28th Annual FSHD Society

International Research Congress

JUNE 24-25, 2021

ABSTRACT BOOK

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Transient *DUX4* expression provokes long-lasting cellular and molecular muscle alterations

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SPEAKER ABSTRACTS

S1.100

Transient *DUX4* expression provokes long-lasting cellular and molecular muscle alterations

Darko Bosnakovski[1], Ahmed Shams[1], Madison Douglas[1], Natalie Xu[1], Christian Palumbo[1], David Oyler[1], Elizabeth Ener[1], Daniel Chi[1], Erik Toso[1], Michael Kyba[1]

[1]*University of Minnesota*

A key problem in the FSHD field is the lack of direct evidence for *DUX4* expression in adult muscle biopsy tissue. The presence of downstream target genes in the absence of *DUX4* protein suggests the possibility that transient *DUX4* expression may lead to downstream effects that are maintained even without continued *DUX4* expression.

The iDUX4pA;HSA FSHD mouse model uses doxycycline-titrable *DUX4* induction in skeletal myofibers. Using low levels of doxycycline, we can induce barely detectible levels of *DUX4* in stochastic myofibers over an extended period, and this recapitulates essential pathologies of the FSHD phenotype. The iDUX4pA;HSA mouse model progressively develops a muscular pathology that is not only due to myofiber damage, but also to depleted regenerative potential, infiltration of profibrotic macrophages, defective microvasculature, a significant expansion of FAPs, and abnormal extracellular matrix deposition. Interestingly, the transcriptional profile of the dystrophic muscle of the iDUX4pA;HSA mouse model shows striking similarities to that obtained from biopsy samples of MRI-guided muscle biopsies of FSHD patients but not to that obtained from random biopsy samples, suggesting that this model represents actively progressing FSHD.

To address whether transient *DUX4* expression can provoke longstanding muscle perturbation that in combination with external insults lead to dystrophic phenotype, the iDUX4pA;HSA animal model, being inducible and reversible, has unique advantages. Here we will present our recent findings of the long-term effect of transient *DUX4* expression on cellular and molecular levels in the skeletal muscle.

S1.101

Identification of the first endogenous inhibitor of *DUX4* in FSHD muscular dystrophy

Paola Ghezzi[1], Valeria Runfola[1], Maria Pannese[1], Claudia Caronni[1], Roberto Giambruno[1], Annapaola Andolfo[1], Davide Gabellini[1]

[1]*San Raffaele Scientific Institute*

Facioscapulohumeral muscular dystrophy (FSHD) is the most prevalent neuromuscular disease affecting children and adults of all ages and both sexes. Unfortunately, no treatment is currently available. FSHD is caused by gain of expression of the double homeobox 4 (*DUX4*) gene, encoding for a transcription factor largely silent in somatic tissues. In FSHD, *DUX4* activates a pro-apoptotic program resulting in muscle wasting. Due to unknown molecular mechanisms, FSHD displays clinical and pathological manifestations overlapping with amyotrophic lateral sclerosis (ALS). While blocking *DUX4* activity is a plausible therapeutic option for FSHD, the mechanism underlying *DUX4*-induced toxicity is poorly understood.

We have identified MATRIN 3 (MATR3) as the first direct endogenous inhibitor of *DUX4*. MATR3 is a nuclear protein mutated in ALS and dominant distal myopathy. We found that MATR3 blocks *DUX4* expression and its ability to activate gene expression. As a result, MATR3 administration rescues cell viability and myogenic differentiation of FSHD muscle cells while it is safe to healthy muscle cells. Notably, we characterized a short MATR3 peptide that is necessary and sufficient to recapitulate full-length MATR3 activity.

Our data promote MATR3 as a therapeutic molecule to develop a rational treatment for FSHD that, in perspective, might be applied to a spectrum of diseases associated to aberrant *DUX4* expression or activity.

S1.102

Use of snRNA-seq to characterize the skeletal muscle microenvironment during pathogenesis in FSHD

Anugraha Raman[1], Anthony Accorsi[1], Michelle Mellion, MD[1], Bobby Riehle[1], Lucienne Ronco[1], L. Alejandro Rojas[1], Christopher Moxham[1]

[1]*Fulcrum Therapeutics*

Facioscapulohumeral muscular dystrophy (FSHD), one of the most common forms of muscular dystrophy, is a rare monogenic disease that currently has no effective treatment. It is caused by deletions of the macrosatellite D4Z4 repeats at the sub-telomeric region of chromosome 4q35. Deletions to repeat numbers less than 10 within this region cause chromatin de-repression resulting in stochastic and aberrant expression of the embryonic transcription factor *DUX4* in skeletal muscle. The expression of *DUX4* is myotoxic and results in muscle weakness in facial muscles and around the shoulder, eventually progressing to lower extremity and truncal weakness. While the root cause of FSHD has been elucidated, the molecular and cellular microenvironmental underpinnings that drive the loss of muscle tissue and function are not well understood.

Here we describe the first characterization of human FSHD skeletal muscle biopsies using single nuclear RNA-sequencing (snRNAseq). Our goal was to understand the dynamic FSHD microenvironment at higher resolution. Analysis of these snRNA-seq profiles suggested an increase in relative populations of infiltrating immune cells and expansion of resident progenitor cell populations in FSHD versus healthy biopsies. In addition to expanded intercellular heterogeneity within the FSHD microenvironment, we also saw a significant intracellular heterogeneity suggestive of altered cell states within diseased muscle. Characterization of these FSHD muscle biopsies will help us understand the processes contributing to FSHD pathogenesis, thereby enabling new target/therapeutic hypotheses.

S2.200

Identification of a druggable epigenetic target required for *DUX4* expression and *DUX4*-mediated toxicity in FSHD muscular dystrophy

Emanuele Mocciaro[1], Roberto Giambruno[1], Stefano Micheloni[1], Cristina Consonni[1], Maria Pannese[1], Valeria Runfola[1], Giulia Ferri[1], Davide Gabellini[1]

[1]*San Raffaele Scientific Institute*

Facioscapulohumeral muscular dystrophy (FSHD) is an inherited progressive neuromuscular disorder that afflicts both children and adults regardless of gender. FSHD has been associated with loss of epigenetic repression of tandemly repeated units in 4q35, leading to aberrant activation of the *DUX4* retrogene. *DUX4* expression is physiologically restricted to early stages of embryogenesis while being silenced in most somatic tissues of the adult. In FSHD, *DUX4* mis-expression triggers the activation of a pro-apoptotic transcriptional program leading to muscle wasting. As of today, no cure or therapeutic option is available to FSHD patients.
Our laboratory previously showed that the long non-coding RNA DBE-T is required for aberrant *DUX4* expression in FSHD. Using affinity purification followed by proteomics, we identified a chromatin remodeling protein as a novel DBE-T interactor and a major player required for the biological activity of the lncRNA. We found that the novel DBE-T binding protein is required for *DUX4* activation in FSHD muscle cells. Moreover, targeting the novel DBE-T binding protein rescues cell viability and myogenic differentiation of FSHD muscle cells without affecting healthy muscle cells. Remarkably, we obtained analogous results by pharmacological inhibition of the novel DBE-T binding protein.
Our results further elucidate the regulation of *DUX4* expression and identify a novel druggable regulator of *DUX4* function opening a new therapeutic perspective for FSHD.

S2.201

Accessing D4Z4 (epi)genetics with long-read sequencing

Quentin Gouil[1], Ayush Semwal[1], Frédérique Magdinier[2], Marnie Blewitt[1]

[1]*Walter and Eliza Hall Institute*
[2]*Aix-Marseille Université*

D4Z4 is extremely refractory to genetic and epigenetic characterisation due to its repetitiveness, size and similarity to other genomic regions. This makes diagnosis of FSHD difficult, and limits our understanding of how the D4Z4 locus is regulated.
To address these issues we are using long-read nanopore sequencing to obtain a single-molecule, single-nucleotide evaluation of D4Z4 sequence and DNA methylation simultaneously. This allows us to count repeat numbers, distinguish alleles and reveal the epigenetic state of D4Z4 in patient-derived samples. This technique has the potential to make FSHD diagnosis both much faster and much more informative, and shed light on the mechanisms of macrosatellite repeat epigenetic regulation in health and disease.

S3.300

System biology approach links muscle weakening to alteration of the contractile apparatus in FSHD

Camille Laberthonnière[1], Megane Delourme[2], Raphael Chevalier[1], Elva-Maria Novoa-del-Toro[1], Emmanuelle Salort Campana[3], Shahram Attarian[3], Rafaelle Bernard[3], Karine Nguyen[4], Jérome Robin[1], Anais Baudot[1], Frédérique Magdinier[2]

[1]*Marseille Medical Genetics*
[2]*Aix-Marseille University*
[3]*Centre de référence des maladies neuromusculaires et SLA, Hôpital de la Timone*
[4]*Département de Génétique Médicale, Hôpital Timone*

Facio Scapulo Humeral Dystrophy is the third most frequent genetic neuromuscular disorder and affects specific muscles of the face, shoulders and arms. Muscle weakening typically occurs in the second decade of life but molecular defects leading to the disease remain partially understood.
To get further insight into the muscle phenotype, we derived induced pluripotent stem cells from FSHD patients, differentiated these cells into innervated muscle fibers and analyzed their transcriptome by RNA-sequencing. In order to uncover biological pathways involved in the disease with no a priori, we applied a novel algorithm, MOGAMUN, a multi-objective genetic algorithm that integrates biological pathways, protein-protein interactions and co-expression to find active modules. All findings and networks converge towards decreased expression of genes encoding proteins involved in the functioning of the contractile apparatus and calcium handling. These findings suggest that the muscle weakness that is typical of the FSHD clinical spectrum might be associated with dysfunction of Actin-Myosin interactions, motor activity and mechano-transduction. These pathways, which have been so far overlooked open new perspectives in the definition of biomarkers able to define the disease and muscle weakening but also in the development of novel strategies to improve muscle function by regulating the contractile apparatus.

S3.301

Targeting *DUX4* post-translational modifications in vitro protects against *DUX4*-mediated toxicity

Renatta Knox[1], Jocelyn Eidahl[1], Lindsay Wallace[2], Sarah Choudury[1], Afrooz Rashnonejad[1], Scott Harper[1]

[1]*Center for Gene Therapy, The Abigail Wexner Research Institute at Nationwide Children's Hospital*
[2]*Nationwide Children's Hospital*

While significant progress has been made in determining the transcriptional targets of *DUX4*, the regulation of *DUX4* protein and the molecular consequences of this regulation are unclear. Using mass spectrometry, we identified several *DUX4* post-translational modifications (PTMs) which include phosphorylated serine and threonine residues as well as methylated arginine residues. In prior, unpublished work presented at the IRC, we carried out extensive studies to further characterize *DUX4* PTMs using mutagenesis. Here, we completed our mutagenesis screen to identify serine/threonine phosphomimetic mutants which protect cells against *DUX4*-mediated toxicity. We also identified serine/threonine kinases that, when overexpressed with *DUX4*, mitigate *DUX4* toxicity. Our *DUX4* proteomics screen and follow-up biochemical and mutagenesis analyses also identified an arginine methylation null mutant that is protective and also reduces the ability of *DUX4* to transactivate downstream gene targets, including FSHD biomarkers. Using a proteomics screen, we identified the arginine methyltransferase PRMT1 as a component of the *DUX4* complex. Pharmacologic inhibition of PRMT1 protects myoblasts from *DUX4*-mediated apoptosis. Taken together, these results demonstrate that *DUX4* is regulated by PTMs and that PTMs may be a druggable target for FSHD therapy.

