrespective of the technology used (Table 1). It is also endorsed to add if mutations are de novo, because of the risk of undetected mosaicism. For FSHD2 this information should be accompanied by SMCHD1 pathogenic variant and D4Z4 methylation level documentation. This strategy for FSHD1 and FSHD2 is expected to cover 98, 99% of all genetically confirmed FSHD cases. Complex cases such as individuals with D4Z4 duplications [18], mutations in rare FSHD2 disease genes [13,19] or proximally extended deletions

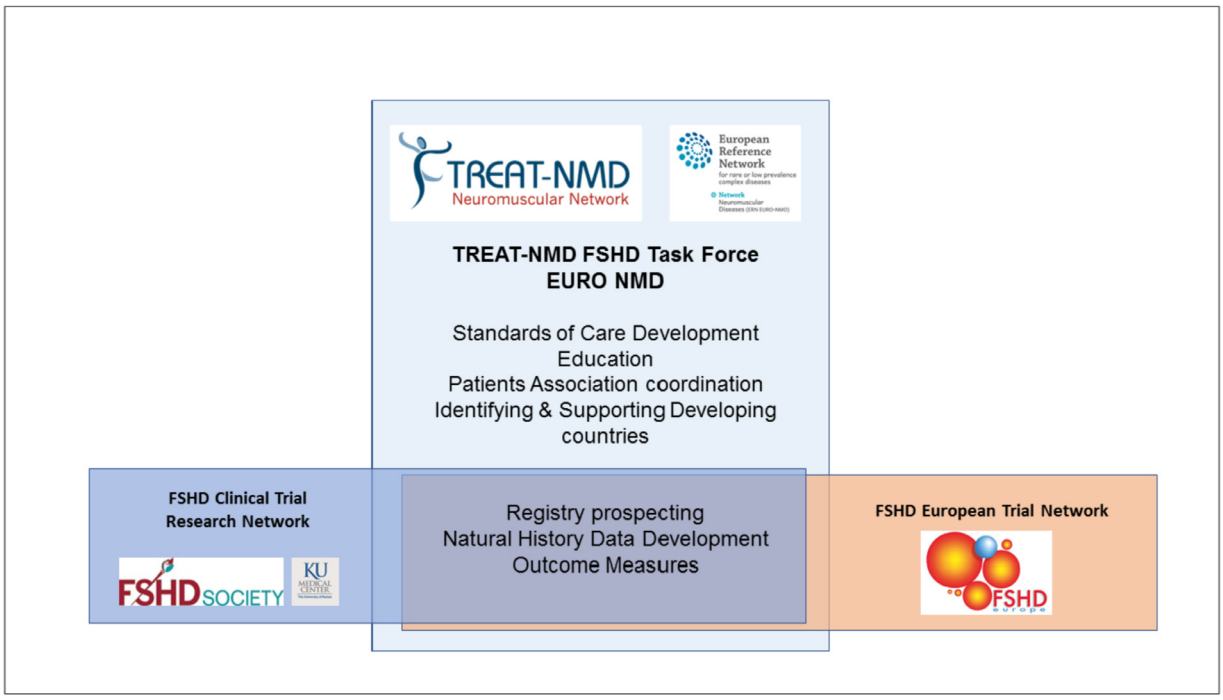

Fig. 1. Partially overlapping and complementary aims of the CTRN, FSHD European Trial Network and TREAT-NMD.

Table 1
Confirmation table of D4Z4 repeat lengths in units and kb with the different techniques.
For registries it is recommended to document the D4Z4 repeat size in repeat units including the genetic background irrespective of the technology used.

Size D4Z4 fragment (in basepair) after digestion				
	4A161S		4A161L	
units	EcoRI	EcoRI/BlnI	EcoRI	EcoRI/BlnI
1U	10,2	7,0	11,8	8,6
2U	13,5	10,3	15,1	15,1
3U	16,8	13,6	18,4	18,4
4U	20,1	16,9	21,7	21,7
5U	23,4	20,2	25,0	25,0
6U	26,7	23,5	28,3	28,3
7U	30,0	26,8	31,6	31,6
8U	33,3	30,1	34,9	34,9
9U	36,6	33,4	38,2	38,2
10U	39,9	36,7	41,5	41,5
11U	43,2	40,0	44,8	44,8
12U	46,5	43,3	48,1	48,1
13U	49,8	46,6	51,4	51,4

1 unit fragments (4A161S = short variant); 4A161L = long variant

[20] should be documented as "genetically confirmed as complex".

