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ABSTRACT BOOK

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Session 1: Disease Mechanisms & Interventional Strategies

S1.01

Engineered FSHD mutations result in D4Z4 heterochromatin disruption and feedforward *DUX4* network activation

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Facioscapulohumeral dystrophy (FSHD) is linked to contraction of D4Z4 repeats on chromosome 4q with *SMCHD1* mutations acting as a disease modifier. D4Z4 heterochromatin disruption and abnormal upregulation of the transcription factor *DUX4*, encoded in the D4Z4 repeat, are the hallmarks of FSHD. However, defining the precise effect of D4Z4 contraction has been difficult because D4Z4 repeats are primate specific, and *DUX4* expression is very rare in highly heterogeneous patient myocytes. We generated isogenic mutant cell lines harboring D4Z4 and/or *SMCHD1* mutations in a healthy human skeletal myoblast line. We found that the mutations affect D4Z4 heterochromatin differently, and that *SMCHD1* mutation or disruption of DNA methylation stabilizes otherwise variegated *DUX4* target activation in D4Z4 contraction mutant cells, demonstrating the critical role of modifiers. Our study revealed amplification of the *DUX4* signal through downstream targets H3.X/Y and LEUTX. Our results provide important insights into how rare *DUX4* expression leads to FSHD pathogenesis.

S1.02

Identification of novel druggable activator of *DUX4* expression in FSHD muscular dystrophy

Emanuele Mocciaro[1], Davide Gabellini[1]

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Previously, we identified the chromatin remodeling protein WDR5 as a key factor required for *DUX4* expression. Since WDR5 is involved in multiple gene regulatory complexes controlling a variety of key cellular activities, its targeting could lead to unwanted side effects. To identify a safer target, we performed a focused genetic screening to determine if a specific WDR5-containing complex is required for *DUX4* expression. We combined RNA silencing, chromatin immunoprecipitation, myogenic differentiation, and apoptosis assays in primary muscle cells derived from FSHD patients or healthy donors. We set up a humanized mouse model of FSHD to evaluate safety and efficacy of drug candidates *in vivo*. Our results show that one specific complex is required for *DUX4* expression in FSHD. In particular, silencing the novel *DUX4* activator inhibits the expression of *DUX4* and *DUX4* target genes, and rescues both cell viability and myogenic differentiation of primary FSHD muscle cells. Importantly, several small molecule drug inhibitors of the novel *DUX4* activator are commercially available with high picomolar to low nanomolar activity, favorable PK profile, and high oral bioavailability. Two of these compounds are currently in clinical trial. Thanks to our humanized animal model of FSHD, we will evaluate safety and efficacy of targeting the novel *DUX4* activator in a relevant setting, providing a possible novel therapeutic strategy for FSHD patients.

S1.03

Understanding and treating inflammation in FSHD muscular dystrophy

Beatrice Biferali[1], Mara Salomè[1], Daniele Campolungo[1], Davide Gabellini[1]

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Muscle inflammation is a prominent FSHD feature and anticipates muscle loss and its fibro-fatty substitution, suggesting that inflammation contributes to FSHD pathogenesis and is not just a non-specific event, unlike other muscular dystrophies in which inflammation is generally secondary to muscle wasting. It is tempting to speculate that the nature of the inflammatory insult in FSHD is different from that of other muscular dystrophies and should be hence treated differently. The main direct *DUX4* targets are repetitive sequences. Their activations cause the accumulation of double strand RNA (dsRNA), which might lead to the induction of a "viral mimicry" status encompassing innate and adaptive immune responses. We found that dsRNA, viral mimicry, IFN-γ, STAT-1, STAT-3, and IL-6 are activated by *DUX4* in primary muscle cells of FSHD patients and in muscle tissues of the FSHD mouse model. *In vivo*, this is associated with the expansion of fibro-adipogenic progenitors and their conversion to a pro-fibrotic state. Notably, *in vitro* and *in vivo*, IFN-γ treatment is sufficient to activate the same pathway. Importantly, *in vivo* treatment with an anti-IFN-γ neutralizing antibody reduces inflammation, decreases FAPs accumulation, and preserves muscle tissue. Collectively, our results indicate that *DUX4* directly activates a pro-inflammatory pathway contributing to muscle wasting in FSHD. Elucidation of this pathway could provide novel approaches to improve FSHD pathophysiology.

S1.04

Creating an immune cell atlas of the peripheral blood for facioscapulohumeral muscular dystrophy (FSHD)

Chantal Coles[1], Ian Woodcock[2], Peter Houweling[1], Katy de Valle[2]

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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 recruited 23 patients with childhood-onset FSHD through the neuromuscular clinic at Royal Children's Hospital (Melbourne, Australia), as part of the infantile-onset FSHD Longitudinal Outcome Study (iFSHD-LOS). Blood samples have been collected and screened using high-dimensional flow cytometry to identify 55 subtypes of immune cells. Our initial analyses identified alterations in immune cell subtypes in patients with FSHD. This study aims to create an immune cell atlas of the peripheral blood of patients with FSHD over the next 5 years using the iFSHD-LOS study. This will involve a yearly blood sample for immune cell phenotyping, and transcriptomic and plasma cytokine analyses which will be coupled with key clinical outcome measures to determine severity.

S1.05

Establishing a clinical candidate for FSHD RNAi gene therapy

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We previously published and presented preclinical efficacy and safety studies for various iterations of a *DUX4*-targeted RNAi-based gene therapy for FSHD using a product called mi405. Every iteration was designed to enhance distribution, restrict expression, or optimize transduction, thus resulting in a safer, more efficient product. Here, we are presenting the lead clinical candidate of Armatus Bio, ARM-201, a construct containing the extensively tested mi405 sequence in the clinically proven second-generation myotropic AAV capsid, SLB-101. In this study, we performed a dose-finding protocol using the FSHD TIC-*DUX4* mouse model. Here, we assessed *DUX4*-associated mouse biomarkers and behavioral assays to establish a potential minimal effective dose 1 log lower than first-generation products. Our data demonstrate that combining SLB-101 with the highly efficacious mi405 product will enable lower and potentially safer AAV doses while maintaining or improving the therapeutic efficacy achieved using high-dose first-generation vectors.

S1.06

Developing a potent gene therapy candidate for facioscapulohumeral muscular dystrophy (FSHD) through high-throughput AAV capsid and cargo engineering

Sharif Tabebordbar[1], Saurav Seshadri[1], Kyle Brown[1], Shayan Tabebordbar[1], Edward Marsh[1], Jana Jenquin[1], Janelle Stricker[1], Brian Ferguson[1], Mark Fielden[1]

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal-dominant genetic disorder affecting an estimated 1 in 8,000 individuals. Patients with FSHD typically exhibit progressive wasting of muscles in the face, shoulders, upper arms, legs, and abdomen; approximately 20% of patients will eventually require the use of a wheelchair. De-repression of the transcription factor *DUX4* has been identified as the pathogenetic mechanism in both FSHD1 and FSHD2. Insufficient epigenetic silencing of the *DUX4* gene in patient muscles results in aberrant expression of DUX4, which is toxic to mature myofibers. Therefore, knockdown of the *DUX4* transcript has been advanced as a therapeutic strategy for FSHD and has shown efficacy in preclinical models. Here, we demonstrate efficacy of a novel MyoAAV-mediated RNAi therapy for FSHD both *in vitro* (in human patient-derived myotubes) and *in vivo* (in the ACTA1-MCM;FLExDUX4 mouse model). First, we conducted a tiling screen to identify the most potent DUX4-targeting artificial miRNA sequences, using an engineered HEK293 cell line stably expressing a tagged mutant DUX4 transgene. Top hits from this screen were validated in FSHD patient-derived myotubes. These cells contain a disease-causing contraction in the D4Z4 region of chromosome 4 that is responsible for epigenetic silencing of *DUX4*, and upon differentiation, show expression of DUX4 and its transcriptionally regulated target genes. Candidate miRNA sequences were ranked based on the magnitude of DUX4 and target gene knockdown achieved in these cells, and selected sequences were carried forward for additional characterization. We performed small RNAseq in human myotubes to assess processing efficiency of the miRNA-containing transcript. To ensure that our candidate miRNA sequences were specific for DUX4 and did not cause knockdown of unintended transcripts, we adopted an unbiased, empirical approach and performed RNAseq in human primary myotubes. Our lead sequence showed optimal potency and processing, with minimal off-target effects. In separate experiments, we evolved liver de-targeted, muscle-tropic MyoAAV capsid variants in non-human primates. We packaged our lead miRNA sequence in our top liver de-targeted, muscle-tropic MyoAAV and tested the resulting candidate *in vitro* and *in vivo*. We confirmed potent knockdown of DUX4 and target genes, efficient processing, and minimal off-target effects at a relevant dose in human myotubes. To assess *in vivo* efficacy, we used ACTA1-MCM;FLExDUX4 mice, a bi-transgenic line with inducible expression of DUX4 in skeletal muscles, which reproduces disease-relevant biochemical, histological, and functional phenotypes of FSHD. We dosed these mice with our candidate or vehicle, and induced DUX4 expression in all mice by injecting tamoxifen. Treatment with MyoAAV delivering DUX4 miRNA protected mice from severe impairment in treadmill time to exhaustion, prevented expression of markers of histopathology, and blocked elevations in DUX4 and target genes, all of which were observed in vehicle-treated mice. Taken together, these data support further preclinical development of our gene therapy candidate for FSHD.

S1.07

Efficacy and safety of an investigational single-dose epigenome editing therapy, EPI-321, targeting D4Z4 in facioscapulohumeral muscular dystrophy

Alexandra Collin de l'Hortet[1], Abhinav Adhikari[1], Sid Boregowda[1], Hao Zheng[1], Vishi Agarwal[1], Andrew Norton[1], Nalinda Wasala[1]

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EPI-321 is an investigational drug product for the treatment of FSHD. It is a single vector AAVrh74 encoding an ultracompact, dead Cas protein fused to gene-suppressing modulators, and a gRNA-targeting D4Z4 locus to permanently suppress *DUX4* expression through re-methylation of the locus. Our preclinical studies showed that EPI-321 leads to dose-dependent suppression of *DUX4* and *DUX4*-downstream gene expression in 10 FSHD patient-derived myoblasts *in vitro*, irrespective of the number of D4Z4 repeats, and showed decreased apoptosis. EPI-321 showed re-methylation of the D4Z4 target locus leading to suppression of *DUX4*. *In vivo* evaluation of EPI-321 in humanized FSHD mice showed a dose-dependent suppression of *DUX4*-pathway, and anti-apoptotic activity in muscle. Additionally, twitch and tetanic forces were improved in 3D engineered FSHD muscle tissues upon EPI-321 treatment assayed more than 46 days. The safety of EPI-321 was assessed in both mice and NHP. This comprehensive examination encompassed in-life observations, clinical and anatomic pathological examinations, immunogenic response, biodistribution studies, spatial distribution in the gonads, and off-target effects. These analyses underscored the favorable safety profile of EPI-321. Our findings provide robust evidence for EPI-321 as a potential gene therapy for treating FSHD by permanently suppressing *DUX4*. We intend to submit an IND application and are looking forward to commencing first-in-human trials in 2024.

S1.08

Translating AAV-delivered CRISPR-Cas13 therapy for FSHD may require overcoming size and immune challenges

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We are developing a CRISPR-Cas13-based gene therapy approach to knock down the *DUX4* gene. We utilized Cas13b and Cas13d proteins from different bacterial species. We first identified lead Cas13b gRNAs and tested the system *in vivo*. After intramuscular injection in a rapidly progressing AAV-based FSHD mouse model, we measured significant *DUX4* mRNA reductions in AAV-CRISPR-Cas13b/gRNA1 co-treated muscles. Knockdown was accompanied by histological improvements, and reductions in DUX4 protein, *DUX4*-activated biomarkers, and apoptotic nuclei at early timepoints (2 wks). In our genetic mouse model of FSHD (TIC-*DUX4*), Cas13b/gRNA1 also reduced *DUX4*-induced myopathy, but protection waned over time. In wild-type mice, loss of Cas13b expression was accompanied by robust cytotoxic T cell responses in skeletal muscle, suggesting an immune response to the Cas13b protein. For the smaller Cas13d enzyme, we designed and tested 50 gRNAs, all of which caused significant *DUX4* knockdown using several *in vitro* assays. We then selected 3 leads, based on their ability to consistently reduce *DUX4* transactivation activity and FSHD biomarker expression, and knockdown DUX4 protein by 80% to 90% *in vitro*. *In vivo* testing of the potency and durability of single AAV-Cas13d+gRNAs in our FSHD mouse models is ongoing. We conclude that CRISPR-Cas13 is a robust *DUX4* RNA-targeting tool *in vitro* and a promising strategy for *DUX4* mRNA silencing *in vivo*, if immune response challenges can be overcome.

Session 2: Preclinical Models

S2.01

Mstn is a reliable biomarker for monitoring therapy effect in FSHD

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Blood-based biomarkers of disease severity and/or therapeutic efficacy are cruelly lacking for FSHD. Here, we investigated if circulating myostatin could be a quantitative biomarker for treatment efficacy in FSHD. Myostatin is a secreted protein, produced and released by the skeletal muscle, which acts as an inhibitor of muscle growth. We have previously demonstrated that circulating myostatin levels are dramatically reduced in patients affected by muscle wasting or atrophying disease, including FSHD. In this study we used the Acta-Cre/FLExDUX4 mouse model, which expresses *DUX4* at low levels in the absence of tamoxifen, thus mimicking what happens in FSHD patients. Myostatin levels were measured at both protein (in the blood) and mRNA (in different muscles) levels. An inverted correlation was observed between circulating Mstn levels and the age of the animals, reflecting disease progression. At the mRNA level, Mstn levels were also reduced. The Acta-Cre/FLExDUX4 mice were also intravenously injected with an AAV-sh*DUX4*. One month after injection, vector delivery significantly reduced all the pathological signs of the *DUX4* expression including molecular, histological, and functional changes. An increased expression of Mstn was observed in muscle and the blood, both in the presence and absence of tamoxifen. The data obtained 1 year after AAV-sh*DUX4* injection will be also presented. These data show that circulating Mstn is a reliable biomarker for monitoring therapy effect.