S3.302

Genetic engineering and characterization of isogenic FSHD mutant myocytes

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Facioscapulohumeral Muscular Dystrophy (FSHD) is one of the most prevalent muscular dystrophies. FSHD is linked to derepression of the transcription activator *DUX4* embedded in the D4Z4 macrosatellite repeats on the chromosome 4q. Most of the FSHD patients exhibit a low copy number of D4Z4 repeats, which disrupts the D4Z4 heterochromatin structure, leading to *DUX4* derepression (termed FSHD1). Less than 5% of FSHD patients have normal D4Z4 repeats but display similar clinical phenotype and are also associated with the loss of heterochromatin at D4Z4 and derepression of *DUX4* (termed FSHD2), which are often linked to the mutations of SMCHD1. To understand the effect of *DUX4* in FSHD pathophysiology, several in vitro and in vivo models have been developed with the inducible recombinant *DUX4* protein. These models have been highly instrumental in obtaining many important molecular insights and development of possible therapeutic strategies. However, overexpression of the recombinant *DUX4* is toxic in human myocytes, which appears to differ from the consequence of the endogenous *DUX4* expression in FSHD patient cells. Furthermore, because *DUX4* and some of the major *DUX4* target genes are missing, it is difficult to completely recapitulate FSHD in mice. Importantly, it has not been explicitly tested whether D4Z4 contraction is sufficient to cause the FSHD phenotype. To address the consequence of D4Z4 contraction, we created isogenic FSHD mutants from an immortalized control myoblast cell line using CRISPR-Cas9. Three types of mutant clones were generated: D4Z4 contraction, SMCHD1 deletion, and double mutations to simulate FSHD1, FSHD2 and severe FSHD1, respectively. The effects of these mutations on the cellular phenotype, D4Z4 chromatin structure, *DUX4* and target gene expression are characterized and compared to those in FSHD patient cells. The potential of these mutant cells as a tool to study the mechanism of FSHD pathogenesis and therapy development will be discussed.

S3.303

IL-6 and TNFα are key inflammatory cytokines in facioscapulohumeral muscular dystrophy

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Although a consensus has been reached clarifying the underlying (epi)genetic background of FSHD, still little is known about its pathogenesis. In this study, we aimed to explore if inflammatory cytokines are associated with FSHD and which innate immune cells may be involved in its pathophysiology. Therefore, we carried out a large translational case-control study including FSHD patients (N=190, 48 ± 14 years, 49% men) and sex- and age-matched healthy controls (N=135, 44 ± 15, 47% men). We measured multiple clinical outcomes, circulating inflammatory cytokines, and the cytokine production capacity of monocytes, Natural Killer (NK) cells, and muscle specimens in patients and controls. We found significantly higher levels of IL-6 and TNFα in the circulation of FSHD patients, as well as after ex-vivo stimulation of NK cells and muscle specimens compared to healthy controls. Importantly, we found that IL-6 and TNFα concentrations were significantly associated with FSHD duration, severity, and muscle weakness. We propose that innate immunity cytokines such as IL-6 and TNFα may be significantly associated with FSHD pathogenesis and progression. Additionally, NK cells may be activated in FSHD patients with a consequent more prone inflammatory phenotype whose implication for muscle disease in FSHD warrants further investigation.

Special Session: A phase 2, randomized, double-blind, placebo-controlled, 48-week, parallel-group study of the efficacy and safety of Losmapimod in treating subjects with facioscapulohumeral muscular dystrophy (FSHD) with open label extension (OLE): ReDUX4

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Primary Objective: Evaluate the efficacy of losmapimod in inhibiting the aberrant expression of *DUX4*, the root cause of FSHD. Secondary objectives are to evaluate the safety, tolerability, PK, and TE in blood and muscle, and muscle health with MRI.
Background: FSHD is caused by aberrant expression of *DUX4* due to loss of repression at the D4Z4 locus. *DUX4* activates a downstream transcriptional program that causes myofiber death, maladaptive tissue remodeling characterized by replacement of muscle with fat ultimately resulting in progressive motor disability. Losmapimod is an orally active, selective, small molecule inhibitor of p38α/β. Preclinical studies demonstrated that losmapimod reduces *DUX4* in differentiating FSHD myotubes across multiple genotypes. Losmapimod rapidly distributes in muscle and engages p38α/β. Losmapimod has been tested across 12 indications resulting in >3,500 human exposures with satisfactory safety and tolerability.
Methods: Eighty subjects age 18 to 65 years with genetically confirmed FSHD1, clinical severity score of 2 to 4 (range 0-5) and MRI-eligible skeletal muscles for needle biopsy were randomized 1:1 to receive 15 mg losmapimod or placebo tablets PO twice daily for 48 weeks. Subjects were followed for approximately 53 weeks, including 4-week screening period and 7-day safety follow-up period. Participants had muscle biopsies pre-treatment and on treatment at Week 16 or 36 to measure effects on *DUX4* activity. MRIs were performed at screening, Week 12, and Week 48 to assess changes in muscle. Potential emerging clinical changes were measured using performance measures of the function of shoulder and proximal arm function (reachable workspace), mobility (FSHD-TUG), physical function

(MFM domain 1) and muscle strength (manual dynamometry). Patient reported outcomes were assessed with the FSHD health index and PGIC questionnaire.
Results and Conclusions: The results of this Phase 2 study will be presented.

S4.400

Identifying biomarkers for facioscapulohumeral muscular dystrophy using Olink Proteomics

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Mis-expression of *DUX4* in skeletal muscle causes facioscapulohumeral dystrophy (FSHD), a progressive muscle disease for which there are no approved therapeutics. Developing robust molecular biomarkers for FSHD is essential for quantitatively assessing disease severity and progression, and for evaluating treatment strategies. Identifying FSHD biomarkers in blood plasma or serum would additionally allow for quick and simple sample collection. We used the Olink Proteomics Proximity Extension Assay (PEA) to assess the levels of select *DUX4*-induced target proteins in the cell lysate and cell culture supernatant of human myoblasts expressing an inducible *DUX4* transgene, and in FSHD patient-derived muscle cells. We identified Placental Alkaline Phosphatase (ALPP) as robustly distinguishing *DUX4*-expressing samples from controls in both lysates and supernatants. Inhibition of *DUX4* via siRNA-mediated knockdown or small molecule inhibition leads to a corresponding drop in ALPP levels. Studies using serum from FSHD-affected and control individuals are ongoing. Overall, these results identify ALPP as a potential secreted biomarker for FSHD.

S4.401

SLC34A2 as a protein biomarker of FSHD

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Our laboratory has been studying SLC34A2 as a potential protein biomarker of FSHD, for use in studying progression of the dystrophy and the efficacy of treatments now in development. SLC34A2 is responsible for sodium-dependent phosphate uptake into cells and as a *DUX4* target gene it is upregulated at least 10-fold in FSHD patients. It is normally expressed in epithelial cells, such as those that line the lungs, gut and kidney, and, because those cells are sloughed off when they die rather than being degraded in the blood, SLC34A2 is usually not present at high levels in healthy human serum. We have reported that SLC34A2 is detected by immunofluorescence at ~10-fold higher levels in biopsies of human FSHD muscle than in controls, and in xenografts of FSHD tissue that we generate in mice (Mueller et al., Exp. Neurol. 320:113011 [2019]). We report here that we also detect it in immunoblots of the FSHD xenografts and in the serum of mice carrying FSHD xenografts. Again, levels are higher than in controls. Preliminary results from immunoblotting show that SLC34A2 is present at 3-9 times higher levels in the serum of FSHD patients than in a commercial control. Additional controls will be examined shortly. Our results suggest that SLC34A2 may prove useful as a serum protein biomarker for FSHD.

S4.402

Serum interleukin-6 levels as severity biomarker in FSHD1

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common myopathies in adults, displaying a progressive, frequently asymmetric involvement of a typical muscle's pattern. FSHD is associated with epigenetic derepression of the polymorphic D4Z4 repeat on chromosome 4q, leading to *DUX4* retrogene toxic expression in skeletal muscles. Identifying biomarkers that correlate with disease severity would facilitate clinical management and assess potential FSHD therapeutics' efficacy.
To this end, we retrospectively measured the levels of 20 pro-inflammatory and regulatory cytokines in sera from 100 genetically confirmed adult FSHD1 patients ranging in severity. We investigated associations between cytokine concentrations and various clinical severity scores. We then measured serum and muscle interleukin-6 (IL-6) levels in a validated FSHD-like mouse model, ranging in severity and *DUX4* expression.
We identified IL-6 as the only cytokine with a concentration following the disease severity: the more severe the disease is, the more elevated the IL-6 concentration is. We correlated IL-6 levels with several clinically established severity tests (Clinical Severity Score, Manual Muscle Testing sum score, Brooke and Vignos scores), demonstrating for the first time the direct link between FSHD severity and IL-6 levels. Further analysis in an FSHD-like mouse model confirmed that IL-6 levels positively correlate with disease severity and *DUX4* expression.
In conclusion, serum IL-6 levels show promise as a serum biomarker of FSHD severity in FSHD patients. Further, our results highlight the potential use of IL-6 levels as a suitable tool for phenotypic stratification and a candidate target for therapy in FSHD. Further studies will be crucial for therapeutic development since anti-IL-6 receptor monoclonal antibodies have already been approved for the treatment of rheumatoid arthritis and some other IL-6-related pathologies.

S5.500

Persistence of p38-independent *DUX4* target gene expression in FSHD xenografts

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p38 MAP kinase drives *DUX4* mRNA expression in differentiating FSHD myocytes and inhibitors of p38alpha/beta suppress *DUX4* expression in a xenograft model of FSHD (1,2). Fulcrum Therapeutics has taken the p38alpha/beta inhibitor losmapimod into a Phase II clinical trial in patients (NCT04003974). Interim trial results show that the *DUX4* target gene expression was decreased in the losmapimod treatment group only in patients with the highest levels in pre-treatment biopsies (3). To understand the response of *DUX4* and *DUX4* target gene expression to p38 inhibition in vivo, we first generated gene expression profiles of human FSHD myoblasts transplanted into the tibialis anterior muscle of immunodeficient mice over a four-week period. Sequential peaks in expression of markers of early and late differentiation indicated successful integration of xenograft tissue. *DUX4* mRNA levels declined from the peak at day 4 after xenotransplantation through day 9 and were not detectable at day 14 or later. Levels of *DUX4* target mRNAs decreased from peaks at day 5 through day 14, where low levels were still detectable. These low levels persisted through 28 days after xenotransplantation. We previously showed that when xenograft mice are treated with p38 inhibitors for the first four days (through peak of *DUX4* expression), *DUX4* and *DUX4* target mRNA levels were reduced by 80% at that time point. Importantly, when xenograft mice were treated with p38 inhibitors for 14 days, the low levels of persistent *DUX4* target mRNA levels present at that time were unaffected by treatment. These data suggest that p38 inhibition suppresses differentiation-dependent increases in *DUX4* mRNA levels in vivo but that a low level of p38-independent *DUX4* target mRNAs persists.

