

An Adjunct Scientific Workshop Sponsored by the FSHD Society to Familiarize the FDA with Biomarkers and Clinical Assessments of Potential Use in FSHD Clinical Trial

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About the FSHD Society

The FSHD Society is the world's largest research-focused patient advocacy organization for facioscapulohumeral muscular dystrophy (FSHD), one of the most prevalent forms of muscular dystrophy. For over 30 years, the Society has driven major advancements in research and treatment development, with the mission to end the pain, disability, and suffering endured by one million people worldwide living with FSHD. The Society supports families affected by this disease through programs like BetterLife, which empowers patients with tools to track symptoms and connect with researchers, and FSHD Navigator, a service providing personalized assistance to help handle the challenges of living with FSHD. Our promise to patients is that no family need ever face this disease alone.

Workshop Goals

This Adjunct Scientific Workshop enabled the FSHD community to share our progress and collaborate with the FDA on clinical trial endpoints. This meeting follows productive interactions with the agency including a Patient Focused Drug Development (EL-PFDD) meeting held on June 29, 2020 and a Patient-Led Listening Session (PLS) on Upper-body mobility on August 23, 2024.

The goals of the Workshop were to discuss FSHD clinical trial endpoints from the patient perspective and to have leading experts provide an overview as well as report on progress in FSHD clinical knowledge including: clinical outcome assessments, musculoskeletal MRI and biomarkers. Experts provided presentations on the background and advancements of each assessment, and indicated strategies to address existing challenges.

Full recording available here: <https://youtu.be/EfKWakL7a7I>

Slide deck available here: <https://www.fshdsociety.org/adjunct-slides>

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Speakers

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Opening Remarks

Michelle Mellion, MD

Medical Advisor, FSHD Society

The number of clinical trials in FSHD is increasing, with several ongoing and completed trials using different therapeutic approaches. Most of these focus on the root cause of disease, expression of DUX4 or closely associated processes. **The toxic gain of function due to de-repression of DUX4 causes relentless progression of cranial to caudal muscle weakness, significant morbidity, and early mortality.**

FSHD is a Relentlessly Progressive Disease resulting in significant Morbidity and Early Mortality



Figure 1. FSHD impacts all skeletal muscles of body in heterogenous pattern and with profound morbidity and early mortality.

FSHD has been clinically diagnosed in an estimated 8,000-10,000 US citizens. It is a genetic condition, which can be either inherited or occur spontaneously. The onset of symptoms can begin at any age, and anyone can be affected. **Given the relentless nature of the disease and that the cause of the disease resides in DUX4 mis-expression in muscle regardless of age of onset or clinical phenotype, treatments for FSHD should not be restricted based on arbitrary inclusion criteria, but rather clinical trial results should be appropriately extrapolated so that the entire community may have access to a treatment once approved.** Now is a critical time for our community to work collaboratively with our regulatory partners to understand these important clinical trial endpoints that support the development of disease modifying or potentially curative therapies.

Patient Voice

Summary of learnings from EL-PFDD and PLS

James Valentine, Consultant

Hyman, Phelps & McNamara

A patient-focused FSHD approach will align with the FDA's Patient-Focused Drug Development (PFDD) initiative, launched in 2012, which acknowledges that patients, as those living with the disease, have a critical role in informing regulatory decisions. While FDA approval is grounded in scientific analysis of benefits and risks, there

is also a subjective component that involves value judgments. These judgments depend heavily on understanding how much risk patients are willing to accept for potential benefits, including the uncertainty that comes with early access to treatments based on biomarkers rather than direct clinical outcomes. The FDA's regulatory framework explicitly incorporates patient experience data, particularly focusing on the burdens of daily living and the inadequacies of current treatment options. This information directly influences decisions around trial design and outcome measures. For instance, patient input helps determine which symptoms to target, how to best measure them (e.g., using digital tools versus clinic visits), and what level of improvement would be considered meaningful. The FSHD community has had two significant engagements with the FDA previously: the 2020 EL-PFDD meeting, and a 2024 Patient Listening Session (PLS) meeting which focused on upper body mobility.

- The EL-PFDD meeting involved over 600 patient and caregiver survey responses, and more than 300 participants in live discussions. It revealed that impaired mobility, fatigue, and difficulties using arms and hands are among the most burdensome symptoms. These limitations significantly impact walking, socializing, and independence. Patients also reported that current treatments offer minimal relief, highlighting a major unmet medical need. Notably, 62% of patients indicated that slowing or stopping muscle function loss—rather than a complete cure—would be the most meaningful outcome.
- The PLS highlighted the often-overlooked impact of upper body mobility loss, which affects 96% of people with FSHD. Patients described how these limitations can be more disabling than walking difficulties, with simple tasks like lifting a cup or typing becoming impossible. Even small functional improvements were valued highly, as they contribute to maintaining independence and quality of life.

We urge all stakeholders to keep these patient insights at the forefront of ongoing discussions, particularly when considering outcome measures and therapeutic goals for FSHD.

Patient Perspective

Grace Fairchild Fisher

FSHD patient advocate

When you live with a disease that eats your muscles slowly, you feel an ever-present torture both bodily and mentally. The pain, the fire you feel in your muscles and the anxiety of what small or large abilities you may lose – and when. **When is the ever-present question.** You don't know if tomorrow you won't be able to brush your own hair, physically do your job, hold your baby, get up the stairs. Each day you feel the urgency of science – the hope for some therapy that will help slow, stop or restore function. It is so many little things we lose that many researchers and doctors have trouble seeing and understanding – before we lose the big things- swimming, running, walking, even breathing.

It is the little things about this disease that are hard to describe, hard to measure. These are not insignificant. When you lose your muscles, you are keenly aware of every slight loss

of strength and function. With FSHD, many of us lose abilities at the prime of our lives; or before. In the cases of the kids who experience early onset, and are never able to play a childhood game the disease process can impact our ability to play sports, go on hikes, swim in the ocean. Imagine what it is like to lose the ability to walk at the height of career advancement, or to lose the ability to pick up a child, a pot or chop vegetables when you need to feed your kids. The timing of this loss for many of us means we can't live life to its fullest, or we struggle immensely to do so. The world is not built for the disabled.

