Abstract Book

Session 1: Population Genetics and Modifiers

S1.01 FSHD in sub-Saharan Africans

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Previous studies have suggested differences in susceptibility to FSHD1 among populations, based on typical repeat unit (RU) sizes found in patients with European (1-10 RU) and Asian (1-7 RU) ancestry. So far, only two patients with sub-Saharan African (SSA) backgrounds have been reported, but without genetic confirmation. Underreporting of FSHD in this region may be due to limited access to diagnostic tools and a shortage of neurology specialists. However, it could also indicate that the SSA population is less susceptible to FSHD compared to European and Asian populations. In this study, we provide a detailed clinical evaluation and genetic overview of FSHD in eight unrelated individuals clinically affected with FSHD from Senegal, Côte d'Ivoire, Ethiopia, South Africa, Curaçao, and the USA. Genetic analysis confirmed FSHD1 in seven cases, with FSHD1 alleles ranging between 2 and 6 RU. One case was identified as FSHD2, characterized by pan-D4Z4 hypomethylation, a pathogenic variant in SMCHD1, and an 11 RU 4qA allele. We determined the genetic background of all cases by using a global screening array analysis, confirming SSA ancestry in six cases, while two showed more admixture. This study confirms that FSHD can occur in individuals

with SSA ancestry, although likely at a lower incidence than in those with European and Asian ancestry. Further analysis of patients with different ethnic backgrounds might reveal the genetic and epigenetic modifiers of FSHD severity.

S1.02 Analysis of genetic diversity and phylogenetic relationships of D4Z4 repeats: Implication for health and disease

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Repetitive DNA represents about half of the human genome, but its function has been underestimated due to methodological/technical limitations. We developed an *ad hoc* computational workflow to finely characterize the size, distribution, and evolution of the FSHD-linked D4Z4 repeats. We analyzed the T2T genome assembly, 94 haplotype resolved telomere-to-telomere assemblies from the Human Pangenome Project, and a selection of non-human primates. We identified hundreds of D4Z4 sequences in the human genome, with wide inter- and intra-individual variability, not only at the subtelomeres of chromosomes 4q and 10q, but also at the short arms of acrocentric chromosomes, interspersed with rDNA operons, and at the pericentromeric chromatin of chromosome 1. This heterogeneity has significant implications for FSHD diagnostic tests that assess 4q35 methylation via PCR. The information from T2T shows that 4q/10q specific primers designed based on hg38 can also amplify other D4Z4 sequences elsewhere in the genome, potentially producing misleading results. Based on these findings, a systematic reanalysis and a critical review of previous studies on FSHD pathogenesis and diagnostics considering the information in T2T is needed. Our study offers an integrated approach for the study of repetitive elements and highlights the importance of telomere-to-telomere assemblies offering new insights for the interpretation of the biological role of D4Z4 repetitive elements.

S1.03 Correlation of methylation, severity, and parent-of-origin effects in large, multigenerational kindred with FSHD

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In one of the earliest descriptions of FSHD, Tyler and Stephens described a six-generation kindred (K1462) segregating FSHD, descended from a single individual born in 1775 in Lancashire, England, who emigrated to Utah in the 1850s. Members of K1462 have been followed for decades, with DNA samples collected since the 1980s. As is typical of FSHD, members of K1462 show marked variability in onset, severity, and progression, including siblings with either severe or mild disease. The mechanisms underlying this variability, including potential roles for genetic modifiers, CpG methylation levels, and parent-of-origin effects, remain poorly understood. Since all affected individuals carry the same 5RU D4Z4 contraction and surrounding genomic context, K1462 is an ideal cohort for studying modifiers of FSHD severity. We assessed parent-of-origin effects and the relationship between methylation and severity across generations V-IX using a nanopore Cas9-targeted sequencing (nCATS) assay that resolves sequence, length, and methylation status of the D4Z4 array at base-pair resolution. We demonstrated stability of the founder mutation across nine generations and complete penetrance of the 5RU allele. Variable severity was seen both within and between families, and was correlated with methylation levels, with a moderate parent-of-origin effect.

S1.04 Exploring the boundaries of the diagnostic spectrum of FSHD: Complex genetic findings and their implication for the molecular genetic model of the disease

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Despite the often characteristic clinical phenotypes of FSHD, the molecular genetic diagnosis of FSHD remains a challenge. This is due to the high complexity of the chromosomal locus, which is only accessible to molecular genetic testing to a limited extent, and the not fully understood linkage between phenotype, genetics, and epigenetics. With the help of methylation profile analysis, we identified 18 patients with rare or complex genetic findings, some of which being of risk to receive an incorrect diagnosis. These patients were comprehensively characterized clinically and genetically. Several patients displayed global hypomethylation indicative of FSHD2, despite lacking variants in known epigenetic suppressor genes or carrying borderline (gray zone) alleles with 11 repeat units. In one case, a 7-unit hybrid allele could be excluded as origin of the pronounced clinical phenotype. In a family with overlapping features of FSHD1 and FSHD2 across different population backgrounds, methylation profiling allowed accurate determination of carrier status. These results highlight methylation analysis as a precise and reliable tool for the molecular genetic diagnosis of FSHD. Distal methylation appears to best reflect disease status, shaped by repeat length and suppressor gene variants. However, as the latter two parameters do not fully explain differences in methylation and disease status, other yet unknown genetic modifiers need to exist.

Session 2: Measures of Disease Activity and Progression

S2.01 FSHD1 in Italy: A 20-year follow-up study of the Italian Clinical Group for FSHD (AIM-FSHD)

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Long-term data on motor-respiratory function in facioscapulohumeral muscular dystrophy type 1 (FSHD1) remain limited. The Italian FSHD Myology Group (AIM-FSHD) analyzed 10- to 20-year follow-up data of 219 genetically confirmed FSHD1 patients (mean age at T0: 41.3±16.3y). Reported onset occurred at 27±15y (95% as scapular-facial) with over 30% manifesting <18y. Initial Comprehensive Clinical Evaluation Form (CCEF) categories were A: 73.8%, B: 11.6%, C: 6%, D: 8.6%. Mean age at loss of ambulation was 57.5±19.7y, increasing from 3% at T0 to 23.8% at T20, primarily within category A (73.8%). One-third of patients initially in category C (asymptomatic/mildly symptomatic) transitioned to categories A/B/D over time. Despite low numbers in categories B-D, CCEF FSHD scores differentiated progression in the first decade, notably in category A (+2 pts mean increase, max +7 pts; $p < 0.01$), while B and D remained more relatively stable. Arm abduction declined by ~20° in T0-T10 interval ($p < 0.01$), stabilizing thereafter, alongside distal lower limb Medical Research Council grading decreasing from 4.1±1.1 to 3.1±1.6 in the same time range ($p < 0.01$). Forced vital capacity percentage only mildly declined (~10%) in 20y ($p = 0.78$). Non-invasive ventilation began at mean age 62.3±12.7y, rising from 1.3% at T0 to 19.1% at T20 (83.3% category A). Overall mortality was low (2.2%), mostly unrelated to FSHD. These findings confirm the slowly progressive nature of FSHD1 and underline distinct clinical trajectories across CCEF categories, relevant for patient stratification especially in clinical trials.

S2.02 Integrating MRI, RNAseq, and pathology to predict fat fraction change over 1 year in FSHD

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Prior studies show association between fat, elevated short-tau inversion recovery (STIR+), and RNA sequencing measures of *DUX4*. It remains unclear what features are most predictive of fat progression. To address this question, a cohort of 34 FSHD subjects, 16 female and 18 male, aged 21-69 (mean 47.1±14, standard deviation), was analyzed. Bilateral biopsy of the texture analysis (TA) at baseline generated measures for RNA (*DUX4*, complement, and immunoglobulin G [IgG]) and pathology (total score, fibrosis, central nucleation, among others). MRIs at baseline, 1 year, and 2 years were analyzed to quantify TA fat fraction, fat pattern variables (e.g., kurtosis, skew), and lean muscle STIR+ %. Input into a random forest model along with descriptive data (e.g., D4Z4 length, methylation, age, sex). The goal was to predict change in fat fraction over 1 year. Results demonstrated a highly significant model (root mean squared error [RMSE] of 1.67 [95% CI: 1.36–1.97]) with a calibration correlation of 0.41 ($p < 0.001$) on the testing set. Variable importance analysis showed percent RMSE reductions of 0.11 (95% CI: 0.09–0.13) for kurtosis, 0.08 (95% CI: 0.06–0.09) for IgG, 0.05 (95% CI: 0.04–0.07) for skewness. STIR+, complement, and pathology metrics were all below 0.05, with most *DUX4* measures being below 0.025. Fat pattern metrics and immune features appear most related to progression in FSHD fat fraction over 1 year. Ongoing analyses of previous cohorts with varied muscles biopsied will extend and refine these observations.

S2.03 Multi-scale machine learning model predicts muscle and functional disease progression in FSHD

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This study introduces a multi-scale machine learning framework leveraging whole-body magnetic resonance imaging (MRI) and clinical data to predict regional, muscle, joint, and functional progression in FSHD. The goal of this work is to create a "digital twin" of individual FSHD patients that can be used in clinical trials. Using a dataset of more than 100 patients from 7 studies, the researchers integrated MRI-derived metrics – including fat fraction, lean muscle volume, and fat spatial heterogeneity at baseline – with clinical and functional measures. A 3-stage random forest model was developed to predict annualized changes in muscle composition and a functional outcome (timed up-and-go [TUG]). All model stages revealed strong predictive performance in separate holdout datasets. For example, the models predicted fat fraction change with a root mean square error (RMSE) of 2.16%, lean volume change with an RMSE of 8.1ml, and change in TUG with an RMSE of 0.6s, all in a holdout testing dataset. Feature analysis revealed that fat heterogeneity within muscle was the strongest predictor of muscle-level progression. This study demonstrates the machine learning model's incorporating individual muscle and performance data can effectively predict disease progression and functional performance. Further studies will incorporate the model into clinical trials that include longitudinal cohorts, effects of treatment, as well as comprehensive biological, clinical, and functional measures.

S2.04 Identification of potential plasma extracellular vesicle-associated protein biomarkers for facioscapulohumeral muscular dystrophy (FSHD)

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FSHD is a debilitating muscular dystrophy affecting skeletal muscles and lacking any effective treatment. Recently, multiple targeted therapies are emerging. However, the complexity of the disease, along with the limitations of clinical outcome measures, pose significant challenges for therapy approval. Identifying reliable, circulating molecular biomarkers is crucial to accelerating this process. Circulating extracellular vesicles (EVs) are emerging as promising disease biomarkers. In this study, we analyzed the plasma EV proteome from FSHD1 patients and healthy controls across two independent cohorts (N=11-25) and correlated it with confounding factors (i.e., disease severity, age, and sex). Our findings revealed significant overlaps in potential FSHD1 biomarkers, particularly a metalloprotease and complement pathway proteins. The metalloprotease, which processes certain muscle-enriched dipeptides and GPLD1, linked to glucose metabolism, was downregulated in all or a subset of FSHD1 patients aged 55 and older. Furthermore, we found that FSHD-COM (Composite Outcome Measure) severity scores correlated with the abundance of proteins involved in inhibiting (i.e., C4BPB) or activating the complement pathway, further supporting the involvement of EV-associated complement proteins in FSHD1. The identification of these biomarkers establishes a strong foundation for longitudinal studies aimed at validating their clinical relevance by monitoring their levels with disease progression.

S2.05 Myostatin is a biomarker of disease severity in facioscapulohumeral muscular dystrophy

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FSHD is driven by aberrant expression of the toxic transcription factor *DUX4*. Recent therapeutic developments targeting *DUX4* have progressed to clinical trials. However, a major hurdle in the field remains the absence of blood-based biomarkers, which limits both disease monitoring and the evaluation of therapeutic efficacy. We previously showed that myostatin (MSTN) is lower expressed in neuromuscular patients (Mariot, 2017). The regulation of the MSTN network is disease dependent, the patients affected by the most atrophying disease showing the strongest extinction of the MSTN pathway, suggesting that MSTN could be used as a marker of disease severity. This study aimed to evaluate MSTN as a potential biomarker of FSHD disease severity. We retrospectively measured serum MSTN levels in 81 FSHD patients and >100 healthy controls in 2 independent cohorts. Correlations between MSTN levels and clinical severity scores were evaluated. FSHD patients exhibited MSTN levels approximately twofold lower than in healthy controls. MSTN levels correlated with D4Z4 repeat size and showed strong inverse associations with clinical severity scores, including the Vignos, Ricci, and FSHDeval scores (Pearson r score up to -0.74). MSTN levels identified patients requiring assistance with walking or walking unassisted with up to 90% specificity. These findings support MSTN as a robust and reliable circulating biomarker for FSHD disease severity.

S2.06 Extracellular vesicle RNA profiling reveals candidate biomarkers of disease activity and progression for FSHD patients

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The development and evaluation of therapies for facioscapulohumeral muscular dystrophy (FSHD) are challenged by the lack of circulating biomarkers correlating with disease activity and progression. In FSHD, disease activity is characterized by short-tau inversion recovery (STIR)-positive signal at muscle magnetic resonance imaging (MRI), while disease progression is usually assessed using T1 MRI signal. To address this critical gap, extracellular vesicles (EVs) have emerged as a promising source of biomarkers. Their RNA cargo reflects the physiological state of muscle tissue and remains stable after being released into the bloodstream. In this study, we profiled muscle-derived EVs from FSHD STIR+, FSHD STIR, non-penetrant gene carriers, and healthy control muscles using RNA sequencing. By correlating differential expression analysis with MRI signal, we identified specific muscle EV signatures (microRNAs, isomiRs, and long RNAs) that distinguish STIR+ muscles. These biomarkers were associated with pathways relevant to the pathology and demonstrated the ability to predict T1 progression over a 1-year MRI follow-up, highlighting their potential as longitudinal prognostic biomarkers. Our findings provide the first RNA-based characterization of muscle-derived EVs in FSHD. The potential to explore these EV biomarkers in the bloodstream paves the way for non-invasive liquid biopsies, while complementing MRI for patient stratification and therapeutic monitoring.

Session 3: Novel Clinical Outcome Measures

S3.01 Deep learning-based facial movement analysis for automated detection and severity classification of facioscapulohumeral muscular dystrophy (FSHD)

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Facioscapulohumeral muscular dystrophy (FSHD) is characterized by progressive muscle weakness, prominently affecting facial muscles. Early and accurate diagnosis is crucial for patient management and monitoring disease progression. This study investigates deep learning techniques, specifically employing facial movement analysis, to identify facial features indicative of FSHD. We used a dataset of 113 unique patients collected by Nice University Hospital, comprising 284 long videos of 15 facial exercises recorded with two cameras. We employed a VideoMAE-based model pre-trained on VoxCeleb, leveraging its transformer encoder as a feature extractor. The extracted facial motion descriptors were refined using an UpConv module, composed of a deconvolution layer for temporal resolution enhancement and a multi-layer perceptron for descriptor refinement. A lightweight transformer classified the clips using a multi-task learning approach. Our model achieved 79.1% accuracy in FSHD detection on individual clips and 72% when aggregating predictions across all exercises. Inference on the test set was completed in under a minute. The confusion matrix analysis indicates strong specificity (79.2%) but limited sensitivity (63.2%). This study demonstrates the potential of deep learning for facial features associated with FSHD detection in videos, highlighting the importance of data quantity and quality. These methods pave the way for more precise automated diagnostic tools in FSHD patient monitoring.

S3.02 Reassessing training thresholds in FSHD: The diagnostic value of compensatory movement patterns

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Fatigue is a common and debilitating symptom in individuals with facioscapulohumeral muscular dystrophy (FSHD), limiting daily functioning. While exercise may reduce fatigue and improve fitness, determining appropriate training intensity remains difficult. Ventilatory thresholds (VT1 and VT2) are typically used to guide training but may overestimate optimal intensity in FSHD due to muscle weakness. Fatigue can trigger compensatory mechanisms such as altered muscle activation and movement patterns. Insight into the type and timing of ventilatory thresholds may support individualized training guidance. This study aimed to 1) identify and compare compensatory mechanisms during maximal incremental cycling in healthy individuals and neuromuscular disease (NMD) patients, including FSHD, and 2) assess the timing of ventilatory thresholds in relation to kinematic and surface electromyography EMG (sEMG) change points. Eighteen healthy controls and 17 NMD patients underwent cardiopulmonary exercise testing with sEMG and 3D motion analysis. A sports physician identified ventilatory thresholds. Regression analysis determined normalized power at the first and second change points. Preliminary results showed VT1 and VT2 occurred earlier in patients, while kinematic change points (pelvic rotation, thoracic flexion, ankle dorsiflexion) appeared later. These findings suggest kinematic, and sEMG change points may complement ventilatory thresholds in tailoring training intensity for FSHD.

S3.03 Current abilities score (CAS) is a valid and a reliable patient-reported functional rating scale in FSHD: Data from MOVE natural history study

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Patient-reported rating scales are important to measure the impact of FSHD on function. If such scales are sensitive to change over time, they can be helpful for planning patient care as well as recording patient-reported experiences in both natural history studies and clinical trials. Current abilities score (CAS) is a rating scale used in two large multinational natural history studies in FSHD: ReSOLVE and MOVE. The CAS has six items including functions of face, arms, hands, mobility, and chair and bed transfers, with a maximum score of 25 points indicating severe disability. Here we report the results from the MOVE study. Baseline CAS from 300 FSHD patients were studied. A total of 135 were female with an average age of 47.31yr (range 5-83) and average Functional Capacity Scale (FCS) of 7.32 (standard deviation ± 4.05). CAS positively correlated with FCS ($p=0.79$, $p <0.001$), Mini-Mental Test Score (MMTS) ($p=0.74$, $p <0.001$), negatively with Upper Extremity Functional Index (UEFI) ($p= -0.78$, $p <0.001$). CAS had excellent reliability in both in-person and remote completions (ICC day 1 vs. day 14 ICC = 0.979 ($n = 15$). Change over time data up to 24 months will be presented. We present CAS data from a large FSHD natural history study MOVE and propose that CAS is valid, and a reliable outcome measure that can be used to track functional response in a clinical trial, serve as a bridge between in-person assessments and home reports, allow for tracking the time course of response to interventions, or used in large registries to track clinical change and response to interventions.