S5.501

Human miRNA mir-675 inhibits *DUX4* expression and may be exploited as a potential treatment for facioscapulohumeral muscular dystrophy

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Double homeobox protein 4 (*DUX4*) emerged as an important gene because of its linkage to facioscapulohumeral muscular dystrophy (FSHD), a devastating disease affecting up to 870,000 people worldwide. The *DUX4* locus is normally repressed by heterochromatin in adult muscle, but silencing mechanisms are absent or reduced in FSHD muscle, thereby permitting *DUX4* expression. When present in muscle, the *DUX4* gene product, a transcription factor, activates genes involved in cell death and dysfunction, leading to muscular dystrophy. The most direct route to FSHD therapy will likely involve inhibiting *DUX4* in muscle, but currently there are no approved *DUX4*-targeted treatments that slow disease progression or improve muscle weakness. In prior work, we designed artificial microRNAs targeting *DUX4* mRNA for degradation through the RNAi pathway and demonstrated that RNAi-based gene therapy could improve FSHD-associated phenotypes in FSHD mice and human myotubes. Here we pursued an alternative and completely novel strategy to treat FSHD with RNAi therapy, using a natural microRNA, mir-675. Accordingly, we provided the first evidence that a natural human microRNA, mir-675, could silence *DUX4* expression via RNAi and counteract *DUX4*-associated phenotypes. We then developed two novel FSHD therapies relying upon mir-675 upregulation with gene therapy and using small molecules. Both strategies resulted in decreased expression of *DUX4* and *DUX4*-associated outcomes in FSHD mice and human FSHD myotubes. To our knowledge, this is the first study demonstrating the use of small molecules to suppress a dominant disease gene using an RNAi mechanism.

S6.600

Systemic delivery of a *DUX4* targeting antisense oligonucleotide reduces *DUX4*, *DUX4* responsive genes, and pathology in skeletal muscles of ACTA1-MCM;FLExDUX4 mice

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by aberrant expression of the transcription factor *DUX4* in skeletal muscles. Previously, antisense oligonucleotides (ASOs) targeting the *DUX4* transcript could efficiently repress *DUX4* expression in FSHD myocytes and after intra-muscular injections in a mouse model for FSHD. In this study, a systemically delivered *DUX4* targeting ASO was tested in ACTA1-MCM;FLExDUX4 mice that express the full-length *DUX4* transcript in skeletal muscles and develop a progressive skeletal muscle phenotype. The *DUX4* ASO was well tolerated and could repress the *DUX4* transcript, *DUX4* protein and *DUX4* responsive genes in all tested skeletal muscles. Functional tests showed that the *DUX4* ASO reduced muscle fatigue but did not improve muscle strength in ACTA1-MCM;FLExDUX4 mice. The *DUX4* ASO further alleviated the severity of skeletal muscle pathology and reduced the percentage of fibers with central nuclei and the percentage of macrophage infiltration. RNA-sequencing analysis showed that the *DUX4* ASO reduced biological processes involved in fibrosis and inflammation in the quadriceps muscle compared to control ASO treated ACTA1-MCM;FLExDUX4 mice but did not completely restore the dysregulation of genes compared to ACTA1-MCM mice that do not express *DUX4*. In conclusion, systemically delivered ASOs targeting the *DUX4* transcript are a promising therapeutic strategy for FSHD but improving the *DUX4* repression efficiency in skeletal muscles is warranted.

S6.601

DUX4 siRNA optimization for the development of an antibody-oligonucleotide conjugate (AOCTM) for the treatment of FSHD

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Despite the promise of oligonucleotide-based therapeutics for over four decades, the realization of FDA approved medicines has required advances in genomics, chemistry, pharmacology and drug delivery that are still largely limited to the liver. The main challenge limiting clinical development of oligonucleotide therapeutics for muscular diseases has been the delivery of oligonucleotides to muscle. To solve this problem for the FSHD indication, Avidity is developing an Antibody-Oligonucleotide Conjugate (AOC™) that combines the selectivity of monoclonal antibody directed towards the transferrin receptor 1 (TFRC) with the specificity of an siRNA targeted to *DUX4*. *DUX4* is an aberrantly expressed transcription factor responsible for the pathophysiology of FSHD. The antibody component binds to the TFRC on the cell surface and results in internalization of the AOC through TFRC-mediated endocytosis, while the siRNA component provides the pharmacological mechanism of action which is siRNA-mediated degradation of the *DUX4* mRNA. We have conducted an extensive in vitro screening of a *DUX4* siRNA library in 11 FSHD patient-derived muscle cells, that allowed us to select highly potent siRNA sequences with minimal off-target profile. The selected lead *DUX4* siRNAs were conjugated to the TFRC antibody and are being further characterized in mouse models of FSHD. The best performing *DUX4* siRNA conjugated to human anti-TFRC mAb will be selected as the therapeutic candidate for patients living with FSHD.

S6.602

Lipid-conjugated *DUX4*-targeting siRNA therapeutic to treat FSHD

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Background: We have identified a *DUX4*-targeting siRNA, DU01, that potently inhibits *DUX4* biomarker expression in FSHD patient primary myogenic cells in vitro. Our collaborators in the RNA Therapeutics Institute at UMMS have developed a platform of siRNA lipid conjugates that are systemically deliverable to skeletal muscle, as shown by the functional efficacy of DCA-conjugated myostatin siRNA to increase muscle volume in mice.
Objectives: To develop a *DUX4*-targeting siRNA therapeutic that is systemically deliverable and potently inhibits *DUX4* and *DUX4* biomarker expression in vivo in FSHD patient muscle xenografts.
Results: We performed experiments utilizing two systemic DCA-DU01 dosing strategies in muscle xenografts generated by engraftment of primary FSHD patient myogenic cells. Both dosing strategies decreased *DUX4* biomarker expression in DCA-DU01 treated mice compared to DCA-NTC treated controls. Ongoing experiments are assaying the efficacy of DCA-DU01 in xenografts of multiple FSHD patient cells and evaluating drug safety by DCA-DU01 dose escalation studies.
Conclusions: We are scheduling a pre-IND meeting with the FDA to inform and enable our plan to bring DU01 siRNA forward to FSHD clinical trials.

S6.603

FORCE platform enables muscle targeted delivery of antisense oligonucleotide and silencing *DUX4* activity in an FSHD patient cell line

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by aberrant activation of the Double Homeobox 4 (*DUX4*) transcription factor in muscle cells, leading to skeletal muscle loss, progressive muscle weakness and wasting. Despite significant progress in understanding the molecular events that drive disease progression, no specific treatment has been developed to suppress *DUX4* expression in muscle and address the genetic basis of disease. Antisense oligonucleotides (ASO) have shown promise as potential therapeutics capable of repressing *DUX4* mRNA expression. However, achieving therapeutically relevant concentrations of ASO in muscle has proven challenging. To overcome the current limitations in delivery to muscle tissue, we have developed the FORCE platform which consists of three essential components: a Fab targeting TfR1 to enable targeted delivery to skeletal, cardiac and smooth muscle; a clinically validated cleavable Val-Cit linker; and a therapeutic payload rationally selected to target the genetic basis of disease. For FSHD we are developing a conjugate that includes a PMO that targets the polyadenylation signal of the *DUX4* transcript. We evaluated the activity of this conjugate in the AB1080FSHD26 C6 immortalized FSHD1 cell line (Association Institut de Myologie) which has significant levels of surface TfR1 expression and activation of *DUX4* transcriptome markers (MBD3L2, TRIM43, ZSCAN4).

We demonstrate that receptor-mediated delivery of PMO by the FORCE platform into muscle cells results in ~75% reduction of *DUX4* transcriptome biomarkers at an 8 nM PMO concentration, whereas equivalent unconjugated PMO shows no significant biomarker reduction compared to vehicle treated cells. In conclusion, the FORCE platform has the potential to enhance delivery of therapeutic oligonucleotides to muscle cells to address the genetic basis of disease for the treatment of FSHD and other muscle disorders.

S6.604

Systemic antisense therapeutics inhibiting *DUX4* expression ameliorate FSHD-like pathology in an FSHD mouse model

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Aberrant expression of the double homeobox 4 (*DUX4*) gene in skeletal muscle causes muscle deterioration and weakness in Facioscapulohumeral Muscular Dystrophy (FSHD). Since the presence of a permissive pLAM1 polyadenylation signal is essential for stabilization of *DUX4* mRNA and translation of *DUX4* protein, disrupting the function of this structure can prevent expression of *DUX4*. We and others have shown promising results using antisense approaches to reduce *DUX4* expression in vitro and in vivo following local intramuscular administration. Here we demonstrate that further development of the antisense chemistries enhances in vitro antisense efficacy. The optimal chemistry was conjugated to a cell-penetrating moiety and was systemically administered into a double-transgenic mouse model of FSHD. After four weekly treatments, mRNA quantities of *DUX4* and target genes were reduced by 50% that led to 12% amelioration in muscle atrophy, 52% improvement in in situ muscle strength, 17% reduction in muscle fibrosis, and prevention of shift in the myofiber type profile. Systemic *DUX4* inhibition also significantly improved the locomotor activity and reduced the fatigue level by 22%. Our data demonstrate that the optimized antisense approach has potential of being further developed as a therapeutic strategy for FSHD.

S6.605

Development of an RNAi therapeutic, ARO-*DUX4* for the treatment of FSHD

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Aberrant expression of *DUX4* is considered the cause of facioscapulohumeral muscular dystrophy (FSHD); however, there is no effective therapy targeting the myotoxic *DUX4*. With the goal of developing a *DUX4*-specific therapy for FSHD patients, we have developed ARO-*DUX4*, an RNAi therapeutic specifically targeting *DUX4* transcripts. We first assessed the pharmacodynamic (PD) effects of ARO-*DUX4* in a well-characterized transgenic model of FSHD, FLExDUX4 mice. Treatment of FLExDUX4 mice with ARO-*DUX4* greatly decreased the tamoxifen-induced *DUX4* mRNA, and corrected elevated expression of Wfdc3 and Myo1g, both transcription targets of *DUX4* in mice. We then evaluated the pharmacological effects of ARO-*DUX4* by treating FLExDUX4 mice before and after tamoxifen induction of *DUX4* expression. Under the conditions of prophylaxis and intervention, ARO-*DUX4* preserved body weight, decreased muscle atrophy, resolved muscular dysfunction, and mitigated the severity of histopathological lesions in skeletal muscle caused by the expression of *DUX4*. To explore the translatability of the observed effects in the FLExDUX4 mouse model, we analyzed the PD effects of ARO-*DUX4* in FSHD patient-derived skeletal myocytes. ARO-*DUX4* treatment caused a dose-dependent decrease in the expression of *DUX4* and a set of established *DUX4* target genes in the differentiated patient-derived myocytes. The preclinical evidence provided in this abstract suggests that ARO-*DUX4* is a strong drug candidate for clinical development

S6.606

Translating *DUX4*-targeted RNAi-based gene therapy for FSHD

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Background: Previously, our lab demonstrated efficacy and safety for AAV.mi405, a *DUX4*-targeted RNAi-based gene therapy for FSHD.
Objectives: 1) To assess the long-term durability of the mi405 product, 2) perform pivotal pre-clinical dosing studies to support clinical trial design, 3) evaluate human biomarkers for potential outcome measures.
Results: We performed multiple long-term studies in TIC-*DUX4* mice to assess durability and dosing requirements for AAV.mi405. At 1-year post-injection, which is to date the longest timepoint studied, we find sustained mi405 expression and histological muscle protection in TIC-*DUX4* mice. Ongoing systemic dosing studies will conclude at 6 months post-injection where monthly total cage activity, rearing, molecular and histological outcomes will be used to determine a safe and effective dose range. Finally, we have determined a panel of mi405-responsive biomarkers using an FSHD xenograft model.
Conclusions: We expect that these final pre-clinical studies will support our planned first-in-human clinical trial of AAV.mi405 gene therapy for FSHD.

