Abstract Book

Session 1: Biomarkers of Disease Activity and Progression

S1.01 Global dysregulation of major immune cell subsets in the peripheral blood of an Australian paediatric FSHD cohort

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Peripheral immune dysregulation plays a central role in the pathogenesis of chronic infections, autoimmune, and inflammatory conditions. Alterations in circulating immune cell populations, such as skewed $CD4^+$ and $CD8^+$ T cell ratios, expansion of effector/exhausted T cell subsets, or perturbed B cells can contribute to disease progression. An immune infiltrate in FSHD is thought precede the replacement of fat. However, very little is known about how the immune system influences disease pathogenesis. This work aims to generate a detailed immune profile of the peripheral blood in an Australian paediatric FSHD cohort (FSHD longitudinal outcome study [FSHD-LOS]) and compare it to age/sex-matched controls. High-dimensional spectral flow cytometry (45-colour panel, Cytek Aurora) was used to identify >100 immune subsets in peripheral blood mononuclear cells of paediatric FSHD. Plasma was screened for 34 cytokines/chemokines to identify novel pro-inflammatory markers of disease. We found global dysregulation of major immune cell subsets (monocytes, dendritic, natural killer, T, and B cells) in the peripheral blood of paediatric FSHD. For the 34 cytokines/chemokines screened, we identified novel pro-inflammatory markers of disease. These novel markers also correlated with alterations in immune cell subsets. Understanding the drivers of peripheral immune dysregulation is critical for identifying biomarkers, and the development and monitoring of immune therapies for FSHD.

S1.02 A cell-free DNA biomarker for facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by aberrant expression of *DUX4* in muscle, yet no blood-based biomarker exists to diagnose FSHD or track disease progression. Cell-free DNA (cfDNA), short fragments of DNA shed into plasma from tissues throughout the body, carries chromatin structural information that reflects the transcriptional state of cells of origin. We hypothesized that *DUX4* activity in FSHD muscle would leave a detectable signature in cfDNA fragmentation patterns at *DUX4*-regulated gene promoters, enabling a minimally invasive diagnostic and disease-monitoring tool. We profiled cfDNA from a cohort of FSHD-affected (n = 51) and unaffected donors (n = 48) using whole-genome sequencing. By analyzing subnucleosomal fragmentation patterns genome-wide, we identified a signature that distinguishes FSHD-affected from unaffected individuals with promising accuracy in cross-validation analysis. Several *DUX4*-induced genes emerged as candidates whose cfDNA signatures correlate with clinical severity, particularly in individuals with active disease. These findings demonstrate that cfDNA fragmentation encodes enough information to detect FSHD status and track disease severity from a blood draw, providing a foundation for biomarker development in future clinical trials and treatment response monitoring.

S1.03 Integrating whole-body MRI across the developmental spectrum to model lifespan progression in facioscapulohumeral muscular dystrophy (FSHD)

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Facioscapulohumeral muscular dystrophy (FSHD) is characterized by significant heterogeneity across individuals and muscles. To capture this complexity, integrating whole-body MRI data across the developmental spectrum – from pediatric onset through late adulthood – is essential for advancing predictive data models. This study builds upon an existing adult data lake (N = 137; Blemker et al., 2025) by incorporating new longitudinal samples from the MOVE+ adult cohort (N = 55) and the US National Institutes of Health MOVE pediatric study (MOVE Peds, N = 15). These integrated datasets enable the exploration of 3 critical concepts which will be discussed: 1) Characterization of fat infiltration patterns across the age range, with specific focus on pediatric-onset markers. 2) Evaluation of bone-muscle volume relationships, determining if the linked correlations observed in healthy adults (Riem et al., 2026) are preserved in FSHD. 3) Differentiation of FSHD-specific pathology from natural aging, investigating whether sarcopenia and diffuse adiposity coexist with or masquerade as disease expression. With target recruitment of N = 200 (MOVE+) and N = 80 (MOVE Peds) by early 2027, this aggregated framework will facilitate high-fidelity lifespan progression modeling. These initial insights establish a path toward improved understanding of developmental progression and the identification of targets for stage-specific therapies.

S1.04 Growth impacts MRI biomarkers of disease progression in pediatric FSHD

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Early-onset facioscapulohumeral muscular dystrophy (FSHD) presents challenges for interpreting MRI biomarkers, as ongoing growth may influence measures of muscle composition. We analyzed longitudinal MRI data from 22 pediatric subjects (55 scans; ages 6 to 20 years; 1 to 6 timepoints per subject) to examine how muscle volume and fat fraction evolve during development. Growth-related increases were observed in raw and lean muscle volume across all major muscle groups, with consistent within-subject trajectories over time. However, fat fraction patterns were more complex and did not exhibit a consistent increase with age. While some muscles showed increases, others remained stable or declined. Negative rates of change in fat fraction were found in several muscles, including the hip flexors ($r = -0.87$, $p < 0.001$) and hamstrings ($r = -0.45$, $p = 0.006$), with similar trends in adductors and gluteals. These decreases occurred during adolescence and are attributable growth-related increases in lean muscle that reduce the proportion of muscle composed of fat. These findings indicate that fat fraction must be interpreted within the context of growth in early-onset FSHD. These results highlight the need for normative reference data defining expected trajectories of muscle growth and composition across development, which will be essential for distinguishing physiological maturation from disease-related change and for improving interpretation of MRI endpoints in pediatric clinical trials.

S1.05 An innate immune cell/FSHD patient muscle xenograft model to investigate the role of the innate immune system in the initiation of FSHD muscle pathology

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To investigate a role for innate immunity in the initiation and propagation of FSHD muscle pathology, we developed a human innate immune FSHD muscle xenograft mouse model. The NSG-SGM3 mouse strain has been engineered to selectively expand human innate immune cell lineages following engraftment of umbilical cord blood derived hematopoietic stem cells (HSCs) and co-engraftment of patient-derived FSHD (or unaffected control) muscle stem cells into the mouse tibialis anterior (TA) muscle. FSHD muscle xenografts in HSC engrafted NSG-SGM3 mice preferentially accumulate human macrophages and B cells, and express RNAs encoding activators of both the classical and alternative complement pathways and complement factor C3, which is the mediator of complement response. Notably, immunofluorescence studies of immune/muscle xenograft TA muscle cryosections showed FSHD xenografts expressed C3 protein, and muscle fibers expressing high levels of C3 lacked co-staining with the membrane protein spectrin β1. These C3+/spectrin β1- muscle fibers were also observed in FSHD patient muscle biopsies. Experiments are ongoing using our innate immune cell/FSHD muscle xenograft model to investigate the efficacy of complement targeting therapeutics for the treatment of FSHD.

P1.01 TMT quantitative proteomics identified protein biomarker candidates that are associated with disease progression

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This study aims to identify and validate circulating proteins associated with FSHD disease progression. Tandem mass tag (TMT)-based quantitative proteomics were conducted to quantify protein abundance in human plasma and mouse sera samples. Human plasma samples from 20 individuals (10 females and 10 males) genetically confirmed with FSHD1, and sera of FLExDUX4 mice treated with antisense oligonucleotides targeting *DUX4*, were studied. Human samples collected at an earlier visit were compared to samples from the same individuals collected at a later visit. Validations were performed using immunoblotting. Using the TMT proteomics approach, we identified 1 protein in males and 27 proteins in females, which were significantly different between the early and late time points. Five proteins were found to be also changed in the FLExDUX4 mouse samples. *DUX4* reduction by antisense oligonucleotides corrected the protein level differences. The results show that these protein changes correlate with disease progression, which can be corrected by *DUX4* reduction in the mouse model of FSHD.

P1.02 Identification of extracellular vesicle-associated plasma protein biomarkers for facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is a common and debilitating neuromuscular disorder with no approved therapies. As multiple treatments enter clinical trials, there is a growing need for reliable biomarkers to monitor disease progression and therapeutic response. Currently, outcome assessment relies largely on functional and patient-reported measures, while circulating molecular biomarkers remain unvalidated. Here, we profiled the plasma extracellular vesicle (EV) proteome to identify protein biomarkers relevant to FSHD prognosis, patient stratification, and treatment response. Plasma samples from 45 FSHD1 patients and 28 healthy controls across 2 independent cohorts were analyzed using integrated, batch-corrected mass spectrometry. This approach identified Lumican (LUM), along with several complement and extracellular matrix-associated proteins, as consistently upregulated EV proteins in FSHD. EV-associated LUM levels correlated with disease severity (FSHD-COM score; sex-adjusted partial Pearson $R = 0.454$, $p = 0.039$) and continuous functional outcomes, including self-selected gait speed (partial Pearson $R = -0.485$, $p = 0.022$) and timed up-and-go (partial Pearson $R = 0.463$, $p = 0.034$). These findings demonstrate that circulating EV profiling can reveal candidate protein biomarkers that warrant further validation in larger cohorts, supporting their potential as minimally invasive surrogate molecular biomarkers for FSHD.

P1.03 Circulating extracellular vesicle small RNAs associate with radiological and clinical features in FSHD: A discovery analysis

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The identification of non-invasive biomarkers reflecting disease activity and severity remains a major unmet need in facioscapulohumeral muscular dystrophy (FSHD). We previously reported that muscle extracellular vesicles (EVs) carry RNA signatures associated with imaging-based disease features; however, whether disease-related RNA signals are detectable in circulating EVs remains unexplored. In this exploratory study, we profiled the small RNA cargo of plasma-derived EVs from FSHD patients, non-penetrant gene carriers, and healthy controls by RNA sequencing. FSHD patients were stratified according to radiological and clinical parameters, including the number of short tau-inversion recovery (STIR)-positive muscles, global T1-score, and clinical severity score. Using an integrative approach combining differential expression and correlation analyses, we identified plasma EV signatures, including both canonical microRNAs and their isomeric variants (isomiRs), showing associations with radiological and clinical measures of disease activity and muscle degeneration. Enrichment analysis of predicted microRNA targets highlighted biological processes relevant for FSHD pathology. These preliminary findings extend our previous muscle EV RNA profiling by suggesting that circulating EVs carry disease-relevant molecular information. While independent validation in larger cohorts is ongoing, this work supports the potential of plasma EV-RNAs as minimally invasive candidates for disease monitoring in FSHD.

P1.04 Bone composition is impaired in FSHD and shows a steep decline at low muscle levels

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Facioscapulohumeral muscular dystrophy (FSHD) is characterized by progressive muscle degeneration, yet its impact on bone composition remains poorly defined. The goal of this study was to quantify bone composition in FSHD and determine its relationship to muscle involvement. We analyzed a cohort of N = 52 individuals with FSHD from the FSHD Global Patient Registry and N = 115 age- and sex-matched controls. Bone composition was assessed using MRI-derived measures validated against dual-energy X-ray absorptiometry and peripheral quantitative computed tomography. Femur bone density was significantly reduced in FSHD (12% *vs.* controls, $p < 0.001$), indicating impaired bone composition. Decreases in bone density were associated with reduced normalized lean muscle ($r = 0.32$, $p < 0.001$). This relationship showed a sharp decline in bone density at low lean-to-bone ratios, suggesting a threshold effect potentially related to reduced mechanical loading or loss of ambulation. Bone density also declined with age ($r = -0.35$, $p < 0.001$), with a steeper slope in FSHD (interaction $p < 0.001$). Bone asymmetry mirrored muscle asymmetry ($r = 0.33$, $p < 0.05$), supporting localized muscle–bone interactions. These results demonstrate that bone composition is compromised in FSHD and tightly linked to muscle degeneration. Given the high prevalence of falls in FSHD, these changes may contribute to increased frailty and fracture risk.

P1.05 MRI-derived lean muscle volume as a predictor of strength and ambulatory function in FSHD

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This study examines how MRI-derived muscle metrics relate to functional performance in individuals with facioscapulohumeral muscular dystrophy (FSHD). Using quantitative muscle testing (QMT) and the 2-minute walk test (2MWT) as functional outcomes, we evaluated associations with MRI-derived measures of lean muscle volume (LMV) and fat fraction (FF) across major lower limb muscle groups in 30 patients with FSHD. LMV demonstrated strong positive correlations with QMT ($r = 0.5$ to 0.86, $p < 0.01$). For ambulatory function, LMV was normalized by the product of height and mass to account for the body-loaded nature of the 2MWT, providing a more physiologically relevant measure of muscle capacity. Normalized LMV showed strong associations with 2MWT performance ($r = 0.69$ to 0.87, $p < 0.01$). In contrast, FF exhibited moderate negative correlations with QMT and 2MWT but consistently explained less variability in function than LMV. When torque was normalized by LMV (*i.e.*, torque efficiency), relationships with FF ranged from weak to strong positive associations ($r > 0.7$, $p < 0.001$), suggesting that muscles with higher FF may compensate by utilizing their remaining contractile tissue more effectively. Across analyses, LMV was the strongest predictor of functional outcomes; however, at higher FF, the relationship shifted, consistent with increased torque efficiency and suggesting altered utilization of the remaining muscle tissue.

P1.06 Defining MRI-based biomarkers for separating muscle fat neogenesis and atrophy in FSHD

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Muscle fat fraction (MFF) is often used to describe muscle fat replacement in FSHD by normalizing the amount of intramuscular fat (muscle fat volume, MFV) to the total muscle volume (TMV). However, FSHD is a disease characterized by both muscle fat replacement (increasing MFV) and atrophy (decreasing TMV), causing an ambiguity in interpreting changes in MFF. By normalizing MFV with the baseline TMV at all points in time, referred to as MFF*, the biomarker will be more directly linked to fat neogenesis. To inform on the clinical applicability of MFF*, we used MRI data from the ReDUX4 clinical trial (baseline and 48 weeks), which we analyzed using AMRA Researcher. We measured lean muscle volume (LMV), muscle fat infiltration (MFI), MFF, and MFF* in 36 muscle groups. Muscles were categorized at baseline as A (MFI <10% and MFF <50%); B (MFI ≥10% and MFF <50%); or C (MFF ≥50%). Longitudinal composites for baseline B muscles were derived. At week 48, there were significant (mean ± SE) changes from baseline (treatment/placebo groups) in both LMV (-6.15 ± 0.93/-5.57 ± 0.95 %) and MFF (1.16 ± 0.44/1.87 ± 0.35 pp). However, when analyzing MFF* we found significant changes in the placebo group (0.95 ± 0.46 pp), but not the treatment group (-0.11 ± 0.48 pp). This indicates that the treatment group MFF increase was driven by atrophy without fat neogenesis (significant decrease in LMV, but not in MFF*), while the increased MFF* and decreased LMV in the placebo group indicate atrophy with co-occurring fat neogenesis.

P1.07 Responsiveness to change of quantitative whole-body MRI: Results from ReDUX4 OLE

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To detect disease progression in FSHD, despite challenges with heterogeneity and slow progression, a standardized and highly reproducible methodology for quantitative whole-body MRI has been developed. By combining muscles identified to have high probability of disease progression into composites, we aim to provide responsive biomarkers suitable as endpoints in multisite clinical trials. MRI data from the ReDUX4 clinical trial (baseline and at week 48, 96, and 120) were analyzed using AMRA Researcher quantifying lean muscle volume, muscle fat fraction, and muscle fat infiltration for 36 muscle groups. Muscles were categorized (A, B, C) based on baseline fat content (reflecting probability of progression), and longitudinal whole-body composites were calculated for A and B muscles. Mean change from baseline was analyzed using paired t-tests, and standardized response mean (SRM) determined responsiveness. B-muscle composites showed significant, progressive changes across all timepoints, with SRMs increasing from 0.4 to 1.1 (range across composites) at week 48 to 0.8 to 1.5 at week 120. Initially, A-muscles showed little to no change, but were significant by week 96 with lower SRMs throughout. Using whole-body MRI to identify and combine muscles likely to progress results in higher SRM, enabling earlier detection of disease progression or treatment effects in clinical trials.

