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Background: In order to optimize the participation in the therapeutic advances, knowledge on the natural history and clinical research tools for children are highly needed.

Objectives: We performed a nationwide, single-investigator, natural history study on FSHD in childhood.

Results: Thirty-two children were identified (estimated prevalence of 1:100.000 in the Netherlands). FSHD in childhood consisted of facial weakness with normal or only mildly affected motor performance, decreased functional exercise capacity, pain and fatigue, lumbar hyperlordosis, and abnormalities on muscle ultrasound. Systemic features such as hearing loss, retinal, and cardiac abnormalities were infrequent and subclinical. Patients had a mean D4Z4 repeat array of units (range 2-9 units) and 14% of the mutations were de novo.

Conclusions: FSHD in childhood is more prevalent than previously known and the genotype resembles classic FSHD. Importantly, FSHD mainly affects functional exercise capacity and quality of life in children.

S1.2 Clinical examination of scapula function in patients with FSHD.

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Background: Scapular dyskinesis (SD) is a distinct feature of the FSHD phenotype. Limitations in use of the upper extremity and pain in the shoulder neck and arms are common problems in these patients. SD can be caused by mainly serratus anterior and trapezius muscle weakness but it also occurs in patients with normal muscle strength. Clinical examination of SD is difficult, but needed to distinguish patients that can or cannot benefit by exercise interventions or who need orthoses or surgical management of upper extremity problems.

Objectives: To develop a clinical method for evaluation of SD in patients with FSHD.

Results: We have collected video data from 3 patients with FSHD and varying scapular muscle function. We will show how to discriminate patients that show SD due to loss of muscle strength from those who have SD due to loss of motor control.

Conclusion: FSHD patients with scapular dyskinesis can have sufficient serratus anterior and trapezius strength which is not adequately recruited for maintaining scapular posture. Some patients might benefit from scapular coordination training.

S1.3 Facioscapulohumeral muscular dystrophy (FSHD) has a talk with endocrinologic parameters: estradiol, progesterone and testosterone.

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Background: Facioscapulohumeral Dystrophy manifests earlier and more severe in men; in women symptoms aggravate after menopause.

Objective: We tested the effects of estradiol and other hormones on severity, by using facial 3D morphology scan and clinical severity score.

Results: Luteinizing hormone, follicle-stimulating hormone, free-estriol, estradiol, free-total testosterone, progesterone, 17-OH-progesterone, prolactin, albumin, fibrinogen levels were analyzed in 38(23 Males/15 Females) patients with clinical severity score and in 11 (6 Males/5 Females) patients with facial 3D morphology scan. There was a negative correlation between clinical severity score and estradiol($p = 0.031$/ $r = -0.556$), progesterone($p = 0.029$/$r = -0.561$), estradiol/free-testosterone ratio($p = 0.001$/ $r = -0.759$), estradiol/total-testosterone ratio($p = 0.028$/ $r = -0.567$) in 15 females; there was no correlation in 23 males. Maximal closing of eyes($p = 0{,}002$) and whistling($p = 0.000$) were significantly different than control group and correlated with free-testosterone($p = 0.010$/$r = -0.800$) in addition to detected hormones with clinical severity score. Besides, high fibrinogen levels were detected in of 38 patients.

Conclusion: This is a pilot study revealing endocrinologic background in Facioscapulohumeral Dystrophy. Having restricted number of patients, there is a need for far-reaching studies to verify the effect of hormones in the course of disease. After verification and detailed research, hormonal therapies can be suggested to enlighten Facioscapulohumeral Dystrophy.

Keywords: Facioscapulohumeral Muscular Dystrophy(FSHD); Estradiol; Testosterone; Progesterone; Facial 3D morphology scan

S1.4 High frequency of keratinocyte-related skin diseases in FSHD.

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Introduction: FSHD is caused by derepression of the D4Z4-encoded transcription factor DUX4 in skeletal muscle.

Objective: This study investigates the clinical features that may help to distinguish FSHD1 from FSHD2.

Methods: We examined 57 FSHD1 patients, 20 FSHD2 patients, 7 FSHD1+2 patients and 8 patients with other NMD.

Results: We observed an increase in the frequency of skin diseases including moderate-severe atopic dermatitis, prurigo nodularis, nummular eczema, psoriasis, lichen simplex chronicus, actinic keratosis, all characterized by keratinocyte involvement, a cell type that expresses DUX4. Dermatological diseases were especially found in patients with 7-10 RU. Their presence does not correlate with FSHD disease severity (CSS, age-corrected CSS, Brooke score, Vignos score), disease duration or age at examination. A significant correlation was found with DR1 methylation.

Conclusions: The presence of keratinocyte-related skin diseases is frequent in FSHD patients with >7 RU and correlates with their epigenetic status.

S2.1 Large scale genotype-phenotype correlation study in 1,703 carriers of D4Z4 reduced alleles from the Italian National Register for FSHD.

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Background: The majority (95%) of people affected by facioscapulohumeral muscular dystrophy (FSHD) carry a reduced number of tandemly arrayed repetitive DNA elements, named D4Z4. Clinical reports show high phenotypic heterogeneity in carriers of D4Z4 reduced alleles (DRA) ranging from healthy carriers to typical FSHD to complex muscular phenotypes. 3% of healthy people carry one DRA.

Objectives: To clinically describe subjects carrying one DRA with 1-10 repeats including family members.

Methods: Clinical evaluation of 1703 individuals carrying one contracted alleles with 1-10 D4Z4 repeats from the Italian National Registry for FSHD using the FSHD Comprehensive Clinical Evaluation Form (CCEF) that defines nine clinical categories aimed at capturing clinical diversity. The CCEF classifies: 1) typical FSHD (subcategories A1-3), 2) incomplete phenotypes (subcategories B1, B2), 3) asymptomatic/healthy subjects (subcategories C1,C2), 4) atypical phenotypes (subcategories D1,D2).The CCEF category was assessed in 846 index cases and 857 relatives from 267 unrelated families.

Results: Clinical evaluation shows that 541probands (63.9%) present the classical FSHD phenotype (CCEF categories A1-3), 87 (10.3%) do not display facial weakness (CCEF category B1), and 188 (22.2%) show atypical clinical features (CCEF categories D1,2); 14 (1.6%) present signs such as scapular winging or horizontal clavicles without muscle weakness (CCEF category C1). The large phenotypic variability observed among probands is greater among the 857 relatives from 389 families: 227 (26.5%) were assessed as category A, 71 (8.3%) as subcategory B1, 100 (11.7%) have facial weakness as unique symptom (subcategory B2), 79 (9.2%) show atypical clinical features (subcategories D1, D2); whereas 380 (44.3%) have no muscle weakness (subcategories C1, C2). Subjects were subdivided in[5] groups on the basis of DRA size. We found clinical categories distributed in all groups. Disease penetrance is reduced with 44.3% of healthy relatives. The age at evaluation of healthy relatives is not significantly

different from that of relatives who developed the full disease. The facial-sparing FSHD phenotype (subcategory B1) was observed 10,2% of index cases and 8.3% of relatives. A[5] year follow-up study conducted on 246 subjects revealed that disease trajectory observed in probands is steeper than that observed in relatives. The subcategory B1 phenotype is associated with milder motor disability and slower progression when compared with category A phenotype, regardless the size of the DRA.

Conclusion: These results highlight the importance of building a precise phenotypic classification of probands and families for a correct stratification of patients. Collectively, our analyses demonstrate that clinical variability is a critical element that needs to be considered together with the family history for the creation of suitable clinical practice guidelines, proper genetic counseling and for defining criteria for trial readiness.

S2.2 Exploring the pathogenicity of DUX4 permissive and non-permissive 4qA haplotypes in FSHD.

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Introduction: Somatic derepression of DUX4 on disease-permissive 4qA haplotypes, but not on 4qB or 10q haplotypes causes FSHD. Permissivity for DUX4 expression is mainly based on sequence differences in the DUX4 3'UTR, most notably the presence of a DUX4 polyadenylation signal (PAS). Many 4qA haplotypes have been identified, all of them associated with FSHD, except for the DUX4 PAS-containing 4A166 haplotype as shown in multiple unaffected carriers of FSHD1-sized D4Z4 repeats on a 4A166 allele.

Methods: Analysis of the DUX4 3'UTR sequence and DUX4 transcription in carriers of an FSHD1-sized 4A166 allele.

Results: DUX4 expression is strongly reduced in FSHD1-sized 4A166 muscle cells compared to FSHD1 muscle cells. Haplotype-specific sequence variants other than the DUX4 PAS may affect DUX4 mRNA biogenesis.

Conclusion: Haplotype specific SNPs may contribute to DUX4 mRNA processing and/or stability.

S2.3 SMCHD1 mutation spectrum for facioscapulohumeral muscular dystrophy type 2 (FSHD2) and Bosma arhinia microphthalmia syndrome (BAMS) reveals disease-specific localization of variants in the ATPase domain.

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Background: Variants in SMCHD1 result in quantifiable local DNA hypomethylation and can cause FSHD2 and the unrelated BAMS. In FSHD2, pathogenic variants are found anywhere in SMCHD1 while in BAMS, pathogenic missense variants are restricted to the extended ATPase domain. We compared FSHD2-, BAMS- and non-pathogenic SMCHD1 variants to derive genotype-phenotype relationships.

Objectives: Examination of SMCHD1 variants and D4Z4 methylation in 187 FSHD2- and 41 BAMS families and in the control population (n=58). Analysis of variants in a 3D model of the ATPase domain of SMCHD1.

Results: D4Z4 methylation analysis is essential to establish pathogenicity of SMCHD1 variants. FSHD2 missense variants are significantly enriched in the extended ATPase domain. The position of the (recurrent) variants in the 3D model suggest disease-specific residues for BAMS and FSHD2.

Conclusions: The localization of missense variants in SMCHD1 may contribute to the differences in phenotypic outcome.

S2.4 Trans-generational effects in FSHD: clinical and lab evidence for imprinting, possibly cumulative?

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Background: Understanding epigenetic factors in FSHD will help identify potential therapeutic targets. One possible epigenetic factor could be if D4Z4 might be differentially methylated in egg and sperm, and any difference not fully erased/reset, resulting in sex of transmission imprinting.

Objectives: To analyse clinical severity and methylation data for trans-generational sex influences.

Method: Age-at-onset data, recorded between 1985-90 from personal interview with subjects from >20 3-generation UK FSHD1-families, was reanalysed for the 105 affected adult grandchildren (ages 18-70 yrs) where sex of the transmitting grandparent and parent was known, by grouping into the 8 possible "transmission-sex combinations" (eg. FMM is "female-to-male-to-male"). Comparison of grandchild age-at-onsets between these groups has been made using 2-tailed parametric (t-test) and non-parametric (Mann-Whitney) methods. A subset of 58 of these adult grandchildren, being from the 9 families with 5-6 D4Z4 repeats (22-25 kb on EcoR1-digest), was chosen for more detailed analysis, and for direct comparison with similar analysis on published D4Z4 methylation data in 9 US kindreds, mostly from Utah, who also have 5-6 repeats (Jones et al., 2017 NMD:27,221-8).