S3.04 Gait-based biomarkers in facioscapulohumeral dystrophy (FSHD): Establishing the validity of inertial measurement units (IMU) to augment in clinic assessments in FSHD

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Mobility is a significant issue in FSHD, and is a key outcome measure. Inertial measurement unit (IMU) sensor-enabled clinic-based gait analysis may improve precision in detecting changes over time. IMU digital mobility outcomes have been proposed for several neuromuscular conditions. Objectives – To investigate the ability of IMU measures to: 1) Differentiate between FSHD and a healthy control group. 2) Differentiate between subgroups of FSHD severity. 3) Detect change over time. Twenty-eight participants with FSHD were recruited at baseline, and 18 completed a 12-month visit. All completed an instrumented (with 6 IMU sensors) 10-meter walk task. Data were extracted using a previously validated algorithm (van Gelder et al., 2023). Results were compared with a control group (n = 24). Paired analyses were used to assess change between visits. Mean age was 47.6 (standard deviation (SD) 15.6), with mean Functional Capacity Scale 8.7 (SD 3.5). Baseline spatiotemporal (ST) measures were significantly greater in the FSHD group than in control, and with increased severity ($p < 0.001$). Upper body movements were less smooth in the FSHD group ($p < 0.001$) than in control, and with increased severity ($p < 0.001$). Significant differences were found at 12 months in ST measures ($p=0.04$), and upper body movements ($p = 0.04$). IMU gait assessments were able to differentiate between FSHD and control group, and among subgroups of FSHD severity, with evidence of responsiveness at 12 months. IMU represents a promising tool for assessment in FSHD.

Session 4: Mechanisms of Disease and Interventional Strategies

S4.01 SUMOylation regulates SMCHD1 activity and DUX4 expression in FSHD muscle

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Facioscapulohumeral dystrophy (FSHD) is caused by de-repression of the *DUX4* transcription factor in skeletal muscle. Structural maintenance of chromosomes hinge domain containing 1 (SMCHD1) is a chromatin-bound protein involved in the repression of *DUX4*. Pathogenic variants in SMCHD1 are associated with FSHD type 2 (FSHD2), and increased amounts of SMCHD1 decrease *DUX4* levels in FSHD muscle cells. Therefore, understanding the regulation of SMCHD1 activity might provide therapeutic targets for FSHD. Post-translational modifications of proteins, like SUMOylation (SUMO), modulate protein activity. Several studies have shown that SMCHD1 can be modified by SUMO, but little is known about how this modification regulates SMCHD1 activity. We identified the SUMO acceptor sites in SMCHD1 and the deSUMOylase enzyme, SENP5, that acts on SUMO-modified SMCHD1. Our mass spectrometry analysis shows that SMCHD1 interacts with other D4Z4-relevant chromatin repressors, like TRIM28 and HNRNPK, in a SUMO-dependent manner. Furthermore, we show that modulating global SUMOylation or SENP5 activity impacts *DUX4* expression in FSHD muscle cells. Altogether, our results suggest that modulating SMCHD1 SUMOylation has therapeutic potential in FSHD.

S4.02 Mechanism of SMCHD1 and engineering for FSHD treatment

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SMCHD1 is a non-canonical SMC protein important for X inactivation and the silencing of specific autosomal genes. Loss-of-function mutations in SMCHD1 cause facioscapulohumeral dystrophy type 2 (FSHD2). Although recognized as an epigenetic repressor, its structural characteristics and mechanism of action remain largely elusive. Using atomic force microscopy, we showed that the SMCHD1 homodimer is remarkably flexible and dynamic in solution. Interestingly, our findings demonstrate that SMCHD1 can directly bridge and compact DNA, in the form of naked DNA or nucleosome arrays, independently of other proteins, forming large protein-DNA clusters. Surprisingly, DNA compaction by SMCHD1 does not require ATP. Paradoxically, compaction rate is reduced with the addition of ATP. Collectively, these insights suggest that SMCHD1 is a novel compactor of DNA and chromatin, operating through ATP-independent bridging rather than ATP-dependent loop extrusion. Building on our structural and biophysical understanding of SMCHD1, we performed protein engineering to generate a smaller version that retains its repressive capabilities. We present a mini-SMCHD1 that is small enough to be packaged in an adeno-associated virus. As overexpression of SMCHD1 is effective at reducing *DUX4*-associated expression in both FSHD1 and 2, mini-SMCHD1 holds promise as a potential gene therapy for FSHD patients.

S4.03 Understanding and treating inflammation in FSHD muscular dystrophy

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Muscle inflammation plays a central role in FSHD. However, specific inflammatory pathways remain poorly characterized. This gap of knowledge is critical, as understanding the inflammatory mechanism in FSHD could lead to the development of targeted therapeutic interventions. Recent data suggest that an immune response to FSHD muscle cell antigens might have a role in disease progression. Even so, the nature of these “FSHD antigens” is currently unknown. The main direct targets of *DUX4* are endogenous retroviruses and other repetitive sequences which, through the accumulation of double strand RNA (dsRNA), might lead to the induction of a “viral mimicry” status, culminating with the activation of an interferon (IFN)-mediated inflammatory response. We found that the IFN-γ response pathway is the most significantly upregulated inflammatory pathway in muscles of FSHD patients and the FLExDUX4 mice. We also found that *DUX4* is required and sufficient to activate this inflammatory pathway in FSHD. Notably, IFN-γ treatment is sufficient to activate the same pathway in healthy human and mouse muscles. Importantly, we found that IFN-γ neutralization in vivo significantly reduces muscle inflammation and fibro-fatty substitution, preserving muscle size in FLExDUX4 mice. Collectively, our results indicate that *DUX4* directly activates a pro-inflammatory pathway contributing to muscle wasting in FSHD. Importantly, we found that this pathway can be targeted with clinically approved treatments.

S4.04 Single-cell RNA sequencing identifies ferroptotic stress in FSHD myoblasts

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We previously showed FSHD myoblasts have significant delays in plasma membrane (PM) repair compared to healthy myoblasts. However, the molecular mechanism of this deficit is not clear. Here, using single-cell RNA sequencing, we report that PM injury triggers a significant increase in *DUX4* mRNA expression, which is lowly expressed and associated with activation of novel potential FSHD biomarker genes. Additionally, FSHD myoblasts show elevated levels of cell death at 6 and 24 hours post-injury, as well as enrichment of genes in the ferroptosis signaling pathway by 6 hours post-injury. Follow-up experiments confirmed that FSHD myoblasts display signs of ferroptotic stress, including elevated labile iron, lipid peroxidation, and expression of ferroptotic biomarkers at baseline, and during the recovery from injury. Treating cells with ferric ammonium sulfate revealed FSHD myoblasts have poor tolerance for increased labile iron, which resulted in a disproportionate and significant increase in FSHD lipid peroxidation and failed repair compared to myoblasts from their healthy siblings. Treatment of FSHD myoblasts with the membrane-permeable iron chelator 2'2'-Dipyridyl, and the ferroptosis inhibitor Ferrostatin both improved membrane repair and reduced cell death in FSHD myoblasts. The results suggest excessive lipid peroxidation secondary to increased levels of reactive labile iron and ferroptotic stress may contribute to defects in PM repair and disease mechanisms of FSHD.

S4.05 Decoding the cellular and epigenetic landscape of affected and spared muscles in FSHD patients through single nucleus multi-omic analysis

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The mechanisms driving muscle degeneration in FSHD remain only partly understood. We hypothesize that dysregulated crosstalk among cells in skeletal muscle contributes to disease progression. To clarify what distinguishes muscles in FSHD patients, we analyzed biopsies derived from affected and spared muscles of FSHD patients, following MRI guidance, as well as from non-penetrant gene carriers and healthy controls. We performed single-nucleus assay for transposase-accessible chromatin sequencing (snATAC-seq) on whole muscle tissue to define cellular muscle heterogeneity and identified gene expression signatures linked to pathogenesis. Our single-nucleus multi-omic approach includes qualitative and quantitative assessment of all muscle cell populations, the identification of expression patterns in each subtype, and a bottom-up analysis of dysregulated pathways. Preliminary results revealed at least 12 distinct cellular clusters and muscle-specific variations in cell population composition, suggesting a distinct distribution across samples. Chromatin accessibility analyses uncovered gene-specific expression variability across clusters. Thanks to the integration of public snRNA-seq data, we were able to assign clusters to specific cell types and identify subpopulations potentially involved in degenerative or regenerative processes. This integrative approach reveals key cellular and regulatory differences between affected and spared muscles, providing novel insights into FSHD pathogenesis.

S4.06 DYNE-302 leads to functional improvement and resolves muscle transcriptomic changes in mouse models of FSHD

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FSHD is a severe autosomal dominant disorder characterized by progressive loss of skeletal muscle and debilitating weakness. The muscle pathology is caused by aberrant expression of the *DUX4* transcription factor in myonuclei, which leads to dysregulation of downstream genes collectively known as the *DUX4* transcriptome (D4T). To develop a potential therapy for FSHD, we leveraged the FORCE^{TM} platform to create DYNE-302, a molecule comprising a fragment antigen binding specific for human transferrin receptor 1 (TfR1) conjugated to an siRNA against the human *DUX4* mRNA via a cleavable linker. To determine the potential of DYNE-302 to achieve functional improvement in FSHD, we generated mice expressing human TfR1 and a tamoxifen-inducible human *DUX4* transgene (hTfR1/iFLExD). These mice develop an acute myopathy with impaired muscle function upon *DUX4* induction. At the nadir of locomotor function, hTfR1/iFLExD mice were subjected to a single DYNE-302 injection. DYNE-302 suppressed D4T expression in skeletal muscle and restored ability to run on a treadmill, suggesting that pre-existing, severe skeletal muscle disease can be corrected by targeting *DUX4* mRNA. Accordingly, whole-transcriptome analysis in skeletal muscle revealed dose-dependent changes in gene expression that were indicative of reduced muscle damage and inflammation. Collectively, these data demonstrate that DYNE-302 has high potential to achieve functional improvement in FSHD.

S4.07 SRK-015 improves muscle mass, strength, and endurance in the FLExDUX4.Cre mouse model of FSHD

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Selective inhibition of myostatin activation with SRK-015 (apitegromab) is a promising approach for safely building skeletal muscle and strength in neuromuscular disorders. We previously demonstrated in the SMNΔ7 model of spinal muscular atrophy (SMA) that SRK-015 improves muscle strength when combined with a therapy to stabilize the motor neuron, an approach now validated in multiple clinical trials in SMA. Facioscapulohumeral muscular dystrophy (FSHD) is a neuromuscular disease in which sporadic *DUX4* expression causes a mosaic pattern of muscle atrophy, with the remaining motor units remaining intact. We hypothesized that selective myostatin inhibition with SRK-015 may have the potential to improve muscle function, even in the absence of *DUX4*-suppressing therapies. We here evaluated the effect of SRK-015 as a standalone therapy in both 2-month and 8-month-old FLExDUX4.Cre mice and observed increases in both muscle size and force. We additionally observed increases in treadmill endurance in the older cohort. These results support the potential of SRK-015 to improve muscle function in FSHD, and warrant future studies aimed at a greater understanding of the effects of selective myostatin inhibition on FSHD muscle.

S4.08 Preclinical evaluation of a small molecule inhibitor of WDR5 in facioscapulohumeral muscular dystrophy (FSHD)

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Because of the pivotal role of *DUX4* in FSHD pathology, targeting its expression using small molecules is an attractive solution. We previously demonstrated that WDR5, a chromatin remodeling protein, is required for *DUX4* expression in FSHD. Interestingly, we identified a novel WDR5 inhibitor (OICRX) with high potency and favorable pharmacokinetic properties, making it a strong candidate for preclinical evaluation. We extensively tested OICRX in vitro using primary muscle cells derived from FSHD patients and healthy donors. OICRX effectively suppresses the expression of *DUX4* and its downstream targets. Importantly, muscle cell proliferation or differentiation was unaffected by OICRX treatment, indicating a promising safety profile. To extend our findings in vivo, we took advantage of a humanized mouse model of FSHD. Pharmacokinetic and bio-distribution studies identified the conditions to obtain good OICRX skeletal muscle exposure without obvious signs of toxicity. Intriguingly, OICRX in vivo treatment caused a decrease of *DUX4* expression coupled with significant reduction of *DUX4* target genes without affecting muscle differentiation markers. Notably, this was associated to an improvement in FSHD muscle cell survival in OICRX-treated compared to vehicle-treated mice.

Session 5: Clinical Care and Related Issues

S5.01 Classification, clinical care, outcome measures, and biomarkers in childhood-onset facioscapulohumeral muscular dystrophy (FSHD): An update from the 279th European Neuromuscular Centre (ENMC) Workshop, the Netherlands, November 2024

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FSHD is an autosomal dominant muscular dystrophy with variable age of onset and severity, often affecting children more profoundly than adults. To facilitate clinical trial readiness the 279th European Neuromuscular Centre (ENMC) workshop aimed to standardize classification based on disease severity, address implications for clinical trials and patient access, and improve paediatric clinical management. Key priorities included establishing a working party to address knowledge gaps in management and outcome measures, defining a standardized minimal dataset in both research and clinical environments, and enhancing pharmaceutical engagement. This workshop, brought together 27 participants from 14 countries, including clinicians, researchers, patient advocates, and industry representatives. Because childhood-onset FSHD represents a continuum rather than a discrete subtype, recommending the discontinuation of the term "infantile FSHD" achieved consensus. The need for standardised care considerations, including psychological care and transition support along with the harmonisation of outcome measures to track disease progression, was prioritised. Challenges in paediatric trial design highlighted by industry and clinical experts, including disease heterogeneity and ethical considerations, helped identify knowledge gaps. Consensus was reached on childhood-onset FSHD classification. Two task forces were established to define minimal outcome measure datasets and paediatric-specific care guidelines, marking a crucial step toward improved care and trial readiness.

S5.02 Focus on fatigue: The presence of performance fatigability in childhood-onset facioscapulohumeral dystrophy

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Fatigability, defined as the inability to sustain repetitive physical activities with declining motor performance, is frequently reported in FSHD, but data in childhood-onset FSHD are lacking. Fatigability has been demonstrated using the Endurance Shuttle Box and Block Test (ESBBT) and Endurance Shuttle Walk Test (ESWT) in spinal muscular atrophy. This cross-sectional study explores fatigability and assesses the applicability of ESBBT and the ESWT in children and young adults with childhood-onset FSHD. Genetically confirmed FSHD patients aged 8-25 years with symptom onset in childhood were included. Fatigability was defined as dropout (time to limitation <1200 s) on the ESBBT or ESWT. Tests were video-recorded and analyzed for motor adaptations indicative of fatigability. Disease severity, muscle strength (isokinetic dynamometry for elbow and knee flexion and extension), perceived fatigue, pain, and quality of life were assessed. Preliminary results will be presented. Twenty patients (12 males, age 8-24 years) were included; one was excluded due to poor data quality. Fatigability was observed in 8/19 participants (42%): 1 on the ESWT, 8 on the ESBBT (including 1 on both). Motor adaptations were seen in 60% of all participants and in 86% of those with fatigability. Fatigability appears common in childhood-onset FSHD. The ESBBT and ESWT are promising tools to assess performance fatigability in this population.

S5.03 An updated international standard of care for facioscapulohumeral muscular dystrophy

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Since the last evidence-based clinical care guideline for FSHD was published in 2015, advances in research, therapy development, and patient advocacy have motivated the need for this updated guideline. Several novel therapies are in clinical trials, raising the urgency of diagnosing and optimizing patient care as part of trial and treatment readiness. In 2021, the FSHD Care Considerations Working Group was formed with 40 clinicians from diverse disciplines and nations to develop recommendations aimed at improving care standards and health outcomes for people with FSHD. The FSHD Society and people with FSHD provided input at all phases. The working group identified 13 topics to include in this update, 9 of which were addressed in the 2015 guideline. The 4 new topics are communication, speech, and swallow; mental health; pregnancy, birth, and parenting; and emerging pharmacologic therapies. Here we summarize key recommendations. A manuscript is being prepared for publication in a medical journal. Summaries for patients and families will be disseminated via patient advocacy organizations around the world.

S5.04 Facioscapulohumeral dystrophy (FSHD): In search of a Brazilian epidemiological profile

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FSHD is a highly heterogeneous disease. In the context of emerging therapies, it is crucial to enhance clinical trial readiness by deepening our understanding of its presentation and natural history across diverse populations. Objective: To describe the onset, progression, and severity of Brazilians' FSHD as well as distinguish clinical factors and daily quality of life. It's an observational study, from January 2024 to April 2025 at the University of São Paulo, Brazil. Sixty patients distributed by sex (median: 47y) with onset between ages 3 and 57y (median: 18y; interquartile range (IQR): 10–37), and disease duration from 5 to 62y (median: 19y; IQR: 13–28) were included. Initial weakness was most commonly in shoulder girdle (61.1%), followed by facial (24.1%), pelvic (7.4%), and axial (5.5%). While 44.8% reported facial weakness, 89.6% had examiner-detected facial involvement. In total, 68.3% maintained walking ability, 23.3% required assistance, and 8.3% used a wheelchair. Respiratory dysfunction affected 15%, and 19.2% had dysphagia; 79.3% had spinal deformities and 77.5% reported pain, mainly in the lower back, hips, and shoulders. Mood disorders affected more than 30%. When asked about self-perceived severity, 20% considered it mild, 58.2% moderate, and 21.8% severe – with a median objective clinical severity score of 7. This study highlights FSHD’s clinical heterogeneity and impact, supporting efforts to improve diagnosis, monitoring, and future targeted treatments

S5.05 Addressing the unmet need for advance care planning and palliative care in FSHD

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FSHD is a progressive neuromuscular disease, yet current care guidelines neglect advanced disease management, including palliative care. Unlike Duchenne muscular dystrophy and motor neuron disease, FSHD lacks structured pathways for advance care planning (ACP) and end-of-life support, leaving patients and families without guidance. An upcoming European Neuromuscular Centre workshop aims to establish consensus guidelines on ACP and palliative care in FSHD and myotonic dystrophy. Experts in neurology, rehabilitation, palliative care, and patient advocacy will discuss:

- Barriers to ACP implementation.
- Care prioritization models like the "traffic light" approach and frailty concept.
- Multidisciplinary frameworks for late-stage FSHD care.
- International consensus guidelines to improve practice.

FSHD Global and FSHD Europe, in collaboration with FSHD Society and global advocacy organizations, will conduct a pre-workshop patient survey. Preliminary findings suggest that structured ACP enhances autonomy, symptom control, and resource optimization. Early palliative care integration reduces hospitalizations and invasive interventions, ensuring a patient-centered approach.