As someone who is "too strong" with this disease, I would like to offer some insight. I can climb 3 stairs, I can walk, I can run, I can squeeze a hand, generally lift my legs and point my toes. The assessments aren't able to show the fatigue I feel afterwards for hours, days and weeks. These tests measure our body's ability to perform these functions but often they don't show how well we perform, or the compensation we use to perform. They can't measure that I have to roll over to my side and use my arms to push myself up when I try to sit up. They show that I can raise my arms, but not how long I can hold them up before they collapse.

I know when researchers are trying to measure my Tibialis anterior strength, I feel my quadriceps muscles activate instead. This is part of our compensation in FSHD. It is very difficult to isolate a single muscle. With the irregular patterns of disease as well as compensatory abilities of the human body, the disease is hard to describe, hard to understand, and hard to track in clinical settings.

I want to speak about biopsies. This is a patient population that has been told that any muscle we lose, we will never get back. Each muscle is so precious. While we are so eager for treatments, and we largely will do anything to help progress the science, taking chunks of our precious muscles is not something we take lightly. It would be ideal to find alternative options.

Because of this and the heterogeneity of FSHD, I wanted to underscore **the need to think creatively about how sponsors demonstrate efficacy**. A one-size-fits-all approach may not work, and we must evaluate response on the patient level.

Last, I want to implore the FDA to honor the fact that without any treatment the patient community must live in mental and physical anguish as each day passes. For us, we are so eager to extend the window of our functional abilities- to allow us the time to live our lives to the fullest- to play a game of tag, to have a career, to hold our babies and to swim in the ocean, that even a treatment that might slow progression, as have recently been approved in Alzheimer's, would be a win. We are so eager for therapies, and are so very grateful for the attention the FDA, scientists, physicians and pharmaceutical companies are spending on our disease to ensure that we get treatment as soon as possible.

Clinical Outcome Assessment

Jeffrey M. Statland, MD

University of Kansas Medical Center

Clinical outcome measure development in FSHD has been a truly collaborative international effort between academic centers and industry partners. The publication of the genetic model for FSHD in 2010, which identified DUX4 as a target for therapy, led to a

translational shift in the field from understanding what causes FSHD to determining how to treat it. We are now seeing the fruits of many years of effort as the first molecularly designed and gene-targeted therapies enter clinical trials.

When developing clinical outcome measures, it is essential to consider what we know about FSHD and the patient experience. FSHD is caused by a toxic gain of function due to the sporadic expression of DUX4. The sporadic expression has its clinical correlate in a disease that affects people from birth through old age, with considerable variability in severity and rates of progression between individuals, within single families, across generations, and even within an individual from one side of the body to the other.

On average, FSHD is a relentlessly progressive disorder where about 20% of people over 50 may require wheelchair use or lose their ability to maintain employment. Motor strength or function declines on average about 2 to 4% per year. While there are clinically significant extra-muscular manifestations affecting the eyes or ears, these occur very rarely in an average adult population, perhaps in 1% of cases, making them very challenging to consider as outcome measures. There are currently no FDA-approved therapies, with treatment consisting of multidisciplinary care. While standard of care may be uniform across the United States, it differs from country to country.

Large surveys in both the United States and European Union have assessed the prevalence and impact of different symptom categories on patients' lives. In a recent EU survey of over 1,200 individuals with FSHD, the most prevalent and impactful symptom categories were almost all related to weakness: difficulty lifting arms, mobility limitations, fatigue, and endurance issues. Future facing concerns are centered on developing increased weakness, losing mobility or walking ability, and losing independence. These findings underscore that drugs improving strength or function would be important to patients, and our outcome measures must assess these domains.

Current outcome measures span several categories. Global measures represent what is happening across the whole body, such as the FSHD functional composite. Strength measures can be used regionally or to provide a whole-body average. Regional upper extremity assessments like reachable workspace quantify how far someone can reach: there may be a need for more outcomes measuring shoulders and arms, there are a large number of functional motor tasks related to mobility, including the six-minute walk test that have fairly extensive data available for trial planning in FSHD.

For patient-reported outcomes, two are disease-specific: the FSHD Health Index and the FSHD-RODS. Additional data has been obtained on narrower instruments including the Upper Extremity Functional Index and the Facial Disability Index. Most studies now include standard anchoring questions such as patient global impression of change or severity. A model is developing for disease-targeted therapies regarding which outcomes take primary importance depending on development phase. Early in development, biomarkers take the forefront. These include DUX4-related biomarkers in muscle tissue or MRI fat fraction as early signals of muscle changes. Later in development, heading towards drug registration, the approach broadens to include functional outcomes such as reachable workspace or strength testing.

Several large natural history studies provide valuable data sources for outcome measure development. The data from these have fed directly into industry studies. The RESOLVE

natural history study was designed for trial preparedness, including multiple measures of strength and function plus biological fluid collection for biomarker development. It enrolled 320 individuals who were followed for 24 months at 14 sites in the United States, United Kingdom, and Europe. The follow-up MOVE study allowed RESOLVE participants to continue while broadening inclusion criteria to include children and non-ambulatory individuals. Currently involving 400 individuals followed for over three years at 18 international and US centers, MOVE includes sub-studies that add measures based on clinical trial learnings, including whole body MRI, muscle biopsies, reachable workspace, and digital motion analysis in 100 individuals using stride velocity 95th percentile. Completed FSHD clinical trials include the Acceleron and Fulcrum programs, ranging from 80-90 participants in early phases, and up to 260 in the first Phase 3 study. The REACH study proved we could successfully conduct a Phase 3 study in FSHD, enroll it, and complete it in a reasonable timeframe, though the primary outcome of reachable workspace did not demonstrate treatment benefit. The model for study design progressing from Phase 2 to Phase 3, using biomarkers and MRI in Phase 1-2 studies transitioning to functional outcomes for later phases, was developed in these programs. The current trial landscape shows exciting momentum, with two molecularly designed therapies—RNA therapeutics and gene-delivered therapies—currently in human studies, and five to eight additional studies expected to begin first-in-human trials within one to two years. This makes it a critical time for the field (1).