Results: 1) There is a statistically significant reducing age-at-onset from grandparent to parent to (proband-excluded) grandchild (37-21-18 yrs), as published previously (Lunt et al 1995 HMG:4,951-8 & erratum 1243-4), but no significant sex difference for age at-onset within any one generation. **2)** For the 105 grandchildren together, only between FFF (n = 22, median 17 y) versus MMF (n = 17, median 21 y) did any difference by transmission sex-combination approach significance (p = 0.056). **3)** In the subset of 58 g-children with 5-6 D4Z4 repeats, the g-child age-at-onset means and medians show significant difference between maternal transmission xFx (x = either sex) (n = 36; mdn. 15y; mn. 19.3 y) vs. paternal xMx (n = 22, mdn. 21 y, mn. 26.4 y) (p = 0.04), and which is enhanced where the affected grandparent is of the same sex; FFx (n = 19, median 18 y, mean 17.7 y) vs. MMx (n = 16; mdn. 22 y; mn. 27.6 y) (p = 0.02). For Fxx (mn. 19.1 y) vs. Mxx (mn. 24.2 y), p = 0.2. **4)** The Utah/US family methylation data shows significant difference in g-child D4Z4 methylation between having an affected grandmother Fxx (n = 22, mn. 17.4%) vs. affected grandfather Mxx (n = 10, mn. 34.3%) (p=0.003), and similarly enhanced for FFx (n = 12, mean 14.9%) vs. MMx (n = 5, mn. 36.6%); but xFx (n = 17, mn. 19.9%) vs. xMx (n = 15, mn. 25.7%) did not reach significance (p = 0.30).

Conclusions: Restricting analysis to families with D4Z4 5-6 Units minimises potential ascertainment bias from the combined factors of male-female severity difference and the average 6 yr increase in median onset-age per extra D4Z4 unit, enabling comparison of clinical and laboratory measures in 2 independent large data sets. The results suggest the level of demethylation of D4Z4, affecting onset age, is subject to trans-generational imprinting in FSHD1, but with an additional cumulative effect across 3-generations. Earlier onset and at least halving of methylation occurs if inherited from g-mother through mother, compared with g-father through father. Confirmation and better understanding of this effect, particularly if 2-generations of male-transmission may be partially protective, could open avenues for therapeutic options. The effect may also have bearing on the level of retrospectively deduced non-penetrance in females at larger D4Z4 fragment sizes.

S3.1 Consequences of DUX4 Expression *in vitro* and *in vivo*.

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Background: DUX4 is normally expressed in cells of the cleavage stage embryo where it activates genes characteristic of the first wave of zygotic gene activation. In addition, DUX4 activates the expression of repetitive elements that have been implicated in both normal developmental processes and in disease pathology.

Objectives: Several mechanisms have been identified for the DUX4-toxicity in somatic cells, including the activation of the double-stranded RNA response pathway, but the mechanisms of activating this pathway in muscle cells remains unknown, as is the differential activation of this pathway between somatic cells and early development.

Results: Our studies have identified components of the toxic pathways in DUX4 expressing cultured cells. We have also analyzed RNA expression from MRI-informed biopsies of FSHD skeletal muscle to determine the molecular correlates of FSHD disease and progression.

Conclusions: These studies are beginning to bridge the *in vitro* studies of DUX4 with the disease progression in FSHD.

S3.2 Single-cell transcriptomes of myogenic cells in facioscapulohumeral muscular dystrophy.

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Objective: We hypothesize that single-cell transcriptome analysis of primary cells from FSHD muscle biopsies and iPSC-derived secondary myoblasts may inform regarding the pathophysiology of muscle damage in FSHD and the identification of FSHD biomarkers.

Methods: We induced a PAX3-driven myogenic differentiation program using iPS cells derived from early-onset or adult-onset FSHD or control individuals and performed multiplexed single-cell RNA-seq.

Results: Muscle progenitor cells (S1 cells) with 2-4 D4Z4 repeats (early-onset phenotype) showed higher expression of DUX4 biomarkers compared to S1 cells harboring 5-8 D4Z4 repeats (adult-onset). Proliferating secondary myoblasts and biopsy-derived cells did not express detectable DUX4 biomarkers. Ongoing experiments include trajectory analysis of the iPSC-derived myogenic program and single-nucleus approaches to analyze the transcriptomes of myotubes and acutely isolated biopsy samples from FSHD and controls.

Conclusions: These results may provide insight into mechanisms contributing to the wide variance in disease severity among early-onset, adult-onset, and non-manifesting FSHD muscles and among corresponding iPSC myogenic lineages.

Support: Muscular Dystrophy Association, University of Massachusetts Wellstone MDCRC

S3.3 Generation of an iPSC model of FSHD and unveiled aspects of DUX4 expression under genotoxic stresses.

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Background: FSHD is a type of genetic/epigenetic skeletal muscle disease which causes progressive "asymmetric" muscle weakness with a wide range of clinical manifestations among patients, indicating involvement of environmental factors.

Objectives: We investigated whether such potential factors have influence on DUX4 expression in patient-derived cells.

Results: First, we established a simple FSHD cell model in which patient-derived iPSCs, including gene-corrected FSHD2 isogenic clones, are differentiated into muscle lineages by MyoD overexpression, recapitulating FSHD-specific DUX4 expression. Using this model, we found that oxidative stress and other genotoxic stresses upregulated DUX4 expression. DUX4 reporter analysis revealed that oxidative stress increased the number of DUX4-positive cells which are rare under normal condition.

Conclusions: Our results suggests that these environmental factors can be risk factors to FSHD muscles through gene expression and may explain unique features of FSHD.

S3.4 In vitro challenging of facioscapulohumeral muscular dystrophy macrophages derived monocytes: the role of trained innate immunity in FSHD.

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Background: FSHD is one of the most prevalent inherited myopathies and it is characterized by a progressive and asymmetric muscular weakness and atrophy. Muscle inflammation may play a role in promoting the dystrophic process. In fact, recent studies describe muscle inflammatory infiltrates mainly composed by macrophages derived monocytes and CD8+ T cells in muscles showing hyperintensity features on T2-weighted short tau inversion recovery magnetic resonance imaging (T2-STIR-MRI) sequences. A further understanding of the inflammatory mechanism involved is crucial, especially as it may open up new possibilities for pharmaceutical interventions, anti-inflammatory drugs being available.

Objectives: this study aimed at understanding if an enhanced (also referred as trained) innate immunity could explained the inflammatory infiltrates associated with FSHD histology.

Methods: to unravel the nature of the inflammatory component associated with FSHD, as a first step, we measured serum circulating inflammatory markers such as IL-6, TNFα, IL-1α, IL-β, MCP-1, and VEGF-A in FSHD and healthy control sera. Subsequently, we challenged in vitro PBMCs and monocytes, freshly isolated from FSHD patients and healthy controls. The cytokine production was measured as well after the in vitro challenge to investigate whether innate immune cells displayed an enhanced programme. Moreover, the phenotype of circulating immune cells was analyzed by flow cytometry.

Results: higher serum circulating levels of IL-6 and TNFα were found in the patient group, while MCP-1 and VEGF-A serum concentrations were significantly lower in the patient group suggesting possible new disease markers. However, the cytokine production from in vitro challenged monocytes and PBMCs did not differ between patients and controls.

Conclusion: Our results may suggest that higher serum levels of circulating inflammatory markers in FSHD could be a consequence of local muscle injury and not of an enhanced functional status of the innate immunity.

S3.5 The interplay between myogenesis and inflammation in FSHD.

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Background: Upon injury, healthy muscle, in cooperation with the immune system, initiates a repair program that involves activation of muscle stem cells that proliferate/differentiate to repair the damage. In FSHD however, the immune response is misregulated, and inflammatory changes in muscle are a prominent histological feature in many patients. Such perturbed immune function may also affect muscle repair.

Objectives: To investigate how the immune system affects myogenesis in FSHD.

Results: RNA-seq analysis on muscle cells from FSHD patients revealed suppression of the transcription factor CCAAT/enhancer-binding protein beta-2 (CEBPβ) in FSHD cells, compared to matched controls. CEBPβ is integral to efficient muscle regeneration and immune cell function, but its role in FSHD pathology is unknown. Increasing CEBPβ expression in FSHD myoblasts either by genetic modification or via administering anti-inflammatory non-steroid drugs, enhanced proliferation and viability in FSHD myoblasts, as well as reducing their susceptibility to oxidative stress. Myogenic differentiation of FSHD myoblasts was also significantly improved with transiently upregulated levels of CEBPβ. Thus enhancing the levels of CEBPβ in FSHD myoblasts can rescue several phenotypes that are associated with FSHD. Cytokines produced by the immune system during muscle regeneration are known to influence muscle stem cell behavior. To begin to establish how the immune system interacts with muscle repair in FSHD, we carried out RNA-seq analysis of lymphoblastoid cells from FSHD patients and matched controls. We found that production of certain cytokines by immune cells from FSHD patients are misregulated. Such perturbed cytokine production could then lead to the observed suppression of CEBPβ levels in muscle cells. This in turn likely affects the regeneration potential of FSHD muscle.

Conclusions: CEBPβ expression is suppressed in FSHD and enhancing CEBPβ levels rescues aspects of myogenesis in FSHD myoblasts. CEBPβ upregulation could contribute to a therapeutic strategy in FSHD.

S3.6 Identification of the hyaluronic acid pathway as a novel therapeutic target for facioscapulohumeral muscular dystrophy.

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DUX4 protein causes a number of cytopathologies that likely give rise to FSHD pathology, but the underlying mechanisms that cause these pathologies remain elusive. To investigate these mechanisms, we screened for DUX4-interacting proteins and identified C1QBP, which regulates several pathways that are disrupted by DUX4. C1QBP is regulated by hyaluronic acid, suggesting that HA-dependent signaling pathways play a role in pathology. We demonstrate that DUX4 causes accumulation of HA, which correlates with delocalization of C1QBP and the mitochondria. Inhibiting HA synthesis with 4MU prevents the appearance of these, as well as other DUX4-induced pathologies, while having only a selective effect on DUX4 transcriptional activity. Additionally, we observe that inhibitors of HA signaling protect from toxicity and cause DUX4 levels to decline via a post-transcriptional mechanism, further identifying HA-signaling pathways as mediators of DUX4 pathology and potential targets for therapeutics.

S3.7 The role of mitochondrial reactive oxygen species in pathology of FSHD myogenesis.

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Background: Oxidative stress is characteristic of FSHD muscles. Nevertheless, the exact role of reactive oxygen species (ROS) in FSHD pathogenesis is still unclear.

Objectives: We treated immortalized human myoblasts MB135 expressing DUX4 that exhibit excessive ROS levels and form atrophic myotubes with various antioxidants and studied the changes in expression pattern and myotube formation.

Results: In contrast to classical antioxidants (Tempol, Trolox), the mitochondria-targeted antioxidant SkQ1 did not suppress myotube formation and had a beneficial effect on myogenesis in MB135-DUX4. We have analyzed the transcription profiles of antioxidant-treated MB135-DUX4 cells and found out that this treatment decreased PITX1 expression.

Conclusions: Mitochondria-derived ROS play a role in pathophysiology of FSHD. This allows to envisage new possible treatment strategies.

S3.8 Regulation of DUX4 by natural microRNAs: from a case study to the identification of the DUX4-targeted miRNome from a library of 1,881 natural human miRNAs.