P1.08 Tracking disease progression in FSHD: Change after 1 year of muscle MRI and functional clinical outcomes comparing outcome responsiveness and sensitivity

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Trials need reliable, sensitive metrics to detect early changes in slow-progressing diseases like FSHD. Our aim was to quantify 12-month changes within FSHD patients and relate muscle MRI to function. FSHD patients (N_0 = 68, N_{12} = 56) were evaluated at baseline and 12 months using whole-body MRI (AMRA Researcher; lean muscle volume [LMV], muscle fat fraction and infiltration [MFF, MFI]) and clinical functional metrics. Longitudinal change was assessed using linear mixed-effects models and standardized response means (SRMs), as well as baseline correlations between MRI and functional metrics. All MRI metrics showed significant change after 12 months (excluding right biceps and left triceps brachii); in contrast, the only significant functional metrics were elbow extension, ankle dorsiflexion, and right shoulder abduction. SRMs were greater for MRI metrics than respective functional metrics (excluding elbow extension). For example, right hamstring mean change (SRM) LMV/MFF/MFI/QMT (quantitative muscle test) were -4.2%/1.2pp/0.3pp/-0.7kg (0.7/0.7/0.3/0.1), respectively. At baseline, MRI metrics ranged from moderately to strongly correlated to QMT, excluding triceps brachii, while cross-sectional composite MFF/MFI were moderately correlated to functional metrics. While MRI and functional metrics relate at a single timepoint, MRI is a more sensitive, responsive biomarker, making it a superior longitudinal biomarker, whereas function requires longer study durations for measurable progression.

P1.09 Video-based analysis of longitudinal changes in motor function in FSHD

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Variable progression in FSHD typically manifests as a slow overall decline interrupted by periods of more rapid asymmetrical muscle-by-muscle degeneration. For FSHD therapeutic trials, there is a need to develop more sensitive outcome measures and biomarkers. To conduct a longitudinal pilot study assessing whether 3D video-based approaches using ACTIVE-WorkSpace Volume (WSV) and OpenCap kinematics of upper and lower extremity function may correlate with FSHD clinical severity. Adult subjects (11 FSHD, 5 unaffected controls) were evaluated at 6-month intervals using 1) WSV and 2) OpenCap with Sit-to-Stand-5x (STS-5x) and Timed Up and Go (TUG) protocols. WSV quantifies upper extremity motion and compensatory movements that may contribute to function. Results were correlated with muscle ultrasound (MUS), 10-m and 100-m walk/run, timed TUG, and severity scores, including manual motor function, Ricci and Lamperti scales, and the FSHD-RODS. MUS detected muscle structural abnormalities in a set of 7 muscles examined bilaterally and provided reliable correlation to measures of clinical severity. WSV scores declined over 12 months for half of the FSHD subjects but not for controls. OpenCap maximum lumbar lateral bending during STS-5x correlated with Ricci, RODS, and 100-m time ($r > 0.90$). WSV and OpenCap are promising outcome measures that may be further validated for relevance to future FSHD clinical trials.

P1.10 MRI-based prediction of *DUX4* expression for patient selection and biopsy targeting in clinical trials

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Facioscapulohumeral muscular dystrophy (FSHD) shows heterogeneous muscle involvement, complicating biopsy site selection for capturing active disease biology. We examined relationships between quantitative MRI features and RNA-seq–derived *DUX4* expression to inform biopsy targeting and patient selection using datasets from Wong et al. (2024) (34 subjects, bilateral tibialis anterior; 68 muscles) and Wong et al. (2020) (34 subjects, multiple muscles). MRI metrics correlated with *DUX4* expression, including short tau-inversion recovery (STIR) ($r = 0.66$), fat fraction (FF; $r = 0.46$), Moran's index ($r = 0.55$), and higher-order FF metrics (variance $r = 0.42$, skewness $r = -0.50$, kurtosis $r = -0.40$; all $p < 0.001$). A random forest model integrating MRI features predicted *DUX4* expression (train $r = 0.89$; test $r = 0.63$), with Moran's index and STIR most important. Models excluding STIR remained robust (test $r = 0.56$), supporting use without qualitative imaging. A simple decision tree using FF and Moran's index identified *DUX4*-positive muscles (threshold >0.5477) with 79.1% accuracy, 60.9% sensitivity, and 97.8% specificity. Applying these thresholds to a larger dataset (Blemker et al., 2025) showed ~70% of patients have at least 1 eligible biopsy site. These results demonstrate that MRI captures molecular disease activity and can guide biopsy selection and trial design in FSHD.

P1.11 Automated MRI-based biopsy site selection in FSHD using quantitative Dixon and STIR imaging

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Muscle biopsy remains a critical tool in FSHD research and clinical trials, yet site selection is largely subjective and dependent on clinician experience. Our group has developed and validated an automated pipeline for objectively identifying optimal biopsy candidates and locations using quantitative MRI. In 21 patients enrolled in ongoing clinical trials, lower-limb Dixon and short tau-inversion recovery (STIR) MRI data were processed using our validated automated segmentation framework (Riem, 2024). Across 4 muscles (vastus lateralis, medial gastrocnemius, lateral gastrocnemius, tibialis anterior), automated summary statistics were derived for fat fraction (FF%), lean muscle area, and fat muscle area, expressed as summary values and as a continuous function of position along the muscle axis. Total STIR signal percentage, 3D visualizations, and biopsy depth and distance from bony landmarks were also generated. Optimal biopsy regions, prioritizing confluent fat deposition, retained lean muscle volume >2cm³, and ease of targeting were identified in all subjects and confirmed by an expert rater. Tibialis anterior (52%, 11/21) and vastus lateralis (33%, 7/21) were most frequently selected. Selection criteria like FF% threshold and STIR positivity are customizable, with new metrics supported. These results demonstrate that objective, reproducible biopsy site selection is achievable using quantitative MRI. Future work will expand the pipeline beyond the current 4 lower-limb muscles.

P1.12 Spatiotemporal *DUX4* toxicity in FSHD: From epigenetic derepression to biomarker-driven targeted therapies

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Over the past decade, extensive genetic and mechanistic studies have transformed the FSHD landscape. Despite the mosaic, sporadic, spatially restricted nature of FSHD, a unified genetic model has emerged. Although the exact role of *DUX4* in disease pathogenesis remains to be fully deciphered, a substantial body of evidence implicates it as the central driver of pathology. It is therefore an appealing therapeutic target, and several clinical trials are now underway to determine if suppressing its expression is therapeutic. One of the current challenges in assessing these therapies is the identification of biomarkers capable of capturing disease activity, severity, and target engagement. This is especially challenging with a disease like FSHD that progresses slowly and heterogeneously. Progress in identifying biomarkers for potential clinical use marks a turning point for FSHD studies that can pave the way for therapies targeting the root cause of the disease. Here, we integrate what is known about the genetic and epigenetic mechanisms of *DUX4* derepression with emerging evidence for spatiotemporal *DUX4* toxicity in muscle. We argue that the mosaic expression of *DUX4* has direct consequences for the design of biomarkers, the selection of biopsy muscles and sampling sites, and the appropriate endpoints in clinical trials. Finally, we outline a pragmatic approach to align *DUX4*-targeted and downstream therapies with biomarker strategies that are realistic for FSHD trial design

P1.13 Circulating Mstn as a longitudinal biomarker of therapeutic efficacy in a *DUX4* mouse model

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There is no validated circulating biomarker for FSHD that can reliably monitor therapeutic efficacy over time or indicate whether the treatment effect is maintained or gradually diminishes. We previously showed that treatment with adeno-associated virus (AAV)-sh*DUX4*, provides short-term benefit but has reduced long-term efficacy in an adult *DUX4* mouse model (Sohn et al., 2025). Here, we evaluated whether circulating Mstn could serve as a biomarker of therapeutic efficacy and whether it could detect the maintenance or decline of treatment benefit over time. Adult mice were injected with an AAV-sh*DUX4* or AAV-shCtrl, and circulating Mstn levels were measured longitudinally after treatment. Circulating Mstn levels increased after AAV-sh*DUX4* injection, consistent with an initial therapeutic response, and declined several months later. These changes were examined together with histological, molecular, and functional readouts to assess whether circulating Mstn tracks treatment response over time. Our results identify circulating myostatin as a promising longitudinal biomarker of therapeutic efficacy in FSHD, capable of capturing both an initial response to treatment and its subsequent decline over time.

P1.14 Investigating baseline DEXA lean tissue mass as a predictor of longitudinal functional decline in facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is a slowly progressive skeletal muscle disease. Dual energy X-ray absorptiometry (DEXA) can measure lean muscle mass and has been used in previous neuromuscular clinical trials. From 2018 to 2023, the Clinical Trial Readiness to Solve Barriers to Drug Development in FSHD (ReSolve) study was a prospective, multisite observational study that collected DEXA scans and functional outcomes from 160 patients with FSHD at baseline and 72 patients at the 18- to 24-month visits. We investigated whether baseline percent lean tissue mass predicted measurable strength and functional decline over 1 to 2 years, observing an average decrease of 1.1 percentage points (95% CI, -1.8% to -0.4%) in percent lean tissue over 2 years, with equal decline across the upper extremities, trunk, and lower extremities. A 10-percentage-point increase in baseline percent lean tissue corresponded to reduced Timed Up and Go time at the 18- to 24-month visits (-0.83 s; 95% CI, -1.33 to -0.34) by linear mixed models. Final analyses are ongoing, and additional results on the association of changes in arm and leg lean tissue masses and functional outcome measures will be reported in the coming months. With lower cost and more facile analysis than MRI, DEXA has the potential to serve as a longitudinal biomarker for patients with FSHD.

P1.15 *DUX4*-regulated gene expression variability in FSHD muscle biopsies: Implications for clinical interpretation

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DUX4-regulated gene expression in muscle biopsies has been used as a molecular biomarker in FSHD trials. However, recent trials have highlighted high variability in these signatures. We reanalyzed 3 public datasets from FSHD muscle biopsies: One RNA-seq dataset of longitudinal pairs 1 year apart (n = 26), a second RNA-seq dataset of bilateral pairs at the same timepoint (n = 31), and a third TaqMan dataset on longitudinal biopsies from an FSHD trial placebo arm (n = 38). *DUX4*-regulated gene expression fold change between pairs was quantified in all 3 datasets. *DUX4*-regulated gene expression showed substantial variability in all datasets, with more than half of paired biopsies differing by >twofold and more than 30% by >fourfold in spatial and longitudinal contexts. Variability was highest in the trial placebo arm. Both longitudinal datasets showed regression to the mean, where direction and magnitude of change in *DUX4*-regulated gene expression were dependent on its baseline expression. Differences in cell type markers between paired biopsies in RNA-seq datasets showed minimal correlation with change in *DUX4*-regulated gene expression. High variability in *DUX4*-regulated gene expression in paired muscle biopsies is consistently observed across multiple datasets and does not appear to be explained by tissue composition changes. These findings inform interpretation of *DUX4*-regulated gene expression as biomarkers.

P1.16 Shared and distinct muscle-level patterns of fat infiltration in aging and FSHD

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Muscle fat infiltration increases with both aging and facioscapulohumeral muscular dystrophy (FSHD), but whether these processes follow similar muscle-specific patterns remains unclear. We compared muscles with high average fat fraction (FF) in FSHD (from the FSHD Global Research Foundation Patient Registry) to muscles showing the greatest age-related increases in fat fraction (from a normative age-inclusive database). Several muscles showed similar vulnerability across aging and FSHD, including the hamstrings, gastrocnemius, adductor magnus, and abdominal muscles, suggesting that some patterns of disease involvement may reflect underlying susceptibility also present with aging. Other muscles diverged. Scapular and shoulder-associated muscles, including the serratus anterior, trapezius, latissimus dorsi, and rhomboids, showed high FF in FSHD despite relatively lower age-related increases, indicating selective disease-specific involvement. In contrast, some muscles with stronger age-related increases, such as the quadriceps, were comparatively less involved in FSHD than the hamstrings, highlighting differences even within the same region. These findings indicate that while aging and FSHD share aspects of muscle-specific vulnerability, the patterns are not globally consistent. FSHD appears to combine elements of shared susceptibility with selective, disease-specific involvement, emphasizing the importance of muscle-level analyses in FSHD.

P1.17 Bone-muscle interactions in FSHD: Femoral anteversion and hip external rotator loss

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Bones and muscles form the foundation that enables movement. Variations in bone shape may be influenced by disease progression and result in changes in functional capacity in individuals with FSHD. Femoral neck anteversion angle (FNA), twist of the femur, influences the alignment of the hip and the function of surrounding muscle. Alterations in FNA are associated with abnormal movement patterns, particularly during gait, including instability and increased internal rotation of the feet, ankles, and knees. This study investigated the relationship between lean muscle volume and FNA in individuals with FSHD. MRI scans of 11 FSHD patients (2F/9M; 50.3 ± 16.8 yrs) were analyzed at baseline. The femur and hip musculature were segmented using a deep neural network followed by manual review. FNA was quantified as the angle between 1) a line connecting the center of the femoral head and the centroid of the greater trochanter and 2) a line passing through the centers of the medial and lateral femoral epicondyles. Increased FNA was significantly correlated with decreased muscle volume of the hip external rotators ($R^2 = 0.38$, $p = 0.002$). Specifically, significant associations were observed for the obturator externus ($R^2 = 0.35$, $p = 0.004$), quadratus femoris ($R^2 = 0.35$, $p = 0.004$), and gemellus ($R^2 = 0.35$, $p = 0.032$). These findings suggest that degeneration of key hip external rotators may contribute to increased femoral anteversion in FSHD, potentially influencing gait mechanics and joint stability.

P1.18 Smartphone video–based automated gait assessments for FSHD

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Current clinical outcomes lack sensitivity to the subtle, heterogeneous progression of FSHD. Smartphone-based whole-body biomechanics may offer a more sensitive alternative for clinical trials. We examined whether models using smartphone kinematics can capture FSHD-specific motor patterns better than common clinical features, using FSHD case-control classification, a key step towards sensitive digital biomarkers. Using gait kinematics (*e.g.*, joint angles) from smartphone video-based motion capture (OpenCap), we trained models to differentiate individuals with FSHD (n = 26) from controls (n = 13). As input to the classification model, we used features from a foundational machine learning model trained using gait data from 381 individuals (excluding FSHD), which distills gait kinematics to 16 features, and compared them to nine gait features selected by clinical FSHD experts. The classification model using machine learning-based features distinguished individuals with FSHD from controls (accuracy = 62%) more effectively than the model using hand-selected gait features (accuracy = 44%). Fine-tuning the foundational machine learning model using FSHD gait data improved classification accuracy to 75%. A foundational model applied to OpenCap kinematics outperformed clinician-designed features in detecting FSHD-specific gait patterns. We plan to develop a multi-activity composite digital functional outcome measure for FSHD that could readily be applied in the clinic or at home with a single smartphone.

Session 2: Clinical Care Progress with an Emphasis on Human Studies

S2.01.01 Ten top tips for non-pharmacological management in facioscapulohumeral muscular dystrophy (FSHD): Informed by evidence, clinical practice, and lived experience

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Facioscapulohumeral muscular dystrophy (FSHD) has no cure, making conservative non-pharmacological management the mainstay of treatment. FSHD has variable presentation, and the limited evidence base contributes to insufficient clinical guidance and care variability. This clinical dissemination piece presents 10 top tips for the conservative management of FSHD, synthesising evidence alongside the perspectives of people with FSHD, carers, and clinicians. These tips were derived from key lessons learned across 3 stages of a mixed-methods study: 1) A systematic review and meta-analysis of non-pharmacological interventions in muscular dystrophies; 2) surveys and ethnographic observations of UK FSHD care; and 3) focus groups with adults with FSHD, carers, and clinicians to define high-quality care. The 10 tips address: 1) Physical activity; 2) safe symptom monitoring and management; 3) exercise modalities: Strength training and beyond; 4) personalised exercise prescription; 5) non-pharmacological pain management; 6) fatigue management; 7) psychological support; 8) patient-clinician communication; 9) information and resource sharing; 10) shoulder surgery: Indications and timing. These tips provide accessible, evidence-informed guidance for the conservative management of FSHD, and offer a practical framework to support clinicians in delivering best practice care.