Strength Measurements

Strength was one of the first outcomes developed systematically for FSHD and remains a very important concept for patients. Two widely used methods are manual muscle testing and quantitative myometry. Both use standardized positions to obtain strength measurements and can assess individual muscles or combine them for whole-body composites.

A natural history study in the late 1990s demonstrated good to excellent inter- and intra-rater reliability in multicenter settings (2). Strength was the most sensitive measure to change with an effect size around 0.3, representing approximately 2 to 5% loss of strength per year. Subsequent RESOLVE and MOVE studies have validated these findings, seeing very similar rates of change year to year and similar measurement variability. This enables fairly specific sample size estimates: if a drug simply halted progression in FSHD, about 160 participants per group would be needed. However, if a drug both halted progression and allowed improvement by a similar amount, the number needed becomes quite reasonable at 40 per group.

FSHD Functional Composite

The University of Rochester group developed the FSHD functional composite in response to early clinical trials, recognizing the need for a functional motor outcome representing all affected areas in FSHD. Based on early patient surveys identifying prevalent and impactful functional categories, evaluators chose already validated and well-established functional motor tasks to represent each area.

The composite includes 18 items. Several items have been used as primary outcomes in other muscular dystrophy trials, including the six-minute walk test and ascending/descending stairs. It demonstrates excellent test-retest reliability in multicenter

settings with an intraclass correlation coefficient greater than 0.9. Factor analysis supports a four-factor solution, validating the breakdown into four basic functional categories.

The minimally detectable change required for confident measurement was determined and this threshold is approximately 10%. The composite shows moderate to strong cross-sectional associations with other FSHD features of interest: disease duration, strength, and patient-reported impact. Change over two years is approximately 10% (3).

Reachable Workspace

Reachable workspace (RWS) was designed to fill a critical gap because the field had not previously employed a quantitative measure of shoulder and arm function. Developed by a California group and adopted by Fulcrum for their drug development program, it represents novel repurposing of an existing technology (4). It uses a stereographic Kinect camera to calculate the volume of space someone can reach into, measuring four quadrants around the individual plus a fifth quadrant reaching back and behind. It shows excellent reliability in multicenter settings with standardized training, with an intraclass correlation coefficient greater than 0.95. Good correlations exist between RWS measurements and upper extremity strength measurements as well as ability to perform motor tasks. For example, differences in RWS area correlate with ability to take a shirt on and off or pick up clothes (4).

However, in the RESOLVE natural history study, reachable workspace in adults is fairly static over time, showing only about 1 to 2% change over one to two years. In clinical trials to date, the Phase 2 ReDUX4 study, showed improvement in reachable workspace, but this was not replicated in the Phase 3 REACH study where both placebo and treatment groups improved slightly over one year.

Patient-Reported Outcomes

Two disease-specific patient-reported outcomes (PRO) warrant particular attention. The FSHD Health Index (FSHD-HI) was developed using FDA guidance for industry on patient-reported outcomes. Development of the HI started with individual patient interviews where symptoms were coded based on World Health Organization definitions. The resulting list of items that patients identified as important was surveyed in over 300 individuals who ranked them based on prevalence and impact on their lives. This yielded a 14-domain instrument with 116 items.

The top 14 symptom themes became the instrument domains, recapitulating that top items patients identify are motor-related including: motion in the shoulders, walking ability, and core strength. Ranking uses a consistent six-point Likert scale ranging from "doesn't affect my life at all" to "affects it severely," taking about 15 to 30 minutes to complete.

The FSHD-RODS is a Rasch-built PRO using standard psychometric approaches. As a narrower instrument, it centers on functional activities and participation. The 32 included items show fair overlap with the Health Index. Scoring breaks down from ability to perform without difficulty, to needing adaptations to complete tasks, to being unable to perform tasks. Items are ranked from those affecting people quite early (like losing ability to run) to much later impacts (like ability to fill out a form).

Other instruments used include NeuroQoL and Patient Global Impression of Change. Data from natural history studies and clinical trials are currently being analyzed to understand how these respond to therapeutic interventions.

Digital Outcome Measures

Several studies have explored digital outcome measures, mostly pilot studies or smaller single-site investigations. Digital health outcomes can measure functional activities or patient reports of function. Most work has focused on motion analysis using tiny motion sensors attached to the body or cameras capturing motion, including systems by APDM, BioSensics, and ActiGraph.

Data is currently being obtained in larger datasets through the MOVE study, with 100 individuals being followed for two years. There is substantial interest in this approach and technology, with expectations for expanded use going forward.

Case Study: 10-Meter Walk/Run

The 10-Meter Walk/Run exemplifies how data from studies like RESOLVE and MOVE can inform understanding of outcome measures and their use in clinical trials. This is the most sensitive functional motor task for disease progression, seen in RESOLVE and recapitulated in MOVE.

The test simply asks people to cover 10 meters as fast as they can safely, requiring minimal time and expense to perform in clinical trials. Multicenter training achieves very consistent values from one administration to the next, with an intraclass correlation coefficient around 0.97. Nice cross-sectional associations exist with other important disease aspects: duration, severity, strength testing, and patient-reported impact. Individual muscles from the waist down to the distal lower extremity show clear relationships to performance. Key muscles driving performance are hip extensors and knee flexors, which are important for acceleration and maximal performance tasks and are very commonly affected in FSHD.

The MOVE study data helps contextualize how performance on this instrument fits within changes occurring in FSHD. Healthy population performance ranges from 0 to 4 seconds to perform this task; about 20% of MOVE participants at first visit showed relatively normal performance. When patients perform in the 5 to 10 second range, typically they have experienced changes in performance that relate to their patient-reported community and life activities. At 10 seconds, hard transitions emerge where people start needing orthotics for movement assistance. At 15 seconds, canes or walkers become necessary, and at 20 seconds, wheelchair use for mobility is required. Unlike some other dystrophies, the transition period from complete ambulatory independence to needing equipment can last many years.

