The First Steps in the New Era of FSHD Trials

Trial Readiness, Execution and Evaluation

Joost Kools

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The First Steps in the New Era of FSHD Trials

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Chapter 1

Introduction

Everyday activities, such as grocery shopping, cycling to work, climbing stairs, or responding to compliments with a smile, are generally performed effortlessly. Neuromuscular disorders (NMDs) can transform these routine activities into large obstacles for affected individuals. Facioscapulohumeral muscular dystrophy (FSHD) is an NMD affecting approximately 2000 individuals in the Netherlands[1]. The skeletal muscles of FSHD patients gradually deteriorate, resulting in progressive disabilities[2]. Despite many preclinical studies and a small number of clinical trials, disease modifying therapies are not yet available[3].

This thesis encompasses endeavors to improve clinical trial readiness in FSHD, the results of a phase II clinical trial of losmapimod, and an evaluation of the participants' experiences of the losmapimod trials. The introduction provides a background on the key characteristics of FSHD, the essential components needed for a clinical trial, and an insight in why FSHD poses a challenge for successful clinical trials. Furthermore, it shortly discusses previous trials conducted in FSHD and introduces losmapimod, the study drug currently under examination.

Facioscapulohumeral Muscular Dystrophy

Clinical characteristics

FSHD is a rare NMD affecting approximately 1 in 10,000 individuals[1]. The classical presentation involves asymmetrical weakness in facial, shoulder, and upper arm muscles, manifesting between the ages of 15 and 30 years (Figure 1)[2]. With progression of the disease the pelvic girdle, lower extremity and trunk muscles often become affected[2,4]. Approximately 20% of FSHD patients eventually become wheelchair-dependent[5]. The disease course is highly variable and many patients present themselves with symptomology that differs from the classical presentation. The age of onset and disease severity varies greatly, ranging from wheelchair-dependent children who require non-invasive ventilation to asymptomatic 70-year old genetic carriers[6,7]. Furthermore, the disease progression does not appear to be linear but step-wise, with patients remaining stable for years and suddenly experiencing fast progression[2]. Why certain muscles are affected and when patients will experience disease progression remains unclear.

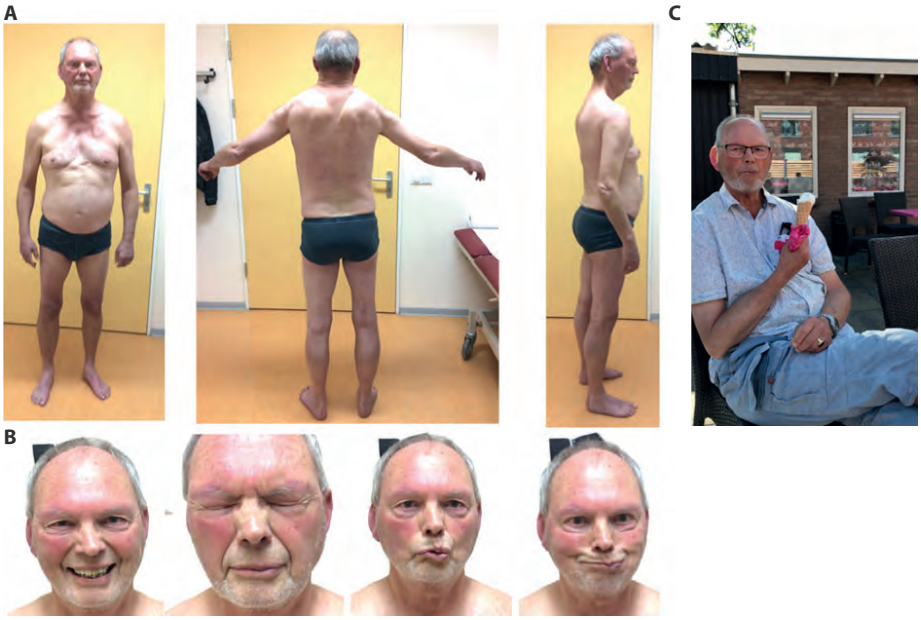

Figure 1. Clinical characteristics of FSHD

A. The three pictures show some of the characteristics of FSHD. The first picture shows atrophy of the pectoralis major muscle and slight asymmetry of the abdomen. The abdomen is protruding due to weakness of the abdominal muscles. In the second picture the participant is asked to raise his arms as high as possible. He is unable to raise his arms higher than 90 degrees caused by shoulder girdle weakness, which is accompanied by scapula alata. Notable is the sparing of the muscles of the forearm. Additionally, slight asymmetry in the volume of the muscles of the legs is noticeable due to atrophy of the hamstring muscles of the right leg and slight atrophy of the left calf muscles. The third picture shows mild lumbar hyperlordosis.

B. These four picture show some of the facial weakness signs that can occur in FSHD patients. Weakness of the right orbicularis oris results in a slightly asymmetric smile, with more clearly pronounced asymmetry when asked to whistle (3rd picture) or purse the lips (4th picture). The second picture shows the inability to completely close the eyes, caused by weakness of the orbicularis oculi. The left eye shows a 'signe de cils', an inability to completely burry the eyelashes when tightly squeezing the eyes.

C. This picture shows the patient enjoying an ice cream in daily life. Despite the muscle weakness, patients are still able to enjoy their daily life. Research tends to diminish patients to symptoms and numbers, it is important to keep reminding ourselves of the persons behind the numbers.
Pictures are used with permission from the patient.

Pathophysiology

FSHD is an hereditary disease following an autosomal dominant pattern, but 10-30% of the patients result from a *de novo* mutation[8]. The underlying genetic cause of FSHD is inadequate closure of the chromatin structure of the D4Z4 repeat array on chromosome 4q35, which results in the production of the transcription factor DUX4[9]. Although the precise pathophysiological mechanism remains unclear,

downstream proteins of the DUX4-pathway prove toxic to muscle cells, inducing apoptosis and inhibiting myogenesis[10]. Subsequently, muscle tissue slowly deteriorates, clinically presenting as progressive muscle atrophy and weakness.

In short, FSHD is genetically categorized into two types, which are clinically indistinguishable[11,12]. In the general population, the size of the D4Z4 repeat array is 8 – 100 units. The structure is supported by chromatin modifiers such as the structural maintenance of chromosomes flexible hinge domain containing 1 (SMCHD1) protein. This results in closure of the chromatin structure, which makes transcription of the D4Z4 repeat array (and thus production of DUX4) impossible. FSHD type 1, present in approximately 95% of the patients, is caused by a shortening of the D4Z4 repeat array to 1 – 10 repeat units (Figure 2). Due to the decrease in the number of repeat units, the chromatin structure of the D4Z4 repeat array is unable to close adequately, enabling transcription of the D4Z4 repeat array and subsequently production of DUX4. A moderate inverse correlation between the number of D4Z4 repeat units and the disease severity has been observed, which partially explains the variable phenotype[7,13]. FSHD type 2 has a digenic etiology requiring a shortening of the D4Z4 array to 8 – 20 repeats and loss-of-function of a chromatin modifier, most commonly the *SMCHD1* gene, which also results in inadequate closure of the chromatin structure.

Management

Currently there is no disease-modifying treatment for FSHD. The management of FSHD is symptomatic requiring a multidisciplinary team consisting of a neurologist, rehabilitation physician, physical therapist, occupational therapist, speech therapist, dietician, and social worker[14].

Muscle strength and stamina should be maintained by performing (individualized) low-intensity aerobic exercises, preferably guided by an experienced physical therapist[15]. Musculoskeletal pain is a common symptom which should initially be treated by physical therapy, followed or supported by the standard analgesic ladder[16]. A limited scapular range of motion might improve after surgical scapular fixation. The evidence on the safety and efficacy of this surgery is scarce and this intervention should only be considered after a careful assessment of the patient[17,18]. Fatigue symptoms can be treated using cognitive behavioral therapy, education and training about energy-management, and aerobic exercises[15,19]. The use of supporting devices such as ankle-foot orthoses, thoracolumbar braces or a walker to improve the mobility and safety of the patients is encouraged.

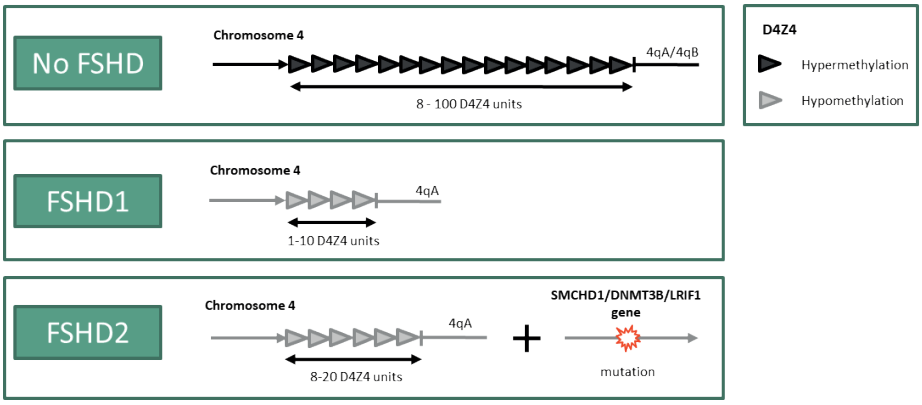

Figure 2. Genetic mechanism of FSHD

No FSHD: The majority of the Western population without FSHD have a hypermethylated D4Z4 repeat array on chromosome 4q35 consisting of 8-100 D4Z4 repeat units. This results in a closed chromatin structure preventing transcription of the D4Z4 repeat array, which contains the *DUX4* gene.

FSHD type 1: 95% of the FSHD patients have a contraction of the D4Z4 repeat array to 1-10 units combined with hypomethylation and a 4qA haplotype. The short repeat length and hypomethylation result in an open chromatin structure, allowing transcription of the D4Z4 repeat array. The 4qA haplotype contains a polyadenation signal (PAS) that stabilizes the DUX4 transcript. Without the PAS, the DUX4 transcript deteriorates before translation can occur, thus preventing FSHD from manifesting.

FSHD type 2: Present in 5% of the FSHD patients, FSHD type 2 is caused by a shortening of D4Z4 repeat array to 8-20 units, hypomethylation and 4qA haplotype combined with a pathogenic variant in a chromatin structure modifier gene. Most often this is the *SMCHD1* (structural maintenance of chromosome flexible hinge domain containing 1) gene, but other genes such as the *DNMT3B* (DNA methyltransferase 3 beta) and *LRIF1* (Ligand Dependent Nuclear Receptor Interacting Factor 1) genes were recently discovered to also involved in FSHD type 2. It is likely that in the future more genes related to FSHD type 2 will be discovered.

The original figure was created by S. Vincenten and is being used with permission.

Box 1. Extra-muscular symptoms in FSHD

Besides the skeletal muscle wasting, patients with FSHD may also experience one or more of the following extra-muscular symptoms: respiratory insufficiency, cardiac abnormalities, retinal vascular disease and hearing loss. The evidence on the prevalence of these symptoms is limited, therefore, the prevalence numbers mentioned below might not be reliable[14].

Respiratory insufficiency occurs in approximately 1-13% of patients with FSHD. The insufficiency can be caused by a combination of severe proximal weakness, kyphoscoliosis, wheelchair dependence, or comorbidities affecting ventilation. Patients with pulmonary insufficiency should be monitored regularly and referred for pulmonary or sleep consultation if necessary. Noninvasive ventilation can be considered to improve the quality of life.

Although some studies reported on supraventricular arrhythmias in patients with FSHD, the consensus is that these symptoms are rare, rendering routine cardiac evaluation unnecessary.

Retinal vascular disease occurs in 0.6% of the total patient population, and exclusively in patients with a D4Z4 repeat array length of 1-3 units (generally patients with childhood onset). FSHD patients with 1-3 D4Z4 repeat units should therefore always be referred to an ophthalmologist. Subsequent monitoring can be determined after the initial screening.

Approximately 30% of the patients with 1-3 D4Z4 repeat units suffer from, sometimes progressive, hearing loss. As untreated hearing loss can impair language development, young children with FSHD should be yearly screened on hearing loss.

Clinical trial readiness

Testing novel therapies on safety and efficacy occurs through clinical trials, typically following a three-phase sequence (Figure 3). To conduct clinical trials successfully, certain components, collectively termed clinical trial readiness, must be in place. These components include well-characterized and registered patients to enable fast and sufficient recruitment, reliable methods to measure target engagement for phase I and II trials, and validated clinical outcome measures (COMs) and patient reported outcomes (PROs) for phase III trials[21,21]. In rare diseases like FSHD, phase III trials are often multisite, international endeavors and thus also require sufficient, adequately equipped study sites[22].

The FSHD community faces several challenges in meeting the components of clinical trial readiness. First, ensuring an adequate number of eligible FSHD patients for drug trials is challenging due to the rareness of the disease, prompting the initiation of national FSHD registries to facilitate access to patients and improve disease characterization[20,21,23-25]. Second, biomarkers tracking disease progression or target engagement on a cellular or molecular level are limited. Currently, the primary method for (indirectly) measuring DUX4 gene expression in muscle tissue is through invasive, burdensome muscle biopsies (Box 2). The reliability of this method is still unclear with studies reporting contradictory results[26-28]. Ideally, a less invasive (e.g. blood) biomarker to measure disease state will become available in the near future. Thirdly, phase III trials encounter challenges due to the heterogeneity and slow, variable disease progression[2]. Common NMD COMs are therefore unable to reliably capture disease progression in the short timeframe of a clinical trial[29]. To address these challenges, natural history studies have been initiated to gain more insight in disease progression and develop and evaluate new FSHD-specific COMs (Box 2)[7,20,30,31]. Lastly, to enhance the trial readiness on an international level, several network initiatives were set-up, such as the FSHD Clinical Trial Research Network (global), FSHD European Trial Network (Europe), and Project Mercury (Global)[32-34].

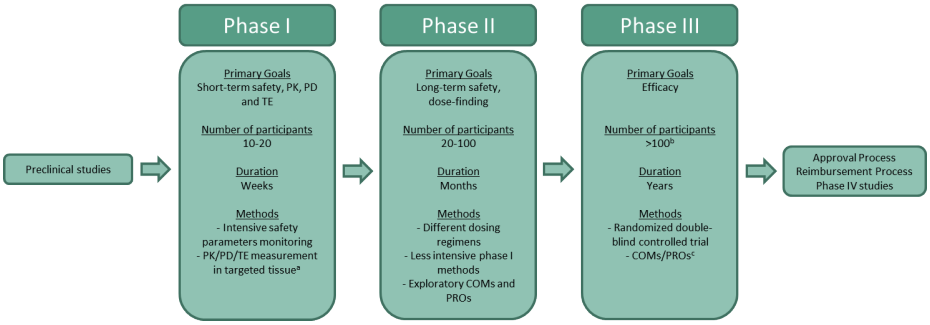

Figure 3. Phases of clinical trials

The primary goals, number of participants, duration of the trials and expected outcomes are presented per trial phase. Preclinical studies generally consist of in vitro and in vivo (animals) studies.
Phase IV studies are surveillance studies performed after a drug received market approval.
a. In the case of FSHD, the targeted tissue is skeletal muscle. Target engagement of DUX4 is currently being determined by taking tissue samples from the muscle using needle biopsies (BOX 2).
b. The number of participants in a phase III study will be determined using power calculations based on previous (phase II) results.
c. Generally, fewer COMs and PROs are included in phase III trials compared to phase II trials. The phase II trials determine which COMs and PROs are the most feasible for a phase III trial.
PK = pharmacokinetics, PD = pharmacodynamics, TE = target engagement, COM = clinical outcome measure, PRO = patient reported outcome

Box 2. Biomarkers and clinical outcome measures in FSHD

The United States Food & Drug Administration (FDA), the organ that amongst others oversees safety and efficacy of drugs in the United States, defines a biomarker as 'a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions'[35]. Biomarkers can be of molecular, histologic, radiographic or physiologic nature.

The majority of the FSHD studies utilizes molecular and radiographic biomarkers. DUX4 mRNA levels in muscle biopsies are indirectly measured to serve as a molecular biomarker. Directly measuring DUX4 mRNA is unreliable because of the unstable mRNA and the stochastic expression[36]. Instead, a panel of more stable downstream genes of DUX4 are measured to indirectly measure DUX4 levels[26].

The measurement of the fat fraction in muscles using Magnetic Resonance Imaging (MRI) is the common radiographic biomarker in FSHD studies[4]. Another possible radiographic biomarker for FSHD could be muscle ultrasound, but will require more validation[37].

The FDA defines a clinical outcome measure (COM) as 'a measure that describes or reflects how a patient feels, functions or survives'[38]. A COM can be a performance outcome, patient-reported, observer-reported or clinician-reported measure.

Performance outcome measures in FSHD initially consisted of muscle strength measurements or functionality tests generalized for all neuromuscular disorders. Recently, new performance outcome measures were developed such as the reachable workspace, which measured upper extremity functionality, or the FSHD Composite Outcome Measure, a composite score of functional tests to measure the FSHD-specific weakness pattern[39,40].

Previous studies used more general patient-reported outcomes (PROs), but recently two FSHD-specific PROs were created: the FSHD Health Index and FSHD Rasch-built overall disability scale[30,31]. These FSHD-specific PROs might be able to capture the subjective symptoms patients with FSHD experience more reliably compared to the more generalized PROs.

Clinical trials in FSHD

Previous clinical trials in FSHD have yielded varying results, with none of the studied therapies being approved for clinical practice[3]. The therapies aimed to improve muscle growth, inhibit muscle inflammation, prevent cell death with anti-oxidative properties or to re-methylate the DNA (Table 1). Negative outcomes may be attributed to the lack of efficacy or, in part, to insufficient clinical trial readiness, particularly concerning insensitive COMs.

With the discovery of FSHD's (epi)genetic and pathophysiological mechanisms, the development of DUX4-targeting drug therapies surged, initiating a new era in FSHD trials. Over twelve companies and academic laboratories were developing DUX4-targeting therapies using multiple strategies such as small molecules, silencing mRNAs, antisense oligonucleotides and CRISPR-CAS (Figure 4). It is therefore essential to keep improving the state of clinical trial readiness, to ensure these new therapies can be tested in optimally designed trials.

The first drug being tested in the new era of DUX4-targeting therapies is losmapimod: a small molecule, selective inhibitor of p38 α/β mitogen-activated protein kinase. By screening large databases of chemical compounds, losmapimod was discovered to have the potential to inhibit DUX4 production. Although the exact mechanism is unknown, losmapimod has shown promising results in preclinical studies, inhibiting DUX4 production in FSHD myoblasts and animal models[41,42]. Clinically, losmapimod already exhibited a favorable safety profile in previous clinical trials for various disorders[43-48]. However, due to a lack of efficacy for those disorders, losmapimod was never approved for widespread use. A phase I trial in FSHD patients confirmed the short-term safety profile, target engagement and appropriate dosage in FSHD patients[49]. Phase II and III trials will be essential to explore long-term safety and assess the potential impact on disease progression in FSHD patients.

Table 1. Previous drug trials in FSHD

Therapy	Year	Outcome	Ref
Promoting muscle growth			
Albuterol (B2-agonist)	1998-2000	Improvement of muscle mass and strength, but no functional improvements.	50
Salbutamol (B2-agonist)	?-2009	No improvement of muscle strength and functionality.	51
Albuterol + exercise	? - 2004	No synergistic effect between exercise and albuterol. Minor improvement of muscle strength.	52
Clenbuterol (B2-agonist)	?	No improvement of affected muscles or daily life activities.	53
MYO-029 (Myostatin inhibitor)	2005-2007	Improvement of muscle mass, but no functional improvements.	54
ACE-083 (Myostatin inhibitor)	2016-2019	Muscle mass improved, but no functional improvements.	55
Testosterone + rHGH (hormones)	2017-2022	*pending*	-
Creatine (supplement)	? - 2000 2017-2021	Short-term improvement of strength and functionality.	56
Anti-inflammatory			
Prednisone (corticosteroid)	? - 1997	No improvement of muscle strength or muscle mass. Long-term treatment might induce severe side-effects.	57
ATYR1940 (Aminoacyl-tRNA synthetase)	2014-2017	Improvement of muscle strength and quality of life.	58[a]
Antioxidative stress			
Antioxidant Capsule (supplement)	2010 - ongoing	Improvement of muscle strength and functionality.	59
Flavomega (supplement)	2016	Improvement of muscle strength.	60
D4Z4 repeat array methylation			
Folic Acid + methionine (hormones)	? - 2006	No change in D4Z4 repeat array methylation.	61

a: It is unclear why the development of ATYR1940 was halted. The available information point towards a positive safety profile and efficacy, yet a phase III study was never initiated. It is also notable that no scientific paper of the ATYR-trials was published, the results are only available in a press release of aTyr Pharma.

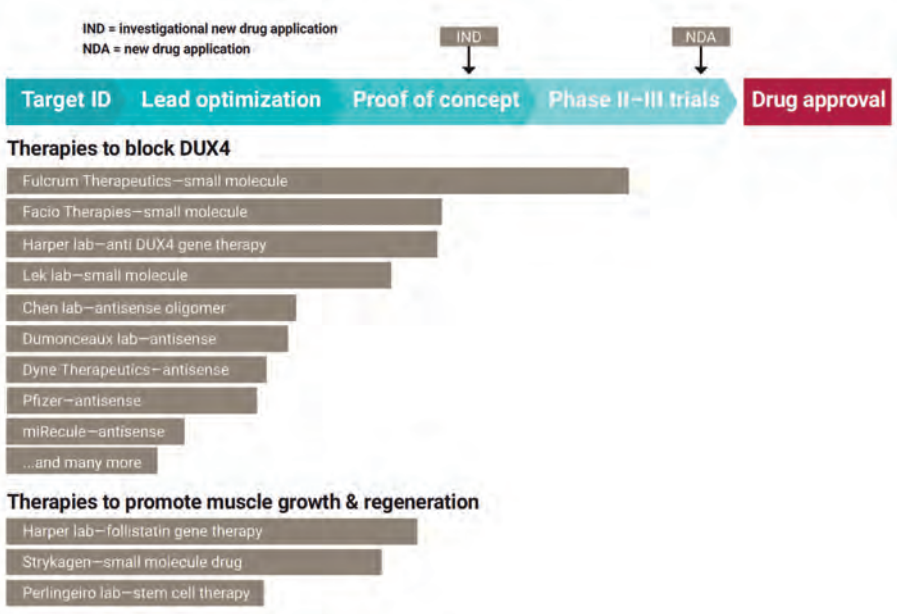

Figure 4. FSHD drug development pipeline of October 2019[62]

This figure lists the various companies and laboratories working on potential DUX4-targeting drugs. It also shows their progression towards drug approval.
The drug pipeline was created and maintained by the FSHD Society and is being used with permission.

Aims and Outline of this Thesis

This thesis aims to enhance clinical trial readiness by analyzing the feasibility of PROs and COMs, advance the development of losmapimod, and evaluate the trials from a patient's perspective.

Part I: Enhancing Clinical Trial Readiness

Chapter 2 details the establishment of the Dutch FSHD Registry and reports on the data captured using the registry. Participants received questionnaires on FSHD symptoms, pain, fatigue and quality of life at registration and every six months thereafter. The data were analyzed to gain more insight into the prevalence of FSHD symptoms and to determine the feasibility of questionnaires for clinical trials.

The utility of the FSHD Registry was clearly demonstrated during the COVID-19 pandemic as reported in **Chapter 3**. In these unprecedented times, we managed to perform a longitudinal survey study on the impact of the COVID-19 pandemic on FSHD symptoms, received care, stress symptoms and COVID-19 incidence.

Besides PROs, we also analyzed COMs to enhance trial readiness. **Chapter 4** describes a five-year follow-up of the FSHD-FOCUS natural history study, focusing on the COMs included in this study. Analyses on statistical significant changes as well as clinically relevant changes were performed to determine the feasibility of the COMs. Additional, power calculations were performed to challenge an often used eligibility criterion which only allows moderately affected patients in clinical trials.

Part II: Clinical Trial and Patient Experiences

Chapter 5 reports on the phase II losmapimod open-label trial, examining safety, pharmacokinetics, pharmacodynamics, target engagement, and efficacy on exploratory COMs in fourteen FSHD patients.

During the phase II losmapimod trial, participants had to undergo two muscle needle biopsies, one at baseline and one during treatment with losmapimod. It became clear that these biopsies had a high burden, but was never studies in FSHD patients. We therefore performed a survey study to determine the burden of muscle needle biopsies which were performed for research purposes **(Chapter 6)**. Additionally, two methods used in research (outpatient clinic biopsies and MRI-guided biopsies) were compared to each other.

After promising results of the phase II open-label trial (**Chapter 5**) and a separate randomized placebo-controlled phase II trial, a phase III randomized placebo-controlled trial (REACH) was initiated to test the efficacy of losmapimod. Most of the phase III trial participants reported an unexpectedly high psychological burden due to the placebo-controlled design. We therefore wanted to gain more insight in how participants experienced participating in a trial. **Chapter 7** describes a qualitative study using semi-structured in-depth interviews with participants from the phase II and III trial about trial participation. This study resulted in key points for patient and site education as well as recommendations for the sponsor.

Chapter 8 starts with a summary of this thesis, followed by a discussion of the results. The main purpose of this discussion is to provide an updated blueprint for upcoming FSHD trials based on the lessons learned in this thesis. Furthermore, it will provide future perspectives based on the current developments in FSHD trials.

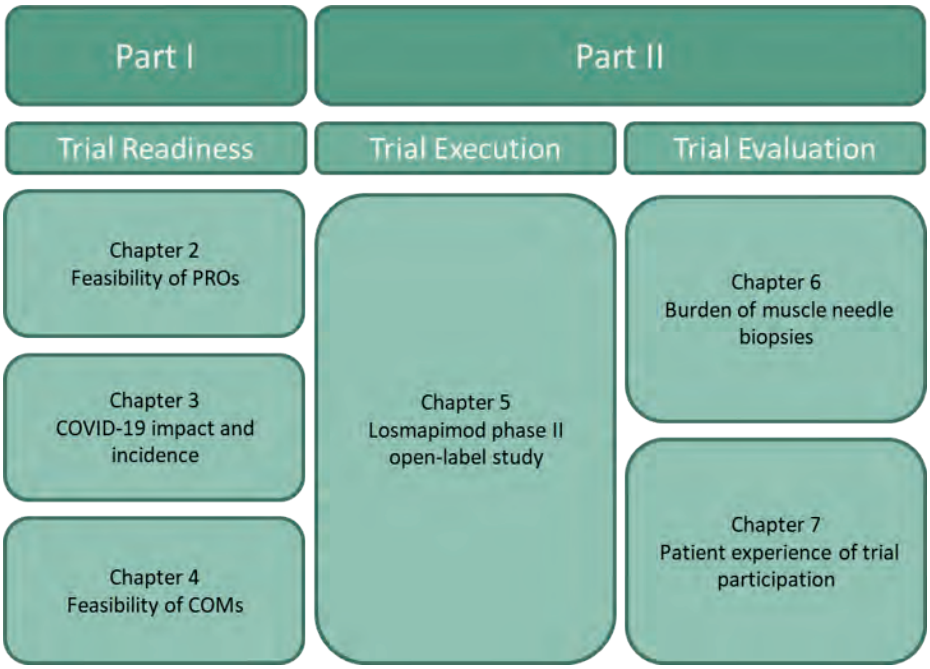

Figure 5. A graphical representation of the chapters in this thesis

PROs: Patient Reported Outcome. COM: Clinical Outcome Measure

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Part I:
Enhancing Clinical Trial Readiness

Chapter 2

The Dutch Registry for Facioscapulohumeral Muscular Dystrophy: Cohort Profile and Longitudinal Patient Reported Outcomes

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Abstract

Facioscapulohumeral dystrophy (FSHD) is the second most prevalent inherited muscular disorder and currently lacks a pharmaceutical treatment. The Dutch FSHD Registry was initiated in 2015 as a result of an international collaboration on trial readiness. This paper presents the cohort profile and six years of follow-up data of the registered FSHD patients. At the time of self-registration and every six months thereafter, participants were invited to complete a digital survey of patient and disease characteristics and the Dutch versions of the Checklist Individual Strength (CIS20R), the Individualised Neuromuscular Quality of Life Questionnaire (INQoL), the Beck Depression Index – Primary Care and the McGill Pain Questionnaire. From March 2015 to March 2021, 373 participants completed at least one survey. At baseline, fatigue and muscle weakness were the most frequently reported symptoms (median CIS20R sumscore 77 [IQR 60-92], median INQoL Fatigue score 58 [IQR 42-68] and median INQoL weakness score 58 [IQR 42-68]). Pain was experienced most often in the head and shoulder region (193, 52%). Nineteen of the 23 (sub)sections of questionnaires showed no significant changes over time. We conclude that the Dutch FSHD Registry was successfully set up, enabling collection of longitudinal data and facilitating recruitment in several studies.

Keywords

Facioscapulohumeral muscular dystrophy, Registry, Longitudinal prospective cohort, Patient reported outcome measure

Abbreviations

FSHD = Facioscapulohumeral Muscular Dystrophy
CIS20R = The Checklist Individual Strength
INQoL = Individualized Neuromuscular Quality of Life Questionnaire
BDI-PC = Beck Depression Inventory - Primary Care
MPQ-DLV = McGill Pain Questionnaire – Dutch Language Version
VAS = Visual Analogue Scale
NWC-T = Number of Words Chosen – Total
PRI-T = Pain Rating Index – Total
MCID = Minimal Clinically Important Difference

Introduction

Facioscapulohumeral dystrophy (FSHD) is a muscular disorder with a wide variability in clinical symptoms, disease progression and functional impairments. Usually, the first symptoms develop in the second decade of life. Approximately 10% of patients present with an infantile onset, where the disease manifests before age 10[1]. In general, patients experience weakness of facial, shoulder and upper extremity muscles and gradually weakness of the trunk and leg muscles will develop. In late adulthood, approximately 20% of the FSHD patients use a wheelchair in daily life (this is 40% in infantile-onset patients)[1,2]. Although FSHD is one of the most common inherited myopathies in western countries, it is still classified as a rare disease with a prevalence of <1/5,000 and an estimated incidence of 0.3/100,000 person-years[3-5].

Currently, no curative treatment for FSHD is available. Management of the disease consists of symptomatic therapy such as cognitive behavioural therapy, physical, occupational and speech therapy, aerobic training and adequate pain medication[6,7]. The increase in pathophysiological knowledge of the disease enables the development of novel therapies for FSHD. A surge of new potential medications has arrived of which the first one reached a phase III trial. It is expected that the number of clinical trials will increase quickly in the near future[8-10]. FSHD registries were set-up across various countries to support these upcoming clinical trials[11,12].

Fast and selective recruitment of patients with FSHD is crucial in order to run successful and well-powered trials in this small patient population. Registries are of great value in this process as they provide access to a large number of FSHD patients. Furthermore, the prospective, longitudinal data collected within these registries are valuable to gain insight in the natural history of FSHD, clinical subtypes and genotype-phenotype associations, and may be helpful in selecting outcome measures that are sensitive to change[13].

This study describes the cohort profile of the Dutch FSHD Registry participants registered between March 2015 and March 2021. Furthermore, longitudinal patient reported outcome measures on fatigue, quality of life, mental status and pain were analysed. Lastly, the studies that made use of the Dutch FSHD registry were reported.

Methods

Registration and recruitment

The Dutch FSHD Registry started in the spring of 2015 by launching the website www.FSHDregistratie.nl. Registration of patients has continued ever since. Patients with FSHD can register themselves or their child by following the guidelines on the website. All forms and questionnaires are in the Dutch language. Foreign/non-Dutch-speaking patients are encouraged to find a registry in their country of residence and/or in a language they master. Genetic confirmation is not obligatory.

Treating physicians and/or the genetic lab are requested to provide the genetic test result of registered patients if permission is provided. Gathering data on genetic information is an ongoing process. Physicians, researchers, nurses and other health care professionals involved, repeatedly encourage FSHD patients to take part in the FSHD Registry. In addition, starting from 2019 onwards, information about the FSHD Registry is provided as standard practice when patients receive genetical confirmation of the disease. Patient advocacy group representatives also play an important role by informing FSHD patients about the FSHD Registry and its significance.

Governance and data access

The FSHD Registry is a collaboration of four parties: The Dutch Association of Neuromuscular Diseases (a nationwide patients association), the Dutch FSHD Foundation (fundraising organisation), Leiden University Medical Center, and Radboud University Medical Center (Radboudumc). The latter two are academic referral centres for FSHD and form the FSHD Expertise Center in the Netherlands. The ownership of the registry is delegated by these parties to Radboudumc. Its daily management and maintenance is carried out by a registry curator (JCWD). A steering committee for the FSHD Registry was installed by the four collaborating parties and consists of delegates from the parties and a fifth independent rehabilitation physician. The committee decides on requests for data access and study recruitment. Requests can be made by filling in a form available on the website. Contact information and pseudonyms of registered patients are stored in a separate secured location accessible only by the registry manager and a backup manager. Research data are stored in Castor, a secured electronic data capture system operated by Radboudumc.

Ethical approval

The Registry, and the analysis of longitudinal patient reported outcome measures, involve medical research that do not fall within the scope of the Medical Research Involving Human Subjects Act, as declared by the local Medical Ethics Review Committee of the Radboudumc (amendment of file 2015-1812 on April 15th 2020). All participants of the FSHD registry provided their written informed consent before they entered the registry. The registry and its databases are in concordance with the General Data Protection Regulation and all other acting laws.

Study design

This study was a prospective cohort study. At the time of registration and every subsequent six months, participants received a digital survey invitation. Data collection ran from March 2015 to March 2021. Participants <16 years old could be registered, either by or with consent of their parents. However, the number of registered minors was limited, and they completed a different set of questionnaires. Therefore, these data were not included in this study. All registered Dutch FSHD patients aged ≥16 years old who completed at least one survey were included in this study.

Questionnaires

The surveys consisted of five Dutch questionnaires: a questionnaire on FSHD disease characteristics in accordance with the global FSHD registry framework, the Checklist Individual Strength (CIS20R), the Individualised Neuromuscular Quality of Life Questionnaire version 1 (INQoL), the Beck Depression Inventory for Primary Care (BDI-PC), and the McGill Pain Questionnaire – Dutch Language Version (MPQ-DLV)[14-18].

The global FSHD registry framework items included questions about demographics, diagnosis, muscle weakness and its time of onset, best motor function, presence of specific comorbidities like retinal vascular disease, hearing loss, retardation and epilepsy, use of (non-)invasive ventilation and FSHD family history.

The CIS20R measures four dimensions of fatigue and consists of 20 questions with a seven-point Likert scale answer option (1-7). The total CIS20R score ranges from 20-140 points with 20 meaning no symptoms and 140 meaning severe symptoms. The CIS20R can be divided into four subsections: 'Fatigue' containing eight items (score range 8-56), 'Concentration' with five items (score range 5-35), 'Motivation' with four items (score range 4-28) and 'Activity' with three items (score range 3-21).

The INQoL measures quality of life and consists of ten subsections with questions on a seven-point Likert scale (0-6 or 1-7). The answers of the subsections are combined and converted to a 0-100% score, with 0% meaning no symptoms and 100% severe symptoms. In total, the INQoL consists of twelve different subscores.

The BDI-PC measures the severity of depression symptoms, consisting of seven questions with four answer options ranging from zero to three points for a possible total of 21 points. A value of ≥4 on the BDI-PC has a sensitivity and specificity of 82% for identifying patients with a major depressive disorder[16].

The MPQ-DLV measures pain symptoms and is divided in three subsections. In the first part, participants are asked to indicate where they experience pain and characterize the pain in more detail. In the second part, participants are asked to enter their current, minimum and maximum pain on a visual analogue scale (VAS), which is converted to a 0-10 score. The third part consists of a list of words that describe pain in increasing severity divided in 20 categories. Participants need to indicate which words describe their pain experience best. The third part results in the number of words chosen (NWC-T) ranging from 0-20, and the severity of the pain expressed as the Pain Rating Index (PRI-T), ranging from 0-36. Generally, a high NWC-T or PRI-T means a high burden of pain.

Data availability and statistical analysis

Incomplete surveys were excluded from analysis. The first completed survey was considered the baseline survey. Baseline data were reported as the median [IQR] value because some questionnaires did not show normally distributed data. Normality of data was determined via visual evaluation of the data. Means (SD) were presented in the tables to make comparisons with other studies more convenient and underpin the mixed models. For the INQoL and MPQ-DLV subsections, the median [IQR] and mean (SD) were calculated using the scores of patients who experienced the concerned symptoms (i.e. subsections with a score of zero were not used for these calculations). The reported percentages of experienced pain and analgesic use were based on the total number of included participants.

Longitudinal changes were analysed using linear mixed effect models with compound symmetry matrices and the restricted maximum likelihood as estimation method. The sum scores were the dependent variables. Survey round was a repeated variable and fixed factor. A p-value <0.05 was considered statistically significant.

Current and upcoming trials usually select moderately affected patients (e.g. Ricci-score between 4-8 on a scale from 0-10), as these patients supposedly have the highest chance of rapid disease progression[8-10,19,20]. To simulate this while lacking actual clinical data, a sub-analysis was performed based on the responders' baseline mobility: ambulant, ambulant with assisting device(s) (e.g. brace, walker, or cane) and wheelchair dependent. For this subgroup analysis correction for multiple testing by the Bonferroni method was applied (statistical significance at $p<0.017$).

Data were collected in CastorEDC[21]. Analysis of the data was done in R (R Foundation for Statistical Computing, Vienna, Austria) and SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Graphs were created using GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, California USA). The data are not publicly available due to privacy or ethical restrictions, but can be requested using the registry's website.

Results

Demographics

From March 2015 until March 2021, a total of 373 participants joined the Dutch FSHD Registry and completed at least one survey. During the first two years the annual number of new registered patients was high: 198 patients in 2015, 75 patients in 2016 and from 2017 onwards an average of 25 (Figure 1). During the six years of follow-up, thirteen participants were reported to be deceased, nine left the registry, and eighteen reported they did not want to receive the questionnaires anymore but remained in the registry. The response rate of the survey was 97% at baseline and gradually diminished to 65% at survey round twelve, with a mean response rate of 80%.

Baseline survey data

At baseline, the median age was 51 [39-63] years and 212 participants (57%) were female (Table 1). A genetically confirmed diagnosis was available for 111 participants (30%). Regarding mobility, 224 participants (60%) were ambulant, 109 (29%) were ambulant with assisting device and 40 (11%) were non-ambulant. The country of residence was the Netherlands for 344 participants (92%), 21 (6%) participants were living in Belgium and the remaining seven (2%) in other countries.

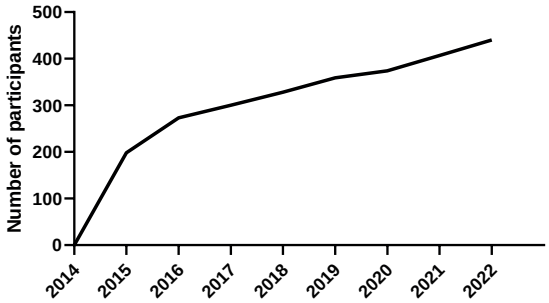

Figure 1. Number of participants in the registry

Table 1. Characteristics of the FSHD patients in the registry at baseline

	n (%*)
n	373 (100%)
Age (Median [IQR])	51 [39-62]
Female	212 (57%)
Age of onset (Median [IQR])	18 [10-30]
Family history with FSHD	91 (25%)
Country of residence	
The Netherlands	344 (92%)
Belgium	22 (6%)
Other	7 (2%)
Self-reported FSHD diagnosis	**363 (97%)[a]**
Type 1	113 (30%)
Type 2	16 (4%)
Unknown	233 (62%)
Mosaicism	1 (<1%)
Mobility	
Ambulant	224 (60%)
Ambulant with assisting device	109 (29%)
Non-ambulant	40 (11%)
Wheelchair / scooter use	
None	225 (60%)
Part-time use	106 (28%)
Full-time	42 (11%)

Table 1. Continued

	n (%*)
Weakness	
Face	227 (61%)
Neck	129 (35%)
Shoulder girdle	344 (92%)
Trunk	267 (72%)
Lower arm	191 (51%)
Hand	129 (35%)
Hip girdle	268 (72%)
Foot extensor	225 (60%)
Ventilation status	
No assistance	360 (96%)
Non-invasive part-time	11 (3%)
Invasive part-time	0 (0%)
Invasive fulltime	2 (1%)
Comorbidities	
Hearing loss	246 (66%)
Coats (retinal vascular disease)	0 (0%)

* unless stated otherwise; [a] remaining responders reported to be undiagnosed at baseline.

The baseline median total score of the CIS20R was 76 [59-92], mainly caused by a high score on the fatigue scale (38 [29-46]), indicating severe fatigue symptoms (Table 2). According to the INQoL scores, muscle weakness and fatigue were the most pronounced symptoms (median scores 63 [47-74] and 58 [42-68] respectively), yet social relations were barely affected (12 [0-33]). The BDI-PC median score was 1 [0-3] with 117 (23.6%) participants scoring ≥4. According to the MPQ-DLV, pain was most often experienced in the head-shoulder area (52% of the participants) (Figure 2). Furthermore, a large difference between the minimum and maximum pain was reported on the VAS (1.8 [1.0-3.0] vs. 7.3 [5.6-8.5]). Analgesics were used by 149 participants (40%), of which paracetamol (N=91, 24%) and nonsteroidal anti-inflammatory drugs (NSAIDs) (N=65, 17.5%) were the most common (Figure 3).