Sabrina Sacconi (France) gave a summary on **clinical diagnosis: terminology, childhood/adult type, criteria, scales, differential diagnosis**. She reviewed the clinical characteristics of FSHD patients. Facial weakness mainly involves the orbicularis oculi and orbicularis oris muscles. Scapular weakness results in inability to lift the arms to carry weight and scapular winging; humeral weakness is most pronounced in the distal part of the deltoid, biceps and triceps (resulting in "Popey forearms"). Peroneal weakness is the initial symptom in 5–10% of the patients. Other striking features are abdominal weakness, lumbar hyperlordosis and pectus excavatum. Furthermore, muscle weakness is typically asymmetrical. Less frequent symptoms are retinal involvement, dysphagia, cardiac and respiratory involvement and CNS involvement (symptomatic only in early onset patients) [21]. Early onset FSHD is characterized by an onset before the age of 10 years, and may be associated with multisystem involvement [22]. FSHD may present with predominant axial weakness [23]. Finally, she presented the data showing that FSHD1 and FSHD2 form a disease continuum rather than two different diseases [17].

This allowed for the highlighting of important aspects to be considered in developing outcomes measures for clinical trial readiness and to stratify patients in trials. Classifications in typical and atypical clinical phenotypes were discussed as well as the list of outcomes measures that can be done on regular bases for these patients (Fig. 2) [6,24].

Teresinha Evangelista *(France)* presented on the **FSHD registries in Europe, their harmonization, the minimal datasets and the relationship with ERN**. There are six specific FSHD registries in Europe. The data elements collected follow the TREAT-NMD core dataset published in 2011. They are patient reported with an online consent form and allow for longitudinal data collection. Clinicians validate data partly and they allow the collection of research questionnaires (https://treat-nmd.org/patient-registries/treat-nmd-core-datasets/).

The FSHD registries from Germany, Netherlands and UK have similar characteristics. The Czech Republic registry is similar to the previous ones regarding the datasets and consent