Determining meaningful change size requires multiple approaches to establish consistency across different methodologies. Cross-sectional examination of relationships to patient-reported function uses the Current Ability Score (CAS), which asks about ability to perform common daily tasks like transitioning from bed or chair (5). Each one-point change in the CAS represents losing a key function. In normal ranges on the 10-Meter Walk/Run, little movement appears on the scale, but then stepwise increases in current abilities burden emerge based on test performance, with one-point changes in current abilities roughly equating to 0.9-second changes on the 10-Meter Walk/Run. This is

also the change size seen annually in large populations of clinically affected adults—about 10%.

This change magnitude also relates to estimates using statistical techniques like minimally detectable change or anchors where people rank whether they have a little more or less trouble over one year. Estimates across strength and functional measures have been fairly consistent at about 10% change being meaningful.

Selection Criteria and Meaningful Change

Several factors have been proposed to potentially impact performance over one to two years, including genetics, gender, and baseline functional status. Of these, only baseline functional performance clearly impacts ability to select people more likely to change.

This theme repeats in MRI findings where muscles already partially affected but not too affected are most likely to change over shorter timeframes.

Analysis using RESOLVE data for trial planning calculated standardized response means (change at one year divided by variability) for each outcome. Values around 0.3 indicate small responsive outcomes, 0.5 moderate, and 0.8 large.

Quantitative myometry shows effect sizes around 0.3, consistent with natural history study findings. Simple selection of individuals in dynamic ranges—between 20 and 80% of predicted for strength testing, or 4 to 10 seconds for 10-Meter Walk/Run performance—produces much more consistent annual change, with response sizes increasing to moderate levels just through selection criteria.

Considering meaningful change, one approach examines hard motor events occurring in people's lives, such as losing walking ability or needing a wheelchair. Data shows rough annualized frequency of these events at about 2% per year. However, this very low frequency makes clinical trial design around hard motor events during trial conduct very difficult. Alternative approaches

to determine meaningful change magnitudes on outcome measures become necessary.

Trials use various techniques, looking for consistency in estimate sizes across different approaches. Statistical approaches include minimal detectable change confirming true change is occurring. Anchors ask people whether they feel a little better, a little worse, a lot better, or a lot worse, identifying change sizes corresponding to feeling "a little better."

Cross-sectional relationships to patient reports of functional abilities can be examined.

Changes seen over time in individuals with the disease provide another perspective.

Finally, when data permits, modeling can predict how current performance on outcome measures predicts hard motor events over the next one to two years, such as wheelchair need or other assistive mobility devices. Generally, estimates have been fairly consistent across strength and functional measures at about 10% change per year being meaningful.

Q: How might clinical trials manage the phenotypic variability in FSHD when assessing muscle function?

Lawrence Hayward, MD

University of Massachusetts Chan Medical School

The clinical heterogeneity in FSHD is extreme. The focus on motor function relates directly back to patient concerns about ability to perform activities and anxiety about future progression. The challenge is that the power of a clinical trial depends on identifying meaningful change, using both global and more specific measures. Natural history studies demonstrate ways to link these measures together, with certain relatively simple-to-perform measures offering more informative change over time. Patients who do not fit usual progression patterns may have baseline characteristics identifiable by MRI that help dictate which FSHD phenotype subgroups warrant closer follow-up with outcomes tailored to specific disabilities. Some outcomes might prove more sensitive for particular patient subsets. However, treatments should ultimately be validated as useful for patients as a whole, creating inherent tension. The exciting development is that measures are becoming more granular—both structurally with whole body MRI and functionally with tasks that go beyond simple measurements to examine combined effects of different muscles, potentially breaking these down into individual muscle function. This is important because we want to capture not only weakness and task inability but also how patients' other muscles compensate. This helps us address other patient concerns such as pain development from overworked muscles that remain relatively unimpaired.

Q: What remains to be done with Reachable Work Space (RWS) and other upper extremity endpoints?

Jay J. Han, MD

University of California, Davis

The field has moved tremendously, yet much remains to be done regarding clinical meaningfulness of these outcome measures and what they truly mean for patients. RWS was developed to overcome strength limitations and to capture functional meaning. Reachability as an upper extremity outcome measure is important because we use our upper extremities to interact with the world: grabbing things, bringing items to our bodies or mouths, brushing hair, putting on clothes.

While reachability represents an important outcome measure, it is not all-encompassing and does not account for all factors. As patients have said, FSHD feels like your arms are tied down with rope and you are unable to move. Independence in activities of daily living and quality of life closely track to upper extremity mobility. Strength measures, and other outcome measures speak to this, yet the connection between strength and what it really means in day-to-day activity remains lacking. Imparting more meaning to strength changes and RWS changes is a necessary element requiring continued work.

Transfer activities are an area that require more study. From a physical medicine and rehabilitation perspective in the neuromuscular realm, transfer activities represent the basis for mobility. Before standing and walking, can you get out of bed? Can you move from lying to sitting position? Can you move from sitting to standing position? Patients voice real difficulty in transfer activities. This represents an area deserving more attention because while we have examined upper extremity, lower extremity, and ambulation

extensively, transfer activities remain less studied despite being elemental for all activities. Transfers directly involve truncal and torso movement and weakness.

Q: How can we better evaluate meaningful muscle function change in FSHD?

Tina Duong, MPT, PhD

Stanford University

Statistical approaches to meaningful change involve not only statistics but the psychometrics of outcomes. Whenever examining minimal detectable change (MDC) and minimal clinically important difference (MCID), we must think specifically about disease and disease stage and function, not broadly. Baseline characteristics strongly predict what these values may be.

Important collaboration between academic natural history studies and drug development partners has shown that increasing sensitivity requires standardization in methodology—whether strength testing or functional testing—to decrease variability. Another approach to increase sensitivity considers not just disease stage but rate of change. Learning from spinal muscular atrophy and Duchenne, particularly Duchenne's recent FDA approval examining rate of change rather than just mean difference between baseline and 12 months, shows how rate of change can anchor to clinically important differences.