Table 2. Median scores of the CIS20R, INQoL, BDI-PC and MPQ-DLV at baseline

Questionnaire (sub)score	Baseline Median [IQR] score[c]	Baseline Mean (SD) score[d]	Possible scoring range[b]	Symptoms experienced by n (%)[a]
CIS20R				
Sumscore	76 [59-92]	76 (24)[N]	20-140	373 (100%)
Fatigue	38 [29-46]	37 (12)[S]	8-56	373 (100%)
Concentration	13 [8-20]	15 (8)[S]	5-35	373 (100%)
Motivation	13 [9-17]	14 (6)[S]	4-28	373 (100%)
Activity	10 [6-14.5]	11 (5)[N]	3-21	373 (100%)
INQoL				
Weakness	63 [47-74]	61 (19)[N]	0-100	351 (94%)
Muscle Locking	47 [32-63]	49 (20)[N]	0-100	138 (37%)
Pain	47 [37-63]	50 (20)[N]	0-100	262 (70%)
Fatigue	58 [42-68]	56 (19)[S]	0-100	314 (84%)
Activities	50 [30-64]	46 (23)[S]	0-100	373 (100%)
Independence	39 [19-56]	38 (26)[S]	0-100	373 (100%)
Social Relations	12 [0-33]	19 (21)[S]	0-100	316 (85%)
Emotions	25 [11-43]	29 (22)[S]	0-100	373 (100%)
Body Image	44 [19-64]	43 (27)[S]	0-100	373 (100%)
Quality of Life	42 [24-56]	40 (20)[N]	0-100	373 (100%)
Perceived Effect of Treatment	33 [17-44]	30 (25)[N]	0-100	190 (51%)
Expected Effect of Treatment	25 [8-42]	26 (25)[N]	0-100	190 (51%)
BDI-PC				
Sumscore	1 [0-3]	2 (3)[S]	0-21	373 (100%)
MPQ-DLV				
VAS current pain	4.0 [2.0-5.5]	4 (2)[N]	0-10	149 (40%)
VAS Minimal pain	1.8 [1.0-3.0]	2 (2)[S]	0-10	149 (40%)
VAS Maximal pain	7.3 [5.6-8.5]	7 (2)[S]	0-10	149 (40%)
NWC-T	12 [9-15]	12 (4)[N]	0-20	259 (69%)
PRI-T	62 [43-83]	63 (26)[N]	0-36	259 (69%)

[a] Number of participants (percentage of total responders) who experienced the symptoms of the concerned subsections of the questionnaires. [b] Possible scoring range for each subscore, a low score correlating to mild symptoms and a high score indicating severe symptoms in all scores. [c] The median and interquartile range [IQR] and [d]mean and standard deviation (SD) were calculated based on the scores of the number of participants in ([a]). [N] Data were normally distributed. [S] Data were skewed.
CIS20R = The Checklist Individual Strength, INQoL = Individualized Neuromuscular Quality of Life Questionnaire, BDI-PC = Beck Depression Inventory - Primary Care, MPQ-DLV = McGill Pain Questionnaire – Dutch Language Version, VAS = Visual Analogue Scale, NWC-T = Number of Words Chosen – Total, PRI-T = Pain Rating Index – Total.

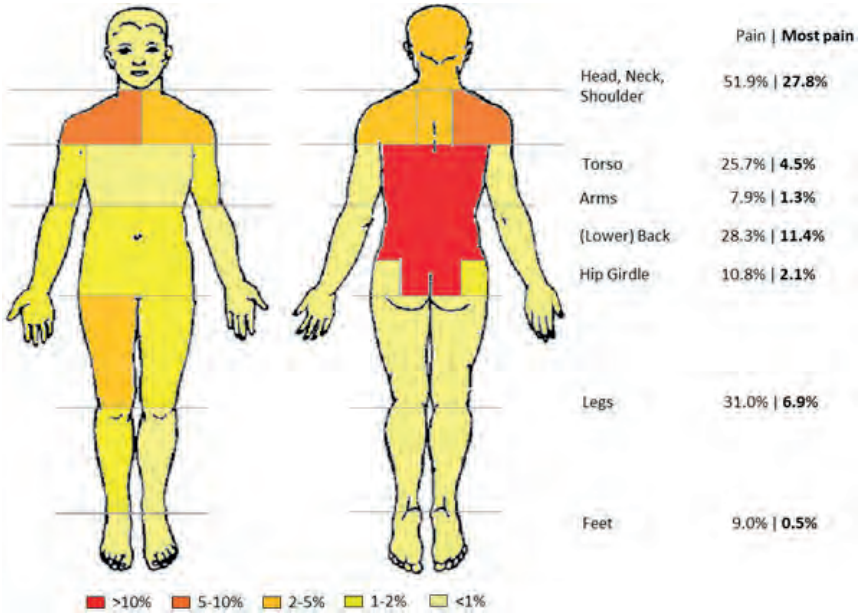

Figure 2. Pain experienced by FSHD participants at baseline

The body areas are colored based on where participants experienced the most pain. Smaller body areas were combined into larger body areas, corresponding participant numbers are given in the right column. The left column shows the percentage of the total number of participants (N=373) that reported to experience pain in that body area. The right column shows where the most pain was experienced as a percentage of the total number of participants.

Follow-up survey data

Including all participants, nineteen out of the 23 (sub)scores showed no significant changes over time as presented in Figure 4 and Appendix A (CIS20R Sumsore, Fatigue, Concentration and Motivation; INQoL Weakness, Muscle Locking, Pain, Fatigue, Activities, Emotions, Quality of Life, Perceived Effect of Treatment and Expected Effect of Treatment; BDI-PC; MPQ-DLV VAS Current, VAS Minimum, VAS Maximum, NWC-T and PRI-T).

The mean CIS Activity score at baseline was 10.6 (SD=5.0, N=373) slowly increasing to 11.6 (SD=3.9, N=46) at survey round 12, indicating slightly more difficulty doing activities. The mean INQoL Independence score increased from 38.2 (SD=25.9, N=373) to 47.3 (SD=24.5, N=46), reflecting loss of independency over time. Unexpectedly, the mean INQoL Social Relations improved from 18.65 (SD=20.7, N=373) to 15.22 (SD=15.9, N=46). Lastly, the mean INQoL Body Image remained mostly stable from the mean baseline score of 43.2 (SD=26.8, N=373), but increased to 45.0 at survey round 11 (SD=23.5, N=121) and 12 (SD=22.6, N=46).

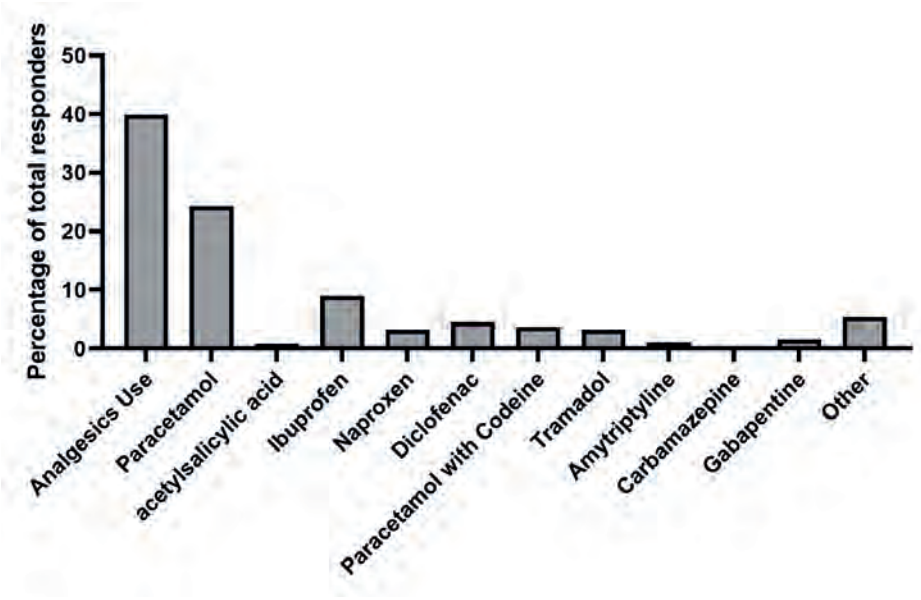

Figure 3. Analgesic usage in the Dutch FSHD registry participants at baseline

Percentages are calculated based on the number of participants reporting usage of analgesics and the total number of participants (n=373). Paracetamol is also known as acetaminophen.

Mobility sub-analysis

At baseline, the mobility subgroup-analysis showed between-group differences in scores on the CIS Fatigue (p=0.044), CIS Activity (p<0.001), INQoL Weakness, Muscle Locking, Activity, Independence, Social Relations, Body Image, and QoL (p<0.001 for all INQoL sub scores) (Figure 4, Supplementary Table 1). The wheelchair-dependent group showed the highest variability, most likely caused by a small number of participants (N=40 at visit 1, N=5 at visit 12) (Supplementary Table 2).

Within the ambulant participants group, 21 out of 23 (sub)scores showed no significant changes over time. The INQoL Social Relations improved from 15.5 (SD=18.4, N=173) at baseline to 11.6 (SD=14.6, N=19) at round 12. However, the INQoL Quality of Life worsened from 35.3 (SD=19.9, N=173) to 39.0 (SD=19.6, N=58) at round 11. It seemed to improve again at round 12 to 36.5 (SD=19.1, N=19), but this might have been caused by the relatively big drop in the number of participants.

In the subgroup of participants ambulant with assisting device, 22 out of 23 (sub) sections showed no changes over time. Only the INQoL Body Image improved from 54.4 (SD=23.6, N=92) to 44.2 (SD=23.8, N=22).

In the wheelchair-dependent group, none of the (sub)scores showed a significant change over time, possibly caused by the small number of participants.

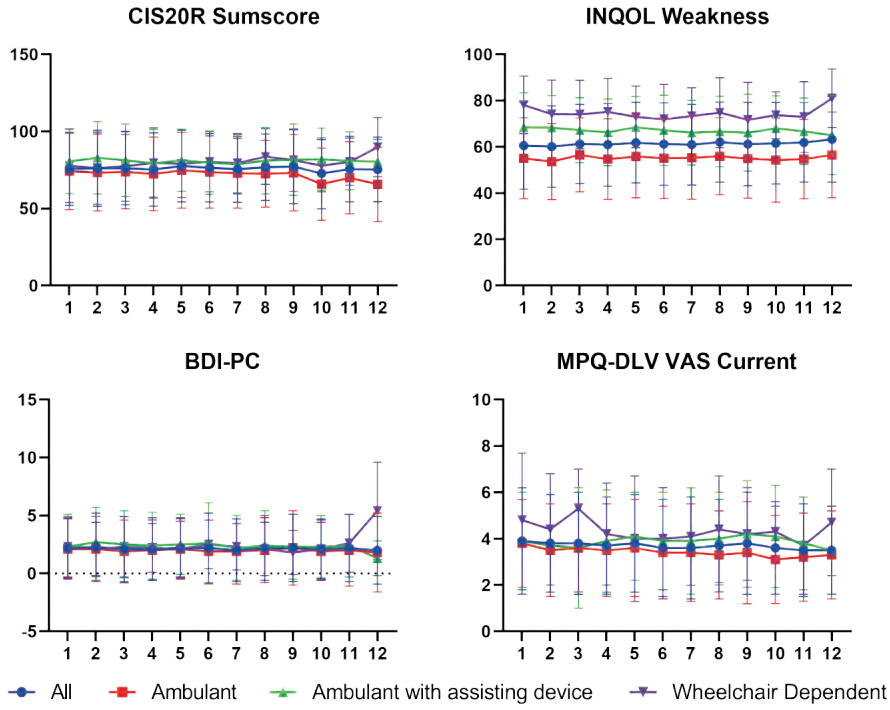

Figure 4. Change in mean (SD) over time

The graphs show the mean (SD) of the (sub)questionnaires for all responders and for the subgroups 'ambulant', 'ambulant with assisting device' and 'wheelchair dependent'. On the y-axis the score of each (sub)questionnaire is given, on the x-axis the survey rounds.
CIS20R= Checklist Individual Strength, INQoL = Individualized Neuromuscular Quality of Life, QoL = Quality of Life, BDI-PC = Beck Depression Inventory – primary care, MPQ-DLV= McGill Pain Questionnaire – Dutch Language Version, VAS = Visual Analogue Scale

Studies facilitated

From 2015 until 2023, the registry received fourteen requests so far for either data (3), facilitating recruitment of participants (8) or a combination of both (3). These requests were all reviewed and approved by the steering board. Studies included patient-reported FSHD symptoms and their impact in daily life, a study on the socioeconomic burden of FSHD, and clinical drug trials and a questionnaire study regarding FSHD symptoms and received care during the COVID-19 pandemic was conducted using the registry (publication pending)[10,20,22,23]. In addition, the registry was used to inform all participants about early access to the first COVID-19-vaccination round in the Netherlands in 2021. A (Dutch) layman summary of all approved requests is posted on the website, accompanied by a results summary and link to the paper as soon as this becomes available (www.fshdregistratie.nl/gehonoreerde-verzoeken/).

Discussion

In 2015, the Dutch FSHD Registry was set up according to the recommendations discussed in the trial readiness workshop (2015) and workshop of the European Neuromuscular Centre (ENMC) on the global FSHD registry framework (2016)[11,12]. The registry has successfully been used to gather cross-sectional and longitudinal data from self-reported questionnaires. Overall, the results showed barely any longitudinal changes on (self-reported) fatigue, QoL, mental status and pain. Furthermore, the registry facilitated targeted patient recruitment for a number of studies, clinical trials and the collection of longitudinal patient-reported outcome measures.

So far, 452 FSHD patients were registered within the Dutch FSHD Registry. As the prevalence of FSHD in the Netherlands is estimated at 2,000 individuals, this represents approximately 23% of the Dutch FSHD population[3]. This finding is similar to the French registry (21%), but lower than in the United Kingdom registry (31%)[24,25]. The Dutch prevalence estimate was based on a capture-recapture calculation, taking into account unobserved persons. Other prevalence estimates were based on observed persons only, resulting in lower prevalence estimates and thus higher registry coverage rates. Therefore, the coverage of the Dutch FSHD registry is probably higher compared to other FSHD registries. Nevertheless, efforts to encourage patients to participate in the registry are ongoing to further improve coverage. Also, we expect a rise of new participants when additional clinical trials will start.

In line with the high level of motivation of the study group, response rates on the half-yearly questionnaires were initially high. Although the response rate did decrease over time, it was still considered relatively high compared to response rates of other surveys[26]. The decrease in response rate was possibly caused by the relatively large time investment for completing all the questionnaires and/or a lack of information about the results. Reducing the number of questionnaires based on usefulness as well as more frequent reporting of the results may be necessary to maintain a high response rate.

The baseline scores on the questionnaires were similar to the scores found in other studies. The high CIS20R scores indicating severe fatigue were also observed in a different Dutch study of 135 FSHD patients, reiterating the high prevalence of fatigue symptoms. We do expect these two cohorts to overlap partly, which may account for the similar outcomes[27]. The different subscores of the INQoL corresponded well with the findings reported by the UK FSHD Registry[28]. Interestingly, the Dutch registry cohort scored lower on the Independent, Emotions, Body Image and QoL subcategories, indicating a lower burden, compared with the UK registry population. This may be caused by the slight difference in disease severity between the two cohorts. The UK cohort seemed to have a higher disease severity with 48% of the cohort being ambulant compared with 60% in the Dutch cohort. Additionally, country-specific cultural and healthcare differences may play a part. For example, a large European survey on chronic pain reported a higher use of analgesics (NSAIDS and opioids as well) in the UK compared with the Netherlands[29]. This corresponds well with the much larger proportion (92%) of UK FSHD patients using analgesics, most commonly NSAIDs or opioids (both roughly 30%), compared with 40% of the Dutch patients using analgesics consisting mostly of paracetamol (24%) or NSAIDs (17.5%)[28].

Lastly, the mean BDI score of the FSHD population corresponds well with the mean score found in screening 120 random outpatient clinic patients (2.15 vs 2.18)[30]. Although we cannot say for certain that the 117 (23.6%) FSHD patients who scored ≥4 on the BDI-PC were all affected by a major depressive disorder, this percentage also corresponds well with the outpatient clinic study (24% were diagnosed with a major depressive disorder).

The majority of the questionnaires in this study showed no (sub)score changes in persons with FSHD over the course of six years. Based on the currently accepted view that the strength and functionality of moderately affected patients decline relatively fast, we expected the 'ambulant with assisting device' group to show the

largest difference over time. However, even in this subgroup almost all (sub)scores remained stable over the six years follow-up. Of the (sub)scores that did show a small change over time, it is highly unlikely that a clinically important difference was reached within this timeframe. Unfortunately, no data are available on what would be the minimal clinically important difference (MCID) of the questionnaires for FSHD. Barely any data were available on the MCIDs of these questionnaires in other diseases and it is questionable if MCIDs correspond well across diseases. The general MCID of the CIS Fatigue is 10 points, which was not reached in our cohort[31]. The MCID of the pain score (0-10) in chronic pain patients was 0.9-2.7 depending on the calculation method used and could be compared to the VAS scores in the MPQ-DLV questionnaire[32]. However, both scores were stable and no MCID was not reached in our cohort. It is clear that the knowledge base regarding the MCIDs of these questionnaires is small and mostly unavailable for FSHD[33]. A currently ongoing natural history study within this research group will provide more knowledge about the clinical progression of FSHD symptoms over a longer period. Combining the clinical data with the FSHD-registry data may enable us to determine clinically important differences of these questionnaires and provide knowledge about MCIDs in FSHD and the responsiveness of specific PROMS. The lack of change in scores on the questionnaires could indicate that: 1) FSHD patients remain stable for a long time, 2) the questionnaires are not sensitive enough to detect the probably small occurring changes, and/or 3) fatigue, QoL, depression and pain are influenced by a wide range of factors and do not directly relate to disease progression. As this study currently does not include sufficient clinical data regarding the disease severity and its changes, we cannot rule out nor confirm any of these hypotheses. However, a longitudinal study in myotonic dystrophy type 1 patients did not find longitudinal changes in the INQoL subscores (or even improvements on some subscores) either, despite worsening of the clinical symptoms in the patients[34]. The authors suggested that quality of life was not directly related to disease progression and could increase by changing external factors (e.g. using assisting devices or a wheelchair when necessary) or internal factors (adaptation of the patient's perspective on what relates to quality of life). Their conclusions point towards the second and third hypothesis. In addition, previous studies pointed to at least mild progressiveness of symptoms within a year, and the Italian FSHD Registry found clinical worsening of disease after five years of follow-up, making it unlikely that the Dutch cohort remained stable over (a maximum of) six year follow-up[35,36].

Although we cannot completely rule out the usefulness of the questionnaires in clinical trials because the subgroup analysis displayed the ability to discriminate between specific mobility subgroups, the data collected from this cohort seem to

suggest a lack of sensitivity to change for all the questionnaires. We are therefore hesitant to recommend the CIS20R, INQoL, BDI-PC and MPQ-DLV to measure drug efficacy in a clinical trial.

Currently, access to longitudinal clinical outcome assessments has been unavailable. Interpretation of the results of the questionnaires will improve with access to longitudinal clinical data and gives the opportunity to calculate the MCID. Furthermore, it will improve the enrolment process by increasing the possibilities for pre-screening (e.g. based on clinical severity scores or muscle strength scores). Lastly, this will enable to start a range of new studies for example about identifying subtypes of FSHD, establishing genotype-phenotype correlations or investigating the relationship between muscle weakness, psychosocial factors, daily functioning and quality of life. We therefore propose that FSHD registries will be expanded to include clinical outcome assessments, either by performing separate study visits, combining registry data with already ongoing natural history studies or by linking the registry to parts of the patient files.

As almost all of the (sub)questionnaires remained stable over the course of six years, we recommend reducing the survey frequency. This will lower the burden on the registered patients and is expected to improve the response rate. Furthermore, recently developed questionnaires such as the FSHD-HI and FSHD-RODS may be more sensitive and specific and be useful to include in the registries as well[37,38]. Together with the Dutch patient advocacy group, we started the process to carefully select which improvements need to be made, what clinical data need to be captured, which questionnaires are to be used and in which frequency, while minimizing the burden on both the participants and clinicians. In this process, we will make sure that the Dutch registry remains harmonised with other national FSHD registries. Additionally, an effort should be made to combine the data of all the national registries as was originally the aim.

The strengths of this study are the size of the FSHD cohort and the long follow-up period with frequent survey rounds and high response rate, resulting in reliable cross-sectional and longitudinal analysis.

There are several limitations. First, selection bias may be introduced by self-registration, and the registry may therefore not be representative of the entire Dutch FSHD population. However, the demographics of the Dutch Registry population were similar to other studies and FSHD registries. Another limitation of the Registry is the lack of clinical data collection. As mentioned before, clinical

data will be useful for interpreting the results of questionnaires, enabling large genotype-phenotype studies, and a more precise preselection of patients for clinical trials. Finally, the process of including the genetical confirmation of the disease was not fully completed at the time of writing. It will become available in the near future to be used for upcoming studies and enable genotype-patient reported phenotype coupling.

Conclusion

The Dutch FSHD Registry has been successfully implemented with a still increasing number of participants. It has been used for fast and selective patient recruitment for several studies and for contacting patients on short notice if important information became available. It will prove to be invaluable for recruitment in future trials. Although the CIS20R, INQoL, BDI-PC and MPQ-DLV questionnaires do discriminate between specific subgroups of this FSHD cohort, these scores detected minimal or no longitudinal changes in these FSHD patients over a six-year period. These questionnaires may therefore not be useful to monitor disease progression in prognostic studies or clinical trials in patients with FSHD. The inclusion of clinical outcome assessments in FSHD registries should be considered.

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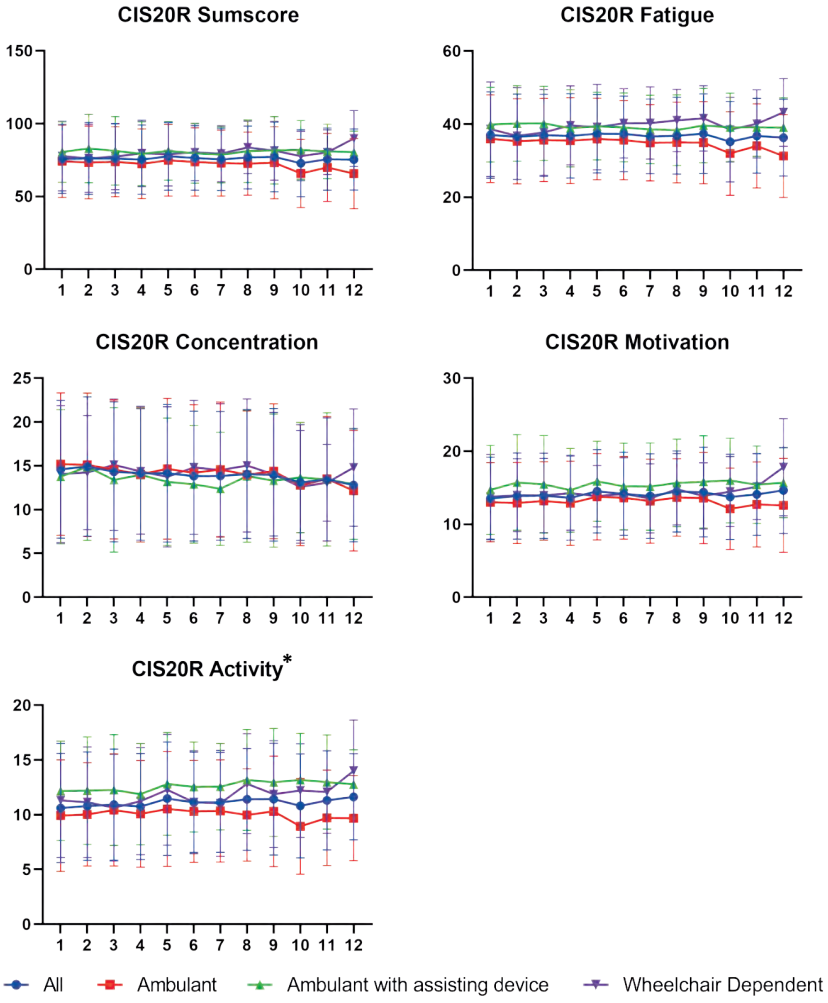
Supplementary Data

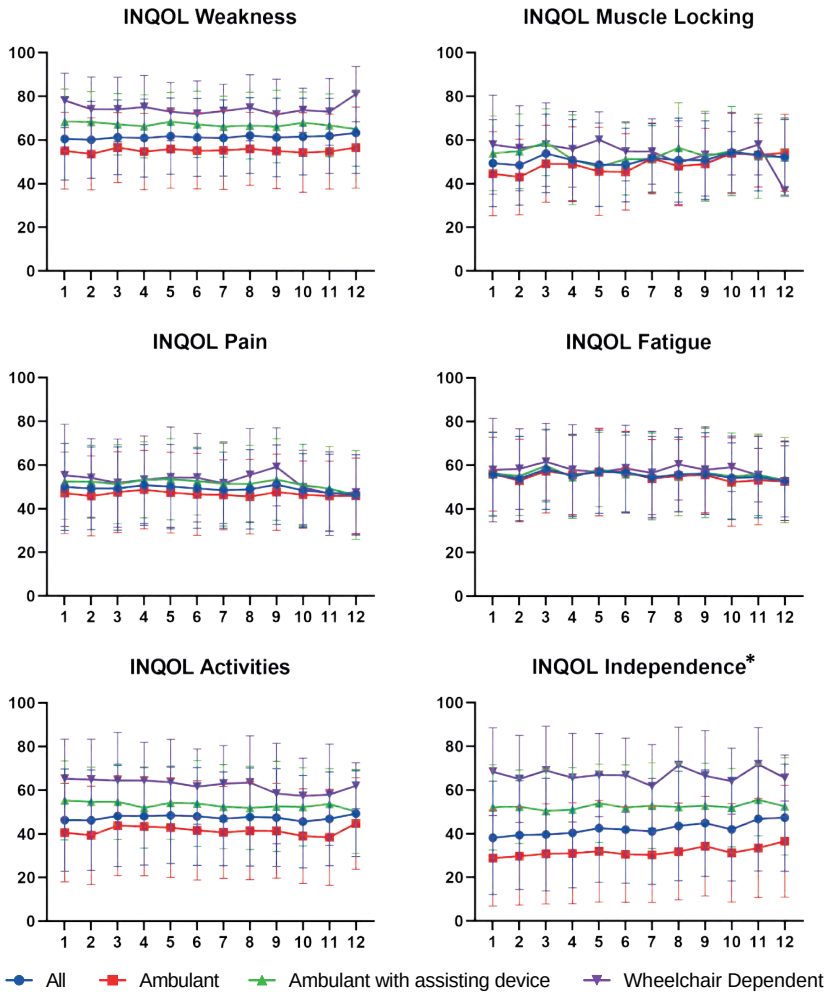


Supplementary Figure 1. Change over time of the CIS20R (sub)questionnaires

The graphs show the mean (SD) of the CIS20R (sub)questionnaires for all responders and for the subgroups 'ambulant', 'ambulant with assisting device' and 'wheelchair dependent'. On the y-axis the score of each (sub)questionnaire is given, on the x-axis the survey rounds.

*A statistically significant change over time was found on the Activity subscore when including all responders (p=0.031).

CIS20R = Checklist Individual Strength

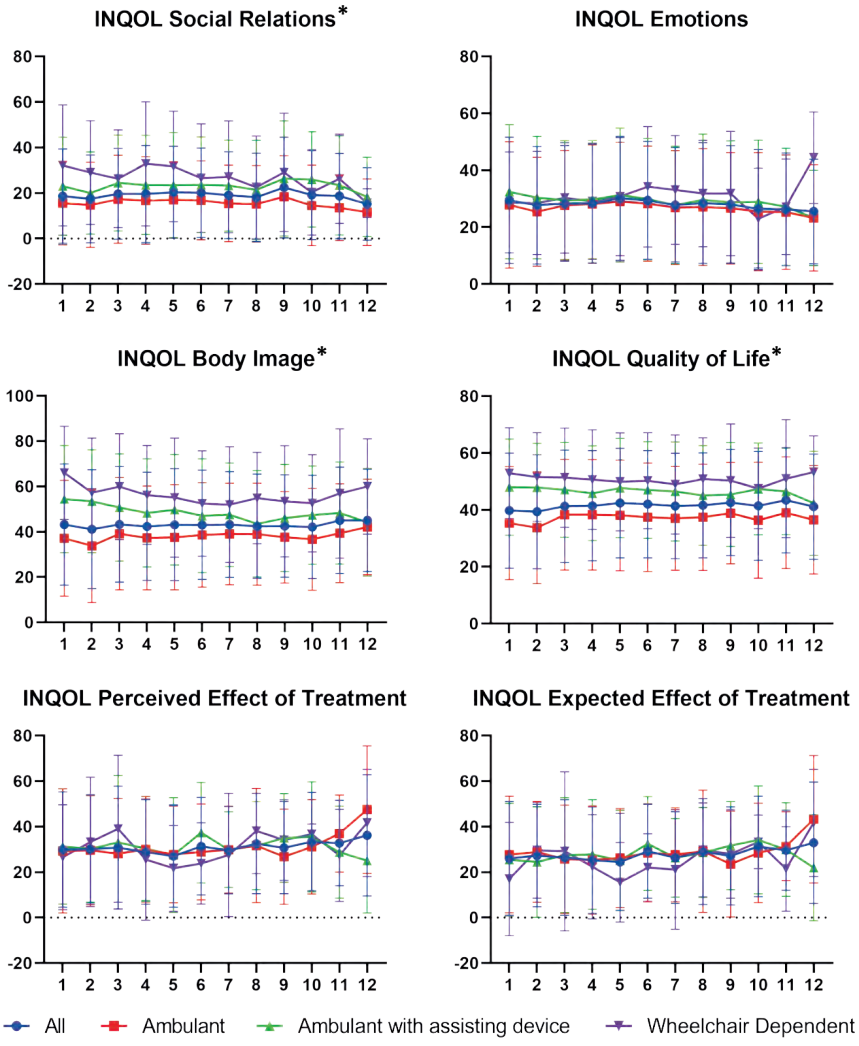


Supplementary Figure 2. Change over time of the INQoL subquestionnaires

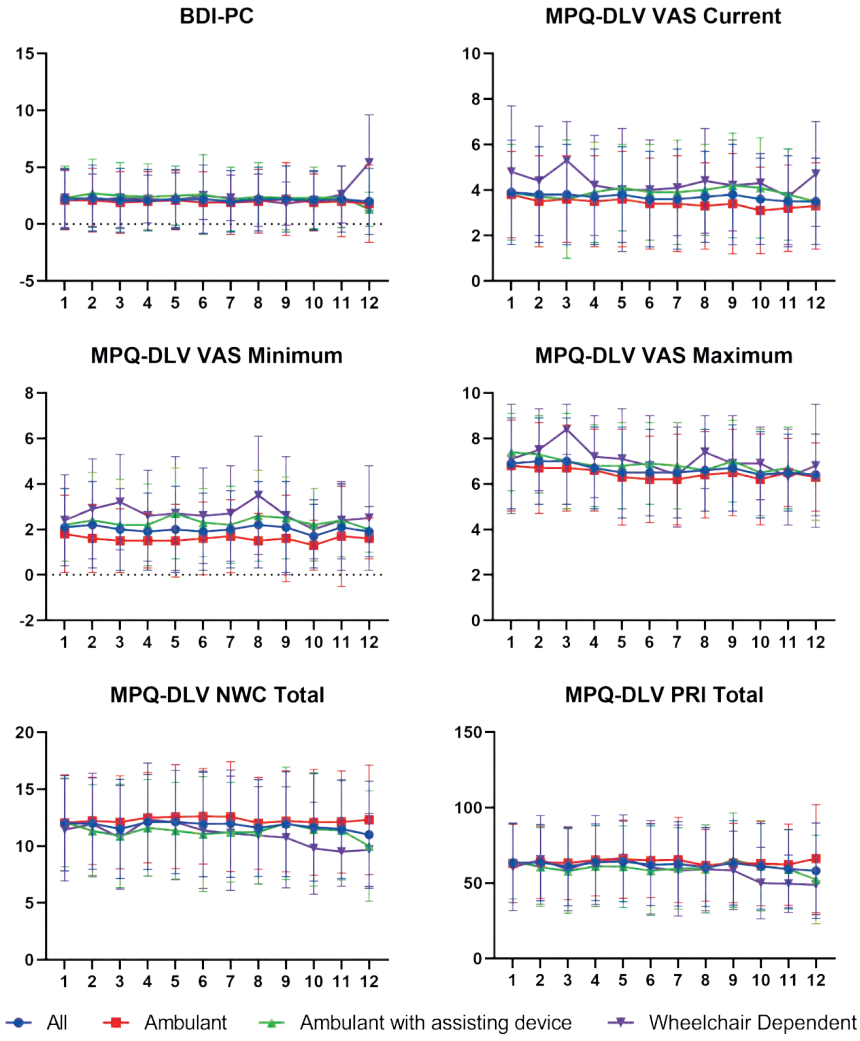
The graphs show the mean (SD) of the INQoL subquestionnaires for all responders and for the subgroups 'ambulant', 'ambulant with assisting device' and 'wheelchair dependent'. On the y-axis the score of each (sub)questionnaire is given, on the x-axis the survey rounds.

*A statistically significant change over time was found on the Independence, Social Relations and Quality of Life subscore for all responders (p=0.007, 0.006, 0.024 respectively). For ambulant responder, Social Relations (p=0.010) and Quality of Life (0.002) changed over time. For ambulant with assisting device responders, Body Image (p=0.000) changed significantly over time.

INQoL= Individualised Neuromuscular Quality of Life Questionnaire



Supplementary Figure 2. Continued



Supplementary Figure 3. Change over time of the BDI-PC and MPQ-DLV (sub)questionnaires

The graphs show the mean (SD) of the INQoL subquestionnaires for all responders and for the subgroups 'ambulant', 'ambulant with assisting device' and 'wheelchair dependent'. On the y-axis the score of each (sub)questionnaire is given, on the x-axis the survey rounds.

BDI-PC = Beck Depression Inventory - Primary Care, MPQ-DLV = McGill Pain Questionnaire – Dutch Language Version, VAS = Visual Analogue Scale, NWC = Number of Words Chosen, PRI = Pain Rating Index.

Supplementary Table 1. P-values of the effect of visit number on the means of the (sub)questionnaires.

Questionnaire	All	Ambulant	Ambulant with assisting device	Wheelchair dependent
CIS20R				
Sumscore	0.866	291	0.698	0.358
Fatigue	0.185	0.04 [b]	0.434	0.195
Concentration	0.317	0.515	0.089	0.615
Motivation	0.197	0.643	0.3	0.775
Activity	0.031 [a]	0.787	0.361	0.019 [b]
INQoL				
Weakness	0.706	0.695	0.233	0.112
Muscle Locking	0.338	0.645	0.148	0.945
Pain	0.78	0.978	0.579	0.541
Fatigue	0.287	0.308	0.492	0.948
Activities	0.14	0.114	0.019 [b]	0.253
Independence	0.007 [a]	0.220	0.496	0.580
Social Relations	0.006 [a]	0.010 [c]	0.452	0.362
Emotions	0.219	0.499	0.357	0.046 [b]
Body Image	0.024 [a]	0.609	0.000 [c]	0.155
Quality of Life	0.492	0.002 [c]	0.466	0.998
Perceived Effect of Treatment	0.62	0.544	0.086	0.122
Expected Effect of Treatment	0.8	0.749	0.316	0.282
BDI-PC	0.799	0.629	0.643	0.399
MPQ-DLV				
VAS current	0.93	0.924	0.775	0.96
VAS Minimum	0.115	0.896	0.219	0.303
VAS Maximum	0.08	0.137	0.461	0.589
NWC-T	0.663	0.931	0.356	0.719
PRI-T	0.644	0.937	0.47	0.81

The p-values of the mixed models are given in the 2nd column. No correction for multiple testing was applied. Number of participants (percentage of total participants) are given in the third column.

a. Statistically significant ($p<0.05$), no correction for multiple testing was applied to the analyses including all responders.

b. Statistically significant ($p<0.05$) before correcting for multiple testing.

c. Statistically significant ($P<0.017$) before and after correcting for multiple testing.

CIS20R = The Checklist Individual Strength, INQoL = Individualized Neuromuscular Quality of Life Questionnaire, BDI-PC = Beck Depression Inventory - Primary Care, MPQ-DLV = McGill Pain Questionnaire – Dutch Language Version, VAS = Visual Analogue Scale, NWC-T = Number of Words Chosen – Total, PRI-T = Pain Rating Index – Total.

Supplementary Table 2. Number of responders per survey round

Visit	All	Ambulant	Ambulant with assisting device	Wheelchair dependent
1	373	173	92	40
2	308	147	81	35
3	284	143	76	33
4	272	137	78	33
5	258	137	80	30
6	236	130	76	29
7	220	125	71	24
8	198	107	67	24
9	188	99	65	24
10	152	82	55	15
11	121	58	49	14
12	46	19	22	5

Chapter 3

Living with Facioscapulohumeral Muscular Dystrophy During the First Two Covid-19 Outbreaks: A Repeated Patient Survey in the Netherlands

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[1]Authors contributed equally; [2] Authors contributed equally;

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Abstract

Patients with facioscapulohumeral dystrophy (FSHD) suffer from slowly progressive muscle weakness. Approximately 20% of FSHD patients end up wheelchair-dependent. FSHD patients benefit from physical activity to maintain their muscle strength as much as possible. The impact of the COVID-19 pandemic on the health of FSHD patients was unknown.

This study assessed changes in daily care received, perceived psychosocial stress, and worsening of FSHD complaints in 2020. Furthermore, we compared COVID-19 infection incidence and severity of symptoms between FSHD patients and non-FSHD housemates.

Three online survey rounds were sent out to all adult participants of the Dutch FSHD registry regarding daily care received, perceived psychosocial stress, COVID-19 infection rate and COVID-19 symptoms severity. They also included COVID-19-related questions regarding the participants' housemates, which served as control group.

Participation rate was 210 (61%), 186 (54%), and 205 (59%) for survey 1, 2 and 3 respectively. Care reduction was reported by 42.7%, 40%, and 28.8% of the participants in the respective surveys. Perceived psychosocial stress increased in 44%, 30%, and 40% of the participants. Compared to the 197 non-FSHD housemates, the 213 FSHD patients reported more possibly COVID-19 related symptoms (27% vs. 39%, p=0.017) of mostly minimal severity (63%). No difference in (possible) COVID-19 infection incidence rates was found (2.0% vs. 2.8%, p=0.527).

The COVID-19 pandemic negatively impacted care received and increased perceived psychosocial stress in FSHD patients. However, COVID-19 infection incidence in FSHD patients was similar to their non-FSHD housemates.

Keywords

Neuromuscular diseases, COVID-19, Epidemiology, Surveys and Questionnaires, Incidence, Registries

Abbrevations

FSHD – facioscapulohumeral muscular dystrophy
COVID-19 – Coronavirus Disease 2019
NMD – Neuromuscular Disorder

QoL – Quality of Life
ENMC – European Neuromuscular Centre
MRS – Modified Ranking Scale
PSS – Perceived Stress Scale

Introduction

The coronavirus disease 2019 (COVID-19) pandemic has affected the health status, daily activities, social participation, care availability and quality of life of individuals all over the world. In the Netherlands, 6.5 million people tested positive in a registered PCR test, and almost 40,000 people died over the course of two years[1,2]. To slow down the rapid spread of the disease, rigorous restrictions were implemented in March 2020 for a prolonged period of time such as social distancing, quarantine, and lockdowns[3].

These restrictions resulted in a decrease of physical activity, available healthcare, and an increase in loneliness, anxiety, and depression[4,5]. For patients with facioscapulohumeral muscular dystrophy (FSHD), a slowly progressive muscle disease, physical activity is crucial to maintain muscular strength, flexibility in joints, and physical endurance to reduce progression of muscle weakness[6,7]. At the time the study was initiated in March 2020, it was unknown what the impact of COVID-19 and the restrictions on FSHD patients would be.

In Italy, research on various Neuromuscular Disorders (NMDs) has shown a subjective worsening of the NMD symptoms and a significant worsening of quality of life (QoL) during the pandemic[8,9]. It is expected that the worsening of disease aspects and QoL will also have occurred in FSHD patients. However, the infection rate and course might differ in FSHD patients. Previous studies hypothesized that the inflammation observed in biopsies and imaging modalities could point to possible alterations in the immune responses[10,11]. On the other hand, a minority of patients does experience respiratory weakness or weakness in coughing, increasing the susceptibility for infections[12,13]. It is unknown whether these changes affect the response to the SARS-Cov-2 virus.

The goal of this study was two-fold. First, we aimed to assess and describe the physical and mental health of the FSHD patients during the pandemic. Second, we aimed to gain more insight in the COVID-19 incidence rate and severity of symptoms compared to a non-FSHD population.

Methods

Study design

This was an observational questionnaire study, performed in an already existing cohort (i.e. the Dutch FSHD registry cohort). A survey was created to inquire about the impact of the COVID-19 pandemic on care received, perceived psychosocial stress, FSHD complaints, the number of COVID-19 infections and the severity of corresponding symptoms (Appendix 1, online available). The survey was electronically sent using CastorEDC to FSHD patients in three rounds in 2020: survey 1 (S1) on May 22nd 2020, survey 2 (S2) on August 26th 2020 and survey 3 (S3) on December 19th 2020[14].

Study population: the dutch registry

The Dutch FSHD registry was set up in 2015 to enable recruitment of FSHD patients for research and to collect patient-reported data about the natural course of the disease, including the core dataset decided upon during the 225th European Neuromuscular Centre (ENMC) workshop[15-17]. The registry was originally intended for Dutch-speaking participants only. Other interested people were encouraged to participate in the national registry in their country. Since 2020, people who still wished to enter the Dutch registry despite geographical and language barriers were accepted in the Dutch registry.

All registered FSHD patients aged 16 years and older, the age of consent in the Netherlands regarding medical decisions, were invited for the surveys. The control group consisted of the housemates of the participants who were ≥16 years old and did not have FSHD. This enabled comparison of COVID-19 infection incidence rate and severity of possible COVID-19 related symptoms. Housemates were defined as: spouses, children, parents, family or other. Housemates with FSHD were excluded from the analysis to prevent any accidental duplications in FSHD patients.

The data concerning the housemates was reported by the FSHD patients instead of the housemates themselves, because , no contact details of housemates were available in the registry. Furthermore, it was a relatively quick process to submit an amendment on the already existing approval of the FSHD-registry. Sending the surveys directly to housemates or other control groups would have required a completely new submission, which would have delayed the study. As time was of the essence during the pandemic, the method for gathering indirect data on housemates was chosen.

Survey

Demographic data regarding age and sex were retrieved from the Dutch FSHD registry. Furthermore, the survey contained a question about risk factors for a more severe COVID-19 disease course known at that time: age >70 years old, respiratory problems, chronic heart disease, severely overweight, and immunodeficiency.

The survey consisted of three parts: (1) Impact of the pandemic on FSHD complaints and care (2) perceived psychosocial stress, (3) COVID-19 infection rate and severity of possible symptoms experienced by the FSHD patients and their housemates.

Specifically, part one consisted of questions concerning the participants' living arrangement, care received pre-COVID-19, change in received care during the pandemic compared to pre-pandemic care received (yes/no answer with option to elaborate on what changed and the consequences of the changes), and the Modified Ranking Scale (MRS)[18]. The MRS asks about the disease severity as experienced by the participants with 0 – 'no symptoms' and 5 – 'severely handicapped, constant need for care'. Participants were asked to report the MRS pre-pandemic and at the time of survey completion.

The second part consisted of questions about the perceived psychosocial stress during the pandemic compared to before (0 'a lot less stress' - 5 'a lot more stress'). It included the Perceived Stress Scale (PSS) ranging from 0 'no stress' to 40 –severe stress', which evaluates how unpredictable, uncontrollable and overloading someone experienced the previous month, and their perceived ability to cope[19]. Furthermore, a set of possible COVID-related stressors used in an ongoing global study were tested on percentage (I do / do not experience this stressor) and their associated burden if experienced (0 'no burden' – 5 'high burden'). Lastly, participants were asked to report on any positive effects of the pandemic (yes/no answer with option to elaborate on what positive effect if present)[19-21].