S7.700

Five-year follow-up study on quantitative MRI in facioscapulohumeral muscular dystrophy

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Recent FSHD research has identified quantitative muscle MRI (qMRI) as a biomarker, because of its ability to characterize muscle changes over time in FSHD patients. How these changes relate to clinical outcome remains unclear, while this information is crucial to use MRI as an imaging biomarker in trials. We aimed to assess changes in leg muscle qMRI and in clinical outcome measures over 5 years in a large cohort of FSHD patients. We included 105 FSHD patients covering the entire disease severity spectrum (41% male, mean age 54±14 years, mean repeat size 6±2 D4Z4 units). All patients were assessed twice: at baseline and 5 year follow up. Clinical outcome measures included the Ricci CSS and Motor Function Measure. qMRI of 19 leg muscles was performed. All clinical outcome measures used showed significant change in 5 years, like most leg muscles. Muscle fat fraction changes were most evident in Ricci scores 4-8 and did not depend on age or repeat size. Cross-sectionally, the MRI total leg fat fraction score correlated strongly with the Ricci score and the MFM (CC>0.864, $p<0.05$). The change in MRI total leg fat fraction and change in clinical outcome measures also correlated significantly (CC0.22-0.387). We are currently performing regression analysis to predict fat fraction changes in 5 years, aiming to determine in which subgroup these changes are most evident. We expect this will provide a better insight in the most optimal use of qMRI for upcoming clinical trials in FSHD.

S7.701

Objective monitoring of facioscapulohumeral dystrophy during clinical trials using a smartphone application and wearables

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Facioscapulohumeral muscular dystrophy (FSHD) is a progressive neuromuscular disease impairing daily living. Currently, FSHD symptom severity is assessed by clinical assessments. Such assessments are limited in their ability to continuously capture changes and to capture the full impact of the disease. Remotely capturing data related to daily activities could provide additional insights in the disease. This study investigated the feasibility of using smartphones and wearables to capture FSHD-related symptoms. Additionally, we identified features that could distinguish between FSHD patients and control subjects.

Thirty-eight FSHD patients and 20 non-FSHD controls were monitored using a smartphone for 6 weeks. At the first and last day of the study period, clinicians assessed the subjects' FSHD Clinical Score and Timed Up and Go time. Subjects installed the monitoring app on their smartphones, were given a smartwatch, and were instructed to record their weight and blood pressure (BP) weekly using a scale and BP monitor. The user experience of the app was assessed at week 6 using a questionnaire. We built a model based on the remote data to distinguish between FSHD and control subjects, and identified features that could distinguish between the two groups.

Overall, the app was well tolerated, but 67% of the subjects noticed reduced battery life. Data completeness was more than 75% for all sensors. We classified FSHD and non-FSHD controls with 93% accuracy, 100% sensitivity, and 80% specificity. Features relating to smartphone acceleration, app usage, location, physical activity, sleep, and call behavior were the most salient features for the classification.

Remote monitoring data collection allows for the collection of daily activity data in FSHD patients and control subjects for 6 weeks. We demonstrated the ability to detect differences in features in FSHD patients and non-FSHD controls using smartphones and wearables based on data related to physical and social activity.

S7.702

FSHD European Trial Network

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FSHD European Trial Network
FSHD is on the doorstep of clinical therapeutic trials, there is interest in FSHD from several pharmaceutical industries and a few trials are currently performed. The guidelines for clinical trials, pharma regulation and participation, and health care provisions in European countries differ in various subtle ways and would benefit from an overall strategy specifically catered to the European situation. In order to offer the FSHD patient community in Europe the best position in discussions with clinical and basic science researchers and with pharma, FSHD Europe has initiated the FSHD European Trial Network. The network organizes a virtual meeting in Spring 2021 with the following aims:
•Establish the foundation of European FSHD Trial Network
•Increase the commitment of clinicians and researchers in clinical research in Europe
•Harmonize criteria for clinical and genetic diagnosis, for registries and outcome measures
•Exchange clinical experience and genetic reference material
•Bring Europe on a par with the USA on trial-readiness in FSHD
•Engage Pharma and EMA for a Europe wide collaboration
•Harmonize treatment and care for all European FSHD patients
This will be done in collaboration with the Clinical Research Trial Network, TreatNMD and the European Reference Networks for Rare Diseases. At the IRC, we would like to present the network, its members, the main results of the spring meeting and aims for the next years.

POSTER PRESENTATION ABSTRACTS

P110

p38 mediates differentiation-dependent *DUX4* expression in facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by epigenetic de-repression and inappropriate transcription of the *DUX4* gene in skeletal muscle. We have shown that p38alpha and p38beta MAP kinases independently contribute to drive *DUX4* mRNA expression in differentiating FSHD myocytes and that inhibitors of p38alpha/beta suppress *DUX4* expression in a xenograft model of FSHD (Oliva et al. 2019). Fulcrum Therapeutics has also reported on the role of p38 in *DUX4* regulation (Rojas et al. 2020) and has taken the p38alpha/beta inhibitor losmapimod into Phase II clinical trials in patients (NCT04003974, NCT04264442). Importantly, the roles of p38 isoforms in *DUX4* expression during different stages of myogenesis remain largely unknown. To address this, we used CRISPR technology to knock out p38alpha and p38beta, individually and in combination, in healthy and FSHD myoblasts and monitored *DUX4* and *DUX4* target gene expression during proliferation and during myotube formation in vitro. Knockout of p38alpha alone or p38alpha in combination with p38beta resulted in FSHD myoblasts that retained a low level of *DUX4* expression during proliferation. This level was slightly lower than in non-knockout FSHD myoblasts. During differentiation, non-knockout cells exhibit increased *DUX4* expression coincident with myotube formation. While knockout FSHD cells exhibited a delay in differentiation, myotubes did fully form with equal expression of differentiation markers as compared with non-knockout myotubes, suggesting that p38alpha and p38beta are not essential for myotube formation. However, *DUX4* expression remained low in knockout cells. This data demonstrates that p38 mediates differentiation-dependent increases in *DUX4* expression in FSHD muscle cells.

Oliva, J. et al. (2019). J Pharmacol Exp Ther 370(2): 219-230.
Rojas, L. A. et al. (2020). J Pharmacol Exp Ther 374(3): 489-498.

P111

A longitudinal, multi-omics approach to study the temporal effects of *DUX4* expression

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Expression of *DUX4* in skeletal muscle cells results in extensive alterations in the transcriptome of the cell. This causes a variety of phenotypes and ultimately leads to cell death. There is little understanding of how specific changes in gene expression results in cell stress, death, and the disease process. Understanding what stress responses become activated and the timing of their activation is critical to defining the mechanism of *DUX4*-induced cytotoxicity in FSHD muscle. To address this, we induced *DUX4* expression in MB135 myoblasts and performed longitudinal RNA sequencing paired with proteomics and phosphoproteomics with fine time resolution to gain insight into the changes in cellular physiology brought on by *DUX4* expression.

Here we demonstrate that the cellular response to *DUX4* expression is dynamic. We characterize how expression patterns of *DUX4*-affected genes change over time at the RNA and protein level. We also identify *DUX4*-affected pathways by gene set enrichment analysis and alterations in the phosphoproteome. Validation of these affected genes and signaling pathways using orthogonal methods in primary FSHD patient-derived myotubes may identify targets for modulation to rescue the muscle pathology in FSHD patients.

P112

Finding the balance: dual-target inhibition to inhibit *DUX4* and preserve myotube fusion

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Misexpression of *DUX4* in muscle tissue as an underlying cause of FSHD is a well-established concept. Consequently, many attempts are underway to identify treatment options based on *DUX4* inhibition, thereby preventing the progressive loss of muscle tissue. Modulation of the cAMP signaling pathway, beta-2 adrenoreceptor agonists or p38 kinase inhibitors have all been reported as potential pathways to inhibit *DUX4* expression in myotubes.
However, using our high-content imaging platform in a differentiating culture of primary FSHD myoblasts we found that pharmacological modulation of those pathways interferes with the formation of fused multi-nucleated myotubes. Since *DUX4* expression only occurs in differentiated myotubes, any mechanism that inhibits this process would be associated with the risk of false positive results regarding *DUX4* repression. Moreover, as fusion of myotubes is an essential process in muscle hypertrophy and regeneration, such a mechanism could counteract the desired therapeutic efficacy.
To overcome these limitations, as part of a diverse portfolio, we are developing unique dual-kinase inhibitors, which efficiently inhibit *DUX4* expression without affecting myotube fusion. We generated an integrated mechanism-of-action model for these compounds by building a custom deep data processing strategy integrating multi-omics datasets including transcriptomics, proteomics and phosphoproteomics, as well as public datasets, high-content imaging and biochemical kinase profiling. Our model suggests that specific and balanced inhibition of two kinases triggers at least two independent sequences of events at the level of gene expression and protein phosphorylation, leading to *DUX4* repression while preserving signaling linked to myotube fusion.

P113

Relationship of *DUX4* and target gene expression in FSHD myocytes

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Facioscapulohumeral dystrophy (FSHD) is associated with the upregulation of the *DUX4* transcription factor and its target genes. However, low-frequency *DUX4* upregulation in patient myocytes is difficult to detect, and examining the relationship and dynamics of *DUX4* and target gene expression has been challenging. Using RNAScope in situ hybridization with highly specific probes, we detected the endogenous *DUX4* and target gene transcripts in primary and immortalized FSHD2 patient skeletal myotubes. We found that the endogenous *DUX4* transcripts are expressed only in one or two nuclei (while *DUX4* protein is in almost all nuclei) in ~1-2% of myotubes. The endogenous *DUX4* RNA localizes as foci in the nucleus in contrast to the accumulation of the recombinant *DUX4* transcripts in the cytoplasm. During 13-day differentiation of immortalized FSHD2 myocytes in vitro, we found the continuous increase of *DUX4* and target gene-positive myotubes beyond day 3, arguing against immediate cytotoxicity of *DUX4*. Interestingly, we observed that *DUX4* and target gene expression becomes discordant later in differentiation and not all *DUX4*-positive myotubes express target genes, indicating the different states of *DUX4*-induced myotubes. Similar results were obtained in FSHD1 myocytes, indicating that this is not restricted to FSHD2 with SMCHD1 mutation. Later in differentiation, the expression of two *DUX4* target genes, LEUTX and KDM4E, becomes more concordant than *DUX4* and KDM4E expression in the same myotube. Consistently, depletion of LEUTX or DUXA, which we previously demonstrated to regulate LEUTX, repressed KDM4E later in differentiation. The results indicate that after the initial activation by *DUX4*, LEUTX and DUXA contribute to the upregulation of KDM4E, suggesting that target genes themselves participate in the maintenance of the *DUX4* gene network. Together, the study provides important new insights into the dynamics of the *DUX4* transcriptional network in FSHD patient myocytes.

P114

A patient-focused survey to assess the effects of the COVID-19 pandemic and social guidelines on people with muscular dystrophy

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In this study, we examined the social and health impacts of the coronavirus disease 2019 (COVID-19) pandemic and social guidelines on people with muscular dystrophy (MD).
A prospective de-identified electronic survey was distributed to adults with self-reported Facioscapulohumeral Muscular Dystrophy (FSHD), Myotonic Dystrophy (DM), or Limb-Girdle Muscular Dystrophy (LGMD) enrolled in registries or patient advocacy groups.
The COVID-19 Impact Survey was developed by MD experts in association with patients and advocacy groups. The Perceived Stress Scale was used to measure stress.
Respondents (n=774: 56% FSHD; 35% DM, and 9% LGMD) were mostly women and middle-aged. Rates of COVID-19 infection were low (<1%), compliance with local social distancing guidelines high (98%). Major challenges reported during the pandemic included: obtaining treatment (40%), managing stress (37%), social distancing (36%) and obtaining essentials (34%). The majority reported a slight worsening in their disease state. Respondents reported moderate stress levels, with higher stress reported by women and those under age 30 years. Three-quarters of participants who had telemedicine visits were satisfied with the encounters; however, most reported preference for in-person visits.
Interventions like exercise and stress-coping strategies, including strategies specific to women or people <30 years, may be important. Further investigation is needed into the role of telemedicine in the care of those with MD.