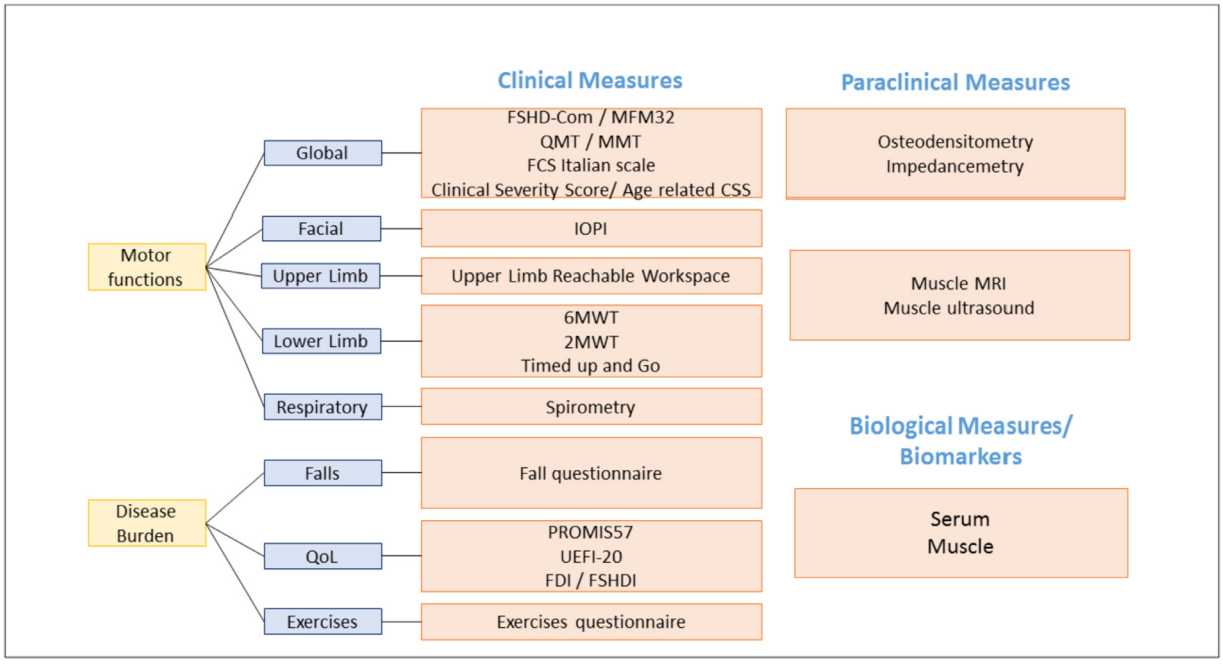

Fig. 2. Overview of the clinical outcome measures applied in routine clinical practice.

process. It covers two countries Czech Republic and Slovakia. The French National registry has a comprehensive number of data elements collected by clinicians and allows for patient reported data. The consent process is classical, not on an online format. It uses well-known ontologies, allows for collection of longitudinal data and has potential for partial interoperability. The Italian registry is a clinician reported registry. The data elements are different from the ones in the previous registries, which makes interoperability difficult.

The TREAT-NMD core data set from 2011 has been updated during the 225th ENMC workshop; the registries will have to be updated [25]. To progress towards interoperability the registries should also consider the common data elements set for rare diseases registration developed by EU Rare Diseases Platform (https://eu-rd-platform.jrc.ec.europa.eu/set-of-common-data-elements_en) and use a pseudonymisation tool (EUPID) to avoid duplication of data (https://eu-rd-platform.jrc.ec.europa.eu/erdri-description_en#inline-nav-3). Furthermore, registries should improve their visibility with use of the European Directory of Registries (ERDRI) and the Central Metadata Repository (ERDRI.mdr) (https://eu-rd-platform.jrc.ec.europa.eu/erdri-description_en). The ERN EURO-NMD is developing a generic registry (https://registry.ern-euro-nmd.eu/) for all neuromuscular diseases including undiagnosed patients, consisting of standardized common and disease specific data sets. It will allow for the generation of a Privacy Preservation Record Link through EUPID and it will use internationally accepted ontologies (HPO) and ORPHA codes. As such it will allow linking and extraction of data from different sources (FAIR data principles). An interoperability layer will be freely available to all registries that aim to become interoperable with the EURO-NMD registry (Fig. 3).

4. Muscle imaging

This session was chaired by Shahram Attarian and Giorgio Tasca.

Robert Carlier *(France)* presented **the role radiologists can play in FSHD:** in the diagnosis process of a suspected inherited muscle disorder; during the follow-up of FSHD patients; and in the evaluation of the periscapular involvement before scapula fixation.

Whole body MRI is the most appropriate tool to detect the muscle involvement of scapular and pelvic girdle, lower limbs, and also of the trunk, face and upper limbs. Like for others whole body MR examinations (cancer, sepsis….) a classical network of head and neck, posterior table, large body surface and lower limbs coils, is used. The arms and forearms and hands have to be placed, in prone position, upon the thorax, abdomen and pelvis in order to be in the field of view. Axial 5 to 8 mm thick, contiguous slices obtained in successive stacks from head to toes are sufficient. T1 and STIR (Short Tau Inversion Recovery) or Dixon T2 with fat and water (in the same acquisition) images are necessary in order to detect sign of fibrous-adipose replacement and dystrophy (bright signal on STIR or water images.) which can be associated or isolated in the same muscle.

Tasca has described the pattern of involvement of scapular girdle and lower limbs [26]. The mostly affected (peri) scapular muscles are trapezius anterior serratus, latissimus dorsi, major and minor pectoralis whereas the most preserved are the rotator cuff muscles. At abdominal level, pelvic girdle and lower limbs the abdominal wall muscles, erector spinae, gluteus minimus, adductor magnus, hamstring, tibialis anterior, soleus and gastrocnemius medialis muscles are mostly affected, whereas iliopsoas, obturators, pectineus,