Session 6: Clinical Studies and Trial Designs

S6.01 Introducing BetterLife FSHD, an innovative new patient-reported data repository and engagement tool

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BetterLife FSHD is a novel, patient-reported data platform, launched by the FSHD Society, that transcends the engagement limits of a traditional registry by providing direct benefit to participants. Co-created with the patient community, BetterLife offers participants personalized resources, matching to clinical trials and studies, a community forum, and disease tracking. Participants enter structured survey data, which are stored in an auditable, modern data platform under IRB approved protocol. Surveys cover domains including diagnosis and genetics, disease progression, symptom management, pain, sleep and fatigue, mental health, diet and exercise, activities of daily living, and more. More than 500 participants have enrolled and completed more than 2,000 surveys in BetterLife in the 7 months since its launch in the United States. All data collected by BetterLife are available to the research community upon request and approval by the Steering Committee. To this end, we are thrilled to launch and reveal the new BetterLife FSHD data explorer at the 2025 International Research Congress. This tool enables researchers to dynamically search, filter, visualize, and request aggregate or row-level data. The research community is also invited to leverage and collaborate with the BetterLife team to administer new survey-based studies or recruit to other studies. Together, we aim to accelerate research by improving the participant experience and simplifying access to patients and patient data.

S6.02 Natural history of muscle volume and muscle fat content biomarkers in FSHD based on whole-body fat-referenced MRI

Markus Karlsson[1], Per Widholm[1], Jonathan Pini[2,3], Angela Puma[4], Luisa Villa[4], Michele Cavali[2], Andra Ezaru[2], Guillaume Bassez[5], Benjamin Marty[5], Teresinha Evangelista[5], Romain Thomas[6,7], Loïc Danjoux[6,7], Céline Tard[6,7], Sabrina Sacconi[2,3]

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Showing treatment effects in FSHD trials is difficult due to disease heterogeneity and slow progression. Fat-referenced MRI biomarkers can identify intermediately affected muscles at risk of progression, but natural history data are limited. We aimed to examine 1-year fat-referenced MRI biomarker progression in FSHD and assess if selecting intermediate muscles accelerates biomarker progression. Whole-body Dixon MRI from the CTRN FSHD France natural history study was analyzed using AMRA® Researcher. Thirty-six muscle groups were evaluated, quantifying lean muscle volume (LMV), muscle fat fraction (MFF), and muscle fat infiltration (MFI), and composites were calculated. Muscles were categorized at baseline as Normal Appearing, Intermediate, or End-Stage based on baseline fat content. Responsiveness was assessed using standardized response mean (SRM). Fifty-six patients were enrolled (mean±SD 50.1±12.2 yrs old, CSS median [min, max] 6 [2,9]). One-year change in whole-body composite LMV/MFF/MFI was mean±SE(SRM) -2.33±0.47%(-0.66)/0.93±0.16pp(0.77)/0.21±0.06pp(0.46). Change in Intermediate muscles was -4.10±0.67%(-0.86)/1.89±0.33pp(0.8)/0.37±0.11pp(0.47), and -1.59±0.49%(-0.43)/0.58±0.12pp(0.64)/0.19±0.06pp(0.43) in Normal Appearing muscles. Changes in Intermediate muscles (LMV and MFF) were significantly higher. Whole-body composites of intermediate muscles showed the highest progression rate, making them a highly responsive biomarker for slowly progressing, heterogeneous diseases like FSHD.

S6.03 Motor Outcomes to Validate Evaluations in Facioscapulohumeral Muscular Dystrophy (MOVE FSHD): Interim baseline data and potential predictors for FSHD

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The MOVE FSHD study aims to determine the predictive value of clinical and motor assessments, patient-reported outcomes, and imaging and tissue biomarkers on disease progression in FSHD. This prospective observational study will evaluate 450 FSHD participants over 24 months with 200 participating in the MOVE+ sub-study. Visits include physical examination, patient-reported outcomes, FSHD history, strength, timed functional tests (TFTs), and spirometry. Sub-study participants have additional biomarkers collected, including reachable workspace at each visit, whole-body MRI at baseline and 12 months, optional muscle biopsy occurring at baseline and (n=40) at 4 months, and optional wearable device every 6 months. The MOVE FSHD study has enrolled >400 participants across 24 international sites: Ninety are enrolled in the MOVE+ sub-study, ~30 are pediatric, and ~30 are non-ambulatory. TFTs, such as the 10-meter walk run (10mwr) and timed up-and-go (TUG), correlate well with disease severity (>0.6), change from baseline in 12-24 months, and may predict a shift in other TFTs. The current abilities scale (CAS), a patient-reported outcome, also has a strong correlation to disease severity and strength (>0.8), as well as a moderate correlation to function (>0.5). The MOVE FSHD study has improved our understanding of FSHD,

impacted direct patient care, and refined inclusion/exclusion criteria for clinical trials, as well as identified outcomes and biomarkers for FSHD.

S6.04 Motor Outcomes to Validate Evaluations in Pediatric facioscapulohumeral muscular dystrophy (MOVE Peds): Protocol for an observational study

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FSHD severity and its impact on function are often more severe in children, and measurement tools must account for the effects of growth and maturation on motor function. Cross-sectional studies have suggested an association between earlier onset and greater clinical severity. This population is of interest because treating FSHD in a more rapidly progressing population may allow faster detection of treatment effect. While the adult FSHD field has coalesced around outcome measures (FSHD-Composite Outcome Measure [FSHD-COM], reachable workspace [RWS], and quantitative MRI [qMRI]), academic and industry experts agree there is an urgent need for data in pediatric FSHD. The primary goal of this study is to validate outcomes and refine trial strategies for pediatric-onset FSHD. This 24-month prospective observational study will enroll 80 symptomatic, genetically confirmed pediatric FSHD participants (at least half meeting early-onset criteria). Visits will occur every 6 months and collect history, results of neurological exam, patient-reported outcomes, functional performance (FSHD COM Peds, strength, and respiratory parameters), RWS, and whole-body MRI. We hypothesize that baseline features in qMRI will predict 24-month changes in FSHD-COM Peds or RWS. Validating measures in children with FSHD will improve trial readiness. Outcomes that can span the lifetime would offer advantages for clinical trials, enable longitudinal studies, and support effective health monitoring in integrated lifespan care models.

S6.05 Topline data from dose escalation cohorts A and B in FORTITUDE™, a Phase 1/2 trial evaluating *del-brax* (delpacibart braxlosiran) in adults with facioscapulohumeral muscular dystrophy (FSHD)

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Delpacibart braxlosiran, or *del-brax* (AOC 1020) is an antibody-oligonucleotide conjugate (AOC™) composed of a *DUX4*-targeting siRNA conjugated to a humanized anti-transferrin receptor 1 antibody to enable targeted delivery to muscle. *Del-brax* is designed to target the root cause of FSHD by degrading aberrantly expressed *DUX4* mRNA and reducing expression of *DUX4*-regulated genes. FORTITUDE™ (NCT05747924) is a Phase 1/2 randomized, double-blind, placebo-controlled trial evaluating safety, tolerability, pharmacokinetics, and pharmacodynamics of del-brax in individuals with FSHD. FORTITUDE includes three cohorts with a 1-year treatment and follow-up period. The first two cohorts (Cohort A and B; n=12 and 27, respectively) were multiple-ascending dose cohorts that evaluated 2 mg/kg (first dose 1 mg/kg) and 4 mg/kg given every 6 weeks for three doses followed by two quarterly doses. Cohort C (n=51) is a biomarker cohort evaluating 2 mg/kg given every 6-7 weeks. Cohort A and B have completed, and Cohort C is ongoing. Data will be presented for Cohorts A and B, including *DUX4*-regulated biomarkers, serum creatine kinase, exploratory efficacy endpoints, and long-term safety.

FORTITUDE data from Cohorts A and B support del-brax's continued clinical development as the first investigational therapy targeting the root cause of FSHD via the ongoing biomarker cohort and the initiation of a global Phase 3 study.

POSTERS
Session 1: Population Genetics and Modifiers

P1.01 Genetic epidemiology of facioscapulohumeral muscular dystrophy in Hungary

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Facioscapulohumeral muscular dystrophy (FSHD) is the third most common hereditary muscular dystrophy, following an autosomal dominant inheritance pattern. The most common symptom is a progressive bulbar, shoulder girdle, and proximal lower limb muscle weakness. The 4q35 chromosomal region was assessed by digesting the DNA by EcoRI/HindIII, EcoRI/BlnI, and XapI restriction enzymes and by a confirmatory Southern blotting using the p13E-11 probe. Pathogenic repeat numbers ranged from 1 to 9, while reduced penetrance alleles had 10 or 11 D4Z4 units. Altogether 152 genetically confirmed Hungarian FSHD1 patients (72 females and 80 males) were collected from our database. All patients are currently alive. The mean age at onset was 26 ± 15.32 years, 16 patients showed infantile FSHD phenotype. The most common presenting symptom was shoulder girdle weakness followed by proximal lower limb weakness. A positive family anamnesis was noted in 68% of our patients; 33 cases showed anticipation. There was a weak correlation between shorter repeat lengths and earlier symptom onset. The most common shorter allele in the Hungarian FSHD patients was 5 D4Z4 units followed by 6 repeats. FSHD is more common in the central part of the country than in the peripheral counties; the highest prevalence data were 0.0037% in Jász-Nagykun-Szolnok county. With our results we contribute to shedding light on the population-specific genetic background of FSHD.

P1.02 Estrogen rescues muscle regeneration impaired by DUX4 in a humanized xenograft mouse model

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Facioscapulohumeral dystrophy (FSHD) shows a wide range of clinical signs, with modifying factors contributing to this variability, especially in patients with mild disease. Among these factors, the beneficial activity of estrogen hormones is controversial. We investigated the effect of 17β-estradiol (E_2) – more prevalent in fertile females – and the 5α-dihydrotestosterone-derived 3β-androstenediol (3β-diol) – more prevalent in males – on muscle regeneration. To recapitulate human hormone sensitivity, we developed a humanized heterokaryon FSHD mouse model by engrafting human immortalized myoblasts or human primary muscle mesenchymal stromal cells into surgically treated murine muscle. By employing a volumetric muscle loss model, we could evaluate the contribution of human cells to murine muscle regeneration. Inducible lentiviral expression of the pathogenic FSHD gene, *DUX4*, in human cells impaired the structural and functional recovery of murine muscle, providing a humanized mouse model of *DUX4*-mediated pathogenicity and proving that the biological effect of *DUX4* spreads across the neighboring murine nuclei. Both hormones counteracted *DUX4* transcriptional activity and rescued structural and functional muscle performance impaired by *DUX4* expression, while not affecting control engrafts. The beneficial activity of estrogen in this heterokaryon model supports the hypothesis that these hormones contribute as a modifying factor in FSHD.

P1.03 Progesterone may be a regulator and B12 could be an indicator of the proximal D4Z4 repeat methylation status on 4q35ter

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FSHD patients have hypomethylation; different course in males and females was linked to sex hormones. We hypothesized that sex hormones, estradiol, testosterone, progesterone, and prolactin might be associated with methylation status of proximal part of D4Z4. We also investigated fT3, folic acid and vitB12 levels. DNA was extracted from 28 FSHD patients and 28 controls for bisulfite methylation analysis, and serum was separated for biochemical analysis of estradiol, testosterone, progesterone, prolactin, fT3, folic acid, and B12. Methylation analysis was specified to DR1, 5P regions, and proximal region covering both DR1 and 5P. Methylation was compared between patients and controls. Then correlation of methylation with estradiol, testosterone, progesterone, prolactin, fT3, folic acid, and B12 was investigated. We found that 5P and proximal region, but not DR1, were significantly hypomethylated in patients compared to controls. Male patients had significant hypomethylation compared to male controls. Older FSHD patients exhibited notable decrease in fT3 levels and hypomethylation of 5P region. Analyses of each CpG revealed seven hypomethylated positions significantly different from controls. Two of the positions demonstrated correlation with progesterone in controls. Except for one position, methylation levels were inversely correlated with B12 in patients. The results indicate that methylation of proximal D4Z4 region, particularly specific positions, may be associated with progesterone.

P1.04 FSHD onset before 18 years of age: A retrospective longitudinal study

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Childhood-onset FSHD1 is often associated with a severe phenotype, but its full spectrum remains poorly understood. While early-onset cases have been linked to rapid progression, data are limited and longitudinal studies are scarce. This study focuses on assessing disease severity and progression in FSHD1 patients with symptom onset before 18 years. In this retrospective longitudinal study, clinical and genetic data were collected from FSHD1 patients with onset before 18 years (mean follow-up 13.2 years) recruited from Policlinico Gemelli of Rome and the John Walton Muscular Dystrophy Research Centre in Newcastle upon Tyne. A subset of severely affected individuals was identified based on age at loss of ambulation and age at lower limb involvement (clinical severity score ≥3). Among 107 patients with onset before age 18, 26 exhibited an earlier disease onset (median 5 vs. 10 years, p=0.0115), fewer D4Z4 repeats (median 3 vs. 5, p=0.00036), higher prevalence of respiratory abnormalities vs. 2% p=0.0107). Patients in this group showed a significantly steeper decline in age-corrected clinical severity score (-46.97 vs. -9.90, p=0.007) and faster FSHD score progression (0.90 vs. 0.50, p=0.013). Notably, among these severe patients, 9 had ≥4 D4Z4 repeats, whereas 3 patients meeting early-onset Brouwer criteria did not align with expected severity. This study highlights the complexity of early-onset FSHD1 progression and emphasizes the need for larger studies to refine prognostic markers beyond traditional genetic and clinical criteria.

P1.05 Beyond the classical definition: Unusual genetic patterns and clinical diversity in FSHD

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FSHD genetics is traditionally defined by three key features: D4Z4 contraction or epigenetic de-repression, a permissive haplotype, and compatible clinical features. However, some patients present with symptoms despite lacking these criteria or show additional complexities such as mosaicism, rearranged alleles, or atypical repeat structures. These findings suggest a broader genetic spectrum and challenge current diagnostic models. We analyzed 147 individuals from 117 unrelated families, all with suspected FSHD. Molecular combing and optical genome mapping were used to assess D4Z4 repeat unit (RU) size and 4qA haplotype status. For individuals without contraction, bisulfite sequencing and next-generation sequencing were performed to evaluate epigenetic status and screen for FSHD2-related variants. FSHD1 was confirmed in 96 index cases (82%), including three somatic mosaics with variable clinical severity. Of the 21 uncontracted cases, 16 had low to borderline methylation. This subgroup showed diverse genotypes: One with a complex rearrangement and SMCHD1 variant, one low-level mosaic with 1 RU, and 12 with contracted 10qA alleles despite >11 RU on 4qA. Many showed FSHD-like features with discordant genotype-phenotype patterns. Our findings underscore the need to go beyond the classical definition of FSHD genetics. We aim to resolve the region's complexity through long-read sequencing to better understand genotype-phenotype correlations in relation to the underlying genetic defect.

Session 2: Measures of Disease Activity and Progression

P2.01 Whole-body muscle MRI in children: Two-year follow-up of the iFSHD-LOS cohort

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FSHD presenting in childhood has a more variable course in terms of groups and severity of muscle involvement and the rate of progression as compared to the adult-onset phenotype. Utilizing techniques to measure fat fraction (MFF), infiltration (MFI), and lean muscle volume (LMV) developed by AMRA, we aimed to compare disease progression patterns between pediatric and adult populations as measured on whole-body muscle (WB)MRI. Eighteen young people with FSHD underwent at least two WBMRI scans 12 months apart as part of the iFSHD-LOS natural history study performed in Melbourne, Australia. Of those enrolled, 11 had three scans over two years. Data showing change over 12 months can be compared to an adult-onset disease cohort from Fulcrum's ReDUX4 clinical trial of losmapimod. Participants were separated by repeat size into Early-onset (1-3) or Classic onset (≥4). The results show that growth remains a significant confounder in analysis: LMV increased across the whole cohort in normal muscles, but intermediate muscles showed a decrease in LMV. In normal muscles, a percent reduction in MFI and MFF suggests that growth of the muscles is greater than the speed of disease progression. In the early-onset cohort, the MFF and MFI increase at a rate greater than those seen in adults, particularly in intermediate muscles. This suggests that in individuals with early-onset disease, the rate of progression is more aggressive compared to adult population, particularly in intermediate muscles.

P2.02 Electrical impedance myography captures features of muscle structure measured by MRI and transcriptomic analysis in facioscapulohumeral muscular dystrophy

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Electrical impedance myography (EIM) has been proposed as an efficient, non-invasive biomarker of muscle composition in facioscapulohumeral muscular dystrophy (FSHD). We performed a multicenter cross-sectional study enrolling 33 patients with FSHD. EIM measurements were recorded from bilateral vastus lateralis, tibialis anterior (TA), and medial gastrocnemius muscles and compared to quantitative muscle volume measures by MRI as well as knee extension and ankle dorsiflexion strength by quantitative muscle testing. EIM measurements of the bilateral TA were further compared to histology and transcriptomic analysis (RNAseq) of muscle and fat content. EIM phase at multiple frequencies was positively associated to the amount of muscle measured by MRI (ρ = 0.48 to 0.70, p 0.001) and negatively associated to the amount of fat replacement of muscle (ρ = -0.53 to -0.73, p 0.001). EIM phase of the vastus lateralis and TA was positively associated with knee extension and ankle dorsiflexion strength normalized to age and sex (ρ = 0.45 to 0.60, p <0.0001). The bilateral TA muscles were analyzed at the microscopic level and also showed that EIM phase was positively associated, with amount of muscle and negatively associated with amount of fat by transcriptomic analysis. This study supports the hypothesis that EIM is associated with the amount of muscle in patients with FSHD that can be measured by MRI, strength, and amount of muscle RNA measured in biopsy samples.

P2.03 Mapping immune dysregulation in FSHD: Toward the first immune atlas and novel therapeutic strategies

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Facioscapulohumeral muscular dystrophy (FSHD) is the third most common muscular dystrophy, characterized by progressive muscle atrophy and fat replacement. Although FSHD is linked to the epigenetic de-repression of *DUX4*, the mechanisms driving disease progression remain poorly understood. Early pathological changes are detectable by MRI as hyper-intense signals on short tau inversion recovery (STIR) sequences, indicative of muscle edema. STIR-positive regions correlate with active immune cell infiltration, including CD4+ and CD8+ T lymphocytes, B cells, and macrophages, resembling features of inflammatory myopathies. However, the role of the immune system in FSHD pathogenesis remains unclear. Preliminary data indicate that FSHD patients display distinct immune endotypes compared to non-penetrant gene carriers and healthy controls, marked by the expansion of activated T and B cell subsets, and the emergence of novel immune populations that discriminate between disease states. Integration of immune signatures with MRI biomarkers suggests a link between specific immune profiles and early-stage inflammatory muscle damage. This study aims to define the immune landscape of FSHD using high-dimensional flow cytometry and cytokine profiling, integrated with MRI analysis, to generate the first comprehensive immune atlas of FSHD and identify targets for precision immunotherapy.