P115

Genotype-phenotype correlation in Chinese patients with facioscapulohumeral muscular dystrophy type 1

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Facioscapulohumeral muscular dystrophy type 1 (FSHD1) has been genetically associated with D4Z4 reduced alleles (DRA) located on chromosome 4q35. While complicated genotype-phenotype correlation among different ethnic populations remains controversial, FSHD intrinsically displays a high degree of clinical variability. Among all FSHD patients, a subgroup of particular severe FSHD cases is characterized by infantile-onset and extra-muscular features and has been associated with 1-3 DRA. In this context, our study is aimed to investigate the overall genotype-phenotype correlation of Chinese FSHD1 patients and analyze differences between genetic subgroups of shortest DRA and relative longer DRA. We enrolled 127 FSHD patients from 120 unrelated Chinese families with D4Z4 repeated array of 1-10 DRA on the 4qA haplotype confirmed by molecular combing. The median DRA size was 4, and 122 (96%) Chinese patients with FSHD carried 1–7 DRA. The mean onset age was 10.62±9.32 years. We observed a positive correlation between DRA size and onset age ($r=0.443$, $P<0.01$) and a roughly inversed correlation between DRA size and FSH-Clinical Score ($r=-0.261$, $P<0.05$). There are 37 carriers with 1-3 DRA in the cohort. In the shortest DRA subgroup (1-3 DRA), the early-onset (onset age earlier than 6 years of age) ratio is 75% (18/24), high-frequency hearing loss ratio is 16.7% (4/24), retinal angiopathy ratio is 4.2%. In comparison, in the subgroup of patients with 4-10 DRA, the early-onset ratio is 43.5% and no hearing loss or retinal angiopathy is observed. Comparing age at onset and disease severity outcome between genetic subgroups, we find a distinction of early-onset morbidity (Cramer's $V = 0.300$, $P<0.05$) but failed to detect statistical significance. In summary, the predictive value of the size of the D4Z4 allele is more appropriate and informative in the age of onset, rather than clinical severity.

P116

Identification and testing of Everolimus as a potential therapeutic compound for facioscapulohumeral muscular dystrophy

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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 or cure currently exists. We sought to use CRISPR screening in order to identify genes and pathways of which modulation leads to apoptosis resistance from *DUX4*, the toxic protein associated with FSHD's pathology. One of the most promising pathways from this screen was the hypoxia signaling pathway and so we explored the potential of compounds that target this pathway as a therapeutic strategy. The mTOR inhibitor everolimus was the most promising of these compounds, which successfully reduced *DUX4* toxicity in vitro. We are currently testing the efficacy of everolimus in vivo using xenograft and *DUX4*-inducible mouse models. These results are currently mixed but demonstrate the utility of using CRISPR screening to identify novel therapeutic targets for FSHD. Importantly, emphasis on FDA-approved compounds would be a major boon for patients as any successful candidate would have reduced time in the clinical trial pipeline.

P117

SMCHD1's ubiquitin-like domain is required for N-terminal dimerization and chromatin localization

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Structural Maintenance of Chromosomes flexible Hinge Domain-containing 1 (SMCHD1) is an epigenetic regulator that mediates gene expression silencing at targeted sites across the genome. Our current understanding of SMCHD1's molecular mechanism, and how substitutions within SMCHD1 lead to the diseases, facioscapulohumeral muscular dystrophy (FSHD) and Bosma arhinia microphthalmia syndrome (BAMS), are only emerging. Recent structural studies of its two component domains – the N-terminal ATPase and C-terminal SMC hinge – suggest that dimerization of either domain plays a central role in SMCHD1 function. Here, using biophysical techniques, we demonstrate that the SMCHD1 ATPase undergoes dimerization in a process that is dependent on both the N-terminal UBL (Ubiquitin-like) domain and the ligand, ATP. We show that neither the dimerization event, nor the presence of a C-terminal extension past the transducer domain, affects SMCHD1's in vitro catalytic activity as the rate of ATP turnover remains comparable to the monomeric protein. We further examined the functional importance of the N-terminal UBL domain in cells, revealing that its targeted deletion disrupts the localization of full-length SMCHD1 to chromatin. These findings implicate UBL-mediated SMCHD1 dimerization as a crucial step for chromatin interaction, and thereby for promoting SMCHD1-mediated gene silencing.

P118

Analysis of *DUX4* expression in bone marrow and re-discussion of *DUX4* function in the health and disease conditions

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DUX4 re-expression in somatic tissue is revealed to be present in pathologic conditions such as facioscapulohumeral muscular dystrophy (FSHD) and in most cancer types such as B cell acute lymphoblastic leukemia. However, *DUX4* expression hasn't been revealed before in healthy bone marrow. In addition, *DUX4* expressing cells that are in fact pluri/multipotent cell types led us to question, Could *DUX4* be a transcription factor active in certain types of somatic potent cells? As a perfect reflection of a potent cell pool, we aimed to reveal *DUX4* expression in bone marrow aspirates. Bone marrow aspiration materials of seven healthy donors aged between 3 and 32 (2 males/5 females) had been investigated with qPCR analysis after RNA isolation for presence of *DUX4* full length mRNA expression. Bone marrow aspirates had also been investigated for protein existence of *DUX4* via immunohistochemistry in two donors that had sufficient aspiration material. *DUX4* mRNA expression had been observed in all samples and it was higher compared to B-actin. In immunohistochemistry, not all but some of the cells exhibited positive staining with *DUX4* antibody. In this study, active *DUX4* expression in bone marrow was reported for the first time. With this data in somatic progenitor hematopoietic cells, we suggested a novel role for *DUX4* in addition to its role in embryonic and germline cells or in pathologic conditions such as hematologic/other malignancies and FSHD.

P210

High resolution breakpoint mapping of proximally extended D4Z4 deletions in FSHD1 reveals evidence for a founder effect

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Genetic diagnosis for FSHD is generally based on the sizing and haplotyping of the D4Z4 repeat array on chromosome 4. This analysis can be performed by Southern blotting (SB), molecular combing or single-molecule optical mapping. Following these methods, the identification of FSHD1 is usually straightforward, but sometimes atypical rearrangements of the D4Z4 repeat occur which might result in a false negative test result. One of the more common atypical rearrangements found in FSHD1 individuals is a so-called D4F104S1 deletion (or D4Z4 proximally-extended deletion, DPED) allele. In these individuals, not only the D4Z4 repeat is partially deleted, but also sequences immediately proximal to the repeat are lost, which can trouble accurate identification of the FSHD allele in all diagnostic methods.
Previously, we identified several DPED alleles in FSHD and roughly mapped the proximal deletion breakpoints for some of them by a complex pulsed-field gel electrophoresis (PFGE) and SB strategy. Here, using next generation sequencing, we have defined the proximal and distal breakpoints of these DPED alleles at the base pair resolution in 12 FSHD families and 4 control individuals. We set up a PCR-based method for the identification of these DPED alleles in a diagnostic setting.
Our results show that half of the DPED alleles are derivates of an ancient founder allele. We discuss the possible rearrangement mechanism underlying these deletions, give insight in the characteristics of the deleted sequences in DPED alleles and show that some pathogenic DPED alleles carry a D4Z4 repeat beyond the FSHD1 threshold.

P211

Synonymous MYH9 mutation was identified in a FSHD family with thrombocyte number change: is it more than a coincidence?

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Background: Recently, we reported a coincident thrombocythemia in a 67-year-old male FSHD case. This case had a pathogenic JAK2 mutation in his blood sample.
Objective: To clarify whether this is a co-incidence or common pathway indicator, we investigated other family members for FSHD and for their thrombocyte counts.
Material-Methods and Results: One son and one daughter of the JAK2 mutated FSHD case were investigated. They had FSHD clinical symptoms. Because of this, they were genetically tested for FSHD and found to be carrying a 6 repeat unit with a qA allele on chromosome 4, and they were diagnosed as FSHD1. In the son's and the daughter's whole blood investigation, macrothrombocytopenia had been detected. The father's pathogenic JAK2 mutation had been screened in these two cases and found to be negative. For macrothrombocytopenia, an MYH9 gene investigation had been further carried out and as a result a synonymous MYH9 mutation had been detected.
Conclusion: MYH9 is one of the proteins that was revealed to directly interact with *DUX4* protein in FSHD studies. Coincidence of FSHD and thrombocyte number abnormality with MYH9 synonymous mutation might provide valuable information and further studies from this family might contribute to a more thorough understanding of the pathogenesis of FSHD.

P310

Estrogenic hormones counteract FSHD features in a mouse model of muscle regeneration

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The wide range of clinical symptoms in FSHD patients suggests the presence of modifying factors, still partially explored. Our group demonstrated that estrogens improve in vitro muscle differentiation of myoblasts from FSHD patients. Estrogens, through estrogen receptor beta (ERβ), displace *DUX4* from its target promoters and antagonize its transcriptional pathogenetic activity during myoblast differentiation.
In this work, we confirm these data in vivo by analyzing the effect of estrogen on the regenerative potential of human muscle-precursor cells (PVCs) derived from healthy individuals and engineered to express *DUX4* (*DUX4*-PVCs) or derived from FSHD patients. *DUX4*-PVCs were implanted into injured hindlimb muscle of NOD-scid-gamma (NSG) mice treated with 17β-estradiol (E2), 3β-diol (a specific ligand of ERβ present in males), or EtOH (vehicle). Animals were monitored by a functional treadmill test and at the molecular levels by immunohistochemistry, gene, and protein expression.
Our data demonstrate that human PVCs participate in mouse muscle regeneration and form functional heterokaryon muscle fibers that increase run ability recovery. The expression of *DUX4* impairs this function and determines the increased formation of fibrotic tissue. Of relevance, both E2 and 3β-diol rescue this impairment, enhancing muscle formation and reducing the fibrotic response. Accordingly, both mouse hormone treatments rescue functional running ability reduced by *DUX4* expression.
Overall, these results suggest that estrogen hormones improve *DUX4*-PVCs regeneration ability and support the hypothesis of their beneficial activity in humans. In addition, our data substantiate the usefulness of the implantation/regeneration system here developed as an FSHD experimental model.

P311

Sarcolemmal Injury in the pathophysiology of FSHD

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Background: We previously demonstrated that immortalized myoblasts from individuals with FSHD demonstrate significantly worse sarcolemmal repair than their unaffected siblings. Sarcolemmal repair relies on the activation of cellular pathways that stimulate *DUX4* expression, but it is unclear if plasma membrane itself triggers *DUX4* expression.
Objective: To determine if plasma membrane injury leads *DUX4* expression. If so, to determine the time-course of *DUX4* expression during the recovery from injury.
Approach: We generated stable myoblast lines expressing a fluorescent reporter of *DUX4* expression (producing a nuclear GFP signal) using a third-generation lentiviral vector system. We subjected the reporter cells to stretch-induced sarcolemmal injury using a custom-built cell stretching device. Cellular injury was assessed via accumulation of cell-impermeant FM-1-43 dye into the cytosol post-injury. Reporter expression was monitored for 24 hours after injury using time-lapse, live-cell imaging. qRT-PCR was used to assess for *DUX4* expression, and expression of its downstream targets ZSCAN4, MBD3L2, and TRIM43, 24 hours post-injury.
Results: Five mm of stretch was needed to induce sarcolemmal injury. FSHD myoblasts demonstrate an increase in nuclear GFP fluorescence that peaks ~2-5 hrs post-stretch injury, and remains elevated for approximately 24 hours. There was no change in nuclear GFP fluorescence above background after injury in healthy myoblasts. Confirming our previous findings, FSHD myoblasts were more susceptible to membrane injury – evidenced by the greater percentage of cells that failed to repair ($p<.05$). Likewise, we observed significantly increased *DUX4* expression at 24 hours post-injury ($p<.05$), and elevated (though non-significant) ZSCAN4 expression at the same timepoint.
Conclusion: The results suggest that membrane injury may contribute to *DUX4* expression and could therefore be an important mechanism of disease progression.