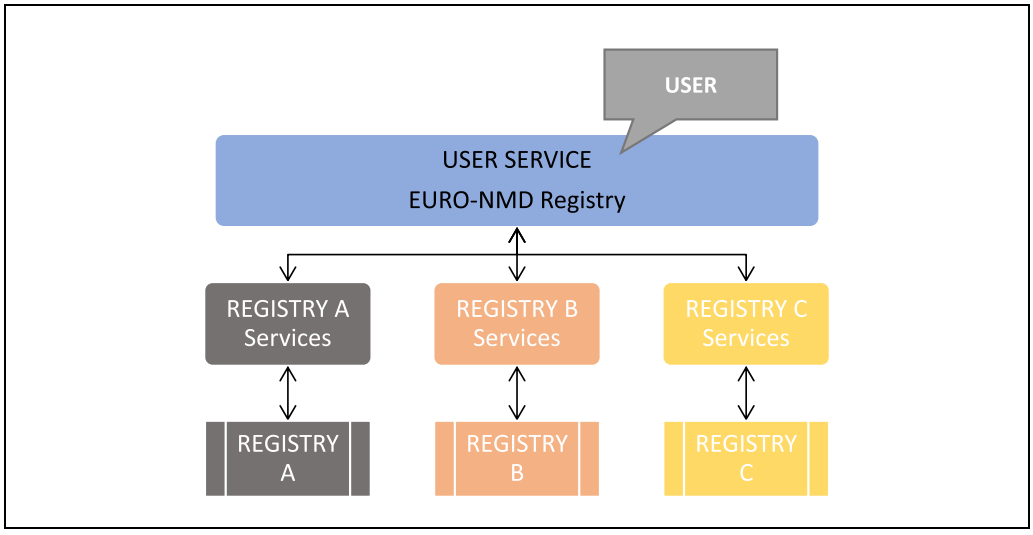

Fig. 3. Schematic representations of the EURO-NMD registry.

tibialis posterior and flexor digitorum are well preserved. Asymmetric distribution for fatty replacement and dystrophic activity is very suggestive of FSHD [27].

Finally, Wang has demonstrated that STIR bright signal has predictive value in identifying muscles with active disease. MRI might thus be useful in early phase therapeutic trials to demonstrate target engagement in therapies aiming to suppress DUX4 expression [28].

John Vissing *(Denmark)* presented data on the use of **quantitative MRI as outcome measure in FSHD**. First, he showed evidence for a severe affection of paraspinal muscles in FSHD (29), and showed data on a close correlation between muscle fat fraction levels in paraspinal muscles and back extension strength as well as correlation between the 6MWT and fat fractions in leg muscles. These findings suggest a close link between MR-assessed fat fraction and muscle function. A 1-year follow-up study of 45 patients with FSHD showed significant progression in fat fraction by 3.9% yearly, while functional measures such as 6MWT, timed-up-and-go and stair climbing tests did not change [30]. Muscles with the greatest progression in fat fraction had an intermediary level of fat replacement (40–60%). Two other longitudinal studies following fat fraction and edema/inflammation in muscles, assessed by STIR and T2 mapping during sequential MR scans, showed that high progression of fat replacement was paralleled by STIR-positivity and high T2 relaxation times [31,32]. The longitudinal assessments showed that once the muscle was STIR positive or had high T2 relaxation time the muscle would progress towards end-stage, complete fat replacement before muscle edema disappeared. It remains to be shown whether muscle edema/inflammation plays a part in the pathogenesis of the disease or whether it is just a marker of disease activity. The studies collectively indicate that quantitative MRI is a sensitive surrogate marker for disease progression in FSHD and that identification of muscles with edema, using STIR or T2 mapping, can be used to distinguish muscles with active disease.

Next, **Nens van Alfen *(the Netherlands)*** explained that **muscle ultrasound is a valuable addition to the neuromuscular toolkit** in both the clinic and research settings. It is patient-friendly and non-invasive and can easily be used in an outpatient setting, at the bedside, and in wheelchair-bound patients. In FSHD, both visual analysis and scoring and quantitative grayscale analysis have a good correlation with clinical measures and a very good responsiveness to change [33,34]. Ultrasound and MRI have a similar sensitivity in FSHD. For use in natural history studies and treatment trials, a standardized acquisition protocol across centres and a centralized reader are recommended.