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Background: Here, we hypothesize that miRNAs expressed in skeletal muscles could target DUX4 mRNA, and therefore, miRNAs could function as modifiers of FSHD severity. Previously, we found that miR-675 could directly inhibit DUX4 expression and counteract DUX4 pathogenicity in skeletal muscle and non-muscle cells (manuscript submitted).

Objectives: We aim to test the role of miR-675 as a modifier of DUX4-induced damage, in a loss-of-function in vivo study, and subsequently identify similar miRNAs using a library screen strategy.

Results: Using a loss-of-function study, we crossed a miR-675 knock-out mouse with our Tamoxifen inducible Cre-DUX4 mouse model (TIC-DUX4), and measured total cage activity and rearing behavior. As a result, we showed earlier weakness in the TIC-DUX4 mice lacking miR-675 when compared to TIC-DUX4 mice. These results point towards a protective function of miR-675 against DUX4-induced muscle weakness, and consolidates our in vitro findings. On the other hand, this proof-of-concept encouraged us to look for other miRNAs similar to miR-675. To do so, we designed a miRNA library screening approach, in which we transduce a pooled lentiviral library containing all 1,881 known human miRNAs into a stable myoblast cell line expressing a mCherry-DUX4 fusion mRNA. We then select, by flow cytometry, cells with reduced mCherry fluorescence, and isolate their gDNA for Next Generation Sequencing (NGS) to identify DUX4-targeted miRNAs. So far, we prepared the library and mCherry-DUX4 stable cell line, and optimized the gDNA extraction method for NGS, and by the meeting, we expect to have identified a set of miRNAs specifically targeting DUX4 mRNA.

Conclusions: The results of the miR-675 loss-of-function study encourage us to look for a correlation between miR-675 expression and severity in FSHD manifestation. In order to do so, we will measure the expression of miR-675 and DUX4 in muscle cell lines taken from FSHD patients showing a wide range of phenotype severity. On the other hand, the identification of additional miRNAs targeting DUX4 mRNA would pave the way for their use in a miRNA-based gene therapy approaches and/or for the identification of small molecule drugs increasing their expression in muscle.

S4.1 Transgenic mice expressing tunable levels of DUX4 develop characteristic facioscapulohumeral muscular dystrophy-like pathophysiology ranging in severity.

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Background: There is large variability in the FSHD patient population; thus, there is a need to control the timing and severity of pathology in FSHD-like models.

Objective: To identify conditions to consistently generate varying levels of FSHD-like pathology using our DUX4 transgenic mice.

Results: The FLExDUX4 mouse crossed with the skeletal muscle-specific and tamoxifen inducible ACTA1-MerCreMer mouse generates a highly versatile bi-transgenic model with chronic, low-level DUX4-fl expression and mild pathology, that can be induced to develop more severe FSHD-like pathology in a dose-dependent response to tamoxifen. Here, we assayed DUX4-fl mRNA and protein levels, fitness, strength, global gene expression, histopathology, and immune response and compare with FSHD.

Conclusions: The ACTA1-MCM;FLExDUX4 bi-transgenic mouse model expresses a chronic low level of DUX4-fl and has mild pathology and detectable muscle weakness. The onset and progression of moderate to severe pathology can be controlled via tamoxifen injection to provide consistent mosaic expression and readily screenable FSHD-like phenotypes for assessing therapies targeting DUX4-fl mRNA and protein.

Supported by the Chris Carrino Foundation, the Muscular Dystrophy Association, the FSH Society, and the National Institutes of Health.

S4.2 Muscle xenografts reproduce key molecular features of FSHD.

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Background: The pathogenic mechanism that leads from DUX4 expression to FSHD is unknown. Transgenic and viral overexpression models do not fully replicate FSHD, and studies of endogenous DUX4 have been limited to biopsies and immature muscle cultures. Other approaches are limited, as they require fresh human tissue.

Objective: Using methods we reported earlier (Sakellariou et al., Skeletal Muscle 6:4, 2016), we have engrafted immortalized human muscle precursor cells from patients with FSHD and their close relatives into immunodeficient mice to generate mature human muscle xenografts, with the goal of using them to study FSHD pathogenesis and to test potential therapeutics.

Results: We report that FSHD and control human myogenic cells develop into fully mature, organized and innervated human muscle tissue with minimal murine myonuclear contamination. They also reconstitute the satellite cell niche within the xenografts. FSHD but not control xenografts express DUX4 and DUX4 downstream targets, retain the 4q35 epigenetic signature of their original donor, and express a novel protein biomarker of FSHD, SLC34A2. Recent evidence shows that an inhibitor of p38 suppresses the DUX4 pathological program in xenografts of FSHD cells, with no apparent toxic effects on grafts of control cells.

Conclusions: Ours is the first scalable, mature in vivo human model of FSHD. It is already proving useful for studies of the pathogenic mechanism of FSHD and for tests of therapies that target DUX4 expression.

Supported by the Friends of FSH Research, the FSH Society, the Chris Carrino Foundation for FSHD, and the NIH.

S4.3 Clinically advanced p38 inhibitors suppress DUX4 expression in cellular and animal models of FSHD.

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Background: Facioscapulohumeral muscular dystrophy (FSHD) is characterized by mis-expression of the DUX4 developmental transcription factor in skeletal muscle where it is responsible for muscle degeneration. Preventing expression of DUX4 mRNA is a disease-modifying therapeutic strategy with the potential to reverse the course of disease. We previously reported that agonists of the beta-2 adrenergic receptor suppress DUX4 expression through an adenylate cyclase/cAMP mechanism. Efforts to further explore this signaling pathway led to the identification of p38 MAP kinase as a major regulator of DUX4.

Objectives: To determine the role of p38 MAP kinases in DUX4 expression and to evaluate the therapeutic potential of p38 inhibition for FSHD.

Results: In vitro experiments demonstrate that clinically advanced p38 inhibitors suppress DUX4 mRNA synthesis in FSHD type 1 (FSHD1) and FSHD2 myoblasts and differentiating myocytes with exquisite potency. Myocyte differentiation was not blocked as evidenced by myotube formation and the expression of differentiation markers. Individual siRNA-mediated knockdown of either p38alpha or p38beta suppresses DUX4 expression, demonstrating that each kinase isoform plays a distinct requisite role in activating DUX4. Finally, systemic administration of p38 inhibitors effectively suppress DUX4 expression in a mouse xenograft model of human FSHD gene regulation at clinically relevant doses.

Conclusions: These data support the repurposing of existing clinical p38 inhibitors as potential therapeutics for FSHD. A surprise finding emerged that p38 alpha and beta isoforms each independently contribute to DUX4 expression. This offers a unique opportunity to explore the utility of p38 isoform-selective inhibitors to balance efficacy and safety in skeletal muscle. We propose p38 inhibition as a disease-modifying therapeutic strategy for FSHD.

S4.4 Examining the aetiology of myopathy mediated by the transcription factor Dux.

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Introduction: While mutations that promote expression of the double homeobox (Dux) transcription factor have been linked to Facioscapulohumeral dystrophy, the cellular mechanisms by which Dux mis-expression promotes myopathy warrant further elucidation.

Objectives: This research sought to examine the acute effects of Dux activation in mammalian skeletal muscle, by developing in vivo models of regulatable Dux expression.

Methods: Wild-type mice administered intramuscular injections of AAV vectors expressing inducible Dux constructs were examined across dose and time conditions for effects on muscle morphology, transcriptome and proteome signatures.

Results: Ectopic Dux expression in mouse muscles produced a progressive degenerative myopathy. Robust changes in muscle transcriptome and proteome signatures were observed prior to the onset of pathology, implicating alterations in a range of important biological processes.

Conclusions: The findings demonstrate methods for examining early cellular responses to Dux in mammalian skeletal muscle, offering prospects to study disease etiology and test prospective therapies.

S4.5 Cellular pathway disruptions in mouse muscle expressing low levels of DUX4 protein.

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Introduction: FSHD is caused by a DNA repeat deletion that leads to expression of the DUX4 gene. Low-level DUX4 mRNA in FSHD biopsies is associated with muscle toxicity.

Objectives: Since recent studies from inducible DUX4 transgenic mice support the importance of DUX4 levels for accurate modeling, we expressed DUX4 in mouse muscle at low-levels for molecular analysis of global gene expression changes in vivo.

Methods: We examined mRNA profile changes following adeno-associated virus (AAV) delivery of DUX4 and the DUX4 promoter to wild type mouse muscle.

Results: Intramuscular injection of AAV-DUX4 produced a dose-dependent toxicity with local histopathological changes. RNAseq revealed involvement of the innate immune system, muscular system, cell cycle, cell death, NFKB cascade, and lipid metabolism.

Conclusions: Our model of DUX4 toxicity produced expression patterns consistent with other models and provides a rapid, inducible platform for studying pathways involved in ongoing damage and testing therapeutic interventions in established disease.

S4.6 Study of regenerative potential of human perivascular cells expressing DUX4.

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Background: In vitro data demonstrate that estrogens improve differentiation of myoblasts deriving from FSHD patients, counteracting differentiation impairment caused by DUX4.

Objectives: We aim to assess in vivo estrogen activity on the regenerative potential of muscle precursor cells (perivascular cells, PVCs) derived from healthy individuals and engineered to express DUX4, or derived from FSHD patients.

Results: Fluorescent-PVCs were implanted into injured hindlimb muscle of NSG mice treated with 17β-estradiol (E 2) or fulvestrant. Animals were monitored by fluorescence emission, by a functional treadmill test, and molecularly by IHC, gene and protein expression. Human PVCs are able to participate in muscle regeneration of injured muscle. Preliminary data show that exogenous DUX4 reduces the performance of implanted PVCs whereas 17β-estradiol is able to recover it.

Conclusions: Implant of human PVCs is a useful model to study muscle regenerative process of FSHD.

S4.7 Retrotransposon-mediated repression of Dux in early mouse development

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Background: De-repression of the DUX4 homeodomain transcription factor drives the muscular dystrophy, FSHD, yet its roles in normal cells have previously remained a mystery. Recently, DUX4 and its murine homolog, Dux, were found to be specifically and transiently activated in early cleavage stage embryos, where they drive a programme closely tied to zygotic genome activation (ZGA). In the mouse, this occurs at the 2-cell embryonic stage and includes Dux-mediated activation of the transposon, MERVL, along with hundreds of MERVL-driven ZGA genes.

Results: The mechanisms that regulate Dux/DUX4 and its targets in development are poorly understood. Strikingly, Dux is rapidly shut down upon 2-cell exit and remains repressed throughout subsequent embryonic development. We discovered that another highly expressed transposon, LINE1, is essential for this process and the specific repression of Dux and MERVL-driven 2-cell genes. Knockdown of LINE1 results in *Dux* and MERVL reactivation, a loss of ESC self-renewal, and in embryos, arrest at the 2-cell stage. LINE1 RNA acts as a nuclear scaffold that binds two other Dux repressors, Nucleolin and Kap1, and is essential for their direct recruitment to *Dux* repeats.

Conclusions: Together these data provide unprecedented insight into Dux regulation in development and highlight the importance of retrotransposons in early embryonic gene control.

S5.1 The crystal structure of SMCHD1's hinge domain and the pathway for SMCHD1 chromatin association reveal a model for how SMCHD1 is targeted to chromatin.

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Background: SMCHD1 is an epigenetic regulator required to silence D4Z4. Previous studies have implicated SMCHD1's hinge domain in chromatin association, but without high resolution structural information, the underlying mechanism has remained unclear.