Clinical meaningfulness varies considerably, varying even more for heterogeneous diseases like FSHD. We must consider context of use: Are we examining change related to quality of life, function, or changes in healthcare or standards of care? These yield important differences. Recent work in using the Revised Hammersmith Scale (a similar scale to FSHD COM) examined different cohorts—pediatric, adult, ambulatory, non-ambulatory, and different evaluator experience levels—finding MDC varied between 2 to 7 points. Defining change in different patient cohorts helps statistical modeling and power analysis.

Biomechanics offers another avenue to increase sensitivity by decreasing variability. Understanding why quantitative muscle testing does or does not correlate with function matters. Quantitative muscle testing and manual muscle testing typically occur in open-chain fashion where hands or feet are not planted on the ground. Functional tasks relate to closed-chain activity requiring different muscle activation. In FSHD, when different muscle parts are affected, this can alter function. Additionally, truncal deviations from abdominal and trunk extensor weakness affect arm-lifting ability through very small movements. Technology examining these explanatory variables through motion analysis or kinematics can better explain differences, improve sensitivity and better reflect clinical meaningfulness.

Q: What are the considerations for electronic patient-reported outcome measurement approaches in FSHD, and is this currently being evaluated to complement performance measurements?

Johanna Hamel, PT, PhD

University of California, Irvine

Patient-reported outcomes are extremely important and key in evaluating meaningful change. However, they are only as good as their design. Questions are often not phrased as participants would have chosen, even when researchers make best efforts to include patient input during development—they are never perfect. Taking these limitations into consideration acknowledges they do not capture the full picture, though they remain extremely helpful.

People are capable of filling them out and will complete them. Making the process as easy as possible is critical based on participant feedback, whether on iPad or other devices. Technical issues can cause frustration. Avoid deploying PRO tests too frequently to prevent automatic answering. There is an art to this: How should it be displayed? What should it look like? When moving from paper to electronics, should there be one question per page or not?

Deploying them daily, for instance, when we get greedy wanting more data, risks fatigue in completion. For symptoms that do not change much day to day, repeatedly asking the same question becomes problematic. Including patients as part of the development process, thinking about what filling out forms looks like on their end and appropriate frequency, is extremely important.

What has been incredibly helpful in trial experience is the investigator-participant conversation at each visit, asking how things are going and truly exploring—not with just one question and a patient-reported outcome, but with follow-up questions getting to the bottom of experience. While global impression of change (patient-completed or clinician-completed) captures some of this, much gets lost. This connects to exit interviews, which can be extremely important in capturing information because we only capture what we ask about. Many things happen that we may not know are FSHD-related or treatment-responsive. In early trials, keeping limitations in mind and remaining open to learning with each participant as we progress is important.

Q: How should we think as clinicians about trends in clinical evaluations of novel therapeutics that may not necessarily meet statistical endpoints?

Michelle Mellion, MD

Fulcrum Therapeutics

FSHD is a heterogeneous disease, so people experience it differently depending on where they are in their disease course. It is difficult to stage this disease specifically to fit people in specific stages, although there is equality data showing where milestones may be.

As a community considering this myriad of endpoints and developed data, we should think about evaluating endpoints in terms of trends and not necessarily statistical significance alone. To understand potential impact of new and innovative therapies, we may need to consider evaluating trends on different markers that are important to patients and consider them alongside statistically significant endpoint assessments. In this way we work; to identify what is important to patients, how disease affects them and their experience.; Following this over time within clinical trials may prove more meaningful ultimately than finding MCID or statistical significance on another endpoint that may not be as meaningful for patients.

As a community moving we must include patient experience and learn how to quantify, at least by trends, potential treatment impact on patients.

Q: If therapeutic benefit is seen in a clinical trial population, is it reasonable to extrapolate to a more diverse FSHD population who did not meet inclusion criteria?

Jeffrey M. Statland, MD

University of Kansas Medical Center

We must remember that ALL FSHD arises as the result of toxic gain of function expression of DUX4 in patient skeletal muscle. Therefore, any therapeutic modality that reduces DUX4 levels will benefit all FSHD patients. Further, this question arises frequently, appearing in other neuromuscular disorders where new therapies have been approved and efforts emerge to limit access to trial-included populations. However, the entire concept and infrastructure of experiments gaining evidence to support drug marketing always assumes we conduct experiments in more limited groups, and if our assumptions are correct, results should generalize across broader populations.

For disease-targeted drugs expected to hit their target and modify disease, you might examine ambulatory measures, but the question arises: if someone uses a wheelchair, would this benefit them? The answer is yes—it will benefit them to the degree they have function to preserve, save, and benefit from treatment.

We must remember as we move forward that trials are always meant to support broader generalization when going to market. Part of Phase 3 programs may attempt to gather information about other individuals, but sometimes that information is gained post-market while following people in registries to truly understand who benefits and who may not.

MRI

Seth Friedman, MD, PhD

Seattle Children's Hospital

Magnetic resonance imaging has evolved significantly over nearly 20 years to become a critical tool for understanding and tracking FSHD (5). MRI provides unique insight into disease progression by revealing muscle changes that bridge the gap between cellular-level pathology and whole-body function.

The primary MRI techniques used in FSHD research is Dixon imaging, which provides separate water and fat contrasts, and short tau inversion recovery (STIR) imaging for assessing inflammation. While other contrast methods have been explored for measuring fibrosis and diffusion, Dixon-based fat quantification has emerged as the most useful biomarker to date.

MRI research has progressed through distinct phases. Early work utilized qualitative rating scales that could assess involvement across the whole body but required expert readers and were insensitive to change over time. The field has since moved to quantitative techniques that objectively track fat infiltration and lean muscle volume. Recent advances in artificial intelligence have solved longstanding challenges in analyzing entire muscles from their proximal to distal extent, enabling comprehensive whole-body assessment.

Several key findings have emerged from cross-sectional MRI studies. FSHD is definitively a whole-body disease with high variability as the rule rather than the exception. Patients show substantial differences in disease progression, muscles vary considerably in their degree of involvement, and asymmetry is very common. Surprisingly, while many muscles show fat infiltration, only about 5% demonstrate inflammation on STIR imaging.