Part three inquired whether participants and housemates experienced COVID-19 related symptoms suggestive of an infection and the severity of these symptoms, as well as if they were tested for COVID-19 and the result of the test.

COVID-19 timeline and survey modifications

Each country reacted differently to the COVID-19 pandemic with restrictions and opportunities changing over time. A timeline with the number of COVID-19 infections and the most important events in the Netherland in 2020 is shown in Figure 1. During the first months of the pandemic, testing facilities were only

available in case hospitalization was needed and primary healthcare availability was limited due to lockdown restrictions. This period coincided with survey 1. From June 2020 on, access to both testing facilities and primary healthcare became available again across the country. Furthermore, barely any restrictions regarding the pandemic were present when survey 2 was sent. At the time of the last survey, new restrictions in the form of a soft lockdown were present and (self)testing on COVID-19 was widespread available. Because of these changes, slight modifications to questions concerning COVID-19 incidence and testing were made in survey 2 and 3 to fit the new situation, mostly concerning questions regarding testing of COVID-19 (Appendix 2, online available).

During survey 1, a large portion of the participants reported reduced physical activity in the comment sections of questions. Therefore, a question was added to capture this in survey 2 and 3.

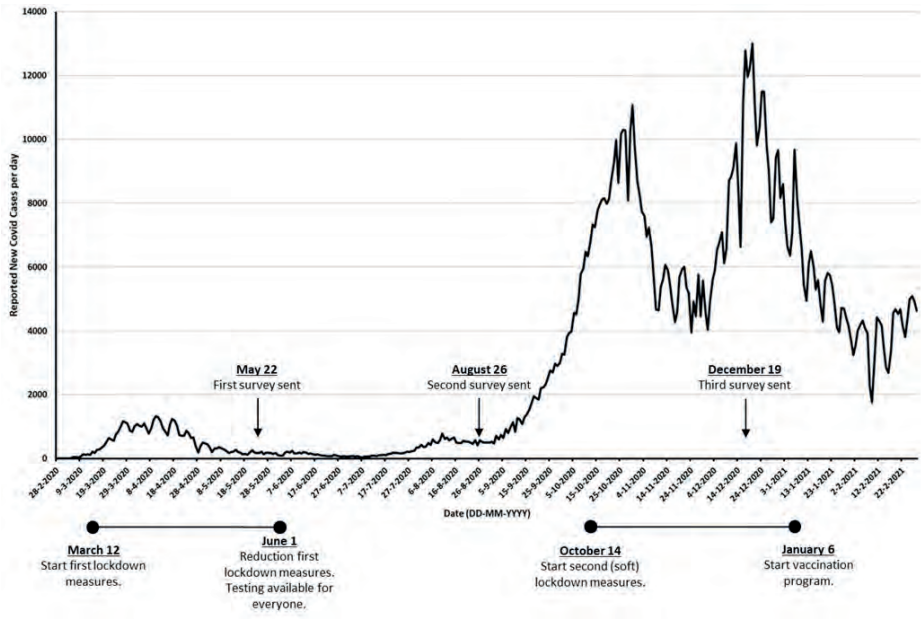

Figure 1. New COVID-19 infections per day in the Netherlands during the pandemic

The timepoints when the surveys were sent are pictured in the graph. The most important restrictions and developments regarding testing are stated below the graph[29]. Dates are given as dd-mm-yyyy.

Data availability and analysis

The data supporting the findings of this study are available on request from the Dutch FSHD registry. The data are not publicly available due to privacy or ethical restrictions[14]. Data was collected in CastorEDC[14]. Analysis of the data was done in R (R Foundation for Statistical Computing, Vienna, Austria) and SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Figures were created using GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, California USA).

Demographics, impact of the pandemic on care and perceived psychosocial stress are reported using descriptive statistics. The received care pre-pandemic is reported as a pooled group of all unique patients across the three surveys. Data is reported as mean (SD) or median [IQR] depending on normality of the data. Pearson's chi-square was used to test for differences between FSHD patients and the non-FSHD housemates concerning COVID-19 infection rate and severity of the symptoms with a p-value <0.05 considered as statistically significant. These analyses were done using only data of survey 3, because for this survey patients had to report on the whole period since the start of the pandemic, including the timespans of survey 1 and 2. Furthermore, for this comparison only housemates ≥16 years were included.

Ethical approval and informed consent

This study involved clinical research that did not fall within the scope of the Medical Research Involving Human Subjects Act, as declared by the local Medical Ethics Review Committee of the Radboud university medical center (amendment of file 2015-1812 on April 15th 2020). All participants of the FSHD registry provided their written informed consent before they entered the registry. The registry and its databases are in concordance with the General Data Protection Regulation and all other acting laws.

Results

Demographics and clinical features

Of the respectively 339, 341 and 343 invited patients for each for the three surveys, 210 (62%) completed the first, 186 (55%) the second, and 205 (60%) the third survey. In total, 261 participants completed at least one survey. The mean age per survey ranged from 54.6 (14.1) to 56.0 (14.5) years and 39-44% of the population was male (Table 1). Almost half of the participants in each survey (47.6% (S1), 49.5% (S2),

46.8% (S3)) belonged to one or several risk groups for a severe course of COVID-19 when infected with the SARS-CoV-2 virus.

COVID-19 impact on received care, FSHD complaints and physical activity

Pre-pandemic care was received by 86 (33%) participants across the three surveys, mostly consisting of care from their partner (18.4%) and/or homecare (12.6%) (Figure 2). At the time of surveys 1 and 2, 41.7% and 40% of the patients receiving care reported a decrease in care received compared to pre-pandemic care, reducing to 28.8% at the time of survey 3. The following changes were most often reported: home care unavailable, physical therapy unavailable, care personnel having less time, and domestic help unavailable. This reportedly led to a higher burden for informal caregivers, more symptoms, and less activity in general. Although an increase in FSHD related symptoms was reported by participants, the pre-pandemic MRS did not differ from the MRS at time of the survey (p=0.99 (S1), p=0.99 (S2), p= 0.90 (S3)). In surveys 2 and 3, 45% and 53% of the participants respectively were a little to a lot less active compared to before the pandemic.

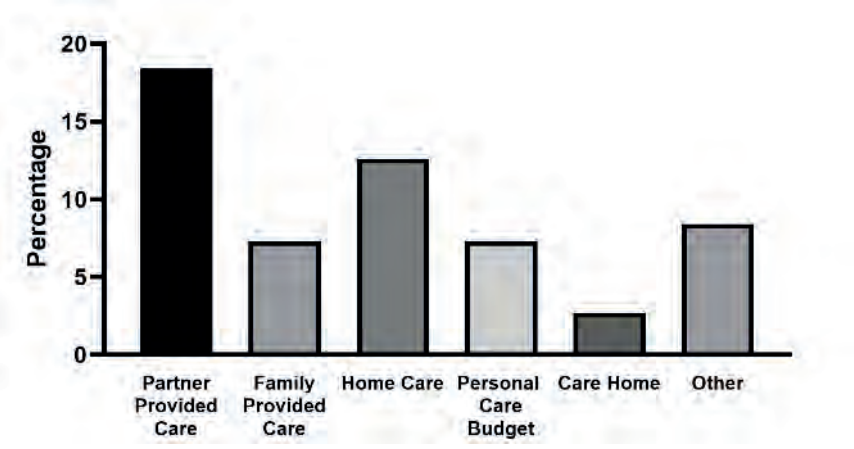

Figure 2. Types of care participants (N=86) reported to receive under normal circumstances

Of the 261 unique responders across the three surveys, 86 (33%) reported that they received care before the COVID-19 pandemic.

Partner provided care: partner of patient provides daily care; Family provided care: family provides daily care; Home care: care at home provided by an organization, consisting of healthcare, nursing, domestic help and guidance in everyday life; Personal care budget: a budget provided by the government with which a patient can buy their own care or assistance; Care home: a house or institution in which the patient lives and is provided with daily care, such as a nursing home.

Table 1. Demographics by survey round

	Survey 1 (22 May 2020)	**Survey 2** (26 Aug 2020)	**Survey 3** (19 Dec 2020)	**Non-FSHD Housemates**
N	210	186	205	204
Age, mean (SD)	54.6 (14.1)	56.0 (14.1)	55.7 (14.5)	49.9 (18.3)[b]
Male	82 (39)	78 (42)	90 (44)	106 (52)
Living arrangement[a]				
Independent	169 (81)	155 (83)	170 (83)	
Home care or personal care budget	25 (12)	19 (10)	24 (12)	
Assisted living or care facility	7 (3)	5 (3)	5 (2)	
Other	9 (4)	7 (4)	6 (3)	
Risk factors severe COVID-19				
>70 years old	33 (16)	36 (19)	36 (18)	22 (11)
Respiratory problems	27 (13)	24 (13)	21 (10)	5 (2)
Chronic heart disease	18 (9)	15 (8)	12 (6)	7 (3)
Severely overweight[b]	7 (3)	5 (3)	6 (3)	6 (3)
Immunodeficient	7 (4)	7 (4)	6 (3)	1 (1)
Other	36 (17)	32 (17)	34 (17)	13 (6)
Relation				
Spouse				153 (75)
Parent				10 (5)
Child				14 (7)
Brother/Sister				2 (1)
Other				7 (3)
Missing				17 (8)

Data is shown as N (%) unless given otherwise.
a: Independent – living independently in their own home, by themselves or with their partner/family. Home care – care at home provided by an organization, consisting of healthcare, nursing, domestic help, and guidance in everyday life; Personal care budget - a budget provided by the government with which a patient can buy their own care or assistance; Assisted living or care facility: a house or institution in which the patient lives and is provided with daily care, such as a nursing home.
b: Housemates were significantly younger compared to FSHD patients of survey 3 ($p<0.001$)

Impact of the pandemic on perceived psychosocial stress

Compared to pre-pandemic perceived psychosocial stress (PSS), 44% (S1), 30% (S2), and 40% (S3) of the participants reported a little to a lot more stress. Nevertheless, the perceived stress scores were low, with a median PSS of 11 [6-16] (S1), 9 [6-15] (S2), and 10 [6-15] (S3) (Figure 3). Stressors most often reported were 'loss of social contact' (86% - 91.4%) and 'COVID-19 related media coverage' (89.3% - 90.3%). The stressors that were most burdensome for FSHD patients were 'being unable to attend a funeral of a loved one' (3.06 (1.25) – 3.57 (1.16)) and 'being restricted in visiting family, friends or loved ones in the hospital' (3.03 (1.00) – 3.23 (1.16)) (Appendix 3, online available).

Positive effects of the pandemic were reported by 32.4% (S1), 26.3% (S2), and 27.8% (S3) of the participants. The most often reported positive effects were fewer social obligations and more time to rest resulting in less pain, less fatigue, less stress, and the opportunity to spend more time with their partners and children.

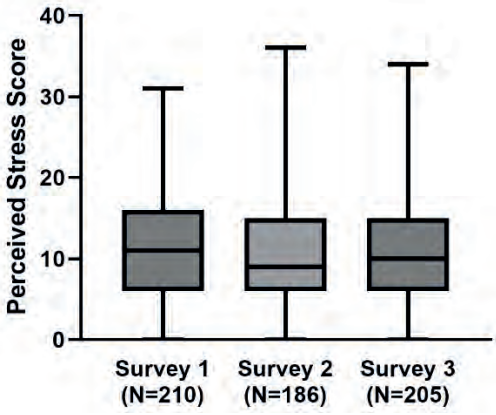

Figure 3. Perceived Stress Scale of participants from three consecutive survey rounds

A total score of 0-13 is considered low stress, 14-26 moderate stress and 27-40 high stress.

Comparison FSHD patients and their housemates

In survey 3, 216 housemates were reported on of which 12 housemates were also FSHD patients, resulting in 204 non-FSHD housemates (table 1). The housemates were significantly younger compared to the FSHD patients (49.9 (18.3) vs. 55.7 (14.5) years old, $p<0.001$). The majority of the housemates were the spouse of the FSHD patients (n=153, 75%), followed by their children (n=14, 7%) and parents (n=10, 5%).

FSHD patients had more possible COVID-19 related symptoms (38% (n=80) vs 27% (n=55), $\chi 2=6.73$, p=0.012). No differences were found in the number of patients and housemates that were tested (34% (n=70) vs 36% (n=74), $\chi 2=0.203$, p=0.68) or tested positive (3% (n=6) vs. 2% (n=4), $\chi 2=0.558$ p=0.53) (figure 4). The severity of possible COVID-19 related symptoms differed significantly between patients and their housemates (N=135, $\chi 2=9.11$, p=0.03) (figure 5).

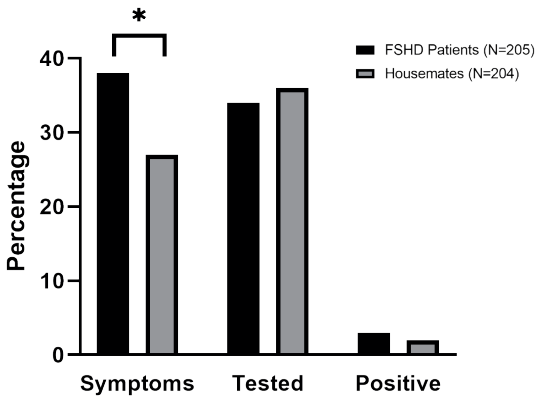

Figure 4. Comparison of possible COVID-19 symptoms, COVID-19 tests performed and number of positive tests in FSHD patients versus their housemates

Results are based only on survey 3. FSHD patients reported significantly more possible COVID-19 related symptoms (38.5% vs 27.4%, $\chi 2=5.68$, p=0.017). There was no difference between the number of tested participants (regardless of positive or negative result) (33.3% vs 35.5%, $\chi 2=0.219$, p=0.639) and number of positive tests (2.8% vs. 2.0%, $\chi 2=0.40$ p=0.527).

Figure 5. Severity of possible COVID-19 related symptoms in FSHD patients compared to their housemates

The percentages were calculated based on the number of FSHD patients and housemates who experienced symptoms (N=80 and N=55 respectively. The severity differed significantly between the two groups (N=136, $\chi 2=10.34$, p=0.016).

Discussion

This study investigated the impact the COVID-19 pandemic had on FSHD patients and the incidence of COVID-19 infections in the Netherlands. The COVID-19 pandemic reduced available care, physical activity and increased the psychosocial stress in FSHD patients. The COVID-19 infection rate in FSHD patients did not differ from their housemates without FSHD, but they did report more symptoms of minimal severity.

At surveys 2 and 3, nearly 50% of the patients reported to be less active during the pandemic than before. This is a considerable difference with findings in the general population, where no decline of physical activity was observed[22]. We hypothesize that people without physical challenges can easily change to outdoor activities, which may be harder to do for patients with FSHD or other NMDs. Since physical activity is known to be an important factor to stay in shape for FSHD patients, it is important to educate and support patients in maintaining their levels of physical activity during another pandemic. Even though face-to-face interactions are preferred by patients, during a pandemic this might not be possible and telemedicine approaches should be considered for the continuity of physical therapy and rehabilitative care[23-25].

Patients reported to have more psychosocial stress than before the pandemic. This was not reflected by the PSS scores reported in our study, which were low compared to worldwide studies in the general population as well in NMD patients during the pandemic (PSS scores of 15.4 to 17.4)[13,26,27]. However, similarly low PSS scores were also reported from the general population in the Netherlands in the same time during the pandemic[22]. The lower stress scores might be due to a higher social security and relatively mild course of the pandemic in the Netherlands compared to other countries. Studies with longer follow-up periods will need to confirm if the stress levels of patients normalize to pre-pandemic levels.

The most prevalent and most burdensome stressors in our study were similar to stressors in healthy individuals (DYNACore-C) and in Parkinson's patients, indicating the stressors perceived by FSHD patients were not disease-specific[20,28]. Findings from large studies on these stressors such as the DYNACore-C may therefore be applicable to FSHD patients, which might help with creating therapies to cope with these stressors. Interestingly, more than 25% of the FSHD patients from each survey reported various positive effects of the pandemic, for instance being well rested. A more detailed, possibly qualitative, follow-up on what these positives effects

were may help us to improve the quality of life of FSHD patients within as well as outside of a pandemic period. We did not find a difference in infection incidence rates between FSHD patients and their non-FSHD housemates. One international study in 1243 NMD patients reported a higher infection rate of 8% compared to our findings, but only a minority of those infections (20%) were found in European patients bringing it more in line with our incidence rate[3]. Another international study mentioned an infection incidence of <1% but lacked details[13]. Our data did show a higher incidence of possible covid-related symptoms in FSHD patients compared to their housemates. However, we suspect this is due to reporting bias as recalling one's own minimal symptoms is different from identifying and recalling when housemates experienced such symptoms. We also suspect that the higher number of minimal symptoms in the FSHD patients caused the difference in severity of symptoms between the patients and their housemates.

Due to the limitations of social distancing and lockdowns as well as the lack of contact details of participants' spouses in the registry and limitations in the survey system, the study was limited to data reported by the registry participants, including the data about the housemates. Therefore, a drawback of this method is that the data on housemates is secondhand information and might be more biased. In addition, although we did inquire about the exposure by asking participants about measures taken, we failed to ask about the situation of the housemates. Therefore, we cannot rule out possible exposure differences between participants and housemates.

This study assessed the changes in health(care) during the pandemic. The healthcare system changed after the pandemic, most noticeably in the higher frequency of telemedicine approaches. A study comparing pre- and post-pandemic healthcare received and the satisfaction regarding the new telemedicine approach would be interesting to perform.

Conclusion

This study showed that care received, physical activity and perceived psychosocial stress were negatively impacted by the COVID-19 pandemic. Although an increase in FSHD complaints was reported by participants, the pre-pandemic MRS did not differ from the MRS at time of the survey. We did not find evidence for a different susceptibility to COVID-19 infections in FSHD patients compared to the control group and differences in the number and severity of possible COVID-19-

related symptoms could well be attributable to reporting bias. Since the COVID-19 pandemic is characterized by cyclical outbreaks and given the possibility for other future pandemics, an adequate approach for the support and continuity of care of these patients is essential.

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Chapter 4

A Five Year Natural History Cohort of Patients with Facioscapulohumeral Muscular Dystrophy Determining Disease Progression and Feasibility of Clinical Outcome Assessments for Clinical Trials

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Abstract

The number of clinical trials in Facioscapulohumeral muscular dystrophy (FSHD) is expected to increase drastically in the near future. There is a need for clinical outcome assessments (COAs) that can capture disease progression over the relatively short time span of a clinical trial. In this study, we report the natural progression of FSHD and determine the feasibility of COAs for clinical trials. Genetically confirmed FSHD patients underwent various clinical assessments at baseline and after five years. COAs consisted of the Motor Function Measure (MFM), manual muscle testing using the medical research council (MRC) score, 6-minute walk test (6-MWT), quantitative muscle strength assessment (QMA) of the quadriceps muscle, clinical severity score (CSS) and FSHD evaluation score (FES). Statistical significance and the minimal clinically important difference (MCID) were calculated and power calculations were performed.154 symptomatic FSHD patients were included, with a mean (SD) age of 51.4 (14.6) years old. All COAs showed a statistically significant progression after five years. MCID was reached for the MFM Domain 1(D1), MFM Total score, and FES. The MFM D1, MFM Total score and FES showed the lowest sample size requirements for clinical trials (185, 156 and 201 participants per group for a trial duration of two years respectively). The captured FSHD disease progression rate in five years was generally minimal. Extended trial durations or novel outcome assessments might be necessary to improve trial feasibility in FSHD.

Keywords

FSHD, disease progression, clinical outcome assessments, minimal clinically important difference, power calculation.

Abbreviations

6-MWT – Six-minute walk test
CSS – Clinical Severity Score
COA – Clinical Outcome Assessment
FES – FSHD Evaluation Score
FSHD - Facioscapulohumeral muscular dystrophy
MCID – Minimal Clinically Important Difference
MFM – Motor Function Measure
MFM D1 - Motor Function Measure Domain 1
MFM Total – Motor Function Measure Total score
MMT – Manual Muscle Testing
MRC – Medical Research Council
QMA – Quantitative Muscle Assessment

RWS – Reachable Workspace
SD – Standard Deviation
SEM – Standard Error of the Measurement

Introduction

Currently, no disease-modifying therapy exist for facioscapulohumeral muscular dystrophy (FSHD), but a drastic increase in the number of clinical trials for new therapies is expected[1-6]. Due to the large phenotypic variability and slow progression of the disease, it is of the utmost importance to carefully select the study population and clinical endpoints to reliably demonstrate the potential efficacy of the investigational drugs[7]. Although many clinical outcome assessments (COAs) have been tested in FSHD, evidence of the validity, reliability and sensitivity to change of these COAs is scarce[8].

The feasibility of a COA should be determined not only by their ability to capture a statistically significant change, but also by their ability to identify a clinically important difference. The minimal clinically important difference (MCID) is defined as 'the smallest difference in score in the domain of interest which patients perceive as beneficial and which would mandate, in the absence of troublesome side effects and excessive cost, a change in the patient's management'[9]. Two different approaches to calculating the MCID have been used: a statistical (distribution-based) approach and a patient-reported (anchor-based) approach[10]. As neither of these methods proved superior, using both is currently deemed to be the best approach to calculate the MCID.

In this study, we measured the clinical disease progression of FSHD patients over a period of five years using several commonly used COAs in FSHD. Additionally, the MCIDs and estimated required sample sizes for clinical trials were calculated. We aimed to provide knowledge about the feasibility of COAs to capture disease progression and drug efficacy for use in clinical trials.

Methods

Study design

Genetically proven FSHD patients ≥ 18 years old were invited for a baseline visit and a follow-up visit after five years. No exclusion criteria were maintained, if participants could not complete any of the assessments they were excluded from that particular assessment. The following COAs were tested at both visits: Motor Function Measure (MFM), Manual Muscle Testing (MMT), a quantitative muscle strength assessment (QMA) of the m. quadriceps and the six-minute walk test (6-MWT). The overall disease severity was scored using the clinical severity score (CSS) developed by Ricci et al. and the FSHD Evaluation score (FES) developed by Lamperti et al[11,12]. At the follow-up visit, participants had to answer multiple anchor question using the patient global impression of change with answer options ranging from '0 – much worse' to '4 – much better' (supplemental questionnaire). If a participant was unable or unwilling to visit the clinic, a home visit was performed. In the case of a home visit, the MFM, MMT, CSS and FES were tested following the same protocol as a clinic visit, the QMA and 6-MWT were not performed as these were only performed in a standardized setting. All the baseline visits were performed by one assessor (Mul), the follow-up visit by a second assessor (Vincenten). The second assessor received extensive training from the first assessor before performing the follow-up visits and received supervision on the first follow-up visits from the first assessor.

Clinical outcome assessments

The MFM consists of 32 items divided in three domains: D1) standing position and transfers; D2) axial and proximal limb motor function; D3) distal limb motor function[13]. The mean of the sumscores of the left and right side was calculated for Domain 1 (MFM D1) and the total score (MFM Total), reported in percentage of the maximum score with 0% meaning no function and 100% maximum function, despite knowing its deficiencies. D2 and D3 were not included in the analyses separately, because these domains are known to have a significant ceiling effect in FSHD patients[14-16].

MMT was scored using the Medical Research Council (MRC)-score ranging from 0-5, with 0 meaning no visible contraction and 5 normal power and range of motion[17]. The mean (ranging from 0-5) of the following muscle movements was calculated: neck flexion, neck extension, and bilaterally the shoulder abduction, shoulder adduction, shoulder exorotation, elbow flexion, elbow extension, wrist flexion,

wrist extension, hip flexion, knee flexion, knee extension, ankle dorsal flexion, ankle plantar flexion.

The 6-MWT was performed using a 40-meter track in a standardized setting. Participants had to walk as far as possible in six minutes. The total distance walked is reported in meters[18].

The QMA of the m. quadriceps was performed for both legs. Participants were seated in a standardized set-up and had to push against a force transducer (KAP-S, AST®) with maximum force. The mean score of both sides was calculated in Newton.

The CSS ranges from 0-10 with 0 having no symptoms and 10 being wheelchair-dependent[11]. The FES separately scores facial muscles (0-2), scapular girdle muscles (0-3), upper limb muscles (0-2), leg muscles (0-2), pelvic girdle muscles (0-5) and abdominal muscles (0-1). A sum score was subsequently calculated, ranging from 0-15, with 0 having no symptoms and 15 being severely affected in all areas[12].

Data analysis

Non-penetrant gene carriers (CSS of zero at baseline and follow-up) were excluded from the analyses to reduce the dilution of the data. Longitudinal continuous data was analyzed using the paired t-test or Wilcoxon sum rank test, depending on normality of the data. Binary or ordinal data was analyzed using the McNemar test. Normally distributed data were reported as mean (standard deviation), and skewed data was reported as median [interquartile range]. Missing data were pairwise excluded. All statistical analyses were performed using IBM SPSS Statistics (SPSS Inc., Chicago, IL), version 25. Figures were created using GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, California USA).

The MCID was calculated using both the distribution and anchor-based method. The distribution method consists of calculating Cohen's d, and comparing the difference between baseline and follow-up to half its SD and the standard error of the measurement (SEM). Usually, the SEM is calculated using the test-retest method. However, this method was unavailable because the baseline visit did not include retesting of the measurements. As a substitute, the SEM was calculated using participants who reported stability on all anchor questions (n=21). This method has been used and deemed acceptable in earlier studies on other medical conditions[19-21]. Based on the anchor question "Compared to five years ago, how is your ability to move?" two groups were created. Participants who scored 'much worse' or 'worse' were categorized as 'progressed participants'. Participants who

scored 'no change', 'better' or 'much better' were categorized as stable patients. The mean (SD) progression on the COAs was calculated for both groups and compared to each other.

Power calculations were performed using the following assumptions: 1) The treatment and placebo group will have the same size, 2) the treatment group will remain stable, 3) the placebo group will progress according to the natural progression observed in the current study, 4) α=0.05 using two-sided testing and β=0.8. First, the number of participants needed in each group for a five-year trial was calculated. Second, the number of participants needed for a trial duration of two years was calculated, assuming a linear progression while maintaining the same SD. Third, the necessary duration of a trial and the number of participants to reach an MCID based on the anchor question were calculated, assuming linear progression. Lastly, we performed power calculations for a two-year trial with different ranges of the CSS as this is an often used eligibility criterion in clinical trials[2-6].

Results

Demographics

Of the 204 eligible patients seen at baseline, 162 participants were seen for the follow-up visit (Figure 1). Eight participants were classified as non-penetrant and therefore excluded from the analyses, resulting in 154 participants. Patient characteristics are shown in Table 1. The number of home visits was significantly higher at the follow-up visit (12.3% vs. 24.0%, $p<0.001$). The increase in disease duration is less than expected based on our follow-up period, most likely caused by recall bias.

Change over time

All COAs showed a statistically significant change over time, even though some COAs (MRC, 6-MWT, QMA) showed minimal changes over a five year period (Table 2, Figure 2). A large effect size was reached for the MFM D1 (d=0.7), MFM Total (d= 0.8), and FES (d=0.7). A mean difference between baseline and follow-up larger than 0.5*SD and the SEM was reached in the MFM D1, MFM Total, CSS, and FES (Table 3).

The anchor question was completed by 130 (84%) participants with the same demographic profile as the total group (Supplementary Table 1). Based on the anchor question, 78 (50.6%) participants reported (much) worsening of their ability to move compared to five years ago, 52 (33.8%) reported stability or improvement, and

the other 24 (15.6%) participants had missing data. The anchor-based MCIDs were reached for the MFM D1, MFM total and FES after five years follow-up (Table 3).

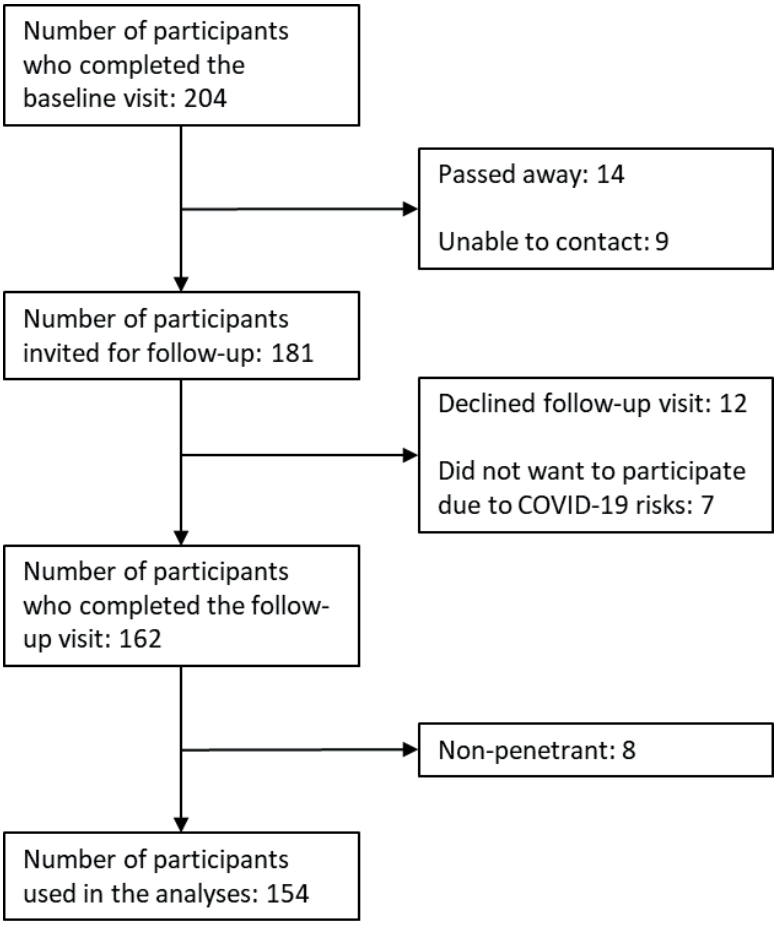

Figure 1. Flowchart of participants

Table 1. Demographics

Variable	Baseline (N=154)	Five year follow-up (N=154)
Age (y)	51.4 (14.6)	55.9 (14.4) *
Height (m) (n=128)	1.76 (0.1)	1.76 (0.1)
Weight (kg)(n=128)	78.2 (14.3)	80.0 (13.7) *
BMI (kg/m^2)(n=127)	25.3 (4.1)	25.9 (4.1) *
Abdominal Circumference (cm) (n=73)	96.5 (13.6)	94.0 (14.8) *
Sex		
Male	80 (51.9%)	80 (51.9%)
Female	74 (48.1%)	74 (48.1%)
FSHD Type		
1	146 (94.8%)	146 (94.8%)
2	8 (5.2%)	8 (5.2%)
Repeat size type 1		
Mean	6.11 (1.65)	6.11 (1.65)
1-3	9 (5.8%)	9 (5.8%)
4-6	73 (47.4%)	73 (47.4%)
7-9	64 (41.6%)	64 (41.6%)
CSS	6 [3.5-8]	7 [6-9]*
Duration of symptoms (y) Visit	**22 [10-36]**	**23 [12-36]***
Home	19 (12.3%)	37 (24.0%)*
Clinic	135 (87.7%)	117 (76.0%)*

Continuous data is presented as mean (SD), except for duration of symptoms and CSS which is median [IQR].

* Statistically significantly different from baseline ($p<0.05$). BMI = Body Mass Index. CSS = Clinical Severity Score.

Table 2. Median scores of the clinical outcome assessments at baseline and follow-up

	Baseline		Follow-up		
COA	**n**	**Median [IQR]**	**n**	**Median [IQR]**	**p-value**
MFM D1 (0-100%)	154	73 [31-96]	154	63 [22-92]	<0.001
MFM Total (0-100%)	154	86 [63-97]	154	78 [55-96]	<0.001
MRC (0-5)	150	4.3 [3.7-4.7]	146	4.3 [3.6-4.7]	<0.001
6-MWT (m)	103	488 [399-545]	75	465 [368-534]	0.014
QMA (N)	107	288 [205-407]	107	244 [176-399]	0.002
CSS (0-10)	154	6 [4-8]	154	7 [6-9]	<0.001
FES (0-15)	154	7 [3-11]	154	9 [4-12]	<0.001

Some participants were excluded for certain COAs (e.g. wheelchair-bound participants were excluded from the 6-MWT), therefore the number of participants for each COA is given.
COA = Clinical Outcome Assessment. IQR = Interquartile Range. MFM D1 = Motor Function Measure Domain 1. MFM Total = Motor Function Measure Total score. MRC = Medical Research Council. 6-MWT = Six-Minute Walk Test. QMA = Quantitative Muscle Assessment. CSS = Clinical Severity Score. FES = FSHD Evaluation Score.

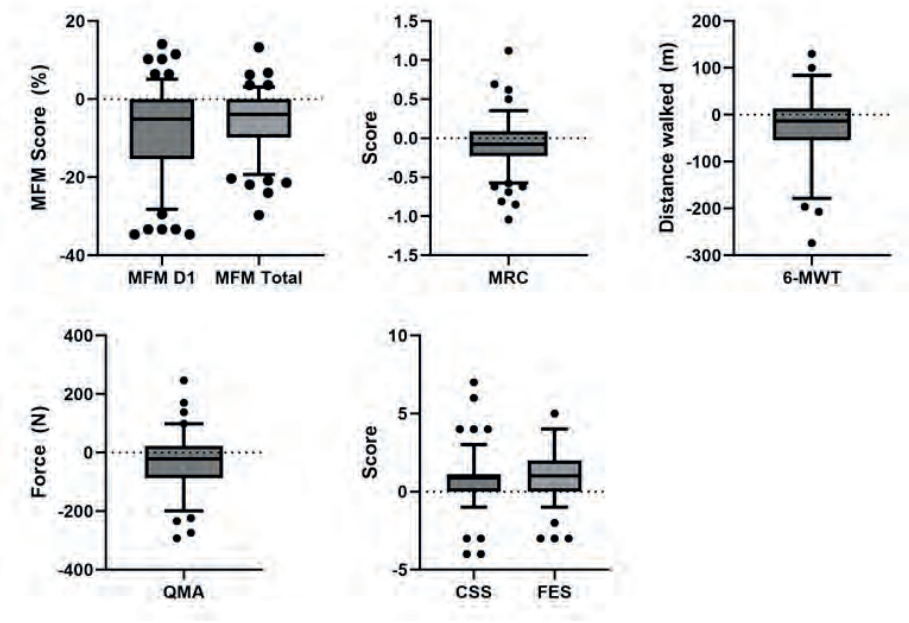

Figure 2. Boxplots of the change over time of the clinical outcome assesments

The boxplots show the median [IQR] with 5-95% confidence interval, each dot is one individual outlier. MFM D1 = Motor Function Measure Domain 1. MFM Total = Motor Function Measure Total score. MRC = Medical Research Council. 6-MWT = Six-Minute Walk Test. QMA = Quantitative Muscle Assessment. CSS = Clinical Severity Score. FES = FSHD Evaluation Score.

Table 3. Minimal Clinically Important Difference

COA	Mean (SD) Progression	Distribution Based			Anchor Based			
		Effect size	0.5 SD	SEM	Progressed patients N=78	Stable patients N=52	Difference Stable and progressed patients	Number of Participants reaching Anchor MCID
MFM D1 (0-100%)	**7.7 (10.6)**	**0.7**	**5.3**	**5.7**	9.8 (11.4)	4.7 (9.2)	**5.1 (1.9)**	**78 (50%)**
MFM total (0-100%)	**5.7 (7.2)**	**0.8**	**3.6**	**4.3**	7.8 (7.5)	2.8 (5.5)	**5.0 (1.1)**	**73 (47%)**
MRC (0-5)	0.08 (0.29)	0.3	0.15	0.16	0.11 (0.35)	-0.01 (0.19)	0.12 (0.05)	59 (40%)
6-MWT (m)	24.4 (73.0)	0.2	36.5	40.8	46.7 (81.1)	-1.57 (53.8)	48.2 (18.2)	20 (27%)
QMA (N)	30.4 (91.3)	0.3	45.7	51.1	38.9 (88.1)	21.2 (100.9)	17.7 (21.0)	50 (47%)
CSS (0-10)	**0.8 (1.4)**	**0.6**	**0.7**	**0.7**	0.9 (1.4)	0.7 (1.7)	**0.2 (0.3)**	**86 (56%)**
FES (0-15)	**1.0 (1.4)**	**0.7**	**0.7**	**0.7**	1.2 (1.4)	0.7 (1.4)	**0.5 (0.3)**	**92 (60%)**

The bold numbers show the COAs that reached MCID after five years. The data of the anchor based question is reported in Mean (SD). The percentage of the participants reaching the anchor-based MCID is calculated based on the number participants who completed the COA at both visits, not the total number of participants.

COA = Clinical Outcome Assessment. MCID = Minimal Clinically Important Difference. MFM D1 = Motor Function Measure Domain 1. MFM Total = Motor Function Measure Total score. MRC = Medical Research Council. 6-MWT = Six-Minute Walk Test. QMA = Quantitative Muscle Assessment. CSS = Clinical Severity Score. FES = FSHD Evaluation Score. SD = Standard Deviation. SEM = Standard Error of the Measurement.

Power calculations

A trial with a five-year follow-up would need an estimated 30 participants per group for the MFM D1, 25 for the MFM Total, 32 for the FES and 48 for the CSS (Table 4). The MRC (N=195 per group), 6-MWT (N=178 per group), and QMA (N=142 per group) would require a much larger study population. Recalculating the estimated study population for a trial duration of two years, the MFM D1 would require 185 participants per group, MFM Total 156, CSS 297, and FES 201 participants.

To reach the anchor-based MCID of the outcome measures a trial would need to run for 3.3 years with 67 participants per group for the MFM D1, 4.4 years with 32 participants for the MFM Total, 1.2 years with 769 participants for the CSS, and 2.6 years with 119 participants for the FES. As the MFM D1, MFM Total, and FES required the lowest number of participants in each group, the additional power calculations using different CSS ranges and a trial duration of two years were performed using these three COAs. If only the number of participants needed in each group is considered, a CSS range of 5-8 would be the most optimal for the MFM D1 (n=127 per group) and MFM Total (n=114 per group). A CSS range of 3-8 would be optimal for the FES (N=141 per group) (Table 5).

Table 4. Power calculations for different trial durations

COA	5-year trial	2-year trial	MCID (anchor)	Duration to reach MCID (years)
MFM D1	30	185	67	3.3
MFM Total	25	156	32	4.4
MRC	195	1222	94	7.2
6-MWT	178	878	36	9.9
QMA	142	886	417	2.9
CSS	48	297	769	1.2
FES	32	201	119	2.6

Number of participants needed per group (rounded up) for each COA.
COA = Clinical Outcome Assessment. MFM D1 = Motor Function Measure Domain 1. MFM Total = Motor Function Measure Total score. MRC = Medical Research Council. 6-MWT = Six-Minute Walk Test. QMA = Quantitative Muscle Assessment. CSS = Clinical Severity Score. FES = FSHD Evaluation Score. MCID = Minimal Clinically Important Difference

Table 5. Power calculations for a two-year trial based on different CSS as an inclusion criterion

COA	All N=154 (100%)	3-8 N=106 (69%)	5-8 N=89 (58%)	6-8 N=83 (54%)	3-9 N=122 (79%)	0-3 N=38 (25%)
MFM D1	185	138	127	129	139	280
MFM TOTAL	156	126	114	114	114	207
FES	201	141	144	166	158	272

Number of participants needed per group using different ranges of the CSS at baseline as an inclusion criterion. Number of participants (% of total study population) scoring in the specific CSS-ranges are reported.
COA = Clinical Outcome Assessment. MFM D1 = Motor Function Measure Domain 1. MFM Total = Motor Function Measure Total score. CSS = Clinical Severity Score. FES = FSHD Evaluation Score

Discussion

This paper described the natural progression of FSHD over five years using multiple COAs. Although a statistically significant change was reached for all COAs, the changes were minimal and clinically relevant in only four of the seven assessments. Due to the minor changes yet relatively high standard deviations, clinical trials using these COAs would require a large number of participants. The COAs tested in this study are therefore unlikely to be feasible for clinical trials in FSHD considering the number of available patients and high costs of long-term trials.

Whether our findings were similar to results of previous natural history studies, was dependent on the analyzed COA. Our MFM data is in accordance with another, albeit smaller natural history study with a shorter duration on the MFM in FSHD[22]. They found a mean decrease in D1 of 1.7% over 1 year (8.5% over 5 years given linear progression) and a slightly larger decrease in the total score of 1.5% over 1 year (7.5% over 5 years given linear progression). In contrast, the MRC scores in this cohort decreased significantly less compared to other studies. The FSH-DY group reported a mean decrease of approximately 0.07 per year, while a recently published natural history study by Varma et al. reported a mean decrease of 0.03 after a year[23,24]. In this study, we did not allow plus or minus scores on the MMT, while the FSH-DY group did, which may have reduced the sensitivity to detect minor changes. Furthermore, because we did not maintain any exclusion criteria regarding disease severity, our cohort may contain more minimally- and severely-affected patients whose disease course is considered to be more stable compared to moderately affected patients. Whether our number of stable patients differs from the number of the FSH-DY study is hard to determine based on their data, but this is most likely the case for the study from Varma et al. considering their inclusion criteria.

The data of all the measurements in this paper were highly skewed and warranted the reporting of the median[IQR] and use of non-parametric testing. This complicated comparison to previous studies, as those generally reported the mean (SD) and used parametric testing. The skewness of the data also introduces an inaccuracy to our MCID and power calculations, which require the mean (SD) to calculate. Additionally, some assumptions for the power calculations might not fully represent reality. First, we interpolated the two-year data assuming a linear progression. Based on MRI data and our clinical experience, FSHD does not follow a linear progression[25,26]. Instead, muscles can remain unaffected for a long period and suddenly decline. However, in a larger sample size a mean linear progression should approximate reality. Secondly, we assumed that the standard deviation of the two-year data would be the same as the five-year data. It is more likely that the standard deviation would decrease as well, although not relative to the mean. One year trial duration power calculations would deviate even more from reality, which is why we refrained from performing this analysis. Thirdly, the assumption was made that the placebo group would follow the disease progression of this natural history study. However, a review of three previous clinical trials reported that the placebo groups showed less progression compared to natural history data[26]. It remains challenging to perform power calculations that exactly fit the reality, in our case some assumptions will overestimate the number of patients (e.g. 2-year standard deviation is the same as 5-year standard deviation), while other assumptions underestimate the effect (e.g. placebo group follows natural history data instead of performing better). Nevertheless, the power calculation presents the approximate feasibility of these outcome assessments and how these COAs compare to each other. Combining our data with other natural history data will most likely give the most reliable power calculations.