Compared to MRI, ultrasound has the advantage of detecting early disease stage muscle fibrosis sooner. Ultrasound can also provide images from body regions that are very relevant to patients (arms, hands, face) but that are more difficult to image using conventional MRI. However, in end-stage muscles with complete fatty degeneration the MRI will be more obviously abnormal than for example muscle echogenicity. QMRI seems preferable in assessing deep muscle layers and muscles with inhomogeneous abnormality distributions [35]. Hence, quantitative muscle MRI and ultrasound for facioscapulohumeral muscular dystrophy are very complementary imaging biomarkers, with their own specific value, and ideally the FSHD clinical trial toolkit should contain both [33].

5. Clinical outcome measures and biomarkers

This session was chaired by Adolfo López de Munaín and Benedikt Schoser.

Karlien Mul (*the Netherlands*) gave an update on **Patient reported outcome measures (PROMs) in FSHD**. For late phase clinical trials and drug approval PROMs are valuable

to measure the impact of an intervention on a patient's life. PROMs are particularly useful to measure at the level of daily activities, social participation and quality of life. Useful outcome measures should be reliable, valid, highly responsive to change, and easy to implement across multiple sites.

Two disease specific PROMs have recently been developed. The FSHD-Health Index (FSHD-HI), is intended to measure a patient's perception of the total disease burden. It consists of 116 question in 14 subscales, and shows good reliability and correlation with the 6 min walk test and manual muscle testing [36]. The FSHD-Rasch built Overall Disability Scale (FSHD-RODS) consists of 32-items and measures the level of daily activities and social participation on an interval scale [37]. Most PROMs are ordinal scales that only provide a structured order. In contrast, the scores of the linear-weighted FSHD-RODS questionnaire have a numerical value and are well suited for parametrical statistical testing. For both these PROMs responsiveness still has to be established in longitudinal studies. Finally, FSHD researchers worldwide should aim at uniformity in clinical outcome measures across studies, centres and countries. In general, the development of PROMs usually focuses on clinical relevance of the questions asked. However, clinimetric aspects of the scale are often overlooked, but are just as important in creating an adequate measurement instrument.

Subsequently, **Federica Montagnese (*Germany*)** presented an overview of the **Functional outcome measures (FOMs): 6MWT, TUG, MRC, VC, reachable workspace (used in ReSolve Study)**

The identification of the best FOMs is complicated by the slow progression, the heterogeneous clinical spectrum, and the asymmetrical muscular involvement of FSHD. The quality of studies assessing the clinimetric properties of FOMs (FSHD-COM, 6MWT, RWS, MFM, QMT, MMT and handgrip) is low to very low [38]. The Resolve study therefore aims to identify and validate the best FOMs in FHSD. In particular, it will determine the multi-site reliability and validity of FSHD-COM and assess its responsiveness compared to other FOMs (MFM32, 6MWT, 2MWT, TUG and reachable work space (RWS)) [6].

The strengths and limitations of composite score in clinical trials were discussed. The major advantage is the higher statistical efficiency. Furthermore, it is 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 [39]. Other limitations for the MFM32 is the high ceiling effect [40]. For the 6MWT the reliability, validity and MDC95 have been calculated for FSHD, and the ReSolve study will give further data on its sensitivity to change [41]. The reachable work space (RWS) has proven its validity, reliability, sensitivity to change and clinical meaningfulness in FSHD and is considered a valuable FOM. Potential limitations arise from the technical equipment needed and the complex data analysis [42].

Respiratory impairment is another important aspect of FSHD and its assessment should be included in clinical trials, especially for the more severely affected patients. The pulmonary function tests should minimally include FVC, MIP and MEP. Additionally, the use of diaphragm ultrasound (thickening ratio and excursion amplitude) should be validated further [43].

Overall, the ReSolve study will certainly provide valuable data for the evidence-based selection of the best FOM for FSHD, taking the clinimetric properties into account. The categories that should be investigated further are those not included in Resolve: patients $<$ 18 years and $>$ 75 years and non-ambulatory patients.