Longitudinal studies reveal that composite measures show relatively modest annual change rates of a couple percent. However, individual muscles progress at much faster rates, with some following an escalated course. Importantly, muscle identity matters: two muscles with identical 20% fat fraction at baseline will progress differently based on muscle type. The spatial pattern of fat distribution is also one of the best predictors of future change over one year. For pediatric populations, bone volume becomes crucial as it constrains muscle growth and must be accounted for to distinguish normal development from disease progression.

MRI demonstrates clear relationships with function: higher fat levels correlate with worse function (5,6). However, the heterogeneity of the disease means that patients present with different affected muscle groups. A patient with transitional shoulder muscle involvement would be best assessed with shoulder-specific tasks, while someone with leg weakness might be better evaluated with walking tasks. This suggests the need for more personalized approaches that link MRI data to appropriate functional outcome measures for each individual.

Looking ahead, the field is moving toward weighted composites that account for both MRI and functional heterogeneity. By tracking the specific muscles affected in each subject and linking those to the tasks those muscles perform, researchers aim to decrease required subject numbers, create more efficient trial durations, and better understand how interventions modulate disease course.

Case Study: Whole Body MRI in Clinical Trials

Per Widholm, PhD

AMRA Medical, Sweden

The development of whole-body quantitative MRI for use in multicenter clinical trials represents a significant advancement in FSHD research. This work, at Fulcrum Therapeutics, aimed to create biomarkers that could objectively describe disease progression before clinically apparent effects become evident (6).

Implementing MRI biomarkers in clinical trials requires meeting stringent regulatory and operational requirements. Minimizing variability across all analysis stages is paramount. This involves standardizing site training, MRI scanner certifications, scan protocols, and settings. Proprietary image calibration techniques ensure consistent results between scanners and protocols. Fully automatic segmentation with dual operator quality control maintains consistency over time and between patients. Continuous image quality control of incoming data throughout studies ensures protocol adherence and enables timely re-scans when needed. All processes must ensure data integrity and traceability suitable for regulatory review and audits.

The methodology addresses key FSHD characteristics including intramuscular fat accumulation, muscle tissue loss, disease heterogeneity, and slow progression (7). Two complementary measures capture muscle fat: muscle fat fraction quantifies what proportion of a muscle consists of fat, while muscle fat infiltration assesses fat present in remaining muscle tissue, excluding completely fat-replaced regions. Lean muscle volume measurement removes all fat to quantify remaining contractile tissue.

To address disease heterogeneity, while leveraging the observation that muscles with increased fat tend to progress faster, endpoints were defined as composites where muscles with similar disease characteristics are grouped together. This enriches the analysis of change and treatment effects while accounting for heterogeneity. For each individual, a personalized set of intermediate-affected muscles is defined and tracked, with these muscles highlighted as most likely to progress in the short term.

Validation studies demonstrated strong technical performance and correlation with muscle function and timed function tests (8). The methodology has been published in peer-reviewed literature and successfully deployed in over 10 clinical trials across more than 100 certified sites worldwide, with over 1,500 datasets analyzed.

Data from Fulcrum's ReEDUX-4 Phase 2 study confirmed that normal-appearing muscles progress much more slowly than intermediate muscles, which show significantly higher progression rates. By classifying muscles based on disease state, this heterogeneous disease becomes more statistically well-behaved with muscles showing aligned progression patterns. The responsiveness of lean muscle volume, expressed as standardized response mean, exceeded 1.0 and approached 1.5 depending on follow-up duration, indicating a highly effective efficacy biomarker.

Correlation between MRI changes and functional outcomes like time up and go was evident, though somewhat hampered by variability in functional tests. Notably, outliers consisted primarily of patients with very poor baseline function (11-14 seconds in various functional tests), suggesting that excluding individuals with severely limited function yields even stronger correlations (8,9).

In summary, whole body quantitative MRI is feasible for use in multicenter clinical trials with the rigor required for regulatory endpoints. These biomarkers correlate with both muscle pathology and function while demonstrating high analytical performance that enables detection of small changes compared to clinical outcome measures. Composite biomarkers have proven effective in describing muscle changes consistent with disease progression even in heterogeneous diseases like FSHD.

Digital Twins and Machine Learning Approaches

Silvia Salinas Blemker, PhD

Springbok Analytics

Understanding how FSHD influences movement requires connecting multiple scales of biology. Impaired DUX4 signaling impacts muscle degeneration and regeneration at the cellular level, but the ultimate concern is whole-body function and mobility. The challenge lies in the complexity between these scales: tissue quality, muscle architecture and size, and how muscle groups are organized all influence how cellular changes manifest as

functional changes. MRI fills this critical gap by explaining how cellular mechanisms affect movement.

Leveraging whole body MRI scans and FDA-cleared artificial intelligence technology, it is now possible to analyze all muscles from neck to feet at the individual muscle level. This AI-based approach, developed and validated through rigorous testing of repeatability, precision, and variability effects, addresses the time-consuming challenge of quantifying every muscle in every slice while maintaining quality assurance through expert review. Comprehensive analysis of pooled natural history data from multiple studies including MOVE+, the Wellstone Project, and the Phase 2 clinical trial ReDUX-4, reveals the profound full-body impact of FSHD. Nearly every muscle shows elevated fat fraction compared to the general population, with the semimembranosus (a hamstring muscle) most frequently affected in adults. Very few muscles remain preserved across all individuals, demonstrating that this is not a selectively targeted muscle disease despite traditional nomenclature. Extreme heterogeneity exists both between muscles and within the same muscle across different individuals.

Equally important is quantifying remaining lean muscle tissue, which directly determines functional capacity and force generation. Analysis shows that muscles most severely depleted of lean tissue are not always the same ones with highest fat fraction, indicating that both measures capture complementary aspects of disease. The spatial distribution and pattern of fat infiltration adds further complexity that influences both progression prediction and muscle function.

The vision is to harness this wealth of MRI data to create digital twins—personalized representations that capture the unique impact of disease on each individual. These models can incorporate molecular-level data, regional heterogeneity within muscles, and muscle or group-level characteristics. Machine learning models can then predict personalized progression for comparison with observed changes, essentially providing each patient with their own surrogate placebo. This approach has shown success in other heterogeneous diseases like ALS and MS.