S2.02

SLC34A2: A biomarker for FSHD in muscle *in situ*

Robert Bloch[1], Maria Traficante[1], Andrea O'Neill[1], Alexia Smith[1]

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SLC34A2, one of many genes upregulated by *DUX4*, is unusual because its protein product, SLC34A2, a Na+,PO42- cotransporter, is readily detected in FSHD tissue and cells. Losmapimod and other p38K inhibitors reduce *SLC34A2* mRNA and protein levels, suggesting that it may be a useful biomarker for FSHD. Here, we show that we can detect *SLC34A2* on the surfaces of mature human FSHD myofibers with great specificity and sensitivity. We used the monoclonal antibody MX35 to the extracellular domain of *SLC34A2* (Sloan-Kettering Inst.), which we tagged with an infrared fluorescent marker. After injection into mice carrying xenografts of FSHD or healthy control muscle, prepared by methods we have described, and imaging with a Xenogen instrument, MX35 labeled engrafted FSHD muscles more brightly than controls. Non-immune antibody, tagged similarly, failed to distinguish between FSHD and control muscles. Thus, labeling was highly specific. Differential labeling of FSHD versus control grafts persisted for several days. Moreover, FSHD grafts with as few as 3 myofibers labeled by MX35 were readily detected by Xenogen imaging, indicating that the method is also highly sensitive. *SLC34A2* on the surfaces of FSHD muscle cells in culture is also reduced twofold by losmapimod. Our results suggest that antibody labeling of *SLC34A2* in FSHD patients can be used to track disease progression and therapeutic outcomes. Supported by The FSHD Society, SOLVE FSHD, AFM Telethon, and Friends of FSHD Research.

S2.03

The FORCE™ platform achieves robust and durable *DUX4* suppression and functional benefit in FSHD mouse models

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FSHD is a severe muscle disorder resulting from aberrant *DUX4* mRNA expression in skeletal muscle, which affects expression of downstream genes collectively known as the *DUX4* transcriptome (D4T) and leads to progressive myofiber loss and debilitating weakness. We leveraged the FORCE platform to develop DYNE-302, a Fab-drug conjugate targeting the human transferrin receptor 1 (TfR1) for muscle delivery of an siRNA designed against *DUX4* mRNA. The DYNE-302 siRNA payload is highly specific for *DUX4* mRNA, and DYNE-302 demonstrated high *in-vitro* potency in FSHD patient-derived myotubes. To establish the *in-vivo* efficacy of DYNE-302, we crossed mice expressing human TfR1 (hTfR1) with mice expressing tamoxifen-inducible human *DUX4* (iFLExD). The resulting hTfR1/iFLExD mice develop a slowly progressive myopathy due to sporadic *DUX4* expression, or an acute myopathy with impaired muscle function upon tamoxifen induction of *DUX4*. A single intravenous dose of DYNE-302 resulted in robust D4T inhibition that lasted up to 3 months, with reduced myofiber pathology. Moreover, DYNE-302 demonstrated profound benefit on muscle function in the tamoxifen-induced acute model. Our data provide the preclinical foundation for therapeutic development of DYNE-302 for the treatment of FSHD.

Session 3: Outcomes Assessments

S3.01

Strength and functional correlates of reachable workspace in facioscapulohumeral muscular dystrophy

Leo Wang[1], Maya Hatch[2], Michael McDermott[3], Bill Martens[3], Katy Eichinger[3], Leann Lewis[3], Michaela Walker[4], Doris Leung[5], Kathryn Wagner[5], Sabrina Sacconi[6], Karlien Mul[7], Perry Shieh[8], Bakri Elsheikh[9], Nicholas Johnson[10], Jay Han[11], Rabi Tawil[3], Jeff Statland[4]

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Facioscapulohumeral muscular dystrophy (FSHD) is an asymmetrical, slowly progressive muscular dystrophy, with predilection for proximal shoulder muscles. Previously published studies have shown that proximal shoulder function in patients with FSHD can be measured with a 3-dimensional sensor-based outcome called the reachable workspace. However, the relationship between reachable workspace and muscle strength, and how reachable workspace relates to patients' self-reported health and function, have not been studied within the same subjects and in detail, and in a large-scale FSHD cohort. The Clinical Trial Readiness to Solve Barriers to Drug Development in FSHD (ReSolve) study, a prospective international multisite observational study, allowed for an opportunity to investigate these potential associations. The final study cohort consisted of 168 adults with FSHD across 10 sites. Results indicate moderate (Spearman rho = 0.70 to 0.73) correlations between total relative surface area, RSA (i.e., upper extremity reachability in the frontal plane, on each side with or without 500 gram wrist weight) and upper extremity quantitative muscle testing (QMT). For each quartile loss of total RSA, there is a corresponding ~20% decrease in upper extremity QMT predicted strength and 10-point loss on the 80-point patient-reported outcome measurement of upper extremity function, the Upper Extremity Function Index.

S3.02

A 5-year natural history cohort of patients with facioscapulohumeral muscular dystrophy determining disease progression and feasibility of clinical outcome assessments for clinical trials

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Introduction: There is a need for clinical outcome assessments (COAs) that can capture disease progression over the relatively short time span of a clinical trial. In this study, we report the natural progression of FSHD and determine the feasibility of COAs for clinical trials. Methods: Genetically confirmed patients underwent various clinical assessments at baseline and after 5 years. COAs consisted of the Motor Function Measure (MFM), manual muscle testing, 6-minute walk test (6-MWT), quantitative muscle strength assessment (QMA) of the quadriceps muscle, clinical severity score (CSS), and FSHD evaluation score (FES). Statistical significance and the minimal clinically important difference (MCID) were calculated and power calculations were performed. Results: A total of 154 symptomatic patients with FSHD were included, with a mean (SD) age of 51.4 (14.6). All COAs showed a statistically significant progression after 5 years. MCID was reached for the MFM Domain 1(D1), MFM Total score, and FES. The MFM D1, MFM Total score, and FES showed the lowest sample size requirements for clinical trials (185, 156, and 201 participants per group for a trial duration of 2 years, respectively). Discussion: The captured FSHD disease progression rate in 5 years was generally minimal. Extended trial durations or novel outcome assessments might be necessary to improve trial feasibility in FSHD.

S3.03

Exploring individual muscle progression and *DUX4* associations in FSHD

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Background: MRI has enabled detailed disease characterization in FSHD. Prior work suggests that fat within an individual muscle is related to higher *DUX4* muscle expression. As part of ongoing work, we aimed to answer 3 questions: 1) Do baseline *DUX4* levels predict fat change over 1 to 2 years? 2) How does fat progress in individual muscles over shorter intervals (3 to 6 months)? 3) Do independent cohorts have similar frequencies/patterns of fat change at 1 to 2 years? Methods: Subjects were part of a Wellstone sample (I N=36; II N=35; biopsy at baseline and MRI follow-up at 1 and 2 years (P50 AR065139, Tapscott PI) and a Kennedy Krieger cohort (N=30, MRIs at baseline, 3, 9, 15, and 21 months (1K23NS091379, Leung PI). Data were analyzed with AI methods as part of a Friends of FSH Research grant (Friedman/Leung) to yield individual muscle metrics. Assessments of change were operationalized using confidence intervals to generate heat maps. Collapsing across subjects generated frequency estimates. Results: RNAseq analyses are in process and will be presented at the meeting. Sporadic muscles showed change at 3 months. Across 6-month intervals, progression was seen across muscles, with the typical individual muscle frequency being <3%, 6%, 13%, and 20% at 3, 9, 15, and 21 months, respectively. The Wellstone cohort demonstrated a similar pattern of results. Discussion: These analyses will provide an improved understanding of how individual muscle measures progress in FSHD and could be used for therapeutic monitoring.

S3.04

Interleukin-6 as a biomarker for disease activity, progression, and muscle composition in facioscapulohumeral dystrophy: Insights from longitudinal studies

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Since inflammation plays a central role in FSHD pathophysiology, we are particularly interested in inflammatory biomarkers and have previously identified interleukin-6 (IL-6) as a promising severity biomarker. To validate this, we conducted 2 longitudinal studies, the CTRN-FSHD France (NCT04038138) and the Cytokine FSHD (NCT04694456), which include 30 adult ambulant FSHD1 patients each (N = 60, 53.2 ± 15.05 years, 53% men, age at onset 32.8 ± 16.1, D4Z4 repeat number 6.6 ± 0.2). Baseline and 12-month assessments included clinical evaluations, IL-6 measurements, and whole-body MRI scans. Our results show a strong correlation between IL-6 levels and clinical severity metrics, confirming IL-6 as a serum biomarker for FSHD activity, severity, and progression. Moreover, we found a significant association between IL-6 levels and MRI composite scores, suggesting that IL-6 may influence muscle composition through its pro-inflammatory effects. Longitudinal analysis revealed that changes in IL-6 levels over time correlate with changes in MRI composite scores, indicating a dynamic relationship between IL-6-mediated inflammation and the alterations in tissue structure detected by MRI. Our findings confirm IL-6 as a reliable serum biomarker for FSHD activity and severity. The correlation between IL-6 levels and MRI findings highlights IL-6's potential for monitoring disease progression and evaluating treatment response in FSHD patients.

S3.05

Developing a cell-free DNA-based biomarker for FSHD

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FSHD is characterized by the death of muscle cells due to aberrant expression of *DUX4*. Current means to monitor the progression of FSHD and response to treatments at a molecular level require invasive muscle biopsies. Availability of FSHD biomarkers in blood can significantly reduce patient hardship and allow for more frequent tracking of disease status. To this end, we are developing FSHD biomarkers from cell-free DNA (cfDNA). Free-floating DNA in our blood, or cfDNA, promises a non-invasive means to monitor an individual's health status. Our genome is packaged with proteins called histones, which form discrete units called nucleosomes. cfDNA arises because nucleases chew the genome when the cell dies, but nucleosomes block the action of nucleases. Thus, cfDNA is a snapshot of where the nucleosomes were arranged on the dying cell's genome. Protected fragments shorter than 147 bp, "subnucleosomes," represent DNA unwrapping from the histone complex during active transcription. Here, with a pilot dataset, we used the length of the cfDNA fragments near the start of genes to infer whether the observed subnucleosome profiles are indicative of *DUX4* gene expression program. Preliminary analysis of 100 to 130 bp fragments shows *DUX4*-specific chromatin signatures in FSHD plasma. These results can be further refined with larger sample sizes and cfDNA library design that enriches for shorter fragments to enable tracking FSHD progression from a blood test at scale.

Session 4: Genetics & Discovery Research

S4.01

Single-cell spatial transcriptomics reveals a dystrophic trajectory following a developmental bifurcation of FSHD myoblast cell fates

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Facioscapulohumeral muscular dystrophy (FSHD) is linked to abnormal de-repression of the transcription activator *DUX4*. This effect is localized to a low percentage of cells, requiring single cell analysis. Using MERFISH, we examined spatial distributions of 140 genes, including 24 direct *DUX4* targets, in *in vitro* differentiated control, isogenic D4Z4 contraction mutant and FSHD patient myotubes, unfused mononuclear cells (MNCs), and individual nuclei. We find myocyte nuclei segregate into 2 clusters defined by expression of *DUX4* target genes, which is exclusively found in patient/mutant nuclei, while MNCs cluster based on developmental state. Patient/mutant myotubes are found in “FSHD-hi” and “FSHD-lo” states, with the former signified by high *DUX4* target expression and decreased muscle gene expression. Pseudotime analyses reveal a clear bifurcation of myoblast differentiation into control and FSHD-hi myotube branches, with variable numbers of *DUX4* target expressing nuclei in multinucleated FSHD-hi myotubes. Gene co-expression modules related to extracellular matrix and stress gene ontologies are significantly altered in patient/mutant myotubes compared to control. We also identify distinct sub-pathways within the *DUX4* gene network that may differentially contribute to the disease transcriptomic phenotype. Taken together, our MERFISH-based gene network profiling of multinucleated cells identified FSHD-induced transcriptomic alterations during myoblast differentiation.

S4.02

Clinical variability in FSHD: The importance of robust clinical information for reliable interpretation of genetic data

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FSHD exhibits significant incomplete penetrance and clinical variability. Moreover, recent population studies highlight the variability in penetrance and clinical severity of D4Z4 repeat contractions. FSHD is caused by the de-repression of the transcription factor *DUX4* located within the D4Z4 macrosatellite repeat on chromosome 4. De-repression can occur through 2 distinct mechanisms: Either by shortening of the D4Z4 repeat (FSHD1) or by pathogenic variants in chromatin modifiers, primarily *SMCHD1*, which normally suppress *DUX4* expression (FSHD2). Several genetic and epigenetic factors seem to affect penetrance and clinical variability in FSHD, amongst which are D4Z4 repeat length and D4Z4 DNA methylation. Genetic diagnosis of FSHD is complex due to overlap between the repeat lengths predominantly found in FSHD1 (1 to 10 units) and FSHD2 patients (8 to 20 units), and in control individuals (8 to 100 units). Moreover, complex rearrangements in the D4Z4 repeat can further complicate genetic diagnosis. In collaboration with genetic and clinical FSHD experts worldwide, representing a comprehensive study group, we have recently updated the guidelines for accurate genetic diagnosis of FSHD. One of the key observations is that laboratories conducting genetic analyses often lack or receive insufficient phenotypic and ethnic information about the patient. This can sometimes lead to incorrect or incomplete genetic diagnoses, which will be discussed in the context of the updated guidelines.

S4.03

Enhancing FSHD diagnosis: A 1-year follow-up study for validating the methylation assay in the clinical practice

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The study aimed at validating the molecular test for discriminating FSHD subjects according to the DNA methylation profile of D4Z4 and to verify its practical utility in clinical settings. A total of 218 subjects with clinical suspect of FSHD collected from 2022 to 2023 were tested for the 4q genotype and methylation levels of the *DUX4*-PAS and DR1 regions. The assay correctly predicted 112 subjects as FSHD, consistently with the presence of FSHD genetic signatures. The remaining 100 subjects were predicted as non-FSHD. Among them, 76 were concordant with the absence of FSHD genetic alterations, and 24 patients displayed borderline methylation levels, including patients with 4qA/4qA, asymptomatic and peculiar cases requiring a clinical evaluation. This issue raised the need for refining the thresholds for methylation levels considering the 4q genotype. Applying these refined thresholds to the previously misclassified samples significantly narrowed the grey zone between true positive and true negative patients, reducing the number of misclassified subjects from 24 to 9 subjects. After adjusting the thresholds, the recalculated performance metrics showed excellent values for 4qA/4qA subjects (sensitivity = 0.96, specificity = 1.00, accuracy = 0.97) as well as on the overall study cohort (sensitivity = 0.93, specificity = 1.00, accuracy = 0.96). The test showed an excellent performance in rapidly discriminating FSHD patients and providing clinicians with a powerful tool for supporting the clinical diagnosis.