The chairs led a short inventory discussion about biomarkers. **Giorgio Tasca *(Italy)*** mentioned his pilot studies on biomarkers in microdialysates (i.e. muscle interstitial fluids obtained after a microcateter insertion in the muscle) from MRI targeted muscles, in particular comparing STIR+, STIR- and control muscles. A first study focused on inflammatory mediators analysed by xMAP technology, and identified two chemokines (CXCL13 and CXCL5) and G-CSF as significantly modulated in affected muscles [44]. A second study more broadly explored changes in the composition of intramuscular milieu by proteomic approach. Three potential circulating biomarkers of disease activity were identified: S100-A8, S100 A-9 and Dermcidin [45]. Interestingly, S100-A8 was simultaneously identified in an independent study as significantly upregulated in the blood of early-onset FSHD patients by mass spectrometry and ELISA [46]. Studies to validate these findings in patients' blood and correlation with radiological markers of disease activity are ongoing.

Enrico Bugiardini *(UK)* further highlighted the importance of inflammatory biomarkers in blood. A previous study has showed an increased cytokine production by peripheral blood mononuclear cells [47] and multiplex screening of serum biomarker in FSHD identified CCL2 (MCP1) as potential inflammatory marker [48]. However, some of these findings were not followed-up, number of patients was limited and it is difficult to determine whether patients recruited were in an active phase of disease. Further exploration of inflammatory markers in blood and correlating them with measure of disease activity such as whole muscle MRI is definitely worthwhile. An additional interesting point was that of **Silvère van der Maarel** on the large variability of DUX4-related biomarkers in muscle biopsies that raises some questions on best biomarkers to use for future clinical trials [49].

Valeria Sansone shared her presentation on **Biomarkers: Old and new: which downstream effects need to be explored?** She started with the presumption that DUX4 is a possible biomarker for FSHD, supported by the correlation between the number of D4Z4 units and disease severity. However, several studies have shown that the levels of DUX4 are very low, almost undetectable in most cells from muscle biopsy samples from FSHD patients, and that DUX4 is expressed in high abundance in nuclei, but at very low frequency. This raises the question as to what are anti-DUX4 therapies actually targeting.

On the other hand, DUX4 may inhibit the function of the myogenic master regulator PAX7, whose repression

is a hallmark of FSHD skeletal muscle when considering RNA-Sequencing data from magnetic resonance imaging-guided muscle biopsies [50,51]. PAX7 target gene repression correlates with active disease, independent to DUX4 target gene expression. In fact, at the single-cell level, PAX7 target gene repression can efficiently discriminate FSHD cells, even when no DUX4 target genes are detectable. Hence, PAX7 appears as a new biomarker for FSHD.

Finally, the relatively slow progression and the extreme clinical variability of FSHD emphasize the importance for patients, clinicians, researchers to define a non-invasive, physiological biomarker(s) of muscle progression early in the disease process thus contributing to pharmaceutical upcoming trial design [52].

6. Current and expected trials, and what do we need to consider

This session was chaired by Baziel van Engelen and John Vissing.

Julie Dumonceaux *(UK)* presented on **the ongoing and upcoming trials, and discussed how we can prepare for this.** To date, 24 clinical trials on FSHD have been terminated and 14 are on-going (Clinicaltrials.gov; updated May 4, 2021). Most of the trials have been conducted in Europe and in the USA and more than 15 sites in Europe were or are inventoried, including sites in France, Denmark, Italy, Spain, UK, the Netherlands, Sweden, and Poland.

Previous trials focused on non-specific approaches targeting muscle homeostasis, immune responses to muscles (ATYR1940, NCT02603562), inhibition of myostatin (ACE-083, NCT02927080), increased anabolic effect on muscle (Albuterol, NCT00027391) or reduction of reactive oxygen species and oxidative stress (antioxidant supplementation, NCT01596803). Today, most trials still address muscle homeostasis to improve muscle mass and strength, including creatine monohydrate (NCT02948244), combination therapy with recombinant human growth hormone (rHGH) and testosterone (NCT03123913), or antioxidant supplementation (NCT02622438). However, the recent advances in the understanding of the pathophysiological mechanisms of FSHD and the identification of the DUX4 gene as the main FSHD causal gene, led to the development of promising small molecules, antisense oligonucleotides and gene therapy vectors targeting DUX4 at DNA, mRNA or protein levels.