Using random forest machine learning models that accommodate multiple data types, predictions incorporate patient characteristics like D4Z4 repeat length, muscle name as a classifier (since different muscles progress differently), initial fat fraction, fat variation (heterogeneous patterns indicate active change), and composite features from other muscles. Models are trained on 80% of available data with the remaining 20% used for validation (10).

Current models demonstrate strong prediction performance that improves substantially when grouping muscles together, reducing noise in individual muscle predictions.

Predictions work across all relevant muscle groups throughout the body for both fat fraction change and lean volume change.

Comparing model-based muscle selection to the original intermediate muscle concept reveals important insights. While intermediate muscles (those with moderate fat fraction at baseline) do capture many changing muscles, they also include many that do not change significantly and miss muscles that are progressing, particularly for lean volume loss. Machine learning models can discern which muscles are most likely to change

across the full range of fat fractions by incorporating multiple muscle and patient characteristics, representing an evolution of the original concept.

Data-driven modeling also reveals which muscle groups most influence specific functional tasks. For the upper body functional assessment RWS, certain muscle groups prove far more influential than others. Patients experiencing changes in these highly influential muscles would reasonably show corresponding changes in RWS, while those with changes in less influential muscles might not demonstrate functional decline in that particular domain.

MRI measures provide critical intermediate information that helps target clinical trials, addresses individual heterogeneity, and clarifies the relationship between treatment and patient function. By creating comprehensive digital representations of each patient's unique disease presentation, the field moves toward more personalized and efficient clinical trial designs.

Q: How do other imaging modalities compare to MRI for sensitivity and reliability in FSHD?

Seth Friedman, MD, PhD

Seattle Children's Hospital

Single-slice measurements from various modalities generally show good reliability.

However, FSHD's heterogeneous expression within muscles creates high likelihood of missing injured areas or changes over time when sampling limited regions. While ultrasound is inexpensive and has been explored, it can be finicky regarding acquisition and analysis.

Whole-body assessment appears crucial for proper evaluation, including the trunk, shoulder girdle, and identifying which specific muscles are involved. Dual-energy X-ray absorptiometry (DEXA) offers an inexpensive whole-body composition assessment but would be very difficult to link back to function.

MRI can detect changes that will not have immediate functional consequences for specific tasks. Different time scales likely apply, with MRI probably changing faster than functional outcome measures. However, MRI can inform which functional outcome measure will serve as the best reporter for an individual patient. The key is understanding for each task which set of muscles contributes most heavily to that function, then mapping the quality of motion onto granular MRI data.

Q: What are the challenges of evaluating the complex FSHD population with MRI, including pediatric, rapid progressors, slow progressors, and ambulatory versus non-ambulatory patients?

Seth Friedman, MD, PhD

Seattle Children's Hospital

MRI offers value across diverse FSHD populations, though each presents unique challenges. Pediatric populations require understanding how to correct for normative growth, as bone volume constrains muscle changes. If bone development is abnormal, it may substantially alter muscle growth patterns. Current

data remain insufficient to fully address these complexities, making ongoing natural history studies in children crucial.

For non-ambulatory patients, MRI effectively evaluates the whole body, but different functional outcome measures are needed to assess treatment efficacy. The real challenge lies in understanding which muscle sets weight most heavily for each specific task. Patients with FSHD move differently than healthy individuals, presenting an opportunity to map quality of motion onto detailed MRI data using unsupervised methods. The field is moving toward more personalized approaches that account for both MRI and functional heterogeneity rather than relying solely on composite measures. This would involve weighting composites appropriately or tracking the specific affected muscles and musculature per subject, then linking those to the task-specific behaviors those muscles support. This strategy could decrease required subject numbers, enable more efficient trial durations, and provide better understanding of how drugs modulate disease course.

Biomarkers

Sujatha Jagannathan, Ph. D.

University of Colorado

Biomarkers can serve many purposes: diagnosing disease, stratifying patients, or tracking response to therapy. For FSHD, we've overcome early hurdles in diagnosis and are now focused on developing reliable response biomarkers to determine whether a treatment is truly effective.

An ideal response biomarker for FSHD would be detectable in affected muscle or, preferably, in circulation. It should correlate with DUX4 activity, be consistently measurable despite the mosaic nature of its expression, and most importantly, reflect changes in disease severity or treatment response in a meaningful way for patients. There are three major categories of biomarkers under investigation: muscle status markers, inflammation-related markers, and DUX4-regulated markers. Muscle status markers like creatine kinase are well-known and widely used but are not specific to FSHD and can be affected by confounding factors like exercise and age. Inflammation-related markers, such as interleukin-6 and complement components, show consistent group-level differences between patients and controls, but have significant individual variability, complicating their interpretation over time.

DUX4-regulated biomarkers are perhaps the most specific and have been widely adopted in clinical trials. RNA sequencing is used to measure expression of DUX4 target genes in muscle biopsies, and these levels correlate with disease severity. However, challenges remain: the success of this approach heavily depends on where the biopsy is taken and whether DUX4 is being expressed at that site. For example, while some trials have shown modest functional improvements without significant changes in DUX4-driven gene expression, others have shown reductions in DUX4 activity with treatment—highlighting variability, the need for extensive biomarker characterization and the need for cautious interpretation.

Looking ahead, there are several exciting biomarkers in development. One example comes from the Avidity trial, where a new DUX4-regulated biomarker was detected in plasma—

though the identity of the biomarker hasn't been disclosed yet. The biomarker has since been identified as KHDC1L, a DUX4-regulated protein (11).