S2.01.02 Quality of life, psychological burden, and their association with motor function in facioscapulohumeral muscular dystrophy: A nationwide cross-sectional study

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Facioscapulohumeral dystrophy (FSHD) is a chronic neuromuscular disorder with major functional and psychosocial impact, but the relationship between motor impairment, quality of life, and psychological burden is not fully understood. We performed a cross-sectional study in Italy including 124 individuals with FSHD and asymptomatic carriers of a *D4Z4* reduced allele (68 females; mean age 50.9 ± 14.2 years). Mean age at diagnosis was 33.9 years. Motor severity was assessed with the FSHD score and Comprehensive Clinical Evaluation Form categories. Participants completed validated questionnaires on quality of life and psychological burden (WHO-QoL, INQoL, DASS-21, UCLA, PSWQ, RRS, IUS, BUT). Mean WHO-QoL score was 83.7 ± 14.2, lower in females (80.8 *vs.* 87.1 in males) and progressively worse with increasing disease severity (94.8 in carriers, 89.6 mild, 83.9 moderate, 75.8 severe). Muscle weakness was reported by 91.9% of participants, fatigue by 82.5%, and pain by 56.5%. Psychological burden was substantial: 57% reported at least moderate anxiety/worry, and DASS-21 scores increased with motor severity and were higher in females. Loneliness, rumination, worry, intolerance of uncertainty, and body image concerns were all prominent, especially in patients with more severe motor involvement. FSHD is associated with reduced quality of life and marked psychological distress, closely linked to motor impairment. These findings support integrating structured psychological assessment and targeted interventions into FSHD care.

S2.02 Epigenetic silencing in FSHD: Preliminary clinical safety and efficacy of the novel gene therapy EPI-321

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EPI-321 is a novel, adeno-associated virus (AAV)rh74-delivered gene therapy designed to treat FSHD by durably suppressing toxic *DUX4* expression in skeletal muscle after a single intravenous administration. It is the only investigational therapy in clinical trials which aims to address FSHD via epigenetic editing at the causative *DUX4* genomic locus. Study EPI-321-02 is an ongoing Phase 1/2, open-label, first-in-human trial to evaluate the safety and efficacy of EPI-321 in adults with FSHD type 1. As of April 1, 2026, 6 participants received a dose of 2×10^{13} vg/kg IV and 2 received 4×10^{13} vg/kg IV with varying follow-up duration, including some through 6 months. Safety was evaluated by reported adverse events (AEs), and preliminary efficacy was assessed by changes from baseline across various outcomes. No serious AEs had occurred. Most EPI-321-related AEs were transient, mild to moderate, and consistent with AAV-delivered therapies. One participant experienced 2 Grade 3 or higher AEs, both of which were resolving. Early assessments revealed that EPI-321-treated participants outperformed external comparators in key clinical measures. These preliminary data demonstrate a manageable safety profile and provide early evidence of therapeutic activity of EPI-321. Continued participant follow-up and longitudinal clinical assessments are ongoing to further characterize its profile.

S2.03 Impact of autoimmune comorbidities on disease severity in FSHD: A retrospective cohort analysis

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Facioscapulohumeral muscular dystrophy (FSHD) shows marked clinical heterogeneity not fully explained by its genetic basis. Increasing evidence suggests that non-genetic modifiers, including immune mechanisms, may contribute to disease variability. We conducted a retrospective analysis of adult patients with genetically confirmed FSHD1 to assess the prevalence and impact of autoimmune diseases. Clinical, demographic, and genetic data (including *D4Z4* repeat units and Comprehensive Clinical Evaluation Form classification) were analyzed. Among 299 patients, 27% had at least 1 autoimmune disease, most frequently thyroiditis, diabetes, and dermatological conditions. Autoimmune diseases were more common in older patients and those with higher *D4Z4* repeat numbers. Multivariate analysis showed that autoimmune disease was independently associated with increased disease severity ($\beta = 1.25$, $p < 0.0001$), after adjustment for age, sex, disease duration, genetic factors, and clinical category. These findings support a role for autoimmunity as an independent modifier of FSHD severity. The heterogeneity of autoimmune comorbidities and their association with more severe phenotypes highlight the need to better characterize immune involvement in FSHD. Recognizing and managing autoimmune conditions may have clinical implications and should be considered in natural history studies and therapeutic trials. Future work will extend these analyses to patients with FSHD2 to determine whether similar associations are observed.

S2.04 The natural history of childhood-onset FSHD in an Australian cohort: The iFSHD-LOS study

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Disease progression in childhood-onset FSHD and optimal functional outcome measures remain poorly defined, limiting pediatric trial design. We conducted a 2-year, single-site, prospective study in children with FSHD1. Performance-based assessments at baseline, 12, and 24 months included FSHD-Composite Outcome Measure (FSHD-COM Peds), Performance of the Upper Limb 2.0 (PUL-shoulder), 6-minute walk distance (6MWD), timed function tests, and reachable workspace (RWS). Eighteen symptomatic children (6 to 19 yrs) with genetically confirmed FSHD, onset <18 yrs, and mean FCS 5.8 ± 3.9 (1 to 15) completed follow-up. Participants were stratified by *D4Z4* repeat contraction: Group A (1 to 3 repeats; n = 7); Group B (4 to 7 repeats; n = 11). Fifty-eight percent were male; 95% facial/scapular weakness, 74% Beevor sign, 47% pelvic weakness, and 32% sensorineural hearing loss (all Group A). Across the cohort, Sit-Stand and 4Stair Climb times, RWS, and PUL outcomes changed significantly over 12 and 24 months, with greater deterioration in Group A. Between-group comparisons showed clear divergence: Over 2 years, Group A exhibited worsening FSHD-COM Peds, 6MWD, 10m Walk/Run, Descend 4 Stairs and Supine-Stand, whereas Group B improved in these measures. The iFSHD-LOS study supports that large *D4Z4* contractions confer more aggressive childhood-onset FSHD, and identifies outcome measures sensitive to change over 12 to 24 months, providing natural history data to inform inclusion of children in future clinical trials.

S2.05 MANOEUVRE: Primary analysis of emugrobart in FSHD

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There is currently no approved therapy for facioscapulohumeral muscular dystrophy (FSHD); thus, there is a high unmet medical need as the disease can cause significant morbidity and reduce the quality of life of affected people. Emugrobart is an investigational, recycling, and antigen-sweeping monoclonal anti-myostatin antibody aimed at promoting muscle growth by binding to latent myostatin and preventing its conversion to active myostatin. MANOEUVRE (NCT05548556) is a Phase 2, multicenter, randomized, placebo-controlled, double-blind study assessing the safety, pharmacodynamics, pharmacokinetics, and efficacy of emugrobart in ambulant adults with FSHD1 or FSHD2. Participants were randomized (1:1) to emugrobart or placebo. The primary endpoints were the safety of emugrobart and percentage change from baseline in contractile muscle volume (CMV) of the quadriceps femoris muscles, assessed by magnetic resonance imaging at Week 52. Secondary endpoints included the percentage change from baseline in CMV of various muscle groups at Weeks 28 and 52, as well as the change from baseline in serum myostatin. Efficacy was evaluated through exploratory performance-based and clinician-reported endpoints. Fifty-one participants were enrolled and all completed the 52-week placebo-controlled period. We present the primary analysis from MANOEUVRE for the first time.

S2.06 Targeting *DUX4* by myocyte-selective siRNA conjugate to treat FSHD

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Soufflé Therapeutics, USA

Soufflé Therapeutics has leveraged a proprietary discovery platform to enable muscle-selective functional delivery of genetic therapeutics. This receptor-mediated delivery pathway offers a differentiated selectivity and safety profile compared to other approaches. Our proprietary antibody to the myocyte receptor was used to generate antibody-siRNA conjugates that can be administered intravenously or subcutaneously. In mouse, a single administration achieves both potent (IC50 <0.05 mg/kg) and sustained silencing of target transcripts in striated muscle, while sparing off-target tissues. This muscle-specific delivery has been confirmed in non-human primates. SFL-0821 is a covalent conjugate composed of Soufflé's myocyte-targeting antibody and a *DUX4*-targeting siRNA. We administered SFL-0821 into Acta-MCM;FLExDUX4 mice, which express human *DUX4* and show FSHD-like pathophysiology. A single intravenous administration of SFL-0821 resulted in potent and dose-dependent silencing of *DUX4* mRNA (>80%) in skeletal muscle that persisted for 3 months. Immunohistochemical analysis revealed a substantial reduction of nuclear DUX4 protein expression, consistent with an observed downregulation of *DUX4*-regulated transcripts. Knockdown of *DUX4* was associated with increased muscle mass and improved muscle function. Our non-clinical data support the potential of SFL-0821 as a novel therapeutic to help people living with FSHD, and we are planning to initiate a clinical trial in 2026.

P2.01 DX5057: An orally bioavailable small molecule *DUX4* inhibitor advancing toward clinical development in FSHD

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Altay Therapeutics Inc., USA

Facioscapulohumeral muscular dystrophy (FSHD) is caused by aberrant expression of the *DUX4* transcription factor, yet therapeutic targeting of DUX4 protein has proven challenging. Here we report DX5057, the first oral small molecule DUX4 inhibitor in clinical development. DX5057 binds DUX4 and inhibits its transcriptional activity in multiple *in vitro* systems, including patient-derived FSHD myoblasts. Mechanistically, DX5057 blocks *DUX4* function by inducing time-dependent ubiquitination and degradation of DUX4 protein, a phenomenon not observed with antisense approaches. This novel mechanism is expected to confer prolonged pharmacodynamic effects *in vivo*, potentially enabling sustained therapeutic benefit. DX5057 demonstrates exceptional *in vivo* efficacy in FSHD mice, with improved muscle function, suppression of *DUX4* target genes, and reduction of KHDC1L secretion in FSHD cells, a clinical biomarker in FSHD. Significantly, DX5057 as an orally bioavailable small molecule offers advantages to IV-administered antibody-oligonucleotide conjugates, including improved patient convenience, reduced healthcare burden, and broader accessibility. We have successfully completed GLP toxicology studies with a favorable safety profile and received FDA orphan drug designation, recognizing its potential to address this serious unmet medical need. First-in-human studies are planned to initiate in 2026, positioning DX5057 as a potentially transformative oral therapy for FSHD patients.

P2.02 Muscle fat fraction (MFF) progression in facioscapulohumeral muscular dystrophy (FSHD) assessed by whole-body MRI: Prognostic factors identified via machine learning

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Clinical trials evaluating treatment effects on muscle composition in FSHD have enriched for target muscles likely to worsen on MFF. Considering the heterogeneity in progression rates in FSHD, we evaluated the extent to which this enrichment could be improved by machine learning approaches. Data from 70 patients (1,393 muscles) from the ReDUX4 trial were used. We compared classification of muscles likely to worsen in MFF over 48 weeks using 2 approaches: 1) Fixed thresholds for intermediate MFF and muscle fat infiltration at baseline, and 2) machine learning-based predictions drawing additionally on patient-level factors including age, sex, Ricci score, and D4Z4 repeat length as well as muscle-level factors including muscle type and MFF. The prediction model explained more than twice the variation in MFF progression compared to the fixed thresholds (R^2: 11% *vs.* 4%) and identified more muscles as likely to worsen (average 10 *vs.* 7 muscles per patient), with similar mean 48-week MFF change (1.5% *vs.* 1.4%) and greater precision (SE: 0.1% *vs.* 0.2%). Most of the improvement was attributable to better classification of upper-body muscles. These findings indicate that machine learning-based classification, applied to whole-body skeletal muscle MRI, can improve enrichment for target muscles likely to progress during a study period, thereby improving power to detect treatment effects on slowing such progression in FSHD.

P2.03 Epigenetic suppression of *DUX4* using CRISPR-GNDM technology as a therapeutic for FSHD

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The FSHD disease-causing *DUX4* gene is typically kept epigenetically silent in adult muscle, but if repression is lost and certain *cis*-acting factors are present, the pathogenic DUX4 protein can be produced. Thus, the *DUX4* gene would seem to be an ideal gene therapy target, but its location within the chr4 *D4Z4* repeat array makes targeting the disease-associated copy of *DUX4* with traditional CRISPR untenable. However, *DUX4* remains an excellent target for our proprietary CRISPR-GNDM epigenome editing technology, which uses a dCas protein fused to an epigenetic payload and can target the *D4Z4* array for repression. We incorporated 2 *D4Z4*-targeting CRISPR-GNDM constructs into a myogenic cell line derived from an FSHD-affected individual and demonstrated that *DUX4* and *DUX4*-target gene expression could be repressed to background levels *in vitro*. Next, we used adeno-associated virus (AAV) vectors to deliver our CRISPR-GNDMs to 3D muscle tissue models, and again demonstrated repression of *DUX4* and its target genes. Critically, we delivered AAV-CRISPR-GNDMs to an *in vivo* xenograft model of FSHD via either intramuscular or tail vein injection. Both showed robust reduction in the expression of the *DUX4*-target genes *KHDC1L*, *TRIM43*, and *ZSCAN4* without inhibition of the myogenic markers *MYOG*, *MYF6*, *MYH8*, or *DMD,* and repression was observed at both high and low systemic doses. Combined with a favorable off-target profile, these data demonstrate the potential for CRISPR-GNDM technology to be an epigenetic FSHD therapy.

P2.04 Russian FSHD patient registry

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The Russian FSHD patient registry was created in 2019 after a PCR-based genetic diagnostic method became available. Clinical data are collected via a 196-item form including FSHD severity scores, plus a 62-item patient self-assessment questionnaire. The registry enrolled 470 participants (51% male). Genetic confirmation was obtained for 76% (n = 356); others were included based on clinical and anamnestic data. Clinical forms and patient questionnaires were analyzed for 310 and 142 patients, respectively. D4Z4 repeat unit (RU) distribution mirrored European cohorts, with most patients having 3 RUs. Periscapular weakness was the most common first symptom (46.8%), followed by facial weakness (31.6%), often unnoticed by patients. Mean age was 37.8 years (range 0 to 97), indicating a younger cohort than international data. Delta-adjusted cluster analysis (n = 215) identified 3 groups of patients with different clinical course trajectories: A classic phenotype with onset before age 14 and early involvement of various muscle groups (n = 177), and 2 clusters with either facial or periscapular onset and slow progression. The Russian FSHD registry provides comprehensive characterization of a large national cohort, showing a predominance of patients with 3 *D4Z4* repeats and a younger demographic profile versus international data. Cluster analysis revealed 3 heterogeneous disease trajectories, offering a framework for improved patient stratification.

P2.05 Safety and exposure of SRP-1001, an αvβ6 integrin-targeting siRNA therapy for patients with FSHD1: Interim findings from a Phase 1/2 study

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SRP-1001 is an investigational αvβ6 integrin-targeting ligand-based siRNA therapy designed to silence aberrant *DUX4* expression in facioscapulohumeral dystrophy type 1. First-in-human trial preliminary data are presented. This ongoing, Phase 1/2, randomized placebo (PBO)-controlled trial (NCT06131983) included patients aged 16-70 years. In part 1, patients had a single ascending dose of siRNA (approx. 1 mg/kg, n = 5; 2 mg/kg, n = 4; 4 mg/kg, n = 4; or 8 mg/kg, n = 5) or PBO (n = 9). In the 12-month part 2, patients had multiple ascending doses (approx. 4 mg/kg, n = 11; 8 mg/kg, n = 11) or PBO (n = 7). Primary/secondary endpoints were safety and tolerability/plasma pharmacokinetics. Exploratory endpoints were siRNA muscle concentration, levels of *DUX4*-regulated gene expression, and serum creatine kinase (CK). The mean (standard deviation) age at baseline was 44.9 (12.75; N = 56) years. Overall, 43 patients experienced treatment-emergent adverse events (TEAEs). Most were mild to moderate in severity, with 1 serious, unrelated TEAE (chest discomfort) reported. SRP-1001 showed consistent dose-dependent increases in plasma and muscle exposure with no evidence of saturation. Compared with PBO, pooled SRP-1001 single doses suppressed *DUX4*-regulated gene expression by ~90% and reduced CK levels by ~33%. SRP-1001 suppressed *DUX4*-regulated genes while showing an acceptable safety profile and dose-dependent increases in plasma and muscle concentration.