During the last few years, about 10 companies have announced the development of a specific program on FSHD. Fulcrum Therapeutics is the only one with phase 2 clinical trials (NCT04003974, NCT04004000, NCT04264442) with their lead candidate Losmapimod, a selective p38α/β inhibitor that reduces DUX4 expression [53]. Few other companies have announced being at the final preparation of the investigational new drug stage, and a phase 1/2 clinical trials are expected in the next coming years (Table 3). Several therapeutic approaches are also currently being developed by academic laboratories (for review see: [54]). The clinical trial design, including cohort homogenization, patient stratification or the inclusion of a run-in period are reflexions to consider to increase the performance of these future clinical trials.

Eva Stumpe from SMA Europe ***(Germany)*** subsequently summarized the **experiences with EMA in the route to approval**, discussing why, when and how a patients association should interact with EMA.

As FSHD is a rare disease with an underlying genetic cause, most probably sponsors of medicines in the pipeline will have to apply for approval through the centralized procedure with EMA. Therefore, FSHD organizations should empower themselves and undertake all necessary steps to be eligible for being involved in EMA procedures. During the medicines lifecycle at EMA patient organizations and patient advocates can get involved in all three phases (pre-submission, evaluation and post-authorization) by presenting the patient perspective in meetings with EMA committees and scientific advisory groups as well as be assuring by reviewing documents meant for publication (package leaflet, EPAR summary, safety communications) that the information given is accurate also from a patient perspective and if patients are the target readers of the documents, the content is understandable for lay person.

Patient organizations can get involved by sending their own patient advocates to EMA consultations and workshops. Beforehand, the organization should apply to become an EMA eligible organization by handing in documents that give proof of its legitimacy, transparency, mission with clear interest in medicines and representation of patients throughout the EU. Individual patient advocates also apply through the EMA stakeholder databank to become involved. They must comply with EMAś robust conflict of interest policy and should not have interests in the pharmaceutical industry that could affect their impartiality. EMA has a dedicated department for public engagement and is always very helpful if questions on possible involvement arise and status of advocates or organizations must be cleared.

Alisa Rahilly from Fulcrum Therapeutics ***(USA)*** presented the high-level **lessons learned from the Fulcrum Therapeutics Trial**: A Phase 2, Randomized, Double-Blind, Placebo-Controlled, 48-Week, Parallel-Group Study of the Efficacy and Safety of Losmapimod in Treating Subjects with Facioscapulohumeral Muscular Dystrophy (FSHD) with Open-Label Extension (OLE). A summary of the high-level lessons is summarized in Table 2. In summary, the study team was consistently looking for a balance between flexibility to adapt the protocol due to COVID and rapid enrolment while keeping safety, quality and oversight a priority.

The next presentations were of **companies who are now in the investigational new drug stage**. They were invited to pitch their plans for phase 1/2 clinical trials in Europe in the next one to three years. Their contributions are summarized in Table 3.

Faciotherapies is a biology-oriented company with different discovery programs based on (i) phenotypic disease platform built on primary patient cells and (ii) biophysical platforms to identify direct inhibitors of the ‘undruggable’

Table 2
Challenges and solutions during Losmapimod trial.

Aspect	Challenge	Solution
Genetic screening process	Variation in testing and reporting results, resulting in confirmation delays	Ensure testing labs are pre-emptively approved for use. Ensure sites are aware translations add 1 business week.
Biopsy training and collection	Large variation in techniques globally, hard to obtain same location of biopsy during 2nd biopsy	Biopsy instruction video should be used. Meetings b/w MRI vendor, site and sponsor before biopsies to review biopsy location will aid in guidance of location selection.
Recruitment	Rapid recruitment in USA centres, creating limited participation for EU sites, also resulting in large boluses of data at sponsor	Strategize enrolment plan to ensure all geographical regions can enrol, considering regulatory approval guidelines and high unmet need in patients/willingness to participate.
COVID-19 challenges	COVID impacted patient's ability to complete primary endpoint assessments. Limited dataset due to pandemic.	Build in flexibility into protocol and extended study. High need for virtual remote safety visits, home health vendor, e-monitoring, digital questionnaires.

Table 3
Companies that have announced being at the final preparation of the investigational new drug stage. Companies that have not contributed but are also working towards clincial trials are: Avidity Biosciences (plans to begin clinical trials in 2022), Myocea (file IND in first quarter of 2021 suggesting clinical trials will commence soon afterwards), and Arrowhead Therapeutics (expected IND clearance in third quarter of 2021).