Objectives: We sought to determine the structure of the SMCHD1 hinge domain, and the mechanism through which SMCHD1 is recruited to chromatin.

Results: Here, we report the first crystal structure of the Smchd1 hinge domain. Using structure-guided mutagenesis, we have defined structural features of the hinge domain that are crucial for nucleic acid binding. In parallel, we have studied chromatin association in the nucleus. Interestingly, SMCHD1 binds nucleic acid without sequence specificity. Instead, SMCHD1's chromatin association and protein stability rely on PRC1-mediated ubiquitination.

Conclusions: Together these studies suggest a model for SMCHD1 targeting, which depends on chromatin state but is enhanced by an affinity for nucleic acids. Moreover, they provide a template for interpreting how patient polymorphisms in the SMCHD1 hinge domain compromise function and lead to FSHD.

S5.2 Methylation of the region distal to the D4Z4 array is lower than predicted in FSHD1.

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Background: Epigenetic changes in FSHD can be investigated using methylation assays specific for permissive chromosomes 4qA (PAS+).

Objectives: Taking advantage of the ability of this assay to directly measure methylation levels of a single allele, we deeply investigated the correlation between methylation and the repeats number. To minimize methylation variability due to sequence variations, we restricted the analysis to a large homogeneous subgroup of individuals carrying a single permissive haplotype 4A161.

Results: Analysis of single CpGs in the region distal to the D4Z4 array showed that methylation levels correlate with the D4Z4 units number. Despite this correlation, the methylations levels detected in FSHD1 and FSHD2 patients are significantly lower than those predicted by control individuals analysis.

Conclusions: Although obvious for SMCHD1 mutation carriers, this observation suggests that, also in FSHD1, methylation levels are affected by factors other than D4Z4 units.

S5.3 Identification of a new epigenetic factor required for the aberrant expression of DUX4 in FSHD muscular dystrophy.

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Background: While there is significant information about the factors needed to maintain the FSHD locus repressed in healthy subjects, little is known regarding the activation of DUX4 in FSHD. We previously showed that the lncRNA DBE-T is required for aberrant DUX4 expression in FSHD.

Objectives: We decided to better characterize the mechanism through which DBE-T activates DUX4 expression.

Results: Using proteomics, we identified the chromatin remodelling protein WDR5 as a novel DBE-T interactor. WDR5 is a core component of the MLL/SET1 epigenetic activation complex. Notably, WDR5 interaction with specific lncRNAs is essential to maintain an active chromatin. We found that WDR5 binds directly and specifically to DBE-T, is recruited to the FSHD locus in a DBE-T dependent manner, and is required for transcription activation by DBE-T. Importantly, WDR5 knockdown or pharmacological inhibition block expression of DUX4 and its target genes in FSHD muscle cells.

Conclusions: We identified a druggable factor required for the expression of DUX4 in FSHD promoting WDR5 as a potential therapeutic target.

S5.4 The D4Z4 macrosatellite sequence as a prototype element for formation of long distance loops: implication in pathologies.

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Topological organization of the human genome follows specific rules and participates in the regulation of genes through long-distance interactions between distant cis regulatory elements and genes. This organization can be altered in diseases, including those linked to mutations in genes involved in this topological organization or upon chromosomal rearrangements. Among them, Facio Scapulo Humeral Dystrophy remains enigmatic in term of pathophysiological mechanisms. In most cases, the disease is linked to shortening of an array of repetitive macrosatellite D4Z4 elements at the 4q35 subtelomeric locus. This element protects against position variegation[1], tethers telomeres at the nuclear periphery 2 and contributes to telomeric position effect over long distances (TPE-OLD)[3], a looping regulatory effect that depends on telomeric chromatin and telomere length. In a subset of patients, the disease is associated with mutation in the SMCHD1 gene that encodes a protein belonging to the cohesin family. This gene mainly characterized for its role in X inactivation and formation of long distance loops at multigenic loci such as Hox genes loci is also involved in de novo methylation of the D4Z4 repeat at the pluripotent stage 4 . By exploring the 3D organization of the 4q35 locus in FSHD cells, we found that D4Z4contractions and/or SMCHD1 mutations impact the spatial organization of the 4q35 region and trigger changes in gene expression. Using induced pluripotent stem cells, we further showed that folding of the 4q35 region is modified upon differentiation. These results highlight the role of the D4Z4 macrosatellite as a prototype sequence involved in the organization of chromatin in human cells and formation of long distance loops.

1. Ottaviani et al. PLOS Genetics, 5(2):e1000394. 2009
2. Ottaviani et al. EMBO. J. 28(16):2428-36
3. Robin et al. Genome Res. 2015. 25(12):1781-90
4. Dion et al. Nucleic Acid Res. 2019

S5.5 Apabetalone, a CVD Phase 3 clinical-stage BET inhibitor, opposes DUX4 expression in primary human FSHD muscle cells.

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Background: Facioscapulohumeral dystrophy (FSHD) pathophysiology is attributable to epigenetic de-repression of double homeobox 4 (DUX4) that is activated in myocytes during differentiation. Bromo- and extraterminal domain protein inhibitors (BETi), including Resverlogix's clinical lead apabetalone, prevent DUX4 activation through an epigenetic mechanism.

Objectives: To quantify global gene expression changes during muscle differentiation and assess BETi effects on disease associated markers and differentiation in order to evaluate BETi's therapeutic potential in FSHD.

Methods: Transcriptome analysis of primary FSHD myoblasts during differentiation, with and without BETi treatment, using RNA sequencing, real-time polymerase chain reaction, and pathway analysis.

Results: Over 10,000 genes exhibited significant changes in expression during differentiation. DUX4 downstream markers were potently downregulated with apabetalone treatment. Differentiation markers were minimally impacted at clinically-relevant concentrations.

Conclusions: BET inhibition is a promising therapeutic opportunity for FSHD with limited risk of off-target effects.

S6.1 Membrane repair deficits in facioscapulohumeral muscular dystrophy.

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Background: We recently identified that repair of injured muscle cells is critically regulated by redox signaling, defect in which is associated with muscular dystrophies.

Objectives: As redox imbalance and myofiber damages are features associated with FSHD, in this study we examined if FSHD muscle cells exhibited poor repair and if this could be a targeted therapeutically.

Results: Healthy and FSHD myoblasts were injured using the laser injury assay. The results showed that FSHD myoblasts exhibited reduced ability and kinetics of cell membrane repair. As therapeutic approaches to address this deficit we examined the efficacy of suppressing DUX4 expression using DUX4-targeting antisense oligonucleotides and of antioxidant treatment on improving the repair ability of the FSHD myoblasts.

Conclusions: Our study is the first to demonstrate membrane repair deficits in FSHD myoblasts and assess the role of DUX4 and oxidative stress in treating the deficits.

S6.2 Muscle ultrasound is a responsive biomarker in FSHD.

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Background: Responsive, relevant, and patient-friendly biomarkers are highly needed.

Objective: Our objective was to assess muscle ultrasound (MUS) as a biomarker in patients with FSHD.

Methods: One-year observational, longitudinal study investigating both quantitative and qualitative MUS.

Results: Twenty-two patients with symptomatic FSHD 1 underwent a clinical examination and MUS at baseline and after one-year follow-up. The qualitative MUS sum score increased from 18.59 to 20.32 (p=0.005), the quantitative MUS sum z-scores increased from 19.96 to 24.72 (p=0.003). The clinical scores did not change over time. Muscle echogenicity correlated with the FSHD evaluation score (R2=0.61, p=0.008).

Conclusions: Muscle ultrasound can detect disease progression in FSHD over 1 year. Both uantitative and qualitative muscle ultrasound correlate with clinical severity in FSHD, and they identify muscle deterioration earlier than clinical assessments. MUS is a responsive biomarker which can be standardized between centres.

S6.3 Long-term follow-up of MRI changes in thigh muscles of patients with facioscapulohumeral dystrophy: a quantitative study.

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Background: Facioscapulohumeral muscular dystrophy (FSHD) has a slow and highly variable disease progression. Quantitative muscle MRI (qMRI) has shown a great potential for the noninvasive assessment of disease progression and the characterization of treatment efficacy.

Objectives: The aim of the present study was to investigate longitudinally the time-dependent changes occurring in a large set of individual muscles in lower limbs muscles of FSHD patients using quantitative MRI and to assess the potential relationships with the clinical findings.

Results: Thirty-five FSHD1 patients were enrolled. Clinical assessment were recorded each year for a period ranging from 1 to 2 years. Clinical scores did not change significantly over time whereas fat infiltration increased significantly.

Conclusions: qMRI could be used as a reliable biomarker and outcome measure of disease progression.

S6.4 PATCHS MRI score correlates with clinical severity in facioscapulohumeral muscular dystrophy.

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Background: Muscle MRI provides an objective measure of disease progression in patients with facioscapulohumeral muscular dystrophy type (FSHD). Quantitative MRI can be a helpful end-point in follow-up and therapeutic trial.

Objective: We aim to identify the muscle MR involvement pattern of both upper and lower parts of body in FSHD patients and its correlation with clinical evaluations.

Results: PATCHS MRI scans consist of 6 muscle groups including scapular girdle, humeral, pelvic girdle, thigh, calf and axial muscles was performed in 30 Chinese FSHD1 patients. The total fat infiltration score correlated highly with the motor function measure, FSHD clinical score, Ricci score, 6-minute walking test and manual muscle testing. Cluster analysis indicated two distinctive patterns of muscle involvement, suggesting a group of untypical FSHD patients with early and more severely involvement of pelvic girdle and leg muscles.

Conclusion: We showed a strong correlation between the fat infiltration score of PATCHS MRI and clinical outcome measures. We also identified a group of untypical FSHD patients.

S7.1 Clinical categories to describe the phenotypic complexity associated with D4Z4 reduced allele.

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Background: Wide phenotypic variability has been observed in D4Z4 reduced allele (DRA) carriers, sometimes with unexpected mode of inheritance. In some cases, the high frequency of FSHD molecular signature might have generated a biased evaluation of families in which a myopathy and a DRA were detected influencing diagnosis and interpretation of clinical and genetic data.

Objectives: Starting from the Italian National Registry for FSHD, in order to capitalize on the rich repository of material from FSHD subjects, we have designed a Comprehensive Clinical Evaluation Form (CCEF).

Results: The CCEF defines phenotypic subgroups by combination of different clinical features. Conclusion: We believe that the precise phenotypic and genetic classification of patients and families will be central to define the natural history of disease, to propose suitable measure of outcome and to identify new susceptibility/causative factors contributing to FSHD.

S7.2 Differentiating phenotypes in carriers of 7-8 D4Z4 reduced alleles: experience of the Italian National Registry for FSHD.

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Background: in FSHD1 alleles with 1-3 D4Z4 repeats are associated with a severe form, 4-8 with the classical form, and 9-10 with a milder disease and reduced penetrance.

Objectives: we investigate phenotypic features of individuals carrying 7-8 repeats.

Results: we examined 187 index cases and 235 relatives with CCEF. Only half of probands display a classical FSHD phenotype, whereas the other half present incomplete or atypical phenotype. The majority of carrier relatives are asymptomatic. The study of 103 families revealed that in one third of families the index case was the only subject presenting a myopatic phenotype.