P2.06 BetterLife FSHD: 1-year descriptive cohort analysis

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BetterLife FSHD is an innovative patient health platform and research registry with a mission to help patients live a better life with FSHD while accelerating FSHD research. Through short survey instruments, participants self-report demographics, health history, diagnosis and progression of FSHD, management strategies, and quality-of-life domains including pain, fatigue, mental health, and physical activity. The platform also delivers personalized support tools (health tracking, data visualizations, trial-matching, community polling, curated resources) to enrich the participant experience and strengthen engagement. Here we detail an initial cohort overview and descriptive analysis of BetterLife data to characterize the participant population over the first 12 months of the registry's operation. We report descriptive statistical analyses of participant demographics, diagnosis characteristics, symptom onset and progression, functional status, and quality-of-life measures derived from patient-reported outcomes. Healthcare utilization, assistive device use, and engagement metrics (survey completion and follow-up rates) will also be presented.

P2.07 Analysis of women's health using BetterLife FSHD data

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Our study will generate a descriptive analysis of self-reported women's health survey data and disease severity in women with FSHD. Analyses will include descriptive statistics of participant demographics such as age, sex, household income, and ethnicity/race. Analysis will also include relevant health history, including menstrual cycle characteristics, menopause status (natural or induced), hormonal oral contraceptive use, and mental health history. Results will provide the first focused characterization of women's health characteristics within the BetterLife FSHD cohort, comparing outcomes to current literature and the general population, and will inform future investigations into sex-specific disease mechanisms and progression. Follow-up analysis on these findings may include examining participants' cycle lengths, pregnancy history, and menopause status to see how these variables may impact symptom progression.

P2.08 Chromosome 4q D4Z4 contraction status in Turkish FSHD patients: Insights from a retrospective study in the Mediterranean region

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FSHD is a rare neuromuscular disorder with limited molecular data available from Turkey. This study aimed to provide an updated overview of the genetic and clinical features of patients with FSHD1 followed between 2006 and 2025. A total of 46 patients diagnosed as having FSHD1 through Southern blot analysis were included. The cohort consisted of 26 males and 20 females, with a mean age of 32.93 ± 17.01 years. Clinical severity scores (CSS) and age-corrected CSS (ACSS) were assessed based on neurologic examinations. Statistical analyses were performed using the SAS 9.4 for group comparisons, and correlation and regression analyses between age, D4Z4 repeat units (D4Z4RU), and severity scores. The most frequent D4Z4 repeat size was 4 units (30.4%). Although CSS and ACSS appeared lower in female patients, the difference was not statistically significant. Patients aged under 30 years exhibited significantly lower CSS and ACSS compared with those aged over 30 years. A strong correlation was observed between age and both CSS and ACSS; no significant correlation was found between D4Z4RU and clinical severity. Among the 46 patients, 20 families were represented, and 1 patient had *de novo* mosaic mutation. Our findings highlight the importance of longitudinal and population-specific data in understanding FSHD. Increased molecular diagnosis and regular follow-up of patients may facilitate future research and the development of targeted therapies.

P2.09 Gut microbiota and short-chain fatty acid profiles in facioscapulohumeral dystrophy: Associations with epigenetic alterations

Ceren Hangul[1], Ozlem Koyuncu Ozyurt[2], Simone Baldi[3], Dilek Colak[2], Oznur Tokta[1], Toygar Pekcan[2], Sara Bertorello[3], Marco Pallecchi[4], Filiz Koc[5], Cumali Dalokay[5], Hilmi Uysal[6], Kemal Hakan Gulkesen[7], Filiz Ozcan[8], Gianluca Bartolucci[4], Sibel Berker Karauzum[1], Amedeo Amedei[9,3]

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Gut microbiota (GM) affects muscle homeostasis, and growing evidence indicates dysbiosis of GM may be a contributing factor in the dystrophies. Furthermore, GM metabolites can interact with DNA methylation. FSHD is the second common dystrophy with hypomethylation of *D4Z4* repeat on 4qter. We investigated 1) GM alterations, and 2) the correlation of microbial-derived free-fatty acids (FFAs) with methylation of DR1 and 5P regions in FSHD. Twenty-eight FSHD patients and 28 gender- and age-matched controls were included. GM characterization was performed through 16S-rRNA sequencing. Methylation levels of DR1 and 5P regions were assessed by bisulfite sequencing. FFAs were analysed with gas chromatography-mass spectrometry. Altered GM was observed in patients, along with distinct profiles of faecal and circulating short-chain fatty acids (SCFAs), medium-chain fatty acids, and long-chain fatty acids. DR1 and 5P regions exhibited significant hypomethylation in FSHD compared to control. Hypomethylation correlated with *faecal and circulating* FFAs in patients, while no correlation was identified in healthy controls. The severely affected patients exhibited a notable increase in the prevalence of *Pasteurellaceae*. This is the first study revealing that FSHD patients showed compositional and functional GM dysbiosis. A strong association between proximal *D4Z4* hypomethylation with microbial-derived SCFAs was identified. These findings suggest that GM modulation with its metabolites could be promising strategy for interventions in FSHD management.

P2.10 Trial design for the Phase 2 FORGE study evaluating apitegromab in adults with facioscapulohumeral muscular dystrophy

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Apitegromab (SRK-015) is an investigational, muscle-targeted treatment that selectively inhibits activation of myostatin, a negative regulator of muscle growth and strength. Phase 2 (NCT03921528) and 3 (NCT05156320) clinical trials for spinal muscular atrophy showed apitegromab improved motor function and was well tolerated. Preclinical work showed the apitegromab murine analog (muSRK-015) significantly increased skeletal muscle mass, muscle force, and endurance in a FLExD murine model of FSHD. FORGE (NCT07435129) is a randomized, double-blind, placebo-controlled, multicenter Phase 2 study that will assess the efficacy, pharmacokinetics/pharmacodynamics, safety, and tolerability of apitegromab in patients with FSHD. FORGE will recruit ~60 ambulatory adult patients (aged 18 to 60 y) with genetically confirmed FSHD (FSHD1/2), a clinical severity score of 1.5 to 3 (Ricci score), and baseline 10MWRT time ≤5s. Key exclusion criteria include any medical condition, clinically significant laboratory result, or ECG value that may compromise patient safety, interfere with study compliance, or confound result interpretation. Patients will be randomized to receive apitegromab 10mg/kg monotherapy or placebo (IV) Q4W. FORGE will consist of up to 4 weeks of screening, 52 weeks of treatment (13 doses), and 20 weeks of safety follow-up for patients not entering the OLE study. The primary endpoint will be percent change from baseline in total lean muscle volume by full-body MRI (wk 52). FORGE is expected to be conducted across 20 sites in North America and Europe.

P2.11 Identifying and solving barriers to FSHD registration in the Netherlands

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The Dutch FSHD registry, established 10 years ago, facilitates patient access for research, enables collection of natural history data, and informs patients about relevant developments. Currently, 513 patients are registered, representing approximately 25% of the estimated Dutch FSHD population. Increasing this number is an important priority. We conducted an online survey to assess awareness of the registry and identify barriers and facilitators to registration. The survey was co-developed with representatives of 2 Dutch patient organizations (FSHD Stichting and Spierziekten Nederland) and FSHD Europe. Invitations were distributed to all registered individuals, patients known at Radboudumc and Leiden University Medical Center, members of Spierziekten Nederland, and via open and closed social media channels. In total, 516 participants (aged 7 to 90 years) were included in the analyses; 216 identified as male and 241 as female. Preliminary results indicate that concerns about security and privacy of personal (coded) data were the main reason for non-registration among nonregistered participants (n = 69). Across all respondents, the most important motivation for registration was contributing data to improve knowledge about FSHD (51%). These findings highlight the need for clearer communication about the purpose and structure of the Dutch FSHD registry. Targeted information tools – such as webinars, brochures, and newsletters – will be developed and designed to be accessible for diverse patient groups.

P2.12 The need for and experiences with psychosocial support among adolescents and young adults at the multidisciplinary facioscapulohumeral muscular dystrophy (FSHD) outpatient clinic in the Netherlands

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Facioscapulohumeral muscular dystrophy (FSHD) substantially affects quality of life due to progressive muscle weakness, changes in appearance, pain, and fatigue. Previous research in children and adolescents with FSHD showed reduced quality of life, partly related to physical limitations and fear of social exclusion. Many young people attempt to appear "normal," highlighting the need for accessible psychosocial support that strengthens coping and social participation. Furthermore, discussions during a European Neuromuscular Centre workshop emphasized that psychological support should be tailored to the individual and family, and that e-health approaches may improve accessibility. The aim of this study was to explore psychosocial support needs among adolescents and adults with FSHD. A semi-structured interview guide, co-developed with patient researchers, was used to explore experiences with psychosocial support, unmet needs, preferred types of support, and perceived barriers. Three online focus groups with patients and 1 with relatives were conducted (age range 22 to 80 years). Preliminary analysis identified key themes including emotional and social consequences of the disease, coping strategies, acceptance, experiences of grief, and dealing with uncertainty. The results are expected to inform the development of a tailored mental training program designed to improve disease acceptance, resilience, and an improved quality of life. Findings might increase preparedness for clinical trial participation.

P2.13 Mental and Intellectual Neurodevelopment in childhood-onset FSHD studies: The MINDS-FSHD study protocol

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The MINDS-FSHD study aims to understand more about cognitive and psychological functioning in patients with childhood-onset FSHD to optimise patient-centred clinical care for children and young people. This prospective, mixed-methods international, multisite study will run at the Murdoch Children's Research Institute (MCRI, Melbourne, Australia), Children's Hospital of Eastern Ontario, Ottawa, Canada) and Amalia Children's Hospital (Radboudumc, Nijmegen, the Netherlands). We plan to recruit 30 to 40 children and young people with genetically confirmed FSHD aged 5 to 21 years. The Weschler Intelligence Scales, NEPSY-II comprehension scales, and RAVLT memory scales will examine cognition. NEPSY Verbal Fluency test and FAS test will examine executive function. The Emotion Recognition Task and NEPSY-II will examine the ability to read facial expressions. The PROMIS (Anxiety, Depression, Peer Relationships), CASP, Kidcope, Child Behaviour Checklist, and Adaptive Behaviour Assessment System will evaluate psychological symptoms. Qualitative interviews conducted at MCRI by a young person with lived experience will examine how physical health impacts on the mental health and well-being of children and young people with FSHD. An improved understanding and ability to identify cognitive and psychological issues in children with FSHD will improve clinical care and enhance quality of life in childhood as they transition through adolescence into adulthood.

P2.14 Self-reported disease burden in childhood-onset FSHD: Reliability and validity of the paediatric FSHD-Health Index (FSHD-HI Peds)

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FSHD causes progressive, asymmetrical muscle weakness. Patient-reported outcome measures (PROMs) are needed to quantify perceived burden in childhood-onset FSHD. The FSHD-Health Index (FSHD-HI), developed for adults, has been adapted for children. This study evaluated the test-retest reliability, validity, and responsiveness of the FSHD-HI Peds. Data came from 2 cohorts with confirmed FSHD: A randomized controlled trial (RCT) of creatine monohydrate and a longitudinal outcomes study. Test-retest reliability used RCT data collected 2 weeks apart. Convergent validity used Spearman correlations between FSHD-HI Peds and FSHD Clinical Score (FCS), FSHD-COM Peds, PedsQL NMM and Severity Scale (CSS). Intraclass correlation coefficients (ICC2, 1), descriptive statistics, and standardized response means were calculated; responsiveness was examined using an anchor-based approach. Seventeen participants (6 to 18 yrs, 47% female) contributed to validity analyses and n = 15 reliability. FSHD-HI Peds showed excellent reliability: ICC = 0.97 (95% CI: 0.92 to 0.99). Convergent validity was moderate to strong: FCS (r = 0.80 p <0.001), FSHD-COM Peds (r = 0.70), PedsQL NMM (r = -0.67, p = 0.003), and CSS (r = 0.67, p = 0.003). During 2 years, mean FSHD-HI Peds scores increased by 6.67 (95% CI: -1.1 to 14.4) p = 0.08, paralleling FCS increase of 0.33 (95% CI: -0.85 to 0.18) and self-rated deterioration. FSHD-HI Peds is a valid, reliable PROM of disease burden in childhood-onset FSHD and is sensitive to deterioration over time, supporting its use in future pediatric research.

P2.15 Motor Outcomes to Validate Evaluations in Pediatric facioscapulohumeral muscular dystrophy (MOVE Peds): Baseline characteristics for the first 20 participants enrolled

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The primary goal of this proposal is to hasten therapeutic development by validating outcomes and refining trial strategies for childhood-onset FSHD. Cross-sectional studies have suggested an association between earlier onset and greater clinical severity. Childhood-onset FSHD is a population of interest, as treating a more rapidly progressing population may detect a treatment effect earlier. While the adult FSHD field has coalesced around outcome measures (FSHD-COM, reachable workspace [RWS], and qMRI), academic and industry experts agree there is an urgent, unmet need. A prospective observational study will enroll 80 symptomatic, genetically confirmed pediatric FSHD participants over 24 months. Visits occur every 6 months, and collect patient-reported outcomes and functional motor performance (FSHD-COM Peds), RWS, and whole-body MRI. Since recruitment began in June 2025, 20 participants have been recruited across sites in the USA and Australia, with additional sites expected to begin recruitment in the first half of 2026. Baseline characteristics including baseline severity scale, FSHD-COM Peds, MRI data, and RWS scores for this initial cohort will be presented. FSHD severity and its impact on function are often worse in children, and measurement tools must account for the effects of growth and maturation on motor function. Validating measures in children with FSHD will improve trial readiness. Recruitment is ongoing.

P2.16 Toward a therapy-agnostic FSHD disease progression model for health technology assessment: Work plan and progress from PaLaDIn

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As disease-modifying therapies for FSHD advance toward approval, the lack of validated health economic evidence risks delaying patient access. Health Technology Assessment (HTA) bodies require robust disease-progression models to inform reimbursement decisions, and patient advocacy organizations (PAOs) often have limited involvement in the process. PaLaDIn (Patient Lifestyle and Disease Data Interactium), an Innovative Health Initiative-funded initiative, is building the Interactium, a patient-centric, real-world data collection platform for neuromuscular disease. Within the PaLaDIn framework, FSHD and Project Mercury serve as a case study to demonstrate how advocacy organizations can leverage Interactium data to lead cross-sector collaboration and generate HTA-ready evidence. PaLaDIn will deliver a therapy-agnostic FSHD disease-progression model that integrates a conceptual framework with a mathematical model to describe, predict, and analyze disease progression over time. The model will use existing international datasets to quantify patient, economic, and societal burden, and will enable extrapolation of outcomes over time. PaLaDIn will also produce a toolkit to support PAOs in engaging with HTA and reimbursement decisions. This poster outlines the model and toolkit work plans and reports on early progress. Together, these tools will form a critical foundation for evidence and advocacy to accelerate reimbursement and patient access to FSHD therapies.