P2.04 Characterization of FSHD1 model mice carrying 5Mb chromosome 4q35 on mouse artificial chromosome

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disorder characterized by muscle weakness and wasting. *DUX4* is a primate-specific gene that is located in 3.3 kb D4Z4 repeat unit at the subtelomeric region of chr4q35. It is normally expressed in testis, while it is repressed in somatic tissues including skeletal muscles. The contraction of the D4Z4 repeat in FSHD1 leads to abnormal *DUX4* expression in skeletal muscles. To understand the molecular mechanisms for the tissue-specific *DUX4* expression, a FSHD animal model carrying human chr4q35 is needed. Here, we generated transchromosomic mice with 5Mb chr4q35 ranging from the *SLC25A4* gene to telomeric regions derived from a FSHD1 patient who carries disease (one D4Z4, 4qA) and control allele (4qB) using a mouse artificial chromosome vector and chromosome transfer technique. The transchromosomic mice with disease allele showed abnormal *DUX4* expression in skeletal muscles and smaller body size with low survival rate than those with control allele during neonatal period. Among adult tissues, *DUX4* was highly and slightly expressed in testis and skeletal muscles of those with disease allele, respectively. These results suggest that our transchromosomic mice could be useful to understand molecular mechanisms for the tissue-specific *DUX4* expression and disease progression.

P2.05 Quantitative whole-body MRI biomarker relation to muscle strength and function in FSHD patients

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Disease onset in FSHD is heterogeneous, and clinical assessment is variable and inconsistent – making assessment of function challenging to reliably interpret in clinical trials. Whole-body MRI provides an objective, quantitative assessment of muscle composition. Our aim was to explore the relationship between muscle function and MRI biomarkers in patients diagnosed with FSHD. Sixty-eight patients (23F:45M; 50±13yrs; CTRN FSHD France Natural History Study) underwent whole-body MRI (AMRA® Researcher), quantitative muscle strength, and 6-minute walk test (6MWT). Muscle strength and 6MWT were correlated to MRI biomarkers (total muscle volume [TMV], lean muscle volume [LMV], muscle fat fraction [MFF], muscle fat infiltration [MFI]) for individual muscles and leg composites, respectively. For arm muscles (biceps, triceps), muscle volume correlations were strong (TMV/LMV: 0.68/0.75, 0.61/0.65); muscle fat correlations were moderate (MFF/MFI: -0.53/-0.58, -0.45/-0.45). For leg muscles (hamstrings, quadriceps, tibialis), muscle volume correlations were strong, except hamstrings TMV (TMV/LMV: 0.43/0.79, 0.64/0.82, 0.73/0.79); muscle fat correlations were moderate to strong (MFF/MFI: -0.75/-0.73, -0.59/-0.60, -0.71/-0.66). Composite correlations to 6MWT were moderate (LMV/MFF/MFI: 0.53/-0.57/-0.54). MRI biomarkers correlated with muscle function, especially LMV (contractile), providing a valid alternative muscle function assessment in FSHD clinical trials to improve our understanding of treatments.

P2.06 Patient-derived stem cell models of childhood-onset facioscapulohumeral muscular dystrophy (FSHD) mirror disease severity *in vitro*

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As part of the childhood-onset FSHD longitudinal outcome study (iFSHD-LOS) we have generated induced pluripotent stem cell (iPSC) lines from four patients that were classified as mild, moderate, and severe, based on established performance-based functional measures. These unique patient-derived iPSC lines were compared to unrelated healthy controls to explore the utility of these models to study disease severity and pathobiology. Using established methods, we differentiated each iPSC line to skeletal muscle progenitors and assessed key disease pathologies in 2- and 3-dimensional fused muscle culture systems to define the potential disease-specific features associated with FSHD in vitro. Our analyses show that iPSC-derived muscle cultures from FSHD patients show an increase in *DUX4* mRNA that correlates with disease severity, as well as reduced muscle volume in 2D terminally differentiated myotubes. Reduced muscle force/strength is also observed in 3D muscle cultures, and also segregates with disease and correlates with severity. Our data support the use of iPSC-derived skeletal muscle cultures to study childhood-onset FSHD and can now be used to assess potential treatments and therapeutic strategies in the future.

P2.07 Associations between muscle strength and MRI biomarkers in FSHD: Toward imaging-based functional classification

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Muscle MRI is increasingly used to monitor disease progression and treatment response in facioscapulohumeral muscular dystrophy (FSHD). To support its use as a surrogate marker of clinical benefit, its relationship to functional muscle performance must be established. Objective: To assess whether manual muscle testing (MMT) can define clinically significant muscle function and to evaluate MRI's accuracy in identifying such muscles. Data from 67 individuals with genetically confirmed FSHD in the CTRN-FSHD France Natural History Study were analyzed. Participants underwent MMT, quantitative muscle testing (QMT), and quantitative muscle MRI. Biceps brachii, triceps brachii, quadriceps, hamstrings, and tibialis anterior were evaluated. Muscles were considered to have clinically significant function if MMT >3. MRI features were categorized as normal-appearing, intermediate, or end-stage. Of 653 muscles evaluated, 516 (79%) were classified as having clinically significant function. Muscles with normal-appearing MRI features correctly classified 538 of 653 muscles, with 82% overall accuracy and 96% specificity for identifying muscles with clinically significant function. MMT enabled consistent classification of clinically functioning muscles across muscles groups. Normal-appearing MRI features demonstrated high specificity in detecting these muscles, supporting the potential of developing surrogate biomarkers of clinical benefit based on MRI.

P2.08 Expanding the UK FSHD Patient Registry Dataset: Improving data collection and amplifying "patient voice"

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Since 2013, the UK FSHD Patient Registry has collected the TREAT-NMD Core Dataset for FSHD, a minimal list of data items agreed to at a 2010 European Neuro Muscular Centre workshop and updated in 2016. The dual-reported registry collected patient-entered data including this core dataset, creating a valuable repository of longitudinal data. The 2016 dataset contains mandatory questions on diagnosis, family history, symptoms, motor function, wheelchair use, and pregnancy history. An optional question on ethnicity is also included. The UK registry also included detailed questions on pain and scapular fixation, and collects clinician-entered genetic test results where available. In 2023, the UK registry identified the need to expand and refine the questionnaires within their dataset. A Working Group was established comprising academic, genetic, physiotherapy, clinician, and patient advocate experts to update the dataset, ensuring data collection remains relevant and appropriate. As clinical trials arrive in the UK, the ability to collect data on treatments or therapies becomes crucial. The expanded dataset allows for real-world data studies to support PMS, and facilitates Privacy Preserving Record Linkage, greatly increasing the usability and value of the data for research. The expanded dataset is available to view on the UK FSHD Registry website and can be adopted by other national FSHD registries and data collection initiatives to ensure harmonization and alignment of data collection.

P2.09 The UK Facioscapulohumeral Muscular Dystrophy Patient Registry: A powerful tool to support clinical research and patient voice in the translational research pathway

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The UK Facioscapulohumeral Muscular Dystrophy (FSHD) Patient Registry is an online database where individuals with FSHD1 and FSHD2 can self-enroll to share clinical and genetic data. Established in May 2013 with support from Muscular Dystrophy UK, it is managed by Newcastle University. The registry supports research, improves understanding of FSHD, amplifies the patient voice, and shares updates on new studies. By collecting real-world data, the registry is a key resource for researchers and industry partners. Participants can enter self-reported data through an online portal. If additional clinical or genetic details are needed, patients can invite their neuromuscular specialist to contribute. The registry is a Core Member of the TREAT-NMD Global Registries Network for FSHD. To enhance data collection, the registry accepts secure digital uploads of genetic reports. It has supported more than 30 research inquiries, including studies on health economics, patient preferences, and experiences with dysphagia, pregnancy, sleep, and caregiving. As one of the largest national FSHD registries, it is a cost-effective research tool advancing FSHD studies. Ongoing improvements focus on better genetic data reporting, patient-reported outcomes, and trial recruitment. In response to patient feedback, the registry is transitioning to a custom platform with improved usability and built-in data quality checks.

P2.10 Linking mechanical strain to progression of fat infiltration in FSHD

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Due to wide variation in FSHD progression among muscles and individuals, the development of tools to understand the mechanical effects of emerging therapeutics is essential. Finite element models (FEMs) offer the ability to map anatomical fat patterns and simulate isometric contraction to predict regions of high strain. This study leverages MRI-derived FEMs to investigate how heterogeneous strain distributions impact longitudinal progression of fat infiltration in the tibialis anterior (TA) of FSHD patients. MRIs from a Wellstone sample (n = 4; P50AR065139, Tapscott PI) were taken at baseline and 12 months. TA muscles and aponeuroses were segmented using a deep neural network and manual review, and muscle-fat fraction values were calculated from Dixon images. Subject-specific geometry and established tissue material properties enabled simulation of isometric contractions to estimate local fiber strain. By integrating MRI-based fat fractions, we examined how baseline strain patterns relate fat diffusion between timepoints, and results suggest non-uniform strain may contribute to regional fat progression. Generally, regions with increased compressive strain during contraction experienced higher increases in fat content. However, the relationship between strain and fat progression was weaker in muscle with low initial fat fraction. Further analysis across additional patients and muscles is needed to identify additional factors influencing regional mechanical environments.

Session 3: Novel Clinical Outcome Measures

P3.01 Quantitative muscle ultrasound as a clinical correlate in facioscapulohumeral muscular dystrophy: Validation of a rapid assessment protocol

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We reported previously that quantitative sonographic assessment of muscle thickness using a brief standardized protocol (<10 minutes) demonstrated strong correlations with clinical findings across various neuromuscular disorders. In this study, we aimed to specifically evaluate the utility of this protocol in patients with facioscapulohumeral muscular dystrophy (FSHD). Twenty-seven genetically confirmed adult FSHD patients (19 males), followed at the Neuromuscular Clinic of Tel Aviv Sourasky Medical Center, were included. Muscle thickness was measured by ultrasound in eight upper and lower limb muscles. The sum of muscle thickness measurements was correlated with the following clinical outcomes: Medical Research Council Sum Scores (MRCSS), six-minute walk test (6MWT), FSHD Clinical Severity Score (CSS), and FSHD Clinical Score (CS). The total ultrasound muscle thickness showed strong correlations ($r > 0.60$, $p < 0.01$) with all clinical measures: MRCSS ($r = 0.77$), 6MWT ($r = 0.74$), FSHD-CSS ($r = 0.86$), and FSHD-CS ($r = 0.86$). This brief, generic ultrasound protocol strongly correlates with clinical outcomes in FSHD and may be applicable to other neuromuscular disorders. Unlike echogenicity-based assessments, which often require dedicated software, our protocol is quick, simple, and equipment-independent – facilitating its use in diverse settings, including in pediatric populations.

P3.02 Quantitative muscle ultrasound as a clinical correlate in facioscapulohumeral muscular dystrophy: Validation of a rapid assessment protocol

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Muscle ultrasound is a complementary imaging technique to MRI that augments clinical assessment in facioscapulohumeral dystrophy (FSHD). Deterioration in muscle structure might explain the observed difference between the loss of specific strength in FSHD muscle fibers and whole muscle groups. The influence of affected muscles on contractile performance remains relatively unexplored. This pilot study assesses the feasibility and repeatability of ultrasound-defined muscle contractile performance in upper and lower extremity muscles as a potential biomarker in FSHD. In healthy individuals (n=15) and patients with FSHD (n=10), we performed dynamic ultrasound during standardized isotonic contractions. We used offline speckle-tracking imaging (STI) to assess the muscle contractile performance. For each muscle, the feasibility and inter- and intra-rater reliability were determined. Preliminary results revealed a higher maximum displacement in most muscles of healthy individuals compared to patients, with good reliability. These data indicate the potential of some of our advanced imaging measures as possible biomarkers. Our data suggest that STI can quantify biomarkers of muscle contractile performance in FSHD. Next, we will compare our measurements with clinical measures and assess their 1-year responsiveness for a comprehensive view of muscle function in follow-up and clinical trials.

P3.03 Measuring upper and lower limb movement with Syde® in patients with facioscapulohumeral muscular dystrophy (FSHD): Analytical validation in a controlled environment

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FSHD is a slow, progressive disease, and currently used endpoints are limited by poor sensitivity to detect changes in clinical function. A solution to this is the development of more objective and sensitive endpoints. Syde® is a wearable device that measures the 3D trajectory of the limbs. Syde can be used by patients at home, providing digital variables from activities of daily living. The Syde variable SV95C represents the top 5% fastest strides recorded. It's already European Medicines Agency-approved as a primary endpoint for Duchenne muscular dystrophy, where it demonstrated greater sensitivity to change in clinical function compared to existing endpoints. This study aims to analytically validate the performance of Syde in the reconstruction of upper and lower limb movement for patients with FSHD. We hypothesize that the Syde can measure equally accurate movement data as gold standard infrared cameras. Seventeen participants with genetically diagnosed FSHD were included, and FSHD-specific outcome measures (RICCI-scale and FSHD-COM) were performed to assess disease severity. Participants then completed a data collection visit, performing activities of daily living while wearing Syde in a controlled environment with gold standard motion capture technology as a control. Data on the validation of Syde for lower limb activity will be presented at the congress. Future perspectives include the validation of Syde in an uncontrolled environment to develop digital variables specific for FSHD.

P3.04 Episodes of symptoms worsening in FSHD patients

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Patient-reported outcome measures (PRO) are important tools to detect disease burden and monitor its change over time. Patients with FSHD experience heterogeneous symptoms with a variable impact. This preliminary exploratory study investigates the prevalence of subjective episodes of worsening neuromuscular symptoms in FSHD patients. We developed a patient-reported questionnaire and distributed it online to FSHD adults followed at the NeMO Center in Milan, Italy. We classified symptoms of fatigability, focal muscle weakness, and pain as episodic if they occurred in addition to their chronic neuromuscular impairment within a defined period and returned to baseline/near baseline condition. Preliminary data from 40 of 117 questionnaires indicate that 28 (70%) experienced episodic symptoms worsening. Of these, 14 (50%) reported episodes lasting 1–7 days. Nine (32%) reported 7-12 episodes yearly, and 8 (29%) reported 4-6. Emotional stress (32%) and physical exertion (36%) were frequent triggers. Fifty percent rated the episodes as moderate severity, and 17 (61%) reported a negative impact on daily living. These preliminary data suggest that episodes of neuromuscular symptom worsening may be a clinically relevant phenomenon in patients with FSHD. These fluctuations may not be captured by existing outcome measures. Additional analysis on a larger FSHD cohort is ongoing to validate these initial findings and will investigate a cross-disease comparison. All in all, our results strengthen the potential of WDR5 as a druggable target for FSHD.

Session 4: Mechanisms of Disease and Interventional Strategies

P4.01 Quantifying anti-DUX4 therapy for facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is an inherited skeletal myopathy with no cure. Expression of the myotoxic transcription factor Double Homeobox 4 (*DUX4*) is believed to underlie FSHD pathogenesis, and many proposed therapies target *DUX4* generation or function. Which of these therapies will be the most effective is unclear. Here, by constructing a Markov chain-based mathematical model of *DUX4*-mediated myotoxicity in FSHD, we interrogated various anti-*DUX4* FSHD therapeutic strategies. We derived an analytical function for myonuclear life expectancy in terms of the parameters of *DUX4* expression and function. In a biologically relevant parameter regime, therapeutically decreasing the DUX4 protein diffusion rate is, surprisingly, predicted to be more effective at increasing myonuclear life expectancy than reducing the rate of myonuclear apoptosis caused by the expression of *DUX4* target genes. We find that targeting elements of *DUX4* transcription/translation, such as mRNA stability via siRNA therapy, has a limited predicted impact on *DUX4*-meditated toxicity when performed in isolation. However, our model predicts a super-additive effect from combining transcription/translation targeting strategies with approaches that minimize *DUX4* diffusion-mediated import into neighboring myonuclei. Importantly, we provide a computational tool to test and inform therapeutic designs, enabling preclinical screening of FSHD treatment approaches.

P4.02 Characterization of a DUX4-responsive reporter mouse

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DUX4 detection is challenging in both clinical and research settings, making disease progression and interventional strategies difficult to directly track molecularly. Here we describe a *DUX4*-responsive reporter mouse to aid preclinical studies for FSHD therapies and disease pathogenesis. Our new model contains a single copy, dual-reporter transgene expressing *Renilla reniformis* Luciferase-P2A-mScarlet. We show the model produces 1) *DUX4*-responsive luciferase, which can be imaged in live mice over time without requiring euthanasia; and 2) mScarlet fluorescence, for tagging individual myonuclei upon sacrifice. Here we show this mouse model can effectively track expression following adeno-associated virus-delivered *DUX4* vectors or with crosses to an established FSHD mouse model, the TIC-DUX4 mouse. Importantly, we see high sensitivity in live imaging, with significant reduction in *DUX4*-associated signal in muscles of animals treated with a *DUX4*-targeted gene therapy. Ultimately, characterization of this reporter model may help elucidate mechanisms associated with FSHD-related disease progression and inform therapeutic outcome measures for translational studies.

P4.03 Investigating Dux expression in extra-embryonic tissues and whether it modulates maternal-fetal tolerance

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Human *DUX4* and mouse *Dux* induce a totipotent state in the early embryo and subsequent development of both embryonic and extra-embryonic tissues. Prior work has shown that knocking out *Dux* in C57Bl6/J mice results in decreased embryonic viability, primarily due to failure during post-implantation development. This study seeks to determine whether *Dux* is expressed in extra-embryonic tissues, and if it might influence maternal-fetal tolerance because of its role in regulating antigen presentation in somatic cells. To test this, we harvested extra-embryonic tissue from mid-gestation embryos of wild-type and *Dux* knockout mice to assay for expression of *Dux* and known *Dux* target genes. We found that *Dux* is expressed at low levels in the placenta of wild-type mice, together with low-level expression of some *Dux* target genes. To assess the role of maternal-fetal tolerance in the lethality of *Dux* null embryos, we crossed the *Dux* knockout locus from the C57Bl6/J strain into the 129S6 genetic background. We are now testing whether the allogenic challenge of crossing 129S6 and C57Bl6/J mice will result in lower fetal viability of *Dux* null embryos than the previously reported syngeneic crosses in C57Bl6/J. These results will be presented.