Conclusions: carriers of 7-8 constitute a genetic subgroup different from classic FSHD with similar characteristics of that including patients carrying D4Z4 borderline alleles. This observation is highly relevant for clinical management because this clinical variability requires additional parameters to be used in clinical practice for diagnosis ad interpretation of the clinical phenotype.

S7.3 Longitudinal MRI evaluation of muscle involvement in FSHD.

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Background: with clinical trials at the horizon in Facioscapulohumeral muscular dystrophy (FSHD), the knowledge of its natural history is of paramount importance to understand the impact of new therapies.

Objectives: to assess disease progression in FSHD using qualitative muscle magnetic resonance imaging (MRI), with a focus on the evolution of hyperintense lesions on short-tau inversion recovery (STIR) sequences.

Results: 100 consecutive FSHD patients underwent lower limb muscle MRI at baseline and after 365±60 days. T1 weighted (T1w) and STIR sequences were used to assess fatty replacement and muscle oedema. 49 patients showed progression on T1w sequences, and 30 patients showed at least one new STIR+ lesion. Increased fat deposition at follow-up was observed in 13.9% STIR+ and in only 0.21% STIR- muscles at baseline ($p<0.001$).

Conclusions: our study confirms that STIR+ lesions represent prognostic biomarkers in FSHD and contributes to delineate its radiological natural history.

S8.1 Report on National FSHD Registries.

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Patient registries containing clinical and genetic information about FSHD patients are essential to achieve a state of 'clinical trial preparedness' for FSHD. They can provide a valuable tool to quickly identify and contact eligible patients to prevent unnecessary delays in the testing of potential therapies as well as provide knowledge on many aspects of FSHD. Currently 13 national FSHD patient registries have been established and efforts have been made to harmonize data collection among these registries. Over the last few years, the field has seen an increase in collaborative research projects based on data from the registries. Despite this increase in usage of registry data, there are still many opportunities to further expand the use of registry data, optimize enrollment of patients into registries and add new ways of data collection.

S9.1 Dose escalation results from a phase 2 study of ACE-08, a local muscle therapeutic, in patients with facioscapulohumeral muscular dystrophy (FSHD).

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12 Brigham and Women's Hospital
13 Children's Hospital London Health Sciences Centre
14 Montreal Neurological Institute
15 University of Minnesota
16 Acceleron Pharma

Background: ACE-083 is a locally-acting muscle therapeutic based on follistatin, a member of the TGF-β superfamily, that binds myostatin and other negative muscle regulators. In a Phase 1 study, ACE-083-treated healthy volunteers had increases in muscle mass that exceeded those reported for systemic muscle agents. FSHD is characterized by progressive weakness in the upper arm and lower leg (foot drop) in the majority of patients.

Objectives: This is a 2-part Phase 2 study in adults with FSHD with mild-to-moderate weakness of ankle dorsiflexion or elbow flexion. Part 1 is open-label, ascending dose and Part 2 is placebo-controlled (ongoing). In Part 1, ACE-083 (150-240 mg/muscle, n = 6 X6 cohorts) was injected into the tibialis anterior or biceps brachii muscle q3 weeks X5 doses, unilaterally or bilaterally. The primary objective of Part 1 was safety and tolerability. Muscle volume and fat fraction were assessed by MRI 2-pt Dixon scan. Motor function tests and patient-reported outcomes (PRO, FSHD Health Index) will be assessed in Part 2.

Results: In Part 1 of the study, the median (range) baseline age was 46 (19-69) yr and duration of symptoms was 25 (4-55) yr. Baseline fat fraction in the treated muscle ranged from 6 to 82%. Significant linear correlations ($p \leq 0.01$) were observed for baseline fat fraction and strength/function measures, as well as for function tests and PRO score. Increases in mean total muscle volume were dose-dependent, with >15% increase observed at doses of 200 to 240 mg/muscle. Mean fat fraction decreased in the tibialis anterior cohorts. Adverse events included injection site reactions and myalgia.

Conclusions: ACE-083 treatment was well-tolerated over a 3-month period in patients with FSHD, and resulted in increased muscle mass and decreased fat fraction. The placebo-controlled Part 2 of this study is ongoing clinicaltrials.gov NCT 02927080).

S9.2 The discovery of a drug target and development candidate that inhibits the expression of DUX4, the root cause of FSHD.

[Abstract title is "Identification of a drug target…." But it's "The Discovery…" everywhere else]

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Background: Facioscapulohumeral Dystrophy, FSHD, is a rare progressive and disabling muscular dystrophy caused by aberrant expression of DUX4 in skeletal muscle which leads to myofiber death and replacement of muscle by fat. No approved therapies exist for FSHD. A therapeutic approach to reducing the expression of DUX4 has the potential to be a treatment for FSHD at its root cause.

Objectives: To identify a druggable target that reduces the expression of DUX4, and subsequently identify a small molecule development candidate suitable for evaluation in clinical trials.

Results: By modeling the disease in vitro using patient-derived myotubes, and subsequently employing our target identification strategies, we identified p38α MAPK as a tractable target, inhibition of which reduces DUX4 mRNA and protein, reduces expression of downstream DUX4 target genes and inhibits apoptosis of FSHD myotubes. Broad RNA-seq profiling indicated a selective effect on the DUX4 program with minimal impact on healthy human muscle cells. Additionally, the effect of p38 inhibitors on DUX4 pathway genes was concentration dependent. Further target validation indicated that p38α inhibition reduces DUX4 expression in both FSHD1 and FSHD2 patient-derived myotubes. P38 inhibitors have been explored extensively in previous clinical trials for other indications. We identified losmapimod as the preferred developmental candidate for FSHD based on substantial and attractive preclinical and clinical data regarding safety, PK and target inhibition.

Conclusions: We have discovered that p38 inhibition reduces the expression of DUX4 in patient-derived myotubes. We have selected p38α/β inhibitor losmapimod for development in FSHD and plan to evaluate its efficacy and safety in clinical trials.

S9.3 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: To refine the product for use in a rodent toxicology and biodistribution study designed to support an eventual IND application.

Results: We compared AAV6 and AAV9 serotypes expressing mi405 from muscle specific (tMCK) or ubiquitous (U6) promoters. We delivered two doses of each vector via tail vein injection to wild type mice. Animals were assessed at 4 weeks for biodistribution and relative mi405 expression. Mice were also evaluated for multiple organ toxicity at 4 week and[5] month necropsies by an independent veterinary pathologist using a blinded design. Comparing all factors (expression, biodistribution, safety), we identified AAV6.U6.mi405 as our lead target product.

Conclusions: Moving forward with AAV6.U6.mi405, we decided the most conservative approach should involve a NHP toxicology study as a next step in the translational path. Because RNAi-based gene therapy is not well-tested in humans, this is now underway.

S9.4 Inhibition of DUX4 expression with antisense gapmers as a therapy for facioscapulohumeral muscular dystrophy.

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Background: FSHD is genetically linked to aberrant expression of DUX4 in muscle. DUX4, in its full-length form, is cytotoxic in non-germline tissues.

Objectives: To develop a treatment for FSHD, we designed locked nucleic acid (LNA)/ 2'-O-methoxyethyl (2'-MOE) gapmer antisense oligonucleotides (AOs), which knockdown DUX4 in immortalized patient myotubes and the mouse model. We also developed a screening method capable of reliably evaluating the knockdown efficiency against endogenous DUX4 mRNA in vitro.

Results: We demonstrate that several designed LNA/2'MOE gapmers selectively and effectively reduced DUX4 expression in vitro. We also found, for the first time, potential functional benefits of AOs on muscle fusion and viability. Finally, we show that one of the LNA gapmers was taken up and induced effective silencing of DUX4 in vivo.

Conclusions: The gapmers and screening protocol developed here will help facilitate the development of FSHD therapies.

S9.5 Targeting the DUX4 transcriptional mechanism of action.

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Facioscapulohumeral muscular dystrophy is caused by mutations that lead to overexpression of the transcription factor, DUX4. Because DUX4 has been shown to interact with and utilize the histone acetyltransferases, EP300 and CBP, we postulated that inhibition of the acetyltransferase activity of EP300/CBP would counter the cytotoxic effects of DUX4. We describe the activity of a new spirocyclic EP300/CBP inhibitor, which we name iP300w and describe effects that this compound has on the cytotoxicity of the DUX4 protein, as well as expres sion of DUX4 target genes, in cells overexpressing DUX4, both engineered cell lines and FSHD myoblasts, as well as in an FSHD animal model. These results point to the central role that EP300 and CBP play in the transcriptional mechanism underlying DUX4 cytotoxicity and the translational potential of blocking this interaction.

S9.6 Discovery of novel small molecule treatment options for FSHD.

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There is wide support for the pathogenesis model in which gain-of-function of the DUX4 gene in skeletal muscle cells underlies FSHD progression. DUX4 is a transcription factor whose expression is normally restricted to early embryonic development. Expression of DUX4 in muscle tissue of FSHD patients initiates a transcription cascade ultimately resulting in overt pathology.

Drug discovery efforts in FSHD have been hampered by the very low and stochastic expression of DUX4 in a small percentage of myonuclei. Because the regulatory pathways involved in the activation of DUX4 are largely unknown, we implemented a phenotypic approach to identify novel small molecules and drug targets to repress DUX4. In order to build a predictive and translatable assay, we used primary patient-derived myocytes to develop and validate a high-content screening assay allowing automated sensitive detection and quantification of endogenous, non-stimulated DUX4 protein and myotube fusion efficiency. Our assay confirmed that the expression of DUX4 is dynamically regulated during myogenic differentiation and showed that effects on fusion are associated with potentially false-positive signals on the DUX4 readout. The excellent assay performance enabled high-throughput screening, resulting in the discovery and optimization of multiple hit series with confirmed activity in secondary assays.

RNA-seq studies with selected compounds revealed normalization of the FSHD transcription signature. We applied a number of chemoproteomic approaches to deconvolute the mode of action of the phenotypic hit series, resulting in the discovery of novel targets to repress DUX4. In order to support the pharmacokinetic/pharmacodynamic strategy, we developed a xenograft animal model in which we could demonstrate in vivo repression of DUX4 after oral dosing of a selected lead compound from one of the series. Together, these results form the basis for building a diverse FSHD-focused product pipeline.

S10.1 Conceptual framework for measurement of treatment effect on DUX4 in losmapimod Phase 2 trials.

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Background: Aberrant and pathologic DUX4 expression drives the rare and progressive muscular dystrophy FSHD, leading to myofiber death and replacement of muscle by fat. DUX4 is a double homeobox transcription factor and its expression in myofibers initiates profound transcription deregulation in FSHD.

Objectives: We have identified a drug target and development candidate that inhibits the expression of DUX4, the root cause of FSHD. Target engagement and inhibition of DUX4 will be measured in muscle biopsies in a clinical evaluation of the p38α/β inhibitor losmapimod.

Results: Because reliable detection of DUX4 protein and mRNA in FSHD subject skeletal muscle biopsies is challenging, this study conducted RNA-seq profiling of FSHD skeletal muscle biopsies to identify a transcriptional signature indicative of DUX4 expression and activity that is feasible for losmapimod clinical trials.

Conclusions: A conceptual framework for selection of a biomarker of DUX4 activity in losmapimod FSHD phase 2 clinical trials will be presented.