S4.04

Modeling cell type-specific and sporadic *DUX4* gene expression in FSHD

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It is already well known that *DUX4* gene is activated in a cell type- and differentiation-specific manner, and sporadically with low frequency even in FSHD muscle cells, but the upstream regulatory mechanism behind this expression pattern has not been well explained. First we modeled the *DUX4* expression pattern by using patient-derived iPSCs and genetic editing strategy, showing myogenic differentiation-driven sporadic *DUX4* activation, which frequency was suppressed by the *SMCHD1* mutation repair and increased by induction of homozygous identical mutation in FSHD2 clones. Interestingly, genetic deletion of the TAD boundary around *FRG1* gene also increased *DUX4* expression level and activation frequency, indicating potential regulatory element(s) beyond the boundary. We also identified some FSHD-specific ATAC-seq peaks and differentiation-specific H3K27ac peaks beyond the boundary that seem independent of *DUX4* binding. These observations support the hypothesis that long-range 3D contact across the TAD boundary can contribute to *DUX4* activation, which may integrate the previous separated knowledge on *DUX4*-centered FSHD pathology and abnormal 3D chromatin contacts to D4Z4 locus in the FSHD chromosomes.

S4.05

DUX4 double whammy: The transcription factor that causes a rare muscular dystrophy also kills the precursors of the human nose

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Missense mutations in the gene *SMCHD1*, which encodes a master epigenetic repressor, have been implicated in 2 seemingly unrelated conditions: A severe craniofacial malformation called congenital arhinia (absent nose) and FSHD2. In FSHD2, loss of *SMCHD1* repressive activity leads to ectopic expression of *DUX4* in muscle tissue leading to cell death. The pathophysiology of arhinia is unknown; however, deep phenotyping studies of patients with arhinia coupled with *SMCHD1* expression studies in mouse embryos strongly suggest a primary defect in the nasal placode cells, the progenitors of the human nose. Here we show that upon *SMCHD1* ablation, *DUX4* becomes de-repressed in human embryonic stem cells (hESCs) as they differentiate toward a placode cell fate and trigger placode cell death. Arhinia and FSHD2 patient-derived induced pluripotent stem cells (iPSCs) both express *DUX4* when converted to placode cells and demonstrate variable degrees of cell death, suggesting the presence of an environmental disease modifier. We show that herpes simplex virus 1 (HSV-1) may be one such modifier, as HSV-1 infection induces *DUX4* expression in *SMCHD1* KO hESC and arhinia and FSHD2 iPSC. These studies suggest that arhinia, like FSHD2, is due to compromised *SMCHD1* repressive activity in a cell-specific context and provide evidence for an environmental modifier. To our knowledge, arhinia represents the first example of a craniofacial malformation caused by epigenetic de-repression of a toxic protein.

Session 5: Pediatric FSHD

S5.01

Longitudinal insights into childhood-onset facioscapulohumeral dystrophy: A 5-year natural history study

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Objective: Understanding the natural history of childhood FSHD and establishing appropriate outcome measures are crucial to successfully prepare for clinical trials, and benefit patient care and education. We examined the disease's course in children over a 5-year follow-up, identifying outcome measures sensitive to change at different timepoints in a population still undergoing normal growth. Methods:
In a nationwide cohort of 20 type 1 FSHD patients with childhood onset, assessments of impairments and physical disability after 5 years of follow-up were added to the earlier measurements at baseline and 2 years. Results: Eighteen of 20 patients completed the follow-up. Disease progression was variable (mean increase FSHD clinical score 1.6 points). Most sensitive outcome measures for detecting progression were the FSHD clinical score (SRM: 1.07) and FSHD clinical severity scale (SRM: 0.92), followed by muscle ultrasonography (MUS, SRM: 0.68). The progression rate could not be predicted from baseline. Discussion: We observed variable disease progression over 5 years, unnoticed by most participants. The relatively slow progression combined with physiological growth emphasizes the need to identify endpoints sensitive to change within 1 to 2 years before the start of trials. We suggest including MUS and FSHD-COM into future studies, evaluating the reachable workspace in the pediatric population, and exploring the feasibility of creating a larger cohort through international collaboration.

S5.02

Pediatric FSHD in the US: Results from the US National Registry for FSHD

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Background: Pediatric-onset FSHD accounts for ~20% of individuals with facioscapulohumeral muscular dystrophy (FSHD), approximately half of whom meet criteria for early-onset FSHD. Few prospective studies exist to inform our understanding of disease progression in pediatric FSHD, which creates a barrier to clinical care and drug development. Methods: We analyzed prospectively collected data from participants in the US National Registry for FSHD who were diagnosed at 18 years of age. Early-onset was defined as diagnosis at 10 years of age. Results: A total of 166 individuals with genetically confirmed FSHD were identified. Individuals with 1 to 3 D4Z4 repeats were diagnosed ($p < 0.001$) and progressed to wheelchair use ($p < 0.001$) at a younger age than those with 4+ D4Z4 repeats. When separated by sex, females were more likely to: Be diagnosed at a younger age than males ($p < 0.001$), be independent of D4Z4 repeat size ($p < 0.001$), be diagnosed at 10 years of age ($p < 0.001$), report facial weakness as their initial symptom ($p = 0.001$), progress to wheelchair use ($p < 0.001$) and at a faster rate ($p = 0.007$) than males, independent of genetics. Conclusions: This is one of the largest analyses of prospectively collected data from individuals diagnosed with pediatric-onset FSHD in the US. These data suggest that females with pediatric-onset FSHD may be more severely affected than males. Larger, prospective studies are needed to further understand disease progression and sex differences in pediatric FSHD.

S5.03

Longitudinal outcomes in pediatric FSHD: An Australian cohort study

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Disease presentation and symptom progression in individuals with early-onset FSHD are poorly characterised. A better understanding is urgently required to prepare for pediatric clinical trials. Robust and responsive outcome measures and biomarkers are required to effectively evaluate function, QoL, and disease progression. This single-site Australian study collected functional data using performance-based measures; the FSHD-COM Peds, PUL, 6MWT, timed function, muscle strength, reachable workspace; self-reported data using pain, fatigue, physical activity scales, and disease burden using pediatric FSHD-Health Index; and muscle MRI as a biomarker from 21 individuals with early-onset FSHD. We present baseline and year 1 data of this early-onset FSHD study. Males made up 62% of the cohort, and ages ranged from 6 to 19 years; 55% (11/20) were classified as early-onset according to D4Z4 repeats (1 to 3). A total of 35% had moderate to severe FSHD (FCS 7 to 15), 2 participants were non-ambulant, and a further 2 required wheeled mobility for community ambulation. At baseline, 20/21 (95%) had facial and scapula weakness, 11/21 (52%) UL weakness, 6/21 (28.5%) distal LL weakness, 11/21 pelvic girdle weakness, and 14/21 (67%) positive Beevor sign. Nine (43%) participants reported falling at least once in the past 6 months, and all reported low to moderate levels of pain and fatigue. Change in function and QoL will be summarised in 19 participants who returned for evaluation at 12 months.

Session 6: Clinical Studies & Trial Designs

S6.01

Pain impacts quality of life, psychological disorders, and exercise in a large international cohort of patients with facioscapulohumeral muscular dystrophy

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Although pain is common in FSHD, it has been less well characterized in the literature. This study aims to characterize pain in an international population of FSHD patients and to determine which factors are associated with pain and pain severity. We analyzed data from a prospective multicenter observational cohort of adult patients with FSHD, the ReSolve study, from 2018 to 2021. We compared patient reported data, motor assessments, and the pharmacologic management of FSHD between patients with and without pain. Patient-Reported Outcome Measures Information System (PROMIS) 57 modules were analyzed for their association with pain. Of 219 patients, 83% reported pain, most commonly located in the lower back and shoulders. There were no significant regional differences in pain medication usage between patients in the United States and Europe. Analysis of PROMIS modules identified an association with the presence of pain and physical function, fatigue, and sleep disturbance. Linear regression modeling of the PROMIS scores revealed that pain intensity had a negative impact on physical function, social participation, depression, anxiety, and sleep interference. Additionally, univariate analysis found a significant association between pain and self-reported psychological problems and resistance exercise rates. Taken together, these data point to the significant impact of pain in FSHD patients and the importance of developing therapies to treat pain in FSHD.

S6.02

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

Michaela Walker[1], Channa Hewamadduma[2], Russel Butterfield[3], Rebecca Clay[1], John Day[4], Stacy Dixon[5], Katy Eichinger[6], Bakri Elsheikh[7], Seth Friedman[8], Angela Genge[9], Nicholas Johnson[10], Peter Jones[11], Doris Leung[12], Leann Lewis[6], Hanns Lochmuller[13], Erin O'Ferrall[9], Bill Martens[6], Dennis Shaw[8], Perry Shieh[14], Sub Subramony[15], Jaya Trivedi[16], Leo Wang[17], Matthew Wicklund[18], Rabi Tawil[6], Jeff Statland[1]

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The MOVE FSHD study aims to determine the predictive value of clinical and motor assessments, patient-reported outcomes, imaging, and tissue biomarkers on disease progression in FSHD. The MOVE FSHD study will evaluate 450 FSHD participants over 24 months with 200 participating in an MRI and muscle biopsy substudy to validate FSHD evaluations and biomarkers. Visits collect FSHD history, physical examination, patient-reported outcomes, strength, timed functional tests (TFTs), and spirometry. Substudy participants have additional biomarkers collected, including reachable workspace at each visit, whole-body MRI at baseline and 12 months, and an optional muscle biopsy occurring at baseline and (n = 40) at 4 months. The MOVE FSHD study has enrolled 305 participants across 14 international sites. More than 150 12-month visits and 75 24-month visits have been completed, 25 are enrolled in the MOVE+ substudy, ~20 participants are non-ambulatory, and ~20 enrolled are <18. MOVE FSHD participants span the full clinical severity scale with more than a third of participants having mild to moderate weakness in their lower extremities. TFTs, such as the 10-meter walk run (10 mwr) and Timed Up and Go (TUG), correlate well with disease severity (>0.6), change from baseline in 12 to 24 months, and may predict a shift in other TFTs. The MOVE FSHD study can improve our understanding of FSHD, impact patient care, refine inclusion criteria for trials, and identify outcomes and biomarkers for FSHD.

S6.03

FSHD Global Registry Project: Whole-body MRI muscle-level analysis for advancing research and empowering patients

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The FSHD Global registry project aims to perform whole-body MRI of Australian patients with FSHD to advance research, develop a clinical trial readiness passport for each patient, and assess the utility of providing patients with their own MRI-derived muscle report. An initial analysis of the first 11 patients is presented here. Leveraging accelerated AI-based muscle segmentation, fat fraction was quantified within 59 unique skeletal muscles bilaterally, totaling 118 muscles per patient. The analysis includes patients with a range of disease states (total muscle fat fraction ranged from 11% to 72% across patients). Despite this range, all patients had at least 4 individual muscles with fat fraction above 30%, demonstrating the potential for detecting early signs of disease progression. While the fat fraction of some muscles correlated highly with total muscle fat fraction (e.g., rectus abdominus), other muscles had no correlation with total muscle fat fraction (e.g., soleus). Comparing total muscle fat fraction in the upper body versus the lower body, some patients demonstrated higher average fat fraction in upper body muscles (n = 7), whereas other patients demonstrated higher average fat fraction in the lower body muscles (n = 4). These results illustrate the need for muscle-level analysis across the whole body to capture individual patient disease state. Collected feedback from patients revealed strong support for providing patients with reports of MRI-derived muscle metrics.

S6.04

Analyzing phenotypes in FSHD: An update of the Comprehensive Clinical Evaluation Form

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The significance of the different clinical forms for prognostic prospective and diagnosis/genetic counseling is still not always clear in clinical practice, although in literature growing evidence suggests the need to consider this variability. In fact, the different phenotypes could show a different disease progression and/or imply distinct genetic mechanisms. Moreover, the high heterogeneity of symptoms in association with a variable penetrance further complicates the genetic diagnosis, as well as the genotype-phenotype correlations, considering the complex genetic architecture to be considered. The availability of a sharing and standardized clinical tool able to describe the clinical complexity by collecting key phenotypic features for stratification of patients is needed in the scenario of FSHD. We present a revised and simpler version of the CCEF, with new, friendlier graphics, focused on the key anamnestic and neurological examination data, to facilitate its understanding and use in clinical practice.

S6.05

Reassessing clinical phenotypes in facioscapulohumeral muscular dystrophy: Late-onset FSHD presentations

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Despite the common belief that late-onset facioscapulohumeral muscular dystrophy (FSHD) cases exhibit milder symptoms, clinical experience often contradicts this. To validate these observations, we analyzed data from the ReSolve study (NCT03458832), comparing patients diagnosed before (adult-onset) and after age 45 (late-onset). The cohort comprised 231 FSHD patients – 190 adult-onset and 41 late-onset. Adult-onset patients completed the Time Up and Go task faster ($p = 0.014$). More adult-onset patients had upper body symptoms, e.g., affected eyelid closure (57.9% vs. 43.9%) and frontalis (24.2% vs. 7.3%). Conversely, 54.3% of late-onset patients needed walking assistance, compared to 30.2% of adult-onset patients. About half of late-onset patients reported lower limb weakness as the initial symptom, contrasting adult-onset patients (proximal lower extremities: 8% vs. 17.5%; distal lower extremities: 10.7% vs. 30%). After adjusting for disease duration, significant differences persisted in Time Up and Go ($p = 0.001$) and the 6-minute walk test ($p = 0.002$), suggesting distinct muscle involvement patterns. In summary, late-onset FSHD patients exhibit less severe upper limb symptoms but more pronounced axial and lower limb weakness than early-onset patients. Late-onset patients more often report lower limb involvement initially. These clinical phenotype differences require further investigation in future trials.

S6.06

FSHD disease progression and losmapimod efficacy assessed by reachable workspace in both arms

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This post-hoc analysis explores averaging reachable workspace (RWS) measurements across both arms in Phase 2 studies of losmapimod for FSHD. In the 52-week open-label study (OLS) and 48-week, randomized, placebo-controlled ReDUX4, 14 OLS and 80 ReDUX4 (40 active, 40 placebo) patients aged 18 to 65 with FSHD1 received losmapimod 15 mg BID or placebo (ReDUX4 only). Efficacy included RWS total relative surface area (RSA) across 5 quadrants (0 to 1.25) with weights, dynamometry to assess shoulder abduction strength, and whole-body muscle MRI composites: Lean muscle volume (LMV), muscle fat fraction (MFF), and muscle fat infiltration (MFI). Results for RSA averaged across both arms are compared with previously reported dominant-arm results. In OLS, RSA averaged across both arms improved 0.037 from baseline versus 0.043 in the dominant arm. In ReDUX4, treatment, benefit shown by RSA averaged across both arms was consistent with dominant-arm improvement (change from baseline, diff. 0.047; [P = 0.014], 0.049 [P = 0.016], respectively). RSA averaged across both arms correlated moderately to strongly with shoulder strength, LMV, and MFF. RSA correlated with MFI in the losmapimod group, but not placebo. These results support potential benefits of using total RSA with weight averaged over both arms as a clinical endpoint. This bilateral assessment of RSA provides a robust measure of FSHD functional impairment and disease progression, aligning with secondary endpoints of muscle strength and structure.