P2.17 Prevalence of facioscapulohumeral muscular dystrophy from the Muscular Dystrophy Surveillance, Tracking, and Research Network (MDSTAR*net*)

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Facioscapulohumeral muscular dystrophy (FSHD) is a common form of muscular dystrophy caused by abnormal expression of the *DUX4* gene. Estimating FSHD prevalence is challenging due to late onset and phenotypic variability, with most estimates between 2 and 4 per 100,000. Few studies have estimated FSHD prevalence in the US. We aim to estimate the prevalence of FSHD and examine differences by sociodemographic characteristics. Data came from the population-based Muscular Dystrophy Surveillance, Tracking, and Research Network (MDSTAR*net*) including Iowa, North Carolina, South Carolina, Utah, and western New York. Individuals with FSHD, resident in an MDSTAR*net* site, and healthcare encounter 2008 to 2019 were included. Point prevalence per 100,000 individuals was calculated overall and by site using 2019 midpoint census population estimates. Wilson score interval was used to calculate 95% confidence intervals (CI). FSHD prevalence overall was 2.11 per 100,000 (95% CI: 1.92 to 2.31) and varied by site (0.89 to 3.96). Prevalence was higher among males (prevalence ratio [PR] = 1.29, 95% CI: 1.07 to 1.56) and non-Hispanic White (PR = 4.79, 95% CI: 3.30 to 6.96) compared to other ethnicities. Overall FSHD prevalence from MDSTAR*net* is largely consistent with prior studies with some variation by site. Findings include higher prevalence among males and lower among some ethnic groups. This information is useful for resource allocation and designing clinical trials for FSHD.

P2.18 Dose-finding safety and tolerability study of clenbuterol in facioscapulohumeral muscular dystrophy (FSHD)

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Facioscapulohumeral muscular dystrophy (FSHD) is a *DUX4*-mediated neuromuscular disorder with no FDA-approved disease-modifying therapies. Clenbuterol, a β2-adrenergic agonist with anabolic properties, has demonstrated preclinical activity in reducing *DUX4* expression and promoting muscle growth. We are conducting a 6-month, non-randomized, open-label, dose-escalation trial supported by the US National Institutes of Health Wellstone Grant (P50 AR065139) to evaluate the safety, tolerability, and preliminary efficacy of clenbuterol in adults with genetically confirmed FSHD. Thirty participants are planned across 3 sequential dose cohorts (20, 40, and 60 mcg twice daily) at 3 US clinical sites. Safety assessments include adverse event monitoring, laboratory testing, vital signs, and electrocardiography; exploratory outcomes include functional and RNA sequencing/MRI-based measures. Cohort 1 enrollment is complete (n = 10), with the first 7 participants having completed 6 months of treatment at the time of this interim analysis. Clenbuterol has been generally well tolerated. Most adverse events have been mild and consistent with the known pharmacologic profile, including nervous system, musculoskeletal, and cardiac-related symptoms. No protocol-defined stopping criteria have been met resulting in treatment discontinuation or dose interruption. These interim findings demonstrate acceptable tolerability of clenbuterol at the initial dose and support continued evaluation across sequential dose cohorts.

P2.19 Assessing non-recommended guideline pharmacologic treatments in patients with facioscapulohumeral muscular dystrophy (FSHD): Analysis of US real-world data

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FSHD patients often experience acute or chronic musculoskeletal pain and chronic respiratory failure. The American Academy of Neurology (AAN) clinical guideline advises against the use of albuterol, corticosteroid, or diltiazem for improving muscle strength or slowing disease progression (AAN, 2015).This study examines off-guideline use of beta 2-adrenergic agonists (B2As) and oral corticosteroids in FSHD patients. B2As were further classified as short-acting (SABAs), long-acting (LABAs), and ultra-long-acting (ultra-LABAs). A retrospective observational approach using Electronic Health Record data-linked claims was employed. Patients with an FSHD diagnosis between 1 January 2020 and 30 June 2025 (earliest event = index) were included. Patients were required to have ≥6 months baseline and follow-up continuous enrollment. Demographic characteristics were described as of index. A total of 2,068 patients with a diagnosis of FSHD met the eligibility criteria. Mean age was 51, and most patients were female (57%) and White (60%). Overall, use of SABAs (11.5% and 12.0%) and corticosteroids (13.9% and 14.8%) was observed at baseline and follow-up. The most common B2As used were albuterol, salmeterol, vilanterol, formoterol, levalbuterol, and olodaterol, while prednisone was the most used oral corticosteroid. Gaps in guideline-recommended pharmacologic interventions to manage FSHD suggest opportunities for optimizing the use of effective treatment strategies to improve the real-world management of the disorder.

P2.20 Strengthening the European FSHD ecosystem: Bridging clinical care, research, and patient-centered outcomes

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FSHD Europe unites and amplifies the voice of people living with FSHD across Europe. Representing 14 national patient organisations from 12 countries, it established itself as a unifying platform connecting patients, clinicians, and researchers. Despite ongoing scientific progress, significant disparities remain across Europe in clinical care, access to specialised expertise, and integration of research – challenges that are shared with other neuromuscular diseases. To address these gaps, FSHD Europe has defined a comprehensive European strategy to accelerate FSHD drug development, structured around 4 strategic pillars: Patient Advocacy and Education, Clinical Trial and Treatment Readiness, Drug Approval and Access, and Stakeholder Engagement. These pillars aim to empower patient advocates, expand and coordinate trial infrastructure, integrate patient input into regulatory and health technology assessment processes, and promote European coordination and global collaboration. The FSHD European Trial Network, a component of FSHD Europe, comprises 5 active working groups, each co-led by recognised experts, and uniquely links a pan-European patient community with a coordinated network of clinicians. FSHD Europe advances clinical care and patient-centered research through coordinated stakeholder engagement, data harmonisation, and advocacy for standardised care pathways. These approaches reduce fragmentation, enhance trial readiness, and strengthen research impact across Europe.

P2.21 A randomized, double-blind, placebo-controlled, single and multiple ascending dose trial to assess the safety, tolerability, pharmacokinetics, pharmacodynamics, and preliminary efficacy of SFL-0821 in adults with facioscapulohumeral muscular dystrophy (FSHD)

Herb Stevenson, David Strochlic, Mark Angelino, Vadim Dudkin

Soufflé Therapeutics, USA

This presentation will provide an overview of the clinical study, Dayrise (SFL-0821-101), a first-in-human study for adults living with FSHD. SFL-0821 is a monoclonal antibody–siRNA conjugate designed to enable targeted functional delivery to striated muscle, resulting in downregulation of aberrant *DUX4* expression. In nonclinical studies, high potency and specificity, broad safety margins, and functional improvement all support clinical use of SFL-0821 as a potential therapeutic agent for patients with FSHD. Dayrise is a randomized, double-blind, placebo-controlled study evaluating the safety and tolerability of escalating single and multiple doses of SFL-0821 in adults aged 18 to 65 years with FSHD types 1 or 2. Key secondary and exploratory objectives include characterization of pharmacokinetics/pharmacodynamics, immunogenicity, and preliminary efficacy (MRI, reachable workspace, manual muscle testing, timed function tests, and patient-reported outcomes). Participants will be randomized 2:1 to SFL-0821 or placebo treatment. Part A (SAD) will include multple single dose-escalation cohorts with a 24-week follow-up. Part B (MAD) will be 48 weeks in duration and evaluate selected doses from Part A, administered every 12 to 24 weeks. MRI guided needle muscle biopsies will be obtained at baseline and post-dose. Key eligibility criteria include ambulatory adults; genetically confirmed FSHD; FSHD Clinical Severity Score ≥1.5 to ≤4.0; MRI-measurable muscle suitable for biopsy; and no prior exposure to oligonucleotide, gene therapy, or other investigational FSHD treatments.

P2.22 Time is muscle: Preliminary retrospective evidence of early disease burden in childhood-onset FSHD

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FSHD is a debilitating genetically defined neuromuscular disease driven by toxic *DUX4* expression that causes progressive muscle degeneration and weakness. About 20% of cases are diagnosed in childhood, and 55% of people with FSHD report symptoms beginning before age 18. Childhood-onset FSHD is particularly devastating, as symptoms begin earlier and progress faster, often resulting in loss of mobility. Children and young adults with ongoing musculoskeletal development are most likely to benefit from therapy, yet most clinical trials focus on adults with preserved ambulatory function. Participants diagnosed with FSHD by age 18 submitted medical records, which were securely stored, de-identified, and reviewed per IRB protocol. Pertinent clinical data were extracted and compiled for analysis. Enrollment is ongoing. Preliminary findings (N = 9) show mean ages of symptom onset of 4.8 ± 2.5 years and of diagnosis of 7.7 ± 4.1 years. This gap represents a time of unmonitored progression without treatment options. All subjects demonstrated widespread muscle weakness including facial, shoulder/back/core, and lower body, with altered gait in 8 of 9 subjects. Pulmonary function decline was observed in 6 of 9 subjects. This ongoing retrospective natural history study aims to describe the progression of childhood-onset FSHD, and to address the urgent need to identify endpoints for clinical trials in the pediatric population.

P2.23 Video assessment for FSHD: Adapting validated methodology as a functional outcome measure across age and disease severity

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FSHD is a progressive neuromuscular disease affecting muscles throughout the body. People with FSHD experience a decline in functional abilities due to weakness in the muscles affected. To maintain function, individuals develop compensatory movements. An innovative outcome measure (OM) capturing these compensations would benefit FSHD clinical trials. The Duchenne Video Assessment (DVA) is a home-based video assessment that measures ease of movement and has demonstrated strong reliability and validity, detecting clinically meaningful changes, and has been used as an endpoint in clinical trials. We are adapting the DVA for FSHD to create an OM applicable across the range of FSHD functional ability. Physical therapists will identify meaningful tasks, and scorecards will be developed and used to rate compensatory movements in the recorded tasks. Video assessment has been created for other neuromuscular diseases, showing broad adaptability. It offers advantages for FSHD: Sensitivity to subtle changes; applicability across age groups and disease severity; real-world data; reduced burden through home capture; and direct assessment of compensations. Feasibility work has been initiated, and meaningful functional tasks have been identified. Video-based assessment represents a promising approach to expand FSHD OM options by reducing assessment burden and promoting inclusive participation in clinical trials while emphasizing compensatory movements and real-world performance.

P2.24 Motor Outcomes to Validate Evaluations in Facioscapulohumeral muscular Dystrophy (MOVE FSHD): A 3-year prospective study in pediatric FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is a progressive, genetic neuromuscular disease with symptom onset often in childhood. Characterizing the pediatric phenotype is critical to defining the burden of disease, informing clinical trial design, and providing essential insight into the full clinical spectrum of FSHD. Emerging therapeutic approaches targeting *DUX4* expression may have broad applicability and benefit individuals across all ages and stages of disease. Pediatric participants aged 5 to 17 years underwent annual study visits for 3 years. Data collection included: Medical history, patient-reported outcomes, and standardized functional assessments including manual muscle testing, FSHD clinical severity score, timed function tasks, and respiratory function measures. Descriptive statistics and Pearson correlation tests were performed. Thirty-nine participants ranged from 5.0 to 17.8 years with full range of clinical severity scores (0 to 10) and 41% were female. Symptom onset ranged from birth to 13.5 years, and age of diagnosis from 2 to 14 years. Mean functional abilities scores declined over time: 8.97 at baseline (n = 36), 9.80 at 12 months (n = 15), and 12.11 at 24 months (n = 9). Additional analysis is ongoing. Participants demonstrated worsening functional abilities scores over 24 months indicating ongoing, measurable functional decline. These data support the need for ongoing pediatric studies and validation of pediatric-specific functional measures.

P2.25 Motor Outcomes to Validate Evaluations in Facioscapulohumeral muscular Dystrophy (MOVE FSHD): A 3-year prospective study in non-ambulatory FSHD individuals

Ally Roets[1], **Michaela Walker**[2], Brianna Brun[3], Leann Lewis[3], Natalie Katz[4], Michelle Mellion[5], Russell Butterfield[6], Katy Eichinger[3], Jeffrey Statland[2,7]

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Facioscapulohumeral muscular dystrophy (FSHD) is a progressive, genetic neuromuscular disease driven by aberrant *DUX4* expression with approximately 23% of individuals losing ambulation. Despite high disease burden, non-ambulatory individuals are underrepresented in research and excluded from clinical trials. Characterizing disease across the full severity spectrum is critical to defining burden and informing inclusive trial design, particularly as *DUX4*-targeted therapies may benefit individuals at all stages. Participants underwent annual study visits over 3 years. Data collection included: Medical history, patient-reported outcomes, and standardized functional assessments including manual muscle testing, FSHD-specific clinical severity scores (CSS), timed function tasks, and the Upper Extremity Functional Index (UEFI). Descriptive statistics and Pearson correlation tests were performed. Thirty-seven non-ambulatory participants aged 16 to 77 years were included. Of these, 59.5% were female (n = 22) and 92% had CSS of 9 to 10. Mean UEFI scores had a clear decline over time: 30.0 at baseline (n = 38), 28.7 at 12 months (n = 29), and 22.3 at 24 months (n = 13). Additional analysis is ongoing. While a floor effect is observed and expected in the CSS of non-ambulatory individuals, the worsening UEFI scores indicate ongoing, measurable functional decline.

P2.26 Clinical characterization of swallowing function in FSHD

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Dysphagia has traditionally been considered uncommon in facioscapulohumeral dystrophy (FSHD), but it was poorly explored in the literature. We aimed to characterize the swallowing function in FSHD patients and detect the prevalence and pattern of dysphagia using clinical assessment and objective orofacial myofunctional evaluation (AMIOFE). The study was conducted at the University of São Paulo, Brazil. Fifty patients with genetically confirmed FSHD underwent systematic screening through a structured questionnaire regarding dysphagia and speech dysfunctions, followed by comprehensive speech-language pathology assessment using the orofacial myofunctional evaluation with scores protocol. Dysphagia was present in 23.33% of patients and dysarthria/dysphonia in 21.11%. AMIOFE scale demonstrated mild impairment overall with a predominant involvement of oral-phase components, particularly mastication and oral control: Mastication behaviors were markedly reduced, cheek performance showed mild involvement, and swallowing-related lip behavior was also impaired, indicating compromised bolus preparation and oral containment. Swallowing efficiency remained preserved. Dysphagia and speech involvement in FSHD were frequent in this cohort, predominantly affecting the oral phase and mastication. Swallowing efficiency remained preserved, which highlights the need of systematic screening to improve detection of bulbar involvement in FSHD.

P2.27 Cas13d-mediated RNA targeting of *DUX4*: Evaluating *in vivo* delivery strategies for FSHD therapy

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We developed a CRISPR/Cas13d RNA-targeting strategy to suppress *DUX4*. Guide RNAs (gRNAs) targeting *DUX4* mRNA were screened using luciferase reporter assays and validated by Western blot, identifying 3 lead gRNAs that robustly reduced DUX4 protein levels. These candidates maintained strong knockdown across a range of gRNA and Cas13d concentrations *in vitro* and significantly reduced *DUX4* biomarker expression in FSHD myogenic cells. We next evaluated Cas13d delivery in mouse skeletal muscle. Intramuscular injection resulted in marked muscle inflammation, immune cell infiltration, and a decline in Cas13d expression between 3 and 10 weeks post-injection, suggesting immune responses associated with local delivery. To improve delivery and expression in skeletal muscle, we tested systemic retro-orbital (RO) administration of Cas13d. In contrast to intramuscular delivery, RO injection showed promising results with no detectable inflammatory infiltration and increased Cas13d expression from 3 to 10 weeks post-injection. These findings suggest that systemic delivery may provide improved tolerability and sustained Cas13d expression in skeletal muscle. Based on these encouraging results, we are currently evaluating the therapeutic efficacy of systemic Cas13d delivery via RO injection with lead *DUX4*-targeting gRNAs in an FSHD mouse model.