S10.2 Elucidating the extent and pattern of longitudinal upper extremity reachability decline in FSHD, using Kinect sensor-based reachable workspace outcome measure.

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Background: The extent and specific pattern of upper extremity reachable workspace decline in FSHD is unknown. Kinect-based reachable workspace outcome measure (RSA) was tracked longitudinally for 5 years in a cohort of 18 FSHD subjects.

Results: Averaged upper extremity (right+left) reachable workspace demonstrated -1.63%/year decline in total RSA ($p=0.144$); with most notable decline in the above-the-shoulder level quadrants' reachability (upper-lateral Q3: -9.5%/year, $p<0.001$ and upper-medial Q1: -6.8%/year, $p=0.063$) with no significant changes in the lower quadrants (Q2,Q4). RSA declined more significantly when loaded with 500g wrist weight: total: -1.82%/year; Q1: -7.20%/year; Q3: -8.09%/year, all $p<0.05$. When dominant and non-dominant sides were analyzed separately, dominant side's reachable workspace declined more significantly (total RSA: -3.24%*/year vs. +0.78%/year; Q1: -7.4%/year vs. -3.61%/year; Q3: -9.09%*/year vs. -4.34%*/year; with *$p<0.05$).

Conclusions: This study elucidates natural history of longitudinal reachable workspace declines found in FSHD, especially involving above-the-shoulder level upper-lateral quadrant, and more significantly affecting the dominant upper extremity.

S10.3 Development of an optimized Timed-Up-and-Go Test for the FSHD population.

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Background: Despite development of many new therapies, the FSHD field is still limited in outcome assessment tools for use in clinical trials. Many of the currently available outcomes were developed long ago and are borrowed; thus not specific to FSHD. FSHD is a slowly progressive disease with unique presentation and distribution, thus necessitating measures tailored specifically for their unique needs.

Objectives: To fill the gap in outcome measures, our group has modified the classic timed-up-and-go (TUG) test and tailored it to the FSHD population.

Results: We utilized a 3-phased iterative approach to modifying the TUG. The finalized measure, the optimized TUG (oTUG), developed by our group included specific patient-identified daily activities that challenged impairments in the upper extremities and trunk/core, in addition to lower extremity impairments. Preliminary results on a small group of individuals show our oTUG is feasible and reproducible in a range of FSHD.

Conclusion: Although preliminary work is promising, larger reliability and correlation studies on the oTUG are required to fully validate this new outcome measure. Additionally, to further advance translatability of the measure and gain additional kinematic data, wireless sensor technology with the oTUG is being considered.

S10.4 The facioscapulohumeral muscular dystrophy specific Rasch-built Overall Disability Scale (FSHD-RODS).

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Background: In FSHD, there is an urgent need for clinimetrically sound patient-reported outcome measures for trials.

Objective: To design a patient-reported Rasch-built interval scale on activity and participation for FSHD.

Methods: A pre-phase scale consisting of 159 items was completed by 498 FSHD patients (Dutch n = 171; UK n = 287; French n = 40). This pre-FSHD-RODS was subjected to Rasch analyses to create an interval measure.

Results: Based on determinants like misfit statistics and -residuals, differential item functioning, and local dependency we removed items from the pre-phase scale until a 47 items FSHD-RODS was constructed achieving Rasch model's expectations. Adequate reliability and (cross cultural and external) validity scores were obtained.

Conclusions: The FSHD-RODS is an interval measure for detecting activity and participation restrictions in patients with FSHD. Its responsiveness is currently being assessed and its cross-cultural validation extended to other countries.

P1 Modelling FSHD by cell transplantation and genetic manipulation in a zebrafish model.

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Background: Facioscapulohumeral Dystrophy (FSHD) is the most prevalent of the primary types of muscular dystrophy, affecting around 1 in 8,333. Mis-expression of the transcription factor DUX4 is the most likely cause of FSHD; however, since the molecular basis for its role in FSHD is still unclear and it is unclear in which cells it induces FSHD, no suitable therapy has yet been developed. A major limitation for understanding DUX4 induced pathologies is the lack of a good animal model to observe how muscle stem cell (muSC) behaviour is affected in vivo and why FSHD muscle is associated with altered behaviour of inflammatory cells.

Objectives: We are generating an animal model for the study of FSHD pathogenesis using the zebrafish, whose
transparency and amenability to drugs has increasingly led to its use as a model organism for the dissecting the importance the role of genes implicated on human diseases, including other muscular dystrophies.

We are developing two zebrafish models of FSHD:

1: A transgenic zebrafish line for drug-inducible DUX4 expression in muscle fibres, muSCs and macrophages. Using live cell imaging, we will then observe the consequences of DUX4 expression on myogenesis and the immune system during muscle repair in vivo.

2: A xenograft protocol to engraft FSHD-derived human myoblasts into transparent zebrafish, which will allow observation of proliferation, migration and differentiation of these cells in vivo. These models will be then used to test the potential of receptor tyrosine kinase (RTK) inhibitors for overcoming DUX4-induced pathophysiology, based on recently published results in mouse.

Results and Conclusions: Preliminary results indicate that over-expression of DUX4 in zebrafish myofibres causes progressive fibre atrophy over a period of days. Immunolabelling of the larvae with antibodies against human DUX4 revealed that expression of DUX4fl in myofibres colocalized with signs of muscle damage, suggesting that expression of human DUX4 in zebrafish muscle leads to muscle deterioration. Experiments are under way to determine whether the muscle wasting arises due to DUX4-induced apoptosis, and to characterize the immune system response to DUX4 overexpression in muscle.

P2 The role of Estrogen Receptor Related beta (ERRβ) in FSHD-1 mechanism.

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Background: We have generated a zebrafish model of FSHD1 by injecting human DUX4-fl (full length) mRNA into fish embryos. By performing an analysis of DUX4 binding sites (CHIP-seq) we have discovered that, at 12h of embryo development, Estrogen Receptor Related Beta (ERRβ) interacts with DUX4. An advantage of our model is that we can monitor DUX4-fl and ERRβ throughout early stages of disease development, which is not feasible in humans.

Results: To characterize this interaction, we modulated ERRβ in a DUX4-fl transgenic zebrafish model and human primary cells and have observed that this leads to DUX4-fl re-localization into cytoplasm at 3-4 dpf. Treatment of transgenic zebrafish with an ERRβ agonist, revealed that swimming performance was impaired in treated fish compared to nontreated animals only at day 4, however at later timepoints their performance improved and the re-localization was no longer observed. We are analyzing the long-term consequences of that treatment as well as the effect of the ERRβ agonist in human myoblasts.

P3 U7-snRNA-Mediated Exon Skipping of the Toxic DUX4 Gene as a Promising Therapeutic Approach for Facioscapulohumeral Muscular Dystrophy (Corrected Title).

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Background: The full-length isoform of DUX4 causes cell death and muscle toxicity, while a short isoform is non-toxic.

Objectives: The goal is to develop a DUX4 exon-skipping strategy designed to bias DUX4 splicing in favor of the non-toxic DUX4-short isoform.

Results: Several U7-snRNAs targeting different parts of DUX4 gene (U7-DUX4) were designed and their ability to suppress full length DUX4 and prevent cell death was investigated. We detected >80% reduction of DUX4 protein in treated cells, and improved their viability. DUX4 mRNA levels and DUX4-responsive biomarkers were markedly decreased in treated FSHD myoblasts/myotubes. As of this writing, in vivo studies are underway, including investigate the capability of AAV-U7-DUX4s to suppress DUX4 long-term in our new TIC-DUX4 mouse model, and improve histopathological, functional, and molecular outcomes.

Conclusions: This study provides proof of concept for silencing DUX4 expression using U7-snRNA mediated exon skipping and has implications for future FSHD gene therapy.

P4 The RNA Binding Protein FRG1 Controls Transcription Landscape Regulating Muscle Maturation and Metabolism.

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Background: Among animal models for FSHD, mice overexpressing FRG1 present progressive myopathy with features of human disease.

Objectives: To investigate FSHD pathogenesis, we analyzed the expression profiles of skeletal muscles of FRG1 mice at 28d (dystrophy onset) and at 96d (full dystrophy).

Results: We found profound transcriptional deregulation with the significant enrichment of genes expressed during embryogenesis and inflammation. Study of FRG1 mice in the postnatal period revealed that FRG1 overexpression induces the impairment of muscle maturation. In this context we observed the deceleration of growth curve and reduction of muscle cross-sectional area associated with lack of adult isoforms of sarcomeric proteins and transcriptional downregulation of glycolytic enzymes. At older age we detected the transcriptional activation of inflammatory pathways. Our findings indicate that delayed maturation and alteration of energy metabolism precedes dystrophy.

Conclusion: Our studies shed new light on molecular mechanisms leading to muscle dystrophy and provide insights for research on effective therapeutics.

P5 Circulating Biomarkers for Facioscapulohumeral Muscular Dystrophy.

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Background: Circulating biomarkers can be used to evaluate disease states and responses to therapeutic interventions. Blood samples from a clinically well characterized cohort of individuals with early-onset FSHD were collected.

Objectives: The goal of this study is to identify protein and miRNA biomarker candidates using omics approaches.

Results: For protein biomarkers, we removed the top 12 most abundant proteins before obtaining the protein profiles using the Q Exactive HF mass spectrometer (22 FSDH and 14 controls). miRNA profiling was conducted using TaqMan Array Human MicroRNA assays (28 FSHD and 16 controls). We identified 28 proteins and 14 miRNAs that were differentially expressed between FSHD and controls; 13 proteins and 3 miRNAs correlated with disease severity. Several identified proteins and miRNAs were reported to be altered in other neuromuscular diseases.

Conclusions: The identified proteins and miRNAs will be further validated and prioritized to be used as biomarkers for therapeutic trials.

P6 Measurement properties of performance-based outcome measures of physical functioning in individuals with facioscapulohumeral dystrophy (FSHD) - A systematic review.

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Aim: This review aims to appraise evidence of measurement properties of performance-based outcome measures of physical function in individuals with FSH.

Method: Using established systematic review protocol, a search of selected electronic health data bases was undertaken. Two authors independently screened studies for eligibility and extracted psychometric data. The methodological quality of studies was appraised using the consensus-based standards for the selection of health measurement instrument (COSMIN).

Results: Of 12 identified outcome measures, four required high technology equipment, three were FSH specific. The FSH clinical score had moderate quality evidence and remaining measures had low to very low quality evidence supporting their measurement properties. Low recruitment in middle-aged, ambulant individuals make results hard to generalise across lifespan and severity.

Conclusion: There is a paucity of valid, accurate and responsive outcome measures in FSH. Further research is required to ensure trial readiness.

P7 Process Abstract: Implementing Kinect sensor-based Reachable Workspace (RWS) measurement system in a multi-site, international FSHD clinical study.

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Background: Kinect sensor-based reachable workspace (RWS) assessment system utilizes motion capture technology to quantitatively track upper extremity skeletal motion, and reconstruct an individual's upper-extremity reachability. The developed RWS outcome measure has shown excellent reliability and validity in FSHD. However, much of the development and testing has been done at one site with small sample size.

Objectives: Test the scalability and implementation of the RWS system in a large, multi-site, international FSHD clinical study (3 E.U. and 8 U.S.-based ReSolve sites; Fulcrum, SRA002-2018).