P312

Exploring the relationship between *DUX4* and hypoxia-inducible factor (HIF1α)

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Studies examining FSHD skeletal muscle molecular networks revealed pathways involved in hypoxic response and oxidative stress to be disturbed, with HIF1α being of particular interest. Our goal is to decipher mechanisms underlying the contribution of the *DUX4*-HIF1α crosstalk to muscle dysfunction in FSHD. To this aim, the effect of a sustained HIF1α activation on myogenic differentiation was first investigated in human skeletal muscle cell lines. Hypoxia enhances early myogenic differentiation and fusion of myocytes into multinucleated myotubes. Similar results were obtained in HIF1α gain of function studies using CoCl2. In *DUX4*-inducible human myoblasts, *DUX4* inhibits differentiation and reduces cell viability in a dose dependent manner. In proliferating myoblasts expressing *DUX4*, the expression of HIF1α and its direct target PDK1 are downregulated at the mRNA and protein level. In myocytes expressing *DUX4*, no change in HIF1α (mRNA and protein level) and target gene expression (VEGFA and PDK1) was detected but PDK1 protein level was downregulated. In vivo studies on a murine model based on the electroporation of a *DUX4* expression vector into mature muscle did not show any significant difference in Hif1A and Pdk1 gene expression at 1, 3, 7 and 14 days post-injection. However, at one day post-injection, an increased VegfA expression was observed in the plasmid control group as compared to the saline injected group. This increase seems delayed in the *DUX4* group. In conclusion, the HIF1α pathway is modulated upon *DUX4* expression but these changes are dependent on the differentiation stage of muscle cells. Moreover, *DUX4* induction and HIF1α sustained activation differentially impact the myogenic process.

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*DUX4*c and PAX7 interfere with *DUX4*-induced perturbation in skeletal myogenesis

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Aberrant expression of *DUX4* is linked to pathogenesis in facioscapulohumeral muscular dystrophy. Induced expression of *DUX4* in human myoblasts suppresses cell proliferation and myogenic differentiation then induces cell death, potentially affecting muscle development and repair in FSHD (Banerji et al. 2020). Centromeric to the D4Z4 macrosatellite array that encodes *DUX4* is a single, inverted, mutated D4Z4 unit that encodes a closely related protein called *DUX4*c. *DUX4* binds DNA via homeodomains that are present in *DUX4*c and have high sequence similarity to that of PAX7, a master transcriptional regulator of myogenesis. Here, we investigated how *DUX4*c or PAX7 interfere with *DUX4* function in human myogenesis. Constitutive expression of *DUX4*c of PAX7 rescues *DUX4*-induced defective proliferation in *DUX4*-inducible human myoblast models. We also show that *DUX4* promotes β-CATENIN nuclear translocation in myogenic cells, a process reverted by concomitant expression of either *DUX4*c or PAX7. Functionally, *DUX4*c robustly suppresses expression of *DUX4* target genes TRIM43, PRAMEF1 or MBD3L2, but enhances *DUX4* repression on myogenic regulators MYF5 and MYOD. In summary, *DUX4*c or PAX7 can counteract aspects of *DUX4*-mediated perturbation in human myoblasts.

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P314

A live-cell drug screening platform for FSHD therapeutics

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Even though several *DUX4*-dependent pathogenic mechanisms for FSHD have been described, the translation of these discoveries into therapeutics has been slow. Progress has largely been hindered by the limitations of existing model systems for evaluating therapeutics. In vitro models mostly rely on patient biopsy-derived cell cultures. These are highly relevant, but *DUX4* expression is quite rare, rendering many molecular techniques inaccessible. Engineered cell models are an alternative, but these are also limited due to their non-physiological nature. Mouse FSHD models have been developed, but they are expensive, labor intensive, and the relevance of expressing *DUX4* in mice is unclear. Thus, there is an urgent need for a drug testing platform that is physiologically relevant, high-throughput, economical, and suitable for testing a variety of therapeutics. To this end, we have designed a platform that combines patient-derived myogenic cell lines carrying a previously published *DUX4* reporter system (Rickard et al. 2015) with an imaging system capable of taking time-lapse images in a 96-well format. Our platform can measure several aspects of *DUX4* expression, including activation frequency and the interval between activation and apoptosis, and thus can be used to evaluate drugs that target *DUX4* synthesis, such as p38 inhibitors, ASOs, and CRISPR gene editing, and drugs that target downstream processes, such as rapamycin or HIF1A inhibitors, in a highly disease-relevant system.

P510

AAV.U7-snRNA targeting *DUX4* polyA prevents muscle damage in the TIC-DUX4 FSHD mouse model

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In this study, we developed a strategy to accomplish *DUX4* inhibition using U7-small nuclear RNA (snRNA) antisense expression cassettes (called U7-asDUX4). These non-coding RNAs were designed to inhibit production or maturation of the full-length *DUX4* pre-mRNA by masking the *DUX4* start codon, splice sites, or polyadenylation signal. In so doing, U7-asDUX4 snRNAs may operate similarly to antisense oligonucleotides. However, in contrast to oligonucleotides, which may be limited by poor uptake in muscle and a requirement for lifelong repeated dosing, U7-asDUX4 snRNAs can be packaged within myotropic gene therapy vectors and may require only a single administration when delivered to post-mitotic cells in vivo. One construct targeting the *DUX4* polyA signal significantly silenced *DUX4* in vitro and in muscles of TIC-DUX4 mice. For the latter, we packaged four copies of our lead U7-asDUX4 expression cassette into AAV6 particles and performed dose-escalation experiments for efficacy and toxicology outcomes. In our first in vivo studies in gastrocnemius muscles of TIC-DUX4 FSHD mice, our lead construct significantly protected from *DUX4*-associated histological deficits, including significant reduction in myofibers with central nuclei, an indication of muscle damage and repair. As of this writing, additional in vivo studies are underway, with expected outcomes using histopathological, functional, and molecular methods. These results support translation of a new *DUX4*-targeting gene therapy for FSHD that could be used alone or in combination with other strategies, like RNAi therapy, to maximize *DUX4* silencing in individuals with FSHD.
Keywords: Facioscapulohumeral Muscular Dystrophy, Double Homeobox 4, *DUX4*, U7-snRNAs, exon splicing, adeno-associated virus vectors, muscle, exon skipping

P511

Apabetalone, a clinical-stage cardiovascular disease drug, inhibits *DUX4* expression in FSHD Cells

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Introduction: Facioscapulohumeral muscular dystrophy (FSHD) pathophysiology is attributable to epigenetic de-repression of *DUX4* that is activated in myocytes during differentiation. *DUX4* is activated via an epigenetic mechanism dependent on bromo- and extraterminal domain (BET) proteins. Apabetalone is a late-stage clinical BET protein inhibitor (BETi) for cardiovascular disease, with a well-established clinical safety record.
Objectives: To quantify differential gene expression in FSHD muscle cells during differentiation and activation of *DUX4*. To assess BETi effects on disease and differentiation markers and to evaluate apabetalone's therapeutic potential in FSHD. To investigate functional properties of BETi-treated cells, including cell viability and apoptosis.
Methods: Transcriptomic analysis of BETi treatment on primary FSHD myocytes by RNA seq and RT-PCR, followed by Ingenuity Pathway Analysis (IPA) to characterize treatment impact on key pathways and cellular functions. Luminescent- and fluorescent-based viability and apoptosis assays to understand treatment impact on cells.
Results: *DUX4* downstream markers, including ZSCAN4, MBD3L2, and TRIM43, were robustly downregulated with apabetalone treatment, IC50: 0.51, 0.46, and 2.19 µM, respectively. Differentiation markers in myotubes (MYOG and MYH2) were not significantly impacted (IC50 > 50 µM). Dozens of canonical pathways were significantly changed during differentiation and activation of *DUX4*, while apabetalone treatment countered downregulation of key pathways associated with muscle function, including ERK/MAPK and CNTF signaling, with pathway z-scores of 2.4 (p=0.01) and 2.7 (p=0.01), respectively. Apoptosis and cell viability were unaffected with treatment.
Conclusions: By preventing *DUX4* activation and downstream signaling, apabetalone treatment is a promising potential therapeutic for FSHD with limited risk of off-target effects.

P512

Molecular deficits in the FLExDUX4 mice are improved by aerobic exercise

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Background: Exercise is a non-invasive intervention shown to improve skeletal muscle health and function in animals and humans. However, the role of exercise as a treatment for FSHD is poorly understood, including the molecular transducers of exercise adaptation.
Objective: To characterize the transcriptional response to aerobic exercise in FLExDUX4 vs wild type (Wt) mice.
Approach: Five-month old male FLExDUX4 (n=15) and Wt (n=9 M) mice were randomly assigned to voluntary wheel running (Ex, FLExDUX4 n=8, Wt, n=5) or no-wheel-running control (Con, FLExDUX4 n=7, Wt n=4) for six weeks. RNA sequencing of the triceps was used to identify differentially transcriptional differences between study groups. DEseq2 was used for differential gene expression. Gene Set Enrichment Analysis and Ingenuity Pathway Analysis were used to identify gene pathways and upstream regulators of the exercise response.
Results: DEseq2 identified 775 genes (416 upregulated, 358 downregulated) that were differentially expressed between FLExDUX4 Con vs Wt Con mice ($p<.05$). There were 1060 genes (515 upregulated, 545 upregulated) differentially regulated between FLExDUX4 Con vs FLExDUX4 Ex groups. The top enriched signaling pathways between FLExDUX4 Con vs FLExDUX4 Ex groups included Sirtuin Signaling, Fibrosis Signaling, Estrogen Receptor Signaling, and Oxidative Phosphorylation (all $p<.001$). Top upstream regulators included the Peroxisome proliferator-activated receptor-gamma coactivator (PGC-1α) ($p<.001$) and vascular endothelial growth factor ($p<.001$). Top canonical pathways demonstrating normalized activity after exercise in FLExDUX4 mice included interleukin-1 (IL-1), Integrin, hepatocyte growth factor (HGF), and mammalian target of rapamycin (mTOR) signaling (all $p<.05$).
Conclusion: Exercise stimulates transcriptional reprogramming in FLExDUX4 mice consistent with increased mitochondrial function and vascularity, as well as reduced muscle inflammation, fibrosis, and oxidative stress.

P710

Integration of UK and USA FSHD registries identifies 7 presentations and associates smoking with earlier onset

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Facioscapulohumeral muscular dystrophy 1 (FSHD1) is an inherited skeletal myopathy linked to truncation of the D4Z4 macrosatellite at chromosome 4q35 on a permissive 4qA haplotype. Though classically presenting first in facial musculature before progressing to the shoulder girdle and lower limbs, muscle weakness in FSHD1 is highly heterogeneous. Here we investigate 511 participants with FSHD1 included in the USA FSHD Registry from 2001 to 2020. Outcomes included 129 clinical and FSHD-related patient-reported features in annual surveys (average follow-up 8 years), as well as D4Z4 small allele size. By categorising self-reported functional limitations as involving facial, upper or lower limbs, we performed multivariate time-to-event analyses on an amalgam of 10 common FSHD symptoms. D4Z4 repeat length inversely associated with earlier involvement of all muscle groups, in line with previous studies. Importantly, patients who reported smoking cigarettes experienced earlier facial and lower body involvement, while obese patients experienced earlier upper body involvement, suggesting roles for smoking cessation and weight loss in FSHD1. Investigation of medication use revealed that patients who experienced earlier symptom onset were more likely to take analgesics, while patients with later onset were more likely to take anti-hypertensives. Lastly, clustering on integrated data describing 213 UK and 114 USA FSHD1 patients revealed 7 distinct clinical presentations of FSHD1: mild, moderate and severe classical presentations independent of D4Z4 repeat length, two upper body sparing presentations, a facial sparing presentation and an outlier group. In summary, we propose smoking and obesity as modifiable risk factors for FSHD progression and identify 7 distinct clinical subtypes of FSHD for use in clinical trial stratification, disease modifier identification and therapeutic development.