The majority of the outcome assessments are ordinal based, but are reported as a continuous sum or mean score. While many COAs following this format are used in clinical studies, the disadvantages of these outcome assessments are usually neglected. First, the distance between the categories is unknown or unequal, only a relative ranking of the data is possible[27]. Second, summing these ordinal scales assumes that all parts contribute equally to the scale, which is highly unlikely. Third, some items may test multiple domains, which could inflate the final sum score. This means that treating these ordinal-based scores as a continuous scale, may lead to false positive or negative results. The Rasch model can be used to transform these ordinal scales into an interval scale, providing that the data fit the model. Unfortunately, neither the MFM D1 nor the total score fit the model for FSHD patients, even after multiple adjustments to either the scoring system or included

items[14]. The MRC score also fails to fit the Rasch model in several neuromuscular disorders other than FSHD[28]. This warrants caution when using these outcome assessments to determine the efficacy of novel therapies in FSHD. Furthermore, this indicates that additional analysis of ordinal-based outcome assessments, such as the FSHD-HI or the novel FSHD-COM, with the Rasch-model is needed.

The in- and exclusion criteria of the ongoing and upcoming clinical trials allow inclusion of FSHD patients who score between 4-8 on the CSS[2-6]. Additionally, most trials require patients to show weakness or fatty infiltration in at least one lower extremity muscle, so practically patients need to score a CSS of 5-8 to be eligible. Based on this cohort, approximately 42% of the FSHD population cannot be included in these trials if this criterion is used. If this CSS criterion is maintained in future trials, we anticipate difficulty with recruiting sufficient eligible patients. Based on our data, expanding the CSS criterion to a CSS of 3-9 will increase the percentage of eligible to 79% of all FSHD patients, without losing a significant amount of power. Naturally, these numbers might differ for other COAs; careful considerations for each specific trial on this criterion is necessary if trials in FSHD will be sustainable. For example, the reachable workspace (RWS) is often used as an important COA, which solely measures upper extremity range of motion, and thus, a minimal CSS of 3 should be sufficient as a criterion[29]. Apart from improving sustainability of the future trials, the expansion of the CSS criterion will also increase the generalization of the data towards the complete FSHD population.

Ideally, the COAs in a clinical trial show both a statistically significant and clinically relevant difference between the treatment and placebo arm. Directly related to the slow disease progression, the majority of the outcome assessments in this study would require a trial duration longer than three years to reach the anchor-based MCID assuming upcoming therapies will slow or halt disease progression. Only the CSS and FES would reach an MCID in approximately two years, but in reality, this is not probable, because these scales measure large changes and are thus unable to capture the minimal two year disease progression. Open-label extension phases with reduced visit frequency and outcome measures might provide the long-term data while minimalizing the cost and burden for the participants, trial sites, and sponsors. Remote monitoring, such as the stride velocity in Duchenne, could also allow for extended trial durations while minimizing the burden[30]. Extrapolation of the data could be another solution, but it might lead to overestimation of minor effects. Regardless of the solution, clear guidelines on approaching this conundrum would increase the feasibility of upcoming trials.

The follow-up period of five years of this study is longer than other natural history studies. Although this is longer than the duration of a clinical trial and may introduce recall bias regarding the anchor questions, the long follow-up period makes our results less prone to be affected by the variable disease course of FSHD and increases the chances of capturing disease progression. The single center design minimizes variability introduced by country-specific effects (e.g. difference in healthcare availability), increasing the uniformity of the data but reducing the generalizability towards international multicenter trials. The first assessor trained the second assessor extensively and was also present at the first couple of follow-up visits, but double measurement were not performed, rendering us unable to determine the inter-rater reliability. Because home visits were allowed, even the most severely affected FSHD patients could be included, reducing selection bias and drop-out that is usually present in other studies. At follow-up, significantly more home visits took place generally caused by the natural progression of the disease that prevented participants from traveling to the site or due to COVID-19 restrictions. We think it is unlikely that the MFM, MRC, CSS and FES are scored differently at a participants home compared to at the clinic and thus we do not expect that the increase in home visits influenced our results.

Inherent to long natural history studies, we were unable to included novel outcome assessments such as the RWS or the FSHD-RODS questionnaire[29,31]. These outcome assessments were added in the follow-up visit and will be analyzed in the ten-year follow-up study. The ongoing ReSolve multi-center natural history study also included some of the novel FSHD outcome assessments which were tested on multiple timepoints instead of two[32]. The collected data allows for determining the MCID and sensitivity to change of the new COAs for clinical trials.

The power calculations using the different ranges of CSS gave interesting insights to improve the sustainability of future trials by including patients with a wider spectrum of disease severity. Combining COA, imaging and biomarker data in a future study might enable us to create a reliable model that predicts which patients will show disease progression in the near future[26,33,34].

In this paper we assumed that novel therapies for FSHD will at best stabilize the patients. However, if novel therapies actually improve the disease status of the patients, the feasibility of the COAs will improve and the MCID can be reached faster than we calculated. In that case, the MFM D1 could have a place in in FSHD trials, although it still has its disadvantages by transforming ordinal data to a continuous scale and having a ceiling effect. The CSS and FES are unlikely to be

sensitive to change due to their large incremental steps, making them more useful as eligibility criteria and not as outcome measures. This is also the case for the MRC score, but muscle strength testing with either handheld dynamometry or QMA could be useful. The 6-MWT does not seems to be feasible for FSHD trials, even if novel therapies can improve the disease status of patients.

To conclude, FSHD patients showed overall minimal progression after five years. The clinical outcome assessments investigated in this study are unlikely to be sufficiently sensitive to capture disease progression in clinical trials assuming novel therapies will at best stabilize the patients. Longer duration of clinical trials and novel, FSHD-specific, outcome assessments are expected to provide the solution.

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Supplemental Data

Supplemental Questionnaire: General health in the last 5 years

Compared to five years ago, how is your ... (see options 1 to 14 in the table below)?

	Much worse	Worse	The same	Better	Much better
General Health					
Quality of life					
Ability to move					
Mobility and strength in the hands and forearms					
Mobility and strength in the upper arms and shoulders					
Mobility and strength of the back, chest and abdomen					
Ability to perform activities					
Ability to think					
Satisfaction in social situations					
Performance in social situations					
Fatigue					
Pain					
Ability to communicate with others					
Ability to swallow or eat					

Supplementary Table 1. Baseline demographics of participants who completed the anchor question

Variable	Total study populationn=154	Completed Anchor questionn=130
Age (y)	51.41 (14.6)	51.33 (14.7)
Length (m)	1.76 (0.09)	1.75 (0.08)
Weight (kg)	78.22 (14.3)	77.6 (14.1)
BMI	25.26 (4.07)	25.1 (4.0)
Abdominal Circumference (cm)	96.52 (13.6)	96.46 (13.1)
Sex		
Male	80 (51.9%)	66 (50.8)
Female	74 (48.1%)	64 (49.2)
FSHD Type		
1	146 (94.8%)	123 (94.6%)
2	8 (5.2%)	7 (5.4%)
Repeat size type 1		
Mean	6.1 (1.7)	6.1 (1.7)
1-3	9 (5.8%)	8 (6.5%)
4-6	73 (47.4%)	62 (50.4%)
7-9	64 (41.6%)	53 (43.1%)
Duration of symptoms Visit	21.5 [10.0-36.0]	23.6 [23.0-37.0]
Home	19 (12.3%)	17 (13.1%)
Radboudumc	135 (87.7%)	113 (86.9%)

Continuous data is presented as mean (SD), except for duration of symptoms which is median [IQR].
BMI = Body Mass Index

Part II:
Clinical Trial and Participant Experiences

Chapter 5

An Open-Label Pilot Study of Losmapimod to Evaluate the Safety, Tolerability, and Changes in Biomarker and Clinical Outcome Assessments in Participants with Facioscapulohumeral Muscular Dystrophy Type 1

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Abstract

Facioscapulohumeral muscular dystrophy (FSHD) is a genetic disease caused by aberrant DUX4 expression leading to progressive muscle weakness. No effective pharmaceutical treatment is available. Losmapimod, a small molecule selective inhibitor of p38 α/β MAPK, showed promising results in a phase I trial for the treatment of FSHD, prompting additional studies. We report the findings of an open-label phase 2 trial (NCT04004000) investigating the safety, tolerability, pharmacokinetics, pharmacodynamics, and exploratory efficacy of losmapimod in participants with FSHD1.

This study was conducted at a single site in the Netherlands from August 2019 to March 2021, with an optional, ongoing open-label extension. Participants aged 18 to 65 years with FSHD1 took 15 mg of losmapimod twice daily for 52 weeks. Primary endpoints were measures of losmapimod safety and tolerability. Secondary endpoints were assessments of losmapimod pharmacokinetics and pharmacodynamics.

Fourteen participants were enrolled. No deaths, serious treatment-emergent adverse events (TEAEs), or discontinuations due to TEAEs were reported. Losmapimod achieved blood concentrations and target engagements that were previously associated with decreased DUX4 expression *in vitro*. Clinical outcome measures showed a trend toward stabilization or improvement.

Losmapimod was well-tolerated and may be a promising new treatment for FSHD; a larger phase 3 study is ongoing.

Keywords
FSHD, open-label study, losmapimod, DUX4, efficacy, safety

Abbreviations

6-MWT – Six-minute walk test
AE – Adverse Event
ALT – Alanine Transaminase
AST – Aspartate aminotransferase
BID – Twice Daily
COA – Clinical Outcome Assessment
ECG – Electrocardiogram
FES – FSHD Evaluation Score
FEV1 – Forced Expiratory Volume in 1 second
FSHD – Facioscapulohumeral muscular dystrophy
FSHD-HI – FSHD Health Index
FSHD-RODS – FSHD Rasch-built Overall Disability Scale
FVC – Forced Vital Capacity
HHD – Handheld Dynomametry
IC50 – half-maximal inhibitory concentration
MAPK – Mitogen-Activated Protein Kinase
MCID – Minimal Clinically Important Difference
MFM – Motor Function Measure
MFM D1 – Motor Function Measure Domain 1
NE – Number of Events
OLE – Open-Label Extension
PGIC – Patient Global Impression of Change
PD – Pharmacodynamics
pHSP27 – phosphorylated HSP27
PK – Pharmacokinetics
PRO – Patient Reported Outcome
Q – Quadrant
QMT – Quantitative Muscle Testing
RSA – Relative Surface Area
RWS – Reachable Workspace
SD – Standard Deviation
SE – Standard Error
STIR+ – Short Tau 1 Inversion Recovery–Positive
TEAE – Treatment-Emergent AE
TUG – Timed Up-and-Go
VC – Vital Capacity

Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is a genetic muscle disease caused by aberrant expression of DUX4 protein in skeletal muscle[1]. FSHD is one of the most common types of muscular dystrophy, with an estimated prevalence of 1 in 8,000 to 31,000 people[2-8]. There are two types of FSHD, FSHD1 and FSHD2, that have similar clinical presentations yet are genetically distinct. The two distinct genetic mechanisms lead to a reduction of epigenetic silencing at the D4Z4 locus and increased DUX4 expression, which causes cellular toxicity. Approximately 95% of people with FSHD are diagnosed with FSHD1[1]. The key feature of FSHD is progressive skeletal muscle weakness, usually starting in the facial muscles and upper extremities, followed by trunk and leg muscle weakness[9]. Currently, no disease-modifying or curative therapy for FSHD is known. Several different treatments have been tested in clinical trials, such as β-agonists and myostatin inhibitors because of their muscle growth promoting properties and corticosteroids due to their anti-inflammatory properties[10-12]. Unfortunately, none showed a long-lasting improvement of muscle function. Losmapimod (GW856553), a small molecule selective inhibitor of p38 α/β mitogen-activated protein kinase (MAPK), has shown promising preclinical and early phase clinical results and may be a potential new treatment for FSHD[13]. It is hypothesized that p38 α/β MAPK plays a role in regulating DUX4 expression[14]. Inhibiting p38 α/β MAPK activity could consequently inhibit DUX4 production[15]. Multiple p38 α/β inhibitors showed robust reduction of DUX4 expression, activity, and cell death in patient-derived FSHD1 and FSHD2 myotubes *in vitro*[14]. In a mouse model of FSHD, treatment with losmapimod decreased *DUX4* expression and DUX4-driven gene expression in a dose-dependent manner without inhibiting myogenesis[13]. Losmapimod was initially developed for its anti-inflammatory properties because p38 MAPKs play a role in cellular stress responses from a range of sources, including the environment, intracellular insults, and pathologies[16]. Thus, losmapimod has been tested in several diseases such as chronic obstructive pulmonary disease, rheumatoid arthritis, cardiovascular-related diseases, major depressive disorder, and neuropathic pain from lumbosacral radiculopathy[17-22]. In total, more than 3500 healthy participants and participants with the above-mentioned diseases have received losmapimod in non-FSHD clinical studies, which showed a favorable safety profile, although anti-inflammatory effects did not translate to clinical efficacy in these conditions[18,22]. The transient anti-inflammatory effect of p38 inhibitors tends to dissipate due to redundancies in the inflammatory network; as such, one pathway could activate additional pathways that perpetuate inflammation[23]. A phase 1 trial of losmapimod in participants with FSHD demonstrated dose-dependent target engagement

in blood and muscle and a favorable emerging safety profile[24]. Furthermore, a population pharmacokinetics (PK) and target engagement model was used to determine losmapimod 15 mg twice daily (BID) as the optimal dose to treat FSHD[24]. Subsequently, two phase 2 clinical trials were initiated in 2019: a single-site open-label study with an open-label extension (OLE) to evaluate the safety and tolerability of long-term treatment with losmapimod (NCT04004000), the results of which are presented here, and a multi-site placebo-controlled trial to assess the efficacy and safety of losmapimod in participants with FSHD1 (ReDUX4; NCT04003974)[25,26].

Here, we report the findings from the phase 2, single-site, open-label study that investigated the safety, tolerability, PK, pharmacodynamics (PD), and exploratory efficacy of losmapimod in participants with FSHD who were treated with losmapimod for 52 weeks.

Methods

Ethical approvals

This study was conducted in compliance with the US federal regulations and was approved by the Regional Medical Ethics Committee (nr. 2018-4627) before participants were enrolled. The study was performed in accordance with the ethical principles in the Declaration of Helsinki, the International Council for Harmonization guidelines for good clinical practice, the laws and regulations of the Netherlands, and in accordance with the Medical Research Involving Human Subjects Act. Written informed consent was obtained from each participant at the start of the study.

Study design

This study is an open-label treatment study conducted at a single center in the Netherlands. After an initial screening visit, an 8-week period to establish baseline observations was followed by a 52-week losmapimod treatment period (Figure 1). After finishing the treatment period, participants were given the option to enroll in the OLE with clinic visits approximately every 12 weeks until study drug approval or until study termination. Participants who chose not to enroll in the extension discontinued losmapimod and were evaluated for safety approximately 4 weeks afterwards. During each visit, a range of measurements was assessed, including patient-reported outcomes (PROs), muscle biopsies, and motor function outcomes (Supplementary Table 1). The main portion of the study, which consisted of the 52-week treatment period, was conducted from August 2019 through March 2021; the extension portion is ongoing. Results from the main portion of the study are reported here.

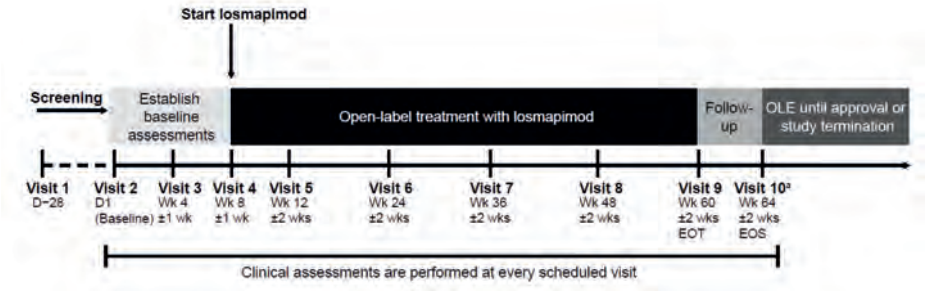

Figure 1. Study design

Enrolled participants received losmapimod for 52 weeks, with an optional OLE until losmapimod approval or study termination.

[a]Only participants who terminated the study after Week 60 attended the EOS visit (Visit 10).

D: day, EOS: end of study, EOT: end of treatment, OLE: open-label extension, Wk: week.

Participants

Eligibility criteria included a diagnosis of FSHD1 with a genetically confirmed D4Z4 repeat length of 1 to 9 repeats. Eligible participants were aged 18 to 65 years and were moderately to severely affected by FSHD, as determined by a Clinical Severity Score (Ricci score) between 2 and 4 (on a 0-5 scale). Furthermore, a biopsy-eligible leg muscle showing a short tau 1 inversion recovery–positive (STIR+) signal with some, but not excessive, fatty infiltration on the screening MRI, as determined by the central reader, was required[27]. Candidates were excluded if they experienced other diseases or comorbidities or used medications that could interfere with losmapimod's bioavailability, affect muscle functionality, or result in safety issues. Individuals that used a wheelchair or walker for any activity were not permitted to enroll in the study. Pregnant or lactating candidates were excluded.

Treatment

The treatment regimen consisted of two 7.5 mg tablets (15 mg total) taken orally BID with food for up to 52 weeks. Participants in the extension portion of the study continue to receive losmapimod 15 mg BID until study drug approval or study termination. Participants self-administered losmapimod by mouth BID with food (preferably meals). Treatment compliance was calculated based on pill count.

Endpoints and assessments

The primary endpoints were losmapimod safety and tolerability measures. Safety assessments were based on adverse events (AEs), vital signs, electrocardiograms (ECGs), and blood parameters. AEs were assessed on severity (mild, moderate, or

severe), relation to losmapimod intake (not related, possibly related, or related), treatment emergence[1], and serious adverse event status. Vital signs (blood pressure, heart rate, respiratory rate, and body temperature) were measured in the supine position after the participant rested for 5 minutes. ECGs were conducted in supine position after resting for 5 minutes. Once dosing with losmapimod started at Visit 4, ECGs were conducted 3 hours (±90 minutes) after morning losmapimod dosing. Blood tests consisted of hematology, clinical chemistry, and coagulation parameters. A urine pregnancy test was performed for female participants of childbearing potential.

Secondary endpoints were plasma and muscle concentrations of losmapimod and changes from baseline in phosphorylated HSP27 (pHSP27) in blood and in the ratio of pHSP27 to total HSP27 in blood and muscle. Losmapimod PK and PD were tested on blood samples that were taken at pre-dose and post-dose timepoints from Visit 4 onwards. Post-dose PK samples were taken within 15 minutes after the ECG, which was scheduled for 1.5 to 4.5 hours after administration of losmapimod. Post-dose PD blood samples were taken 3-4 hours after administration of losmapimod. PK and PD parameters were also measured in STIR+ muscle samples that were biopsied in the outpatient clinic using a Bergstrom needle, as described in the phase 1 losmapimod FSHD study[24]. The region for muscle needle biopsy in the STIR + muscle was informed by MRI coordinates and the same muscle was biopsied twice. The second biopsy occurred within 0.5 cm of the first biopsy.

Exploratory endpoints included changes from baseline during the dosing period in *DUX4*-regulated gene transcripts in skeletal muscle. The exploratory clinical outcome assessments included reachable workspace (RWS), muscle strength, physical function (Motor Function Measure Domain 1 [MFM D1]), ambulatory function (timed up-and-go [TUG] and 6-minute walk test [6MWT]), spirometry, and participant-reported outcomes (PROs) on disease impact.Six *DUX4*-regulated gene transcripts (*CCNA1*; *KHDC1L*; *MBD3L2*; *PRAMEF6*; *SLC34A2*; *ZSCAN4*) were measured by quantitative polymerase chain reaction (qPCR) of skeletal muscle biopsies both at baseline and post-treatment. The DUX4 composite score consisted of the mean normalized gene expression value across all six genes for each participant at each timepoint. The DUX4 composite score was summarized as change from pre-treatment to on-treatment. The individual components of the composite DUX4 score were also summarized at each timepoint, along with the change from baseline for on-treatment visits.

RWS, a three-dimensional, motion sensor-based outcome assessment, measures the range of movement of the upper extremities expressed as the surface area covered (in m^2) in each quadrant[28]. This value is then converted to a relative surface area (RSA), with 0.25 being the maximum for each quadrant, resulting in a total RSA value of 1 for all four quadrants. As the RWS also assesses reaching backward, a fifth quadrant was added, resulting in a maximum total RSA of 1.25 for all five quadrants. The RWS was performed both with and without a 500 g weight on each wrist and the RSA measure was calculated as the average of both arms.

Muscle strength was measured by handheld dynamometry (HHD) using the Microfet 2 (Hoggan Scientific, Salt Lake City, UT), quantitative muscle testing (QMT) using a bedframe myometry set-up and the Jamar device (Patterson Medical, Warrenville, IL). HHD was used to measure upper extremity strength, which included the hands, shoulders, and elbows combined; and lower extremity strength, which included right and left ankle dorsiflexors combined (Supplementary Table 2). Furthermore, QMT was used to measure upper extremity strength, which included the hands, shoulders, and elbows combined; and lower extremity strength, which included knee extension, knee flexion, and ankle dorsiflexors combined. Handgrip strength was measured using the Jamar device. The maximum weight in kilograms per visit as well as the change from baseline and percentage change from baseline were calculated.

The MFM consists of three domains focusing on standing and transfers (Domain 1), axial and proximal motor function (Domain 2) and distal motor function (Domain 3)[29]. Because Domain 2 and 3 have been shown to have a ceiling effect in participants with FSHD, only Domain 1 (D1) was assessed[30]. D1 consists of 13 exercises which are scored from 0 to 3 with 0 meaning 'cannot initiate task' and 3 meaning 'able to perform fully without compensatory movements'[29]. The participants' total scores were reported as a percentage of the maximum score of D1 with 0% meaning 'severe functional impairment' and 100% meaning 'no functional impairment'.

The TUG was performed using a standard chair (fixed height of 46 cm) with armrests[31]. Participants started in the chair and were directed to walk 3 meters, turn around, walk back and sit down again. They were instructed to walk at a comfortable and safe speed. The use of armrests or assistive devices such as ankle foot orthoses (AFOs) or crutches were allowed. The TUG was performed twice, and the mean duration in seconds of both tests was used for analysis.

For the 6MWT, two cones were placed 20 meters apart[32]. Participants had to walk as far as possible in 6 minutes, turning around the cones. Participants were not allowed to run; resting was allowed but time during rest would be accounted toward the 6 minutes. Assistive devices such as AFOs and crutches were allowed. The distance in meters walked after 6 minutes was recorded. Respiratory function was measured using a spirometer in a seated position preferably with a mouthpiece. If the participant could not use the mouthpiece (e.g. could not close their lips around the piece causing air to escape during the measurement) a silicone mask was used. Vital capacity (VC), forced vital capacity (FVC), and forced expiratory volume in 1 second (FEV_1). The highest value was recorded in liters and as a percentage of the expected value. Due to the COVID-19 pandemic, there were variable small sample sizes throughout the study; therefore, clinical meaningfulness could not be fully assessed. The FSHD-Health Index (FSHD-HI) has been developed to evaluate disease severity as experienced by patients with FSHD[33]. The questionnaire consists of 14 sub-scales that evaluate FSHD-related burden. Each item is scored on a 6-point scale ranging from '1: I do not experience this' to '6: This severely affects my life'. The total score for each domain is converted into a percentage with 0% representing no limitations and 100% representing severe disability[34].

The FSHD Rasch-built Overall Disability Scale (FSHD-RODS) is a patient-reported, linearly weighted scale that measures activities of daily living in participants with FSHD[35]. Each item is scored '0: not able to perform', '1: hard to perform', '2: easily performed' or 'not applicable'. The total score is converted into a percentage ranging from 0% to 100% using the Rasch-method, with 0% representing severe disability.

The Patient Global Impression of Change (PGIC) is a single item 7-point Likert scale questionnaire asking, 'Since the start of the study, my overall status is:' with possible answers ranging from '1: very much improved' to '7: very much worse.'

Data analysis

All safety data analyzed in this study originate from the safety analysis set, which was defined as participants who received ≥1 dose of losmapimod. All analyses were conducted using SAS software (SAS Institute, Inc, Cary, North Carolina) version 9.4. All descriptive statistics for continuous variables were reported using mean, standard deviation (SD), median, minimum, and maximum. Standard error (SE) was calculated for all change from baseline tables. Categorical variables were summarized as the number and percentage of participants. The sample size was not based on statistical considerations given the open-label design of this study. A sample size of 14 was considered sufficient to estimate safety, tolerability, PK, and

target engagement properties of losmapimod tablets during long-term dosing and to assess the potential impact of treatment on biomarkers and clinical outcome assessments compared with the pre-treatment period.

Results

Participants

Twenty-six participants were screened of which 14 were eligible for participation. Reasons for exclusion were no STIR+ eligible muscle for biopsy (n=8), ineligible medical history (n=2), and inability to complete the assessments (n=2). All 14 enrolled participants received at least 1 dose of losmapimod and completed the study. The median (range) age at enrollment was 50.5 years (range, 23-58 years) and most participants were male (79%; Table 1). Two participants declined participation in the OLE for reasons unrelated to losmapimod. Treatment compliance was approximately 98.5% across participants. Treatment was delayed for two participants by 12 weeks due to the start of the COVID-19 pandemic; therefore, these participants did not complete all RWS assessments or muscle biopsies.

Table 1. Participant demographics and clinical characteristics

	15 mg BID N=14
Age, median (range), years	50.5 (23-58)
Sex, n (%)	
Male	11 (79)
Female	3 (21)
Race, n (%)	
White	13 (93)
Other	1 (7)
BMI, mean (SD), (kg/m^2)	24.0 (2.9)
D4Z4 repeat category, n (%)	
1-3	3 (21)
4-9	11 (79)

Table 1. Continued

	15 mg BID N=14
Clinical severity score (Ricci score), n[a,b] (%)	
2	0
2.5	1 (7)
3	5 (36)
3.5	2 (14)
4	6 (43)

The majority of participants enrolled were male and had 4-9 D4Z4 repeats and a clinical severity score between 2.5 and 4.

[a] The Ricci score accounts for the extent and location of muscle weakness, and scores range from 0 (least severe) to 5 (most severe, wheelchair bound). Higher scores were assigned to participants who demonstrated pelvic and leg muscle weakness, as weakness of such muscles indicates disease progression (36).

[b]Individuals that used a wheelchair or walker for any activity were excluded from the study because of a Ricci score >4. One participant used crutches at enrollment and for the duration of the study; this participant's results were excluded from the analyses of change from baseline in TUG and 6MWT.

BID, twice daily; BMI, body mass index; SD, standard deviation.

Safety and tolerability

No deaths or serious treatment-emergent AEs (TEAEs) were reported. The most common TEAE was increased alanine transaminase (ALT) (n=5, 36%), all such events were mild and transient, and resolved with continued dosing (Table 2). All participants had ≥1 treatment-related (i.e., probably or possibly related) AE. Four participants had TEAEs that were assessed as probably related; one participant experienced myalgia and paraesthesias, 1 participant had nausea and headache, 1 participant had dizziness, and 1 participant had ALT increased and aspartate aminotransferase (AST) increased. The most frequently (≥3 participants) reported possibly related TEAEs were ALT increased (n=4 [29%]), dry skin (n=4 [29%]), eczema (n=3 [21%]), COVID-19 (n=3 [21%]), neutrophil count increased (n=3 [21%]), and white blood cell count increased (n=3 [21%]). Most TEAEs were considered mild to moderate in severity, and the majority of the TEAEs resolved. No TEAEs led to discontinuation of losmapimod treatment, and no participants withdrew from the study.

All severe AEs (N=7) occurred in a single participant, except for dry skin, which occurred in two participants. Additional severe AEs included hyperkeratosis, upper abdominal pain, back pain, intervertebral disc protrusion, and onychomycosis. Besides increased ALT and AST levels, no clinically significant changes in the vital signs, laboratory studies, or ECG results were observed.

Table 2. Treatment-emergent adverse events (TEAEs)

	NE	n (%)
≥1 TEAE	98	14 (100)
Any treatment-related TEAE	77	14 (100)
Any serious TEAE	0	0
Most commonly occurring TEAEs (≥10%):		
Alanine aminotransferase increased	5	5 (36)
Dry skin	5	4 (29)
Myalgia	4	4 (29)
Eczema	5	3 (21)
Aspartate aminotransferase increased	3	3 (21)
Neutrophil count increased	3	3 (21)
White blood cell count increased	3	3 (21)
Abdominal pain upper	3	3 (21)
COVID-19	3	3 (21)
Headache	3	3 (21)
Nail discoloration	2	2 (14)
Rash	2	2 (14)
Diarrhea	2	2 (14)
Fall	2	2 (14)
Back pain	2	2 (14)
Musculoskeletal stiffness	2	2 (14)
Pain in extremity	2	2 (14)
Nasopharyngitis	2	2 (14)
Dry eye	2	2 (14)
Nasal congestion	2	2 (14)

No serious TEAEs occurred. The most common TEAE was increased alanine aminotransferase.
AE, adverse event; BID, twice daily; NE, number of events; TEAE, treatment-emergent adverse event.

Pharmacokinetics and pharmacodynamics

The mean pretreatment baseline plasma losmapimod concentration was below the detectable limit. The mean predose plasma losmapimod concentrations ranged from 16.38 ng/mL at Week 44 to 27.90 ng/mL at Week 4 (Figure 2). Mean postdose losmapimod plasma concentrations (taken within 15 minutes after the ECG, which was scheduled for 1.5 to 4.5 hours postdose) ranged from 65.08 ng/mL at baseline to 94.52 ng/mL at Week 4. Mean losmapimod concentrations in muscle (74.1 ng/g at Week 44 to 85.9 ng/g at Week 4) were within the range observed in plasma.

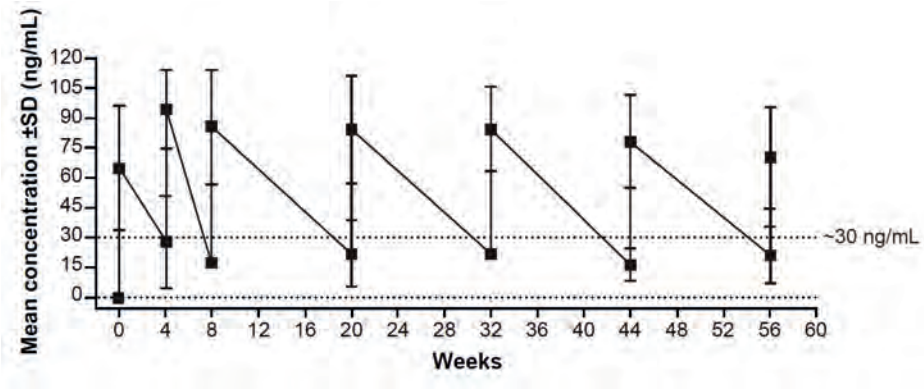

Figure 2. Losmapimod plasma concentration

Losmapimod concentration was measured in blood, and the mean concentration ranged from 16.4 ng/mL to 94.5 ng/mL A concentration of 30 ng/mL was determined to be sufficient for robust target engagement based on prior studies (Barbour et al. demonstrated an IC50 of 37.4 ng/mL in blood[37]). In cultured FSHD myotubes, a concentration of approximately 30 ng/mL resulted in a significant reduction of DUX4-driven gene expression and myocyte apoptosis)[24]. In an *in vitro* model of FSHD, losmapimod reduced DUX4 protein expression with an IC50 of approximately 11.5 ng/mL (30 nM)[14,39].
FSHD: facioscapulohumeral muscular dystrophy, IC50: half maximal inhibitory concentration, SD: standard deviation.

In whole blood, the ratio of pHSP27 to total HSP27 decreased from baseline to each predose and postdose timepoint, indicating target engagement. The percentage reductions from baseline in the pHSP27/total HSP27 ratios were −38.5% (95% CI: −46.0, −29.9) postdose after the first dose of losmapimod; −23.3% (95% CI: −34.2, −10.6) and −33.1% (95% CI: −40.7, −24.5), at predose on Weeks 4 and 44, respectively; and −39.5% (95% CI −48.3, −29.2) and −48.3% (95% CI: −55.4, −40.1) at postdose on Weeks 4 and 44, respectively. The ratio of pHSP27 to total HSP27 at Week 8 was not interpretable given the small sample size (n=2) and large variability (95% CI: −98.8-4282.5).

In muscle, the ratio of pHSP27 to total HSP27 decreased from baseline to Week 4 (−10.8%), indicating target engagement at that timepoint. The ratio of pHSP27 to total HSP27 at Week 8 was not interpretable given the small sample size (n=2) and large variability (95% CI: −78.3-726.5).

The muscle biopsy samples of ten participants were of high enough quality to examine DUX4 activity. DUX4-driven gene expression was highly variable; no meaningful mean changes from baseline in DUX4 activity in muscle biopsies were

observed during the dosing period for the composite score of selected DUX4-regulated gene transcripts (Figure 3A and B) or for individual gene transcripts.

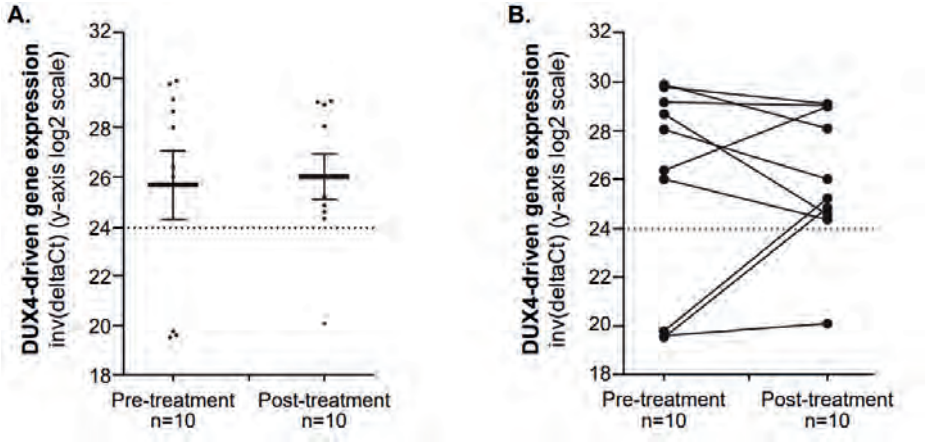

Figure 3. Mean (A) and individual participant (B) DUX4-driven gene expression in muscle biopsies
Expression of DUX4-regulated gene transcripts was measured from muscle biopses of 10 participants at baseline and during the dosing period. No changes in DUX4-driven gene expression were observed. inv(deltaCT): inverted delta cycle threshold.

Exploratory efficacy outcomes

Overall, improvement in RWS was observed after 52 weeks of treatment with losmapimod as shown by RSA measures averaged over both arms, with and without a 500 g weight (Figures 4A and B). The mean (SE) change from baseline in total RSA (Q1-Q5) with wrist weights was 0.037 (0.0185) (Figure 4A). The mean (SE) change from baseline in total RSA without wrist weights was 0.039 (0.0158) (Figure 4B). The mean (SE) change from baseline in RSA by individual quadrant, with and without weights, indicated stability or improvement in each quadrant (Table 3).

A post hoc analysis demonstrated increased annualized RSA for all quadrants (Q1-Q5). When assessed with a 500 g weight, the mean total RSA change per year was 4.39% (Figure 5). After 52 weeks of treatment with losmapimod, dynamometry analyses showed stability or improvement in muscle strength from baseline in most muscles evaluated (Figure 6A). Quantitative myometry data showed no change in muscle strength from baseline at Week 52 (Figure 6B). On the PGIC 10 of the 12 participants (83%) reported stability or improvement after 52 weeks of treatment (Figure 7). The other two participants reported their status as minimally worse.

Minimal or no change from baseline was observed on the TUG, 6MWT, MFM, FSHD-RODS and FSHD-HI (Supplementary Table 3). TUG times after 52 weeks of losmapimod treatment showed a mean (SE) change from baseline of 0.018 (0.35) seconds, and FSHD-RODS showed a mean (SE) percent change from baseline of 0.5% (1.08). Similarly, FSHD-HI measures showed a minimal change from baseline with a mean (SE) change from baseline of −1.67 (1.82) points. The greatest improvement in the FSHD-HI measure was seen in shoulder and arm function (decrease of 4.66 points).

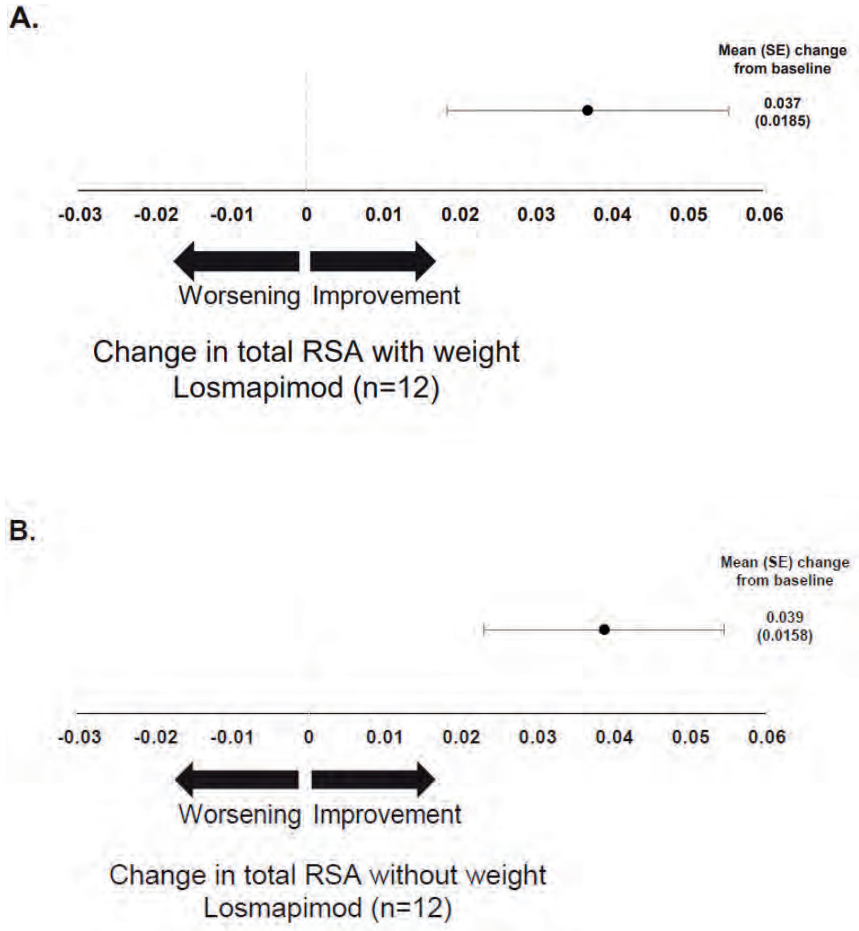

Figure 4. Average total RSA at 52 weeks with (A) and without (B) a 500 g weight

The mean change in average total RSA improved over 52 weeks of losmapimod treatment.
RSA: relative surface area, SE: standard error.

Table 3. Change from baseline in RSA by quadrant, with and without weights, after 52 weeks

RSA change from baseline, mean (SE)	Change from baseline with a 500 g weight (n=12)	Change from baseline without a 500 g weight (n=12)
Upper		
Q1	0.006 (0.0048)	0.006 (0.0033)
Q3	0.020 (0.0081)	0.020 (0.0086)
Lower		
Q2	0.002 (0.0044)	0.000 (0.0039)
Q4	0.006 (0.0047)	0.003 (0.0017)
Behind		
Q5	0.003 (0.0056)	0.010 (0.0053)

RSA improvement was observed in Q3 and stability was observed in the remaining quadrants.
Q: quadrant, RSA: relative surface area.

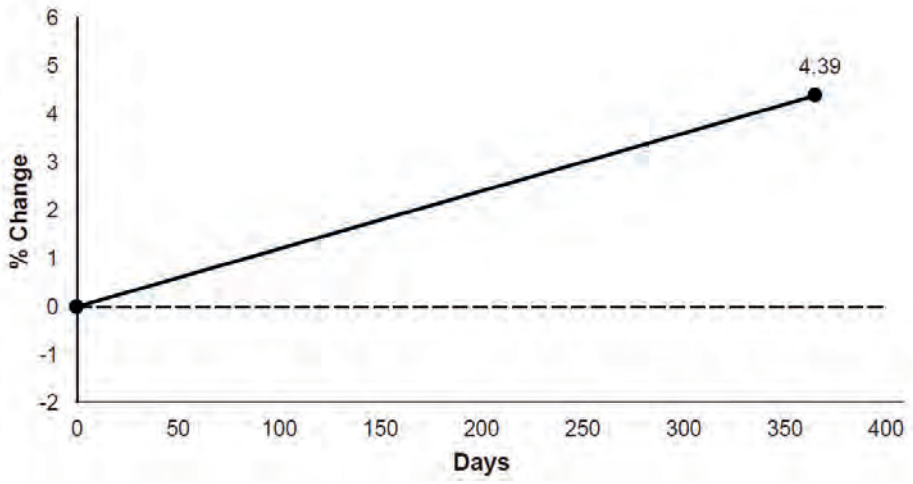

Figure 5. Exploratory annualized mean total RSA

The mean total (Q1-Q5) RSA, with a 500 g weight, change per year was 4.39%.
Q: quadrant, RSA: relative surface area.