S6.07

Safety and tolerability study of clenbuterol in facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is a progressive muscular dystrophy with no currently approved FDA treatments. The muscle disease is due to a de-repression of the *DUX4* gene contained in the D4Z4 repeat. Clenbuterol has been found to be a potent inhibitor of *DUX4* activity in FSHD patient-derived muscle cells and has anabolic effects on the muscle. We hypothesize that clenbuterol can slow disease progression and improve performance. As part of a P50 AR065139 (NIH Wellstone Study), this project will be a dose-finding/safety study to find the optimal dose that is safe, well tolerated, decreases *DUX4* activity, and increases contractile muscle volume. We propose a prospective 6-month non-randomized open-label study at 3 sites (Kansas City, Rochester, and Seattle) with 3 sequential cohorts of 10 participants each who are clinically affected and have had their FSHD genetically confirmed. The cohorts will be given ascending doses of clenbuterol at 20 mcg, 40 mcg, and 60 mcg, taken orally twice daily. The primary endpoints include safety/tolerability, whereas the secondary endpoints include changes in MRI, molecular candidate, and functional biomarkers. The goal is to determine the maximum tolerable dose of clenbuterol in FSHD, potential side effects, and preliminary signs of efficacy. We aim to start recruiting at the end of summer 2024.

S6.08

ReInForce: A bicentric, randomized, double-blind, placebo-controlled pilot study to evaluate the efficacy and safety of satralizumab in FSHD1

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Facioscapulohumeral dystrophy (FSHD) is marked by progressive muscle weakness in facial, shoulder girdle, upper arms, lower limbs, and abdominal muscles, causing considerable morbidity and decreasing quality of life. There are currently no approved therapies for FSHD. The primary form, FSHD1, is linked to harmful overactivity of the *DUX4* gene, resulting in muscle atrophy and weakness. Studies indicate that abnormal *DUX4* expression triggers inflammatory processes in the initial stages of the disease. Patients with FSHD1 have increased inflammatory and reduced anti-inflammatory cytokines, indicating chronic inflammation. IL-6 levels strongly correlate with clinical severity in patients, and with functional scores in patients and FSHD-like mouse models. Here we present the study design of ReInForce (NCT06222827), a bicentric, randomized, double-blind, placebo-controlled, Phase 2 study to investigate the safety and efficacy of satralizumab, an IL-6 receptor inhibitor, in adults with FSHD1. Patients (N = 40) will receive 120 mg satralizumab or placebo subcutaneously at weeks 0, 2, 4, and then every 4 weeks until week 48. The study will evaluate efficacy by assessing changes in muscle composition and function, as well as measures of clinical disease progression. Given the pathological relevance of inflammation in FSHD, and correlation of IL-6 levels with disease severity, satralizumab may reduce muscle and systemic inflammation, thereby reducing fibro-fatty degeneration in FSHD.

S6.09

Interim results from FORTITUDE™, a randomized Phase 1/2 trial evaluating AOC 1020 in adults with FSHD

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[6] *University of Colorado*
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Background: AOC 1020 is an antibody-oligonucleotide conjugate (AOC™) comprising a *DUX4*-targeting siRNA conjugated to a humanized anti-transferrin receptor 1 (TfR1) antibody to facilitate delivery to muscle. Methods: FORTITUDE (NCT05747924) is a Phase 1/2 randomized, double-blind, placebo-controlled trial whose primary objective is to assess the safety and tolerability of AOC 1020 in adults with FSHD. This study has 3 parts (part A, B, and C), which include a 12-month treatment period where 4 doses of AOC 1020 are administered quarterly, plus a booster at 6 weeks. Part A is a dose-titration design where patients received a first dose of 1 mg/kg, then 2 mg/kg for remaining doses. Part B is a single-/multiple-ascending dose design evaluating 4 mg/kg, and part C is a placebo-controlled design to further assess outcomes at selected doses. Results: This preliminary data analysis will include safety data and month 4 pharmacokinetic and pharmacodynamic data. Conclusions: Preliminary results from FORTITUDE support the continued development of AOC 1020.

POSTER PRESENTATIONS

The main poster session is on Thursday, June 13, 5:30-8:30 p.m. Please plan to present your poster on the following schedule: Odd-numbered posters from 5:30-7:00 p.m. Even-numbered posters from 7:00-8:30 p.m. The poster hall will also be open at lunch on both days of the meeting.

Disease Mechanisms & Interventional Strategies

P1.01

A novel proviral plasmid reduces cross-packaging and ITR promoter activity in AAV vector preparations

Pranali Mistry[1], Noah Taylor[1], Scott Harper[1], Oliver King[2], Matthew Guggenbiller[1]

[1] The Abigail Wexner Research Institute at Nationwide Children's Hospital
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We previously reported an FSHD-targeted gene therapy using AAV vectors as a therapeutic delivery system. AAV preps largely contain the intended therapeutic DNA payload but also contain a small percentage (~3.5% avg) of DNA contaminants, mostly derived from plasmid raw materials required for manufacturing. Thus, AAV contaminants include sequences required to propagate plasmids in bacteria (cross-packaged). We were concerned about a recent IND-enabling study in monkeys showing brain toxicity arising from expression of bacterial-derived cross-packaged DNA contaminants in AAV. We hypothesized that AAV vector safety could be improved by reducing cross-packaging of bacterial sequences in AAV preps, and interfering with transcription of any remaining cross-packaged bacterial DNA. We developed a new plasmid for AAV manufacturing, which resulted in: 1) 70% reduction in bacterially derived cross-packaged material; 2) improved ratio of packaged insert to backbone DNAs across 8 serotypes; and 3) blunting of bacterial gene expression without significantly impacting vector yield or expression of our therapeutic (mi405). We believe this study represents an important step forward in improving AAV safety.

P1.02

Anti-fibrotic approach ameliorates muscle pathology in FSHD animal model

Haseeb Ahsan[1], Ana Mitanoska[1], Kenric Chen[1], David Oyler[1], Michael Kyba[1], Darko Bosnakovski[1]

[1] *Lillehei Heart Institute and Department of Pediatrics, University of Minnesota*

The hallmark histological features of affected muscles include myofiber loss, immune cell infiltration, and deposition of fat and fibrous tissue. This excessive accumulation of collagenous extracellular matrix disrupts muscle physiology, diminishes regenerative capacity, and fosters dystrophic outcomes. Hence, our study aims to evaluate the potential therapeutic approach of targeting fibro-adipogenic processes in *DUX4*-affected muscles. We investigated the effects of several anti-fibrotic drugs, known for their efficacy in acute muscle injury models, using a doxycycline-regulated *DUX4*-expressing FSHD mouse model. iDUX4pA mice were induced with low levels of dox to express barely detectable *Dux4* in myofibers and promote moderate muscle injury similar to that observed in patients. The effectiveness of the drugs was evaluated based on the severity of muscle damage, regeneration, and fibrosis. Our findings revealed one drug that moderately improved muscle composition, evidenced by increased myofiber size and enhanced muscle regeneration. Treated mice exhibited reduced infiltration of fibro-adipogenic and macrophages in affected muscles. Furthermore, the expression of genes associated with fibrosis and inflammation was diminished, supporting the beneficial effect of the drug. In conclusion, our study sheds light on the potential benefits of anti-fibrotic drug treatment in mildly *DUX4*-affected muscle in a *DUX4*-based FSHD animal model.

P1.03

Characterization of the *DUX4*-MATR3 complex to design a possible therapy for FSHD

Chiara Zucchelli[1], Andrea Berardi[2], Giacomo Quilici[3], Valeria Runfola[1], Maria Pannese[1], Paola Ghezzi[1], Davide Gabellini[4], Giovanna Musco[1]

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[4] *San Raffaele Scientific Institute*

Matrin 3 (MATR3) binds to *DUX4* DNA-binding domains (*DUX4*dbd) and blocks *DUX4* activity, rescuing cell viability and myogenic differentiation of FSHD muscle cells (Cell Rep. 2023 Sep 26;42(9):113120). Thus, MATR3 represents a novel therapeutic target in FSHD. We discovered that a 55-residue-long MATR3 fragment (MATR3_55) binds to *DUX4*dbd and inhibits *DUX4* activity as full-length MATR3. Our goal is to obtain structural insights into MATR3_55-*DUX4*dbd interaction to design shorter MATR3-based mimicking peptides (mPeps) selectively binding to *DUX4*dbd and inhibiting *DUX4* activity. We measured the affinity between MATR3_55 and *DUX4*dbd by BioLayer Interferometry, and we identified the MATR3_55 key residues for *DUX4*dbd interaction by NMR spectroscopy and site-directed mutagenesis. Guided by these data and by the structural model of MATR3_55-*DUX4*dbd complex calculated by AlphaFold2 Multimer, we designed mPeps and tested their binding to *DUX4*dbd. We identified 2 mPeps that bind to *DUX4*dbd similarly to MATR3_55. We are studying their ability to block *DUX4* activity in FSHD cellular models. To increase affinity for *DUX4*dbd we are also studying mPeps with changes in sequence, to boost new contacts with *DUX4*dbd, and with chemical modifications stabilizing mPep structure into the bound conformation. At the end of this project, we expect to identify at least one mPep inhibiting *DUX4*, herewith providing proof of concept for a drug-like approach to block *DUX4* activity for FSHD treatment.

P1.04

Deficits in the unfolded protein response in FSHD myoblasts

Adam Bittel[1], Yi-Wen Chen[2]

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[2] *Children's National Hospital*

Background: Activation of the unfolded protein response (UPR) is required to mitigate proteotoxic stress. This study investigated the UPR signaling in FSHD myoblasts in response to endoplasmic reticulum (ER) stress. Approach: FSHD and healthy primary myoblasts were collected at 0, 6, and 24 hrs after scrape injury for single cell RNA sequencing to evaluate transcriptional UPR responses. Myoblasts were also treated with 1 uM of ER-stressor thapsigargin (TG) for 24 hrs. Expression of UPR proteins ATF4, CHOP, and pEIF2α was determined via Western blot at 0, 6, and 24 hrs. mRNA expression of UPR-targeted heat shock chaperones were measured using qRT-PCR. Results: Expression of ER-stress marker genes was elevated at 6 and 24 hrs post-injury. mRNA expression of ATF4 anti-stress targets was significantly downregulated in FSHD at 24 hrs. Expression of pro-apoptotic target of CHOP, BAX, was significantly elevated ($p < 0.001$) in FSHD at 24 hrs. TG increased ATF4, CHOP, and pEIF2α protein expression over 24 hours in all myoblasts (all $p < 0.05$). However, phosphorylation of pEIFα was prolonged in FSHD (higher at 6 hrs, $p < 0.05$). ATF4 protein levels were significantly lower in FSHD at 24 hrs ($p < 0.05$), as was the expression of HSP90AA1, HSPD1, and HSPA9 (all $p < 0.05$). Treatment with TG did not induce *DUX4* expression. Conclusions: FSHD myoblasts display blunted UPR responses to ER stress induced by plasma membrane injury and TG treatment, which may trigger apoptosis via BAX expression.

P1.05

DUX4-associated hypoxia signaling impairs oxidative metabolism and shifts towards less oxidative muscle fibers

Justin Cohen[1], Vincent Ho, Keryn Woodman, Angela Lek, Alec DeSimone, Monkol Lek

[1] *Yale School of Medicine*

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common myopathies, affecting an estimated 1 in 8,000 individuals. Despite major progress in understanding the underlying genetics behind the pathology, no treatment exists. We previously performed CRISPR screening to identify genes of which modulation leads to apoptosis resistance from DUX4, the toxic protein associated with FSHD pathology. Hypoxia signaling was identified as one of the most promising targets, and follow-ups using the mTOR inhibitor everolimus, which acts upstream of hypoxia signaling, successfully reduced DUX4 toxicity *in vitro*. As mitochondria are major consumers of oxygen, we further explored the impact of this signaling and its inhibition by everolimus on oxidative metabolism. DUX4 induction reduced mitochondrial complex I levels and oxygen consumption rates (OCR), which were attenuated by everolimus or genetic inhibition of hypoxia signaling. This was further explored *in vivo* using the DUX4-inducible ACTA1-MCM/FLExDUX4 mouse model. The highly oxidative soleus showed a fiber type shift towards glycolytic IIB fibers post-induction and had reduced NADH staining, suggesting a reduction in oxidative metabolism. Promisingly, NADH staining on FSHD patient bicep biopsy sections showed the same pattern, demonstrating physiological relevance. These results demonstrate a shift in oxidative metabolism as a component of FSHD pathology and provide a pathway to identify novel therapeutic targets for FSHD.

P1.06

DUX4 level-dependent sarcolemmal repair deficits in FSHD

Adam Bittel[1], Aiping Zhang[2], Ze Chen[2], Yi-Wen Chen[2]

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Background: Previously, we reported plasma membrane repair (PMR) deficits in immortalized FSHD human myoblasts and FLExDUX4 mouse myofibers. This study determined if PMR deficits can be modulated by different *DUX4* levels in myofibers, and if a threshold level of *DUX4* expression is required to trigger PMR deficits. Approach: We evaluated sarcolemmal repair in myofibers of murine models of FSHD expressing increasing levels of *DUX4* (FLExDUX4 and ACTA1-MCM-cre/FLExDUX4 induced with s.c. injections of 5 mg/kg and 10 mg/kg tamoxifen) using a focal laser ablation system, with wild-type (WT) and ACTA1-MCM-cre mice as controls. To determine the effects of antisense oligonucleotide (AON) therapy on PMR *in vivo*, we treated FLExDUX4 mice with 20 mg/kg AON for 48 hrs. *DUX4* mRNA expression was measured using qRT-PCR. Results: We observed stepwise increases in *DUX4* expression from WT and ACTA1-MCM-cre, to FLExDUX4, uninduced ACTA1-MCM-cre/FLEx*DUX4*, then induced ACTA1-MCM-cre/FLExDUX4. Repair capacity worsened as *DUX4* expression increased. In mice receiving a high dose of tamoxifen, >90% of myofibers failed to repair. Treatment of FLExDUX4 mice with 2'MOE AON significantly improved PMR toward WT levels ($p < 0.05$). ACTA1-MCM-cre also showed slight but significant PMR deficit. We also observed sex differences in PMR. Conclusions: These results indicate PMR deficits are dependent upon *DUX4* expression, which can trigger PMR impairments at low levels. PMR deficits can be reversed via *DUX4* knockdown.