P711

Long-term follow-up of respiratory function in facioscapulohumeral muscular dystrophy

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OBJECTIVE – To evaluate the 5-year change in respiratory function in facioscapulohumeral muscular dystrophy (FSHD).
METHODS – Genetically confirmed FSHD patients aged 18 years and older were examined twice over a period of approximately five years. The forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) were measured using handheld spirometry with a face mask. Manual muscle testing (MMT), the Motor Function Measure (MFM), spinal deformities, Ricci score and FSHD Clinical score were used as measures to correlate respiratory function to clinical features.
RESULTS – Ninety-two patients were included (57% male, age 18-75 years). The spirometry outcomes of 25 patients classified as restrictive pulmonary involvement at baseline. The mean FVC decreased during follow-up, from 88.4% predicted to 85.3% predicted ($p = 0.003$). The difference in FVC% predicted between baseline and follow-up did not correlate with the change in clinical outcome measures. Eighteen patients had a deterioration of FVC of more than 10% predicted and they had higher FSHD clinical scores and lower MMT sum scores at baseline than the other patients, more frequently suffered from kyphoscoliosis at follow-up and had a larger decline in MFM scores between baseline and follow-up.
CONCLUSIONS – Pulmonary function, measured by spirometry, deteriorates slowly in FSHD. Patients with severe muscle weakness at baseline, or a relatively fast decline in functioning, are at higher risk of an accelerated decline in pulmonary function.

P712

Functional outcomes and complications following scapulothoracic arthrodesis in patients with facioscapulohumeral dystrophy

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Background: Scapulothoracic arthrodesis is a successful treatment approach for patients with loss of shoulder elevation. The purpose of the present study was to assess functional outcomes and complications following scapulothoracic arthrodesis.
Methods: We retrospectively reviewed the records of 40 patients (64 shoulders) in whom scapulothoracic arthrodesis was performed. To achieve fusion, multiple multifilament cables were used together with autologous bone and allograft bone. Preoperative and postoperative shoulder elevation and abduction; Disabilities of the Arm, Shoulder and Hand (Quick version, qDASH) scores; and pulmonary function were compared. Recorded complications were classified as pulmonary or scapular.
Results: The mean age of the patients at the time of the operation was 25.4 years (range, 15 to 60 years), and the mean duration of follow-up was 71.2 months (range, 12 to 185 months). When the preoperative values were compared with those at the latest follow-up, significant improvement was noted in terms of elevation (from a mean [and standard deviation] of 60.6 ± 17.2 to 123.7 ± 26.7; $p < 0.001$), abduction (from 52.7 ± 15.8 to 98.8 ± 20.3; $p < 0.001$), and qDASH scores (from 34.7 ± 11.4 to 13.3 ± 13.1; $p < 0.001$). The overall complication rate was 26.6%. There were 7 pulmonary complications (4 pneumothoraxes, 2 pleural effusions, and 1 major atelectasis), and 5 chest tube placements were required. Ten complications (including 3 rib fractures, 1 brachial plexus palsy, 2 cases of implant irritation, 2 nonunions, 1 delayed union, and 1 scapular fracture) were related to the scapular fixation, and 7 revision procedures were required. Scapulothoracic fusion was achieved in all patients but 1, who had a scapular fracture.
Conclusion: Scapulothoracic arthrodesis with use of multifilament cables is a successful surgical technique with high fusion rates and low morbidity. Pulmonary complications are common but resolve with careful attention.

P713

Estimation of the clinical severity of facioscapulohumeral muscular dystrophy (FSHD) using smartphone and remote monitoring sensor data

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Background: Facioscapulohumeral muscular dystrophy (FSHD) is a progressive, asymmetric neuromuscular disease. The slow and variable rate of progression of FSHD makes the development of new treatments for FSHD highly dependent on validated biomarkers that can quantify disease progression and response to drug interventions. The objective of this study was to build a tool that estimates FSHD clinical severity based on common behavioral features captured using smartphone and remote sensor data.
Methods: Thirty-eight genetically confirmed FSHD patients were enrolled in this study. The FSHD Clinical Score and the Timed Up-And-Go (TUG) test were used to assess FSHD symptom severity at day 1 and day 42 of the trial. The remote sensor data were collected using an Android smartphone, Withings Steel HR+, Body+ and BPM Connect+ for 6 continuous weeks. We created two single-task regression models that estimated the FSHD Clinical Score and TUG separately. In addition, we built one multi-task regression model that estimated the two clinical assessments simultaneously. The performance of the three models were compared by means of the R2.
Results: The single-task regression models correlated with FSHD Clinical Score and TUG (R2 of 0.57 and 0.59, respectively). The multi-task model achieved an R2 of 0.74 and therefore outperformed the single-task models in estimating the clinical severity.
Conclusions: We created a novel composite score by using a combination of monitored behaviors that correlates with FSHD clinical severity. We have shown that smartphone and remote sensor data could therefore complement the assessment of FSHD symptom severity outside of the clinic. Longitudinal follow up studies should be conducted to further validate the reliability and validity of the remote score as a predictive tool to monitor disease progression over a longer period of time.

P714

Pediatric facioscapulohumeral dystrophy (FSHD) natural history study – planned protocol in an Australian cohort

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Aims: This project aims to: i. contribute to standards of care guidelines for children and young people world-wide with FSHD; ii. contribute to outcome measure and biomarker development to enhance clinical trial readiness and; iii. establish the cognitive and psychological profile of children and adolescents with FSHD to establish any genetic links.
Method: Children aged 0-18 years with a confirmed genetic diagnosis of FSHD and clinical features in keeping with this diagnosis will be eligible to participate. Patients will be recruited to the study and followed up for 2 years. During the study, medical, performance-based physical function and self-reported quality of life assessment data and muscle MRI images will be collected annually. Each participant will undergo neuropsychological and behavioral assessment on a single occasion.
Results: Results will help to: i. estimate the need for development of pediatric standards of care and formalise the most clinically relevant evaluations required to serially monitor children with FSHD from a functional and psychological perspective; ii. establish correlations between genetic data, disease severity and function; iii. estimate the validity and responsiveness of currently available performance-based measures of physical function (including FSHD-COM Peds) and self-reported measures of quality of life and perceived disease burden (including FSHD-HI Peds); iv. examine correlations between serial muscle MRI data and performance-based and self-reported measures of functioning and disease burden to help establish biomarkers of disease progression and; v. establish correlations between genetic data, disease severity and function in a pediatric cohort.

P715

Relationship of strength to functional assessments for individuals participating in the ReSolve FSHD study

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Introduction: Standardized clinical outcome assessments may serve as endpoints for late phase FSHD clinical trials. However, as weakness is the primary impairment resulting in functional changes, it is important to understand the relationship between strength and function.
Methods: Individuals with FSHD participating in the ReSolve study underwent measures of strength and function. Strength assessments included manual muscle testing (MMT) and quantitative muscle testing (QMT). Functional assessments were performed including donning and doffing a coat, Timed Up and Go, 10 meter walk/run, gait speed, ascending and descending 4 stairs, and the 6 Minute Walk Test.
Results: A total of 133 participants (56% male) with a mean age of 49.9 (range 19-75) years are enrolled at 9 international sites. They presented with a mean strength of 4.1 (range=2.0-5.0) on the modified MRC scale and a 7.32 (range 1-15) on the FSHD clinical score. The strength of the correlation between overall lower extremity strength to functional assessments ranged from 0.66 to 0.84 (MMT) and 0.49 to 0.64 (QMT). The 10 meter walk/run had the strongest correlation with strength. The correlation between individual lower extremity muscle groups and the 10 meter walk/run ranged from -0.41 to -0.74; with the hip extensors and knee flexors having the strongest correlations. Donning and doffing a coat was significantly correlated with overall upper extremity strength (MMT; ρ=-0.63, p=0.01).
Conclusions: Understanding the relationships between strength and function may help to guide the selection of muscle groups and functional assessments for future clinical trials.

P716

The Russian registry of patients with facioscapulohumeral muscular dystrophy

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Background: Facioscapulohumeral dystrophy (FSHD) is one of the most common hereditary muscular dystrophies, with estimated prevalence of 1:20,000. Due to the recent setting up of a genetic study to determine the number of D4Z4 repeats it became possible to keep the registry of patients with FSHD in Russia.
Objectives: To describe anamnestic and clinical features of patients carrying a reduced number of D4Z4 repeats.
Methods: The Russian Registry of patients with FSHD includes information collecting from both patients and physicians. Self-report questionnaire and clinical evaluation form were designed for that purpose. Clinical evaluation form includes questionnaire for physicians, FSHD clinical score from FSHD Comprehensive Clinical Evaluation Form and Facial Disability Index.
Results: The Registry began to be maintained from March 2020. To date, 98 patients from 76 families participated in the Registry; all of them had FSHD type 1 confirmed by DNA diagnostics performed in our center for 65 probands (85%). All examined patients showed classical FSHD phenotype. Clinical evaluation revealed that 7 probands (10%) presented the mild clinical features as weakness of facial muscles and scapular winging only. There are 5 families with prominent variability of clinical severity.
Conclusions: The Russian Registry as other national registries aims to promote a comprehensive assessment of patients with FSHD and the collection of more complete data to contribute to future clinical research. We plan to continue expanding the number of patients in the Registry and we hope that at some point in the future we will be close to the estimated number of patients with FSHD in Russia.

P717

The UK FSHD patient registry: An important tool linking patients to national and international research projects

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Background
The UK Facioscapulohumeral Muscular Dystrophy (FSHD) Patient Registry is a self-enrolling online database collecting clinical and genetic information about FSHD. The registry aims to facilitate academic and clinical research, better understand the condition, and disseminate information relating to upcoming studies and research advancements. It was established in May 2013 supported by Muscular Dystrophy UK, and is coordinated by Newcastle University.

Method
The registry captures longitudinal, self-reported and clinician-reported data (the TREAT-NMD core dataset and questionnaires on pain, quality of life and scapular fixation) through an online portal.

Results
Between May 2013 and March 2021, 1,035 participants registered with the registry. On average, nine new participants register each month. For those who provided a clinical diagnosis, 96% reported FSHD/FSHD1, and 4% reported FSHD2. Overall, 48% have genetic confirmation of their condition and the most commonly reported weakness was shoulder weakness (88%).
The registry has previously supported 27 research enquiries including the ACTMuS clinical trial and a natural history study of infantile-onset FSHD. In the past 12 months, the registry has facilitated 10 enquiries including three COVID-19 surveys, and various surveys capturing information on dysphagia, pregnancy, sleep and the patient/caregiver experience.

Conclusion
The registry continues to support the FSHD community and help facilitate and advance a range of research.