Results: The total equipment cost for each site was ~$1900 (TV-display, computer, Kinect). Average system assembly time per site is estimated at ~2 hours. Remote web-based evaluator training sessions took ~1 hour. Each evaluator then underwent certification process, with 18 evaluators certified thus far (8 passed on 1st attempt, 6 on 2nd, and 4 on 3rd). Anonymous surveys delivered to each participating site confirmed ease of using the system and setup and high overall satisfaction. Conclusion: Scalability and implementation of the RWS system for large mulit-site studies overall has been successful with reasonable costs, and fairly minimal setup time and training.

P8 Proposal of a neuropsychological protocol to study cognitive functions in FSHD.

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Background: Signs of systemic and cerebral nervous system (CNS)'s abnormalities are occasionally reported in rare paediatric severe form of FSHD; a CNS involvement in the adult-onset classic form of FSHD has not been accurately investigated.

Objectives: Here we present data of e a neuropsychological evaluation protocol aimed to evaluate the cognitive functioning, also in well correlation with the degree of motor impairment and disability, in a cohort of 20 adult patients clinically and genetically characterized. In particular, the protocol includes the evaluation of short- and long-term memory, selective and shift attention and executive functions.

Results: This study shows a possible cognitive impairment in the adult-onset form of FSHD.

Conclusions: In view of these results seems useful encourage the planning of future investigations to better characterized the cognitive profiles of these patients.

P9 Self-report questionnaire vs. clinical evaluation form in the French National Registry on Facioscapulohumeral Dystrophy: a statistical comparison.

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* The list cannot be displayed here due to space constraints but is available online through a QR code on the poster

Background: FSHD, one of the most prevalent neuromuscular dystrophies, has no treatment. The physiopathological mechanism must be further characterized in view of future clinical trials. To this end, national registries on FSHD have been set up. Data are gathered mostly through medical evaluation, which relies on the willing participation of physicians; some databanks are fed with data provided by patients themselves, the reliability of which has never been investigated. To help increase inclusions and data quality, the French registry was designed to combine both a clinical evaluation form (CEF), and a self-report questionnaire (SRQ), thereby allowing for an evaluation of data accuracy and consistency.

Methods: Statistical comparison between CEF/SRQ pairs collected at close time points was made for 281 patients (131 women/150 men; average age 59.5 ± 16.0 years). Kappa or ICC values were calculated to determine the correlation between answers provided in both forms.

Results: Quantitative or objective content such as the Brooke scale show the best agreement (kappa/ICC >= 0.6). Discordance is observed in questions involving symptoms, interpretation or medical technicalities. Errors may originate from either party, but it is safe to assume that patients better answer symptom-related questions, while more technical items are best left to physicians.

Conclusion: Patient answers to questions involving easily understandable objective criteria should be trusted, allowing physicians to focus on items requiring medical expertise. Our results form the basis

for tailoring an optimized collection form, addressing questions to either the patient or the physician. Once online, such questionnaires will facilitate telemedicine care of FSHD.

This work was supported by AFM.

P10 Patterns of muscle involvement, predictive characteristics, and meaningful change for functional motor tasks in FSHD.

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Background: The possibility of molecularly targeted therapies for facioscapulohumeral muscular dystrophy (FSHD) create a pressing need to develop functional motor outcomes (FMOs) for clinical trials. The ability to understand muscle input on performance and predictive baseline characteristics can help inform trial design.

Methods: We combined data from 2 studies: one from the Universities of Kansas and Utah (n=44) and the other from the University of Rochester (n=31) to correlate manual muscle testing from 34 upper and lower extremity muscles to performance on FMOs: 6 minute walk test, timed up and go, and 30 foot go. Predictive models were constructed using baseline characteristics. Meaningful changes were approximated using linear regression on patient-reported global impact on daily activities.

Results: Knee flexion showed the strongest correlations to FMOs (r=|0.58-0.72|). Age, gender, baseline clinical severity and knee flexion accounted for half the variability in performance. Increases in FMOs between 16-26% corresponded with one degree more patient-reported impact on daily activity.

Conclusions: Knee flexion strength was the strongest driver of gait FMO performance in FSHD; with changes of 16-26% corresponding to increased impact on daily activity.

Study funding: grant support from the FSH Society; and a CTSA Multi-Institute Pilot grant.

P11 Biomarker identification by high-resolution proteomic approach in FSHD.

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Background: the identification of tissue and circulating biomarkers is of major importance for FSHD.

Objectives: in this exploratory study, we combined muscle imaging, microdialysis and proteomics of muscle interstitial fluids and patients' sera. We had collected microdialysates (i.e., interstitial fluids obtained through a microcatheter) from muscles with different features on MRI (STIR+, STIR- and controls). Microdialysates were analyzed using a high-resolution mass spectrometer, to detect proteins dysregulated, and possibly secreted in the bloodstream, in the early phases of the disease

Results: a total of 1290 proteins were identified, 132 of which only found in STIR+ muscles. The majority belonged to the nucleic acid-binding, immune response, enzyme modulator and protein signaling classes. Forty-six were dysregulated in STIR+ compared with STIR- muscles and 79 compared with controls. Ingenuity Pathway Analysis confirmed that these proteins are mostly involved in inflammatory response. Serum analysis is currently ongoing and the results will be presented during the congress.

Conclusions: High-resolution proteomics is a feasible method to analyze muscle microdialysates. The modulation of pathways related to inflammation further supports the role of this process in active disease phases.

P12 Effect of creatine monohydrate on functional muscle strength and muscle mass in children with FSHD: a multi-centre, randomised, double-blind placebo-controlled crossover trial.

Dr Ian Woodcock[1,2,3], Ms Katy De Valle[1,2,3], Dr Eppie Yiu[1,2,3], Dr Kate Carroll[1,2,3], Dr Rachel Kennedy[1,2,3], Monique Ryan[1,2,3] **[Abstract name on spreadsheet and on program is Creatine in paediatric FSHD: an RCT.**

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Creatine monohydrate is a naturally occurring compound involved in energy production in cells, particularly in tissues with high energy requirements such as skeletal muscle, which has been shown to increase strength and function in Duchenne muscular dystrophy. Creatine has not yet been studied in paediatric FSHD, although a previous study in adults showed a trend towards benefit. This multi-centre, randomised, double-blind, placebo-controlled crossover trial will compare changes in strength-related motor function following treatment with creatine monohydrate to treatment with placebo, as measured by the Motor Function Measure, from baseline to 12 weeks. Eligible subjects will be randomised to either creatine monohydrate therapy or placebo for three months, then crossover to a further three months of therapy with either placebo or creatine. Subjects will undergo clinical assessments and study safety assessments at the beginning and end of each treatment period. Recruitment is on-going with plan to complete end of 2019.

P13 Phenotype may predict the clinical severity of facioscapulohumeral muscular dystrophy.

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Background: The Comprehensive Clinical Evaluation Form (CCEF) was reported to facilitate the harmonized phenotypic classification of facioscapulohumeral muscular dystrophy (FSHD) patients and families.

Objectives: We aimed to explore the clinical severity in FSHD patients classified by CCEF. 54 Chinese FSHD1 patients were included and evaluated by structured questionnaire, manual muscle test (MMT), FSHD clinical score (CS), Ricci score (CSS), 6-minute walking test (6MWT) and motor function measure (MFM).

Results: There were 26 males and 28 females, with the D4Z4 repeat number of 4.54 on average. 16 patients belong to category A1, 22 to A2, 4 to A3, 1 to B1 and B2 respectively, and 10 to D1. The A1 patients was younger, with an earlier onset, shorter disease duration, smaller D4Z4 repeat number and more severe clinical disability in CS, MMT and MFM. 6 D1 patients displayed pes cavus.

Conclusions: The patients with severer facial involvement may have aggressive disease progression and worse motor functions. Facial impairment could be a predict factor to clinical severity.

P14 An in situ hybridization-based method for detecting DUX4 RNA expression in vitro.

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Background: Detecting endogenous *DUX4* in patient tissue using conventional methods can be challenging. Developing simple and trustworthy *DUX4* detection methods is an important need in the FSHD field.

Objectives: Our objective is to develop a new strategy to detect endogenous *DUX4* mRNA.

Results: Here, we describe a novel and efficient technique for detecting over-expressed and endogenous *DUX4* mRNA in human cells. which based on a RNA *in situ* hybridization (ISH) technology. We show that a custom-designed RNAscope assay can detect *DUX4* mRNA in transfected HEK293 cells also, it was highly sensitive for detecting lower amounts of endogenous *DUX4* mRNA in FSHD myotubes. Moreover, the RNAscope assay was able to track reductions in *DUX4* mRNA following treatment with our RNAi therapy.

Conclusions: We found that RNAscope was a highly sensitive method for detecting *DUX4* mRNA *in vitro*, and may enable us to develop a new, rapid RNA ISH-based molecular diagnostic assay for FSHD.

P15 Characterization of human perivascular cells as a new cellular model of facioscapulohumeral muscular dystrophy.

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Background: Regeneration process of adult skeletal muscle is mediated mainly by satellite cell activation. However, others cell types as perivascular cells (PVCs, also pericytes, or mural cells) can participate this process. Given their abundance and proliferative potential, PVCs may represent a useful model for studying in vivo FSHD and a promising therapeutic candidate.

Objectives: We aim at characterizing PVCs from FSHD and healthy individuals at molecular and functional levels.

Results and Conclusions: We Isolated and characterized PVCs from muscle and fat tissues from healthy and FSHD patients and analysed their properties compared to those of myoblasts from the same individual. FSHD-PVCs recapitulate properties of FSHD myoblasts, thus indicating they can represent a good model for investigating in vitro and in vivo FSHD properties. Moreover, FSHD-PVCs can differentiate in myotube, thus suggesting the usefulness to evaluate their therapeutic potential.

P16 Investigation of the Effect of Estrogen on DUX4/β-Katenin/PAX3-7 Protein Levels in Facioscapulohumeral Muscular Dystrophy (FSHD).

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Objective: In Facioscapulohumeral Dystrophy(FSHD) It is noteworthy that the findings progressed at an earlier age in men, and the disease worsens in postmenopausal women. Depending on these observations; the role of estrogen was investigated in the pathophysiology of FSHD.

Method: Primary FSHD1 myoblast cell lines prepared from biopsies of four individuals(63 and 71 years old(63yM/71yM)two males; 47 and 58 years old(47yF/58yF) two females) were used. Three different groups i) estradiol untreated control group, ii) 10 nM 30-minutes and iii) 10 nM 4-hours estradiol treatment were generated. Cell lysates from FSHD myoblasts in these groups were examined by western blot for the presence-amount of DUX4, PAX3/7 and β-catenin transcription factors.

Results: After estradiol treatment DUX4 protein level reduced to zero in 71yM, it wasn't detected in 63yM and 47yF. Because of ineffective attachment in 58yF, proteins weren't obtained. The level of β-catenin protein increased with estradiol in 71yM, 63yM and 47yF samples. 3 different protein bands of 80 kDa, 56 kDa and 45 kDa were determined for PAX3/7 proteins. Of these, 80 kDa and 56 kDa forms were observed only in 71yM; after estradiol 80-kDa PAX3/7 form reduced; 56-kDa PAX3/7 form was expressed at the 4th hour. The 45 kDa form was determined in all samples; with estradiol treatment this form decreased in 71yM-47yF and increased in 63yM.