P1.07

Fibro-adipogenic progenitors and FSHD

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Fibro-adipogenic progenitors (FAPs) are muscle interstitial mesenchymal stem cells that regulate muscle regeneration and metabolism. FAPs differentiate into adipocytes and fibroblasts during chronic muscle regeneration. FSHD shows the progressive replacement of muscle fibers with fat and fibrotic tissue. Identifying the cellular and molecular mechanisms underlying the adipogenic and fibrogenic differentiation of FAPs will help us to define novel therapeutic strategies for FSHD. Exosomes are extracellular vesicles released by cells to establish cell-to-cell communications. Our hypothesis is that the expression of *DUX4* in FSHD myofibers reduces the release of FAPs-targeting exosomes that carry anti-fibrogenic and anti-adipogenic inputs. Chronic loss of these signals may result in increased rates of FAPs adipogenic and fibrogenic differentiation during FSHD progression. To verify this hypothesis, we developed the FLExDUX4-/+; ACTA-Cre-/+; hCD63-GFP-/+ transgenic mice, in which *DUX4* and green fluorescent protein (GFP)-tagged exosomes are expressed in muscle fibers upon tamoxifen (TMX) treatment. We are currently analyzing the muscle histology of FLExDUX4-/+; ACTA-Cre-/+; hCD63-GFP-/+ mice, and of ACTA-Cre-/+; hCD63-GFP-/+ mice, treated with and without TMX for 4.5 months. We additionally isolated FAPs and skeletal muscles of the same type of mice treated with and without TMX for 4.5 months to perform an RNA-Seq analysis.

P1.08

Nucleolar stress, apoptosis, and FSHD myopathy

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We still have a partial knowledge of FSHD etiology and progression. We previously found the presence of *DUX4* in the nucleoli of myotubes generated by MB135-i*DUX4*CA (i*DUX4*) cells, a human muscle cell line expressing *DUX4* upon doxycycline (Doxy) treatment. We also found a very sporadic presence of *DUX4* inside the nucleolus of human primary FSHD myotubes. i*DUX4* myotubes treated with Doxy showed the transcription of nucleolar non-coding RNAs (ncRNAs) that are usually expressed by cells exposed to different types of stress. However, we did not detect the expression of these specific nucleolar ncRNAs in FSHD primary myotubes, probably due to the very low number of myotubes expressing *DUX4*. We are currently analyzing an RNA-Seq we performed in i*DUX4* myotubes treated with and without Doxy. With this analysis, we do expect to find additional information about the effect of *DUX4* on the nucleolus-related transcriptome. In parallel, we are performing experiments using human immortalized FSHD myoblasts expressing a nuclear green fluorescent protein (GFP) whose DNA sequence is under the control of a *DUX4*-dependent promoter. Our plan is to separate by fluorescence-activated cell sorting (FACS) GFP+ vs. GFP- myotubes to carry out gene expression analysis. In this manner, we expect to better evaluate the role that the endogenous *DUX4* may play on the transcription of nucleolar DNA regions controlling nucleolar stress and nucleolar-driven apoptosis.

P1.09

Scapulothoracic fusion using high-strength suture tape cerclage for the treatment of facioscapulohumeral muscular dystrophy

Michael McDermott[1], Neil Lancaster[2], Joey Bell[2], Brandon Merryman[2], Anthony Romeo[1]

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Facioscapulohumeral muscular dystrophy (FSHD) is a progressive neuromuscular disorder that can cause substantial deterioration of the muscles in the shoulder girdle, commonly presenting with functional deficits as a sequela of painful scapular winging. Scapulothoracic fusion (STF) has emerged as a viable treatment option, as it has been shown to improve range of motion and quality of life. A retrospective review was conducted of 10 patients with FSHD who underwent STF utilizing high-strength suture tape cerclage with iliac crest autograft and biologic augmentation. The average age was 34.4 ± 8.7 years (range: 19.6 to 42.5 yrs) and an average BMI of 28.2 ± 5.8 kg/m2. Average operative time was 3.3 ± 0.4 hours with an average EBL of 358.3 ± 73.6 mL (range: 300 to 500 mL). Mean length of stay was 3.0 ± 0.8 days with a mean postoperative follow-up time of 7.3 ± 7.9 months. All patients achieved significantly increased range of motion postoperatively compared to their preoperative evaluation ($p < 0.01$). Shoulder range of motion was measured at the 3-month follow-up with 83.3% (7/10) of patients achieving ≥90° FE and 100% reaching ≥90° FE by final follow-up. This cohort also observed clinical and radiographic signs of fusion in 100% of patients by their final follow-up. This STF technique utilizing high-strength suture tape cerclage shows functional range of motion of the shoulder, high fusion rates, and did not have any complications or reoperations in this cohort.

P1.10

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

Maëlle Limpens[1], Aline Derenne[1], Carmen Burtea[1], Sophie Laurent[1], Alexandre Legrand[1], Steve Wilton[2], Anne-Emilie Declèves[1], Alexandra Belayew[1], Frédérique Coppée[1], Alexandra Tassin[1]

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In a therapeutic goal for FSHD, antisense oligonucleotides (ASOs) directed against *DUX4* mRNAs have been developed at the University of Mons. 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 (MSPeps) 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 (UBic, 54-6), hepatocytes (HepaRG, HepG2) or renal cells (HEK293). At all tested doses (10, 40 µM), MSPepIC (from screening #1) and MSPep1-3 (from screening #2) were internalized into myotubes after 2h of incubation. As expected, MSPepIC and MSPep1-3 were not internalized by renal cells that do not express endogenous MMP. MSPep1-3 were only internalized by renal cells when they were transfected with an MMP expression plasmid. MSPepIC 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 MSPep2-3, likely due to its helical tertiary structure. MSPep-ASO complexes were designed *in silico* based on literature data and collaborators' expertise. The next experiments will evaluate the ability of MSPep-ASOs to target skeletal muscle and deliver ASO efficiently into muscle cells.

P1.11

Short- and long-term systemic treatment of the ACTA1-MCM/FLExDUX4 mice with an AAV-shDUX4

Julie Dumonceaux[1], Solene Sohn[1] Sophie Reid[1], Maximilien Bowen[2], Emilio Corbex[1], Baptiste Morel-Prieur[2], Christophe Hourde[2], Virginie Mariot[1]

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FSHD is characterized by the aberrant expression of the DUX4 transcription factor in muscle. DUX4 is a toxic protein, triggering a cascade of events that eventually lead to myofibre death. In this study, we designed an shRNA directed against DUX4 (shDUX4) to knock down DUX4 expression in the cre-inducible DUX4 bi-transgenic mouse model (ACTA1-MCM/FLExDUX4). Four weeks after vector delivery, most of the molecular and histological pathological signs of the DUX4 expression were significantly reduced. Using the force-velocity-endurance model (FoVE) we developed, we were able to *in situ* compare the muscle functional capacities of the ACTA1-MCM/FLExDUX4 transgenic mice after IV injection of the AAV-shDUX4 or AAV-shCtrl. The integrative FoVE model describes the evolution of the muscle force capacity simultaneously as a function of the contraction velocity and the time (i.e., fatigue), which much better mimics what happens in everyday life. The data obtained 1 year after AAV-shDUX4 injection will be also presented.

P1.12

The prevalence of sleep disorders among individuals with facioscapulohumeral dystrophy: A review of the literature

Nedra Whitehead[1], Joyce Alese[2], Michael Enger[1], Christine Hill[1], Jamie Zimmerman[2], Barbara Do[1], Amy Moore[1], Shiny Thomas[3], Julie Royer[4], Swamy Venkatash[5], James Howard[5]

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[2] Centers for Disease Control and Prevention
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[4] South Carolina Department of Revenue and Finance
[5] University of South Carolina

Sleep problems are common comorbidities of muscular dystrophies. Sleep evaluations are recommended for dystrophinopathies and myotonic dystrophy, but not facioscapulohumeral dystrophy (FSHD). Our objective was to examine the prevalence of sleep disorders among individuals with FSHD. We systematically reviewed PubMed- and EBSCO-indexed literature for studies of sleep disorders and muscular dystrophy, including FSHD. Two librarians searched the literature with the same strategies. Two investigators reviewed each citation using preset eligibility criteria: 1) published in English at any time, and 2) provided data on the prevalence of sleep evaluations, disorders, or treatments among individuals with an eligible muscular dystrophy. Abstractors used a standard template, and a senior investigator reviewed each abstraction. We calculated summary prevalence as the total affected/total in the studies. Eight studies reported on individuals with FSHD, and 6 studies reported on sleep disorders among healthy controls. Ten percent of individuals with FSHD had abnormal daytime sleepiness (756 individuals, 5 studies, range: 2% to 17%) compared to 12% of healthy controls (174 individuals, 5 studies, range 6% to 17%). Among 86 individuals with FSHD (3 studies), 27% had sleep apnea compared to 10% of healthy controls (172 individuals, 5 studies). Our findings suggest sleep studies may be useful for individuals with FSHD.

P1.13

The roles of flavones and autophagy in *DUX4* toxicity

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Our objectives are to identify the cell pathways that mediate *DUX4* muscle toxicity in FSHD and discover drugs that target the *DUX4* toxicity pathway as candidate therapeutics. FSHD is caused by epigenetic disruptions leading to misexpression of the *DUX4* gene in skeletal muscle, causing toxicity and muscle degeneration through caspase 3/7 mediated apoptosis. Identification of the pathways that mediate *DUX4* toxicity in muscle and drugs that modulate these pathways are a focus of our investigations. Our studies have focused on small molecule screens using an inducible *DUX4* muscle cell line, *DUX4*i MB135, to identify compounds that reduce *DUX4* muscle toxicity. A group of compounds structurally related to flavones have been identified that function downstream of *DUX4* expression to inhibit *DUX4*-mediated caspase 3/7 apoptosis in the *DUX4*i MB135 model. Suppression of *DUX4*-induced toxicity by these flavones is mediated through an mTOR-independent mechanism that maintains expression of ULK1 protein, an autophagy regulator, and increases expression of an autophagy marker-LC3I/II. Findings support a hypothesis that *DUX4* inhibits expression of ULK1, which leads to a failure of autophagy to remove the accumulation of aberrant proteins known to be produced by a *DUX4*-mediated damage-associated molecular patterns response, resulting in apoptosis. Experiments are in progress to investigate the mechanisms of *DUX4* knockdown of ULK1 expression and flavone inhibition of this *DUX4* function.

P1.14

Unraveling the role of non-myogenic mesenchymal cells in FSHD pathogenesis and investigating the impact of human amniotic stromal cell conditioned medium on non-myogenic mesenchymal cell functionality

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Non-myogenic mesenchymal cells are pivotal in muscle regeneration, but in a degenerative environment they accumulate, causing the aberrant deposition of fibrous and adipose tissue. Our recent study revealed that these cells participate in muscle degeneration in FSHD patients. Currently, we are delineating the *in vitro* features of non-myogenic mesenchymal cells isolated from FSHD muscles, assessing their proliferation, differentiation capacities, *DUX4* activation, and impact on myogenesis. Also, we are exploring how conditioned medium from human amniotic stromal cells (CM-hAMSC), able to modulate the functions of various cells including immune cells, influences the proliferative and differentiation properties of these cells. Our data show increased proliferation and altered differentiation of FSHD-derived cells compared with control cells, with specific *DUX4* target activation. FSHD and control cells differentially influence myoblast proliferation and differentiation in co-culture experiments. We also show that CM-hAMSC significantly reduces the proliferation and differentiation of FSHD-derived cells. Elucidating the role of non-myogenic mesenchymal cells in FSHD pathogenesis could help to clarify disease mechanisms and to identify specific pathways eligible as novel therapeutic targets in patients. In this context, CM-hAMSC could re-educate the altered behavior of FSHD non-myogenic mesenchymal cells, acting on their impaired proliferation and differentiation capabilities.

Preclinical Models

P2.01

A comprehensive functional force-velocity-endurance (FoVE) model reveals unsuspected muscle properties affected by the expression of *DUX4*

Julie Dumonceaux[1], Maximilien Bowen[2], Solene Sohn[1], Virginie Mariot[1], Baptiste Morel-Prieur[2], Christophe Hourde[2]

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Measurements of muscle function are crucial to the assessment of potential treatments for muscular diseases. To date, several force tests have been proposed in the literature such as the grip strength test and *in situ* measure of maximum muscle force-producing capacity. These experiments are realized under isometric conditions at null velocity, which does not reflect what happens in everyday life. Here, we developed a force-velocity-endurance (FoVE) model to *in situ* compare the muscle functional capacities of the ACTA1-MCM/+ (Cre+) and ACTA1-MCM/FLExDUX4 (CD-Cre+) transgenic mice. The integrative FoVE model describes the evolution of the muscle force capacity simultaneously as a function of the contraction velocity and the time (i.e., fatigue). Experimentally, FoVE capacities were assessed during a fatiguing 180-s exercise by repeating maximal (100 Hz stimulation) shortening contractions performed at different velocities (2 to 15 mm.s-1). This test provides a complete mapping of muscular function by describing the force production capacities at all velocity and fatigue levels. This comprehensive functional force-velocity-endurance model reveals muscle properties affected by the expression of *DUX4*, leading to a weaker, less powerful muscle but less quickly fatigable muscle.

Outcomes Assessments

P3.01

A comprehensive analytical description of atypical features in FSHD1 patients from the French FSHD registry

Benoît Sanson[1], Abderhmane Slioui[1], Caroline Stalens[2], Lori Klouvi[2], Céline Guien[3], Hadrien Delattre[2], Sitraka Rabarimeriarijaona[4], Rafaëlle Bernard[4], Christophe Béroud[5], Sabrina Sacconi[6], the French FSHD Registry Collaboration Group

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Facioscapulohumeral muscular dystrophy type 1 (FSHD1) presents highly variable phenotypes with frequent atypical manifestations complicating diagnosis and prognosis. Identifying atypical features and their determinants is crucial to enhance patient care. Using data from 252 French FSHD registry patients, we conducted statistical analyses on FSHD1 atypia. We also explored severity via multivariate analyses, recognizing perceived links with some atypical features. Half of atypical patients showed a discordance between their condition's severity and the number of D4Z4 repeat units (RUs) identified via molecular diagnosis. Three unexpected atypical features emerged: anosmia, predominant axial impairment, and atopic dermatitis. Univariate analysis showed that only older age at onset was significantly associated with atypia (60.7 ± 17.0 years vs. 51.8 ± 16.4 years in typical patients, $p < 0.001$). Multivariate analysis revealed an RU number in the top FSHD1-compatible range (8 to 10) as a significant predictor (OR = 3.90, 95% CI [1.81 to 8.39], $p < 0.001$). In some multivariate models, the presence and number of systemic impairments, along atypia, contributed to severity. This study will aid in validating or refining the classification of atypia within the French FSHD registry, in turn facilitating the development and adoption of an international standardized classification system. Subgrouping FSHD is pivotal for enhancing diagnosis accuracy and informing future therapeutic interventions.