P4.04 Modeling cell-type-specific sporadic DUX4 activation in FSHD

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How *DUX4* is activated in FSHD muscle cells remains unclear. Whereas sole *DUX4* promoter's open state with significant DNA hypomethylation ubiquitously observed across various cell types in FSHD cannot explain infrequent leakage of expression in muscle cells, atypical clinical cases with extended proximal deletion beyond D4Z4 repeats challenges necessity of reported myogenic regulatory elements close to the *DUX4*/D4Z4 locus. This study aims to uncover the (mis)regulatory mechanism of *DUX4* activation using patient-derived iPSC models including gene repair FSHD2 controls free of copy number variations, genetic/epigenetic engineering, the *DUX4* reporter system, and series of high-throughput sequencing methods. In this presentation I will give an update of the study and the hypothesis to be proved, that is, a disease-specific long-range regulatory mechanism that activates *DUX4* in the muscle-specific context.

P4.05 SMCHD1 enzymatic activity is promoted by bidentate binding to DNA

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SMCHD1 is an epigenetic silencer in which heterozygous variants are reported in FSHD as well as a second distinct disorder, bosma arhinia microphthalmia syndrome (BAMS). However, the mechanism by which SMCHD1 pathogenic variants lead to these disorders are not fully understood. There has therefore been a growing interest in unveiling SMCHD1's atomic structure and the molecular mechanisms underlying its function. While we have previously shown that SMCHD1 is able to interact with nucleic acids via its C-terminal hinge domain, we have now identified a second DNA-binding site that is located downstream of the ATPase module. This is formed by two domains, which we termed the bromo-adjacent homology (BAH) and immunoglobulin-like 1 (IGL-1), based on their structural homology with other proteins. Using site-directed mutagenesis and structural analysis via cryogenic electron microscopy, we demonstrate the mode of DNA-interaction by the BAH and IGL-1 domains, and we present the first high-resolution structure of the *wild-type* human SMCHD1 ATPase. Excitingly, we have discovered that DNA binding stimulates the ATPase activity of full-length SMCHD1 *in vitro.* This finding raises further questions about how exactly DNA is able to influence SMCHD1's catalytic function, and why this mechanism may be required for its molecular role in gene repression. It additionally highlights a potential avenue for boosting SMCHD1 enzymatic function as a therapeutic strategy for FSHD.

P4.06 Detailed analysis of inflammatory and ultrastructural changes in TIRM-MRI guided muscle biopsies of FSHD patients

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FSHD is an inherited myopathy with complex epigenetic pathogenesis and no causal treatment. Inflammation is believed to contribute to muscle pathology, with inflammatory infiltrates found in one-third of FSHD biopsies, but the nature of the inflammatory response is unclear. **Objective:** To identify and characterize inflammatory infiltrates and morphological changes in targeted MRI-guided FSHD biopsies and healthy control (HC) biopsies. We conducted turbo inversion recovery magnitude (TIRM) and Dixon MRI analysis of 43 FSHD patients (50 ± 12 years old, 51% men) to evaluate inflammation and fatty muscle infiltration. From these, 24 patients with TIRM+ muscle were selected for two MRI-guided biopsies (TIRM+ and TIRM-). Biopsies from 8 HC (36 ± 12 years old, 62% men) were also collected. All biopsies were stained for CD3, CD4, CD8, CD56, CD68, HLA-ABC+, HLA-DR, and MAC. Electron microscopy was performed. TIRM+ FSHD samples had significantly higher histopathology and inflammation grades compared to TIRM- and HC muscles. Inflammatory infiltrates, predominantly CD8 lymphocytes and CD68 macrophages, were found in 67% of TIRM+ and 20% of TIRM- samples. Electron microscopy revealed myofibrillar disorganization in TIRM+ samples. TIRM hyperintensity is a reliable biomarker for active disease, correlating with inflammation. Inflammation is more frequent in FSHD than previously thought, supporting its role in disease pathogenesis and therapeutic targeting.

P4.07 Selection of peptides for a muscle-targeted delivery of ASOs directed against DUX4 *mRNAs* through complementary approaches *in silico*, *in vitro*, and *in vivo*

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In a therapeutic goal for FSHD, antisense oligonucleotides (ASOs) directed against *DUX4* mRNAs have been developed at UMONS. However, ASO use is limited by its restricted tissue delivery, lack of tissue selectivity, and rapid clearance. By screening a phage-display library of linear peptides against #1 myotubes or #2 a muscle-membrane protein (MMP), we selected peptides (MSPep) that specifically bind to muscle surface proteins. The 4 most promising MSPeps were synthesized with rhodamine conjugation and added to cell culture media to study their internalization into myotubes, hepatocytes, or renal cells. At all tested doses (10, 40 µM), $MSPep_{IC}$ (from screening #1) and MSPep1-3 (from screening #2) were internalized into myotubes after 2h of incubation. As expected, $MSPep_{IC}$ and MSPep1-3 were not internalized by renal cells that do not express endogenous MMP. MSPep 1-3 were only internalized by renal cells when they were transfected with an MMP expression plasmid. $MSPep_{IC}$ was internalized by hepatocytes but to a lesser extent as compared to myotubes. MSPep1 is internalized by hepatocytes at a bigger extent than MSPepIC and MSPep 2-3, likely due to its helical tertiary structure. We designed MSPep-ASO complexes *in silico* based on literature data and collaborators' expertise. Ongoing experiments aim to evaluate the ability of MSPep-ASOs to target skeletal muscle and deliver ASO efficiently into muscle cells.

P4.08 DUX4-sPAS base editing gene therapy technology in FSHD

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FSHD is caused by inappropriate *DUX4* expression in skeletal muscle. We already demonstrated that disruption of the *DUX4* somatic polyadenylation signal (DUX4-sPAS) by base editing significantly downregulates expression of *DUX4* and its target genes in patient-derived immortalized muscle cell lines (Šikrova et al., 2021). Our aim was to 1) further optimize this technology by testing novel base editors and different methods of delivery, and 2) test the application of base editing in primary muscle cells of a broad spectrum of FSHD1 and FSHD2 patients. Induced transduction by osmocytosis and propanebetaine transfection of base editor ABE8e resulted in efficient base editing of the DUX4-sPAS concomitant with significant reduction in *DUX4* and *DUX4* target gene expression in most of the primary muscle cells of FSHD patients tested. This further confirms the potential of DUX4-sPAS base editing as gene therapy for FSHD. Editing patterns and efficiency were evaluated by next-generation sequencing, and the impact of the base editor on the individual positions in the DUX4-sPAS sequence was independently assessed by creating single nucleotide mutations. We next successfully applied DUX4-sPAS base editing on 3D tissue-engineered skeletal muscles (3D-TESMs) and successfully investigated an alternative delivery of base editors to skeletal muscle using the latest generation of the adenoviral particles. These results establish DUX4-sPAS base editing as a potential therapy to treat FSHD, and future studies will focus on *in vivo* delivery in FSHD animal models.

P4.09 Unlocking the potential of 3D-TESMs: A promising drug testing tool for FSHD therapies

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Patient-derived 3D muscle bundle models represent a promising avenue for disease modeling and drug testing of targeted therapies. To this end, we have generated and validated a 3D-tissue engineered skeletal muscle (TESM) model generated from myogenic progenitors, differentiated from human induced pluripotent stem cells, that originates from skin fibroblasts of mosaic FSHD patients (*Franken et al., 2024*). The 3D-TESM shares the genetic background of the patients and overcomes many limitations of 2D cultures, offering additional insights into the morphology and functionality of the muscle bundles. Our FSHD 3D-TESM recapitulates the molecular signature of *DUX4* expression observed in affected tissues and shows impaired twitch and tetanic contraction compared to its isogenic control. Using our 3D-TESM model, we investigated the effect of several small molecules: A CK1 inhibitor, pamapimod (an analogue of losmapimod) and rebastinib, a small-molecule inhibitor of multiple tyrosine kinases. While showing promising results in 2D cultures we found a negative outcome for *DUX4* expression, morphology and contractile forces in our 3D-TESM model[1]. Currently, we are evaluating RNA based therapeutic approaches for FSHD and first results show the importance of the need for proper delivery methods towards skeletal muscle. With this research we lay the basis for the applicability of our 3D-TESM model as a potential tool in the pipeline of advanced in vitro testing of targeted therapies for FSHD.

P4.10 Increased *METTL3* expression and m6A methylation in myoblasts of facioscapulohumeral muscular dystrophy

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RNA methylation is a post-transcriptional modification that affects RNA stability, splicing, transport, and translation. The most common RNA modification in mRNAs is N6-methyladenosine (m6A), which is regulated by a set of proteins classified as writers, erasers, and readers. In this study, we detected increased mRNA and protein levels of the main m6A writer, methyltransferase-like protein 3 (METTL3), in immortalized human FSHD myoblasts compared to their unaffected siblings (UASb) (n=4). Additionally, total m6A quantification in the FSHD myoblasts was elevated compared to their UASb counterparts, which was reversed to the UASb levels following treatment with an antisense oligonucleotide targeting *DUX4* mRNA. Using Nanopore direct-RNA sequencing, we mapped m6A across the transcriptome and identified genes harboring differentially methylated m6A sites, including several involved in iron homeostasis. Western blotting was used to determine whether the modifications affect the protein levels of the modified transcripts. Our data showed higher levels of two proteins that exhibited differential m6A methylation in FSHD myoblasts. Furthermore, analysis of human FSHD muscle biopsies revealed differential expression of six m6A regulators, including writers, readers, and eraser proteins. These findings suggest that aberrant expression of *DUX4* may affect m6A RNA modifications and contribute to disease mechanisms in FSHD.

P4.11 DUX4 overexpression in proliferating myoblasts induces an early response of the metabolic sensor AMPK

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Among *DUX4*-induced pathophysiological mechanisms, increased attention is being given to metabolic stress and mitochondrial dysfunction characterizing FSHD muscle cells. Among the key regulators of these processes, a decrease in *PPARGC1A* expression, which encodes the transcriptional coactivator PGC-1α, was previously reported in immortalized FSHD patient-derived muscle cells. PGC-1α activity is dependent on post-translational modifications, notably phosphorylation by AMPK, a key metabolic sensor known to play a beneficial role in several myopathies. Here, given our expertise on AMPK, we further studied the impact of a boost of *DUX4* expression on the AMPK-PGC-1α axis with a first focus on the early response following the induction of *DUX4* expression in LHCN-M2-iDUX4 myoblasts treated with doxycycline (6, 18, and 24h). *DUX4* induction increased *ZSCAN4* (a direct target gene) and decreased *MYOD1* and *MYF5*, validating our myoblast model. Moreover, oxidative stress was measured after *DUX4* induction, and we saw a significant increase in our groups. Interestingly, *PPARGC1A* expression was increased under our experimental conditions along with upregulation of AMPKα2 and regulatory subunits β1 and β2 in induced myoblasts. This could be a result of a compensatory response to cope with mitochondrial alterations. Further analysis must be performed to determine the exact role of these key factors.

P4.12 Application of the Cuore to analyze effect of training on FSHD 3D tissue engineered skeletal muscles

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Our research group has developed 3D tissue engineered skeletal muscles (3D-TESMs) for modeling facioscapulohumeral muscular dystrophy (FSHD), a genetic muscle disorder characterized, amongst others, by asymmetric weakness of the facial, shoulder, and upper arm muscles. 3D-TESMs are generated from myogenic progenitors differentiated from human induced pluripotent stem cells originating from skin fibroblasts of three mosaic FSHD patients. Genetically affected 3D-TESMs recapitulate pathological features of FSHD which are *DUX4* and *DUX4* target gene expression and reduced contractile forces upon electrical stimulation. With no current *DUX4*-targeting treatment available for FSHD, training has become a promising approach for optimizing daily functioning and reducing pain and fatigue. Nevertheless, little is known about impact of exercises on mechanisms maintaining muscle capacity in FSHD patients. To explore this, I will investigate the effects of various muscle training regimens on FSHD 3D-TESMs cultured in the Cuore platform. The Cuore is a 3D muscle contractility modeling platform. It can electrically stimulate and real-time measure contractile forces of 24 3D-TESMs in a noninvasive manner during the whole life span in the incubator. Therefore, the platform can be used to expose 3D-TESMs to periodical electrical stimulations mimicking exercises. As an outcome, I will study the effect of exercises on 3D-TESMs maturation, morphology, strength, longevity, and disease hallmarks of FSHD.

P4.13 Baseline expression of DUX4-regulated pathogenic genes across 74 different human muscles in healthy controls

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Aberrant expression of *DUX4* is a well-established driver of facioscapulohumeral muscular dystrophy (FSHD). Previous studies have identified sets of up- and downregulated genes targeted by *DUX4*. However, most existing reference expression data originate from limited biopsy sites (e.g., quadriceps) or cell line models, potentially obscuring muscle-specific variation. To expand our understanding beyond these limitations, additional data on gene expression across different muscles are needed. Recently, CAGE-sequencing data from 222 samples representing 75 distinct muscles across four healthy autopsy donors were compiled in FANTOMUS, an atlas of transcribed regulatory elements and gene expression. With FANTOMUS data, we assessed the baseline expression levels of *DUX4*-targeted genes. Additionally, we identified a set of genes with the most divergent expression between muscles frequently affected in patients with FSHD and those rarely involved in the pathological process. For FSHD type 2, we found that SMCHD1 exhibited stable expression levels across muscles (mean = $\log_2$CPM=4.8; CPM [counts per million]; IQR [interquartile range: 4.57–5.05) in healthy controls, with no significant muscle-specific fluctuations. This stability supports the clinically indistinguishable presentation of FSHD types 1 and 2. We thank Gusev and Kulakovskiy for establishing an advance access to the FANTOMUS data, and Mr. Buan for his help in extracting the gene expression profiles of individual genes.

P4.14 Fibro-adipogenic progenitors and FSHD

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FSHD is characterized by the progressive accumulation of fibrous and adipose tissue in skeletal muscles. Fibro-adipogenic progenitors (FAPs) are muscle mesenchymal stem cells that have the potential to differentiate into fibroblasts and adipocytes. A better understanding of the mechanisms underlying FAP adipogenesis and fibrogenesis is essential for the development of therapeutic strategies for FSHD. Exosomes are extracellular vesicles important in cell-to-cell communication, and play a role in regulating muscle physiology and regeneration. Our hypothesis is that muscle *DUX4* expression alters the release from myofibers of exosome-mediated stimuli that regulate FAP adipogenesis and fibrogenesis. We developed FLExDUX4$^{-/+}$; ACTA-Cre$^{-/+}$; hCD63-GFP$^{-/+}$ transgenic mice that express both *DUX4* and green fluorescent protein (GFP)-labeled exosomes in muscle fibers after tamoxifen treatment. GFP+ exosomes are generated primarily in myosin heavy chain type IIb muscle fibers. *DUX4* expression resulted in reduced distribution of GFP+ exosomes. *DUX4* expression caused muscle necrosis and regeneration, leading to an increased number of FAPs and a broader distribution of perilipin+ muscle areas, indicating intramuscular fat accumulation. RNA isolated from flow-sorted FAPs and quadriceps muscles was processed for RNAseq. We also isolated total proteins from flow-sorted FAPs. We are currently performing transcriptomic and proteomic analyses.

P4.15 A discrete region of the D4Z4 is sufficient to initiate epigenetic silencing

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The transcription factor *DUX4* is typically expressed during the 4-cell stage of human embryogenesis, where it contributes to the initiation of a subset of genes involved in the first wave of embryonic transcription, critical for establishing totipotency. After this brief period of activity, the *DUX4* locus undergoes epigenetic silencing and remains transcriptionally inactive in nearly all somatic cell types. In facioscapulohumeral dystrophy (FSHD), mutations impair the mechanisms responsible for this silencing, leading to inappropriate expression of *DUX4* in skeletal muscle tissue. Although several factors involved in maintaining *DUX4* silencing in muscle have been identified, it has remained unclear whether particular sequences within the *DUX4* locus are sufficient to confer epigenetic repression. In this study, we cloned segments of the D4Z4 macrosatellite repeat – which contains the *DUX4* retrogene – adjacent to a constitutively active promoter driving a reporter. Through this approach, we identified a single fragment of the D4Z4 macrosatellite array capable of epigenetic silencing of reporter gene expression. This silencing effect required the activity of previously identified *DUX4* repressors, including SETDB1, ATF7IP, SIN3A/B, and LRIF1, while p38 MAPK inhibitors further enhanced repression. These results define a critical regulatory element within D4Z4 responsible for epigenetic silencing and provide a functional model for future mechanistic and discovery-based studies.

P4.16 Targeting DUX4 transcriptional activity with engineered DNA-binding repressors: A novel therapeutic approach for FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is a neuromuscular disorder caused by aberrant expression of the *DUX4* transcription factor in skeletal muscle. In adults, full-length *DUX4* (*DUX4*-FL) activates a toxic genetic program that drives muscle degeneration. Both the DNA-binding domain (DBD) and transactivation domain (TAD) of *DUX4*-FL are required for its pathogenic activity. We hypothesized that overexpression of *DUX4-DBD*, a truncated *DUX4* construct lacking the TAD, could compete for *DUX4*-FL DNA binding sites and thereby block aberrant activation of its downstream targets. To enhance repression, we engineered a *DUX4-DBD-KRAB* fusion incorporating the potent *KRAB* epigenetic silencer. We evaluated these constructs in HEK293T and C2C12 cells using a fluorescent *DUX4*-FL activity reporter, and found that increasing *DUX4-DBD* expression results in decreased reporter gene expression, confirming competitive inhibition. Further, *DUX4-DBD-KRAB* resulted in more potent repression, reducing reporter expression to near-background levels. Ongoing studies aim to evaluate these inhibitors in FSHD patient iPSC-derived myoblasts. Collectively, our data suggest that *DUX4-DBD*-based constructs, particularly combined with epigenetic repression, can effectively inhibit *DUX4*-FL transcriptional activity. This approach offers potential for a novel gene therapy approach cemented in the biological root of FSHD that does not require editing the genome or immunogenic proteins.