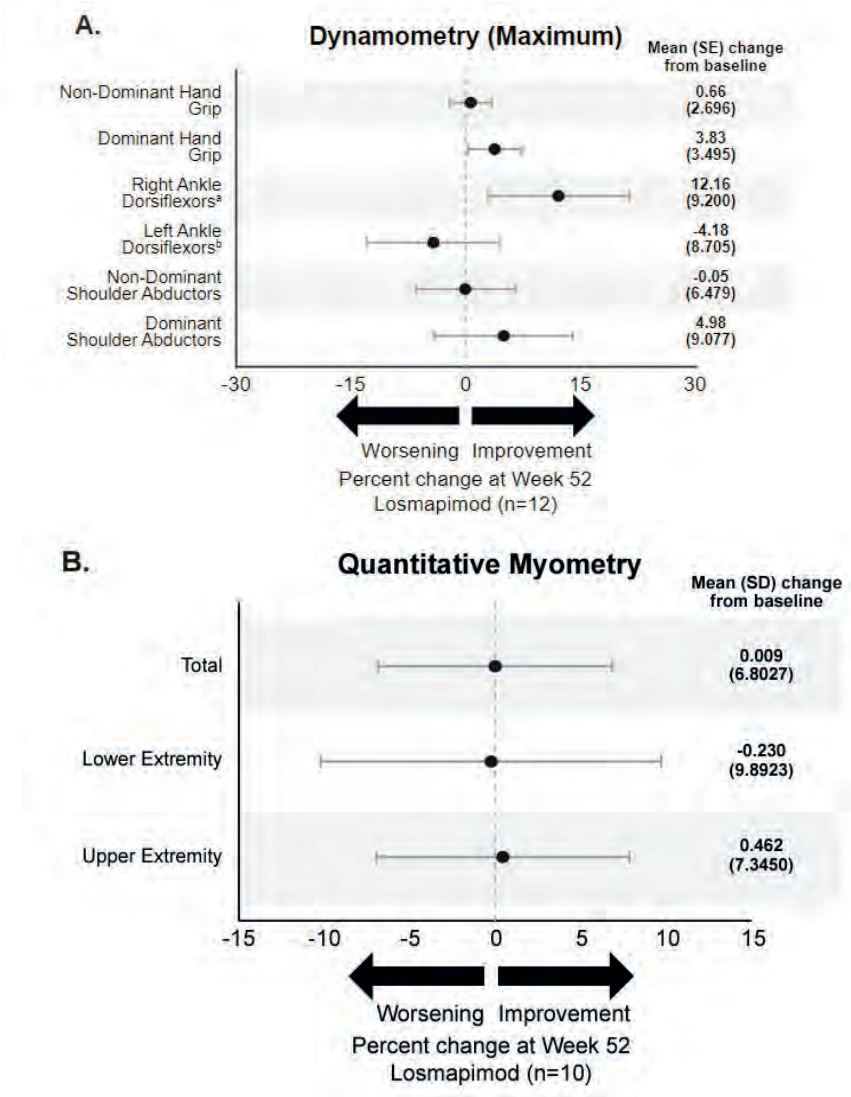

Figure 6. Muscle strength measurements by (A) HHD dynamometry and (B) quantitative myometry

Muscle strength, as assessed by HHD dynamometry (A), improved the greatest in the right ankle dorsiflexors between baseline and Week 52 of losmapimod treatment. Muscle strength, as assessed by quantitative myometry (B), remained relatively stable between baseline and Week 52.

[a]Losmapimod (n=9).

[b]Losmapimod (n=11).HHD: hand-held dynamometry.

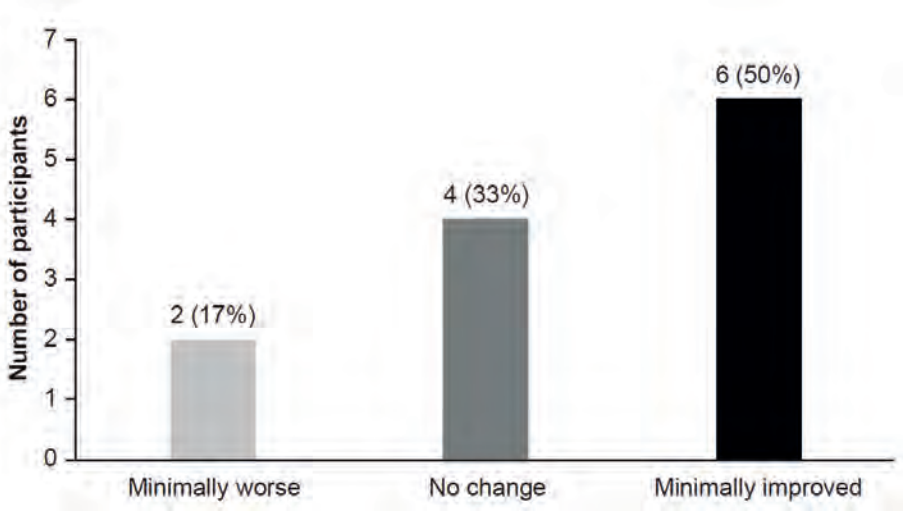

Figure 7. Participant-reported PGIC score after 52 weeks of losmapimod treatment[a]

After 52 weeks of losmapimod treatment, half of the participants reported their status as minimally improved.[a]n=12 completed the PGIC assessment. PGIC, Patient Global Impression of Change.

Discussion

This phase II open-label study demonstrated that long-term treatment with losmapimod was well tolerated by participants with FSHD. The blood and muscle concentrations of losmapimod were as expected based on prior clinical data[24], but changes in DUX4-driven gene expression could not be assessed due to the high variability. Participants remained stable or improved regarding clinical outcomes and PROs. In this long-term (52 weeks) study, losmapimod continued to demonstrate a favorable safety and tolerability profile in patients with FSHD, consistent with observations in >3500 healthy participants and patients with disease exposed to losmapimod in non-FSHD clinical studies[22]. No serious TEAEs, deaths, or TEAEs leading to study discontinuation were observed in this study. The most commonly reported TEAE was increased ALT, which in all cases was mild and resolved with continued dosing. The majority of the possibly related dermatologic TEAEs were of mild severity and were quickly resolved with topical treatment.The proposed losmapimod dose for humans enrolled in FSHD clinical trials is 15 mg BID. Based on prior studies, this dose provides drug concentrations that are sufficient to significantly inhibit p38α/β MAPK and reduce aberrant expression of DUX4 and DUX4-driven gene expression in skeletal muscles. In this study, mean losmapimod plasma concentrations were within a range consistent with that observed in the phase I trial of losmapimod in participants with FSHD[24]. Additionally, there is a clear

relationship between losmapimod plasma concentrations and target engagement for p38α/β MAPK, as measured by pHSP27. PK/PD modeling indicated that the oral 15 mg BID dose should result in 40% to 70% pHSP27 reduction[24,37,38]. In this study, following losmapimod administration, the pHSP27/total HSP27 ratio decreased approximately 40% to 50% from baseline, indicating target engagement. In an *in vitro* model of FSHD, losmapimod reduced DUX4 protein expression with a half-maximal inhibitory concentration (IC50) of approximately 11.5 ng/mL (30 nM)[14,39]. Based on the *in vitro* data and the results of this study, IC50 was reached in the participants enrolled. The DUX4-driven gene expression data that were extracted from the available biopsies were highly variable, which may be due to the small sample size, and no conclusion could be drawn from these data. However, preclinical data, both *in vivo* and *in vitro*, have shown that losmapimod decreases DUX4 activity, DUX4 target gene expression, and markers of apoptosis[13,14,40]. Multiple factors may have contributed to the large variability in DUX4-driven gene expression observed in this study, including small sample size, the stochastic nature of DUX4 expression among differentiated myonuclei (1:1,000 to1:3,000), the diverse composition of cell types in the FSHD skeletal muscle, relative biopsy imprecision, and the fact that muscle biopsy itself (muscle trauma) may alter *DUX4* gene expression in the biopsied tissue[41-46]. High variability of DUX4 activity was also observed at baseline in both the losmapimod and placebo arms of the ReDUX4 trial and no difference in change from baseline between the groups was observed[47]. Currently, it is unknown whether the high variability in *DUX4* gene expression among individuals is a disease characteristic or whether it represents inconsistencies in sampling. The method of targeting 1 to 2 cm STIR+ foci using MRI-informed coordinates may be too imprecise to target the STIR+ muscle twice[42]. MRI-guided biopsies (i.e., biopsies performed while a participant is being scanned) could be a solution to ensure that the STIR+ muscle was targeted[48]. However, MRI-guided biopsies are more burdensome compared to the standard muscle biopsies[42]. Furthermore, not every hospital may have access to the equipment or the expertise for MRI-guided biopsies. FSHD is a serious, rare, progressive, and debilitating neuromuscular disease, characterized by muscle weakness and eventual loss of function. Muscle weakness occurs rostral to caudal, characteristically starting with facial and scapular weakness, followed by weakening in the lower extremities and trunk. Disease progression leads to difficulty using the arms to carry out activities of daily living, which significantly impacts the individual's independence[49]. A natural history study of individuals moderately affected by FSHD, defined as having a baseline RSA >0.2 and <0.7, reported a decline in weighted RSA by 3.0% over one year, as assessed by RWS. The decline was most apparent in the upper quadrants with weighted assessments (upper-lateral Q3: −13.2% and upper-medial

Q1: −15.8%)[50]. In the current study, RSA improvement was detected in Q3 and a trend toward stability was observed in the remaining quadrants. Results from the FSHD-HI and PGIC measures further suggest a trend toward improvement with losmapimod. Similar observations were made in the ReDUX4 trial, in which RSA declined in both the dominant (−6.41%) and non-dominant (−4.02%) arms of participants in the placebo group, while participants in the losmapimod group showed stability or improvement in RSA (26). Taken together, these data show that RSA is sensitive to change in FSHD and change in RWS is expected to be a suitable outcome measure of disease progression for subsequent trials. Results from one natural history study of individuals with FSHD showed no appreciable change (median [IQR] increase of 4 [−30, +21] meters) in 6MWT performance over a 12-month period[51]. In the current study, no effect was observed in 6MWT performance after 52 weeks of losmapimod treatment. The present study showed a trend toward improvement or stabilization in PRO measurements, such as the FSHD-RODS, FSHD-HI, and the PGIC. Although this study is not placebo-controlled, these results indicate a possible stabilization of disease progression with losmapimod treatment compared to natural history data and provide promising data for an ongoing phase 3 trial studying losmapimod in participants with FSHD1 and FSHD2[52,53].

This exploratory study was the first long-term study of losmapimod in FSHD and was conducted in a small number of patients with FSHD1 at a single site in the Netherlands to minimize patient heterogeneity and variability in assessments. Future trials should include a more genetically diverse sample size that includes participants with FSHD1 and FSHD2 as well as participants with a wider range of FSHD severity. Notably, the ongoing phase 3 trial includes participants diagnosed with either FSHD1 or FSHD2[52].

The biggest limitations of this study are the lack of a placebo group and the small sample size. In spite of these limitations, the data are consistent with the results of the larger, placebo-controlled ReDUX4 study[25,26]. Additionally, this study coincided with the COVID-19 pandemic, which caused challenges regarding data collection. For example, treatment was delayed for two participants by 12 weeks due to the COVID-19 pandemic; therefore, these participants did not complete all RWS assessments or muscle biopsies. This open-label, phase 2 study is still ongoing in the form of an extension study. Together with the extension of the ReDUX4 study, these data will collectively contribute to understanding the safety, tolerability, and efficacy of long-term losmapimod treatment in approximately 100 participants with FSHD. Furthermore, an international, phase 3 randomized, placebo-controlled trial to study the efficacy of losmapimod is currently ongoing[52].

Conclusion

Losmapimod is a twice-daily oral therapy that showed a tolerable safety profile after 52 weeks of treatment in participants with FSHD. Although reduction in DUX4-driven gene expression could not be established, clinical outcome assessments showed stability or improvement after 52 weeks of treatment with losmapimod in a disease in which progressive decline of muscle function is inevitable in the absence of an efficacious treatment. A larger phase 3 study is necessary to assess the efficacy of losmapimod.

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Supplemental Data

Supplementary Table 1. Overview of the measurements

Outcome domain	Measurement	Visit number
Screening		
Demographics	Age, sex, race, ethnicity	1
Disease characteristics	First FSHD symptoms, age of onset, age of diagnosis, family history, D4Z4 array repeat size, Clinical Severity score (also completed at Visit 9)	1
General health	Physical examination, vital signs, ECG, genetic confirmation of FSHD, hCG serum test, hematology, serum chemistry, MRI, height, weight, medical history, current use of medication;	1
	coagulation[a], serology, urine drug screen, serum follicle-stimulating hormone test[b]	1, 7 (for muscle biopsy)[a]
Primary outcomes		
Blood tests	Hematology, clinical chemistry	1 and ≥4
	pregnancy	1: serum hCG test 4-9: urine hCG test
Vital signs	Blood pressure, heart rate, breathing rate, temperature	All
Heart function	Electrocardiogram	1, ≥4
Adverse events	Diagnosis, start and stop date, severity, relation to study drug, medication use, consequence on study drug intake, serious?	All
Secondary outcomes		
Pharmacokinetics	Blood sample	4-9
Pharmacodynamics	Blood sample	4-9
	Muscle needle biopsy	4[c], 5[d], 8[d]
Exploratory outcomes		
Functional	Reachable workspace	1, 3-9
	Timed up-and-go	1, 3-9
	Handheld dynamometry	1, 3, 5, 6, 8, 9
	Quantitative muscle testing	1, 3, 9
	Motor Function Measure Domain 1	1, 4, 6, 8, 9
	6-minute walk test	1, 3-9
	Spirometry	2, 6, 8, 9
Questionnaires	FSHD Health Index	1, 3, 6, 9
	FSHD Rasch-built overall disability scale	1, 3, 6, 9
	Patient Global Impression of Change	5-9

Supplementary Table 1. Continued

Outcome domain	Measurement	Visit number
Imaging	Muscle ultrasound	2, 5, 6, 7, 9
	MRI	1, 7, 9
Other	Biomarker discovery	4, 5, 8
	Outpatient mobility assessment using wearables	Intermittently from Visit 2 until EOS

[a]Coagulation test was performed at baseline.
[b]Serum follicle-stimulating hormone was only measured when a female participant was considered post-menopausal.
[c]Muscle needle biopsy at Visit 4 was performed pre-dose.
[d]Some of the participants underwent the follow-up biopsy at Visit 5 and others underwent this biopsy at Visit 8.
ECG, electrocardiogram; EOS, end of study; FSHD, facioscapulohumeral muscular dystrophy; hCG, human chorionic gonadotropin; MRI, magnetic resonance imaging.

Supplementary Table 2. Positioning during HHD and QMT.

Muscle group	Positioning		
	Participant	Extremity	Strap / HHD Device
Shoulder abduction	Lying in supine position	Shoulders at 90 degrees abduction, forearm pronated	On the upper arm, just proximal to the epicondyles
Shoulder external rotation[a]	Lying in prone position	Shoulder at 90 degrees abduction, forearm pronated	Around the wrist, just proximal to the styloid process
Elbow flexion	Lying in supine position	Shoulders at 0 degrees abduction, forearm supinated	Around the wrist, just proximal to the styloid process
Elbow extension	Lying in supine position	Shoulders at 0 degrees abduction, forearm in neutral position	Around the wrist, just proximal to the styloid process
Knee flexion[a]	Sitting at the end of the table	Knees in 90 degrees	Around the lower leg, just proximal to the ankle
Knee extension[a]	Sitting at the end of the table	Knees in 90 degrees	Around the lower leg, just proximal to the ankle
Ankle dorsiflexion	Lying in supine position	Ankle at 90 degrees of dorsiflexion	Around metatarsal heads
Hand grip	Sitting position	Shoulders and wrist in neutral position, elbow in 90 degrees of flexion	Jamar device in second position

[a]These assessments were not performed using the HHD device.
HHD, handheld dynamometry; QMT, quantitative muscle testing.

Supplementary Table 3. Additional outcomes

Outcome Measure	N	Mean (SE) change from baseline at Week 52
TUG[a,b]	11	0.018 (0.35) seconds
6MWT[a,b]	11	0.46 (5.83) meters
MFM (Domain 1)[a]	12	-1.28 (1.28) percent
FSHD-RODS[c]	14	0.5 (1.08) percent
FSHD-HI[c]	14	-1.67 (1.82) points

[a]Two participants were excluded from the analysis due to COVID-19 related delays in starting losmapimod treatment.
[b]One participant was excluded from the analysis due to the use of bilateral crutches.
[c]Due to missed visits related to COVID-19, the FSHD-RODS and FSHD-HI results were presented by protocol visit as opposed to analysis weeks.
6-MWT: 6-minute walk test, FSHD-HI: facioscapulohumeral muscular dystrophy health index, FSHD-RODS: facioscapulohumeral muscular dystrophy-Rasch-built Overall Disability Scale, MFM: motor function measure, TUG: Timed up-and-go.

Chapter 6

Assessment of the Burden of Outpatient Clinic and MRI-guided Needle Muscle Biopsies as Reported by Patients with Facioscapulohumeral Muscular Dystrophy

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Abstract

Muscle biopsies are used in clinical trials to measure target engagement of the investigational product. With many upcoming therapies for patients with facioscapulohumeral dystrophy (FSHD), the frequency of biopsies in FSHD patients is expected to increase. Muscle biopsies were performed either in the outpatient clinic using a Bergström needle (BN-biopsy) or in a Magnetic Resonance Imaging machine (MRI-biopsy). This study assessed the FSHD patients' experience of biopsies using a customized questionnaire. The questionnaire was sent to all FSHD patients who had undergone a needle muscle biopsy for research purposes, inquiring about biopsy characteristics and burden, and willingness to undergo a subsequent biopsy. Forty-nine of 56 invited patients (88%) completed the questionnaire, reporting on 91 biopsies. The median pain score (scale 0-10) during the procedure was 5 [2-8] , reducing to 3 [1-5] and 2 [1-3] after one and 24 hours respectively. Twelve biopsies (13.2%) resulted in complications, eleven resolved within 30 days. BN-biopsies were less painful compared to MRI-biopsies (median NRS: 4 [2-6] vs. 7 [3-9], p=0.001). The burden of needle muscle biopsies in a research setting is considerate and should not be underestimated. MRI-biopsies have a higher burden compared to BN-biopsies.

Keywords

Facioscapulohumeral muscular dystrophy, Muscle biopsies, Burden, Questionnaire, Retrospective

Abbreviations

FSHD = Facioscapulohumeral muscular dystrophy
STIR+ = an increased signal intensity using short tau-inversion recovery sequences
BN = Bergström needle

Introduction

Facioscapulohumeral Dystrophy (FSHD) is one of the most common inherited myopathies affecting roughly 1:10,000 people[1,2]. Symptoms usually start with weakness of the face, shoulder and upper arm muscles, progressing into weakness of the leg, girdle and trunk muscles[3]. FSHD is diagnosed by genetic testing using blood samples, so most FSHD patients have never undergone a muscle biopsy before participating in a trial. However, phase I and II clinical trials are expected to include muscle biopsies to demonstrate proof of target engagement at a molecular level. FSHD is caused by expression of the transcription factor DUX4 and its downstream genes in muscle cells[4]. Thus, examination of treatment effects in FSHD will mainly comprise of comparing pre- and post-dose gene expression profiles in muscles cells. With the improved understanding of the pathophysiology and the development of innovative therapies, a significant increase in the number of clinical trials, and therefore muscle biopsies, is expected in the near future[5].

A muscle biopsy can be performed in the outpatient clinic or in a Magnetic Resonance Imaging (MRI) machine. Muscle biopsies in the outpatient clinic are easier to perform and schedule, but lack the precision of biopsies performed in the MRI which allows targeting of specific areas in the muscle [6,7]. The latter may be beneficial for FSHD, because 1) in some patients muscle atrophy is pronounced and a specific area needs to be targeted to obtain sufficient muscle tissue, 2) affected muscles have small patches of disease activity which can be identified as an increased signal intensity using short tau-inversion recovery sequences (STIR+) in the MRI machine[7,8].

An outpatient clinic biopsy using a Bergström needle (BN-biopsy) starts with marking the targeted area on the skin. After injecting local anesthesia, a small incision (ca. 5mm) is made in the skin through which the BN-needle (5 mm) is inserted[6]. While applying negative pressure, muscle tissue is cut and collected. After gathering enough tissue, the incision is closed with adhesive plasters and a pressure bandage is applied for 24 hours.

An MRI-guided biopsy (MRI-biopsy) starts with scanning the patient in the MRI-machine[7]. Using these images, the appropriate target area and needle trajectory is determined. The biopsy is then performed while the patient still lies on the MRI table. After injecting local anesthesia, a small incision is made (ca. 5 mm) and an MRI-compatible trocar (3.4 mm) is inserted. A repeat scan is performed to verify the position of the trocar and to adjust the position of the trocar if necessary. A

vacuum-assisted needle (3.7 mm) is then used to perform the biopsy. A final scan is made to confirm the biopsy site, followed by closing the incision with adhesive plasters and applying a pressure bandage for 24 hours.

Based on the different methods, we hypothesized that MRI-biopsies are expected to be more burdensome than BN-biopsies, because of the additional scanning, repositioning of the needle and gathering the muscle tissue using suction instead of cutting. However, little is known about the burden and possible complications of muscle biopsies in FSHD patients or the differences in burden between BN-biopsies and MRI-biopsies. In this retrospective, observational study we aimed to assess the burden of muscle biopsies performed in research setting in FSHD patients.

Methods

Study design

This was an observational, retrospective questionnaire study. Eligible patients were invited by e-mail including a link to the electronic questionnaire. After 1.5 months, a reminder e-mail was sent out. After three months, non-responders were invited by phone. After four months, the questionnaire was closed.

Study population

All Dutch-speaking adult FSHD patients who underwent needle muscle biopsies for research purposes were invited to participate. Five different studies involving BN- or MRI-biopsies were identified from which the participants received an invitation[9-13]. Four studies were performed in the Radboudumc (Nijmegen, The Netherlands) and one in the Centre for Human Drug Research (CHDR, Leiden, The Netherlands). In summary, the studies aimed to 1) measure the specific force in FSHD muscle tissue (Radboudumc, BN- or MRI-biopsies), 2) describe the inflammatory response in muscle cells (Radboudumc, BN- or MRI-biopsies), 3) explore eligibility of whole-body MRI and muscle biopsies for clinical trials (Radboudumc, BN-biopsies), or 4) test the safety of losmapimod treatment in a phase I (CHDR, BN-Biopsies) and a phase II clinical trial (Radboudumc, BN-biopsies)[9-13]. All FSHD patients participating in these studies had genetical confirmation of FSHD based on the global guidelines[14].

Questionnaire

A Dutch questionnaire was developed in Castor EDC inquiring about demographic characteristics, muscle biopsy experience and willingness to undergo a subsequent biopsy[15]. The questionnaire was adapted from the questionnaire in a recent

study on the burden of muscle biopsies in Duchenne muscular dystrophy[16]. The questionnaire consisted of 5-point Likert scale questions and open questions (e.g. number of days until pain free). An English version of the questionnaire can be found online in Appendix A.

Demographic characteristics consisted of date of birth, sex, self-reported disease severity (ranging from 1 – 'no symptoms' to 5 – 'very severe symptoms') and the number of muscle biopsies that had been performed. Responders were asked to report on the characteristics, physical and emotional burden on subsequent biopsies separately.

Biopsy characteristics consisted of which muscle had been biopsied, for which study it was performed, whether it was a BN- or MRI-biopsy and the date of the procedure. The physical aspects consisted of: pain level on a numeric rating scale (NRS) from 0-10 during biopsy, one hour and 24 hours afterwards, number of days until pain free, number of days until full use of the biopsied muscle was possible, the development of complications (yes/no complication followed by multiple answer options of the most common complications: hematoma, numbness of the skin, muscle weakness, infection, other) and duration of complications (number of days), the use of analgesics (yes/no analgesics and which one), and if a scar remained (yes/no scar, size of the scar in mm, 1 – 'not burdensome' to 5 – ' very burdensome').

Emotional aspects included fear and reluctance before the biopsy (1 – 'no fear' or 'no reluctance' to 5 –'a lot of fear' or 'a lot of reluctance') and the overall experience of the procedure in hindsight (1 – 'a lot better than expected' to 5 – 'a lot worse than expected).

Lastly, responders were asked about their willingness to undergo a subsequent muscle biopsy with answer options 'willing', 'only willing in case of a drug study', 'not willing', 'do not know').

Data analysis

The data was analyzed using IBM SPSS statistics version 27.0 (IBM Corp, Armonk, NY, USA). Figures were created using GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, California USA). Ordinal outcomes were reported in percentages and continuous outcomes are reported as median [interquartile range] as all continuous data were not distributed normally.

Demographic data was analyzed for all responders and for two subgroups: responders who reported on at least one BN-Biopsy and responders who reported on at least one MRI-biopsy. As some responders reported on multiple biopsies, the number of biopsies is not equal to the number of responders and some responders are in both the BN- and MRI-biopsy group.

Mixed model analyses were used to compare BN- and MRI-biopsies regarding pain scores, days until pain free and full use, and the overall experience in hindsight. Generalized Estimating Equations (GEE) were used to compare BN- and MRI-biopsies regarding the frequency of analgesic use (Binary GEE), the number of responders reporting one or more complications (Binary GEE), and the total number of complications (Poisson distribution GEE). A p-value <0.05 was considered statistically significant. As this study is of an explorative nature, no correction for multiple testing was applied.

Ethical consideration

The protocol was approved by the local ethics committee (CMO-nr: 2020-6981). The data was handled according to Good Clinical Practice guidelines and the local privacy laws.

Results

Demographics

Fifty-six patients who underwent at least one muscle biopsy in one of the studies were identified and received the questionnaire, which was completed by 49 (88%) responders. Two (4%) patients refrained from participation indicating they could not remember sufficient details from their biopsy procedure and five (9%) patients did not complete the questionnaire. The 49 responders reported having undergone a total of 99 biopsies, but complete data of 91 biopsies was available. The median age of the responders was 54 years [44.5-58.5] with 29 (59.2%) responders being male (Table 1). Self-reported disease severity was most often reported as moderately affected (40.8%). Thirty-three (67.3%) responders underwent two muscle biopsies, and biopsies were mostly taken from the vastus lateralis (30.8%) or gastrocnemius (24.2%) muscles. The median time passed since the procedure for 47 biopsies (51.6%) was 18 [13-30] months. Fifty-five (60.4%) of the biopsies were BN-biopsies, 31 (34.1%) MRI-biopsies and five biopsies (5.5%) unknown. Five (10.2%) responders reported on at least one BN- and MRI-biopsy.

Burden of muscle biopsies

The median reported pain score was 5 [2-8] during the biopsy, 3 [1-5] after one hour, and 2 [1-3] after 24 hours. Responders reported to be pain free after 3[1-7] days and could use the biopsied leg fully after 2 [1-5] days (Figure 1a). When comparing the different muscles, biopsies from tibialis anterior were the least painful with a median pain score during procedure of 3 [2-7] and biopsies from the vastus lateralis were the most painful with a median pain score during the procedure of 6 [3-8] (Figure 1b). Analgesics had been taken after 27 (29.7%) biopsies, with paracetamol being the most common analgesic taken (19.8%). Twenty-five complications were caused by 12 (13.2%) biopsies, most commonly local hematoma (n=10, 40%), muscle weakness (n=5, 20%), and numbness of the skin at the biopsy site (n=4, 16%) (Figure 2a). No complications occurred after tibialis anterior biopsies, while two out of nine (22%) biopsies in the vastus medialis and four out of twenty-two (18%) resulted in one or more complications (Figure 2b). The hematomas resolved in 3 - 21 days; and numbness and muscle weakness in 10-30 days. One responder reported permanent (>999 days) muscle weakness of the biopsied muscle. Thirty-two (35.2%) visible scars were reported, with a median size of 5 [3-5] mm causing no to little burden.

Sixty-eight (74.8%) of the biopsies were preceded by no to little fear, and 61 (67.1%) with no to little reluctance. The overall experience in hindsight was a little to a lot better than expected in 50.6% of the biopsies and a little to a lot worse than expected in 31.9% of the biopsies (Figure 3).

Twenty-six (53.1%) responders reported to be willing to undergo another muscle biopsy for research, 5 (10.2%) were not willing, 5 (10.2%) were willing only in the case of a drug trial and 13 (26.5%) were unsure (Figure 4).

Table 1. Responders and biopsy characteristics

	All Biopsies (N=91) N (%)	BN-Biopsies (N=55) N (%)	MRI-Biopsies (N=31) N (%)
Unique Responders	49	25	20
Male	29 (59.2)	18 (72.0)	9 (55.0)
Female	20 (40.8)	7 (28.0)	11 (45.0)
Age, median [IQR]	54 [45-59]	53 [43-57]	55 [44-58]
Disease severity			
No symptoms	2 (4.1)	1 (4.0)	0 (0.0)
Mild	12 (24.5)	6 (24.0)	6 (30.0)
Moderate	20 (40.8)	12 (48.0)	7 (35.0)

Table 1. Continued

	All Biopsies (N=91) N (%)	BN-Biopsies (N=55) N (%)	MRI-Biopsies (N=31) N (%)
Disease severity			
Severe	14 (28.6)	6 (24.0)	6 (30.0)
Very Severe	0 (0.0)	0 (0.0)	1 (5.0)
Number of Biopsies*			
One	9 (18.4)	1 (4.0)	6 (30.0)
Two	33 (67.3)	21 (84.0)	11 (55.0)
Three	4 (8.2)	1 (4.0)	2 (10.0)
Four	3 (6.1)	2 (8.0)	1 (5.0)
Months passed since biopsy, median [IQR]**	18 [13-30] (n=47)	17 [13-22] (n=24)	16 [13-24] (n=19)
Biopsied Muscle			
Vastus lateralis	28 (30.8)	18 (32.7)	10 (32.2)
Vastus medialis	9 (9.9)	6 (10.9)	3 (9.7)
Tibialis anterior	17 (18.7)	12 (21.8)	3 (9.7)
Gastrocnemius	22 (24.2)	11 (20.0)	10 (32.2)
Other	10 (11.0)	5 (9.1)	4 (12.9)
Do not remember	5 (5.5)	3 (5.5)	1 (3.2)
Type of Biopsy			
BN	55 (60.4)		
MRI	31 (34.1)		
Other	5 (5.5)		
Study			
Specific force (9)	9 (6.6)	4 (7.2)	4 (12.9)
Inflammatory response (10)	29 (31.9)	2 (3.6)	25 (80.6)
Eligibility clinical trial (11)	0 (0.0)	0 (0.0)	0 (0.0)
Phase I Losmapimod (12)	21 (23.1)	21 (38.1)	0 (0.0)
Phase II Losmapimod (13)	23 (25.3)	23 (41.8)	0 (0.0)
Do not remember	9 (9.9)	5 (9.1)	2 (6.5)

The data of the responders' characteristics (Sex, Age, Disease Severity, Number of Biopsies) are based on the number of unique responders. The data of the biopsy characteristics (Biopsies Muscle and Type of Biopsy) are based on the number of total biopsies. Five responders are in both the BN-biopsy and MRI-biopsy group as they reported on both.

* Responders underwent a total of 99 biopsies, but not all responders reported on every biopsy. Data of 91 biopsies is available.

** A lot of data was missing regarding time passed since the biopsy procedure. Therefore, the number of biopsies reported on for this specific variable is reported and is different from the total number of biopsies.

BN-Biopsy = A muscle biopsy performed in the outpatient clinic with a Bergström Needle. MRI-Biopsy = A muscle biopsy performed in the magnetic resonance imaging machine. IQR = Interquartile range. BN= Bergström Needle. MRI = Magnetic Resonance Imaging.

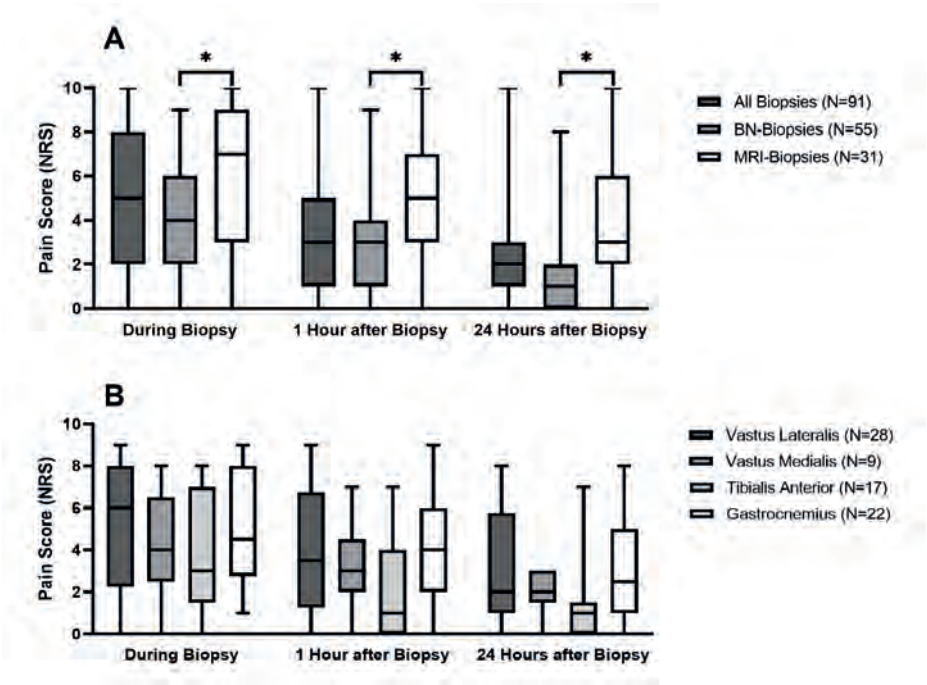

Figure 1. Pain levels during and after biopsy

Boxplots are shown for the reported pain levels during the biopsy procedure, one hour after the biopsy and 24 hours after the biopsy. Each bar represents the median, IQR and total range of the pain scores. The NRS ranged from 0-10.

A. This graph shows the pain scores of all biopsies (N=91) and compares the pain scores of BN-Biopsies (N=55) to MRI-Biopsies (N=31). The data of BN and MR biopsies is not completely independent from each other, as some responders reported on both BN and MR biopsies.

* Mixed models resulted in a significant difference for all pain scores: p=0.003 during the procedure, p=0.000 after 1 hour, and p=0.000 after 24 hours.

B. This graph shows the pain scores per biopsied muscle.

NRS= Numeric Rating Scale. IQR= Interquartile range BN-Biopsy = A muscle biopsy performed in the outpatient clinic with a Bergström Needle. MRI-Biopsy = A muscle biopsy performed in the magnetic resonance imaging machine.

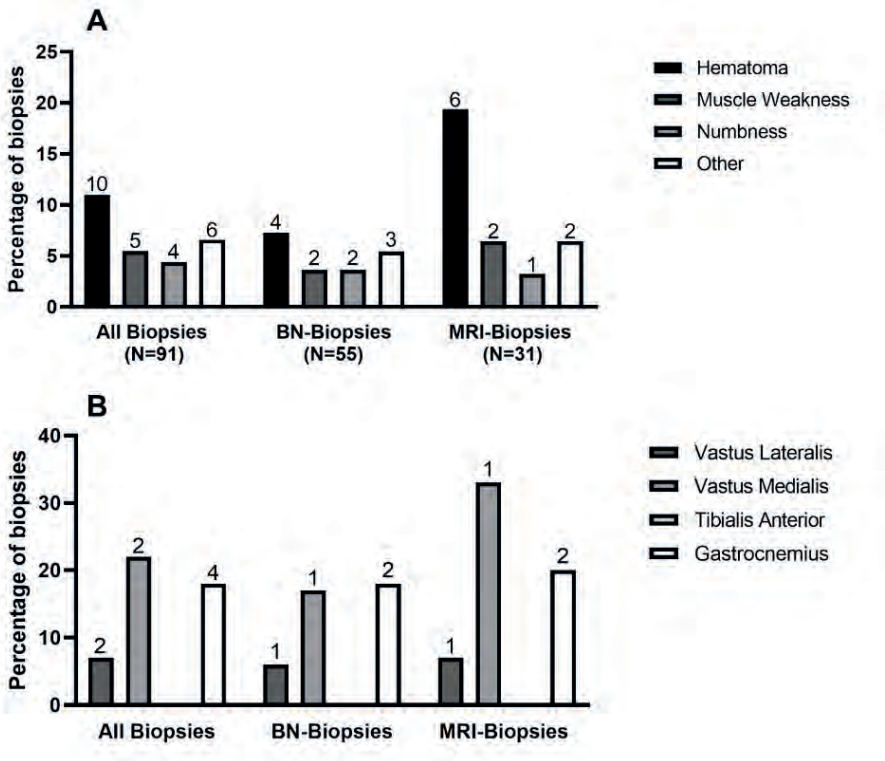

Figure 2. Complication rate of biopsies

A. The height of the bars indicate the percentage of the biopsies that resulted in the concerned complication. The numbers above the bars indicate the absolute number of the reported concerned complication. No significant difference was found in the number of responders reporting one or more complications (5 (20%) vs. 6 (30%), p=0.174) or complication rate (11 vs. 11, p=0.360) between BN- and MRI-biopsies.

B. The height of the bar indicate the percentage of biopsies that resulted in one or more complications per biopsied muscle. Noticeably, none of the tibialis anterior biopsies resulted in a complication.

BN-Biopsy = A muscle biopsy performed in the outpatient clinic with a Bergström Needle.

MRI-Biopsy = A muscle biopsy performed in the magnetic resonance imaging machine.

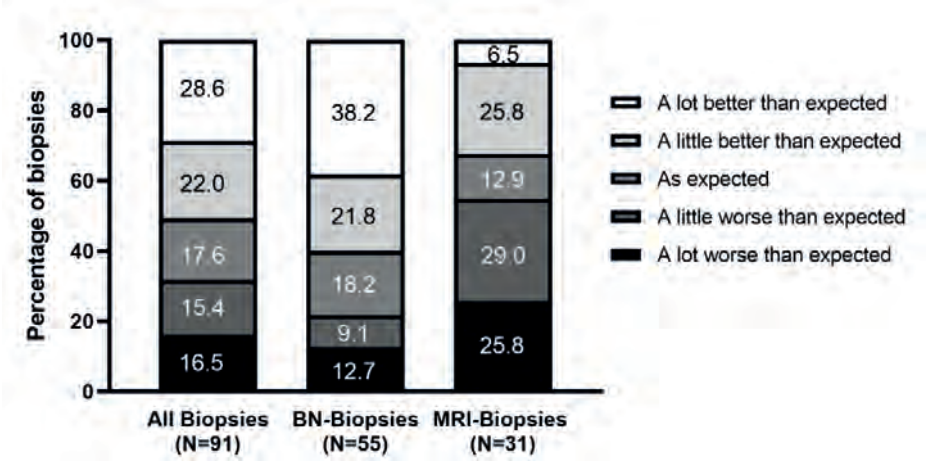

Figure 3. The experience of the biopsies in hindsight

Responders were asked if the biopsy was better, worse or as they had expected it. Data is based on number of biopsies as shown below each bar. The numbers in the blocks are the percentages for each answer option. The experience of BN-biopsies was considered significantly better compared to MRI-biopsies (estimated means (SD): 2.3 (0.2) vs. 3.5 (0.3), p=0.002).

BN-Biopsy = A muscle biopsy performed in the outpatient clinic with a Bergström Needle. MRI-Biopsy = A muscle biopsy performed in the magnetic resonance imaging machine.

Comparison of biopsy technique

Besides the higher male-female ratio in BN-biopsies, the demographic data of the BN-biopsy and MRI-biopsy groups are comparable (Table 1). BN-biopsies were mostly performed on the vastus lateralis (32.7%), tibialis anterior (21.8%) and gastrocnemius (20.0%), while MRI-biopsies were mostly performed on the gastrocnemius (32.2%) and vastus lateralis (32.2%).BN-biopsies caused less pain compared to MRI-biopsies: the median pain scores were 4 [2-6] vs. 7 [3-9] (p=0.003) during the procedure, 3 [1-4] vs. 5 [3-7] (p=0.000) after 1 hour, and 1 [0-2] vs. 3 [2-6] (p=0.000) after 24 hours (Figure 1a). Reported recovery of BN-biopsies did not differ from MRI-biopsies: responders were pain free after 3 [1-5] days vs. 5 [4-8] days (p=0.937) and could fully use the biopsied leg after 2 [1-5] days vs. 3 [2-8] days (p=0.925). Sensitivity analysis in which outliers were excluded gave similar results.

Analgesics were used less frequently after BN-biopsies compared to MRI-biopsies (11 (20%) vs. 14 (45.2%) respectively, p=0.029). No difference was found in the number of responders reporting complications (5 (20%) vs. 6 (30%), p=0.174) or the complication rate (11 vs. 11, p=0.360) between BN- and MRI-biopsies.

The analysis showed that the overall experience in hindsight was better in BN-biopsies compared to MRI-biopsies (2 [1-3] vs. 4 [2-5], p=0.002).

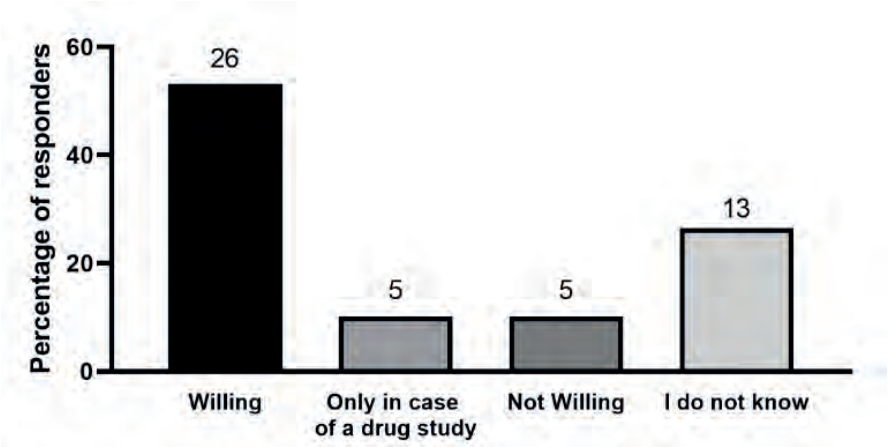

Figure 4. Willingness to undergo a subsequent biopsy

Percentage of responders (N=49) indicating if they were willing to undergo a subsequent biopsy for scientific purposes. The numbers above the bars represent the number of responders.

Discussion

The expected increase in clinical trials in FSHD requiring muscle biopsies calls for a better understanding of the burden of muscle biopsies and the willingness of patients to undergo muscle biopsies. This study showed a relatively high, but short-term burden of biopsies, favoring BN- biopsies compared to MRI-biopsies. Complications were frequent but short-lasting. The tibialis anterior muscle seems to be the most patient-friendly biopsy site. The pain score and number of complications were higher than expected based on our clinical experience with BN-biopsies. However, our data corresponds well with other studies reporting on BN-biopsies. Dengler et al. reported a mean NRS of 4.5 (±2.7) of biopsies in the m. deltoideus from 33 ALS patients[17]. Another study with 17 patients showed a NRS range of 4-6 in m. vastus lateralis biopsies[18]. Unfortunately, these studies did not report on the number of complications, so we cannot verify the complication rate of our study. Possibly important to note is that Dengler et al. stab incised the muscle fascia before entering the muscle with the BN. The BN-biopsies in the included

studies in this paper penetrated the muscle fascia with the BN. It is unclear which method is less burdensome and needs to be investigated in future studies.