P2.28 SAT-3247 improves muscle force in a FLExDUX4.CRE mouse model of FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is a genetically complex, progressive muscular dystrophy with highly variable progression caused by aberrant expression of *DUX4* in skeletal muscle. While no approved disease-modifying therapies exist, current approaches typically focus on suppressing *DUX4* and downstream pathology. Although not the primary initiating event, satellite cell dysfunction is a contributing factor to FSHD pathology. SAT-3247 is a small molecule that enhances regeneration and repair of satellite cells in preclinical models of muscle diseases. As such, we evaluated the effects of SAT-3247 on muscle function in the FLExDUX4.CRE mouse. Mice were assigned based on body weight and sex to receive vehicle or SAT-3247 doses between 3 and 30 mg/kg via oral gavage on a 4-day-on/3-day-off regimen. FLExDUX4.CRE mice were not induced with tamoxifen. *In situ* muscle contractile force was evaluated after 4, 8, and 12 weeks of treatment. Dose-dependent improvements in muscle force frequency (0 to 150Hz) were observed in FLExDUX4.CRE mice, with maximum forces approaching those observed in CRE wild-type controls. *In situ* maximum contractile force in 10mg/kg SAT-3247-treated mice was numerically greater than vehicle after 4 weeks of dosing, and significantly greater after 8 and 12 weeks. These results demonstrate that SAT-3247 improves muscle function in a mouse model of FSHD and support clinical evaluation as a potential *DUX4*-independent therapeutic approach in FSHD.

P2.29 Pharmacological restoration of muscle stem cell health and function in FSHD

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Scintillon Research Institute, USA

Therapeutics in development for FSHD primarily target the causal *DUX4* gene in myofibers. However, *DUX4* is rarely detected in FSHD muscle, rendering it a temporally elusive therapeutic target. Further, as a pioneer transcription factor, brief expression of *DUX4* in muscle stem cells may be sufficient to trigger lasting epigenetic changes that can impair their capacity to differentiate into healthy myofibers. Therefore, restoration of muscle regenerative capacity in FSHD will likely require a resolution of the damage caused by *DUX4* in muscle progenitors. We have identified a first-in-class small molecule that can restore myogenic potential and decrease apoptosis in differentiating FSHD patient myoblasts, as well as inhibit *DUX4* target genes. Specifically, this compound resolves the PAS/DAS ratio of *DUX4* transcripts, reduces oxidative stress, and promotes healthy differentiation. In mouse myoblasts with *DUX4* induced, our compound increases expression myogenic genes, improves healthy myofiber maturation, and reduces cell death, outperforming the Phase 3 FSHD drug losmapimod. This compound is orally available, increasing Pax7 and decreasing *Atrogin1* in limb and diaphragm muscles in old mice. Independent testing revealed increased muscle force in a mouse FSHD model after 28 days of treatment. Together, these studies implicate our small molecule as a potential therapeutic for restoring MuSC function to enhance muscle regeneration in FSHD and other muscular dystrophies.

P2.30 Reconstitution of SMCHD1 for FSHD therapy via split intein technology

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Both FSHD1 and FSHD2 are characterized by epigenetic dysregulation of the *D4Z4* repeat. We hypothesized that overexpressing the epigenetic silencer SMCHD1 would promote hypermethylation and repress the *DUX4* locus in FSHD muscles. However, the 6,018 base pair *SMCHD1* coding sequence exceeds the standard packaging capacity of adeno-associated virus (AAV) vectors. To address this, we utilized a split intein-mediated protein trans-splicing approach, delivering SMCHD1 as 2 separate halves via dual AAV vectors. We engineered 4 AAV vector pairs using 2 different split sites and 2 split inteins derived from *Nostoc punctiforme* and *Rhodothermus marinus* (Rma). In each pair, the first vector carries the N-terminal half of *SMCHD1* fused to the N-intein, and the second vector contains the C-intein in frame with the C-terminal half of SMCHD1. All 4 split intein pairs successfully reconstituted the full-length SMCHD1 protein, which displayed correct nuclear localization and superior stability compared to individual protein fragments. *In vivo* validation via intramuscular injection in wild-type mice demonstrated safe and efficient SMCHD1 reconstitution using the SMCHD1-Rma vector pair at a dose of 3.0E + 9vg. Ongoing studies are assessing the efficacy of reconstituted SMCHD1 in silencing *DUX4* in FSHD myoblasts and D4Z4-2.5 mice. Our findings demonstrate that the split intein system is a highly effective strategy for the AAV-mediated delivery of large therapeutic genes.

P2.31 Developing CRISPR/Cas13-ADAR-mediated RNA editing therapy for FSHD by premature termination of toxic *DUX4*

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Suppressing *DUX4* expression represents a promising therapeutic strategy for FSHD. Here, we describe an approach to silence *DUX4* RNA using CRISPR/Cas13-ADAR-mediated RNA Editing for Specific C-to-U Exchange (RESCUE). With this approach, translation of full-length toxic *DUX4* can be prematurely terminated by converting arginine (CGA) and glutamine (CAA and CAG) codons into stop codons. To identify optimal hotspots for *DUX4* mRNA editing, we generated a library of 360 gRNAs targeting 6 arginine and 34 glutamine codons within the *DUX4* RNA transcript for unbiased screening. Quantification of C-to-U conversion revealed that arginine codons at positions 18 and 145, as well as glutamine codons at positions 30 and 68, are promising candidate sites, with the arginine codon at position 145 exhibiting the highest editing efficiency. To validate the candidates, we established a HiBiT-tagged DUX4 dual-luciferase system in which luciferase activity is detectable from the full-length HiBiT-tagged DUX4 protein but not from truncated variants lacking the HiBiT tag. The luciferase assays demonstrated that gRNAs targeting the arginine codon at position 145 significantly suppressed *DUX4* luciferase activity. Collectively, our study establishes a novel dCas13-ADAR-based RNA-editing strategy to suppress toxic *DUX4* translation *in vitro*. While these results are promising, further optimization is required to enhance the efficacy of this approach.

P2.32 Assessing the multidimensional burden of facioscapulohumeral muscular dystrophy through patient-reported outcomes and experience

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Facioscapulohumeral muscular dystrophy (FSHD) is a rare autosomal dominant disorder that impairs quality of life and remains underexplored in China. This study examined its impact on Chinese patients. We used a convergent parallel mixed-methods design, including an online survey (March to October 2024) and semi-structured interviews. The survey covered demographics, diagnosis, disease progression, treatment, quality of life, mental health, and unmet needs. Thirty-three patients with clinically and genetically confirmed FSHD were recruited nationwide through the FSHD Community Network. Interview data were transcribed and thematically analyzed. Patients reported substantial physical, psychological, and economic burdens. The mean diagnostic delay was 9.8 years, and 57% had been misdiagnosed. Over 70% reported moderate to severe impacts on daily life. Moderate to severe anxiety and depression were reported by nearly half and 69.6% of participants, respectively. The mean EQ-5D-5L utility index was 0.542, and the median visual analog scale score was 50. Key unmet needs included targeted therapies, assistive devices, psychological support, employment, and a more supportive social environment. Chinese patients with FSHD face a high disease burden and major unmet needs. Earlier diagnosis, targeted therapies, and comprehensive support are needed to improve outcomes and quality of life.

P2.33 Harmonizing clinical monitoring in FSHD: The ENMC 295th Workshop

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Routine clinical monitoring in FSHD lacks international standardisation. With disease-modifying therapies approaching approval, harmonised monitoring frameworks are urgently needed. The 295th European Neuromuscular Centre (ENMC) International Workshop (1 to 3 May 2026) convened 25 experts from Europe, the UK, the US, and Oceania, with representatives from the FSHD Clinical Trial Research Network, the European Trial Network, the PaLaDIn Consortium, ERN EURO-NMD, TREAT-NMD, and patient organisations. A pre-workshop survey confirmed substantial heterogeneity across centres, with limited standardisation of outcome measures and inconsistent collection of patient-reported outcomes (PROs) and patient-reported experience measures. Five structured sessions addressed: Current monitoring practices, clinical outcome measure harmonisation and digital tools, patient stratification, unmet clinical needs, and alignment with patient organisations and registries. The principal output is a tiered monitoring framework providing a scalable structure adaptable to diverse clinical settings. Pain, fatigue, and PROs were recognised as systematically under-assessed and prioritised for action. Dedicated working groups agreed to standardise pain assessment and to refine the clinical definition of disease onset in FSHD. Final consensus on individual measures will be formalised in a follow-up meeting and published in the workshop report. Updated results will be presented at the meeting.

P2.34 Humanistic burden of facioscapulohumeral muscular dystrophy: Evidence from a systematic literature review

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Facioscapulohumeral muscular dystrophy (FSHD) is a rare, progressive neuromuscular disorder associated with muscle weakness, functional impairment, and reduced quality of life (QoL). While clinical features are increasingly described, its broader humanistic burden is less well characterized. This review synthesizes evidence on health-related quality of life (HRQoL) symptoms and patient-reported outcomes (PROs). MEDLINE, Embase, and Cochrane databases were searched (inception August 2025), along with relevant conference proceedings. Clinical trials, and observational and qualitative studies evaluating humanistic burden were included. Outcomes were synthesized descriptively by domain. Eighty-nine studies assessed humanistic burden using >90 instruments. General HRQoL was most assessed (n = 37), followed by fatigue (n = 26), pain (n = 20), disease-specific QoL (n = 18), and disability/function (n = 9). Physical outcomes were more impaired than mental components. Fatigue and pain were highly prevalent and interfered with daily activities. Disease-specific measures highlighted impacts of muscle weakness, reduced independence, and activity limitations. Qualitative studies underscored psychosocial impacts, including facial weakness challenges, social interactions, disease uncertainty, and concern of loss of independence. FSHD imposes a substantial, multidimensional humanistic burden. Marked heterogeneity of PRO instruments highlights the need for standardized, clinically meaningful measures.

P2.35 Project Mercury: Three-year progress report on global FSHD trial readiness and patient access

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Despite a promising FSHD therapeutic pipeline, critical systemic barriers threaten to delay or prevent new treatments from reaching patients. Clinical trials in rare diseases often fail, and approved therapies face payor delays and coverage gaps. No single organization has the global reach to address these challenges alone. Project Mercury is a patient-led global initiative designed to ensure that safe and effective FSHD treatments reach patients quickly, equitably, and at scale. Between 2023 and 2025, Project Mercury established FSHD as a leading global ecosystem for trial readiness. More than 10,000 patients are enrolled in patient registries; registries were launched or expanded in 6 countries; qualified clinical trial sites nearly doubled; and a site onboarding toolkit was published. In 2025, a new strategic aim was introduced to prepare healthcare systems and professionals for the arrival of the first FSHD therapies. In patient access, Project Mercury is working to shift the paradigm from sponsor-led, product-level evidence to advocacy-led, disease-level readiness by leading the development of an Health Technology Assessment disease progression model. The Sustainability workstream secured biopharma partnerships and a significant EU Innovative Health Initiative public-private grant. With the first FSHD therapies entering Phase 3 trials, Project Mercury offers a scalable, end-to-end blueprint for rare disease readiness applicable beyond FSHD.

P2.36 Natural history and Outcome measures in facioscapulohumeral muscular dystrophy in India

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common inherited muscle disorders, characterised by progressive asymmetric muscle weakness and functional disability. Most FSHD natural history and biomarker studies have been conducted in Western populations, with very limited data available for South Asian populations. The primary objective is to evaluate longitudinal disease progression in genetically confirmed Indian patients with FSHD using clinical, functional and imaging outcome measures.

The FSHD-India Natural History study is a prospective 10-year longitudinal cohort study enrolling 150 genetically confirmed Indian patients with FSHD, with annual follow-ups. Clinical assessments include MRC grading, quantitative dynamometry, and pinch grip strength testing. Upper limb function is assessed using PUL 2.0, Jebsen-Taylor Hand Function Test, and 9-Hole Peg Test. Global motor performance is evaluated using MFM, NSAD, and FSHD Composite Outcome Measure. Functional mobility assessments include the 6-MWT, TUG Test, Stair Climb Test, and Sit-to-Stand Test. Disease severity is measured using Ricci Score and FSHD Clinical Severity Score, along with Facial Function Scale. Patient-reported outcomes include FSHD-HI, FSHD-RODS, Brief Pain Inventory, nutritional evaluation, SF-36, and PSQI. Respiratory function is assessed using spirometry. Muscle involvement is quantified using standardised muscle MRI protocols. We will generate the first comprehensive natural history dataset for FSHD in the Indian population and define clinically meaningful progression rates to enhance trial readiness.

Session 3: Mechanisms of Disease and Genetics

S3.01 Senescence-driven pathogenesis in FSHD: Therapeutic benefit of senolytic intervention

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by aberrant expression of *DUX4*; however, the mechanisms by which its low and sporadic activity drives progressive muscle degeneration remain unclear. We hypothesized that FSHD-affected muscle harbors senescent cells that secrete a senescence-associated secretory phenotype (SASP), thereby contributing to disease progression. Consistent with this, we found that senescence and SASP programs are significantly enriched in FSHD muscle and strongly correlate with *DUX4* target gene expression and disease severity. Notably, these signatures are also detectable in clinically unaffected, short tau-inversion recovery (STIR)-negative muscle, indicating the presence of an early prodystrophic state. In an inducible DUX4pA (iDUX4) mouse model, *DUX4* expression induces rapid accumulation of senescent cells, with fibroadipogenic progenitors and macrophages emerging as key sources of inflammatory and profibrotic SASP factors. Importantly, pharmacological clearance of senescent cells attenuates inflammation and fibrosis and enhances muscle regenerative capacity. Together, these findings identify cellular senescence as a central mediator linking *DUX4* activity to progressive muscle degeneration in FSHD and support senescence-targeting strategies as a promising therapeutic approach to modify disease progression.

S3.02 Epigenetic dysregulation in FSHD by modeling epigenetic dynamics during early embryonic development

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FSHD exhibits epigenetic disruption such as DNA hypomethylation and heterochromatin loss in the D4Z4 repeat in muscle cells. However, the molecular mechanism remains poorly understood. In general, DNA methylation undergoes dynamic changes during early embryonic development. Pre-implantation embryos exhibit global DNA hypomethylation. Post-implantation embryos exhibit global DNA hypermethylation. Human iPSCs can model this transition where conventional “primed” iPSCs resembling the epiblast stage can convert to “naïve” iPSCs resembling the blastocyst stage with global DNA hypomethylation, and *vice versa*. We established naïve iPSCs from primed iPSCs derived from healthy controls, FSHD2 patients carrying *SMCHD1* mutations, and their gene-repaired isogenic controls. All naïve iPSCs showed DNA hypomethylation and heterochromatin loss in the D4Z4 repeat, which were restored in healthy and isogenic control cells during repriming (naïve-to-primed conversion), but not in FSHD2 cells. D4Z4 region-specific targeted proteomics during repriming revealed binding of nucleolus-associated proteins was reduced in FSHD2 cells. CRISPR imaging demonstrated decreased nucleolus-D4Z4 contact in FSHD2 cells. Knockdown of the nucleolar protein NPM1 delayed DNA methylation during repriming, while H3K9me3 signals were not affected. These results indicate that nucleolar contact of the D4Z4 repeat may be a regulatory layer in the establishment of repressive epigenetic states in D4Z4.