P4.17 Direct RNA sequencing reveals the altered epitranscriptomic landscape in DUX4-expressing myoblasts

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Misexpression of *DUX4* in skeletal muscle is considered the primary cause of FSHD. It disrupts multiple biological processes, ultimately leading to cell death. Altered RNA processing is a well-established hallmark of *DUX4*-affected muscle cells. While RNA modifications are emerging as key regulators of RNA metabolism, their involvement in *DUX4*-driven FSHD pathology remains unexplored. Reanalysis of *DUX4* ChIP-seq data revealed its regulation of a subset of genes involved in N6-methyladenosine (m6A), the most prevalent RNA modification in eukaryotic RNAs. *DUX4* binds upstream of these m6A machinery components, and they are significantly upregulated in *DUX4*+ myoblasts. Using Nanopore direct RNA-seq on *DUX4*-inducible myoblasts, we identified 4,248 and 3,270 m6A-modified genes in *DUX4*- and *DUX4*+ conditions, respectively. Notably, 25 out of 41 expressed core *DUX4* targets harbored m6A. Analysis of m6A dynamics revealed that among the genes expressed under both conditions, 733 genes gained m6A upon *DUX4* induction, and 365 of these showed significant upregulation in their expression. Additionally, among the genes uniquely expressed in the *DUX4*+ myoblasts, 834 contain m6A. Our findings indicate that *DUX4* not only alters the m6A modification patterns of genes already expressed, but also activates genes likely to carry m6A. Our study, for the first time, provides a transcriptome-wide view of m6A in *DUX4*-expressing myoblasts, offering a novel layer of insight into FSHD pathology.

P4.18 ***An update: Creating an immune cell atlas of the peripheral blood for facioscapulohumeral muscular dystrophy (FSHD)***

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The immune system is vital for effective skeletal muscle regeneration, with immune dysfunction known to impair regeneration and impact muscle wasting in chronic muscle disease. An immune infiltrate is present in muscle of FSHD patients, preceding the replacement of fat. However, very little is known about how the immune system influences disease pathology in FSHD. This lack of understanding has limited our ability to provide the best quality of care and hampers the development of the next generation of treatments for patients with FSHD. To address this, we have collected three yearly blood samples from the childhood-onset FSHD longitudinal outcome study (iFSHD-LOS). Applying high-dimensional spectral flow cytometry (44-color flow cytometry panel) to immune cell phenotypes, we can identify up to 100 subtypes in the peripheral blood. Compared to age-matched controls, our analyses have identified alterations in subtypes of innate cells, T cells, and B cells in patients with FSHD. The immune cell phenotyping is completed alongside the same blood samples for transcriptomic and plasma cytokine analyses to further understand immune dysfunction. These data are coupled with key clinical outcome measures to identify subtypes linked to disease progression and severity in patients. This study is halfway through mapping alterations in the immune system to create the immune atlas. Data generated will provide a greater understanding of the impact of the immune system in FSHD and provide targets for therapies.

P4.19 Targeting DUX4 mRNA with anti-TfR NANOBODY® oligonucleotide conjugates

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by epigenetic de-repression of the embryonic transcription factor gene Double Homeobox protein 4 (*DUX4*) in skeletal muscle, resulting in toxic expression of DUX4 protein and its downstream target genes, leading to weakness and atrophy in skeletal muscle, along with inflammation and fibrosis. Reducing DUX4 in skeletal muscle could alleviate disease. To better understand the kinetics of *DUX4* target-gene expression, we used a tamoxifen-inducible (TMX) transgenic FSHD mouse model in which the presence of TMX results in expression of human *DUX4* and mouse *DUX4*-target genes in skeletal muscle. We evaluated post-dose timepoints and saw the most change in *DUX4*-target gene expression occurred between days 7 and 12 post-TMX treatment. We then conjugated a tool *DUX4* siRNA to internally discovered mouse/human cross-reactive anti-TfR1 nanobodies for enhanced muscle delivery and examined in vivo efficacy at different time points post-dosing. Our NANOBODY® molecule-siRNA conjugates confer rapid and lasting *DUX4*-target gene knockdown. Lastly, we generated a humanized transferrin receptor FSHD mouse line in which we can evaluate *DUX4*-target gene reduction while evaluating anti-TfR nanobodies and antibodies that specifically target human TfR. In summary, we describe foundational studies for optimized readouts of *DUX4*-target gene expression and reduction in FSHD mice.

P4.20 Non-myogenic mesenchymal cells shape the degenerative microenvironment in FSHD patient muscles

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Understanding muscle degeneration in FSHD remains a major challenge. In skeletal muscle, multiple cell types cooperate to maintain tissue homeostasis; among them, we aim to define the role of non-myogenic mesenchymal cells in shaping the altered microenvironment driving FSHD degeneration. We have previously observed a significant accumulation of these cells in FSHD muscles, which positively correlates with the extent of intramuscular fibrosis, particularly in rapidly degenerating muscles. We are currently characterizing their in vitro properties by comparing non-myogenic mesenchymal cells isolated from muscles with (STIR+) and without (STIR-) signs of disease activity, as well as from healthy controls. Our analysis revealed enhanced proliferative capacity and altered adipogenic/fibrogenic differentiation potential in FSHD cells. Notably, only cells isolated from STIR- muscles promoted myoblast fusion into mature myotubes in co-culture, suggesting a supportive role in regeneration capacity. To better understand the dysregulated mechanisms, we performed bulk RNA sequencing followed by differential gene expression analysis. The results identified significant gene dysregulation among STIR+, STIR-, and control-derived cells, indicating selective activation of disease-related pathways and altered secretion of factors regulating muscle niche homeostasis. Ongoing enrichment analyses aim to pinpoint key mediators of FSHD pathogenesis, offering new targets for therapeutic intervention.

P4.21 Nucleolar FRG2 lncRNAs inhibit rRNA transcription and translation linking FSHD to dysregulation of muscle-specific protein synthesis

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The 4q subtelomere is organized into discrete chromatin domains with distinct gene expression profiles, which respond differently to chromatin-modifying treatments. Upon genotoxic stress, the 4q35 telomere-proximal genes FRG2A, DBE-T, and D4Z4-derived transcripts are upregulated in a manner inversely correlated with D4Z4 copy number and are stabilized post-transcriptionally. Among them, FRG2A shows the strongest response. FRG2A is a heterochromatin-associated lncRNA that localizes to the dense fibrillar component of nucleoli and associates with centromeric α-satellites and the rDNA intergenic spacer (IGS). FSHD myoblasts exhibit altered nucleolus-associated domains (NADs), with abnormal centromere-nucleolus contacts and increased H3K9me3 at the IGS, leading to reduced rRNA transcription and cytoplasmic translation. These alterations are reversed by FRG2A silencing. Elevated FRG2A levels alter 3D heterochromatin organization at the nucleolar periphery, impair rDNA transcription and cytoplasmic translation, and reduce skeletal muscle protein synthesis during differentiation. The human genome harbors 13 FRG2 paralogs expressed in a subject- and tissue-specific manner. FRG2A appears to belong to a novel family of lncRNAs that modulate nuclear architecture and epigenomic function, contributing to FSHD pathogenesis via disrupted protein synthesis and muscle wasting.

P4.22 The overexpression of the RNA binding protein FRG1 leads to reduced maturation and decreased metabolic efficiency in skeletal muscle

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The development of effective therapies for FSHD is hindered by its genetic complexity and high clinical heterogeneity. Although losmapimod initially was a promising molecule for treating FSHD, it failed to meet the primary endpoint in the Phase 3 REACH trial, and the company is suspending further development of the drug. As a result, no approved therapeutic interventions are currently available. A reliable preclinical model is required to test new effective therapeutic avenues for FSHD and to meet the needs of patients. Mice overexpressing *FRG1* (FSHD Region Gene 1) in skeletal muscles develop selective muscle weakness, fatigability, and appearance of a progressive dystrophic phenotype, characterized by fiber necrosis, decreased fiber size, and connective tissue deposition – all features common to the human disease. Longitudinal multi-omics analysis reveals two phases of disease progression in *FRG1* muscles: early, impaired maturation with metabolic defects; later, stress response activation preceding fiber inflammation and degeneration. *Cdkn1a* emerged as a promising candidate molecule in the transition between these two phases, and our studies demonstrated that its genetic ablation in *FRG1* mice avoided the development of chronic immune response. In conclusion, the induction of *FRG1* expression is causative of a myopathic phenotype that can be treated. These findings open new perspectives on the mechanisms underlying muscular dystrophies and possibilities for treating FSHD.

P4.23 DUX4 as a co-regulator of hormone nuclear receptors in human myoblasts

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Previous studies from our laboratory, using transfected model cell lines (HEK293, HepG2, and T47D), demonstrated that *DUX4* may function as a co-repressor of the progesterone (PR) and glucocorticoid (GR) hormone receptors, physically interacting with GR (Quintero et al., 2022). In additional experiments, we found that *DUX4* also acts as a co-regulator of the estrogen receptors ERα and ERβ. As an initial approach to investigate the impact of *DUX4* expression in the muscle of FSHD patients, we studied its potential co-regulatory activity on PR, GR, ERβ, and ERα in normal myoblasts transfected with *DUX4*, as well as in human myoblasts carrying a doxycycline-inducible *DUX4* transgene. We also developed proof-of-concept experiments, using fluorescent reporter transgenes, to study the impact of endogenously expressed *DUX4* on endogenous hormone receptors in *DUX4*(+) FSHD myoblasts. Preliminary in silico analysis of PR, GR, ERβ, and ERα mRNA levels, from single-cell and single-nucleus RNA-seq datasets (Chromium, SMART-seq, and ddSEQ) published by van den Heuvel et al., 2019; Jiang et al., 2020; and Zheng et al., 2024, shows that GR is the most consistently detected, ERα and ERβ are detected at low levels, and PR is virtually undetectable. GR, ERα, and ERβ levels vary between cultures, with no clear differences in FSHD versus control or DUX4(+) versus DUX4(−) cells. Our studies aim to highlight the potential endocrine role of DUX4 and its possible impact on the muscle endocrine physiology in FSHD.

P4.24 Role of miR-200c and oxidative stress in FSHD

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FSHD is an autosomal dominant disorder characterized by progressive weakness and impaired function of skeletal muscle, typically beginning in the facial, shoulder, and upper arm muscles. The most common type of FSHD (FSHD1) is caused by the dysregulation of the D4Z4 macrosatellite, which leads to the toxic misexpression of *DUX4*. The aberrant expression of *DUX4* determines apoptosis, myogenic defects, atrophy, and oxidative stress. In this regard, a more comprehensive investigation into the role of oxidative stress is urgently needed. The purpose of this study was to dissect the correlation between oxidative stress and FSHD, with a focus on the role of miR-200c, a microRNA strictly correlated with oxidative stress. Moreover, in a paper published by our group, we found that miR-200c overexpression in myoblasts led to myogenic defects. We found that miR-200c expression and oxidative stress were upregulated in immortalized FSHD1 myoblasts compared to the control. Additionally, by taking advantage of publicly available RNAseq datasets of muscle biopsies and immortalized human myoblasts from FSHD patients and healthy donors, we identified high-fidelity miR-200c targets de-regulated in FSHD, suggesting that these targets could play a role in FSHD pathogenesis. The future goal is the study of the effect of miR-200c inhibition in vitro by taking advantage of a locked-nucleic acid (LNA) anti-miR-200c, both in vitro, in human myoblasts, and in vivo, by using an animal model for FSHD.

P4.25 Structural basis for the interactions of DUX4 with Med25 and CBP/p300

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The interactions of *DUX4* with transcriptional activators drive its toxicity. We have previously found that the activation domain of *DUX4* interacts not only with CBP/p300, but also with subunit 25 of the Mediator complex. We ventured to establish the structural basis for these interactions using the experimental approach of chemical cross-linking and mass spectrometry, together with atomic structure prediction based on the AlphaFold platform. The crosslinks identified between *DUX4* and Med25 indicate that its activation domain has at least two structurally distinct binding conformations with Med25. However, one conformation is considerably more dominant and singly explains nearly all the high-intensity crosslinks. This conformation has an extensive inter-protein contact area and involves more than half of the circumference of the Med25 domain. Both canonical binding sites of Med25 are occupied by the two TAD elements from the start and end of the *DUX4* activation domain. Crosslinking between *DUX4* and CBP has identified the KIX domain of the latter to be the site of interaction, but follow-up mutation experiments strongly indicated that additional domain(s) of CBP/p300 are involved. Further characterization of this interaction is ongoing. Interestingly, we observed that immunodetection of p300 in human muscle cells showed a punctate nuclear or perinuclear staining in the majority of cells. Yet, following *DUX4* 6h-induction, nucleoplasmic staining became more pronounced.

P4.26 Evidence for the protective effects of estrogens in a mouse model of FSHD

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Epidemiological studies indicate females with FSHD are less affected than males, which we and others have observed in multiple mouse models of FSHD. We hypothesize these sex differences may be caused by higher circulating levels of estrogen in females, which is known to have beneficial effects on muscle health. In this study, we aimed to determine the effect of acutely elevating plasma 17b-estradiol (E2) in membrane repair capacity of muscles of un-induced male ACTA1-MCM/FLExDUX4 (DT) mice. Oral administration of 1.12 ug E2 for 7 days slightly (did not reach significance) reduced *DUX4* mRNA levels. Laser wounding assays indicated male DT mice (n=4) have significantly reduced sarcolemmal repair capacity compared to their wild-type (WT, n=3) littermates ($p < 0.05$), which was significantly improved by E2 treatment (n=4, $p < 0.05$). Likewise, high-resolution mitochondrial respirometry using the Seahorse XFe24 Analyzer indicated DT mice have significantly reduced skeletal muscle oxygen consumption rates, which were increased by E2 administration ($p < 0.05$). Furthermore, the male DT mice have significantly higher mitochondrial DNA content versus WT mice, which was significantly reduced toward WT levels following E2 administration. These results provide initial evidence that E2 may be beneficial for *DUX4*-affected skeletal muscle in vivo by increasing mitochondrial bioenergetics, enhancing mitochondrial quality control, and improving sarcolemmal repair.

P4.27 Non-viral generation of patient-derived iPSCs for modeling FSHD and screening nanoparticle-based therapeutics

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Facioscapulohumeral muscular dystrophy (FSHD) leads to muscle weakness linked to the aberrant expression of the *DUX4* gene. The mice cell line-based models fail to capture the disease pathology, as they lack the *DUX4* gene, and ectopic expression of human *DUX4* in these systems may not accurately mimic the endogenous regulatory context and pathological effects observed in patients. Therefore, this study aims to establish an in vitro model system based on lentiviral free episomal expression of Yamanaka factor's plasmid to make FSHD patient-derived iPSCs. These will be used to evaluate bio-compatible nanoparticle-based gene therapeutics targeting *DUX4*. At present, patient-specific iPSC lines have been successfully generated, and are being characterized for pluripotency and genomic integrity. Future work will involve non-viral, short, and reproducible protocols for differentiation of iPSCs into myogenic lineage and non-viral delivery of antisense oligonucleotides or siRNA for functional studies. This model will lay the foundation for developing personalized therapeutic approaches and improving our understanding of FSHD pathogenesis. A presentation at IRC will highlight the urgent need for palliative strategies in FSHD care. Addressing these unmet needs will improve quality of life for individuals with advanced FSHD and provide data for economic modeling under Project Mercury.

Session 5: Clinical Care and Related Issues

P5.01 Neuropsychological profiles of children and young people with childhood-onset facioscapulohumeral dystrophy (FSHD)

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FSHD is an autosomal dominant muscular dystrophy, with a prevalence of 1 in 8,000. Symptom onset in childhood (<18 years), though likely underestimated, is reported in around 20% of cases. While adult studies suggest cognitive impacts, few have examined cognitive and psychological profiles in childhood-onset FSHD. Some research suggests that early-onset FSHD, defined by short repeat length and/or symptoms under 10 years, is associated with increased cognitive difficulties. This study aimed to establish the cognitive and behavioral profile of children, adolescents, and young

people (CYP) with FSHD. This prospective single-site cohort study was part of a larger Australian pediatric FSHD longitudinal outcome study. Participants included CYP with childhood-onset FSHD. Formal tests included detailed IQ, executive function, and mental health. Participants included 21 CYP (6-19 years) with FSHD1. Eleven had 1-3 D4Z4 repeats and met the criteria for early-onset classification. Cognitive and executive function was generally intact with some specific verbal comprehension and affect recognition differences observed. Few differences were seen between early-onset and classic-onset. Mental health outcomes suggest a vulnerability to anxiety and depression. This study suggests that IQ and verbal comprehension should be confirmed in a larger group, and that children and young people should be monitored regularly for mental health concerns.

P5.02 FSHD UK: From voluntary group to a registered charity creating a strong multi-stakeholder group to strategically drive clinical trial readiness and co-ordination of FSHD activities in the UK

Rajeshri Badiani

FSHD UK, United Kingdom

The UK has a strong reputation for high-quality clinical services and research for people with FSHD. However, these individually strong centres were mainly working in their own areas without an overarching strategy or central co-ordination. In July 2021 FSHD UK was formed with a strong presence from key stakeholder groups, including clinicians, patients, researchers, the UK FSHD Registry, MDUK, and the FSHD Society. The FSHD UK mission was and is to ready the UK for clinical trials and to become the recognised coordinating group for key FSHD activities in the UK. Within two years, we have clinical trials in the UK plus natural history studies. The way we are organised and the delivery of our engagement strategy have helped drive participation in some key areas of work with major stakeholders. By sharing our approach, our desire is to demonstrate how a small start-up group with no funds can form a co-ordinated task force to make a difference in patient engagement, invite clinical trials, and collaborate extensively with national and international organisations to force the FSHD agenda. Our future focus remains around our key areas of patient engagement in readiness for clinical trials, strengthening our clinician and research network and continuing to work with other FSHD organisations for unmet needs.

P5.03 Strengthening regional neuromuscular care in the Netherlands: Preparing for future FSHD trials and care demands

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In the Netherlands, a nationwide neuromuscular care network is being developed to improve the quality, accessibility, and sustainability of care for people with rare neuromuscular diseases, including facioscapulohumeral muscular dystrophy (FSHD). A defining feature of this initiative is the active involvement of individuals with FSHD and their representatives in shaping the future of care. Together with clinicians and researchers, they co-design care pathways, identify barriers, and set priorities such as access to expert knowledge, interdisciplinary coordination, and continuity of care. A central goal is to strengthen regional neuromuscular centers so that they can provide high-quality care closer to patients' homes and contribute meaningfully to clinical trial readiness and follow-up care. This approach fosters close collaboration between regional teams and national expert centers, and ensures that trial-related infrastructure, knowledge, and support are available across the country. With the increasing number of FSHD patients expected to enter care due to diagnostic improvements and upcoming therapeutic trials, preparing the healthcare system is urgent. By investing in professional training, shared protocols, digital tools, and patient empowerment, the Dutch network aims to create a future-proof, inclusive model for neuromuscular care – one that balances excellence in clinical trials with everyday care needs for people living with FSHD.