P3.02

Cohort profile: Clinical characteristics of patients with facioscapulohumeral muscular dystrophy from Russian Registry

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Background: The Russian Registry for Facioscapulohumeral Muscular Dystrophy (FSHD) was established in 2020 following successful development of genetic analysis to assess D4Z4 repeat numbers in Russia. Methods: The Russian FSHD Registry gathers data from both patients and physicians via self-report questionnaires and clinical assessment forms. The assessment tools include 48-Manual Muscle Testing, FSHD Comprehensive Clinical Evaluation Form (FSHD-CCEF), and Facial Disability Index. Results: Currently, the Registry comprises 352 participants, with 168 individuals undergoing comprehensive clinical assessments. Among all participants, 262 patients from 193 families have genetically confirmed FSHD1, and 9 from 6 families have genetically confirmed FSHD2. The median age at examination is 32.5 years (range 5 to 83 years), with an onset median age of 15 years (range 0 to 53 years). Correlation analysis revealed a weak correlation between D4Z4 repeat numbers, FSHD-CCEF score ($r = -0.23$), and age of onset ($r = 0.35$) in FSHD1 patients. The most common clinical signs include facial weakness and scapular winging (observed in 97% and 92.5% of FSHD1 patients, respectively). Sixty-eight percent of FSHD1 patients exhibit some walking difficulties, with 4 patients (2.5%) experiencing complete ambulation loss. Conclusions: The Russian FSHD Registry, like its counterparts in other nations, aims to facilitate comprehensive patient assessments and gather extensive data to support future clinical research endeavors.

P3.03

Enhancing clinical trial eligibility criteria in FSHD: Validating whole-body MRI as a key outcome measure

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Introduction: The heterogeneous disease progression in FSHD makes assessing treatment response in clinical trials challenging. Quantitative whole-body MRI has been recognized as a promising technique to address this, but there are limited data from its use in natural history studies. Aim: The overall aim of the CTRN FSHD France study (NCT03458832) is to validate new outcome measures, define minimal clinically important change, and establish FSHD characteristics useful for determining clinical trial eligibility criteria. Methods: Up to 70 ambulatory FSHD1 patients aged 18 to 75 with symptomatic limb weakness will be included and followed for 24 months. In addition to whole-body MRI, the study will also assess muscle strength and function, as well as several patient-reported outcome measures. Results: Sixty-eight patients successfully completed the baseline analysis, including MRI. The median (min, max) age was 50 (21, 75) years, with CSS 6 (1, 9) and 6 (2, 10) D4Z4 repeats. The muscle fat fraction (MFF) was 7% (1, 91), 21% (2, 96), 6% (1, 87), and 31% (2, 100) in the arms, legs, rotator cuffs, and torso, respectively. Conclusion: We have successfully implemented quantitative whole-body MRI in an FSHD cohort with baseline characteristics resembling what can be expected in clinical trials. Initial analysis of disease progression after 12 months is expected to be available this fall.

P3.04

Patterns of fat distribution from individual muscles across whole-body MRI (iWBMRI): Visualizing and quantifying features

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Recent work by our group has validated the ability to quantify and visualize individual muscles across whole-body MRI (iWBMRI), leveraging automated artificially intelligent-based segmentation, trained using cohorts of patients with facioscapulohumeral muscular dystrophy (FSHD). In this sample collected in Australia as part of the FSHD Global Registry project, 10 patients with FSHD (age = 41 +/- 12 years, 8 males/2 females) were scanned by iWBMRI, and 108 total muscles were analyzed. We hypothesized that previously observed patterns could be seen across affected muscles, and new visualizations reveal unique features of fat distribution in FSHD. Metrics included: 1) distribution graphs of fat infiltration, fat volume, contractile muscle volume, and Moran's I (a measure of homogeneity of fat) as a function of location along the muscle length; 2) fat infiltration pixel intensity histograms; and 3) 3D pixel-by-pixel color-coded fat infiltration mapping. Five main patterns were confirmed: Distal to proximal gradient, proximal to distal gradient, fat at both muscle ends progressing centrally, fat beginning in the muscle center, and fat distributed through the muscle extent. Moran's I, histograms, and individual pixel examples provide additional advanced characterization. Integrated into larger cohorts, these measures may inform which patterns progress more rapidly, and whether observed patterns relate to muscle function, muscle architecture, or vasculature location.

P3.05

The Dutch Registry for Facioscapulohumeral Muscular Dystrophy: Cohort profile and longitudinal patient-reported outcomes

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Introduction: The Dutch FSHD Registry was initiated in 2015 as a result of an international collaboration on trial readiness. This paper presents the cohort profile and 6 years of follow-up data of the registered FSHD patients. Methods: At the time of self-registration and every 6 months thereafter, participants were invited to complete a digital survey of patient and disease characteristics, and the Dutch versions of the Checklist Individual Strength (CIS20R), the Individualised Neuromuscular Quality of Life Questionnaire (INQoL), the Beck Depression Index – Primary Care, and the McGill Pain Questionnaire. Results: From March 2015 to March 2021, 373 participants completed at least 1 survey. At baseline, fatigue and muscle weakness were the most frequently reported symptoms (median CIS20R sumscore 77 [IQR 60 to 92], median INQoL Fatigue score 58 [IQR 42 to 68] and median INQoL weakness score 58 [IQR 42 to 68]). Pain was experienced most often in the head and shoulder region (193, 52%). Nineteen of the 23 (sub)sections of questionnaires showed no significant changes over time. Conclusion: The Dutch FSHD Registry was successfully set up, enabling collection of longitudinal data and facilitating recruitment in several studies.

P3.06

The facioscapulohumeral muscular dystrophy Rasch-built Overall Disability Scale (FSHD-RODS): Longitudinal assessment of a disease-specific patient-reported outcome measure in a clinical trial timeframe

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Objectives: To determine the change in facioscapulohumeral muscular dystrophy Rasch-built Overall Disability Scale (FSHD-RODS) score over a period of 18 months in a cohort of FSHD patients containing the full disease severity spectrum and to assess its responsiveness in a clinical trial timeframe. Methods: FSHD patients of 18 years or older were included for a baseline and follow-up visit at the outpatient clinic 18 months apart, including disease severity assessment using the FSHD clinical score and Ricci score, and functional assessment using the Motor Function Measure (MFM). Patients filled in the FSHD-RODS and Sickness Impact Profile 68 (SIP68) questionnaire at both timepoints. FSHD-RODS raw sum scores were converted to logits. Responsiveness analyses were done by calculating the minimal clinically important difference based on a distribution-based method (MCID-SE) and anchor-based method. Results: Seventy-nine patients were included, comprising the full disease spectrum. The FSHD-RODS, SIP68 questionnaire, disease severity, and MFM were stable over time. There were strong correlations between FSHD-RODS and SIP68, MFM, and disease severity results at baseline and follow-up. Only 3 patients (4%) had an MCID-SE indicating clinically important deterioration. The calculated anchor-based MCID was -0.53. Conclusions: The FSHD-RODS is a reliable interval scale and showed no deterioration in this clinically stable cohort.

Genetics & Discovery Research

P4.01

4qA D4Z4 methylation test as a valuable complement for differential diagnosis in patients with FSHD-like phenotype

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4qA D4Z4 DNA hypomethylation is proposed as a complement for diagnosis and explanation of disease severity of FSHD, but further validation in an extended population is needed. A total of 247 FSHD-like phenotype patients with 4qA array analysis were enrolled. Two hundred nineteen cases were identified as FSHD1 due to at least one contracted D4Z4 repeat. Two out of the remaining 28 undiagnosed cases were further identified as FSHD2 due to distal and global hypomethylation tested by PCR-based bisulfite sequencing. Whole-exome sequencing and/or muscle pathology confirmed other diagnoses in 14 cases. Distal hypomethylation and global hypermethylation were observed in 3 patients with uncontracted 4qA alleles. A repeat length-dependent increase was observed in both distal and global methylation levels. Compared to linear regression, nonlinear regression model showed lower root mean squared error (6.8097 vs. 7.5915) between repeat size and distal methylation level. Distal methylation distinguished FSHD1 better with a sensitivity of 100% and specificity of 95.45% at a cutoff value of 39.66% from controls compared to global methylation levels. Distal methylation level showed a strong correlation with clinical severity similar to D4Z4 repeat unit size. Finally, distal methylation could distinguish early-onset patients from classic-onset patients compared to D4Z4 repeat size. Mediation analysis revealed that the influence of distal methylation on ACSS was partially mediated by onset age (27.11% of total effect).

P4.02

Comprehensive molecular study of patients with FSHD2

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Facioscapulohumeral muscular dystrophy (FSHD) type 2 constitutes only 5% of FSHD cases. Due to its unique and complex digenic pathogenesis mechanism, it remains a significant challenge for both study and diagnosis. We collected 7 families with cases of FSHD2 from the Russian FSHD Patient Registry and conducted a comprehensive study. This study included physical examination (FSHD-Comprehensive Clinical Evaluation Form [CCEF]), determination of the presence of a permissive haplotype, qPCR-based detection of the number of D4Z4 repeats on the allele with a permissive haplotype, whole-exome sequencing/whole genome sequencing methods for searching pathogenic variants in chromatin modifier genes, functional analysis of identified splicing variants, and methylation analysis. Genetic analysis in 6 families identified unique *SMCHD1* gene variants (3 missense, 2 splicing, and 1 nonsense) associated with FSHD, with D4Z4 repeat unit contractions influencing clinical severity and presentation. Methylation levels correlated with disease severity, as evidenced by the proband's clinical assessment scores. In one family, comprehensive analysis established an FSHD type 1 diagnosis. The complex analysis of these families with FSHD2 provides valuable insights into the disease mechanism. It aids in understanding the correlations among genetic data, disease prognosis, and clinical presentation. Overall, this comprehensive approach contributes significantly to advancing our knowledge of the disease and improving patient care.

P4.03

Evaluation of non-invasive biological sources for assessing methylation levels of FSHD-associated locus

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The selection of the primary source of DNA is crucial to ensure the high quality and reliability of molecular assay results. Moreover, low invasive and easy sampling procedures are highly desirable. The study aimed at evaluating the integrity and stability of saliva and buccal swabs, to be applied as DNA sources for assessing the presence of methylation levels compatible with facioscapulohumeral dystrophy (FSHD). Methylation levels of 60 DNA samples derived from saliva, blood, and buccal swab were assessed in the present study. DNA was extracted at T_0 and T_1 (namely upon sample collection and 10 days after, respectively). Each DNA sample was simultaneously treated with bisulfite conversion and sequenced by capillary electrophoresis to quantitatively evaluate methylation levels. The comparison of results obtained from different biological sources revealed no inter-specimen variability within each patient regarding methylation levels, sequencing quality, and data reliability. Such a result was equally valid at T_0 and T_1, suggesting the employment of biological sources alternative to blood for assessing methylation levels in FSHD patients. These results are paramount, considering the difficulties related to FSHD diagnosis in developing countries and the need for affordable and rapid tests. Although this study needs to be validated in larger cohorts, it encourages the employment of non-invasive DNA biological sources to promote FSHD characterization in worldwide populations.

P4.04

Interleukin 1 beta levels were significantly different in male FSHD patients

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In FSHD, *DUX4* toxicity leads to cell death. Cell death is known to start a process called sterile inflammation through IL-1 family, which includes IL-1β. Serum biomarker studies in the field have shown that there is a significant increase in IL-6 and TNF-alpha levels of FSHD patients compared to healthy controls (HC). In this pilot study, we investigated the potential inflammatory changes in our own patient group. For this purpose, we measured IL-1β, IL-6, and TNF-alpha levels in 8 male patients and 8 age-matched HC. The serum levels of Interleukin IL-1β, IL-6, and TNF-α were quantified using a Simple-Plex cartridge for ELLA Automated Immunoassay System Instrument. As a result, we found that all of the parameters – IL-1β, IL-6, and TNF-α – exhibited higher levels in the FSHD group. However, of those, IL-1β was the only parameter that was significantly higher in FSHD patients compared to controls ($p = 0.012$). What we found was the high inter-individual variation of IL-1β and IL-6. The small number of participants might be a limitation, or geographic heterogeneity may be a contributing factor to this variability. It is noteworthy that the significant difference of IL-1β levels, an interleukin that is specifically associated with sterile cell death, was detected even in our pilot group. Based on this significance, we suggest that IL-1β may provide relevant clues regarding pathophysiology.

P4.05

Is *DUX4*-mediated hormone receptor dysregulation contributing to FSHD?

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Our studies of DUX4 molecular domains contributing to its cellular toxicity, subcellular trafficking, and nuclear localization identified 2 LLXXL-like motifs at the C-terminus of DUX4 that may confer upon this protein a coregulatory role on the activity of nuclear hormone receptors. Aberrant expression of DUX4 in muscle cells could disrupt the activity of certain hormone nuclear receptors, potentially contributing to the clinical phenotype of FSHD. Comprehensive biochemical and cellular studies, using various cell lines (T47D, HepG2, and HEK293), as well as wild-type and mutant versions of DUX4, allowed us to demonstrate that DUX4 acts as a corepressor of progesterone and glucocorticoid nuclear hormone receptors, with a physical interaction observed between DUX4 and the glucocorticoid receptor. In further studies, we explore the potential coregulatory role of DUX4 on estrogen receptors (ER**α** and ER**β**), as well as the impact of cellular presence of DUX4 and these receptors on the subcellular distribution of these proteins. Additionally, using available RNAseq data, we are characterizing the relative levels of hormone nuclear receptor mRNAs present in controls, FSHD1 and FSHD2 myoblasts, and differentiating myotubes. Results from these studies would advance our understanding of the physiology of hormone receptors in human muscle, also shedding light on the proposed novel endocrine activity of DUX4 and its connection with the pathogenesis of FSHD.

Pediatric FSHD

P5.01

Early-onset FSHD natural history and preclinical research pipeline at Melbourne Children's Campus

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FSHD is a genetically complex neuromuscular disorder that typically presents in the second or third decades of life. The manifestation of symptoms in early childhood is less common and thought to correlate with greater symptom severity and more rapid disease progression. Early-onset FSHD is rare, and less is known about disease natural history or factors that may impact disease severity and progression. The Royal Children's Hospital (RCH) and Murdoch Children's Research Institute (MCRI) are in the third year of a childhood-onset FSHD longitudinal outcome study (iFSHD-LOS) involving the collection of biological samples (blood and saliva), functional and patient-reported outcome measures, and whole-body MRI. This project involves 3 pillars of work: 1) the clinical/MRI assessment and longitudinal analysis of children with early-onset FSHD, 2) biobanking of patient material (saliva, blood, and induced pluripotent stem cell [iPSC] lines) for current and future research initiatives, and 3) analysis of the role that other biological or environmental factors play in FSH disease mechanism and outcomes. The ultimate goals of this project are to understand the disease trajectory and the impact of development and childhood growth, validate existing outcome measures, and develop a framework for laboratory-based preclinical drug testing to facilitate holistic patient management and research into childhood-onset FSHD.