In line with the more extensive nature of the MRI-biopsy procedure, a higher patient burden was reported compared to BN-biopsies. MRI-biopsies should therefore only be performed if the additional benefits outweigh the burden, which might be the case for FSHD[7]. The phase I and II trials investing losmapimod used BN-biopsies to target STIR+ lesions (1-2 cm) previously found on MRI[12,13]. Prior to the biopsy procedures, participants would be scanned with markers on the legs to acquire the coordinates to a STIR+ lesion. Before starting the BN-biopsy procedure, the markers (or a grid with marker placement) would need to be placed in the exact same way on the leg to target the identified STIR+ lesion using the MRI-acquired coordinates. This method involves multiple steps that are error-prone, especially considering the small sizes of the STIR+ lesions. Confirmation of the needle in a STIR+ lesion using an MRI-biopsy would have been more reliable, and MRI-biopsies could therefore be considered the superior method when targeting the aberrant DUX4 expression, even if the burden is higher. Furthermore, it was reported that the tissue size of MRI-biopsies was similar or larger than BN-biopsies and of high enough quality for histological evaluation[7].

The biopsies reported on in this study were all performed using Bergström needles or the MRI-compatible equivalent needle. However, different materials and techniques are available which might be more patient friendly. Firstly, in open biopsies an incision of ca. 3-4 cm is made, and muscle samples are cut from the muscle using a scalper or scissor. Open biopsies in the deltoid muscle were reported to be less painful compared to needle biopsies[17]. However, because of the larger incision, a more visibly scar may remain after open biopsies[16]. Secondly, the most similar to the BN-biopsy is a biopsy using a conchotome. After local anesthesia, a 1-2 cm incision is made in the skin and the muscle fascia is penetrated with a scalpel blade. The conchotome is then used to collect muscle tissue[19]. The conchotome may lead to more intramuscular bleeding, but it is easier to target a specific area of the muscle using the conchotome[20]. Thirdly, microbiopsies were not considered painful with NRS ranging from 0-1. However, the obtained sample weight is considerably lower (15-55 mg) compared to BN-biopsies (145-218 mg)[18]. Lastly, automatic biopsy devices can be used. Compared to Bergström needles, they require a smaller incision, and the procedure is faster as the devices quickly ejects and retracts the needle. Just as with microbiopsies, the samples are substantially smaller (15 mg)[21]. A recent systematic review compares the aforementioned methods regarding sample yield, diagnostic contributions and complication rate[22].

Currently, the Bergström needle biopsies are most often used in FSHD trials, because they allow for large samples, are cheap and easy to perform, and do not have long-term burden. Careful consideration about the necessary weight of the muscle tissue samples might enable the use of microbiopsy or automatic device biopsy, resulting in a more patient friendly procedure.

This is the first study directly comparing BN-biopsies and MRI-biopsies. The high response rate on the questionnaire allowed for a reliable comparison between the BN- and MRI-biopsies, although selection bias might be present based on the inclusion criteria of the studies. All the biopsies were performed in the Netherlands with the majority having been performed in the same hospital using the same BN-biopsy or MRI-biopsy protocol. The study at CHDR used the same BN-biopsy protocol as the Radboudumc BN-biopsy, but slight differences in the procedure cannot be ruled out. Still, we estimate a low chance of confounding of the data due to differences in the procedures.

Furthermore, both the Radboudumc and CHDR site have multiple years of experience in performing BN-biopsies, in total >100 BN-biopsies per year. Muscle MRI-biopsies were performed specifically for the two aforementioned studies. The MRI-biopsies were performed by intervention radiologists who performed >250 MRI-guided biopsies of the prostate or mammae per year, of which the latter used the same biopsy system as the muscle MRI-biopsies. Biased results caused by a possible difference in experience is therefore negligible.

The biggest limitation of this study is its retrospective nature. Most of the biopsies reported on were undergone one to two years before the questionnaire was sent, which may introduce recall bias.

We suggest that upcoming trials will inquire about the burden of the performed biopsies prospectively during trials using our questionnaire with some modifications. We propose adding two questions regarding 12-hour and 48-hour after biopsy timepoints to gain a more detailed insight on the course of pain levels and complications. Secondly, subsequent biopsies might be experienced differently based on the experience of the previously undergone biopsies. Prospective trials will allow for a reliable analysis on follow-up biopsies. Thirdly, careful documentation on the tissue sample sizes and the quality of the samples would help in distinguishing between biopsy methods and which muscle would be preferred. Our results show that the tibialis anterior is the most patient-friendly biopsy site. However, without reliable data on the quantity and quality of the

muscle samples taken from the tibialis anterior, we cannot conclude that the tibialis anterior is the most optimal muscle to use for biopsies in clinical trials.

Conclusion

In summary, the burden of muscle biopsies should not be underestimated, but is relatively short-lasting. MRI-biopsies have a significantly higher burden compared to BN-biopsies and should be used only when the benefits of MRI-biopsies are essential for reliable measurements. Despite the high burden, most of the adult responders were willing to undergo a subsequent biopsy for research purposes.

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Chapter 7

The Participants' Perspective on Facioscapulohumeral Muscular Dystrophy Trials in the Netherlands – A Qualitative Study

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Abstract

Facioscapulohumeral muscular dystrophy (FSHD) is a hereditary muscle disease without an available cure. The first drug trials have started, including a phase II open-label study and a phase III double-blind randomized placebo-controlled trial assessing the safety and efficacy of losmapimod. Having a more in-depth understanding of the patient's experience of these trials will further enhance the design and recruitment of future trials. We aimed to explore the motivation, expectations, concerns, and experiences of FSHD patients in the first clinical trials in the Netherlands resulting in recommendations for future trials.Semi-structured interviews with participants of phase II and III losmapimod trials were conducted. The interview guide was based on previous conducted literature reviews and consultation of a patient representative. Participants were selected through convenience sampling. Four main themes were discussed: motivation for participation, expectations regarding study drug and trial visits, trial participation experience, and recommendations for future trials. The interviews were transcribed, anonymized, and analyzed using Atlas.ti version 23.1.1 using a deductive approach.Thirteen participants were interviewed; six phase II participants and seven phase III participants. The primary motivations to participate concerned altruistic motives, contribute to science or improve their own health status. The participants had realistic expectations of the effect of the study drug before trial participation. Overall, participants were positive about their trial participation. Specifically, the personal and transparent communication within a trusting and dedicated trial team was appreciated. The phase III participants reported a higher than expected psychological burden on participating in a placebo-controlled trial. Recommendations consisted of more frequent updates on the overall progress and results of the trials. This study presents the participants' perspective on FSHD trials, providing important key findings for future clinical trial design, study site practices and patient education.

Keywords

Neuromuscular Diseases, Qualitative Research, Patient Participation, Clinical Trials

Abbreviations

FSHD = Facioscapulohumeral Muscular Dystrophy
QOREC = Consolidated criteria for reporting qualitative research

Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is a hereditary muscle disease affecting approximately 1:2,000-10,000 persons[1,2]. Patients with FSHD usually experience weakness of the face, shoulder and upper extremity skeletal muscles initially, followed by weakness of the leg, hip girdle and trunk muscles[3]. Notably, approximately 20% of the patients eventually become wheelchair dependent[4]. The contemporary management of the disease consists of a multidisciplinary approach to reduce the symptoms of FSHD, e.g., by improving muscle endurance and reducing pain and fatigue[5]. Currently, no pharmaceutical treatment for FSHD exists.

From 2010 onwards, the understanding of the underlying genetic and pathophysiological mechanism of FSHD has increased[6]. This allows for the development of drugs that specifically target FSHD pathways. The first trials with possible new disease-modifying therapies have already started and the number of trials is expected to increase drastically in the coming years, with over twenty companies developing new interventions[7]. Two clinical trials are currently being conducted at the Radboud University Medical Center (Radboudumc) in Nijmegen, The Netherlands: a phase II single-center, open-label study and a phase III multi-center placebo-controlled study of losmapimod[8,9].

Several steps have been taken to improve clinical trial readiness: natural history studies were conducted, new clinical outcome measures were developed, and (inter)national patient registries were set-up[10,11]. Furthermore, several international collaborations were initiated such as FSHD Europe, the FSHD Clinical Trial Research Network and Project Mercury. On the contrary, evaluation of the trials from a patients' perspective is lacking while it could provide useful insights to improve future trial design[12]. For example, we evaluated the experienced burden of muscle biopsies performed in the phase II losmapimod trial and other studies using a quantitative survey. The burden of the biopsies experienced by trial participants was higher than expected. We therefore advised for more careful consideration when adding muscle biopsies to a study protocol, minimize the frequency and explore more patient-friendly methods[13]. In addition, we performed a scoping review regarding the experience of clinical study and trial participation in rare diseases. It describes the barriers, facilitators and lessons learned of patients with Duchenne muscular dystrophy and SMA in clinical trials[14]. Unfortunately, we could not find studies on the experience of FSHD patients. Therefore, we aimed to explore the motivation, expectations, concerns, and experiences of FSHD patients in a

clinical trial. The insights from this study are expected to improve future clinical trial design, study site practices and patient education.

Methods

Design and data collection

This study followed a phenomenological, interpretive design using semi-structured in-depth interviews with participants in the two FSHD drug trials conducted at the Radboudumc. Participants were informed verbally and in writing regarding the study's objective, methodology, and procedures of data storage. A junior researcher not involved in the execution of the trials (SB) performed semi-structured interviews with a duration of approximately one hour. The interviews were either performed on-site (generally combined with a study visit) or online via Microsoft Teams, depending on the preference of the participants. All interviews were recorded, and transcribed by the same junior researcher (SB). Findings are reported according to the Consolidated Criteria for Reporting Qualitative Research (COREQ) found online in supplemental data 3[15].

Study population and recruitment

In total, 20 participants of the phase II or phase III trial were invited for an in-depth interview. The participants were selected through convenience sampling by SB and JK. The invitees included 9 participants of the phase II trial and 11 participants of the phase III trial. These were patients with genetically confirmed FSHD, aged 18-65 years and a clinical severity score of 4-8 (i.e. weakness in upper and lower extremities while still being able to mobilize with possibly assistant devices). At the time of the interviews (June-July 2023), the phase II trial was running for approximately four years, while the phase III participants were enrolled for approximately 20-40 weeks. We aimed for an even distribution of phase II and III trial participants and sexes, yet with different ages. All patients were invited for participation in the study by SB through e-mail. When patients were interested in participation, they were contacted by telephone for further explanation and for scheduling the interview. Participants were included until data saturation was reached.

Phase II and phase III losmapimod studies

Losmapimod is a repurposed drug with a favorable safety profile tested in over 3500 individuals[16]. The phase II losmapimod study is an open-label, single center study aiming to assess long-term safety and tolerability of losmapimod treatment

in FSHD patients[8]. Secondary and exploratory outcomes included biomarkers in blood and muscle tissue (collected through two muscle biopsies), changes in imaging biomarkers (using MRI and muscle ultrasound) and changes in several strength and functional outcome assessments, such as the 6-minute walk test and manual muscle testing. Hospital visit duration ranged from approximately 3-6 hours. The baseline measurement consisted of 3 visits over 8 weeks, followed by visits every 12 weeks. After one year, participants had the option to enroll in an extension phase, continuing the visit schedule but with a reduced number of clinical outcome assessments.

The phase III losmapimod study is a randomized placebo-controlled multicenter study aiming to assess efficacy of the drug using the upper extremity function as the primary outcome measure[7]. After a screening visit, participants were randomized into the placebo or treatment group. After randomization, visits took place every 12 weeks. Hospital visit duration ranged from 1.5-3 hours. After 48 weeks of treatment, participants had the option to enroll into the open-label extension phase.

In this qualitative study, we purposefully included participants from both studies as they could provide different perspectives on trial participation. It is not our aim to compare the experience of participants regarding the two clinical trials, but notable observations between the trials will be reported.

Interview guide

The interview guide was developed based on several sources describing the concept of conducting in-depth interviews, previous conducted literature reviews regarding patient experiences, clinical (trial) experience and consultation of the patient representative and chairman of the FSHD Advocacy Group (AL)[12,17-19]. The interviews started with exploring the motivations for participation. Secondly, the expectations regarding participation, drug efficacy and risk of adverse events were discussed. Thirdly, we inquired about the communication related to the trial, including the method of recruitment, informed consent, scheduling of visits, instructions during the trial period and communication from the sponsor about study progression and results. Furthermore, the burden of uncertainty regarding receiving the treatment during a placebo-controlled trial was discussed with phase III participants. Lastly, the trust and hope in clinical trials were discussed as well as recommendations for future trials. The full interview guide is available online in supplemental data 1.

Ethical considerations

The study protocol was approved by the Radboudumc research ethics committee (file number 2023-16354). This ensures that the study is carried out in accordance with the applicable legislation (Medical Research involving Human Subjects Act and Medical Treatment Contracts Act).

Data analysis

Transcripts of the interviews were made by a native speaker (SB) and analyzed with Atlas.ti version 23.1.1 using a framework analysis. All participants were pseudo-anonymized in the transcripts, using numbers to indicate the participants. The transcripts were independently coded by two researchers (SB, LS) using a deductive approach to generate codes, based on the theoretical framework used to develop the interview guide. Subsequently, codes were divided into main themes and subthemes, as discussed by two researchers (JK, LS)[20,21]. The transcripts and interpretation of the findings Statement were not discussed or shared with the participants.

Reflexivity

Three researchers (SB, LS, JK) were involved in performing and analyzing the interviews. SB is a white male in his mid-twenties who performed this study as part of his final internship for his master's degree in Science in Society. He is familiar with neuromuscular diseases as he performed a previous internship on respiratory characteristics of Dutch individuals with a diagnosis of centronuclear myopathy at the Neurology department of Radboudumc. He attended several clinics with neuromuscular patients. He had no relation with any of the participants nor was he involved in the execution of the losmapimod trials. He was trained by a senior social scientist experienced in qualitative research. LS is a white female in her mid-twenties working at the Radboudumc as a PhD candidate after receiving her Biomedical Sciences master's degree. She has prior experience in conducting qualitative studies in neuromuscular disorders, but was not involved with FSHD patients or the losmapimod trials prior to this study. JK is a white male in his early thirties working as a trial physician for over five years conducting the phase II and III trial while finishing his PhD candidacy on trials in FSHD.

Results

Out of 20 participants that were invited, thirteen participants completed an interview. A total of seven males and six females were interviewed, with a median age of 53 years, ranging from 27-62 years old. One participant did not want to participate because she felt she could not reliably contribute due to the low number of weeks she was enrolled in the trial. Six participants did not respond to the initial invitation. Follow-up was not required since data saturation had been reached after 13 participants had been interviewed. Eight out of thirteen interviews were performed online, and five interviews were held face-to-face at the Radboudumc. Six participants were participating in the phase II trial, seven in the phase III trial. The spouse of one of the participants was present during the interview. Further patient characteristics are shown in Table 1.

A total of 718 quotes were identified, divided over the following main themes: Motivation (69 quotes), Expectations (136 quotes), Trial participation (461 quotes), and Recommendations (52 quotes) (Supplemental table 1). Trial participation is the most extensively discussed theme. This theme also had the most identified subthemes, especially Communication and trust, General trial experience, and study visits were extensively discussed (163 quotes each). An overview of the main themes and identified subthemes are presented in Figure 1.

Table 1. Participant characteristics

Participant	Sex	Current age (years)	Age atdiagnosis (years)	Phase trial (weeks in trial)	Work status(hours per week)	Location interview (Radboudumc/ online)
1	F	48	28	III (12)	32	Online
2	F	55	33	II	9	Online
3	M	57	12	III (24)	Not anymore	Online
4	M	27	14	II	32	Online
5	F	42	19	III (24)	15	Radboudumc
6	F	52	32	III (4)	Not anymore	Radboudumc
7	M	55	32	III (24)	<40	Radboudumc
8	M	57	40	II	40	Online
9	M	51	26	III (24)	32	Online
10	F	62	60	III (24)	32	Online
11	M	37	16	II	Not anymore	Online
12	F	53	43	II	24	Radboudumc
13	M	61	33	II	Not anymore	Radboudumc

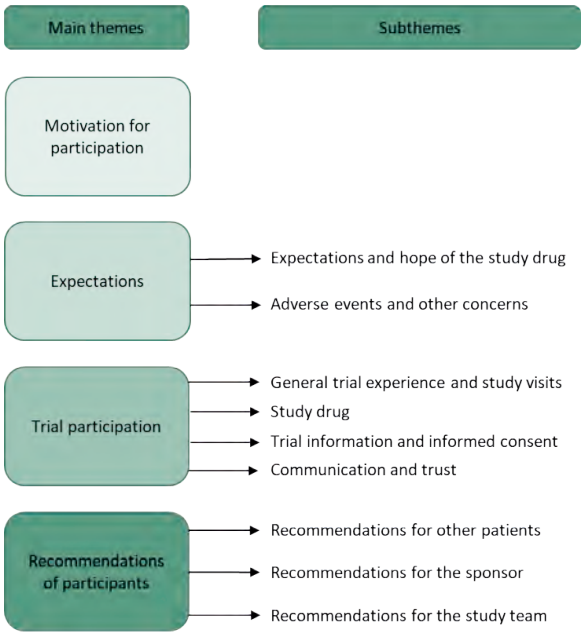

Figure 1. Main themes and subthemes Identified from Interview findings

Motivation for participation

A wide range of motivations for participating in the trials were reported (69 quotes). An overview of these quotes are presented in Table 2. Almost three quarters of the participants had no doubts in deciding to participate. The doubts of the other participants that were mentioned concerned questions regarding the possibility of continuing the drug after the trial was finished, possible side effects mentioned in the informed consent form, the time investment and the invasiveness of the study visits. More than three quarters of the participants participated from an altruistic perspective: to help the development of a therapy for their children or the new generation of FSHD patients. Almost half of them wanted to contribute to scientific research in FSHD, acknowledging that these kinds of studies are only successful if patients are willing to participate. The possibility of being enrolled in the placebo-arm was not considered as a barrier for participation by the Phase III participants. Participants were aware there was a 50% chance of receiving either the study drug or the placebo. They regarded this as something they could accept, which was reinforced by the opportunity of enrolling in the open-label extension study. The certainty of receiving losmapimod after 48 weeks in the open-label extension improved their motivation for participation Phase III participants additionally

reported that they were participating to potentially improve their own disease state and to get early access to a possible new therapy. Besides the primary motivations, more than a quarter of the participants felt a responsibility to participate in trials because of the rareness of the disease. Furthermore, participation gave them a sense of agency. Suffering from FSHD meant a continuous process of losing muscle function, either invoking sadness or fear for the future. Participating in a drug trial gave participants the opportunity to actively try to combat the disease, regardless of actual drug efficacy.

Table 2. Illustrative quotes on Motivation for participation

Subthemes	Quotes
Motivation	*"Yes, self-interest. Yes. That's really the most substantial, self-interest, and I hope that I benefit from it." P2*
	"And yes, it was obviously like this: if you received the placebo, you'd get the real medication in the end. So, either way, you'd receive the medication sooner than if you didn't participate." P9
	"Obviously for myself as well. I really want it to work for me too, but that's not reason number 1 for me. Reason number 1 is truly for my children and others. That there will be a medicine in the future that slows it down." P8

Expectations

In total 136 quotes were identified in this theme, with an overview provided in Table 3. The expectations were divided into two subthemes, including Expectations and hope of the study drug (101 quotes), and Adverse events and other concerns (35 quotes).

Expectations and hope of the study drug

Participants mentioned a clear distinction between their expectations and hopes regarding drug efficacy. In general, participants had realistic expectations about the study drug. They understood the experimental nature and unknown efficacy. The phase III participants understood the necessity for a placebo-arm. More than half of the participants did not expect a cure, but a halt or reduction of the disease progression. Because of the slowness of the disease progression they did not expect to notice any change in disease state during the studies.

Both the preclinical data and the results of the phase II study (through the patient information form, webinars, and trial physician) played a major role in these expectations and hopes while simultaneously increasing the motivation for participation. Besides this specific study, the increasing interest in drug development for FSHD by several pharmaceutical companies gave participants hope for a disease-modifying therapy in the future. For the phase II participants this was reinforced due to the start of the Phase III trial, which is supported by the same sponsor.

Adverse events and other concerns

Expectations on possible adverse events of the drug were based on the patient information provided for the study. In general, participants had no concerns pertaining to the adverse events, as the patient information included information about losmapimod's known, favorable safety profile in healthy individuals. The possibility on drug efficacy study effects outweighed the possibility on adverse events for deciding to participate in the trials.

Almost a quarter of the participants did report concerns about the time investment before starting with the study. Especially the participants with a full-time job mentioned they had concerns about combining their daily life activities with the required study visits. An additional concern mentioned was about the possible psychological effect if the drug turned out to be inefficacious, causing the sponsor to possibly stop the trial.

Table 3. Illustrative quotes on Expectations

Subthemes	Quotes
From/of study drug	*"I actually knew beforehand that there would be no medicine that would miraculously cure the FSHD. I mean, it's something in your DNA. If you could cure it with a pill, that would be very miraculous, but there's still that hope that it would slow it down." P1* *"You shouldn't have expectations of medications; you should go into it blank." P10*
Adverse events and other concerns	*"And in that sense, the doubt that was there was something like, 'Am I going to be able to handle that?' I have quite a busy job and, well, perhaps a bit less energy than the average person, due to FSHD." P2* *"Yes, of course. Because it's a medicine that's already been tested before, I was like, 'Yes, the chance that something could go wrong is relatively small. For example, as compared to a genetic test.' I would have to think about that a bit more carefully." P6*

Trial participation

Trial participation was the most common theme, with a total of 423 quotes, with an overview presented in Table 4. This includes the following subthemes; General trial experience and study visits (163 quotes); Study drug (55 quotes); Trial information and informed consent (80 quotes); Communication and trust (163 quotes). Overall, patients had a positive experience regarding the trial participation within the Radboudumc.

General trial experience and study visits

All participants expressed gratitude for being able to participate in the trial. Especially the phase III participants expressed this, as they were aware of the huge interest in the trial and the fact that not every patient could be included. Overall, it was appreciated that they were the only participant present during visits, receiving full attention from the study team. The guidance for each test was well received, although sometimes reported as too much repetition at subsequent visits. Due to the clearly communicated schedules, patients did not experience large surprises during the studies. Overall, participants of the phase II study mentioned a high intensity of the visits during the first year, sometimes having underestimated the burden on their body. On the contrary, phase III participants experienced the visits as less intensive than expected. In general, the muscle biopsies (phase II) and MRI scans(phase II and III) were reported as the most burdensome tests. Some of the functional tests were also considered burdensome in severely affected patients (e.g. the six-minute walking test for a participant with severely affected leg muscles).

Study drug

Unlike in daily life, almost a quarter of the participants mentioned to be confronted with their disease state and physical limitations every time they took a study pill, reporting this as a drawback for participating. This was most pronounced for phase III participants because of the possibility of receiving a placebo. During the trial, almost half of the participants seemed to have acquired an intense focus on physical functionality, wondering if a certain sensation could be an adverse event suggesting treatment with losmapimod, or experiencing disease progression suggesting taking placebo. Two participants hoped they were being treated with placebo as they did not experience any efficacy at the time. Regardless of phase II or III, participants reported on the difficulty of detecting any change over time due to the slow progression of the disease. This caused uncertainty on the efficacy of the drug and made it hard to reliably complete questionnaires about the disease progression.

Trial information and informed consent

The Dutch FSHD-registry was the most common channel through which participants had received information on the trials. Other channels were the patient advocacy group, treating physician, family, social media or during other study visits. All participants understood the patient information form, although two participants found the length of the letter daunting. Sometimes, participants also searched on the internet for additional information regarding the study, which was hard to find. More than three quarters of the participants shared the information with their partner or close family. There were some uncertainties after reading the information, which were addressed by the study physician via phone, email or during the study visits. This mainly concerned some general questions regarding the placebo, the trial design and reimbursement. On top of that, two participants experienced the language and process of signing informed consent as too formal and therefore difficult to fully comprehend or a hassle to complete for every new amendment.

Communication and trust

All participants reported that both the trial physician and study nurses were easily accessible for questions during the study. They felt the communication was transparent, personal, and non-hierarchical. In general, participants expressed appreciation about the study team setting realistic expectations for both the study drug and visits. Knowing what to expect, via a per visit schedule and a yearly schedule, was of great value for the participants. Due to a small, dedicated study team, participants always encountered the same physician and nurses, reportedly increasing the trust in the study team over the course of many study visits. The need for contact with other study participants differed. The study visits were scheduled individually with the intention of ensuring that patients do not come into contact with each other. More than half of the participants did not want to meet other patients, mainly because they were anxious to meet more severely affected patients, reminding them of what could be their future. More than a quarter of the participants did express the desire to share their trial experience with other participants, mostly wondering if other participants experienced any efficacy from the drug, or if others were inclined to benefit from this form of communication.

Table 4. Illustrative quotes on Trial participation

Subthemes	Quotes
General experience and study visits	*"I actually expected it to be more intense, but yeah, I got through all the tests in a few hours. And then the MRI is once every three months. Yeah, I don't think it's actually that bad." P8* *"But here, I feel exactly as if I'm the only one doing the study, so all the focus is on me when I'm here… but at the other place, you also came together with other participants from other studies, and that felt different. There, I felt more like a number. Yes, and here, that's not the case." P7*
Study drug	*"If you basically feel no side effects at all after a few weeks, then the anxiety will go away as well. After the first two days, it was already a lot less, but in the beginning, it's still somewhat nerve-wracking when taking the first pill. Maybe you won't feel well or whatever, or maybe you suddenly feel deterioration, or whatever side effect." P11* *"I'm a very down-to-earth person, but it still surprises me how much influence whether or not you get a placebo has on your thoughts. Especially in the first few months. Now I'm at peace with it, but I used to worry about it. Yeah, am I feeling something? I'm suffering from that now. Is it mainly because of the disease, or is it because of the medication? There's a bit of uncertainty involved when you're participating." P8*
Trial information and informed consent	*"Yes, they're about the same every time, communication went well… I think I've asked all the questions I wanted to ask, but it just felt right." P5* *"But indeed, during that webinar, I thought, 'Oh, hey, they apparently see something positive here, otherwise they wouldn't continue." P1*
Communication and trust	*"Yes, nice, pleasant people. Appointments are fast and efficient. You're approached personally; you don't feel like a number. I can't say anything other than that it's going very well and feels good." P8* *"So, in that respect, I find it very pleasant and also very important that there was really just one person who was there all the time." P10* *"And I do occasionally want some information on how other people are dealing with it, but often, those people are worse off than I am and then, well, you actually don't want to be confronted with all the troubles of other people." P9*

Recommendations for future trials

A total of 52 quotes were identified regarding the theme on Recommendations, with an overview in Table 5. This is divided over the subthemes Recommendations for other patients (13 quotes), Recommendations for the sponsor (12 quotes), and Recommendations for the study team (27 quotes). Although the participants were positive about the communication during the trial, most of the recommendations involved improving the communication.

Recommendations for other patients

None of the participants would advise against participating in a trial. Almost half of the participants would recommend it, describing it as a unique and pleasant experience and acknowledging the need for sufficient participants in rare disease trials. They also felt that they had contributed to scientific progress. Furthermore, almost half of the participants mentioned that the decision to participate is a personal decision depending on availability(e.g. full-time job), disease severity and travel time to the clinic.

Recommendations for the sponsor

Most of the recommendations were about the lack of communication as well as the lack of sharing study data by the sponsor. First, more frequent updates on the progress and results of the study would be greatly appreciated. Participants suggested the use of a recurrent newsletter from the sponsor. Furthermore, although most participants knew that study data could not be shared ahead of time, they would appreciate a personal data report on their disease progression. Lastly, communication regarding the process of reimbursement of travel costs and overnight stays was not entirely clear and could be improved.

Recommendations for the study team

Even though participants were overall positive regarding the communication with the study team, more updates from the study team would be appreciated. This mainly included more communication on the overall planning and updates concerning the progress of the study.

Table 5. Illustrative quotes on Recommendations

Subthemes	Quotes
For other patients	*"For me, this was a relatively individual consideration, and I think it would be for everyone. But without research, nothing will ever happen. So, yes, if you get the chance, go for it." P1*
	"There are obviously quite a few risks involved, so I would definitely say that everyone should decide that for themselves. Of course, I invite everyone to help in this, whenever they can. But I wouldn't want to force anyone to do this; they really need to weigh the risks and time and such for themselves." P11
For the sponsor	*"Yeah, I don't expect there to be a special presentation for 14 people. But it could simply be a newsletter distributed by the hospital among those participating here, that's also possible. It's not that you have to have direct contact, but I'd really appreciate it if I could get have insight into the status of the research from the pharmaceutical company." P8*
	"Well, maybe you can sign up for some kind of newsletter, or whatever, and then you can choose for yourself whether you want to receive it or not. But that at least some results could be shared." P3
For the study team	*"Yes, of course, the more communication, the better, even if it's just a small message that's published in the muscular disorder newsletter or posted online or shared on Facebook. You see, if there would be a brief update posted once a month, that would be very nice for the research. It doesn't have to be extensive. Or, just to mention something, if there's a new medication coming out and they're looking for new people." P6*
	"Yes, and that's often missing in the processes. That you don't really know when a certain period has ended. Exactly how long will it continue? Am I the last one? Or when can you expect the results? These may also be things that you probably don't know right away at the beginning. But just a brief update?" P5

Discussion

This qualitative study was initiated to gain insight in the trial experience of FSHD patients to improve future trial design, including site practices and patient education. The motivations for trial participation ranged from altruism to hope for improved health status. The participants' expectations regarding study drug efficacy were realistic (stabilizing muscle strength and function), while hope for a potentially higher efficacy was also reported. Trial participation was seen as a positive experience, largely driven by the personalized approach of the study team. Recommendations from the participants included more frequent updates on the progress and results of the trials. Participants acknowledged that the decision about trial participation requires personal considerations, but none of them advised against participation in a clinical trial. A summary of the most important key points are presented in figure 2. We will discuss these main findings below.Although this

study was performed specifically in FSHD patients, we hypothesize that most of the recommendations are also applicable for other neuromuscular disease trials, perhaps even for all rare disease trials. The findings in this paper are supported by our recent scoping review on trial participation in rare diseases and our qualitative study on trial experience of centronuclear myopathies patients[12,14]. The most important facilitators of trial participation were hope for improving the participants' health, altruistic motivations, and gaining a better understanding of the disease. Barriers included unknown efficacy and side effects, the chance of being treated with a placebo, and logistical and financial burdens (e.g., travel time, missed school- or workdays, out-of-pocket expenses). The possibility of receiving a placebo can be a barrier for participants, resulting in a patient's preference for clinical trials without a placebo-arm. In the case of the phase III losmapimod trial, this barrier was overcome by allowing participation in an open-label extension phase after completing the randomized trial phase. If possible, including an open-label extension phase or compassionate use program will benefit the recruitment process of future trials.

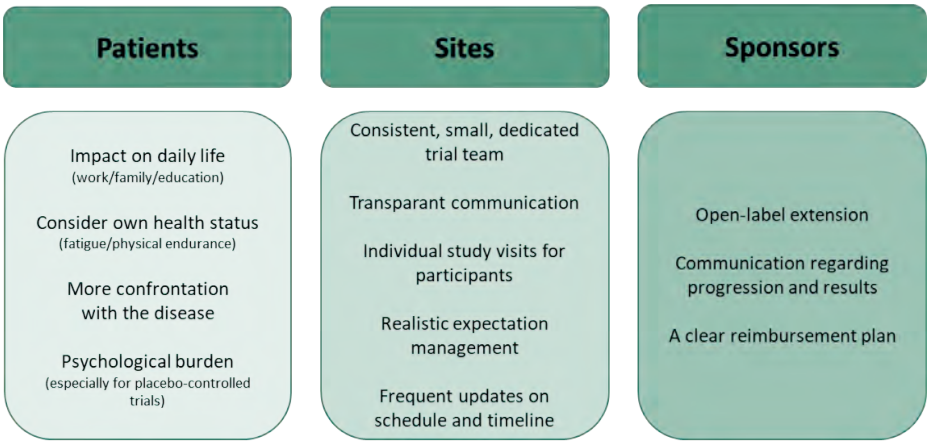

Figure 2. Key points

The logistical burden of future trials may differ depending on the study protocol, study population and the country in which the trial will be conducted. We observed that phase II participants experienced the trial as more burdensome in comparison to phase III participants. This was expected, as the phase II visits included significantly more muscle strength and function assessments compared to those of the phase III trial. Still, as opposed to other neuromuscular disease trials, the logistical burden of the losmapimod trials can be considered relatively low.

They required one visit every twelve weeks after a slightly more intensive study start[8,9]. This is conceivably less burdensome compared to, for example, a myotonic dystrophy study which requires a visit every four weeks (NCT05481879) or a Duchenne trial requiring weekly on-site intravenous infusion (NCT04060199)[22,23].

The losmapimod trials included mostly independent adult patients, which will be different from pediatric patients who require the support or presence of their parents or other caregivers to participate in a trial. Including care-dependent patients will increase the burden on trial participation significantly. For example, in the centronuclear myopathies trial parents often had to take time off from work to support their child during a trial visit[12]. Additional qualitative studies in other neuromuscular disorders will be important to make disease specific recommendations.

Travel time and overnight stays were reported barriers to participation in our scoping review, but not often mentioned by the participants of this study. This is most likely caused by the geographical region of this study (The Netherlands and Belgium); the mean travel time to the site was approximately 90 minutes and an overnight stay was optional based on the participant's preference. The travel time in larger countries will be significantly longer, possibly even include travel by airplane, which will increase the burden on the participants and caregivers.

Both in this study and the study on trial experiences in centronuclear myopathies, participants reported to participate for their own health benefit[12]. While we want to refrain from valuing the motivations for participating in trials, participating for personal health benefit warrants caution. As the efficacy of study drugs are still unknown, participating in trials for personal health benefit is a result of therapeutic misconception. A quantitative questionnaire study in participants with degenerative ataxia also reported on the misconceptions of the efficacy of an experimental treatment[24]. This suggests that therapeutic misconceptions are a widespread issue in rare diseases without an available treatment. Arguably, therapeutic misconceptions may interfere with informed consent and could therefore pose an ethical problem. The nuance of an experimental treatment compared to standard clinical care apparently seem to require additional attention when informing patients about a trial. An exemplary quote in our previous study was "*A trial is not a treatment*"[12]. Assessing the expectations and motivations of the participants before signing informed consent, for example during a pre-screening interview, should therefore become standard practice. Additionally, patient

education on trials should emphasize the difference between standard clinical care and receiving an experimental treatment.

Accurate patient education also serves other purposes. Patients need to be prepared on what trial participation entails to prevent dropouts or unexpected experiences. For example, patients reported a high psychological burden of participating in a trial (with the burden being higher in the placebo-controlled trial) or the discomfort of lying in the MRI machine for an hour. They would have liked to have been informed beforehand. Patient advocacy groups could play an important role in ensuring that the different forms of patient education matches the patients' expectations, is clear and understandable and is easily accessible. The Dutch Muscle Disease Foundation (Spierziekten Nederland) organizes a yearly patient conference to update patients. Patients benefit from the scientific and clinical information and enjoy the social aspect of sharing experiences with other patients. We think that (online) patient education events for new trials would be of great value. Webinars can reach more patients and are rewatchable; use of patient navigators provide a more personalized approach[25].

This study highlighted the patients' preferred interactions with the trial site. This can support site preparations for new clinical trials. The use of a small, dedicated trial team was greatly appreciated by all participants. It allowed for clear communication, personalized care, and building up trust and loyalty, which were all important aspects in maintaining motivation throughout the trial. Using a dedicated trial team also enables clear separation between trial and regular care visits, which is beneficial for both the researcher and the patient. Separation of a trial and care physician may reduce the chance on investigator bias, especially regarding the clinician's impression of change of the disease state. From an ethical perspective, the argument can be made that both the screening and the informed consent procedure will be influenced by the loyalty of the patient and treating physician. Still, we argue that it is beneficial to perform trials in centers with experienced treating physicians in the case of (unexpected) adverse events and to ensure that the trial team has sufficient knowledge on the disease.

Communications from the sponsor was the part that received most recommendations. The participants would have appreciated more frequent updates about the overall progression of the trials, drug efficacy and if possible, their own data. Trials in FSHD, and possibly other neuromuscular disorders, might require a duration of multiple years before efficacy is assessed (e.g. the phase II losmapimod trials is ongoing for more than four years). Keeping participants actively involved

during the trial by frequent updates will help maintaining the participants' motivation and in turn reduce the number of dropouts. To protect the privacy of patients and reduce the influence of the sponsor on the trials, sponsors are not allowed to have direct contact with participants. It is therefore important to identify the correct channels for informing the participants, while adhering to good clinical practice guidelines. Most of the participants suggested using a newsletter, which could be sent from sponsor to the investigators and subsequently be distributed to the participants. Another solution could be the use of a dedicated website as used for the FSHD Fortitude, which removes the need for additional channels to reach the participants[26]. Regardless of the method, a clear communication plan from the sponsor and sites would be highly appreciated by participants and should be in place before the start of the trial.

This is the first qualitative study exploring the experience of clinical trial participation in FSHD patients, resulting in several key points to improve trial readiness. It is important to interpret these results with the strengths and limitations of this study in mind. The interviews in this study were purposely performed by an independent researcher with the notion that the participants might be less inclined to give socially acceptable answers. Nevertheless, participants might have been reluctant to give negative answers because the trials were still ongoing. An aggregate approach in the data analysis was chosen to increase the generalizability of the results. Both phase II and phase III participants were included, which gave insights in the different motivations of participants per trial phase and the additional psychological burden of a placebo-controlled trial. The phase II trial was ongoing for more than four years at the time of this study, which gave better insight into the long-term experiences, but might have introduced recall bias. Although the recruitment of participants continued until data saturation was reached, we cannot rule out that selection bias might be present due to convenience sampling.

With the expected increase of clinical trials in neuromuscular diseases, studies on patient views and experience will become essential to inform the design and implementation of future trials. This study was performed relatively late in the losmapimod trial development process, diminishing the possibility of direct implementation. Therefore, we suggest that future patient view studies should be performed as early in the drug development process as possible. We suggest that prospective trial evaluation from the patient's perspective, quantitative or qualitative, should become an integral part of every future trial. The use of the patient involvement matrix can ensure the incorporation of the patient's perspective in future study/trial design[27]. Additionally, since it is expected that future trials will

expand their focus to pediatric populations, evaluating the views of the pediatric patients and their caregivers on clinical trial design will be essential[28,29].

In conclusion, the overall experience was positive and none of the participants would advise against trial participation. This study resulted in valuable key points to take into account for patients, sites and sponsors. Additional qualitative or quantitative / prospective studies in other geographic regions and patient populations are necessary to optimize the trial design according to the patient's views and experience.

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Supplemental Data

Supplementary Table 1: Total number of quotes for the different themes

Themes	Number of quotes
Motivation for participation	**69**
Expectations	**136**
Expectations and hope of the study drug	101
Adverse events and other concerns	35
Trial participation	**461**
General trial experience and study visits	163
Study drug	55
Trial information and informed consent	80
Communication and trust	163
Recommendations of participants	**52**
Recommendations for other patients	13
Recommendations for the sponsor	12
Recommendations for the study team	27
Total	**718**

Chapter 8

Summary & General Discussion

Facioscapulohumeral muscular dystrophy (FSHD) is a slowly progressing, hereditary muscle disease caused by aberrant production of the transcription factor DUX4 which ultimately results in deterioration of the skeletal muscles. Typically, patients experience weakness of the face, shoulder and upper extremity muscles between the age of 15-30 years. With progression of the disease, muscles of the trunk, hip girdle and lower extremity will also become affected. FSHD has a highly variable disease course, which makes it hard to predict individual progression and results in a heterogenous patient population. Currently, no disease-modifying therapies exist, but can be expected in the future with over twenty companies developing possible new medication. To reliably test these new medications, certain trial components need to be in place, which is called trial readiness. This firs part of thesis aimed to improve the trial readiness by analyzing the feasibility of questionnaires and clinical outcome assessments for clinical trials. The second part of this thesis aimed to contribute towards the development of losmapimod as a disease-modifying therapy for FSHD and evaluate the trials from a patient's perspective. For this aim, a phase 2 open-label study to assess the safety and efficacy of losmapimod, a questionnaire study inquiring about the burden of muscle biopsies and an in-depth interview study to gain more insight in the overall experience of participation in a clinical trial were performed.

Part I: Enhancing Clinical Trial Readiness

In **Chapter 2** we analyzed the data collected using the Dutch FSHD Registry. Initiated in 2015 after an international collaboration on trial readiness, the registry aimed to collect longitudinal data about FSHD symptoms, facilitate data collection and recruitment of FSHD patients for research purposes, and enable rapid spreading of important information. The data collected in the registry existed of a predetermined set of questions sent every six months about disease characteristics, a fatigue questionnaire (CIS20R), quality of life questionnaire (INQoL), depression questionnaire (BDI), and pain questionnaire (MPQ). The data were analyzed cross-sectionally at baseline and the CIS20R, INQoL, BDI, and MPQ were also analyzed longitudinally using mixed models. From initiation until March 2021, 373 patients were registered and completed at least one set of questionnaires. Fatigue, weakness, and pain in the shoulders and (lower) back were the most prominent symptoms. Nineteen of the 23 (sub)questionnaires showed no significant changes over time after six years, the other four showed minimal changes. A sub-analysis between three mobility groups (mobile without assisting device, mobile with assisting device, and wheelchair dependent) showed that some of the (sub)

questionnaires are able to distinguish between the groups, but no difference in longitudinal changes were observed. The registry facilitated fourteen studies with data collection or patient recruitment. Based on these results, we concluded that the questionnaires are not useful for clinical trials.

Chapter 3 reports on the consequences of the COVID-19 pandemic on the physical and mental health of FSHD patients and compares the incidence and severity of COVID-19 infections between FSHD patients and a non-FSHD population. A self-created questionnaire and the validated perceived stress scale (PSS) were send out three times (May, August, and December) in 2020 to capture the evolution of the pandemic. The self-created questionnaire inquired about the participants' physical symptoms, available care and COVID-19 incidence of the patient and their non-FSHD housemates. The three questionnaires were complete by 210, 186 and 205 participants respectively. Participants reported a higher burden of FSHD symptoms and were less active during the COVID-19 pandemic. Participants also reported more stress compared to pre-pandemic levels, but the PSS scores were still considered low. Interestingly, participants also reported on positive effects of the pandemic: due to less social obligations some participants experienced less symptoms and could spend more time with their families. There was no difference in the number of positive tests between FSHD patients and the non-FSHD population. FSHD patients reported to be less affected by COVID-19 infections, but this is most likely caused by reporting bias. This chapter serves as a good example on the effectiveness of the FSHD registry, enabling rapid recruitment of a large number of FSHD patients, even in unprecedented times.