P5.04 Deviating observations when comparing D4Z4 FSHD repeat analysis on the Bionano OGM platform with the linear Southern blot in FSHD diagnostics

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The molecular diagnosis of FSHD type 1 relies on detecting contractions of the D4Z4 microsatellite repeat array at the chr4q35 locus in the presence of a permissive 4qA haplotype. A similar D4Z4 repeat-like structure of the 4qA-type is present on chr10. Several recombination events between these high homologous D4Z4 repeats have been described. Distinction between these compound repeats and the identification of the pathogenic FSHD causing alleles can therefore be a challenge. We validated the use of optical genome mapping (OGM) in a diagnostic setting for testing FSHD cases and compared OGM performance with that of the previously used Southern blotting (SB) with also a focus on elucidating recombination events and other deviating repeat profiles. A total of ~70 samples were tested with both techniques. In the majority we confirmed the observation that was seen on the linear SB (L-SB). Repeats in the range of 1 up to 87 repeat units (RU) could be identified including the 4qA haplotype. However, in some cases the OGM test revealed apparently non-matching repeat results. We will present some of these deviating, often complex results, including an apparent false negative OGM result for a short 4qA D4Z4 repeat that is detected by L-SB, but also the disappearance of an assumed short 4q35 repeat detected by SB and is most likely derived from a large hybrid repeat band. These observations indicate special care with final diagnostic conclusions and the high relevance to always include clinical features when requesting diagnostic FSHD testing.

P5.05 The True Cost of FSHD: A burden of illness study of facioscapulohumeral muscular dystrophy patients in the United States

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant inherited condition that causes lifelong progressive muscle weakness. Despite the significant disease burden, little has been published on the costs associated with a diagnosis of FSHD to patients and their families. While cost of illness studies of FSHD have been performed in Europe, no comprehensive studies have been published in the United States. To our knowledge, efforts that are underway in the United States often only look at insurance claims data. However, these studies underestimate the full economic impact of FSHD because they do not account for out-of-pocket expenses, such as home modifications and special transportation needs, and other indirect costs such as absenteeism and lost opportunities. To better understand the full economic impact of FSHD over the course of a lifetime in the United States, we conducted a survey of the patient community to understand the cost burden carried by patients and families. We report here results from the survey, representing 354 FSHD patients residing in 312 households across the United States, including direct medical, direct non-medical, and indirect cost burden carried by patients and families. The results of this study will help ensure the true burden of FSHD is recognized, supporting advocacy and informing payor and incremental cost-effectiveness ratio (ICER) evaluations of future therapies.

P5.06 Project Mercury: A global platform for accelerating therapeutic development and patient access in FSHD

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Companies developing drugs in rare disease face well-known challenges in clinical trials and global patient access. These can include identifying and optimizing clinical trial sites, defining outcome measures, engaging the patient community, and educating health technology assessment (HTA) bodies and payors on burden of disease and unmet medical need. In response to these challenges, the FSHD Society recently launched Project Mercury – a multi-stakeholder initiative to solve challenges in FSHD clinical trial readiness and therapeutic access through global collaboration. Project Mercury is governed by patient advocacy organizations representing 10 countries; includes representation across FSHD key opinion leaders, subject matter experts, and pharmaceutical companies; and is supported by program management from the FSHD Society. Since its launch in 2023, Project Mercury has succeeded in creating alignment and momentum among numerous global stakeholders; launched key collaborative projects, including an update of the FSHD registry dataset, the creation of an FSHD disease model for HTA decision-making, and the creation of training toolkits for patient advocacy organizations; and helped obtain funding for these projects via a collaborative European Union Horizon grant. Altogether, Project Mercury aims to accelerate and de-risk the delivery of therapeutics for FSHD globally. Project Mercury is also a framework that other rare disease organizations can employ to advance similar goals in their disease areas.

P5.07 AI-driven innovation in FSHD: Next-generation support for patients and providers?

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The increasing integration of artificial intelligence (AI) in medicine is fostering new models of clinical support, with ChatGPT enabling human-like interactions across a range of tasks. Although it does not replace medical professionals, it can support healthcare providers and patients. In complex conditions like facioscapulohumeral dystrophy (FSHD), AI-driven tools may help bridge existing knowledge gaps. In this context, an AI-driven chatbot (AI for FSHD Care) was developed using the GPT-4o model (OpenAI) to provide reliable information on FSHD. The chatbot was trained on the medical and scientific literature from the past 15 years. Through the GPT Builder, the chatbot was customized with precise instructions to tailor interactions to different user types (patients or healthcare professionals). It can also extract relevant content from user-uploaded documents to generate accurate, context-specific responses. The chatbot adjusts language and content depth based on the user. For patients it provides accessible answers on genetic risk, diagnosis, testing centers, and patients' support organizations. For healthcare providers it delivers detailed information on diagnostics, genetic markers, and FSHD clinical guidelines. Overall, AI for FSHD Care is a user-friendly tool that supports both patients and professionals by delivering structured, reliable information and reducing the need to navigate incomplete or outdated sources.

P5.08 Co-occurrence of anti-AChR myasthenia gravis in facioscapulohumeral dystrophy patients: A case series

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Facioscapulohumeral dystrophy (FSHD) and myasthenia gravis (MG) are well-known rare neuromuscular diseases of genetic and acquired origin, respectively. Emerging evidence supports the possibility that FSHD patients having 7-10 repeat units (RU) might require a second condition, either genetic or acquired, to fully express the clinical FSHD phenotype. A recent study showed that among muscular dystrophies, the co-occurrence of MG with FSHD is the most common. Here, we present a retrospective study on FSHD patients with a concomitant diagnosis of anti-AChR MG followed at the Nice University Center from 2020 to 2025. We identified 10 patients (7 females) with a mean age of 70.8±6.7 years at last examination. All patients have a D4Z4 4qA allele of 7-10 RU, a disease onset >45 years, and a mean FSHD score of 9.6±1.95 at the last visit. Apart from uncommon features related to the MG, the phenotype was consistent with FSHD in all cases. The mean age of MG diagnosis was of 68.5±7.6 years. All patients resulted positive for anti-AChR antibodies. All patients benefited from therapy for MG with clinical improvement on MG-Activities of Daily Living (-2.7±1.3) and in 4 of them, unexpectedly, also in the FSHD score (-1.5±0.57). Although we do not know the prevalence of the co-occurrence of MG in FSHD patients, our results underline the need of careful clinical evaluation especially in FSHD patients with borderline RU to exclude the coexistence of other treatable neuromuscular conditions.

P5.09 Starting the Italian Registry for Facioscapulohumeral Muscular Dystrophy

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Disease registries are increasingly recognized as essential tools for characterizing natural history, sharing data within the scientific community, facilitating joint studies, defining standards of care, and preparing for clinical trials. In 2009, Fondazione Telethon, along with several associations of neuromuscular patients, established a legal entity called the Association of the Registry of Patients with Neuromuscular Diseases (ADR). Adopting the guidelines proposed by the international TREAT-NMD network, the ADR has promoted the creation of disease-specific registries, integrating them into an IT platform known as the NMD Registry (www.registronmd.it), compliant with national legislation and General Data Protection Regulation. In Italy, since 2009, the Fondazione Telethon-UILDM projects have funded the creation of a national database dedicated to the collection of clinical and genetic data in FSHD. This initiative has led to several publications on genotype-phenotype correlations in this field. Currently, the Italian Clinical Network for FSHD, established under the auspices of the Italian Association of Myology, is working on the creation of the Italian FSHD registry on the NMD (neuromuscular disease) Registry platform, aiming to collect findings from a large cohort of Italian patients diagnosed with FSHD1 or FSHD2. The enrolled patients will be clinically characterized using an updated version of the FSHD Comprehensive Clinical Evaluation Form with both retrospective and prospective data being collected.

P5.10 Late-onset facioscapulohumeral muscular dystrophy as a differential diagnosis issue

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The aim of this report is to highlight atypical features in late-onset FSHD, which challenges with diagnosis. Medical records of 145 FSHD patients in our center were reviewed retrospectively for those whose symptoms begin at age 40 or older. Patients with genetically confirmed FSHD and patients with category A in the Comprehensive Clinical Evaluation Form (CCEF) with pending genetic analysis were included. Nine (6 females, 3 males) patients (6%) met the study inclusion criteria. Median age at first symptom was 43 (40–53) years and at last visit was 59 (43–73) years. First symptom was related to lower proximal weakness in four patients, shoulder abduction in another four, and tiredness in upper extremity in one. None of those patients presented with facial weakness, and all but one showed asymmetrical facial weakness at last visit. All patients were ambulatory with a median FSHD severity score of 6 (0–7). One patient with pelvic girdle onset had an immunosuppressive treatment for two years with a misdiagnosis of rheumatoid arthritis. Genetic analysis revealed 4qA haplotype with decreased repeat unit (RU) of D4Z4; 7 RU in four patients, 6 in one and 8 in one. One patient showed somatic mosaicism for D4Z4 RU (1-2 RU; 51%). Optical mapping analysis was pending for two who met the CCEF as category A1. Late-onset FSHD may present in women more frequently with atypical symptoms, such as pelvic girdle involvement, leading to misdiagnosis at disease onset until the pathognomonic features of FSHD become overt.

P5.11 Development of a disease progression model for FSHD to support health technology assessment

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Facioscapulohumeral muscular dystrophy (FSHD) is a rare, progressive disease with no approved therapies. As treatments advance, models of disease progression are needed to support Health Technology Assessments (HTA) globally. As part of Project Mercury, a global initiative to speed patient access to future approved therapies, this study assessed the feasibility of developing an FSHD progression model suitable for economic evaluation. We reviewed literature, clinical trials, and three patient registries (UK, Netherlands, Canada), alongside observational (MOVE, ReSOLVE) and interventional studies (e.g., REINFORCE) to understand outcome measures used. No single progression measure is universally used; however, emerging outcomes like the FSHD Composite Outcome Measure may offer promise. Registries provide long-term data to define health states, but mapping will be required to link with clinical trial outcomes. A proposed path forward includes defining health states from registry data, estimating transitions, mapping clinical outcomes, and incorporating resource use and quality of life. This work outlines a roadmap for model development and highlights key gaps and opportunities for advancing HTA readiness in FSHD.

P5.12 Sexual health and pelvic floor function in women with facioscapulohumeral dystrophy

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Limited data exist regarding sexual health and pelvic floor function in individuals with facioscapulohumeral dystrophy (FSHD). This study aimed to describe both in individuals with FSHD. Adults with FSHD and female sex assigned at birth were recruited from the National Registry. Surveys including the Female Sexual Function Index (FSFI), Urinary Distress Inventory (UDI), Pelvic Organ Prolapse Distress Inventory (POPDI), Colorectal-Anal Distress Inventory (CRADI), and Pelvic Floor Distress Inventory (PFDI) were completed. Higher scores suggest more dysfunction in all indices except FSFI. Total FSFI and domain-specific FSFI scores were compared to historic controls using two-sided t-test and linear regression analysis. Of 109 respondents, median IQR (interquartile range) FSFI total score was 18.8 (23.1). Fifty-one individuals (47%) met criteria for female sexual dysfunction and 99 (91%) for hypoactive sexual desire disorder. Median (IQR) for PFDI, POPDI, and CRADI were 35.42 (70.8), 12.5 (31.3), and 12.5 (33.3), respectively, reflecting mild distress range, while UDI was in the no distress range. Individuals with FSHD scored lower on FSFI total score and in all FSFI domains compared to controls ($p < 0.0001$). Individuals with FSHD report higher rates of female sexual dysfunction, specifically hypoactive sexual desire disorder. There was no significant burden of pelvic floor dysfunction reported. Further evaluation using multivariate analysis is planned.

Session 6: Clinical Studies and Trial Designs

P6.01 Making strength training work in FSHD

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This mixed-methods study explored the potential of strength training in individuals with FSHD through two complementary parts. Part 1 (qualitative research) applied Interpretative phenomenological analysis to in-depth interviews with 10 individuals with FSHD: Five who successfully continued strength training and five who had stopped. We found that a prerequisite for strength training is having a good balance across the week. The training must be tailored to the individual's aims, physical condition, and personal context. In addition, a sustaining factor is needed such as social reasons, perceived benefits, or other intrinsic motivation. Participants expressed a need for control during training: Those with strong body awareness want to regulate their own sessions, while others want to rely on external support (e.g., a trainer or therapist). Part 2 (quantitative research) included measurements of 10 individuals with FSHD in their own training environment, including full-body electromyography measurements, submaximal tests (Repetition Maximum test [RM]), and a fatigue assessment. Results showed that these participants train at intensities comparable to healthy controls. Those patients training at a higher intensity (%RM) report a greater perceived benefit. Our findings highlight that patient insight and self-awareness are crucial for safe and effective strength training in FSHD. Contrary to earlier assumptions, training at a higher intensity (%RM) appears safe and potentially more effective.

P6.02 Improving routine care for facioscapulohumeral muscular dystrophy: Effectiveness of personalised antioxidant supplementation on muscle strength and quality of life in a real-world setting

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Facioscapulohumeral muscular dystrophy (FSHD) is a genetic disorder that leads to progressive muscle weakness and is commonly associated with oxidative stress. The PERSPECTYV FSHD initiative aimed to assess the efficacy of individualized antioxidant supplementation on the maximal voluntary contraction of the quadriceps (MVC_{QD}), physical activity, and quality of life (QoL) in patients aged 15-76 with FSHD1. This real-world study (NCT02622438), conducted at Montpellier University Hospital, used an innovative decision-tree algorithm (pending patent) designed to provide personalised dosage recommendations based on individual needs, transferred to KONDREE for further development. A total of 107 patients completed the one-year visit and 74 continued supplementation for two additional years, with the MVC_{QD} as the primary outcome measure. Secondary endpoints included physical activity and QoL metrics. Participants receiving personalized supplementation, including vitamins C and E, zinc, copper, and selenomethionine, significantly increased MVC_{QD} compared to baseline at years 1 (+9.1%), 2 (+14.1%), and 3 (+13.9%; $p < 0.001$). Additionally, significant enhancements in physical activity levels and QoL metrics were reported by participants. These findings underscore the substantial impact that personalized antioxidant therapies can have on mitigating oxidative stress and improving clinical outcomes for patients with FSHD1, highlighting the importance of addressing oxidative stress.

P6.03 A toolkit for new facioscapulohumeral muscular dystrophy trial sites

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Numerous potential treatments are being developed for facioscapulohumeral muscular dystrophy (FSHD). Project Mercury was initiated to overcome challenges that could slow or prevent effective therapies from widespread availability to patients. It is important that upcoming trials include trial sites from different countries. We share our lessons learnt in clinical trials to assist inexperienced sites to become eligible for upcoming clinical trials. To become an eligible site, several key elements need to be in place such as personnel, facilities, and accessible patient populations. Clinical trial networks, patient advocacy groups, and patient registries can support new sites in establishing these elements. As the preparation, execution, and close-out of clinical trials generally involve the same steps every time, it is recommended to create and follow a trial roadmap. Most clinical trials are sponsor-initiated and involve working closely with the sponsor and vendors. It is therefore important to understand each other's perspectives and goals for each trial. Once a drug receives regulatory approval and becomes available for market use, new challenges arise such as patient reimbursement and Phase 4 surveillance of the patients. In summary, we are at a pivotal time for FSHD and other rare neuromuscular disorders with the development of new disease-modifying therapies. It is vital that as many sites as possible can participate in upcoming trials.

P6.04 The FOCUS 3 study protocol: Ten-year follow-up in facioscapulohumeral dystrophy

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Facioscapulohumeral dystrophy (FSHD) is a chronic, slowly progressive muscle disorder with a highly variable phenotype. The clinical variability is not fully explained by the currently known (epi)genetic or environmental factors. Due to this variability, we cannot adequately predict the expected disease course in individual patients, and it complicates the design of clinical trials, as it either leads to large sample size requirements for trials or only includes highly selective subgroups of patients. Long-term natural history data are essential for interpreting results, adequate counseling, and design of future clinical trials. We therefore aim to assess the sensitivity to change of functional and clinical outcome measures and biomarkers, and identify patterns in disease progression to predict personalized disease trajectories and identify disease modifiers. We will include 30 pediatric and 170 adult FSHD patients, including patients who have previously participated in our natural history study and 65 new participants. Disease progression will be assessed using clinical outcome measures such as the Motor Function Measure D1, Ricci score, Medical Research Council score, and FSHD clinical score. Additional assessments include muscle MRI and ultrasound, blood sampling and skin biopsies for (epi)genetic analysis and biobanking. The results of this study are expected to contribute to patient counseling and will improve clinical trial design, facilitating the pathway to the first FSHD-specific therapies.

P6.05 Optimising clinical development programs for facioscapulohumeral muscular dystrophy (FSHD) therapies in preparation for Joint Clinical Assessment (JCA) in Europe

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Facioscapulohumeral muscular dystrophy (FSHD) is a rare, progressive neuromuscular disorder with no approved therapies. From 2028, the European Joint Clinical Assessment (JCA) will become mandatory for orphan drugs, making early preparation essential for manufacturers developing therapies for FSHD. A central aspect of JCA is the selection of population, intervention, comparator, and outcome (PICO), agreed upon with all European Union (EU) member states. Once finalized, manufacturers have only 100 days to submit a complete dossier. Early planning for all plausible PICOs is essential for efficient dossier development and successful pricing, reimbursement, and market access (PRMA). This research aims to identify potential PICOs for a novel FSHD therapy, to inform and support both current and future clinical development programs. A pan-EU landscape assessment was conducted for FSHD to explore possible PICOs relevant to FSHD. Population segments were identified based on genetic subtype, age, disease severity, and biomarker status. The intervention is a hypothetical novel FSHD therapy. As no approved treatments exist, best supportive care was deemed the most appropriate comparator. Outcomes included clinical and patient-reported measures. Aligning PICOs with validated outcomes and molecular insights can optimise FSHD trial design and therapeutic evaluation, supporting regulatory and PRMA success.

P6.06 The FSHD European Trial Network

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FSHD Europe is the voice for FSHD patients across Europe, representing 14 national patient organizations from 12 different countries, and aims to build capacities and strengthen volunteer leadership across Europe. Developments within the international FSHD field are moving quickly. A drastic increase in the number of trials is expected, indicating the urgency of trial readiness, and the importance of Project Mercury in which FSHD Europe is partnering with FSHD Society, FSHD Global, FSHD Canada, and FSHD Brasil (ABRAFEU). Performing trials in Europe is challenging because of Europe's diversity and multilingual situation. Guidelines for clinical trials, pharma regulation, and health care provisions in European countries differ in various ways. Therefore, the FSHD European Trial Network (ETN), initiated by FSHD Europe aims to increase the commitment of clinicians and researchers; harmonize criteria for clinical and genetic diagnosis, clinical outcome measures, biomarkers, and imaging outcome markers; exchange clinical experience and genetic reference material; engage with pharma and the European Medicines Agency; and harmonize treatment and care for all European FSHD patients. The ETN consists of five working groups (WG) on clinical and genetic

diagnosis (WG1), clinical outcome measures (WG2), biomarkers (WG3), imaging outcome measures (WG4), and childhood-onset FSHD (WG5). The ETN WGs work in close collaboration with the CRTN, FSHD Society, TREAT-NMD, the European Reference Network EURO-NMD and the European Neuromuscular Centre.