Researcher, Firm	Product
Joris de Maeyer, **Facio therapies**	Dual-acting novel chemical entity inhibiting DUX4 expression and promoting muscle differentiation
Molly White **Dyne therapeutics**	Proprietary Fab conjugated with linker to an ASO designed to reduce DUX4 expression
Anthony Saleh **Mirecule**	Multi-target RNA targeted therapy
Peter Jones **EpiSwitch Rx**	CRISPR inhibition gene therapy: Single dose AAV vector delivered systemically by IV after immune suppression

DUX4a progressive muscle disease with a very high unmet medical need.

Dyne Therapeutics is building a leading muscle disease company focused on advancing innovative life-transforming therapeutics for people living with genetically driven diseases. They aim to use a specific technique to overcome current limitations of muscle tissue delivery to advance modern oligonucleotide therapeutics.

Mirecule is an early-stage biotechnology company developing RNA-based therapeutics. Their approach to drug design revolves around using genomic patient data to create highly tailored therapeutics – the right drug for the right patient.

EpiSwitch Rx is an FSHD-focused biotechnology company founded by scientists with expertise in epigenetics, gene regulation, muscle biology, and FSHD pathogenic mechanisms in collaboration with experts in translational drug development and commercialization. Our lead program is CRISPR-inhibition gene therapy for FSHD delivered systemically using AAV.

7. Aims of the FSHD European Trial Network

This session was chaired by Enrico Bugiardini and George Padberg.

In the final part of the second session, **Nicol Voermans** summarized the key points of the first afternoon, and presented a plan on subsequent steps of the FSHD European Trial Network. She discussed that the two workshop meetings have offered the start of the network and in ways to collaborate with the other organisations in the field (FSHD Society, FSHD CTRN, ERN EURO-NMD, and TREAT-NMD). Furthermore, the sessions on Imaging and Clinical outcome measures and biomarkers have raised a number of important points that need to be clarified in the way to trial readiness. She explained that although FSHD research is a global process, trials might better be considered as local endeavours: they are bound by the local requirements and trial services. Furthermore, she stressed that one of the important goals of this network would be to attract more FSHD devoted clinicians. They are highly needed to guarantee the treatment of the high number of FSHD patients according to the current treatment guidelines.

The FSHD ETN will install a board with chair, vice chair, secretary and strategic experts (https://fshd-europe.info/core-group-of-experts/). Although participation to this workshop was on invitation only, the network will have an open membership from now on. Members can either be *active members* (who have a role in the EC and/or in one of the WGs) or *associated members* (who want to be kept updated and be connected but who do not play an active role).

The Network will remain closely connected to FSHD Europe, and for the four working groups (WG) on clinical and genetic diagnosis (WG 1), clinical outcome measures (WG2), biomarkers (WG 3), and imaging outcome measures (WG4) clinical researchers will be connected to patient representatives of FSHD Europe. The working groups will work towards an application for two ENMC workshops in 2022(2023). Furthermore, a communication delegate will be appointed. Network members will be appointed to reach out to countries not yet connected and to collaborate with the other organizations mentioned above, including the FSHD CTRN, and the network experts will contribute to the patient expectations survey FSHD Europe is currently performing. The network members will promote the use of agreed FSHD Core dataset across all stakeholder groups and registries, with support of TREAT-NMD.

The network will not take the lead in the development and dissemination of Standards of Care Guidelines or in the organisation of FSHD Educational Programmes, since this is part of the TREAT-NMD objectives and also initiated by the FSHD Society.

This session was concluded by **Pascal Laforêt (*France*)**, who thanked all participants for their constructive participation, thus paving the way to a unique European consortium for FSHD, associating dedicated patient organizations and neuromuscular centres. The previously established collaborations between many participants to this workshop, will certainly help to easily consolidate the FSHD ETN and "put in track" pre-identified investigating sites for clinical trials all over Europe ready to start including patients using standardized tools. Next steps in the coming months will be the harmonization and standardization of genetic diagnosis and minimal data set to collect in various registries and patients databases, in parallel with efforts identification of new clinical outcomes and biomarkers.

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Declaration of Competing Interest

None

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