S3.03 LEUTX binds to *DUX4* target gene promoters and interferes with myotube differentiation

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In FSHD, disruption of D4Z4 heterochromatin leads to abnormal reactivation of an embryonic *DUX4* transcription factor gene embedded in the repeat and its target genes, which is considered the root cause of FSHD. While *DUX4* gene network activation correlates with clinical severity, how those target genes contribute to FSHD pathologies is poorly understood. Our previous study suggested that *LEUTX* and *ZSCAN4* represent distinct *DUX4* target subpathways, possibly differentially contributing to the disease. Using depletion and overexpression of *LEUTX* and *ZSCAN4* in human skeletal myocytes *in vitro*, we found the major role of *LEUTX*, but not *ZSCAN4*, in *DUX4* target gene amplification. Neither of them contributes to *DUX4*-induced cytotoxicity. *LEUTX* binds to multiple *DUX4* target genes only in the FSHD background. *LEUTX* specifically interferes with upregulation of muscle genes and downregulation of cell cycle/mitotic genes resulting in impaired myotube differentiation. When overexpressed in myotubes, *LEUTX* induces aberrant reactivation of DNA replication. A subset of misregulated genes is bound by *LEUTX*, strongly suggesting that they are directly controlled by *LEUTX*. Taken together, our results indicate the unique role of *LEUTX* in *DUX4* signal enhancement as well as interference of proper myotube differentiation, highlighting the differential contributions of *DUX4* target gene pathways in FSHD pathogenesis.

S3.04 Investigating roles of *STEAP3* in iron homeostasis in facioscapulohumeral muscular dystrophy myoblasts

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We previously reported increased methylation at 2 m6A sites of mRNA of six-transmembrane epithelial antigen of the prostate 3 (*STEAP3*) in FSHD myoblasts compared to their unaffected siblings (UASb). RNA methylation is a post-transcriptional modification affecting RNA stability, splicing, transport, and translation. Although expression of *STEAP3* mRNA did not differ between FSHD and UASb myoblasts, STEAP3 protein was significantly higher in FSHD (1.3-fold, $p < 0.05$). *STEAP3* plays an important role in regulating intracellular labile iron level. In this study, we examined how *STEAP3* affected ferrous iron (Fe^{2+}) levels in FSHD and UASb myoblasts (4 sibling pairs). Our data showed that upon silencing *STEAP3* with siRNAs, protein levels of STEAP3 were reduced (-1.5-fold, $p < 0.05$). In addition, ferrous iron levels were significantly decreased by 26% to 70% depending on the sibling pair ($p < 0.05$). *STEAP3* overexpression increased ferrous iron in UASb by 46% to 362% depending on the sibling pairs ($p < 0.05$). To determine whether *DUX4* expression affected STEAP3 levels in FSHD myoblasts, we reduced *DUX4* in FSHD myoblasts using antisense oligonucleotides targeting *DUX4*. Our results showed that reducing *DUX4* led to STEAP3 protein reduction (-1.5-fold, $p < 0.05$). The results suggest that *DUX4* expression may contribute to elevated ferrous iron (Fe^{2+}) via increasing STEAP3 protein.

S3.05 Preliminary evidence for the beneficial effects of estrogens in a mouse model of FSHD

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Epidemiological studies indicate females with FSHD are less affected than males. We recently reported evidence for sexual dimorphism in the severity of muscle pathology in the FLExDUX4 murine model of FSHD, with females exhibiting reduced muscle fibrosis, greater running velocities, and greater weight-normalized grip strength than their male littermates. We hypothesize these differences may be caused by higher circulating levels of estrogens, which have beneficial effects on muscle protein synthesis, inflammation, mitochondrial bioenergetics, and sarcolemmal stability. Therefore, we aimed to determine the effect of acute (oral administration of 1.12 ug 17b-estradiol, E2, daily for 10 days) and chronically (implantation of continuous-release 17β-estradiol pellets for 5 weeks) elevating estrogen in uninduced double transgenic (DT) male ACTA1-MCM/FLExDUX4 mice and ACTA1-MCM controls. Acute elevation of 17b-estradiol significantly reduced expression of *DUX4* and its downstream targets *Wdfc3* and *Trim36* (all $p < 0.05$), improved plasma membrane repair capacity ($p < 0.05$), mitochondrial respiration ($p < 0.05$), and normalized mitochondrial levels to ACTA1-MCM levels. Likewise, chronic 17β-estradiol treatment improved grip strength, body weight, muscle weight, fiber size (all $p < 0.05$), and restored DT mtDNA content to ACTA1-MCM levels. These results provide initial evidence that E2 may be beneficial for *DUX4*-affected skeletal muscle.

P3.01 Mitochondrial respiratory chain function is crucial for muscle toxicity in facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is driven by *DUX4*-induced toxicity, yet the pathomechanisms remain unclear. Here, we identify persistent transcriptional suppression of the mitochondrial respiratory chain and metabolic rewiring in FSHD muscle biopsies and myotubes. Using *DUX4*-inducible human myogenic cells, we show that minute amounts of *DUX4* are sufficient to drive widespread metabolic changes, and that *DUX4* target gene activation is accompanied by mitochondrial function impairment and Caspase 9-mediated apoptosis. Reverse electron transfer (RET) at complex II is the dominant oxidative stress-generating mechanism in FSHD muscle cells, and RET-driven mitochondrial reactive oxygen species (RET-mitoROS) are the trigger for oxidative stress and apoptosis, which is a unique feature of FSHD mitochondria. Pharmacological inhibition of RET suppresses mitoROS, reduces Caspase 9 activation, and rescues abnormal myogenesis in FSHD cell lines. Importantly, *DUX4* is non-toxic in oxidative phosphorylation-deficient human myogenic cells. Our findings identify the mitochondrial respiratory chain as a key mediator of *DUX4* toxicity and highlight RET inhibition as a potential therapeutic approach for FSHD.

P3.02 Fibro-adipogenic progenitors and FSHD

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Muscle affected by FSHD exhibits an accumulation of fibrous and adipose tissue, generated by the differentiation of muscle-resident fibro-adipogenic progenitors (FAPs). A better understanding of the mechanisms underlying FAP differentiation could contribute to the design of new therapies for FSHD. Exosomes are extracellular vesicles that act as messengers in intercellular communication. We hypothesized that *DUX4* expression alters the release by myofibers of exosome-mediated signals that regulate FAP fate. We utilized FLExDUX4; ACTA-Cre; hCD63-GFP transgenic mice, which, following treatment with tamoxifen (TMX), express *DUX4* and exosomes labeled with green fluorescent protein (GFP) within myofibers. *DUX4* expression induced muscle regeneration and an increase in the number of FAPs. Through transcriptomic analysis, we observed a potentially intensified inflammatory response and reduced fatty acid metabolism in the muscle of mice expressing *DUX4*. Conversely, *DUX4* expression resulted in an enrichment of gene expression related to fatty acid metabolism and adipogenesis within FAPs – a finding supported by proteomic analysis. Finally, we purified exosomes released *in vitro* from the quadriceps muscles of TMX-treated FLExDUX4; ACTA-Cre; hCD63-GFP and ACTA-Cre; hCD63-GFP mice. We isolated GFP+ exosomes via flow cytometry to perform small RNA-seq analysis; these data are currently being analyzed.

P3.03 Fibro-adipogenic progenitors enhance human 3D tissue-engineered skeletal muscles: A platform for FSHD research

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Skeletal muscle disease models often lack essential supportive cell types, limiting insight into muscle pathophysiology. We developed a co-culture 3-dimensional tissue-engineered skeletal muscle (3D-TESM) model by combining myogenic progenitors (MPs) with immortalized fibro-adipogenic progenitors (iFAPs). FAPs have roles in myogenesis, tissue remodeling, and extracellular matrix (ECM) formation. Our co-culture 3D-TESMs successfully recapitulated these processes under controlled conditions, resulting in markedly improved contractile force generation, enhanced tissue integrity and longevity, and increased ECM deposition compared with MP-only constructs. We further leveraged this model by inducing fibrogenic and adipogenic remodeling within the 3D-TESMs, pathological hallmarks of muscular dystrophy. Exposure to pro-fibrotic and pro-adipogenic cues resulted in excessive ECM accumulation and fatty infiltration – phenotypes that could not be achieved in 3D muscle models composed solely of myogenic cells. We are currently translating this 3D-TESM platform to the FSHD context using FSHD MPs and iFAPs to dissect the contributions of individual muscle-resident cell types to pathology. This study establishes an advanced human skeletal muscle model with enhanced functional and structural fidelity that captures key pathophysiological processes. It represents a valuable tool for human disease modeling and supports the development of therapies targeting muscle regeneration, fibrosis, and fatty replacement.

P3.04 Context-dependent regulatory functions of *DUX4* G-quadruplexes in FSHD

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Aberrant expression of *DUX4* in skeletal muscle cells is the primary known genetic cause of facioscapulohumeral muscular dystrophy (FSHD). A previous study suggested that guanine-rich regions within the *DUX4* transcript can fold into 4-stranded secondary structures known as G-quadruplexes (G4s), and that stabilization of these structures with a generic G4-binding small molecule can downregulate *DUX4* expression. However, the existence of these structures in cells, the molecular basis of small-molecule targeting, and the functional roles of G4s in *DUX4* regulation have remained unclear. Here, we show that multiple G-rich regions within full-length *DUX4* mRNA (*DUX4*-fl) can indeed fold into G4 structures, and that *DUX4* 3'-untranslated region *G4 formation is critical for DUX4 transcript stability*. Our results further indicate that G4s within the *DUX4* transcript exert distinct regulatory functions through interactions with specific RNA-binding proteins. In addition, using an engineered reporter system, we demonstrate that a *G4 formed within the* DUX4 *promoter enhances its transcription*. Importantly, an *oligonucleotide-based strategy to destabilize the promoter G4 reduces transcriptional output*, highlighting a novel therapeutic avenue for FSHD.

P3.05 Nucleosome-bound *DUX4* activates the FSHD transcriptional program

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DUX4, a double-homeodomain pioneer transcription factor (TF), drives zygotic genome activation in early embryos and causes facioscapulohumeral muscular dystrophy (FSHD) when aberrantly expressed in muscle. Although *DUX4* activates a stereotyped transcriptional program at heterochromatic targets, the chromatin mechanism underlying its pioneer activity remains unclear. Standard assays resolve TF occupancy or nucleosome position independently, leaving TF-nucleosome co-engagement states invisible. To directly visualize these states, we developed a fragment length-resolved cleavage under targets and release using nuclease (CUT&RUN) framework that combines V-plot structural analysis with customized peak calling. Applied to *DUX4*, this reveals that TF binding and nucleosome occupancy co-occur at the same genomic positions: *DUX4* engages nucleosomal DNA at thousands of sites without prior nucleosome displacement. These nucleosome-engaged states are transcriptionally productive, as confirmed by nascent RNA profiling. A spectrum of binding modes is observed: From canonical pioneer activity, where *DUX4* evicts the nucleosome at the binding site and establishes positioned nucleosomes adjacent to the binding sites, to nucleosome-engaged binding, where the nucleosome is retained yet target genes are activated. Both modes operate at FSHD-relevant *DUX4* target loci. These findings suggest nucleosome engagement may be sufficient for pioneer-dependent activation by *DUX4*.

P3.06 *DUX4* induces genes capable of forming viral-like particles

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by misexpression of the transcription factor *DUX4* in skeletal muscle. *DUX4* induces the expression of an early embryonic transcriptional program that includes many endogenous retroviruses and their derivatives, including the domesticated endogenous retroviral genes *PEG10* and *ARC*. *PEG10* and *ARC* retain capsid-forming gag retroviral domains and have been shown to produce viral-like particles (VLPs) that encapsulate their own RNAs. Here, we characterize *DUX4*-induced *PEG10* and *ARC* expression in human muscle cells and assess their association with the release of VLPs. Both *PEG10* and *ARC* are robustly transcribed and translated in *DUX4*-expressing muscle cells. Consistent with their expression described in other cell types, PEG10 and ARC proteins and RNAs are associated with VLP-sized particles in tissue culture supernatant, as determined by ultra-centrifugation and size-exclusion fractionation. Ongoing studies are further characterizing these particles and determining whether they might be transferred between muscle cells as a potential mechanism of disease spread in FSHD.

P3.07 *DUX4*, PARP1 and FUS: A functional triad? Toward new therapeutic strategies

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Our proteomic analyses of the *DUX4* interactome in skeletal muscle cells identified DNA Damage Response (DDR) proteins as major interactors, in agreement with our previous findings in HEK293 cells (Ansseau et al., 2016 and Slavin et al., 2023). Notably, Poly (ADP-Ribose) polymerase 1 (PARP1) was consistently identified as a prominent N-terminal interactor of *DUX4* in both cellular models. Given PARP1's central role in DNA repair pathways, and the reported *DUX4*-induced accumulation of DNA damages in skeletal muscle (Dimitriev et al., 2016), we investigated whether this interaction impacts PARP1 signaling. Using LHCN-M2-i*DUX4* myoblasts, we observed that: 1) *DUX4* expression increases PARP1 catalytic activity despite almost no change in its protein abundance, and a significant time-dependent decrease of its mRNA levels; 2) *DUX4* is in close proximity with PARP1 and the Fused in Sarcoma (FUS) protein. The latter is reported to be one of the first proteins recruited to the auto-modified PARP1 protein. We also confirmed *DUX4*-dependent FUS aggregation as shown in Homma et al. (2016). Our data therefore suggest that the *DUX4*-induced PARP1 activity could be responsible for FUS aggregation. These findings identify a novel mechanism of action for *DUX4* that modulates the PARP1-FUS signaling axis via protein-protein interaction. Interventions in this pathway may open the way for additional therapeutic strategies of the FSHD gain-of-function disease.

P3.08 Human umbilical vein endothelial cells express the DUX4 protein: A basis for further vascular research

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A growing body of evidence suggests a correlation between endothelial cell dysfunction and cancer, as well as facioscapulohumeral dystrophy, both of which are *DUX4*-related diseases. However, the endogenous expression of *DUX4* within endothelial cells (ECs) remains unexplored. This study aims to investigate *DUX4* expression in ECs and examine the presence of DUX4-counteracting proteins named PAX3 and PAX7. This is a cell study in which human umbilical vein endothelial cells (HUVECs) were selected as an *in vitro* representative of the EC population. The presence of the DUX4, PAX3, and PAX7 proteins in the HUVECs was examined using immunofluorescence staining. The mRNA levels of these proteins were investigated using qPCR with specific primers for each transcript. It was observed that 51% of HUVECs expressed the DUX4 protein, whereas only a small number of cells were stained with PAX3/PAX7 antibody. At the mRNA level, HUVECs exhibited positive expression of DUX4, PAX3, and PAX7. The mRNA levels of PAX3 and DUX4 were lower compared to PAX7 mRNA. The high rate of DUX4 protein expression observed in HUVECs is the first positive data and suggests a potential role for DUX4 protein in endothelial cells. Further analyses including the functional analyses of DUX4, PAX3, and PAX7 in ECs could improve our understanding of vascular pathogenesis in *DUX4*-related diseases, particularly in the contexts of cancer and facioscapulohumeral dystrophy.

P3.09 Resistance exercise-induced anabolic signaling is preserved in FSHD

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The progressive loss of muscle mass and strength is a major driver of impaired ambulation in individuals with facioscapulohumeral muscular dystrophy (FSHD). Accordingly, there is an urgent need for therapies that can resolve these pathological features. High-intensity resistance exercise (HIRE) is a potent anabolic stimulus that can produce robust increases in muscle mass and strength. However, the effectiveness of HIRE for individuals with FSHD has been questioned, and there are longstanding concerns that it could accelerate disease progression. Thus, to address this, we combined the FLExDUX4 mouse model of FSHD and a mouse model of HIRE known as weight pulling (WP). Specifically, FLExDUX4 mice were treated with vehicle or tamoxifen to induce mild and moderate forms of FSHD, respectively. Six weeks later, the FLExDUX4 mice and littermate controls performed a bout of WP or a mock training bout as a control. Muscles were collected 3 hours later, and Western blot analyses revealed that WP led to a robust activation of protein synthesis and related anabolic signaling events. Importantly, mild and moderate forms of FSHD did not alter the magnitude of these responses. These initial findings suggest that FSHD does not impair the acute activation of anabolic events that occur in response to HIRE. Given these results, our future studies will focus on whether these acute responses translate into equivalent improvements in muscle mass and strength with chronic HIRE.