P6.07 Comorbidities, medication use, and adverse events in FSHD patients: Insight from the ReSOLVE and MOVE studies

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While treatment options in FSHD are expanding, limited data exist on FSHD-related comorbidities. The aim of this study is to assess comorbidities, medication use, and adverse events in FSHD patients from the ReSOLVE and MOVE cohorts, which reflect clinical trial eligibility criteria. Participants were enrolled in two large natural history studies (ReSOLVE: n=303; MOVE: n=404). Comorbidities were self-reported, medications were categorised by use, and adverse events were tracked over two years. In ReSOLVE (mean age 50.3), common comorbidities included pulmonary (19%), cardiovascular (14%), hearing (17%), and vision (22%). Breathing devices were used by

44%, and hearing aids by 33%. Medications included supplements (33%), analgesics (26%), and cardiovascular drugs (19%); 72% of analgesic use was for FSHD-related pain. In MOVE (mean age 49.2), comorbidities were pulmonary (25%), cardiovascular (19%), hearing (16%), and vision (22%). Breathing devices were used by 52%, and hearing aids by 30%. Medications included supplements (39%), analgesics (26%), and cardiovascular drugs (24%); 25% of analgesic use was for FSHD-related pain. In ReSOLVE, 27% experienced adverse events, mainly musculoskeletal issues (17%), falls (11%), and bruising (5%). Cardiorespiratory comorbidities and device use were common. Analgesic and supplement use was high, particularly in those meeting trial criteria (ReSOLVE cohort). Findings can inform clinical trial design and care planning.

P6.08 A hub-and-spoke model for the French National FSHD Registry: A 2025 update

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Since 2013, the French national FSHD registry – now counting over 1,300 patients – has collected data from both patients and clinicians to serve multiple objectives. Designed to accelerate trial readiness, it is key to refining protocols and rapidly assembling cohorts. Two natural history studies, linked as satellite projects, aim at developing outcome measures for FSHD1 ambulant (NCT04038138) and non-ambulant (NCT05453461) patients. Pediatric and FSHD2 modules are projected. An ancillary project is underway to validate novel biomarkers through real-world data collection. As FSHD's wide phenotypic variability must be thoroughly characterized for future trials, a registry analysis identified atypical features in 19.6% of cases, mainly a mismatch between severity and D4Z4 repeat number (41.7%) and unusual muscle involvement patterns (21.7%). Unexpected features emerged. Higher repeat numbers and older age at onset were associated with atypia. These findings will help refine the classification of atypical manifestations and inform international standards, crucial for enhancing diagnostic accuracy and guiding future therapies. High-quality data are driving machine-learning applications, including predictive models of progression. Going forward, a dedicated smartphone app will boost participation, enable novel data capture, and strengthen the registry's role as both an AI-ready resource and an educational bridge between patients and physicians. This work was supported by the AFM-Téléthon.

P6.09 Early-onset FSHD: An Italian case series

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Early-onset FSHD is defined by Brouwer criteria: Facial weakness before age 5, plus signs and symptoms of shoulder girdle weakness before age 10. These account for about 10% of all FSHD cases. This form is associated with a more severe prognosis, faster progression, and higher rates of extra-muscular symptoms such as hearing and retinal abnormalities, epilepsy, cognitive impairment, cardiac disorders, and respiratory dysfunctions. Shorter D4Z4 fragments have been linked to early-onset FSHD, though a 2015 study by Nikolic et al. reported unaffected individuals carrying very short fragments within affected families. To explore the characteristics of Italian patients, the Italian Association of Myology conducted a multicentre data collection across 14 referral centers. Data from 69 patients aged 8–65 years, all with symptom onset before age 10, were analyzed. D4Z4 fragment length was ≤14 kb in 36.2% cases and >14 kb in 44.9% (including one case >38 kb). De novo mutations occurred in 53.6%. Among the cohort, 1.4% were deceased and 10.1% had lost ambulation. Extra-muscular involvement included hearing loss (29%), respiratory dysfunction (17.4%), cognitive impairment (14.5%), retinal changes (8.7%), cardiac issues (5.8%), and epilepsy (5.8%). Despite being a retrospective study, this study aligns with existing literature and reflects data of most of the Italian referral centres for neuromuscular disorders.

P6.10 The landscape of FSHD data collection: A 2025 expansion to the TREAT-NMD FSHD dataset

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The TREAT-NMD Global Registry Network connects independent patient registries aiming to accelerate treatment to neuromuscular patients, including those with FSHD. Currently, 27 FSHD registries collect 46 core data items, but as clinical trials advance toward marketable treatments, consensus from the registries states that the current dataset no longer fully meets stakeholders' needs.
We have reviewed the current TREAT-NMD FSHD dataset components and compared these to data items currently being collected by registries within the network. We identified the overlap between TREAT-NMD registries and the current dataset, showcasing harmonised data available in registries globally, and assessed the most and least common items collected. We also identified the most collected items not currently present in the standard TREAT-NMD FSHD dataset. This analysis reveals potential areas for dataset expansion and provides insights for developing a dataset that supports post-marketing surveillance studies and bridges the gap between existing and future data needs. TREAT-NMD, building on its previous success of expanding neuromuscular disease datasets through a consensus-based approach, will now support work that is moving toward delivering a FSHD dataset able to meet the needs of multiple stakeholders. This will be conducted in conjunction with key opinion leaders in the field of FSHD, including the TREAT-NMD FSHD registries, patient groups, academics, clinicians, and industry representatives.

P6.11 The natural history of childhood-onset FSHD in an Australian cohort: iFSHD-LOS 2-year data

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Disease progression in childhood-onset FSHD remains poorly elucidated. Improved understanding of how to measure change in function and quality of life (QoL) is required to support design of pediatric clinical trials. This single-site Australian study evaluated prospective longitudinal outcomes in pediatric FSHD over 2 years. Performance-based function (FSHD-COM Peds, PUL, 6MWD, timed function, muscle strength, reachable workspace), self-reported QoL (pain, fatigue, disease burden), and biomarker data (muscle MRI and iPSCs) were evaluated on three occasions 12 months apart. Nineteen children and young adults aged 6-19 years with disease symptoms under 18 years completed the study. Males made up 58% of the cohort. Ten met the criteria for early-onset (1-3 D4Z4 repeats), eight classic-onset (4-7 D4Z4 repeats); one without genetic data was included in classic-onset group due to mild symptoms. In all, 95% had facial and scapula weakness, 74% positive Beevor sign, and 47% pelvic weakness at baseline. In addition, 32% had sensorineural hearing loss, which was only present in early-onset participants. Early- and classic-onset group results indicated mean changes in measures of severity, strength, timed function; QoL worsened, and falls increased in the early-onset group, with relative stability in the classic-onset group. These observational results support the hypothesis that children with early-onset FSHD are more severely impacted by disease progression.

Session 7: Late-Breaking Abstracts

P7.01 Non-viral generation of patient-derived iPSCs for modeling FSHD and screening nanoparticle-based therapeutics

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Facioscapulohumeral muscular dystrophy (FSHD) leads to muscle weakness linked to the aberrant expression of the *DUX4* gene. The mice cell line-based models fail to capture the disease pathology, as it lacks the *DUX4* gene and ectopic expression of human *DUX4* in these systems may not accurately mimic the endogenous regulatory context and pathological effects observed in patients. Therefore, this study aims to establish an in vitro model system based on lentiviral free episomal expression of Yamanaka factor's plasmid to make FSHD patient-derived iPSCs. These will be used to evaluate bio-compatible nanoparticle-based gene therapeutics targeting *DUX4*. At present, patient-specific iPSC lines have been successfully generated and are being characterized for pluripotency and genomic integrity. Future work will involve a non-viral, short, and reproducible protocol for differentiation of iPSCs into myogenic lineage and non-viral delivery of antisense oligonucleotides or siRNA for functional studies. This model will lay the foundation for developing personalized therapeutic approaches and improving our understanding of FSHD pathogenesis.

P7.02 Genetic and epigenetic profiling for FSHD diagnosis using nanopore sequencing

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The genetic diagnosis of FSHD poses a significant challenge. It has been shown that the distal methylation of the D4Z4 repeat of chromosome 4q35 serves as the most reliable marker of disease status and severity. It is influenced by numerous factors, like a contraction of the D4Z4 repeat array (FSHD1) or pathogenic variants in epigenetic suppressor genes (FSHD2). We developed a bioinformatics pipeline based on Oxford Nanopore Technologies (ONT) targeted long-read sequencing for the analysis of allelic haplotypes, repeat unit counts, and allele-specific methylation. We used this workflow for a detailed characterization of 10 patients with FSHD1, two with FSHD2, and two with both FSHD1 and FSHD2. ONT-based sequencing improves the FSHD diagnosis by enabling the detection of allele-specific methylation. Additionally, the analysis of the methylation along the entire repeat shows a gradient which increases toward the distal end. Our data support a lower slope in FSHD2 compared to FSHD1, which could explain why FSHD2 can develop in patients with >11 repeat units but also has an upper limit of ~20 repeat units. Our results reinforce the value of methylation profiling as the most precise biomarker for FSHD diagnostics and suggest the existence of additional unknown methylation modifying factors. The integration of them using long-read sequencing improves diagnostic accuracy and broadens our understanding of the pathomechanisms of FSHD.

P7.03 FSHD-SUM (summarized unified measure) PROM – should we pursue this?

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For facioscapulohumeral muscular dystrophy (FSHD), existing disease-specific outcome measures address distinct aspects of disease burden but can be cumbersome to administer collectively and are time consuming. Within the framework of the EU Horizon 2022 funded PaLaDIN project, we developed a new FSHD-SUM (summarized unified measure) using an AI mapping approach incorporating analyses of four independent agentic AI systems (ChatGPT, Claude AI, Gemini AI, Apple AI). This 15-item patient-reported outcome measure (PROM) synthesizes by cross-mapping domains from FSHD-RODS, FSHD-HI2, PROMIS, SF-36, UEFI, and FDI. The instrument assesses mobility, upper limb function, activities of daily living (ADLs), fatigue, pain, psychosocial health, and body image on a 6-point Likert scale. Total and domain scores are normalized on a 0–100 scale. Integration with REDCap allows immediate non-siloed use in clinical trials and patient monitoring. FSHD-SUM provides a patient-centred PROM designed for efficient, multi-domain assessment of FSHD burden over the long term. It is readily applicable in clinical and research settings, and now requires trial testing for sensitivity to change to achieve final validation in real-world contexts.

P7.04 Investigating the mechanisms of hearing loss in FSHD using a transgenic DUX4 mouse model

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Sensorineural hearing loss is seen in up to 20% of FSHD patients, but the underlying mechanism is unknown. **Objective:** To investigate the cellular and molecular basis of hearing loss in FSHD using a DUX4 transgenic mouse model. We used the iDUX4pA mice, in which a doxycycline-inducible *DUX4* transgene is integrated into the X chromosome. Mice were backcrossed onto an FVB background. Only females were studied, as males do not survive weaning. Auditory brainstem response (ABR) testing was performed at 8, 16, 32, and 45.2 kHz in iDUX4pA mice (n=11) and wild-type littermates (n=4). Cochlear histology was used to assess cellular correlates of dysfunction. Un-induced iDUX4pA female mice exhibited significantly elevated thresholds at 32 kHz ($p = 0.0288$), consistent with high-frequency hearing loss. Histological analysis revealed selective loss of outer hair cells, most prominently in the basal turn of the cochlea. In response to loud sounds, ABR wave I amplitude and latency – reflecting auditory nerve and inner hair cell synapse function – were not significantly different between transgenic and wild-type mice, supporting outer hair cell dysfunction rather than synaptopathy. Our data suggest that *DUX4* expression in the iDUX4pA mouse model is sufficient to cause high-frequency hearing loss through selective outer hair cell loss. Ongoing work aims to map *DUX4* expression and transcriptional changes in the cochlea.

P7.05 Respiratory states and probability surfaces, with application to FSHD evolution
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Recent publications from MD STARnet state that respiratory complications contribute in more than 50% of FSHD deaths. We present a new global, visual, probabilistic, home tool for analysis of a large set (∼300) of respirations. Respiratory states (RS) are instantaneous pairs (x=volume, y=flow-rate). Together with z, the probability of observing a state (x,y), we get the probability surface (PS), known in statistics as the joint probability distribution function of x and y. Preferably, data are recorded in the 25 min Home_Fast_Respiration_Test (see P. Valentin, Milan IRC FSHD Society, 17/06/2023). The origin O is set at mid-tidal volume and null flow rate. PS reference shape looks like a volcano on a plain, with an inner crater lake containing O. PS allows us to detect, at a glance, abnormal RS and their locus, namely apneic, hypopneic, and hyperpneic states. The set of coordinates allows superposition two PS and homothety from the origin with ratio λ to achieve best coincidence and compare tests. For example, a 37% loss (shrinking) in λ is found over a 6-year period for FSHD, compared to only 7% for the reference case. This clearly shows the specific degradation due to FSHD. PS also shows that thoracic and abdominal induction straps remain in phase in FSHD. We recommend using PS for baseline evaluation and follow-up in FSHD. Since FSHD induces a restrictive respiratory syndrome, PS may be a good guide in the use of respiratory aids for improving quality of life and survival time.

P7.06 Incentivizing novel therapeutic approaches for FSHD

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Facioscapulohumeral muscular dystrophy (FSHD), an epigenetically driven myopathy affecting approximately 1 million people worldwide, has seen significant advances in the understanding of causative genes and downstream molecular mechanisms for both FSHD1 and FSHD2. These discoveries have led to a growing pipeline of therapeutic approaches, within a limited scope of mechanisms. To increase engagement, attract new perspectives, and broaden the scope of potential therapeutic approaches, SOLVE FSHD has sponsored XPRIZE's Healthspan competition to include a US$10 million FSHD bonus prize. This 5-year challenge aims to improve physical function and quality of life for people living with FSHD. The FSHD bonus prize runs in parallel with the broader US$101 million XPRIZE Healthspan competition, which focuses on reversing age-related decline in cognition, immunity, and muscle function. The FSHD bonus prize received 54 team applications by its initial December 20, 2024, deadline for the first set of milestone prizes totaling US$2 million. Following review by a dedicated FSHD judging panel, 8 teams were selected as recipients and each awarded a US$250,000 milestone prize. With the competition remaining open to new entrants until 2027, this presentation will outline the competition's structure, judging criteria, and pathway to the US$8 million grand prize to be awarded in 2030.

P7.07 Pilot study of circulating biomarkers in facioscapulohumeral muscular dystrophy 1

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Facioscapulohumeral muscular dystrophy (FSHD) results from epigenetic-driven *DUX4* overexpression, leading to myotoxicity. FSHD1 (D4Z4 contraction) and FSHD2 (epigenetic modifier mutations) show clinical variability, necessitating biomarkers. Analysis of 73 FSHD1 patients (mean age of symptom onset: 23.5±15.5 years) linked D4Z4 repeat numbers to disease severity (FSHD scores, age of ambulation loss). Dysregulated miRNAs were identified. SomaScan 11K proteomics in six FSHD1 patients (3 D4Z4 repeats) versus 70 controls revealed dysregulated proteins in cytoskeletal, epigenetic, metabolic, immune, and survival pathways, with a key biomarker identified. Comparative proteomics analysis of four Duchenne muscular dystrophy (DMD) patients showed shared biomarkers alongside DMD-specific markers, highlighting proteomics' utility in tracking disease progression and therapy response. Non-invasive circulating biomarkers elucidate FSHD pathophysiology and identify therapeutic targets. Expanded cohorts will refine biomarkers to evaluate disease states and responses to therapeutic interventions. Post-treatment expression tracking may assess muscle preservation.

P7.08 Characterization of a promising DUX4-regulated circulating biomarker for facioscapulohumeral dystrophy (FSHD)

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FSHD causes progressive muscle weakness, loss of muscle mass, and severe disability due to aberrant *DUX4* expression in skeletal muscle. While *DUX4*-regulated RNAs have been measured in muscle biopsies, the procedure is invasive and only measures *DUX4* activity in that specific sample of muscle. Measurement of circulating biomarkers is less invasive and allows assessment of *DUX4* activity in the whole-body. To discover candidate circulating biomarkers, we performed a mass spectrometry screen for proteins that accumulated in the supernatant of *DUX4*-inducible immortalized human myoblasts (MB135iDUX4 cells). Hits with ≥2-fold abundance in the doxycycline-treated cell supernatant and containing putative secretion signals were prioritized for further analysis. Multiple potential circulating biomarker candidates were identified. The lead biomarker is a *DUX4*-regulated target found to be elevated in FSHD muscle. Its expression in lysates and supernatants of *DUX4*-expressing cells upon doxycycline induction was confirmed by immunoblot. Intracellular localization of this protein was also evaluated by immunofluorescence. This *DUX4*-regulated biomarker was elevated in plasma from individuals with FSHD compared to healthy volunteers. We identified a *DUX4*-regulated circulating biomarker in FSHD plasma which could be used to further understand disease biology/progression and to support the development of therapies targeting the root cause of FSHD.

P7.09 Introducing BetterLife FSHD, an innovative new patient-reported data repository and engagement tool

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BetterLife FSHD is a novel patient-reported data platform launched by the FSHD Society that transcends the engagement limits of a traditional registry by providing direct benefit to participants. Co-created with the patient community, BetterLife offers participants personalized resources, matching to clinical trials and studies, a community forum, and disease tracking. Participants enter structured survey data, which are stored in an auditable, modern data platform under IRB approved protocol. Surveys cover domains including diagnosis and genetics, disease progression, symptom management, pain, sleep and fatigue, mental health, diet and exercise, activities of daily living, and more. More than 500 participants have enrolled and completed more than 2,000 surveys in BetterLife in the 7 months since its launch in the United States. All data collected by BetterLife is available to the research community upon request and approval by the steering committee. To this end, we are thrilled to launch and reveal the new BetterLife FSHD data explorer at the 2025 International Research Congress. This tool enables researchers to dynamically search, filter, visualize, and request aggregate or row-level data. The research community is also invited to leverage and collaborate with the BetterLife team to administer new survey-based studies or recruit to other studies. Together, we aim to accelerate research by improving the participant experience and simplifying access to patients and patient data.