P718

Validity and reliability of the EMG threshold during incremental cycling in FSHD

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Although muscle fatigue is a common and potentially the most disabling symptom in FSHD, no objective outcome measure is available yet. We evaluated the use of surface electromyography (sEMG) to objectively measure muscle fatigue in 24 healthy participants and 35 patients with a neuromuscular disorder, among which were 13 FSHD patients. In healthy persons, sEMG thresholds (EMG-Ts) during a maximal cycle test are related to the first and second ventilatory thresholds (VTs). We hypothesized that in patients with FSHD, the sEMG thresholds would occur relatively early in time than compared to the VT, compared to healthy subjects, because more muscle fatigue occurs.
All participants performed a cardiopulmonary exercise test (CPET) on a bicycle using a 10 minute ramp protocol, during which we collected ergospirometry (power [W] at the first ventilatory threshold [VT1] and the second ventilatory threshold [VT2]), and sEMG data of lower leg muscles (power at the first sEMG threshold [EMG T1] and the second sEMG threshold [EMG T2]), using the V-slope method. Threshold determination was feasible for VT1, VT2 and EMG T2 (>80%), while feasibility of EMG T1 was low (<43%). Inter-rater reliability of EMG T1 varied between muscles (ICC between 0.169 and 0.990), while inter-rater reliability of EMG T2 was high for all muscles (ICC>0.95). Test-retest reliability was excellent in all participants for VT1, VT2 and EMG T2 (ICC >0.9). Both the VTs are able to differentiate between healthy controls and patients, where healthy controls have thresholds at a higher power ($p<0.001$). In line with our hypothesis, the sEMG thresholds of patients occurred relatively early in time compared to controls, while for the normalized VT, only VT1 shows a significant difference between healthy controls and patients.
In future research projects, we will further explore the underlying mechanism of muscle fatigue in patients with FSHD and develop a protocol for measuring muscle fatigue in daily life.

P719

A novel shoulder disability staging system for scapulothoracic arthrodesis in patients with facioscapulohumeral dystrophy

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Background: Scapulothoracic arthrodesis (STA) is a well-established surgical technique to provide scapular stabilization in patients with facioscapulohumeral dystrophy (FSHD). There are no staging or scoring systems available to guide the surgical decision. The aim of this study was to develop a staging system to evaluate the shoulder disability in patients with FSHD to guide surgical decision-making and assess its reliability among surgeons.
Methods: Fifty-seven shoulders of 29 patients (15 male, 14 female) with an average age of 34.5 years (13–73) were included. Six stages of the disease were defined to create a system consisting of shoulder elevation, deltoid function and scapular winging. Patients were assessed by two independent orthopaedic surgeons who were blind to each other. Statistical analyses included mean and standard deviation for descriptive variables, Pearson's correlation and Cohen's Kappa for inter- and intraobserver agreement.
Results: Measurement of elevation showed an excellent correlation in both inter- and intraobserver assessment. There was substantial agreement on deltoid function and moderate agreement on scapular winging. Decisions on stage showed excellent agreement on interobserver and substantial agreement on intraobserver assessment. Surgical decision using the stage showed excellent agreement on both inter- and intraobserver assessment.
Conclusion: This novel staging system has an excellent inter-observer agreement on FSHD patients' shoulder disability. This would provide surgeons a beneficial tool to define patient groups that would have negatively or positively been affected by STA.

P720

Genotype-phenotype correlation in FSHD-like patients with uncommon features

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Background: Since the first descriptions of the FSHD phenotype, an interesting range of uncommon features has been described by different authors. This clinical variability is captured by the Comprehensive Clinical Evaluation Form (CCEF), a tool validated by the Italian National Registry for FSHD (INRF). Objectives: We investigate the genotype-phenotype correlation in FSHD-like patients, carriers and not carriers of an allele of reduced size, with uncommon features (D1 patients on the basis of CCEF) with the secondary aim of studying the distribution of clinical categories in D1 families. Results: We identified 198 D1 subjects (101 females). Of this cohort 176 were probands (129 single cases, whereas 47 had at least one other member of the family included in the registry) and 22 were relatives. Mean age at onset was 39.8; mean FSHD score was 6.2 (SD 2.8) in probands and 5.3 (SD 2.7) in relatives (p value = 0.2128) and no significant differences were detected between males and women. No particular size of DRA prevails among subjects assessed with category D1. No significant clustering of any uncommon feature was found in the examined cohort. A total of 61.3% of the relatives of D1 patients were asymptomatic (category C). Conclusions: D1 subjects are myopathic subjects with the co-presence of genetic and environmental factors and the D4Z4 reduced allele may be one of the various factors contributing to the clinical picture. The probability for relatives of D1 probands to develop a myopathic phenotype for the exclusive presence of DRA is low. Caution should be exercised in order to avoid uncorrected diagnoses. This observation is highly relevant for clinical management because this clinical variability requires additional parameters to be used in clinical practice for diagnosis and interpretation of the clinical phenotype.

P721

What is the clinical significance of the facial-sparing phenotype in facioscapulohumeral muscular dystrophy? A nation-wide cross-sectional study

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Background: Does the FSHD facial-sparing phenotype have peculiar features, regarding degree of severity, risk of disease in relatives and the size of D4Z4 repeat array? Methods: A multicenter cross-sectional study included 460 subjects (125 with facial-sparing phenotype and 328 subjects with the complete FSHD phenotype) from the Italian National Registry for FSHD. The phenotypic classification was obtained by applying the Comprehensive Clinical Evaluation Form (CCEF) and the degree of muscle impairment was measured as FSHD score (0-15). Results: We found that subjects with facial-sparing phenotype (B1 clinical category) have a significantly milder phenotype in comparison with subjects presenting classic FSHD phenotype (P value <0.001). Out of 33 families having a proband with facial-sparing phenotype in 54.5%, the proband was the only participant expressing a myopathic phenotype. Interestingly, of the 125 clinically assessed B1 subjects, 36% did not carry a D4Z4 allele with 10 or fewer repeat units. Conclusions: This study found that the facial-sparing phenotype defines a distinct nosological entity with different disease course and lower penetrance. It is recommended that clinicians use the CCEF for clinical classification and study the extended family to provide the most adequate clinical management and genetic counseling. The same phenotype was found in participants carrying D4Z4 alleles of normal size in contrast to the indication that a positive molecular test is the only determining aspect for FSHD diagnosis.

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Self-reported sleep quality and daytime sleepiness in patients with FSHD

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Objective: To discover if self-reported lowered sleep quality (SQ) and excessive daytime sleepiness (DS) are prevalent in a large, representative group of patients with FSHD. Background: Facioscapulohumeral muscular dystrophy (FSHD) is a progressive disorder causing muscle atrophy, notably in the upper body. Sleep in patients could be clinically deficient due to causes such as sleep-disordered breathing, anxiety, obesity, and pain. However, to date, only limited, often single-center studies have focused on self-reported characteristics of sleep. Methods: We performed a prospective survey of individuals with FSHD enrolled in the FSHD Society Registry. The survey consisted of demographic and clinical sections, the Pittsburgh Sleep Quality Index (measuring SQ), and the Epworth Sleepiness Scale (measuring DS). The survey was completed by patients or caretakers. We evaluated descriptive statistics, and associations between clinical characteristics and SQ and DS were found using one-way ANOVAs with effect size categorized based on Cohen (1988). Results: Out of a representative sample of 690 respondents, 66% showed reduced SQ (PSQI>5), and 15% showed excessive DS (ESS>10). As SQ deteriorated, DS significantly increased. Nocturnal pain had a large, significant effect on lowering SQ ($p<.001$, $\acute{\eta}2=.192$). Conclusion: Patients should monitor sleep, and physicians should place greater emphasis on sleep in care. Future research should be conducted on physiological effects of pain in sleep.

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Quantitative muscle analysis in FSHD using whole-body MRI: Composite muscle measurements for cross-sectional analysis

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Objective: Develop whole-body MRI protocol and composite analyses that correlate with clinical outcome assessments (COAs) in FSHD.
Background: Functional tests show limited sensitivity to disease progression. MRI can potentially detect muscle changes before COA changes.
Methods: This prospective, observational study enrolled 17 adults with FSHD1 aged 18-65 at 6 sites, imaged at baseline and 4-12 weeks later. Volumetric analysis of 36 muscles included: shoulder girdle/upper arm (upper extremity [UE]), torso, and lower extremities (LE). An automated atlas-based segmentation with manual verification identified muscles. Composite scores included muscles related to COAs: LE for timed up and go (TUG), UE/LE and torso for FSHD-TUG, and UE and trunk muscles for reachable workspace (RWS). MRI measures included MFF, muscle fat infiltration (MFI) and lean muscle volume (LMV).
Results: Total LMV (Pearson's r=0.9984), MFI (r=0.9845) and MFF (r=0.9918) had strong correlations between the two MRIs. The composite total MFF and MFI for the 10 LE muscles correlated with TUG, 0.72 and 0.83, respectively; UE/LE and 36 trunk muscles for FSHD-TUG, 0.73 in both measures and composite total MFF for the 28 UE and trunk muscles with RWS (r=0.85).
Conclusions: There was strong correlation between whole-body MRI-composite measurements and COAs. MRI measures can potentially provide important information about disease severity and progression as it correlates with FSHD relevant clinical endpoints.

P724

Evaluating *DUX4* activity in a phase 2, randomized, double-blind, placebo-controlled, 48-week study of the efficacy and safety of Losmapimod in subjects with FSHD

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Primary Objective: Evaluate the efficacy of losmapimod in inhibiting the aberrant expression of *DUX4* by using a quantitative RT-PCR assay.
Background: FSHD is caused by aberrant expression of *DUX4* due to loss of repression at the D4Z4 locus. *DUX4* activates a downstream transcriptional program that causes myofiber death, maladaptive tissue remodeling characterized by replacement of muscle with fat, ultimately resulting in progressive motor disability. Losmapimod is an orally active, selective, small molecule inhibitor of p38α/β. Preclinical studies demonstrated that losmapimod reduces *DUX4* in differentiating FSHD myotubes across multiple genotypes. Losmapimod rapidly distributes in muscle and engages p38 α/β. Losmapimod has been tested across 12 indications resulting in >3,500 human exposures with satisfactory safety and tolerability. Methods: Eighty subjects age 18 to 65 years with genetically confirmed FSHD1, clinical severity score of 2 to 4 (range 0-5) and MRI-eligible skeletal muscles for needle biopsy were randomized 1:1 to receive 15 mg losmapimod or placebo tablets PO twice daily for 48 weeks. Subjects were followed for approximately 53 weeks, including 4-week screening period and 7-day safety follow-up period. Participants had muscle biopsies pre-treatment and on treatment at Week 16 or 36 to measure effects on *DUX4* activity as the primary endpoint in ReDUX4.

Results and Conclusions: The development of this RT-qPCR assay and results of this Phase 2 study will be presented.

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Australian neuromuscular disease registry

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Recent progress in the understanding of the genetic basis of neuromuscular disorders has led to rapid expansion in clinical research into these conditions throughout the world. Clinical trial readiness for FSHD relies on accurate, well-curated natural history data, which is facilitated by disease registries.
The re-invigorated Australian Neuromuscular Disease Registry (ANMDR) will collect clinical, genetic and personal data on Australians living with neuromuscular disorders, to facilitate clinical research, best practice care and service provision. The ANMDR will include patients with FSHD, SMA, DMD and myotonic dystrophy (DM1). It is a registry that is inclusive of all ages. One in 3000 people in Australia is thought to have a neuromuscular disease. By including adults and children we hope the establishment of this registry has obvious worth for clinicians, patients and pharmaceutical companies. Registrants will be contacted annually to update their information. Anonymised aggregate data from this registry may be used to bring trials to Australia.
We will be heavily promoting the Registry to both support groups and health professionals. We will be recruiting through clinics, neurologists and via links to the Registry on support group webpages.
We have already been able to secure engagement with patients, patient support groups and industry, and expect that the ANMDR will become an invaluable resource to the Australian neuromuscular community.