P5.02

Lower body muscle MRI shows annual progression of fat fraction in Australian children with FSHD

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Pediatric FSHD is a rare genetic muscle disease caused by the aberrant expression of *DUX4*. MRI is a biomarker for disease severity previously found to correlate well with disease-specific functional severity measures. Pediatric FSHD is an area of unmet need and focus for international collaborative efforts to establish better disease measures in the search for a disease-modifying therapy. Participating children were recruited from neuromuscular clinics across Australia into the iFSHD-LOS, a single-site natural history study performed at the Royal Children's Hospital, Melbourne. In this study, lower body MRI data were retrospectively analysed by Springbok Analytics' artificial intelligence platform to determine fat fraction at the individual muscle level in 70 lower limb muscles. Data were quantitatively expressed to examine patterns of disease progression over time and to correlate changes seen on MRI with disease severity as measured using established and emerging clinical measures. To date, lower body muscle MRI taken 12 months apart have been analysed for 15 participants. Preliminary results show there is a mean increase in the average fat fraction across all lower limb muscles of 1.5% (range -1.4% to 5.7% increase) over 12 months, with individual muscle results forthcoming. Further planned analysis includes addition of upper body muscle and detailed integration of pediatric normative growth models as covariates for assessing MRI biomarkers and functional results.

P5.03

Methylation analyses of Australian children with FSHD

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Pediatric FSHD is a rare genetic disease caused by the aberrant expression of *DUX4* because of a hypomethylated D4Z4 area at the telomeric end of chromosome 4. Hypomethylation of the D4Z4 region is a surrogate marker of *DUX4* expression, can be diagnostic of FSHD, and may correlate with disease severity. Participants in the iFSHD-LOS natural history study, run at the Royal Children's Hospital in Melbourne, Australia, had saliva samples sent to the Jones Lab at the University of Nevada, Reno. The relevant methylation status for each sample was determined using targeted bisulfite sequencing of the distal 4qA or 4qAL D4Z4 repeat unit, and data were analyzed using the D4Z4caster program. In addition, the relevant methylation status of all internal D4Z4 repeat units was similarly assessed to determine the presence of epigenetic modifiers of the region. The percent of methylation was low in the first quartile (Q1) of the distal 4qA repeat unit in all 21 participants with a range of 0% to 23.8%, in keeping with the established level of <26% being diagnostic of FSHD1. Of this early-onset cohort, 11 (52%) had undetectable methylation at Q1. Hypomethylation in Q1 showed strong correlation with parent-reported age of symptom onset (r = 0.61). The data from the Australian pediatric cohort show that measurement of D4Z4 hypomethylation can be used to diagnose FSHD. Further studies with larger cohorts are required to establish if hypomethylation could be used to predict rapidity of disease progression.

P5.04

Motor Outcomes to Validate Evaluations in Pediatric FSHD (MOVE Peds) – study outline

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Background: Pediatric FSHD represents early onset of a disease continuum of FSHD across the lifespan. It remains an area of unmet need and the current focus of international collaborative efforts to establish better outcome measures in the search for a disease-modifying therapy. Recently, the disease-specific FSHD-Composite Outcome Measure (FSHD-COM Peds) has been adapted for use in children, with early studies suggesting good reliability and validity. The Reachable Workspace (RWS) is currently the primary outcome measure in a Phase 3 clinical trial in adults with FSHD, and has recently been adapted and used in a small single-site natural history study. Whole-body muscle MRI (WBMRI) is a biomarker for disease severity previously found to correlate well with disease-specific functional measures. Methods: Following on from 2 successful natural history studies (NCT04635891 and ACTRN12621001293853), the authors aim to recruit 80 children with FSHD across 8 sites in the US and Australia to assess clinical trial utility of outcome measures in children to ready the paediatric population for clinical trials. Participants' functional capacity (FSHD-COM Peds and RWS), disease severity (FSHD Clinical Score and FSHD age-adjusted Clinical Severity Scale), and muscle biomarkers (WBMRI) will be evaluated at baseline, 6, 12, and 24 months. Conclusion: Following successful application to the National Institutes of Health to fund this clinical trial preparedness study, we aim to start recruiting at the end of 2024.

P5.05

Preclinical models of childhood-onset FSHD

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FSHD typically presents in the second or third decades of life. The presentation of symptoms in early childhood is less common and thought to correlate with a more rapid disease progression. Early-onset FSHD is typically classified as the presence of symptoms before 10 years of age, and while less is known about the factors that impact disease progression and severity in children, improved diagnosis is resulting in an increase in recognized cases of childhood-onset FSHD. At the Murdoch Children's Research Institute (MCRI) we are in the third year of a childhood-onset FSHD longitudinal outcome study (iFSHD-LOS) involving the collection of clinical natural history data (including functional and patient-reported outcome measures and whole-body MRI) and biological samples (blood and saliva) for research. The biobanking of patient blood has enabled us to generate patient-specific induced pluripotent stem cell (iPSC) lines from children with FSHD, which will be used to model disease and aid in the identification of a novel therapeutic. The ultimate goal of this project is to improve our understanding of the disease trajectory of childhood-onset FSHD. This program aims to link laboratory-based research with patient management to aid in the identification of new pathways to treat patients with FSHD.

Clinical Studies & Trial Design

P6.01

A retrospective cross-sectional clinical study identifies extracellular vesicle-associated circulating protein biomarkers for facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is a prevalent muscular dystrophy leading to muscle weakness and wasting. Recently, influx of FSHD therapies into clinical trials has accelerated. However, disease variability and inconvenience of clinical outcome measures continue to challenge therapy approval. Hence, there is a need to identify reliable blood molecular biomarkers to stratify patients for clinical trials, and to monitor disease progression and response to therapy. Importantly, circulating extracellular vesicles (EVs) are emerging as disease biomarkers, as their content can inform the pathophysiological state of the disease. Here, we used a ReSolve study cohort of 25 FSHD patients and 11 healthy controls to reveal the plasma EV proteome of FSHD patients. As a result, we have identified 3 proteins significantly upregulated in FSHD patients. Notably, males exhibited exclusive expression of 2 striated muscle structural proteins, while females showed a 5-fold upregulation of an activator of the classical/lectin complement pathway. The elevated levels of this activator were more uniform in female patients with moderate to severe clinical severity score, and who are over 50 years old. These changes are FSHD specific, as they are not observed in our dystrophinopathy plasma EVs, indicating their potential implication in FSHD pathogenesis and consolidating their clinical relevance as FSHD plasma biomarkers. We will validate our results in a second independent cohort.

P6.02

Coproducing care quality standards in facioscapulohumeral muscular dystrophy (FSHD) in partnership with people with FSHD, carers, and healthcare professionals: A qualitative focus group study

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Introduction: Facioscapulohumeral muscular dystrophy (FSHD) causes progressive muscle weakness resulting in permanent disability, necessitating lifelong management. Care standards ensure equitable care and measure improvement in FSHD services but are currently lacking. Objective: Develop FSHD care quality standards, using qualitative focus groups with people with FSHD, caregivers, and healthcare professionals. Methods: A 2-stage process: 1) 8 focus groups with separate groups of people with FSHD, caregivers, and clinicians, and 2) 2 focus groups with all 3 participant groups to refine initial findings and co-produce care standards. Focus group transcripts were analysed using thematic analysis. Results: Stage 1 findings from 27 people with FSHD, 4 caregivers, and 20 clinicians identified 11 care quality domains: Education and support at diagnosis; information, education, and support for families and carers; support networks and self-help groups; multidisciplinary team; periodical multidisciplinary review and clinical assessment; access to healthcare professionals with FSHD understanding; named healthcare professional; coordinated care and clinical pathways; service access modalities; self-management; and communication. Conclusions: These findings offer a preliminary framework for developing FSHD care standards to enhance care delivery, standardise practices, mitigate regional discrepancies and health inequalities, and optimise patient health outcomes.

P6.03

Deep phenotyping and comprehensive genetics characterization in atypical FSHD cases

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Introduction: The different FSHD phenotypes could show a different disease progression and/or imply distinct genetic mechanisms. To date, the diagnostic criteria for FSHD are based on the detection of the genetic signature of the disease. However, the molecular diagnosis still needs to be improved in terms of precision, accuracy, required times and costs, and the interpretation of the genetic test cannot ignore a careful correlation with the phenotype. Methods: We present data from a cohort of 43 patients from 24 families selected by phenotypic features, characterized by incomplete penetrance/atypical phenotypes according to the Comprehensive Clinical Evaluation Form (CCEF). The molecular characterization included the assessment of 4q subtype, DNA methylation levels, whole-exome sequencing (WES), and segregation analysis. Results: In our cohort, methylation levels displayed high variability in relation to the disease phenotype. In more than half of the atypical phenotypes, despite the detection of the FSHD genetic signature, WES analysis identified variants of uncertain significance or likely pathogenic/pathogenic variants in other genes or known FSHD-modifying genes. A definitive alternative diagnosis was obtained in 5 families. Conclusion: The results coming from this clinical case study further support the need to perform a detailed phenotypic characterization of patients with a suspect of FSHD, and, in cases of atypical phenotypes, to combine the D4Z4 sizing with other procedures such as WES.

P6.04

Increasing diversity in clinical trial participation: An exploration of clinical trial site engagement

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Increasing diversity among clinical trial participants requires awareness and commitment to change at multiple levels, including clinical trial sites. This study aimed to understand how clinical trial sites approach increasing diversity, access, and inclusion. Avidity surveyed their active clinical trial sites to better understand current practices and plans to increase diversity in muscular dystrophy clinical trials. Of the 92 investigators and research staff invited from 36 sites, 43 individual responses were received. Survey questions focused on awareness of the problem; actions taken at the individual, clinic, and institutional level; feasibility of specific activities; and ideas for next steps for their own site. Descriptive analysis on frequency distributions for close-ended questions illuminated levels of awareness and progress among sites, as well as their perspective on what steps were feasible. Thematic analysis revealed which actions sites have taken and areas they see making further change. Respondents acknowledged the issue of lack of diversity in clinical trial participation, with some action being taken. Sites seemed willing to do more, while recognizing challenges and limitations. Proposed initiatives to address this problem included developing education, tools, and resources, and supporting their implementation to increase diversity in clinical trial enrollment to help bridge community and academic centers and increase access to diagnosis and care.

P6.05

Safety and tolerability of losmapimod for the treatment of FSHD

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FSHD is a relentless, variably progressive disease leading to accumulation of disability over decades. Fulcrum has assessed losmapimod, a small molecule p38 a/b MAPK inhibitor, in FSHD in 1 Phase 1 study (FIS-001-2018) and 2 ongoing Phase 2 studies in the open-label extension period (FIS-001-2019 and FIS-002-2019). Subjects aged 18 to 65 years with genetically confirmed FSHD1, Clinical Severity Score 2-4, and MRI-eligible muscles for biopsy were exposed to losmapimod 7.5 or 15 mg BID PO for 14 days and up to approximately 168 weeks. In FIS-001-2018, 6 subjects were exposed to 7.5 mg and 11 subjects to 15 mg twice daily dosing for 14 consecutive days. In FIS-001-2019 and FIS-002-2019, 14 and 77 subjects, respectively, received at least 1 dose of losmapimod 15 mg twice daily for up to approximately 168 weeks. A total of 108 subjects with FSHD1 have received losmapimod in studies FIS-001-2018, FIS-001-2019, and FIS-002-2019. Most AEs were considered mild to moderate in severity and included eczema, dry skin, alanine aminotransferase increase, rash, headache, and myalgia. Majority resolved with continued dosing. No drug-related SAEs, deaths, discontinuations due to AEs, or clinically significant changes in vital signs, clinical labs, or ECGs were reported. Losmapimod administered up to 15 mg BID in >100 subjects with FSHD1 for up to approximately 168 weeks has been generally well tolerated; the benefit-risk profile of losmapimod for the treatment of FSHD remains positive and favorable.

P6.06

The FSHD European Trial Network

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FSHD Europe is the voice for FSHD patients across Europe, currently representing 10 national patient organizations from 9 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 the FSHD Society. 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 3 years ago, works on: Increasing the commitment of clinicians and researchers; harmonizing criteria for clinical and genetic diagnosis for clinical outcome measures, biomarkers, and imaging outcome markers; exchanging clinical experience and genetic reference material; engaging with pharma and EMA; and harmonizing treatment and care for all European FSHD patients. The ETN consists of 4 working groups (WG) on clinical and genetic diagnosis (WG1), clinical outcome measures (WG2), biomarkers (WG3), and imaging outcome measures (WG4). WG5 will be initiated shortly, focusing on childhood-onset FSHD. The ETN WGs work in close collaboration with the Clinical Trial Research Network, FSHD Society, TREAT-NMD, and the European Reference Networks.

P6.07

The participants' perspective on clinical trials – a qualitative study

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Aim: To create more in-depth understanding of the patient perspective of these trials will further enhance the design and recruitment of future trials. Methods: A retrospective, phenomenological qualitative study was performed through in-depth semi-structured interviews. The interviews were held either at the Radboud University Medical Center or online via Teams. The data were transcribed in Atlas.ti and analyzed through a framework analysis, using a deductive, interpretative approach. Results: A total of 13 participants were interviewed: 6 of the Phase 2 trial and 7 of the Phase 3 trial. Patients did not have very specific expectations of the effect of the study drug before trial participation and mentioned the distinction between the (limited) expectations they had and the hope of an effective cure. Overall, participants were positive about the trial participation experience. Specifically, the personal and transparent communication within a trusting and dedicated study team was appreciated. Participants expressed their strong wish to receive more frequent updates on the overall progress of the trial and on safety issues. Conclusions: This study provides insights from the participants' perspective, providing important insights for future clinical trial design, study site practices, and patient education.

P6.08

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 FSHD Patient Registry is a patient self-enrolling online database collecting clinical and genetic information about FSHD type 1 and 2. The registry was established in May 2013 with support from Muscular Dystrophy UK and is coordinated by Newcastle University. The registry aims to facilitate academic and clinical research, better characterise and understand FSHD, collect patient voice, and to disseminate information relating to upcoming studies and research advancements. The registry also collects real-world evidence and supports data enquiries from industry. The registry captures longitudinal, self-reported data through an online portal available to patients and clinicians. Neuromuscular specialists involved in the patient's care can be invited to add specialised clinical or genetic information. The registry is a Core Member of the TREAT-NMD Global Registries Network for FSHD. As of April 2024, there were more than 970 active, UK-based patient registrations. Almost 60% of patients have had genetic confirmation of their condition. The registry is one of the largest national FSHD patient registries and is an example of a versatile, cost-effective research tool, helping to facilitate and advance a wide range of FSHD research with more than 32 registry enquiries to date. Work continues to be done to improve reporting of genetic information on the registry, and to facilitate the collection of patient-reported outcome measures and trial preferences.