Upcoming biomarkers

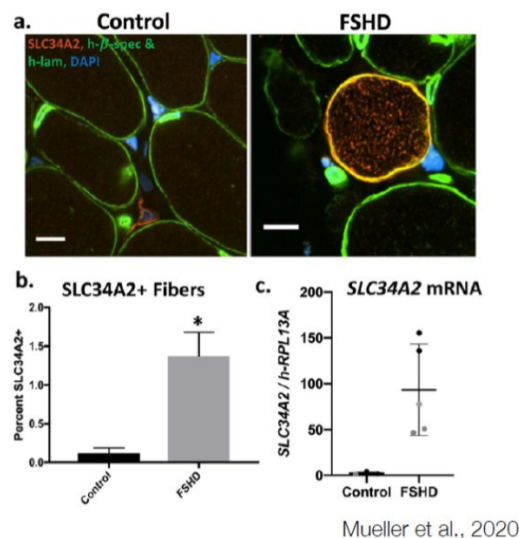


Figure 1. SLC34A2 as a Protein Biomarker for Facioscapulohumeral Muscular Dystrophy (FSHD) *in-vitro* and *in-situ*

Another is SLC34A2, a regulated protein being studied both in muscle and blood. Additionally, my lab is developing a biomarker based on cell-free DNA to potentially track disease presence and progression through a simple blood draw.

As the field moves forward, the goal is to evaluate biomarkers not only for their scientific rigor but also for their clinical feasibility. We want tools that are not burdensome to patients—especially if they can replace or reduce the need for invasive muscle biopsies. It's also important to consider how responsive a biomarker is to therapeutic intervention. If a biomarker lags behind clinical improvement or fluctuates too much day-to-day, it may complicate trial design and interpretation. And of course, we must factor in individual differences like age, sex, and health status, all of which can influence biomarker readings.

This is an incredibly active area of research, with many labs contributing to the effort. With continued collaboration and rigorous validation, there will soon be reliable biomarkers that can help us better track disease and therapeutic progress in FSHD.

Q: How has the community sought to correlate the amount of, and the change of, these molecular markers?

Leo Wang, MD

University of Washington

There is growing value of MRI in identifying molecular biomarkers and guiding muscle biopsies, particularly for assessing DUX4 activity. MRI changes in specific muscles over time can correlate with biomarker fluctuations, though variability remains a significant challenge. Improvements in the areas of biopsy targeting, analysis techniques, and study design are evident in recent studies, including those from Wellstone, international collaborations, and trials involving Losmapimod and del-brax. However, linking MRI and biomarker data to patient-reported outcomes is still difficult due to the localized nature of biopsies and the heterogeneity of the disease. Ongoing correlative analyses of existing data sets are underway.

Q: Has there been research on the effect of hormones on muscles, particularly looking at correlation to severity and progression of disease?

Jeffrey M. Statland, MD

University of Kansas

There's been a lot of interest in this question because there is some difference in the age at which men and women are diagnosed with FSHD. Some studies suggest a protective role for estrogen and a role in the trafficking of DUX4 in the cells. There was a retrospective study on hormone replacement therapy, but investigators were unable to determine any effect. There was a prospective study of testosterone and recombinant growth hormone. This was thought to be an anabolic approach to increase muscle growth. It needs to have some follow-up, which is currently being pursued.

Tina Duong, MPT, PhD

Stanford

When we look at menopause and sarcopenia, we know that estrogen does have an influence on satellite cells that will be responsible for the regeneration and repair of some of these muscles, as well as an impact on inflammation of muscle tissue. These things are worth exploring, and the FSHD field can apply learnings from other areas.

Q: What are the new and future areas of research in molecular biomarkers?

Nizar Saad, PhD

Nationwide Children's Hospital

An important area to investigate are the circulating molecular biomarkers, because they will cover the whole-body effect of disease progression. The field should focus on biomarkers that we can track and can demonstrate are coming from skeletal muscles.

Two important areas of focus are the variability of biomarkers, and how sensitive the detection method would be. With the sporadic expression of DUX4, you might need to have a very high, sensitive method of detection. Reducing the variability of other outcome measures can give us a better chance of identifying circulating biomarkers that are highly sensitive and responsive to disease progression.

Q: What do we need to do to understand how well a molecular biomarker reflects disease?

Sujatha Jagannathan, MD

University of Colorado

There are many ways to meaningfully track the disease. The most important questions about biomarkers are:

- What is the source of it?
- How does it relate to disease?
- How variable is it in a healthy individual versus in an individual with disease?
- How quickly does it track with disease activity or response to treatment?
- How persistent is that signal in the blood?

Patients could lose a muscle function overnight, but the actual process of that muscle deterioration has likely happened over time. We need to understand how a biomarker performs in longitudinal studies to be able to see the change. Also, we are mostly looking for signals that are coming from muscle, but we could also look for signals that come from the immune cell population that is potentially responding to DUX4. But a better understanding of the interplay between FSHD and the immune system is necessary to be able to do that.

Closing remarks

Lucienne Ronco, PhD

FSHD Society

FSHD is a relentlessly progressive disease. The muscle loss is quixotic and leaves patients and their families in a state of constant dread and in mourning for what they repeatedly lose. Weakness and pain are broadly experienced by people living with FSHD. Respiratory insufficiency may shorten the lifespan and certainly impacts daily living.

We know that:

1. FSHD patients are supportive of treatments that halt or slow the progression of the disease.
2. FSHD patients welcome restorative treatments that improve muscle strength and function.
3. People with FSHD have clearly demonstrated they are eager to work with drug developers and regulators by providing muscle biopsies, by undergoing repeat MRI procedures and by enlisting in patient registries as we work together to expedite the approval of new safe and effective treatments.

Currently, there is no FDA-approved treatment for FSHD that prevents or slows muscle wasting and weakness.

If a therapeutic benefit is seen in a clinical trial population, it is reasonable to extrapolate that a more diverse FSHD population, who did not meet trial inclusion criteria, would also benefit. We know that the same disease mechanism, DUX4 driven muscle loss, is present in all patients. FSHD is relentlessly progressive. Someone who is too mildly affected to be included in a trial will inevitably worsen over time. Likewise, someone who is too severely

affected, for example by being non-ambulatory, will also progress, losing upper body, core, and breathing functions, which will decrease their quality of life and lifespan. It's therefore reasonable to expect such patients will receive clinical benefit even if their condition did not allow their functional changes to be measured by the outcome assessments used in the trial. **There is no evidence that people with FSHD have only a limited window in which they would benefit from a DUX4-targeting therapy.**

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