P3.10 Acute responses to an exercise-challenge test in individuals with FSHD

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FSHD is a neuromuscular disease affecting different muscles across the body. It is unclear how muscle morphology (assessed via ultrasound), specifically muscle thickness (MT) and echo intensity (EI), varies across the muscle's length in FSHD. Additionally, the effects of a single bout of resistance exercise on muscle function and morphology in FSHD are unknown. Our aim was to 1) evaluate MT and EI across the length of affected muscles, and 2) evaluate acute responses to an exercise-challenge test in individuals with FSHD. FSHD patients with clinical diagnosis had their physical function (Timed Up and Go test; short physical performance battery; stair climb, and upper and lower limb maximal voluntary isometric torque – MVIT) evaluated. Ultrasound measurements across the length of the muscle, from the biceps brachii (BB), vastus lateralis (VL), rectus femoris (RF), and vastus intermedius (VI) were performed. MT was greater at 30% versus 70% of the muscle's length for VL, RF, and VI (P <0.05). Muscle quality (MQ) was also greater at 30% versus 70% of the muscle's length for VL and RF (P <0.05). BB MT and MQ were greater at 50% versus 70% of the muscle's length. Elbow flexion MVIT decreased from pre- versus post-exercise (P <0.05), while knee extension MVIT was unchanged. Our preliminary results show significant intra-muscle heterogeneity and an acute decrease in muscle function in patients with FSHD. We will further evaluate changes in blood-related biomarkers in response to the exercise-challenge test.

P3.11 Genome-wide analysis of mRNA expression and modification profiles in FSHD cell lines

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Myogenesis is orchestrated by precise pre- and post-transcriptional regulation. Dysregulation of these mechanisms drives RNA metabolic defects that contribute to many muscle diseases, causing structural instability, metabolic dysfunction, and loss of muscle mass and function. RNA modifications are widespread regulators of gene expression, influencing alternative splicing, polyadenylation, RNA stability, and other co- and post-transcriptional processes that shape protein levels and phenotype. In skeletal muscle, reversible modifications such as m6A and m5C, along with irreversible changes, including A-to-I editing, pseudouridylation, and 2′-O-methylation, regulate myogenesis, homeostasis, and regeneration. We used ONT bulk long-read direct RNA sequencing to characterize the epitranscriptomic landscape of control and engineered facioscapulohumeral dystrophy (FSHD) human skeletal myotubes. Preliminary analysis of FSHD targets identified more than 20 genes with disease-specific differential modification patterns at individual sites, with *ZSCAN4* and *H3Y1* exhibiting markedly distinct modification profiles between mutant and control samples. Ongoing analyses will investigate transcriptome-wide modification differences, including non-FSHD disease-specific profiles, and will leverage genomic DNA isolated from these conditions for Fiber-seq to generate a more comprehensive view of overlapping epigenomic and epitranscriptomic changes between control and mutant states.

P3.12 Adaptive response to electrical pulse stimulation is impaired in FSHD myotubes by *DUX4* gene network activation

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FSHD is linked to abnormal expression of the embryonic *DUX4* transcription factor in skeletal muscle, but at very low frequency. How this relates to the disease process and the exact nature of functional defects of FSHD muscle cells remain obscure. We used electrical pulse stimulation (EPS) substituting motor neuron activation to perform quantitative structural, functional, and gene expression analyses of *in vitro* differentiated control and FSHD skeletal myocytes. We found that EPS effectively stimulated muscle contractile gene expression and contractility activation in control cells. However, these adaptive responses are impaired in FSHD patient cells, accompanied by exacerbated organizational differences of sarcomeric striation. Importantly, this FSHD patient cell phenotype was confirmed in engineered mutant cells carrying *D4Z4* repeat contraction and *SMCHD1* mutation. Notably, FSHD patient/mutant cells fail to activate musclin/OSTN, an exercise-responsive muscle-protective myokine. Overexpression of LEUTX, a major downstream *DUX4* target, was sufficient to recapitulate this phenotype, indicating that the phenotype is linked to *DUX4* gene network activation. The results demonstrate *DUX4*-induced cell intrinsic functional defects of FSHD muscle cells in adaptive response to electrical stimulation, suggesting the possible activity-stimulated pathological mechanism of FSHD.

P3.13 Exploring molecular mechanisms of FSHD pathology by weighted gene co-expression network analysis

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Facioscapulohumeral muscular dystrophy (FSHD) is a complex genetic disorder driven by *DUX4*; however, the downstream transcriptional programs underlying the pathology remain incompletely understood. In this study, we performed weighted gene co-expression network analysis on RNA-seq data from MRI-guided FSHD patient muscle biopsies (GSE115650) to identify disease-associated modules and regulatory hubs. Reconstruction of the co-expression network from expression data resulted in distinct gene clusters (modules). The module-trait association analysis identified the clusters that significantly correlated with FSHD pathology, including 4 positively and 2 negatively associated modules. Additionally, network centrality highlighted key hub genes, including *FKBP10*, *PLOD3*, and *COLGALT1*, alongside previously reported genes, *MMP14* and *COL3A1*. Functional enrichment analysis revealed previously reported extracellular matrix organization, immune/inflammatory pathways, and regulation of actin organization as dominant processes. Overall, this study provides a systems-level framework to study coordinated gene networks in FSHD and highlights candidate regulatory hubs for dissection of disease mechanisms. Future work will focus on refining network architecture through community detection, assessing condition-specific topological rewiring, and performing network-perturbation analysis to prioritize regulatory drivers of disease pathology and find potential therapeutic targets.

P3.14 Human Satellite II noncoding RNA drives protein aggregation, impacting RNA processing pathways in FSHD

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RNA-driven protein aggregation disrupts cellular homeostasis and promotes disease and tumorigenesis. We show that double homeobox 4 (*DUX4*), an early embryonic transcription factor and the causal gene of facioscapulohumeral muscular dystrophy (FSHD), drives accumulation of stable intranuclear RNAs, including human satellite II (HSATII) noncoding RNA, which promotes protein aggregation in muscle cells. Specifically, HSATII noncoding RNA acts as a scaffold to sequester RNA methylation factors. Sequestration of factors is dependent on HSATII single-stranded or double-stranded RNA structure. Aberrant HSATII RNA condensates disrupt RNA processing, notably RNA splicing. The differential splicing of HSATII-ribonucleoprotein (RNP)-mediated transcripts aligns with pathways already known to be perturbed by *DUX4*. These results position HSATII RNA as a potential key mediator of RNA processing regulation. Understanding how HSATII-RNP formation influences RNA processing offers insight into the molecular mechanisms driving FSHD.

P3.15 *KLF18* is a necessary component of the *DUX4*-initiated transcriptional network and a candidate locus for phenotypic diversity

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DUX4 initiates the first wave of zygotic genome activation (ZGA) in the human embryo, and its misexpression in skeletal muscle causes facioscapulohumeral muscular dystrophy (FSHD). However, other factors that help regulate ZGA-like transcription in human development and disease are still not fully understood. We identified Krüppel-like factor 18 (*KLF18*) as a component of a *DUX4* feed-forward network that is necessary for expression of *KLF17* and a subset of *DUX4*-regulated totipotency associated genes. We mapped the genome-wide binding profile of *KLF18* downstream of *DUX4* and showed that its activity is influenced by *DUX4* and chromatin accessibility. We find that in contrast to the rodent ortholog, human *KLF18* has a predicted beta-solenoid structure composed of a variable number of tandem repeats (VNTRs) with multiple different structural variants in the human population. Expression of different *KLF18* variants in combination with *DUX4* showed similar transcriptional activity, whereas assessment of *KLF18* structural variants in individuals with FSHD1 showed inconsistent association with disease severity that requires further study as a modifier locus. Together, these findings establish the polymorphic transcription factor *KLF18* as a critical component of a *DUX4*-initiated feed-forward network and a candidate locus for human diversity.

P3.16 Characterizing FSHD-specific co-expression networks at single-nucleus resolution

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A key challenge in dissecting FSHD pathomechanisms is the sporadic and transient expression of *DUX4*, which escapes detection in bulk RNA-seq, thereby limiting the inference of disease-associated regulatory networks. Recent single-cell/nucleus studies offer an unprecedented opportunity to decipher FSHD pathology at systems level, which remains less explored. To address this gap, we utilized single-nucleus RNA-seq data derived from multinucleated myotubes (EGAS00001007635) to reconstruct networks in FSHD and control nuclei. We performed high-definition weighted gene co-expression network analysis (hdWGCNA) to identify FSHD-relevant modules. The analysis revealed 2 modules enriched with detectable *DUX4* expression. These modules comprised *DUX4* target genes as hubs (*ZSCAN4*, *PRAMEF5*, *MBD3L5*) and less-characterized hub genes – *PRDX1*, *CFL1*, *TPM2*, and *TNNC2*. One module exhibited a transitional expression pattern, being active in both *DUX4*-positive nuclei and a subset of late-myonuclei, with *SIPA1L1* as an uncharacterized hub. Importantly, these novel hubs are predominantly identified in snRNA-seq data, underscoring their potential as key disease regulators. Building on these findings, future work will focus on identifying transcription factors and downstream regulatory networks driving these modules. Furthermore, we aim to characterize these networks through virtual gene knockouts and network perturbation analyses to facilitate the identification of potential therapeutic targets.

P3.17 Reciprocal interplay between *DUX4* and estrogen receptors α and β in human myoblasts

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We have previously demonstrated that *DUX4* can function as a co-repressor of progesterone and glucocorticoid hormone receptors (Quintero et al., 2022), as well as estrogen receptors ERα/β. In this study, we extend our analysis in human myoblasts carrying a doxycycline-inducible *DUX4* transgene to explore crosstalk between *DUX4* and ERα/β. Our results confirm that *DUX4* represses ER transcriptional activity, while ERα/β protein levels, in the presence or absence of β-estradiol, remain unaffected. In addition, *DUX4* and the downstream target *ZSCAN* show no changes in mRNA levels in the presence of ERα/β. Strikingly, however, ERα/β markedly reduce cellular DUX4 protein levels, both in the presence and absence of ligand. The physiological and clinical significance of these findings in FSHD, suggesting a reciprocal interplay between *DUX4* and ERα/β, remains unknown. This is particularly relevant in light of our *in silico* analyses of ERα and ERβ mRNA levels indicating that there are no major differences in ERα/β mRNA levels between *DUX4*(+) and *DUX4*(−) FSHD myoblasts. As an extension of these studies, and to validate our findings in a disease-relevant cellular model, we have generated immortalized control (17U) and FSHD (17A) myoblast lines using lentiviral constructs expressing hTERT and CDK4. Overall, our work highlights a potential endocrine dimension of *DUX4* biology and further supports a role for ERα/β signaling in modulating *DUX4* activity and muscle physiology in FSHD.

P3.18 Emerging non-apoptotic cell death mechanisms in muscle degenerating disorders and in FSHD

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Skeletal muscle mainly consists of multinucleated cells known as muscle fibers (myofibers). Upon injury, muscles are typically infiltrated by inflammatory cells, exacerbating damage through the release of death ligands. *In vivo*, myofiber death exhibits typical necrotic morphology, including in FSHD. However, the activation of the apoptotic machinery has been reported in multiple studies. Since its identification in the 1970s, apoptosis has been opposed to necrosis on 2 main criteria: Cell death morphology (plasma membrane integrity *versus* permeability), leading to opposite immunological consequences on the tissue, and biochemical regulations (intracellular regulations *versus* accidental processes). Last year, a conceptual revolution in the cell death field arose from the discovery of genetically programmed forms of necrosis, such as necroptosis. In some cases, pathways of apoptotic and necrotic programmed forms of cell death can be interconnected, leading the scientific community to redefine terminology based on these advancements. This presentation will summarize recent developments in the field. Non-apoptotic regulated cell deaths (RCDs) are now recognized as key contributors to cell degeneration in a wide range of diseases, including those in neuroscience and cardiovascular disorders. We will discuss necrotic RCDs affecting myofibers in muscular dystrophies, with particular emphasis on FSHD and Duchenne muscular dystrophy. Potential clinical targets for these pathways will also be addressed.

P3.19 A telomeric translocation causing facioscapulohumeral muscular dystrophy in a family with normal *D4Z4* size

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FSHD results from aberrant *DUX4* expression associated with *D4Z4* chromatin relaxation, caused by repeat contraction (FSHD1) or mutations in chromatin repressors such as *SMCHD1* (FSHD2). Standard testing confirms diagnosis in most cases, but a small subset remains genetically unsolved, suggesting mechanisms undetectable by conventional diagnostics. Two affected siblings presented with asymmetric shoulder girdle and distal leg weakness, scapular winging, and facial involvement; muscle MRI showed a characteristic FSHD pattern. However, Southern blotting, methylation analysis, and whole-genome sequencing neuromuscular panels were unremarkable. Optical genome mapping detected a structural rearrangement involving chromosomes 4q and 7 on the 4qA-17 allele, present in affected but absent in the unaffected sibling. Long-read sequencing demonstrated that this translocation results in a ~115 kb insertion within the telomeric region, shortening (TTAGGG) n from ~3 kb to <1 kb, with focal hypomethylation of distal D4Z4 units. Aberrant *DUX4* expression was confirmed in fibroblasts from both affected siblings. We report a novel FSHD mechanism whereby a telomeric translocation causes focal *D4Z4* hypomethylation and aberrant *DUX4* expression, independent of repeat contraction and chromatin modifier mutations, undetectable by gold-standard diagnostics. This underscores the utility of advanced genomic technologies in genetically unsolved cases with high clinical suspicion of FSHD.

P3.20 Generating engineered muscle tissues with myoblasts from FSHD1 patients of similar D4Z4 repeat length to characterize variability in function and *DUX4* gene signature

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by the derepression of the transcription factor *DUX4* in skeletal muscle. Contraction of D4Z4 repeats in FSHD1 patients within the *DUX4* gene leads to incomplete silencing of the locus and bursts of sporadic *DUX4* expression in skeletal muscles. Variable expression of *DUX4* downstream target genes, both across patients and within the same patient over time, and inconsistent loss of muscle function, are hallmarks of FSHD that make both treatment and preclinical modeling difficult. To better understand disease biology, immortalized myoblasts from several FSHD1 patients with a similar number of D4Z4 repeats were used to generate engineered muscle tissues (EMTs). Over several weeks in culture, EMTs were electrically stimulated to assess force production and analyzed for the expression of *DUX4* target genes. Despite each patient having a similar number of D4Z4 repeats, EMTs from each had a unique response over time to changes in force, the *DUX4* gene signature, and how these correlated with each other. These data support how EMT models can be leveraged to gain insights into the complex mechanisms of disease progression and potentially screen therapeutics in a system that accurately represents the variability that will ultimately be faced in the clinic to treat FSHD.

P3.21 Chromatin accessibility and SIX2 binding profiles of *DUX4*-expressing nuclei

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A unique and poorly understood feature of FSHD muscle cells in culture is the burst of *DUX4* transcription during myogenic differentiation in a small fraction of nuclei. Theories to account for this restriction on *DUX4* transcription include random nucleosome positioning to make accessible key *DUX4* regulatory sequences, restricted expression or activation of specific transcriptional regulators that then act directly on *DUX4,* and inefficient enhancer-promoter communication over long genomic distances. To distinguish chromatin accessibility changes associated with *DUX4*, we used ATAC-seq to compare differentiating healthy to FSHD myocytes. We further compared the small fraction of *DUX4*-expressing nuclei to the majority *DUX4* non-expressing nuclei in FSHD myocytes. Our data suggest a general reorganization of chromatin at 4q35 in FSHD versus healthy myocytes with further reorganization in the small fraction of FSHD myocytes that express *DUX4*. Preliminary data also indicate that SIX2, a transcription factor important for *DUX4* expression, binds to a region upstream of the D4Z4 repeats only in nuclei that express *DUX4*. These data are consistent with chromatin conformation and long-range interactions playing a role in the rare transcriptional bursts of *DUX4* in FSHD.