Late-Breaking Abstracts

P7.01

Characteristics of the enrolled population in the Phase 3 REACH trial in facioscapulohumeral muscular dystrophy (FSHD): Preliminary results

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Losmapimod, a small molecule p38α/β MAPK inhibitor, was well tolerated, and associated with improved upper extremity function and structural improvements in people living with FSHD1 in the Phase 2 ReDUX4 controlled trial (N = 80). REACH, the Phase 3 trial, is designed to assess efficacy and safety of losmapimod in a larger population of people with FSHD (including FSHD1 and FSHD2). We report preliminary baseline characteristics for participants in REACH. Adults with genetically confirmed FSHD1 or FSHD2 with Ricci score 2 to 4 were enrolled. Participants were randomized 1:1 to losmapimod 15 mg or placebo BID. Demographics and baseline characteristics are summarized; corresponding ReDUX4 data are included for comparison. A total of 260 people were enrolled: 242 with FSHD1 and 18 with FSHD2. Mean age was 43.9 (SD 12.2) years. Most were male (56%) and White (89%); 60% enrolled in Europe and 40% in North America; mean BMI was 25.4 (SD 4.6). Of participants with FSHD1, 14% had 1 to 3 D4Z4 repeats and 86% had 4 to 9 (in ReDUX4, 16% had 1 to 3; 84% had 4 to 9). At baseline, 57% had Ricci score 2 to 3 and 43% had 3.5 to 4 (in ReDUX4, 61% had 2 to 3; 39% had 3.5 to 4). Mean total relative surface area (RSA) of reachable workspace with weight, Q1-Q5, average of both arms was 0.521 (SD 0.165) (0.536 [SD 0.233] in ReDUX4). In sum, baseline characteristics from REACH are similar to those from ReDUX4. Data are subject to updates upon database lock. Top-line efficacy and safety data from REACH are anticipated Q4 2024.

P7.02

Characterizing *SLC34A2* as a biomarker for FSHD

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FSHD biomarker characterization remains a high priority to evaluate therapeutic response in the context of each unique patient trajectory. Our laboratory is investigating the protein SLC34A2 as a potential FSHD biomarker. As a downstream target of *DUX4*, *SLC34A2* is not typically expressed in healthy skeletal muscle, but its protein is increased significantly in FSHD muscle and cell lysates, making it a potential readout of FSHD progression. SLC34A2 encodes a sodium-phosphate co-transporter expressed in lung, kidney, and gut epithelia, but not in mature muscles. Our research thus far shows that *SLC34A2* expression, as well as protein level, is significantly increased in FSHD matured myotube cultures compared to control cultures. These levels decrease when myotubes are exposed to losmapimod and other p38 kinase inhibitors. Previously, we have shown that *SLC34A2* antibodies co-stain FSHD muscle biopsies, FSHD cultured myotubes, and our model of FSHD xenografts, where *SLC34A2* is present in about 1% to 2% of FSHD-affected fibers (Mueller et al., Exp. Neurol. 320: 113011, 2019). Additionally, we can label *SLC34A2*-positive FSHD xenograft fibers *in situ* by using an antibody specific to the exposed extracellular domain of *SLC34A2* and tagged with IR-647. This opens the prospect of tracking disease progression and the efficacy of different experimental therapies for FSHD in the same individuals over time, without the need for muscle biopsies.

P7.03

Small molecule augmentation of Notch signaling rescues models of DMD and FSHD

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Loss of Notch signaling is attributed to regenerative failure of liver ducts in Alagille syndrome (ALGS) and skeletal muscles in DMD, leading to >50% lethality by adulthood. To address these therapeutic needs, we identified a Notch agonist, NoRA1, and found that it directly increases Notch receptor signaling. Treatment of zebrafish and mouse ALGS models with NoRA1 enhances liver regeneration/function and survival. Intriguingly sarcopenia is also found in ALGS patients, a disorder with reduced *JAG1*, a Notch ligand, whereas increased *JAG1* was implicated in attenuating dystrophic pathologies in DMD zebrafish, mice, and dogs. In muscular dystrophies, compromised renewal of muscle stem cells (MuSCs), normally maintained in a quiescent state by Notch-regulated *PAX7*, is thought to contribute to muscle wasting. We find that NoRA1 dosing increases expression of *JAG1*, *PAX7*, and other stem cell regulatory genes in treated mice, primary mouse MuSCs, C2C12 myoblasts, and zebrafish dystrophin homozygotes. In all of these models, including cardiomyocytes derived from human iPSCs, NoRA1 increased expression of Utrophin, a Dystrophin paralog with a compensatory role. Further, dystrophin-null zebrafish, dosed with NoRA1 after significant muscle damage, show locomotor behavior rescue. Moreover, in a myoblast model of FSHD, NoRA1 rescues *Pax7* target gene repression and toxicity caused by *Dux4*. These discoveries demonstrate the therapeutic potential of NoRA1 for treating ALGS and myopathies.

P7.04

DUX4 protein partners in muscle cells are linked to DNA repair, transcription and *DUX4* post-translational regulation

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Last year, we presented interactors of DUX4, a highly polarized protein, using specific AP-MS measurements that reduce electrostatic artifacts. We now confirmed in muscle cells the previous interactors found using HEK293 cells. The C-terminal activation domain of DUX4 strongly interacts not only with p300/CBP, but also with the MED15 and MED25 subunits of the mediator complex, indicating that DUX4 can directly recruit the transcription machinery to the promoters of induced genes, further explaining its potency as transcription activator. DUX4 also interacts with PTOV1, a MED25 homologue, that is endogenously expressed in myoblasts, but of unknown function. The N-terminal DNA-binding region of DUX4 interacts most strongly and specifically with factors involved in DNA double-strand breaks (DSB) repair: C1qBP, XRCC5/6, PARP1, and H2AX. Their interaction with DUX4 may impact their functions and explain the DSB stress that follows DUX4 expression. Interestingly, the strongest interaction of DUX4 is with the RFPL4A protein, encoded by *DUX4* target genes. RFPL4A is the first protein we identified as an interactor of the DUX4 disordered region. As RFPL4A was suggested to be an E3-ubiquitin ligase, DUX4-RFPL4A interaction might induce DUX4 degradation, and co-immunofluorescence allowed their nuclear co-detection only in some dox-treated LHCN-M2-iDUX4 cells. Overall, our findings extend the model of DUX4 activation in several important aspects and reveal new regulatory elements.

P7.05

DUX4c preservation: A key consideration in FSHD therapeutic strategies to safeguard muscle regeneration

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Most therapeutic strategies in development for FSHD target the *DUX4* causal gene, aiming to inhibit expression of its encoded protein. *DUX4c*, a *DUX4* homologue with high sequence similarity, is expressed in healthy muscles and induced in FSHD. Several antisense tools targeting *DUX4* mRNA also effectively target *DUX4c* mRNA. Their encoded proteins are identical over 342 residues, including the double homeodomain. *DUX4* pathological expression leads to cell death, muscle atrophy, and disruption of RNA processes enabling synthesis of aberrant proteins. Additional DUX4 protein toxicity may result from competition with *DUX4c*, disturbing its normal functions. 1) *DUX4/4c* competition affects transcription of genes involved in WNT/beta-catenin pathway activation, with *DUX4c* counteracting *DUX4*-mediated toxicity in myoblasts (Ganassi et al., 2022). 2) *DUX4/4c* competition could affect common partners, as shown for C1qBP, a multi-compartmental protein involved in mitochondrial activity and cell differentiation. On FSHD muscle sections *DUX4* and C1qBP are co-detected in abnormal myocytes/fibers with features of regeneration, suggesting an ineffective process that could result from *DUX4* inhibition of normal *DUX4c*/C1qBP interaction. Novel *DUX4/4c* partners will be presented. In conclusion, we think a decrease in *DUX4c* levels, as a side effect of drugs targeting *DUX4*, is a challenge for FSHD treatment. Maintaining sufficient *DUX4c* amounts is crucial to allow its normal functions in patient muscles.

P7.06

Reliability and validity of reachable workspace total score with wrist weights in facioscapulohumeral muscular dystrophy

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Aim: Evaluate reliability and validity of Reachable Workspace (RWS) total score in facioscapulohumeral muscular dystrophy (FSHD). RWS is a 3D sensor system measuring upper extremity (UE) function, scored as relative surface area (RSA) on 5 quadrants. The total score is the mean of both arms with 500g wrist weights. Methods: Blinded post-hoc analysis of the Phase 2 ReDUX4 trial (NCT04003974) was conducted. Test-retest reliability was examined using intra-class correlation coefficients (ICC) on a population with no change in patient global assessment of change from baseline (BL) to week 4. Convergent and known-group validity were examined using correlations and between group t-tests, respectively: UE related patient-reported activities of daily living (ADL), clinician-reported severity (e.g., Ricci scores), and measures of muscle strength (e.g., dynamometry) were utilized. Results: At BL (n = 79) total RSA score was strongly correlated with its 5 RSA quadrants ($r \geq 0.71$) and moderately correlated with change from BL to week 48 ($r \geq 0.64$; n = 66). Almost perfect test-retest reliability (ICC = 0.98); good convergent validity with moderate ($|r| \geq 0.3$) correlations with most ADL items, clinical severity and UE muscle strength; and good known groups validity, with better (higher) RSA scores in low UE severity groups [nominal $p < 0.05$ in 12/15 (80%) of groups tested, were shown. Conclusion: The results support the reliability and validity of the RWS as an endpoint in FSHD trials.

P7.07

Developing an updated standard of care and management for facioscapulohumeral muscular dystrophy

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Since disease-modifying therapies are entering clinical trials for FSHD, there is a pressing need for a unified and multidisciplinary international standard of diagnostics and care. Aim: This project aims to update and expand the current FSHD standard of care (Tawil et al., 2015) to address the knowledge gaps and present the current evidence base for diagnosis and management of FSHD patients. This international guideline is intended for physicians and healthcare professionals who are involved in the diagnosis and care of people with FSHD. Method: An evidence-based approach is used, including a systematic literature search, assessment of methodological quality of included articles using evidence tables, and assessment of the strength of evidence for each outcome measure using GRADE (Grades of Recommendation, Assessment, Development, and Evaluation). For some topics, a consensus-based approach is more suitable, due to limited evidence in the literature. Together with the clinical expertise of 12 working groups, recommendations will be provided on important topics such as (genetic) diagnosis, pain and fatigue, functional impairments, managing pulmonary and sleep impairment, cardiac abnormalities, surgery, pregnancy, retinal vascular disease, hearing loss, communication, speech and swallow impairments, and mental health. Finally, the prospects for therapeutic interventions and current research gaps will be addressed. We aim to submit for publication in June 2024.

P7.08

A new home respiratory polygraphy for FSHD

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We present an extension of our previous work on HFRT (Home Fast Respiratory Test) into a new and cheap home polygraphic assessment of respiration in FSHD. By combining 2 complementary, and somewhat redundant, sets of data, we get a complete polygraphy. Quantitative volumetric PPC data (AS10, ResMed) and SpO2, PPG, inductive straps and body position (RS10, Contec) are synchronized to better than 0.1 sec. Redundancy allows an assessment of quality of heart frequencies and SpO2. Collection time may be anything between a night, and a 25-min relaxation HFRT. Such a major increase in information is obtained with minimal discomfort for the patient. Tests are conducted on demand, for a self-assessment or a remote evaluation. Respiratory disorders such as desaturations, hypopneas, high respiration rate, flow limitations, quasi-periodic respiration patterns, and micro-arousals can be monitored. The last are characterized by specific perturbations of the PPG associated with special flow-rate patterns beginning by a strong inspiration and a coincidental return to consciousness in HFRT. Thoracic and abdominal volumetric comparison monitoring and correction prove to be important in FSHD. No phase delay occurs in natural respiration. These results lead us to recommend this method to establish a basal state early after a FSHD diagnostic or in place of the usual SpO2 nocturnal monitoring when sleep disorders are suspected. Respiratory exercises can be tailored using this tool.

P7.09

An analysis of medical claims costs for individuals with FSHD 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 the healthcare and payor systems. Most previous studies examining FSHD-associated costs were conducted outside of the United States. To better understand healthcare utilization and cost associated with FSHD in the United States, we conducted a retrospective case-matched control analysis of OptumLabs' de-identified administrative claims data from Medicare Advantage and commercially insured health plan enrollees. The primary aim of the analysis was to estimate annualized medical claims cost for FSHD patients and compare these costs to controls. Secondary aims of the analysis included identifying subgroups (e.g., age, gender, ambulation) that may drive higher medical claims costs and identifying any comorbidities that may disproportionately affect FSHD patients. We report here results from the analysis, which included 383 FSHD patients and 1,915 matched controls. FSHD patients with commercial insurance had significantly higher medical claims costs than controls. An accompanying study examined direct and indirect costs to patients and families with FSHD in the US.

P7.10

BetterLife FSHD: A new patient-driven health and research platform

Amanda Hill[1], June Kinoshita[1]

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As therapeutic development in FSHD advances, it is more important than ever for patients to be actively involved in research and clinical trials, in their own care, and in regulator and payor advocacy. BetterLife FSHD is a pioneering health and research platform launching in summer 2024 dedicated to enabling people living with FSHD to do just that. The mission of BetterLife is to provide patients with the tools and resources to better understand and manage their FSHD, while contributing to research and including their voice as part of a powerful story. When a patient joins BetterLife FSHD, they are prompted to respond to a series of short surveys spread out over time. These surveys cover topics including demographics, health history, FSHD diagnosis and progression, FSHD management strategies, and quality of life domains like pain, fatigue, and mental health. Patients can also connect their electronic medical records and upload any FSHD genetic confirmation they have received. As a patient provides this information to BetterLife, they receive a personalized feed of resources and content relevant to them from the FSHD Society's rich ecosystem of blogs, videos, webinars, events, and more. Patients also receive personalized data visualizations and matching to clinical trials and other research studies for which they may be eligible. The information that BetterLife FSHD collects is stored and managed in a modern and secure real-world data infrastructure optimized to facilitate broad research and analytic uses. Data are made available upon request to researchers, clinicians, biopharmaceutical companies, regulator and payor bodies, and other organizations involved in FSHD research and therapeutic development with approval from a steering committee. BetterLife can also be collaboratively leveraged to implement or support a variety of external research initiatives. Altogether, BetterLife FSHD aims to help patients live a better life with FSHD, and to speed and improve FSHD research and therapeutic development.