A large natural history cohort of FSHD patients was initiated in the Radboudumc to gain more knowledge about the natural disease progression and to identify useful outcome measures for clinical trials. In **Chapter 4** we analyzed the clinical outcome measures (COMs) tested during the five-year follow-up of this cohort, focusing on determining the feasibility of the COMs for clinical trials. In this study, six different outcome measures were tested: the motor function measure (MFM), manual muscle testing using the MRC score, six-minute walk test, quantitative muscle strength assessment of the m. quadriceps, clinical severity scale (CSS), and FSHD evaluation scale (FES). The analyses included the change over time, determining the minimal clinically important difference, and several power calculations. After excluding non-penetrant patients (i.e. patients without any symptoms as determined by the researcher), 154 participants completed the baseline and five-year follow-up visit. All COMs showed a statistical significant difference over five years. However, these changes were minimal and only the MFM, CSS and FES showed a clinically important

difference. These three COMs also required the lowest number of participants for a trial. However, the CSS and FES are not feasible for short-term clinical trials because of their ordinal design and the MFM requires a trial duration of at least three years to reach a clinically important difference. The majority of the current trials maintain a CSS score between 4-8 as inclusion criterion, but this can be expanded to 3-9 without losing much power based on our data. Expanding this criterion will enable faster recruitment, increase the generalizability and improve sustainability of future studies. In conclusion, this study emphasized the minimal progression of FSHD and the COMs investigated in this study are unlikely to be sufficiently sensitive to capture disease progression in clinical trials.

Part II: Clinical Trial and Participant Experiences

A phase II open-label study investigating the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and exploratory efficacy of losmapimod is reported in **Chapter 5.** Losmapimod is a p38 α/β mitogen-activated protein kinase inhibitor, which showed an inhibitory effect on the DUX4 production in FSHD myofibers. Participants were treated with losmapimod 15mg twice daily. Vital signs, adverse events (AE) and blood and muscle samples were taken for safety, PK, PD and target engagement analysis. Exploratory outcome measures included the Reachable Workspace (RWS), muscle strength measurements, motor function measure Domain 1, timed up-and-go, 6-minute walk test, spirometry, and three patient-reported outcomes (FSHD Health Index, FSHD Rasch-built Overall Disability Scale, Patient Global Impression of Change). Fourteen participants with FSHD type 1 were included and all participants successfully completed the study. No serious adverse events occurred, the most common adverse events concerned mild, transient elevation of liver enzymes. Stability or minor improvements were seen on the exploratory outcome measures, in accordance to the treatment arm of the simultaneously ongoing placebo-controlled phase II trial (REDUX4). In summary, losmapimod showed a favorable safety profile as well as a possible positive effect on the FSHD disease course. A phase 3 randomized-controlled trial (REACH) to further investigate the efficacy of losmapimod was initiated.

Phase I and II trials aim to determine pharmacokinetics, pharmacodynamics, and target engagement of the investigational product. In the case of FSHD, this means analyzing muscle tissue on the levels of DUX4 and its downstream genes. Therefore, muscle biopsies will likely remain a necessary part of FSHD trials. The burden of this invasive procedure was not yet established in FSHD patients. **Chapter 6** reports on

a retrospective questionnaire study to assess the patient burden of needle muscle biopsies and to compare the burden of two different methods (outpatient clinic biopsies vs. MRI-guided biopsies). In this study, all Dutch-speaking FSHD patients who underwent at least one muscle biopsy for research purposes, either during a natural history study or a clinical trial, received a self-made questionnaire about the burden of the biopsies. Forty-nine patients reported on 91 biopsies, mostly taken from the vastus lateralis or gastrocnemius. The burden of the biopsies was high but of short duration. The MRI-guided biopsies were significantly more burdensome compared to the outpatient clinic biopsies. Overall, we concluded that including muscle biopsies in trials might be necessary, but the burden should not be underestimated. Novel techniques with smaller needles and/or more use of analgesics might lower the burden in future trials.

In the recent years, several steps have been taken to improve the clinical trial readiness of FSHD: multiple natural history studies were conducted, patient registries were initiated and new clinical outcome measures were developed. However, evaluations of the ongoing trials from a patient's perspective were lacking. We therefore conducted a qualitative study to explore the motivation, expectations, concerns, and experiences of FSHD patients in a clinical trial (**Chapter 7**). Participants from the ongoing phase II open-label study and phase III randomized-controlled trial were invited. An independent researcher held semi-structured interviews of approximately one hour to explore the aforementioned themes. Motivations to participate in a drug trial ranged from altruistic perspective to self-benefit. Participants reported realistic expectations towards the drug efficacy and possible side-effects. They reported a positive trial experience mostly due to the individualized approach and personal communication of the small trial team. More frequent updates from the Sponsor on the progress and results of the trials would have been appreciated. None of the participants advised against participating in a trial, while acknowledging that personal factors such as travel time and employment status need to be taken into consideration.

With the completion of this thesis, we gained valuable insight about the separate components that lead to a successful trial. In the discussion, the results of this thesis will be placed into perspective with the current literature to form recommendations for the most optimal trial design using the currently available tools.

Discussion

This thesis aimed to enhance clinical trial readiness, advance the development of losmapimod as a possible disease-modifying therapy and evaluate the patient's perspective of participating in clinical trials. During the creation of this thesis, new outcome measures were validated and the development of new therapies was initiated (Figure 1). The initial results of the phase III losmapimod study were also published in a press release[1]. Unfortunately, losmapimod showed no efficacy on the reachable workspace (RWS), fat fraction on MRI, or shoulder strength measured using handheld dynamometry. Based on these results, the sponsor decided to halt the losmapimod program. While the negative results of losmapimod are disappointing, the lessons learned in this thesis will still be vital for optimally designing the upcoming trials. In this discussion I will reflect on the current state of the FSHD registry and FSHD trials, leading up to recommendations for future FSHD trials. Lastly, I will discuss the necessary changes in trials and clinical care once the first disease-modifying therapy will be approved.

The FSHD registry

Chapter 2 and 3 demonstrate the successful initiation and maintenance of the Dutch FSHD registry: it is able to characterize the Dutch FSHD population, collect longitudinal patient-reported data and has been pivotal in the support of fifteen studies, including the phase II (Chapter 5) and phase III losmapimod trials. The shortcomings of the current state of the registry also became apparent after analyzing the captured data (Chapter 2) and comparing the FSHD registry to the myotonic dystrophy type 1 database (Myodraft) and the registry for Duchenne and Becker (Dutch Dystrophinopathy Database)[3,4].

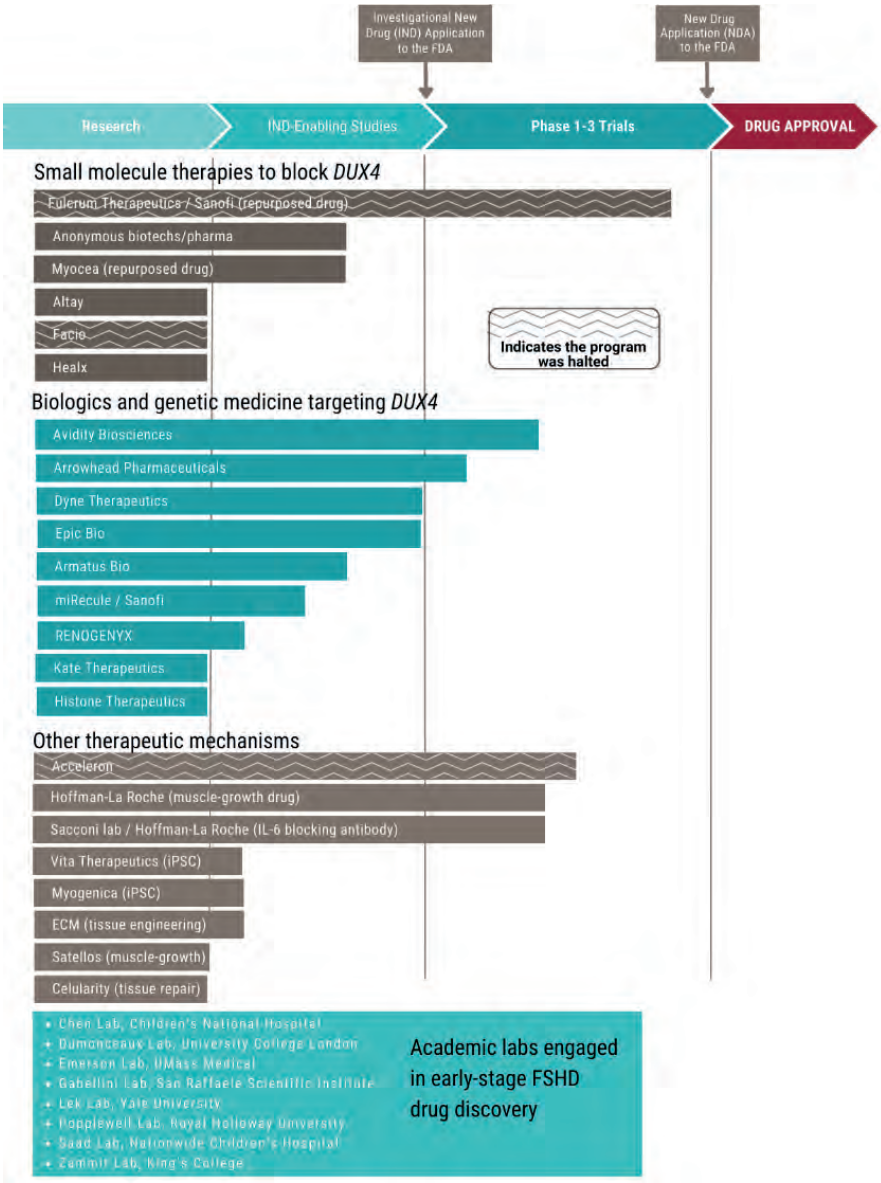

Figure 1. FSHD drug development pipeline of September 2024[2]

This figure lists the various companies and laboratories working on potential DUX4-targeting drugs as well as their progression towards drug approval, categorized as pre-clinical research, IND-enabling studies and Phase 1-3 trials.

Compared to the pipeline shown in the introduction (October 2019), the number of companies and laboratories working on a treatment for FSHD clearly increased. Unfortunately, also three companies (Fulcrum Therapeutics, Facio Therapies and Acceleron Pharma Inc.) had to halt their program due to a lack of efficacy.

The FSHD registry is a fully patient-reported registry. Patients can request registration using the website, which makes it easy for patients to participate. The lack of clinical outcome measures is a big disadvantage though, limiting the analyses of the natural disease progression and reducing the pre-screen possibilities for a clinical trial. The Myodraft is a combination of a natural history study and a registration. Only patients receiving care at one of the myotonic dystrophy expertise centers can join the Myodraft. Clinical data is collected at the national center of expertise in Radboudumc and Maastricht UMC+ and in some cases requested from local hospitals. The extensive data collection allows for more complex analyses and careful pre-screening, at the cost of accessibility, higher financial cost and being more labor-intensive. After the comparison of the registries, the Myodraft-light was initiated: a version that allows patient to register online to be findable for research, just like the FSHD registry. The Dutch Dystrophinopathy Database (DDD) collects data from the outpatient clinic visits and maintains a layered informed consent. Depending on the preferences of the patients, they can be registered without additional requests, receive questionnaires, receive invitations to participate in research, allow the data to be shared with international databases, and allow the data to be shared with international companies.

Based on the lessons learned during the creation of this thesis and the comparison to the other registries, an update of the FSHD registry is ongoing. Amongst other things, the FSHD registry will be updated to include a layered informed consent and a pilot to include clinical data from the natural history study (Chapter 4) will be initiated[5]. Simultaneously, the ongoing natural history study will assess if a patient reported clinical severity scale (CSS) is as reliable as a clinician reported CSS[6] (Box 1). If this is the case, the patient reported CSS can be included in future questionnaire studies to get more insight in the disease state of the study population. Approximately 500 FSHD patients are currently registered in the Dutch online registry, which is definitely an achievement, but is only approximately 25% of the total Dutch FSHD patient population[7]. Considering the surge of upcoming trials and the selective eligibility criteria being used, it will be necessary to increase this number in the short-term. Together with the Dutch FSHD patient organization, a survey study to gain insight on why FSHD patients did or did not register will be performed. Based on the results of this survey, a new marketing campaign will be initiated with the aim to double the number of patients registered.

Lessons learned for FSHD trials

A successful trial requires careful consideration of the three most important elements: study population, study design, and outcome measures. Suboptimal choices in any of these elements will reduce the validity of the results, can lead to false-positive or -negative results and reduce the generalizability of the trial. Due to the scientific devaluing of the results, it is arguably unethical to subject patients to a suboptimal trial. Optimal choices in the trial elements can only be made if knowledge about these elements is available (i.e. clinical trial readiness). The previous FSHD trials might have been optimally designed considering the available knowledge, but new discoveries and large natural history studies call for a careful reflection on the FSHD trial design, showing that maintaining trial readiness requires continuous effort.

Study population

The phase I and II losmapimod studies (Chapter 5) included FSHD type 1 patients with a D4Z4 repeat length of 1-9 units and a CSS between 4-8[8,9]. The decision for these somewhat selective eligibility criteria for these trials was based on several reasons. First, we tried to homogenize the study population as much as possible. Secondly, target engagement (i.e. changes in DUX4-related genes in muscle tissue) needed to be measured in affected lower extremity muscles, necessitating a CSS >4 (Box 1). Lastly, based on previous literature, moderately affected patients (CSS 4-8, Box 1) have the highest chance to show disease progression[10]. Therefore, including moderately affected patients should increase the chance of finding efficacy. While these reasons were valid, it also reduced the generalizability of the results. Based on the current knowledge, I argue that future trials should expand their eligibility criteria without losing significant power.

First, it is unnecessary, even undesirable, to exclude FSHD type 2 from the majority of the clinical trials. Only if the drug specifically targets the D4Z4 repeat, it could be argued that FSHD type 2 patients need to be excluded. FSHD type 1 and 2 both result in aberrant production of DUX4 and are clinically indistinguishable[11]. Including both types should therefore not impact the results of the trial. Furthermore, only including type 1 patients may lead to future therapies being registered for only type 1, while the drug may also be effective for type 2. Because only 5% of the FSHD patients have type 2, recruiting sufficient participants for a subsequent type 2 trial will be very challenging[11]. Fortunately, FSHD type 2 patients are increasingly included in trials. The phase III losmapimod trial did include FSHD type 2 patients,

albeit as a separate population group for the purpose of analysis[12]. Other ongoing trials do not seem to distinguish between the two types[13,14].

Currently, FSHD patients with a D4Z4-array repeat length of ten units are being excluded from clinical trials[8,9,12]. According to the current genetical and clinical guidelines, a repeat length of ten units calls for reconsideration of the FSHD diagnosis[15]. If a patient with ten D4Z4 repeats show clear FSHD symptoms, the diagnosis remains likely true. Considering the other eligibility criteria that require clear FSHD symptoms (e.g. CSS 4-8), it is unnecessary to exclude patients with a repeat length of ten units. Additionally, the disease progression of FSHD patients with a D4Z4 repeat length of ten units does not differ from the patients with a repeat length between 1-9 units. Maintaining this eligibility criterion might lead to medication being unapproved for FSHD patients with a repeat length of 10 units, which is highly undesirable.

FSHD patients can be divided in five categories based on severity (Box 1). Almost all clinical trials include only moderately affected patients, usually selected through a CSS 4-8 and/or MRI muscle fat fraction of the lower extremities between 10-50% (Chapter 5)[9,12-14]. Although moderately affected patients have the highest chance of relatively fast progression, due to the high variability of the disease, capturing disease progression in the time span of a clinical trial remains uncertain. To challenge the notion of only including moderately affected patients, we performed power calculations for several CSS ranges using the five year natural history data (Chapter 4). Based on these results, the CSS range can be expanded to 3-9 without losing much power. This will result in an additional 20% of the patient population becoming eligible, improving the long-term sustainability and generalizability of future FSHD trials.

It is clear that the eligibility criteria of future trials should be expanded to optimize the study population, which will improve the recruitment, sustainability and generalizability. Consequently, this will reduce the chance that new therapies will be approved for only a subset of the patient population. Unfortunately, the aforementioned considerations still have their limits. The analyses performed in Chapter 4 only included several possible CSS ranges. The analysis also did not account for age, sex, D4Z4 repeat or other variables that might influence disease progression. Ideally, a reliable prediction model to identify patients prone to progression will be developed. With new technologies in machine learning, such a model is feasible, as progress towards a prediction model is already being made for Myotonic Dystrophy[16]. The accuracy of these models will be determined by the available data, therefore, performing large natural history studies and maintaining

FSHD registries will remain vital to keep enhancing clinical trial readiness. We aim to generate enough data to build this model by performing a ten-year follow-up study of the natural history cohort (Chapter 4).

Box 1. Categories of FSHD disease severity and the clinical severity scale.

FSHD Severity Categories

Non-penetrant: show no symptoms of FSHD (CSS 0)

Asymptomatic: do not experience symptoms of muscle weakness but a clinician can see signs of FSHD (CSS 0-2)

Mildly affected: weakness in face and shoulders but not in lower extremities (CSS 1-3)

Moderately affected: weakness in the face, shoulders and lower extremities. Walking long distances is possible, sometimes using supportive devices (CSS 4-8)

Severely affected: widespread weakness in most of their skeletal muscles. often wheelchair-dependent (CSS 9-10).

Clinical Severity Scale[4]

0: No symptoms
1: Facial Weakness
2: Mild Scapular involvement without limitation of arm abduction; no awareness of disorder symptoms is possible
3: Moderate involvement of scapular and arm muscles or both (arm abduction $>60^0$ and strength ≥3 in arm muscles); no involvement of pelvic and leg muscles
4: Severe scapular involvement (arm abduction $<60^0$ on at least one side); strength ≤3 in at least one muscular district of the arms no involvement of pelvic and leg muscles
5: Tibioperoneal weakness; no weakness of pelvic and proximal leg muscles
6: Mild weakness of pelvic and proximal leg muscles or both (strength ≥4 in all these muscles); able to stand up from a chair without support
7: Moderate weakness of pelvic and proximal leg muscles or both (strength ≥3 in all these muscles); able to stand up from a chair with double support; able to walk unaided
8: Severe weakness of pelvic and proximal leg muscles or both (strength <3 in at least one of these muscles); able to stand up from a chair with double support; able to walk unaided
9: Unable to stand up from a chair; walking limited to several steps with support; may use wheelchair for most activities
10: Wheelchair dependent

Trial design

Losmapimod followed the traditional road of phase I trial, follow by two phase II trials (Chapter 5 and ReDUX4) and finally a phase III trial (REACH)[8,9,12]. The phase II trial included in this thesis had a single-center, open-label design primarily focusing on long-term safety of losmapimod. The ReDUX4 had a randomized placebo-controlled trial (RCT) design with the primary aim to measure changes in the DUX4 downstream genes after treatment with losmapimod. While the primary aim of the ReDUX4 was not reached, other outcome measures such as long-term safety and changes in fat fraction on MRI, COMs and PROs (Chapter 5) showed enough promise to initiate the REACH trial. The primary aim of the REACH trial was to assess the efficacy of losmapimod treatment on the upper extremity functionality determined by the reachable workspace (RWS) and fat fraction on whole body MRI using a RCT design[17,18]. Even though the phase II trials showed promising results, the REACH trial showed no efficacy of losmapimod. While the losmapimod trials were conducted successfully, based on the assumptions and results in Chapter 4, RCTs would require >250 participants to find a statistical significant difference with a trial duration of two years and >3 year duration to find a clinical relevant difference. This brings the sustainability of the surge of upcoming trials into question. Exploring innovative trial designs might result in opportunities to improve future trials.

New therapies for rare (neuromuscular) diseases are increasingly tested in a mixed phase I/II trial design[13,19]. The first weeks of the trial follow a phase I design, followed by an extension with a multiple ascending dose phase II design. In this way, both short- and long-term safety, pharmacokinetics, pharmacodynamics, target engagement, dose finding and efficacy can be assessed in one trial. There are two reasons why this mixed design is recently being used in rare disease trials. First, for many of the rare disease, we are not yet fully clinical trial ready (e.g. natural history not fully established and a limited knowledge about which COMs are reliable and sensitive to change). The phase II part of the trials usually includes several (exploratory) COMs in order to learn which of the COMs might be the most relevant to disease state or are the most sensitive to change, so they can be used for a phase III trial (Chapter 5). Second, due to changes in the orphan drug law, an accelerated approval program for therapies for rare diseases is available[20]. The organization assessing the safety and efficacy of new therapies, The United States Food and Drug Administration (FDA), will closely monitor and collaborate on the development of therapies which were allowed in the accelerated approval program. The FDA also considers approving new therapies on surrogate biomarkers instead of COMs (e.g. change in muscle fat fraction on MRI instead of change in muscle strength). The European Medicines Agency (EMA) also allow for accelerated

approvals, but these programs only concern faster review times, not guidance during therapy development[21]. Pharmaceutical companies have a high incentive to gain access to the accelerated approval program; a positive mixed phase I/II trial is the most efficient method to gain access to the accelerated approval program.

N-of-1 trials allow for participants to be their own control using a double-blinded crossover design[22]. Participants will have multiple periods of several weeks during which they take either the drug or placebo in a double-blinded design (Figure 2). The N-of-1 design can only be used when certain requirements are met: The disease needs to be relatively stable over the duration of the trial, the drug needs to have a short halftime to ensure complete wash-out to prevent a carry-over effect between periods, the drug should not have a permanent effect, and the intended effect of the drug should be short-term and temporary instead of modifying the long-term disease course. Because participants are their own control and have multiple measurement periods of therapy/placebo, N-of-1 trials require less participants than randomized controlled trials. Additionally, since every block of therapy/placebo can be used as an interim analysis, prospective analysis of the data is possible and may result in shorter trial duration[23]. Although FSHD does not seem to be a good candidate for a N-of-1 trial, based on observations in the natural history studies (Chapter 2 and 4) and phase II trial (Chapter 5), I argue that n-of-1 trials might be feasible. Chapter 2 and 4 clearly show that FSHD remains stable over a long period of time, only finding minor changes after five years. Losmapimod has a half-time of 12 hours and should be washed out after 2.5 days[24]. During the phase II and III trial, some participants noticed a rapid effect (within days) on muscle pain and/or fatigue after halting or (re)starting treatment, thereby meeting all of the requirements of a N-of-1 trial. Still, it is unlikely that N-of-1 trials will be used for FSHD, as most companies aim to impact the long-term disease course which is not a feasible outcome measure for N-of-1 trials. Secondly, a majority of the new therapies being developed are antisense oligonucleotides and DNA-modifying therapies, which have long-term or possibly permanent effects[13].

Another innovative design allow trials to use an external control group, instead of a placebo-group, effectively halving the required sample size[25]. The external control group can come from natural history studies or the placebo-group from previous RCTs[26]. The biggest challenge of this design is to prevent bias between the two groups caused by a lack of randomization. Another issue is that studies in FSHD have shown that a natural history cohort does not follow the same disease progression as a placebo group (which showed minor improvements in several trials), increasing the chance on false-positive findings[27]. An extension of this design would be to maintain a large natural history cohort from which trial arms

can be formed. This would allow for additional analyses, such as comparing the individual slopes of disease progression before and after taking study drug. The biggest challenge would be maintaining a natural history cohort with frequent measurements, as this is an intensive and costly endeavor. Furthermore, clear international consensus on what COMs to test needs to be in place.

A fourth possible design would an umbrella trial in which multiple drugs are compared to each other and one placebo group, originating from the oncology setting[28,29]. This design would drastically decrease the overall number of participants needed because only one placebo group is necessary to compare to multiple treatment arms. Furthermore, it is financially beneficial as it only requires start-up, training and equipment for one trial and the pharmaceutical companies can share the cost of the trial. It also allows for easy comparison between the therapies as the exact same outcome measures were used for each treatment arm. The biggest challenge of this design is to align the multiple pharmaceutical companies on the same methods and to get them to participate. An umbrella trial might pose a risk for pharmaceutical companies; even if a drug shows efficacy compared to placebo, if the efficacy is lower compared to another drug, it might not get approval.

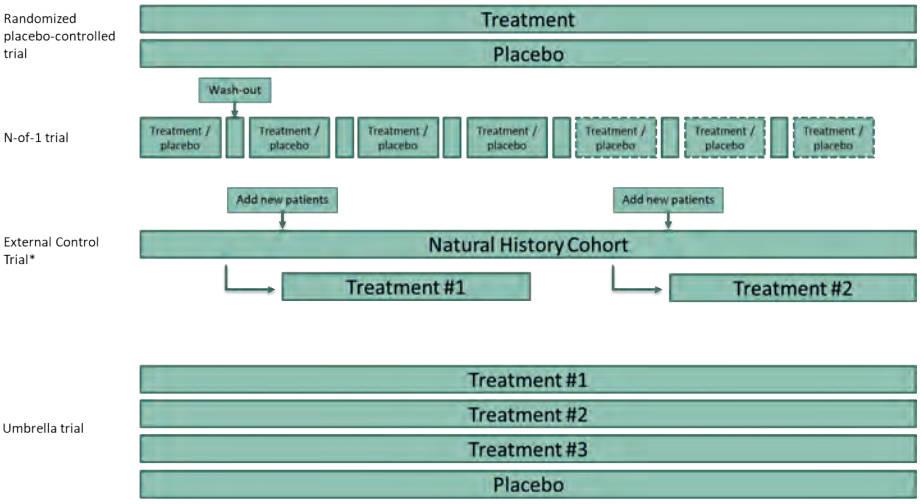

Figure 2. Various trial designs

N-of-1 trials allow for prospective analysis which can determine if data saturation is reached. The last three blocks of treatment/placebo are surrounded by a dashed line to visualize that additional periods can be added to the trial if necessary to reach data saturation.

*External Control Trials can find the control group from multiple sources. In this figure it is visualized as a prospective natural history cohort from which treatment arms can be selected. Alternatively, external controls can come from unrelated natural history cohorts or previous clinical trials.

Biomarkers and clinical outcome measures

Depending on the trial phase, biomarkers (for target engagement) or COMs/PROs are one of the primary outcome measures[8,9,12]. Ideally, before initiating a trial, the validity, reliability, sensitivity-to-change and minimal clinically important difference of the included biomarkers, COMs and PROs are known. In FSHD, this information was not yet readily available, which is why we determined the feasibility of several COMs (Chapter 4) and PROs (Chapter 2) for clinical trials. Unfortunately, most of the commonly used neuromuscular COMs and PROs were unable to detect the small changes occurring in FSHD patients within the timespan of a clinical trial, assuming future therapies will at best halt the disease. Careful selection of biomarkers, COMs and PROs is necessary to prevent false-negative results in future FSHD trials.

Phase I and II trials generally focus on short-term safety and target engagement. For FSHD, target engagement is usually being determined by measuring DUX4-related genes in muscle tissue that was gathered via needle muscle biopsy. Based on the results of the phase II trials and the evaluation of the biopsies (Chapter 6), some restraints toward the inclusion of muscle biopsies is warranted. While many patients are willing to undergo a needle muscle biopsy for the development of new therapies, biopsies had a high burden and should not be underestimated (Chapter 6). On top of that, the results of the muscle biopsies of the phase II losmapimod trials were not useful[9]. Due to unexpectedly high variability of the gene expression in both the treatment and placebo groups, no conclusion about possible efficacy could be drawn. The high variability might be a consequence of the methods used, because a recent longitudinal natural history study compared muscle biopsies of 18 participants after one year showing lower variability[30]. Arguably, the inability to draw a conclusion from the muscle biopsies could have been caused by the lack of efficacy of losmapimod. More potent therapies might be able to overcome the high variability and show significant changes in DUX4-related genes. For example, the first results of del-brax (Avidity Biosciences) show a DUX4 reduction of approximately 50% across several gene panels[31]. Muscle biopsies will likely remain part of the FSHD trial toolkit for the foreseeable future, studies toward improving the biopsy method will therefore remain important. For example, MRI- or ultrasound-guided biopsies might result in more reliable results[32]. Additionally the method used can drastically change the burden of the biopsy. In an ongoing myotonic dystrophy type 1 trial, biopsies are performed using an automated biopsy system and more anesthesia (both subcutaneous and intramuscular)[19]. Based on a shorter version of the questionnaire used in Chapter 6, this method seems to drastically reduce the burden on the patients while the yield is still sufficient.

Ideally, all future studies will incorporate the questionnaire used in Chapter 6 to evaluate the biopsies to keep improving upon the biopsy methods.

Although not a large part of this thesis, imaging plays an important role in measuring the disease progression and drug efficacy in FSHD[10,33,34]. MRI is a commonly used imaging modality in FSHD, determining the disease state of individual muscles and identifying muscles prone to progression in the near future[34]. The phase II and III losmapimod trials included whole body MRI to analyze changes in muscle fat fraction, muscle fat infiltration and lean muscle mass (Chapter 5)[9,12,35]. Muscle ultrasound (MUS) is another imaging biomarker currently being studied for FSHD[36,37]. MUS was also included in the phase II trial, showing stability of the muscles (data not yet published, but presented at multiple congresses). Recent studies suggest that MUS and MRI should not be seen as competitors but as complimentary to each other[34]. MUS seems to be able to detect early changes in early disease stage while MRI is better suited for moderately to severely affected disease stages (Figure 3). Both MUS and MRI show good correlations with muscle strength, they could therefore serve as surrogate biomarkers for therapies in the accelerated approval program[36].

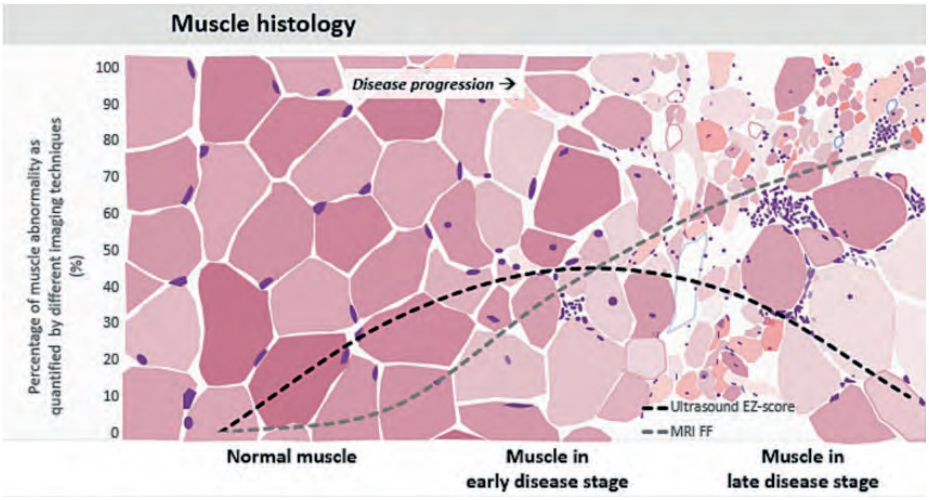

Figure 3. Disease progression in FSHD schematically visualized based on muscle biopsy pathology

The black dashed lines show the hypothesized ultrasound abnormalities, the grey dashed line hypothesized MRI abnormalities. This figure shows that ultrasound is likely more useful than MRI to detect abnormalities in early disease stage, but MRI is more useful in later disease stages.
Adapted from: The complementary use of muscle ultrasound and MRI in FSHD: Early versus later disease stage follow-up[34].

The natural history study in Chapter 4 was initiated in 2014[5]. Recently developed COMs were therefore not included in the analysis. Two new COMs show promising results and are like feasible to be used in FSHD trials. The aforementioned Reachable Workspace (RWS) measures upper extremity functionality and was used as an exploratory outcome measure in the phase II trials (Figure 4, Chapter 5)[17]. The RWS is reliable and correlates well with other scales measuring upper extremity functions, daily activities and muscle strength of the shoulder[38]. It was therefore chosen as the primary outcome measure of the phase III losmapimod trial and is also in studies in other diseases such as neuralgic amyotrophy, Duchenne muscular dystrophy, spinal muscular atrophy, amyotrophic lateral sclerosis and Becker muscular dystrophy[12,39-41]. The FSHD functional composite outcome measure (FSHD-COM) was created to specifically measure FSHD-affected functionality[42,43]. It exists of 18 items involving leg, arm, shoulder, trunk and hand function. The composite nature of the FSHD-COM results in a high correlation to disease severity and strength, but lacks the ability to capture changes on single items. Additionally, some items test the same functionality, which can results in false-positive or -negative results. An international longitudinal history study in FSHD patients is ongoing to determine the validity, reliability and responsiveness of the RWS and FSHD-COM[44].

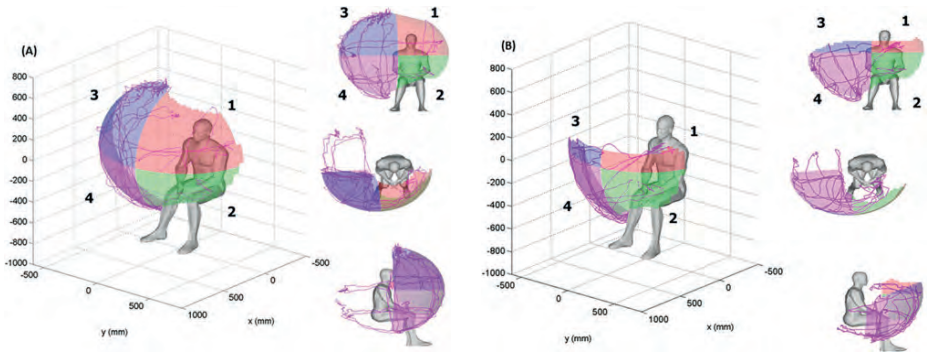

Figure 4. Graphical output of the Reachable Workspace

A: The results of a healthy individual, showing an almost perfect semi-globe range of motion.
B: The results of a patient with FSHD with moderately impaired upper extremity function. The patient is unable to lift his/her arm above shoulder height.
Adapted from: Reachable workspace in facioscapulohumeral muscular dystrophy (FSHD) by kinect[17]

The PROs analyzed in Chapter 2, were generic (neuromuscular) questionnaires. These questionnaires were unable to capture the disease progression of FSHD, and we often received feedback from participants that the questionnaires do not fully encapsulate the FSHD symptoms. Two new FSHD-specific questionnaires were developed and validated in the past years. The FSHD Rasch-built overall disability scale (FSHD-RODS) is a 32-item questionnaire developed using Rasch analysis[45]. This means that the outcome of this ordinal questionnaire can be appropriately converted to an interval scale. Furthermore, it can measure the whole spectrum of severity, instead of other COMs/PROs which have a clear floor and/or ceiling effect in FSHD patients (e.g. motor function measure as described in Chapter 4)[46]. The FSHD-Health Index (FSHD-HI) is a questionnaire developed using FDA guidelines for disease-specific patient reported outcomes[47]. A large list of symptoms were first checked by the researchers and patients on validity and relevance. The FSHD-HI consists of 116 items across fourteen symptomatic themes. The questionnaire showed excellent test-retest reliability and patient reported a low burden on completing the questionnaire despite its many items.

Patient involvement

Besides the scientific elements, patient involvement plays a vital role in designing, performing and closing-out a trial successfully. Patient involvement is the development of partnerships between patient representatives and researchers to ensure the voice of the patients is taken into consideration during conceptualization, preparation, execution and close-out of studies[48]. In this thesis, patient involvement generally consisted of input and feedback from several patient representatives and the patient advocacy group 'Spierziekten Nederland'[49].

In my experience, patient representatives are valuable partners when designing a trial; compared to medical professionals, they are more suited to estimate the burden on the patients and have better insight in which COMs and PROs are valuable to the patients. Furthermore, they have a clear understanding of how eligibility criteria can affect certain subgroups of the patient population. Patient representatives should always be allowed to proofread patient facing documents, like an informed consent form, to ensure the information is understandable for every patient. When a trial is ready to start, patient representatives and patient advocacy groups can aid with recruitment of participants and educate patients on what it means to participate in a trial. Any concerns or unclear information within the patient community can be quickly picked up by patient representatives, so it can be addressed by the medical professionals. They also play an important role in refuting false information spread on social media. Lastly, when a trial is finished the

patient representatives can support the dissemination of the results using websites, newsletters and social media. Additionally, they can organize patient conferences and webinars for educational and social purposes (Box 2).

Box 2. Trial fitness

This thesis focuses on improving trial readiness from a trial perspective. Patients can also improve their individual trial readiness, which we have termed 'trial fitness'. The majority of the eligibility criteria overlap between clinical trials, being trial fit means that you adhere to most of these criteria (provided that this is within the patient's influence). For example, patients should maintain a healthy lifestyle to prevent exclusion due to obesity. Genetical confirmation is always an inclusion criterion, patients interested in clinical trials should therefore ensure that this is available. Any comorbidities should be treated optimally with stable dosage of concomitant medication. Participants or partners of participants are usually not allowed to become pregnant during a trial, adequate contraception is therefore required.

Besides adhering to eligibility criteria, patients also have a responsibility to educate themselves on trial participation. It is important that they understand the difference between a clinical visit and study visit. Furthermore, they need to understand the risks of participating in a trial and know that a trial drug is not a treatment because the efficacy is still unknown. Lastly, they should consider if they are able to handle the burden of trial participation, both in time and energy as well as the psychological burden. Chapter 7 provided some key points for patient education based on the participant's experience. Education about trials should be provided by medical experts and patient representatives.

Naturally, the role of patient participations extend to other type of studies as well. For example, we received clear feedback from the patient representatives on the Beck Depression Inventory (BDI) questionnaire used in Chapter 2[50]. Not only did patients feel that this questionnaire did not align with the symptoms of FSHD, they also found the questionnaire confrontational to complete as this questionnaire inquired about suicidal thoughts. Based on this feedback, we decided to remove the BDI from further survey packages and will replace it with a questionnaire about psychological symptoms that better aligns with the FSHD community. Patient representatives also helped with creating the COVID-19 questionnaire (Chapter 3) and the interview guide in Chapter 7.

A recent ENMC workshop underlined the added value of patient involvement across the spectrum of research[48]. A notable, positive trend in ensuring patient involvement was seen in the past years, although some areas (education, cultural, and structural changes) still require improvement to better support patient involvement. It is important that the patient representatives are well-educated to competently participate in research collaborations. They should understand the background of regulatory issues, know how data is analyzed and viewed by the EMA/FDA, and be taught to voice the community's concern (not individual concerns). Several organizations like Eurordis and Eupati have excellent courses on training patient representatives[51,52]. Furthermore, it should become the standard of practice to prospectively involve patients in future studies. Certain tools can aid with this; the Involvement Matrix was created to assist with prospectively involving patients and to retrospectively discuss whether patient roles were fulfilled satisfactorily (Figure 5)[53]. Structural changes entail allocating budget to compensate patient representatives and hold study leaders (Sponsors or Principal Investigators) responsible for reporting study results back to patients, even if the results are negative.

Lastly, besides being a valuable addition to trial design, patient representatives also play an important part in the approval process of new therapies[54]. Once a drug has been proven to be effective in a phase III trial, governments need to decide if the drug can be prescribed in their country[55]. Several arguments play a role in this decision, such as disease severity, societal cost of the disease, and the expected efficacy and cost of the drug. Patient representatives and advocacy groups will speak on behalf of the patient population in these discussions[56]. As these discussion can take up to several months, it is of utmost importance that the patient representatives are up-to-date on ongoing trials and results so they can initiate these discussions as soon as possible.

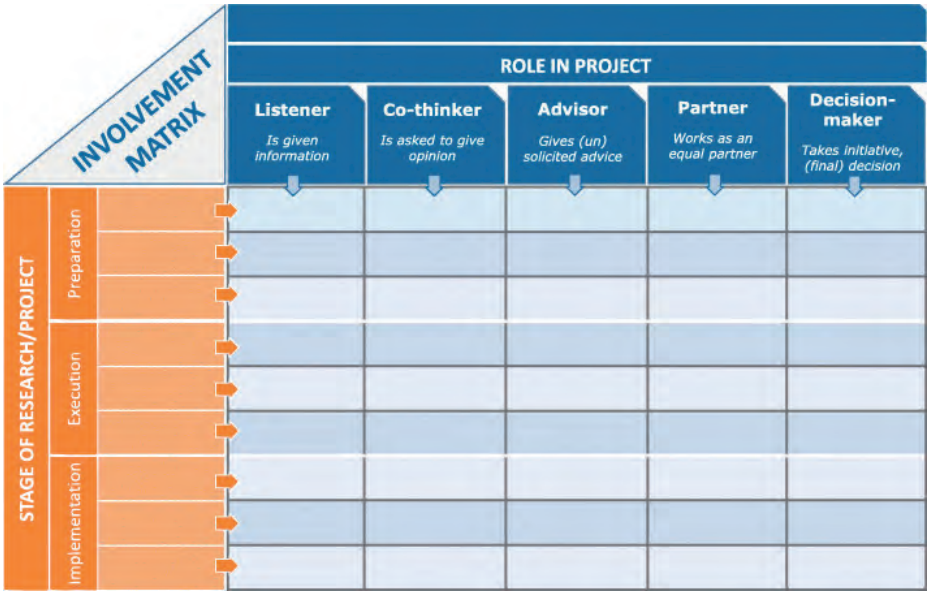

Figure 5. Involvement Matrix

Available from: www.kcrutrecht.nl/involvement-matrix. © Center of Excellence for Rehabilitation Medicine Utrecht, used with permission[53]

8

The optimal FSHD clinical trial

Considering the lessons learned and topics discussed above, the following suggestions would result in the most optimal FSHD trial based on the current knowledge (Figure 6). The study population should (if the specific treatment allows for it) include both genetic FSHD types, regardless of D4Z4 repeat length, with a CSS range of 3-9 (Chapter 4). Although non-traditional trial designs might solve some of the challenges faced in FSHD trials, RCTs will likely remain the most feasible design in the near future. Importantly, all trials should have a low-burden open-label extension phase to be able to capture disease progression over multiple years, while minimizing participant, site and financial burden; the lack of long-term extension phase might lead to false-negative results, due the slow progression of the disease (Chapter 2, 4 and 5). Long-term extension phases also increases the motivation for participation (Chapter 7), facilitating faster recruitment. Additionally, based on the small changes over time (Chapter 2, 4 and 5), it is unnecessary to burden sites and participants with frequent testing of clinical outcome measures: one visit every six months with optional short safety visits in between should be sufficient. The choice of outcome measures is arguably the most difficult decision for

FSHD trials. Phase I trials will need to prove molecular efficacy of the drug, making muscle biopsies mandatory, even with their shortcomings. Traditional functional outcome measures will not suffice; the RWS will most likely be the most optimal functional COM available. Fat fraction on MRI will support clinical findings and may be more sensitive to change compared to COMs. MRI should therefore be included in future trials and could possible serve as a surrogate biomarker for therapies in the accelerated approval program. Commonly used (generic) questionnaires are unable to accurately capture FSHD symptoms, therefore the FSHD-RODS and FSHD-HI may become valuable assets for future trials. Lastly, every trial should be supported by patient representatives, considering their valuable input during trial conceptualization, design, execution, close-out and market-approval processes.

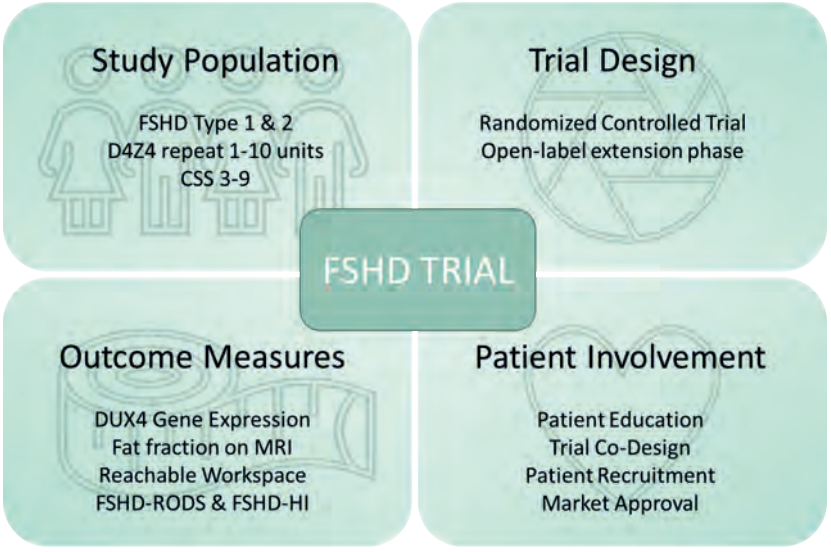

Figure 6. The optimal FSHD trial

Based on the current knowledge, this figure shows the most important elements and criteria to optimally design an FSHD trial.

FSHD: Facioscapulohumeral Dystrophy. CSS: Clinical Severity Scale. MRI: Magnetic Resonance Imaging. RWS: Reachable Workspace. FSHD-COM: FSHD Composite Outcome Measure. FSHD-RODS: FSHD Rasch-built Overall Disability Scale. FSHD-HI: FSHD Health Index. ICF: Informed Consent Form.

The optimal trial team

Through performing the phase II losmapimod trial (Chapter 5), the phase III losmapimod trial and the Dyne-101 myotonic dystrophy trial, I have gained insight in the necessities of a good clinical trial team. In my experience, a small, dedicated trial team consisting of a physician, a study coordinator, several research nurses and optionally a physical therapist will suffice to start trials in FSHD and other neuromuscular disorders. A small team will enable quick and clear communication between the different members. It also ensures continuity in participants visits, which in turn will lead to more personalized communication towards the participants which was highly appreciated (Chapter 7). A clear definition and knowledge of each team member's task will be vital for the efficiency of the trial team. The trial physician will be the main point of contact for the Sponsor, recruit patients and perform physician-related tasks during study visits. The study coordinator will manage the administrative and regulatory tasks and will negotiate about the budget. It is not desirable for the physician to negotiate the budget, as this may influence the relation with the Sponsor. The research nurses will manage the scheduling of the visits, maintain the database, process blood and tissue samples in the laboratory and perform nurse-related tasks during study visits. Lastly, some trials require involvement of a physical therapist depending on the COMs and preferences of the Sponsor. Depending on the number of trials at the site, a dedicated physical therapist might be desirable. Following a standardized sequence of steps, this team can ensure a high quality of the study visits, data capture and smooth recruitment after initialization (Figure 7). Besides the trial-related tasks, a trial team can also support other research-related tasks, such as the maintenance of registries and biobanks or assist with writing protocols and submissions to ethical committees.

Future perspectives

This thesis demonstrated that clinical trial readiness is not an end goal to reach, but an iterative process that requires constant updating based on newly available knowledge. This also means that some of the suggestions in the thesis might eventually become outdated. With the growing number of DUX4-targeting therapies being tested (Figure 1), I expect that the first approved FSHD therapy will become available within a few years. In this last section of the discussion, I will reflect on the necessary changes that need to occur once the first FSHD therapy is approved.

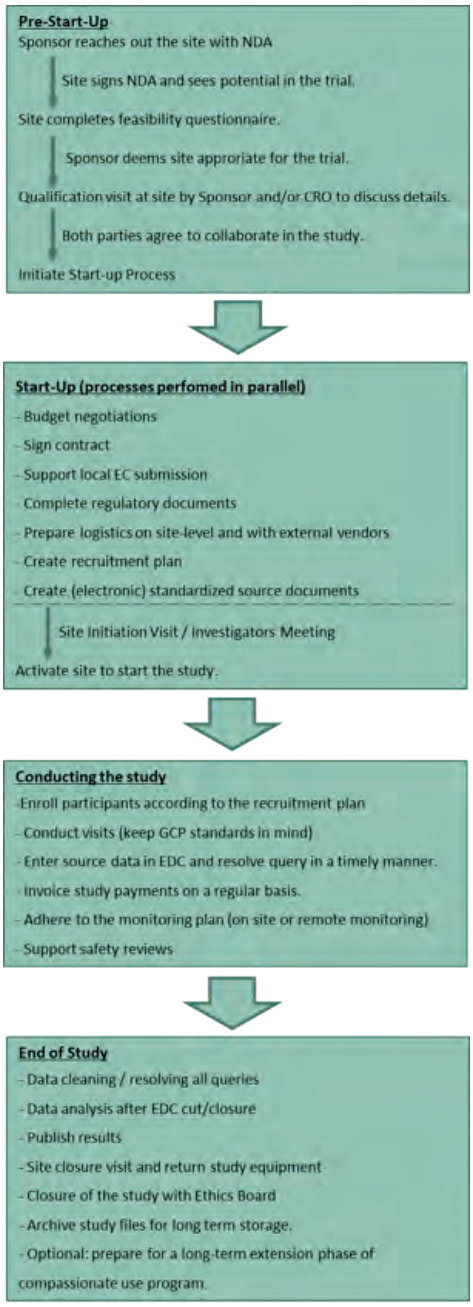

Figure 7. Flowchart of trial tasks categorized per phase

This flowchart details the tasks necessary to initiate, perform, and close-out an industry initiated study at a site. Generally, these steps always follow the same sequence and should be done in a standardized way to reduce mistakes and increase efficiency. In the case of investigator initiated trials, certain tasks are not applicable.

Pediatric trials

Currently, trials involving pharmaceutical treatment in children with FSHD have not been initiated. In general, medication has to be tested in adults before it can be tested in children. While the classic FSHD patients typically manifest muscle weakness between 15-25 years old, a small number of pediatric patients do exist[57,58]. Most likely, the actual number of pediatric FSHD patients is higher, because parents with FSHD currently do not see a reason to get a formal diagnosis for their children with FSHD symptoms as there is no disease modifying therapy. Assuming that full reversal of the muscle deterioration process is not possible, treating FSHD patients as young as possible will maximize the patients' functionality throughout their lives. It is therefore important that approved therapies will also be tested and become available for the pediatric FSHD population.

The clinical trials in children with FSHD will face even bigger challenges than the trials in adult FSHD patients. The laws and ethical considerations surrounding pediatric trials are more strict compared to adult trials. Clinical trial readiness, especially regarding the optimal COMs and PROs, needs to be in place. A trial with many exploratory COMs and PROs to determine which are useful, is unlikely to be allowed in the pediatric population. Furthermore, the natural disease progression of the pediatric FSHD patients is less studied compared to the adult population, with only a handful of natural history studies being available[57,59]. Considering the low number of pediatric patients, it will require international collaborations to ensure clinical trial readiness and sufficient recruitment for a successful clinical trial.

Trial design after the first available FSHD drug

The current trials compare the investigational product to placebo. It is important to consider the necessary changes once the first drug is available for FSHD. Ideally, new therapies will be either compared to already registered drugs instead of placebo, or be tested as an add-on therapy[60]. Considering the multiple different type of medication being developed (Figure 1), with each their own target in the DUX4 pathway, the future treatment regimen of FSHD might consist of multiple medications. Current FSHD trials exclude patients who use medication which possibly affects the muscle function. This will likely need to change once the first disease-modifying drug is approved; it is safe to assume that recruiting sufficient treatment naive in rare disease like FSHD will be nigh impossible.

Upcoming outcome measures

In Chapter 4, we concluded that general neuromuscular COMs are not feasible for FSHD trials. The recently developed RWS shows more promise and will likely be

used in upcoming trials. Other COMs are still being developed, and might replace the RWS eventually. Surface-EMG during a maximum cycling test might be able to capture fatigue of the muscle in a reliable, more sensitive way than the traditional ventilation based methods[61]. It also enables measuring the muscle fatigue without confounding of stamina or cardiopulmonary comorbidities. Surface EMG could become a sensitive biomarker to measure muscle functionality and stamina.

Recordings of body movements will help to gain more insight in the (compensatory) movements of FSHD patients. The RWS is an example of this type of technique. Other techniques used are the VICON system (often used for gait analysis) or the recently developed OpenCap[62,63]. The latter has the advantage of being recorded on smartphones, making it easy to set-up and can be used in out-patient setting or potentially at home. Just like the surface EMG, analyzing (compensatory) movements during functional measurement might enable earlier detection of drug efficacy compared to the actual outcome of the measurement.

There is an increasing interest in measuring the patient at home. It gives more insight in the actual efficacy on their daily life activities and allow for remote trial visits, which decreases the burden on the patient. Wearable devices like a smartwatch can collect the number of hours patient are active or even more detailed information such as 95th centile stride velocity depending on the device[64]. Instead of capturing a snapshot of the participants once every couple of weeks at the hospital, wearables allow for data collection during daily life and for a longer period of time. In the phase II trial of losmapimod (Chapter 5) the actimyo devices (Sysnav, France) were used to measure several upper and lower extremity variables (analysis still ongoing). The actimyo is able to capture stride velocity, which was recently accepted by the FDA as an acceptable outcome measure in Duchenne[65]. Interestingly, during the interviews in Chapter 7, many participants mentioned that wearing the actimyo was the most burdensome part of the trial. Partly due to the relatively large size of the devices, partly due to intensive schedule they needed to adhere to. Besides wearable devices, other measurements could also be completed at home. For example, the developers of the RWS are planning a study to determine the feasibility and validity of performing the RWS at home.

Changes in clinical management

It is reasonable to assume that a drug will be approved for FSHD in the near future, which will require certain preparations for clinical sites. As the developments for disease-modifying therapies are occurring in several neuromuscular diseases, these preparations should be based on the lessons learned from other disease (Box 3).

Most likely, an approved drug will only be available for genetically confirmed patients. Although the exact number of patients without a genetic confirmation in the Netherlands is unknown, we need to anticipate an increase in genetic testing requests. To update the capture-recapture study performed in 2014, we will perform a 4-source capture-recapture study to gain more insight in the incidence number of FSHD patients and number of genetically unconfirmed patients[66]. This study will assist the Dutch FSHD expertise center, FSHD patient representatives and the patient advocacy group (Spierziekten Nederland) in reaching out to Dutch FSHD patients regarding the necessity of genetical confirmation as well as the benefits of registration in the FSHD registry. Ideally, all patients are genetically confirmed before the first drug is approved. Considering the turn-around time of the genetic test is approximately three months, genetic testing should be started preemptively.

It is not expected that upcoming treatments are able to fully reverse the disease state of the current population. Therefore, optimizing the disease management and lifestyle of FSHD patients will remain important. The two available evidence-based guidelines will soon be updated and combined into one guideline to aid physicians in optimizing symptomatic treatment and supporting a healthy lifestyle for FSHD patients[67,68].

It is vital that the necessary logistics for the administration of the new drug are in place. An increase in outpatient clinic visits to gain access to the drug should be expected. It might also be mandatory to monitor the effect and safety of the drug in the initial years after market approval as part of a phase IV study which may require multiple follow-up visits[69,70]. In the case of an oral drug, no large additional logistical measures need to be taken. In the case of IV-administered drugs like Avidity's del-brax[31], several additional measures need to be taken: a location to administer the drugs needs to be available and medical personnel need to be present to monitor the safety. Even for a rare disease like FSHD, it will be difficult to accommodate all the Dutch FSHD patients assuming the first administrations will take place in the two expertise centers. If only half of the estimated 2000 Dutch FSHD patients are eligible for the IV-drug, which needs to administered every eight weeks, the two expertise centers would need to dose twelve patients each day. It will be challenging to fit these administrations in the current daily care, involving a clinical trial team might be the solution for both the administration and phase IV monitoring.

Box 3. Learning from other neuromuscular disorders

Many companies are working on therapies for different neuromuscular disorders simultaneously. Their method of delivering the drug to the muscle cells is their patented product, the drug component itself is often interchangeable. It is therefore likely that other neuromuscular disorders like Duchenne muscular dystrophy and myotonic dystrophy will also have disease-modifying therapies available in the near future. Although not all aspects of these disease are translatable to each other, it is important that we learn from each other, to ensure optimal trial readiness and clinical management. Spinal muscle atrophy (SMA) is a neuromuscular disease for which several disease-modifying therapies were approved in the recent years. Studying the consequences of these therapies will help in preparing the FSHD community for the first approved FSHD therapy[71].

Some of the new SMA drugs showed the most impressive results when the drug was administered before the first clinical symptoms appeared. To enable pre-symptomatic treatment, newborn screening on SMA was developed and is being used worldwide. Early detection and treatment of SMA is of course a beneficial development, it comes with ethical considerations. Parents of asymptomatic babies are confronted with a severe diagnosis and difficult therapy decisions. It is questionable if newborn screening will be necessary for FSHD considering its slow disease progression and relatively onset, but genetic testing of children might be necessary for optimal treatment.

Due the new drugs, severely affected SMA patients have a much longer life expectancy, resulting in the emergence of new phenotypes. This called for new approaches in the clinical care of SMA patients and made the multidisciplinary management of the disease and a healthy lifestyle even more important. While I don't not expect that new FSHD phenotypes will emerge, it does show that clinical care and lifestyle choices remain important even after a disease-modifying drug becomes available. Therefore, studies on optimizing clinical management of FSHD remain important to perform, even with the current developments in new therapies.

Lastly, these therapies were approved on a relatively small amount of data, which makes phase IV trials mandatory. This means that real world data needs to collected systematically and is preferably stored in a standardized (international) registry. To ensure successful phase IV trials, international consensus on standardized outcome measures is of vital importance.

Conclusion

This thesis improved the state of clinical trial readiness of the FSHD field by assessing the results of several questionnaires and clinical outcome measures, and evaluating the trial experience from the participants' perspective. The lessons learned during the creation of this thesis resulted in suggestions for adjustments of the current trends to optimize upcoming FSHD trials. Furthermore, a blueprint of a successful clinical trial team with a standardized workflow was created to assist with establishing new clinical trial sites and to improve the efficiency of current sites. Lastly, it contributed to the development of losmapimod, the first DUX4-targeting therapy, which unfortunately did not show efficacy. With this thesis, the first steps in the new era of clinical trials in FSHD have been taken.

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Chapter 9

Nederlandse Samenvatting

Nederlandse Samenvatting

Facioscapulohumerale spierdystrofie (FSHD) is een langzaam progressieve, erfelijke spierziekte die wordt veroorzaakt door een verhoogde productie van de transcriptiefactor DUX4, wat uiteindelijk resulteert in achteruitgang van de skeletspieren. De typische patiënt ervaart aanvankelijk zwakte van de spieren van het gezicht, de schouder en armen tussen de leeftijd van 15-30 jaar. Naarmate de ziekte vordert, zullen ook de spieren van de romp, bekken en de benen worden aangetast. FSHD heeft een zeer variabel ziekteverloop, wat het moeilijk maakt om de individuele progressie te voorspellen en resulteert in een heterogene patiëntenpopulatie. Momenteel bestaan er geen ziekte modificerende therapieën, maar worden in de toekomst verwacht aangezien meer dan twintig bedrijven nieuwe medicijnen ontwikkelen. Om deze nieuwe medicijnen betrouwbaar te kunnen testen, moeten bepaalde componenten aanwezig zijn, dit wordt trial readiness genoemd. Het eerste deel van dit proefschrift had als doel de trial readiness te verbeteren door de geschiktheid van vragenlijsten en klinische uitkomstbeoordelingen voor klinische onderzoeken te analyseren. Het tweede deel van dit proefschrift had als doel een bijdrage te leveren aan de ontwikkeling van losmapimod als een ziekte modificerende therapie voor FSHD en om de onderzoeken te evalueren vanuit het perspectief van de patiënt. Hiervoor werden een fase 2 open-label onderzoek om de veiligheid en werkzaamheid van losmapimod te beoordelen, een vragenlijstonderzoek naar de belasting van spierbiopten en een diepte-interviewonderzoek om meer inzicht te krijgen in de algehele ervaring van deelname aan een klinisch onderzoek uitgevoerd.

Deel I: Verbeteren van Clinical Trial Readiness

In **Hoofdstuk 2** hebben we de gegevens geanalyseerd die zijn verzameld met behulp van de Nederlandse FSHD Registratie. Het register, dat in 2015 werd opgestart na een internationale samenwerking op het gebied van trial readiness, had tot doel longitudinale gegevens over FSHD-symptomen te verzamelen, de data verzameling en rekrutering van FSHD-patiënten voor onderzoeksdoeleinden te vergemakkelijken en een snelle verspreiding van belangrijke informatie mogelijk te maken. De gegevens die in het register werden verzameld, bestonden uit een vooraf bepaalde set vragen, die elke zes maanden werden verzonden, over ziektekenmerken, een vermoeidheidsvragenlijst (CIS20R), een vragenlijst over de kwaliteit van leven (INQoL), een depressievragenlijst (BDI) en een pijnvragenlijst (MPQ). De gegevens werden bij aanvang cross-sectioneel geanalyseerd en

de CIS20R, INQoL, BDI en MPQ werden ook longitudinaal geanalyseerd met behulp van mixed models. Vanaf de start tot maart 2021 zijn 373 patiënten geregistreerd en hebben zij ten minste één set vragenlijsten volledig ingevuld. Vermoeidheid, zwakte en pijn in de schouders en (onder)rug waren de meest prominente symptomen. Negentien van de 23 (deel)vragenlijsten lieten na zes jaar geen significante veranderingen in de tijd zien, de overige vier lieten minimale veranderingen zien. Uit een subanalyse tussen drie mobiliteitsgroepen (mobiel zonder hulpmiddel, mobiel met hulpmiddel en rolstoelafhankelijk) bleek dat sommige (sub)vragenlijsten onderscheid kunnen maken tussen de groepen, maar dat er geen verschil in longitudinale veranderingen werd waargenomen. Het register faciliteerde veertien onderzoeken met gegevensverzameling of werving van deelnemers. Op basis van deze resultaten concludeerden we dat de vragenlijsten niet bruikbaar zijn voor klinische onderzoeken.

Hoofdstuk 3 rapporteert over de gevolgen van de COVID-19 pandemie op de fysieke en mentale gezondheid van FSHD-patiënten en vergelijkt de incidentie en ernst van COVID-19-infecties tussen FSHD-patiënten en een populatie zonder FSHD. Een zelf opgestelde vragenlijst en de gevalideerde perceived stress scale (PSS) werden in 2020 drie keer (mei, augustus en december) verzonden om de evolutie van de pandemie vast te leggen. In de zelf gemaakte vragenlijst werd gevraagd naar de fysieke symptomen van de deelnemers, de beschikbare zorg en de COVID-19-incidentie van de patiënt en hun huisgenoten zonder FSHD. De drie vragenlijsten werden ingevuld door respectievelijk 210, 186 en 205 deelnemers. Deelnemers rapporteerden een hogere last van FSHD symptomen en waren minder actief tijdens de COVID-19 pandemie. Deelnemers rapporteerden ook meer stress vergeleken met de stress vóór de pandemie, maar de PSS-scores werden nog steeds als laag beschouwd. Interessant genoeg rapporteerden deelnemers ook over de positieve effecten van de pandemie: door minder sociale verplichtingen ervoeren sommige deelnemers minder symptomen en konden ze meer tijd met hun gezin doorbrengen. Er was geen verschil in het aantal positieve testen tussen FSHD-patiënten en de huisgenoten zonder FSHD. FSHD-patiënten rapporteerden minder last te hebben van COVID-19-infecties, maar dit werd hoogstwaarschijnlijk veroorzaakt door rapportagebias. Dit hoofdstuk dient als een goed voorbeeld van de effectiviteit van het FSHD-register, dat een snelle rekrutering van een groot aantal FSHD-patiënten mogelijk maakt, zelfs in ongekende tijden.

In het Radboudumc is een groot natuurlijk beloop studie in FSHD-patiënten gestart om meer kennis te krijgen over het natuurlijke ziekteverloop en om bruikbare uitkomstmaten voor klinische onderzoeken te identificeren. In **Hoofdstuk 4**

analyseerden we de klinische uitkomstmaten die werden getest tijdens de vijf jaar durende studie van dit cohort, waarbij we ons concentreerden op het bepalen van de haalbaarheid van de klinische uitkomstmaten voor klinische onderzoeken. In dit onderzoek werden zes verschillende uitkomstmaten getest: de motor function measure (een schaal die spierfunctie meet), handmatige spiertesten met behulp van de MRC-score, zes minuten looptest, kwantitatieve beoordeling van de spierkracht van de m. quadriceps, clinical severity scale en FSHD-evaluation scale (beide schalen om de ernst van de FSHD symptomen weer te geven). De analyses omvatten de verandering over tijd, het bepalen van het minimaal klinisch relevant verschil, en verschillende powerberekeningen. Na het uitsluiten van niet-penetrante patiënten (d.w.z. patiënten zonder enige symptomen zoals bepaald door de onderzoeker), voltooiden 154 deelnemers het basisbezoek en het vijfjaarlijkse vervolgbezoek. Alle uitkomstmaten vertoonden een statistisch significant verschil over een periode van vijf jaar. Deze veranderingen waren echter minimaal en alleen de motor function measure, clinical severity scale en FSHD-evaluation scale vertoonden een klinisch relevant verschil. Deze drie uitkomstmaten vereisten ook het laagste aantal deelnemers voor een klinisch onderzoek. De clinical severity scale en FSHD-evaluation scale zijn echter niet haalbaar voor klinische onderzoeken op korte termijn vanwege hun ordinale score en de motor function measure vereist een onderzoek duur van minimaal drie jaar om een klinisch relevant verschil te bereiken. Het merendeel van de huidige onderzoeken hanteert een score op de clinical severity scale van 4-8 als inclusiecriterium, maar dit kan worden uitgebreid naar 3-9 zonder veel power te verliezen op basis van onze gegevens. Het uitbreiden van dit criterium zal een snellere rekrutering mogelijk maken, de generaliseerbaarheid vergroten en de duurzaamheid van toekomstige studies verbeteren. Concluderend benadrukt deze studie de minimale progressie van FSHD en het is onwaarschijnlijk dat de in deze studie onderzochte uitkomstmaten voldoende gevoelig zijn om de ziekteprogressie in klinische onderzoeken vast te leggen.

Part II: Geneesmiddelenonderzoek en Ervaringen van Deelnemers

Een fase II open-label onderzoek waarin de veiligheid, verdraagbaarheid, farmacokinetiek, farmacodynamiek en werkzaamheid op verkennende uitkomstmaten van losmapimod werden onderzocht, wordt gerapporteerd in **Hoofdstuk 5**. Losmapimod is een p38 α/β-mitogeen-geactiveerde proteïnekinaseremmer, dat een remmend effect vertoonde op de DUX4-productie in spiercellen met FSHD. Deelnemers werden tweemaal daags

behandeld met losmapimod 15 mg. Er werden vitale parameters gemeten, bijwerkingen bijgehouden en bloed- en spiermonsters afgenomen voor analyse van de veiligheid, farmacokinetiek en -dynamiek, en de DUX4 concentratie. Verkennende uitkomstmaten bestonden uit de Reachable Workspace (een maat om schouderfunctie te meten), spierkrachtmetingen, motor function measure Domein 1, getimed up-and-go, 6-minuten looptest, spirometrie en drie vragenlijsten (FSHD Health Index, FSHD Rasch-built Overall Disability Scale, Patient Global Impression of Change). Veertien deelnemers met FSHD type 1 werden geïncludeerd en alle deelnemers voltooiden het onderzoek met succes. Er deden zich geen ernstige bijwerkingen voor; de meest voorkomende bijwerkingen betroffen een milde, voorbijgaande verhoging van de leverenzymen. Er werden stabiliteit of kleine verbeteringen waargenomen op de verkennende uitkomstmaten, in overeenstemming met de behandelarm van het gelijktijdig lopende placebogecontroleerde fase II-onderzoek (REDUX4). Samenvattend liet losmapimod een gunstig veiligheidsprofiel zien, evenals een mogelijk positief effect op het beloop van de FSHD-ziekte. Er werd een gerandomiseerd placebo-gecontroleerd fase 3-onderzoek (REACH) gestart om de werkzaamheid van losmapimod verder te onderzoeken.

Fase I- en II-onderzoeken zijn gericht op het bepalen van de farmacokinetiek, farmacodynamiek en moleculair effect van het onderzoeksproduct. In het geval van FSHD betekent dit het analyseren van spierweefsel op DUX4 concentratie en andere betrokken genen. Daarom zullen spierbiopten waarschijnlijk een noodzakelijk onderdeel blijven van FSHD-onderzoeken. De last van deze invasieve procedure was nog niet vastgesteld bij FSHD-patiënten. **Hoofdstuk 6** rapporteert over een retrospectieve vragenlijststudie om de patiëntlast van naaldbiopten te beoordelen en om de last van twee verschillende methoden te vergelijken (poliklinische biopsieën versus MRI-geleide biopsieën). In dit onderzoek ontvingen alle Nederlandstalige FSHD-patiënten die ten minste één spierbiopsie ondergingen voor onderzoeksdoeleinden, hetzij tijdens een natuurhistorisch onderzoek, hetzij tijdens een klinische proef, een zelfgemaakte vragenlijst over de belasting van de biopsieën. Negenenveertig patiënten rapporteerden over 91 biopsieën, meestal genomen uit de vastus lateralis of gastrocnemius. De last van de biopsieën was hoog, maar van korte duur. De MRI-geleide biopsieën gaven significant meer last dan de biopsieën op de polikliniek. Over het geheel genomen concludeerden we dat het opnemen van spierbiopten in onderzoeken noodzakelijk zal zijn, maar dat de last niet mag worden onderschat. Nieuwe technieken met kleinere naalden en/of meer gebruik van verdoving kunnen de last in toekomstige onderzoeken verlagen.

In de afgelopen jaren zijn er verschillende stappen gezet om de trial readiness voor FSHD te verbeteren: er zijn meerdere natuurlijk beloop onderzoeken uitgevoerd, patiëntenregistraties zijn gestart en er zijn nieuwe klinische uitkomstmaten ontwikkeld. Evaluaties van de lopende onderzoeken vanuit het perspectief van de patiënt ontbraken echter. Daarom hebben we een kwalitatieve studie uitgevoerd om de motivatie, verwachtingen, zorgen en ervaringen van FSHD-patiënten die hebben deelgenomen aan een klinische studie te onderzoeken (**Hoofdstuk 7**). Deelnemers uit het lopende fase II open-label onderzoek en het fase III gerandomiseerde placebo-gecontroleerde onderzoek waren uitgenodigd. Een onafhankelijke onderzoeker hield semigestructureerde interviews van ongeveer een uur om de bovengenoemde thema's te verkennen. Motivaties om deel te nemen aan een medicijnproef varieerden van altruïstisch perspectief tot eigenbelang. Deelnemers rapporteerden realistische verwachtingen ten aanzien van de werkzaamheid van het geneesmiddel en mogelijke bijwerkingen. Ze rapporteerden een positieve ervaring van deelname aan de onderzoeken, vooral dankzij de persoonsgerichte aanpak en persoonlijke communicatie van het kleine onderzoeksteam. Regelmatige updates van de sponsor over de voortgang en resultaten van de proeven zouden op prijs zijn gesteld. Geen van de deelnemers raadde deelname aan een proef af, maar erkende wel dat er rekening moet worden gehouden met persoonlijke factoren zoals reistijd en werkstatus.

Appendix

Research Data Management

Ethics and Privacy

Chapter 2 and 3 were based on the FSHD registry. Data collection and analyses of longitudinal patient reported outcome measures and was deemed medical research that did not fall within the scope of the Medical Research Involving Human Subjects Act. This was declared by the local Medical Ethics Review Committee of the Radboud university medical center (amendment of file No. 2015-1812 on April 15th 2020 based on the original decision with the file No. 2013/403 on August 28th 013).

Chapter 4 and 5 were based on the results of research involving human participants, which were conducted in accordance with relevant national and international legislation and regulations, guidelines, codes of conduct and Radboudumc policy. The recognized Medical Ethics Review Committee 'METC Oost-Nederland' has given approval to conduct these studies (file numbers: NL68245.091.18 and NL69446.091.19).

Chapter 6 and 7 were based on results from studies that did not fall within the scope of the Medical Research Involving Human Subjects Act. The institutional ethical review committee CMO Radboudumc, Nijmegen, the Netherlands has given approval to conduct these studies (CMO Radboudumc dossier number: 2020-6981, 2023-16354).

For all studies, the privacy of the participants in these studies was warranted by the use of pseudonymization. The pseudonymization key was stored on a secured network drive that was only accessible to members of the project who needed access to it because of their role within the project. The pseudonymization key was stored separately from the research data. If data collection from electronic patient files was needed, it was performed by personnel with a treatment relationship with the patient and by the researcher(s) upon consent by the study participant.

Informed consent was obtained from all participants to collect and process their data for each research project. Permission to share the data was obtained for chapter 2, 3 and 5.

Data collection and storage

Data from chapter 2 and 3 were collected using questionnaires send out to every FSHD patient who registered themselves in the Dutch FSHD Registry. Questionnaires and data were collected using CastorEDC. The data has been archived in the Radboud Data Repository (RDR).

Data from Chapter 4 contained patient information, outcome assessments and questionnaires. Patient information was collected from electronic health records (EPIC). Additionally, informed consent procedure, MRI images and ultrasound images were stored in EPIC. Outcome assessment data were immediately stored in CastorEDC. Questionnaires were send out and stored using CastorEDC. The data has been archived in the RDR.

Data from phase 2 trial in Chapter 5 is stored in several locations. First, clinical outcome assessments were completed on paper and are stored at the UTS Archive. The data was captured in Medrio EDC, a full printout of the completed EDC is stored in the RDR. Additionally, Fulcrum Therapeutics has this data stored. Imaging data are stored in the electronic patient files (EPIC).

The questionnaire data from chapter 6 was collected and initally stored in CastorEDC. The data has been archived in the RDR.

Interview data was initially collected using Microsoft Teams. After analysis, the data of the interviews was archived in the RDR.

Data sharing according to the FAIR principles

Data collected on paper will be stored for 25 years in the UTS archive in accordance with the Radboudumc guidelines. All the digital data collected and used in this thesis have been stored in the RDR. This data will be stored for 15 years or 25 years depending on the study. The table below details where the data and research documentation for each chapter can be found on the RDR.

Chapter	DAC	Storage time
2 & 3	DOI: https://doi.org/10.34973/dxge-bp38	15 years
4	DOI: https://doi.org/10.34973/chmw-nq46	15 years
5	DOI: https://doi.org/10.34973/52t4-mg44	25 years
6	DOI: https://doi.org/10.34973/qdnv-7455	15 years
7	DOI: https://doi.org/10.34973/de07-fp97	15 years

Due to the rareness of the disease it would be possible to identify patients based on a small number of variables (e.g. age, sex and muscle weakness pattern). Hence, to ensure the privacy of the participants, we have decided to store the data in closed access repositories. Chapter 2 and 3 contain data captured in the FSHD registry. Access to data captured in the FSHD registry can be gained by completing a data request form on the FSHD registry website (https://www.fshdregistratie.nl/dataverzoek/data-request2/). Chapter 5 concerned a phase 2 drug trial with Fulcrum Therapeutics as the sponsor. Currently, Fulcrum Therapeutics is collaborating with the patient advocacy group FSHD Society to make the (meta)data available in a FAIR way. As this process is still ongoing, it is not yet known when and where the (meta)data will be available.

Raw data is stored in .sps, .sav or .xlsx files. The files contain explanation of the variables. Data analysis was performed in SPSS (IBM SPSS Statistics Software) or Excel (Microsoft Excel). Text file are stored as .pdf or .docx files. The transcripts of the interview in chapter 7 were analyzed in ATLAS.ti and stored as .atlproj23 files.

List of Publications

Quantitative Muscle Analysis in FSHD Using Whole-Body Fat-Referenced MRI: Composite Scores for Longitudinal and Cross-sectional Analysis.

Mellion ML, Widholm P, Karlsson M, Ahlgren A, Tawil R, Wagner KR, Statland JM, Wang L, Shieh PB, van Engelen BGM, **Kools J**, Ronco L, Odueyungbo A, Jiang J, Han JJ, Hatch M, Towles J, Leinhard OD, Cadavid D.Neurology. 2022 Aug 30;99(9):e877-e889. doi: 10.1212/WNL.0000000000200757. Epub 2022 Jun 24.PMID: 35750498

Assessment of the burden of outpatient clinic and MRI-guided needle muscle biopsies as reported by patients with facioscapulohumeral muscular dystrophy.

Kools J, Aerts W, Niks EH, Mul K, Pagan L, Maurits JSF, Thewissen R, van Engelen BG, Voermans NC.Neuromuscul Disord. 2023 May;33(5):440-446. doi: 10.1016/j.nmd.2023.04.001. Epub 2023 Apr 6.PMID: 37099913 Free article.

The Dutch registry for facioscapulohumeral muscular dystrophy: Cohort profile and longitudinal patient reported outcomes.

Kools J, Deenen JC, Blokhuis AM, Verbeek AL, Voermans NC, van Engelen BG.Neuromuscul Disord. 2023 Dec;33(12):964-971. doi: 10.1016/j.nmd.2023.10.020. Epub 2023 Nov 4.PMID: 38016873 Free article.

Oral ribose supplementation in dystroglycanopathy: A single case study.

Thewissen RMJ, Post MA, Maas DM, Veizaj R, Wagenaar I, Alsady M, **Kools J**, Bouman K, Zweers H, Meregalli PG, van der Kooi AJ, van Doorn PA, Groothuis JT, Lefeber DJ, Voermans NC.JIMD Rep. 2024 Mar 4;65(3):171-181. doi: 10.1002/jmd2.12394. eCollection 2024 May.PMID: 38736632 Free PMC article.

Living with facioscapulohumeral muscular dystrophy during the first two COVID-19 outbreaks: a repeated patient survey in the Netherlands.

Deenen JCW, **Kools J**, Greco A, Thewissen R, van de Put W, Lanser A, Joosten LAB, Verbeek ALM, van Engelen BGM, Voermans NC.Acta Neurol Belg. 2024 Apr;124(2):559-566. doi: 10.1007/s13760-023-02443-3. Epub 2024 Jan 13.PMID: 38218752 Free PMC article.

Safety and efficacy of losmapimod in facioscapulohumeral muscular dystrophy (ReDUX4): a randomised, double-blind, placebo-controlled phase 2b trial.

Tawil R, Wagner KR, Hamel JI, Leung DG, Statland JM, Wang LH, Genge A, Sacconi S, Lochmüller H, Reyes-Leiva D, Diaz-Manera J, Alonso-Perez J, Muelas N, Vilchez JJ, Pestronk A, Gibson S, Goyal NA, Hayward LJ, Johnson N, LoRusso S, Freimer M, Shieh PB, Subramony SH, van Engelen B, **Kools J**, Leinhard OD, Widholm P, Morabito C, Moxham CM, Cadavid D, Mellion ML, Odueyungbo A, Tracewell WG, Accorsi A, Ronco L, Gould RJ, Shoskes J, Rojas LA, Jiang JG.Lancet Neurol. 2024 May;23(5):477-486. doi: 10.1016/S1474-4422(24)00073-5.PMID: 38631764 Clinical Trial.

An open-label pilot study of losmapimod to evaluate the safety, tolerability, and changes in biomarker and clinical outcome assessments in participants with facioscapulohumeral muscular dystrophy type 1. **Kools J**, Voermans N, Jiang JG, Mitelman O, Mellion ML, Ramana V, van Engelen BGM. J Neurol Sci. 2024 Jul 15;462:123096. doi: 10.1016/j.jns.2024.123096. Epub 2024 Jun 15. PMID: 38959779.

A 5-year natural history cohort of patients with facioscapulohumeral muscular dystrophy determining disease progression and feasibility of clinical outcome assessments for clinical trials. **Kools J**, Vincenten S, van Engelen BGM, Voet NBM, Merkies I, Horlings CGC, Voermans NC, Mul K. Muscle Nerve. 2025 Jan;71(1):55-62. doi: 10.1002/mus.28293. Epub 2024 Nov 7. PMID: 39508285; PMCID: PMC11632561.

PhD portfolio of Joost Kools

Department: **Neurology**
PhD period: **01-03-2019 – 10-03-2025**
PhD Supervisor(s): **Prof. dr. N.C. Voermans, Prof. dr. B.G.M. van Engelen**
PhD Co-supervisor(s): **Dr. K. Mul**

Training activities	Year
Courses	
• Graduate School Day	2019
• E-BROK	2019
• GS Introduction	2019
• Scientific Integrity Course	2021
• Graduate School Day 2	2022
• Basic training qualitative research	2024
• E-BROK	2024
Conferences	
• *FSHD International Research Congress*	2019
• *FSHD International Research Congress* Poster: Design of an open-label pilot study of losmapimod to evaluate the safety, tolerability, and changes in biomarker and clinical outcome assessments in subjects with facioscapulohumeral muscular dystrophy 1 (FSHD1)	2020
• *World Muscle Society Congress* Oral Presentation Symposium: Advances in Assessment of FSHD and Clinical Trial Results with Losmapimod	2021
• *FSHD International Research Congress* Poster: Feasibility of measuring functional performance of FSHD patients using wearable sensors to quantify physical activity Poster: Living with FSHD during the pandemic corona outbreak in the Netherlands: pitfalls and challenges of COVID-19 in FSHD Poster: Muscle Ultrasound in an Open-Label Study of Losmapimod in Subjects with FSHD1	2022
• *American Academy of Neurology Annual Meeting* Poster: Muscle Ultrasound in an Open-Label Study of Losmapimod in Subjects with FSHD1 Oral Presentation: Feasibility of Measuring Functional Performance of FSHD Patients Using Wearable Sensors to Quantify Physical Activity	2023
• *FSHD International Research Congress* Poster: Assessment of the burden of outpatient clinic and MRI-guided needle muscle biopsies as reported by patients with facioscapulohumeral muscular dystrophy	2023
• *International Myotonic Dystrophy Consortium Meeting*	2024
• *FSHD International Research Congress* Poster: The participants' perspective on clinical trials – a qualitative study Poster: The Dutch Registry for Facioscapulohumeral Muscular Dystrophy: Cohort profile and longitudinal patient-reported outcomes Oral Presentation: A 5-year natural history cohort of patients with facioscapulohumeral muscular dystrophy determining disease progression and feasibility of clinical outcome assessments for clinical trials Oral Presentation: FSHD disease progression and losmapimod efficacy assessed by reachable workspace in both arms	2024

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Training activities	Year
Conferences	
• *Portuguese Yearly Neuromuscular Congress* Oral Presentation: News in Facioscapulohumeral Muscular Dystrophy Treatment	2024
• *World Muscle Society Congress* Poster: Initial Data From the ACHIEVE Trial of DYNE-101 in Adults With Myotonic Dystrophy Type 1 (DM1)	2024
• *Yearly Neuromuscular Patient Congress* Oral presentations: updates trial FSHD Oral presentations: Updates trials myotonic dystrophy	2020-2024
Other	
• Webcast 'Meedoen aan (medicijn)onderzoek' (Spierziekten Nederland)	2022
• Handheld Dynamometry trainer Reach Study	2022-2024
• Presentation 'Lesson Learned from the OLS' on the Investigator's Meeting Reach Study	2022

Teaching activities	
Lecturing	
• Translation Neuroscience Lectures Myotonic Dystrophy type 1 Tutorship research proposal	2024
• Clinical Trials Lectures FSHD	2023-2024
Supervision of internships / other	
• Willem Aerts 3 months (Assessment of the burden of outpatient clinic and MRI-guided needle muscle biopsies as reported by patients with facioscapulohumeral muscular dystrophy)	2020
• Renée Thewissen 3 months (Assessment of the burden of outpatient clinic and MRI-guided needle muscle biopsies as reported by patients with facioscapulohumeral muscular dystrophy)	2021

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Een promotietraject is een unieke ervaring die deels wordt gevormd door het promotieteam. Baziel, je hebt me vanaf het begin vrij gelaten en het vertrouwen gegeven dat ik de losmapimod studie goed zou uitvoeren. Daarnaast hebben we vele filosofische gesprekken gehad over onderzoek, de huidige zorg en andere onderwerpen die ter sprake kwamen. Nicol, als dagelijkse begeleider was je altijd laagdrempelig beschikbaar, zelfs voor de kleinste vragen of frustraties. Jouw doelgerichte instelling heeft een grote bijdrage gehad aan de succesvolle afronding van dit proefschrift. Karlien, dit avontuur is begonnen met een wetenschappelijke stage bij jou. Ik kon altijd bij je terecht met praktische vragen of even een momentje van reflectie. Het mag duidelijk zijn dat jullie alle drie een andere rol als supervisor hadden en juist dat maakt jullie een geweldig promotieteam.

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Curriculum Vitae

Joost Kools was born on 4 September 1992 in Tilburg, The Netherlands. He followed his secondary education at the Theresialyceum in Tilburg, The Netherlands, graduating in 2010. He started with Biomedical Sciences at the Radboud University in Nijmegen, The Netherlands in 2011, finishing his Bachelor's degree in 2014 with health and technology assessment as specialty. After successfully completing the pre-master for Medicine in 2014-2015, he started his Master's in 2016 which he completed in 2019.

After gaining his MD degree, he immediately started as a PhD candidate at the neurology (neuromuscular) department of the Radboudumc. Initially, his focus was on performing the phase II open-label study of losmapimod in patients with FSHD. Due to the expected surge of upcoming clinical trials, Joost used his clinical trial experience to improve clinical trial readiness (by analyzing the Dutch FSHD Registry data and natural history study data). During his PhD candidacy he had many meetings with pharmaceutical companies, which brought the lack of knowledge about patient experience on clinical trials to light. As a result, he initiated two studies to gain more knowledge about the patient's experience.

After finishing the largest part of his PhD projects, Joost also became involved in Myotonic Dystrophy type 1 (DM1) clinical trials. He became the principal investigator on the ACHIEVE (phase I/II DM1 clinical trial) performed by Dyne Therapeutics. He is also involved as a sub-investigator on the REACH (phase III losmapimod) clinical trial performed by Fulcrum Therapeutics and the HARBOR (phase III DM1 clinical trial) performed by Avidity Biosciences. He aims to stay involved in clinical trials after obtaining his PhD.

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