Conclusion: The decrease in DUX4, the increase in β-catenin, stimulation in the expression of 56 kDa form of PAX3/7 protein family after 4 hours of estradiol treatment support the protective role of estrogen in FSHD pathophysiology. To understand the effect of estradiol on pathophysiology better and to develop treatment alternatives; extensive studies are needed with each transcription factor and related target genes; both at mRNA-protein levels.

Keywords: estradiol, DUX4, β-catenin, PAX3/7, FSHD (facioscapulohumeral muscular dystrophy)

P17 Single-cell transcriptomics reveals DUX4 expression during early stages of myogenesis in FSHD1.

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Background: It is not yet known precisely how the transcriptional de-repression of DUX4 leads to disease pathology in FSHD, a situation complicated by its expression in only a small fraction of muscle cells. To better understand the timing of DUX4 expression and its impact on the transcriptome, we performed single-cell RNA sequencing (scRNA-seq) on myogenic cell-cultures from FSHD1 and control individuals (n=4 each).

Results: Primary myoblasts were cultured in differentiation medium for 3.5 days, at low confluency. The inDrops platform was used to acquire scRNA-seq data for ~3000 cells per library, with 2-4 libraries per individual. DUX4 transcripts were detected in a small set of FSHD cells, and known biomarkers of DUX4 activity (e.g. ZSCAN4, TRIM43, LEUTX) in a larger set of FSHD cells. These findings were broadly consistent with the recent scRNA-seq study by van den Heuval et al. (Hum Mol Genet, 2019), although DUX4 biomarker levels were lower overall in our study. As our study also had lower levels of MYH3, a marker of late-stage myocytes – likely due to the mononuclear cells having low confluency, with little cell-to-cell contact – it provides a complementary view of DUX4 expression at early stages of myogenesis.

P18 Exploring the relationship between DUX4 and hypoxia-inducible Factor (HIF1α).

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Background: Examining FSHD skeletal muscle molecular networks reveals pathways involved in hypoxic response and oxidative stress to be critically disturbed, with HIF1α being of particular interest (Banerji et al., 2015, 10.1098/rsif.2014.0797).

Objectives: Our goal is to investigate potential relationships between DUX4 and HIF1α and its contribution to muscle dysfunction.

Results: Human DUX4 inducible myoblasts were cultured under normoxia or hypoxia. mmunofluorescence studies showed that hypoxia increases HIF1α protein levels, with a concomitant increase in proliferation rate. Hypoxia however, reduced myogenic differentiation into multinucleated myotubes. DUX4 induction reduces differentiation, and preliminary data indicates that HIF1α levels are altered upon DUX4 expression, depending on the differentiation state. 52% of induced myoblast nuclei with DUX4 also contained HIF1α protein. In vivo studies are ongoing, and include a murine model based on an intramuscular injection of a DUX4 expression vector followed by electroporation.

Conclusion: FSHD is linked to a greater sensitivity of muscle cells to oxidative stress. Using transcriptomic studies, we have found that HIF1α signalling is deregulated in FSHD. Expression of DUX4 in human myoblasts associates with HIF1α signalling, and we are investigating this association with the DUX4-induced phenotype.

P19 DUX4 is a co-repressor of the progesterone and glucocorticoid nuclear hormone receptors.

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Background: DUX4 is a nuclear transcription factor that regulates the expression of zygote activated genes in placental mammals. Our laboratory originally showed that DUX4 is a cytotoxic, pro-apoptotic protein. We proposed that DUX4 may contribute to the pathogenic pathway in FSHD. Aberrant expression of DUX4 is now considered a key element in FSHD pathogenesis.

Objectives: To study the potential co-repressor activity of DUX4 on the progesterone and glucocorticoid nuclear receptors (NRs).

Results: Using alternative reporter systems and a reconstituted experimental model in HepG2 cells, which lack endogenously-expressed progesterone NR, we demonstrate that DUX4 has a strong co-repressor activity on the progesterone NR. Additional studies, using a HepG2 reconstituted cellular model, showed that DUX4 is also a co-repressor of the glucocorticoid NR.

Conclusions: Taken together, our results provide evidence indicating that DUX4, a transcriptional activator, could indirectly modulate gene expression via co-repression of hormone NRs, a previously unrecognized endocrine role for DUX4.

P20 Deciphering the mechanism of herpesviral DUX4 induction.

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Background: DUX4 is a transcription factor exclusively expressed during embryonal development and in human testis. Aberrant expression of DUX4 is the cause of FSHD. In a recent study we could show that DUX4 is activated upon herpesviral infection.

Objectives: Accordingly, this project centers on deciphering the role of DUX4 during herpesviral infection.

Results: Using standard molecular biology methods together with modern technologies like RNA-Seq and Chromatin-IP our experiments confirmed the expression of DUX4 upon infection with members of all herpesvirus subfamilies. This leads to the induction of hundreds of DUX4 target genes, such as members of the TRIM, PRAMEF and ZSCAN protein families. We found that DUX4 is still upregulated when using an inhibitor for the viral polymerase, providing evidence that the incoming viral particle can elicit its expression.

Conclusion: Collectively, our results give new insights into the endogenous functions of DUX4 and point at a possible link between herpesviral infection and FSHD.

P21 Genetic and epigenetic analysis of the FSHD-linked 4q35 region in rare female “Coats' disease” patients.

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Background: Sporadic Coats' disease manifests as retinal telangiectasis leading to exudates, detachment and blindness, and mostly affects young boys. However, male and female FSHD patients, especially those with large D4Z4 contractions, are at risk of developing symptomatic “Coats' disease.” FSHD patients sometimes present first to ophthalmologists.

Objective: We analyzed DNA methylation and genomic DNA sequence of the distal-most 4q35 D4Z4 repeat through the polyadenylation site in exon 3 for three females with “Coats'” telangiectasis but no muscle disorder.

Results: “Coats' telangiectasis," can exhibit some FSHD-like D4Z4 genetic and epigenetic characteristics. One patient with severe Coats exudates had D4Z4 deletion (25 kb fragment), hypomethylation, and an unusual 4qA haplotype simulating 4qA161. No patient met the combined genetic and epigenetic requirements of FSHD.

Conclusions: These preliminary data support a relationship between changes in the 4q35 D4Z4 array and Coats' disease in some females. However, overall FSHD-permissive conditions may not be essential.

P22 SMCHD1 plays a pleiotropic role in euchromatin or heterochromatin regulation with consequences in rare diseases.

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SMCHD1 has been of major interest following identification of germline mutations in Facio-Scapulo-Humeral Dystrophy (FSHD) and in an unrelated developmental disorder, Bosma Arhinia Microphthalmia Syndrome (BAMS). By investigating why SMCHD1 mutations lead to these two different rare genetic diseases, we uncovered a role for this factor in de novo methylation at the pluripotent stage using a collection of fibroblasts reprogrammed into hiPSc. SMCHD1 binds to the D4Z4 macrosatellite element and is required for its dynamic methylation upon reprogramming but dispensable for methylation maintenance. We found that FSHD and BAMS patient's cells carrying SMCHD1 mutations are both permissive for DUX4 expression. By analyzing gene expression profiles in FSHD and BAMS patients' cells, we uncovered a role for SMCHD1 in the regulation of genes involved in skeletal muscle differentiation and patterning during development uncovering a pleiotropic role of SMCHD1 in chromatin regulation.

P23 NO66 acts together with SMCHD1 as a co-repressor of DUX4 in facioscapulohumeral muscular dystrophy (FSHD).

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Background: The D4Z4 chromatin modifier SMCHD1 functions as a repressor of the DUX4 locus. Loss of SMCHD1 from D4Z4-repeats leads to DUX4 expression in a permissive 4qA genetic background. We identified nucleolar protein NO66, a demethylase of active chromatin marks H3K4me3 and H3K36me3, as an interactor of SMCHD1.

Objectives: To study whether NO66 plays a role in D4Z4 repression. We did this by depletion of NO66 in primary myocytes derived from healthy controls, FSHD1 and FSHD2 patients, followed by DUX4 expression studies and analysis of SMCHD1 binding to D4Z4.

Results: NO66 depletion resulted in upregulation of DUX4 and DUX4 targets in FSHD1 and 2 myocytes, but failed to derepress DUX4 in controls. Healthy myocytes counteracted NO66 loss by recruiting more SMCHD1 to D4Z4, contrary to FSHD myocytes.

Conclusions: Our study identifies NO66 as a corepressor of D4Z4 together with SMCHD1 and gives us new insights into the epigenetic regulation of D4Z4.

P24 An Innate Immunity Model of FSHD Muscle Pathology

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Facioscapulohumeral muscular dystrophy (FSHD) is the third most frequently diagnosed type of muscular dystrophy which presents with asymmetric progressive muscle weakening of muscles in the face, upper body and shoulder girdle, progressing to loss of ambulation and profound physical disabilities. Misexpression of the transcription factor DUX4 is genetically associated with FSHD muscle pathology, but has highly variable clinical severity and muscle specificity independent of DUX4 misexpression, consistent with findings that implicate innate immunity as a central cause of FSHD muscle pathology. We have developed an FSHD muscle/blood double xenograft model in an immune deficient mouse to investigate the role of innate immunity in FSHD muscle pathology. This mouse model is genetically optimized for engraftment and expansion of innate immune cells from human cord blood. Our immunohistology and gene expression studies in this model show that human CD45+ cells that mediate innate immunity preferentially home to and destroy FSHD xenograft muscle relative to control unaffected muscle, supporting the hypothesis that DUX4 misexpression in FSHD muscle initiates a pathological innate immune response. Our goal is to utilize this FSHD muscle/blood xenograft model to investigate the mechanisms underlying FSHD innate immunity and to develop FSHD therapeutics that block FSHD innate immunity muscle pathology.

Supported by Friends of FSHD Research, NINDS and NICHD UMMS Wellstone MDCRC

P25 An innate immunity model of FSHD muscle pathology.

Katelyn Daman[1], Jing Yan[1], Sonal Jangalwe[2], Jennifer Chen[1], James Windelborn[3], Oliver King[1], Kathryn Wagner[4], Michael Brehm[2], Charles P. Emerson Jr[1]

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Facioscapulohumeral muscular dystrophy (FSHD) is the third most frequently diagnosed type of muscular dystrophy which presents with asymmetric progressive muscle weakening of muscles in the face, upper body and shoulder girdle, progressing to loss of ambulation and profound physical disabilities. Misexpression of the transcription factor DUX4 is genetically associated with FSHD muscle pathology, but has highly variable clinical severity and muscle specificity independent of DUX4 misexpression, consistent with findings that implicate innate immunity as a central cause of FSHD muscle pathology. We have developed an FSHD muscle/blood double xenograft model in an immune deficient mouse to investigate the role of innate immunity in FSHD muscle pathology. This mouse model is genetically optimized for engraftment and expansion of innate immune cells from human cord blood. Our immunohistology and gene expression studies in this model show that human CD45+ cells that mediate innate immunity preferentially home to and destroy FSHD xenograft muscle relative to control unaffected muscle, supporting the hypothesis that DUX4 misexpression in FSHD muscle initiates a pathological innate immune response. Our goal is to utilize this FSHD muscle/blood xenograft model toinvestigate the mechanisms underlying FSHD innate immunity and to develop FSHD therapeutics that block FSHD innate immunity muscle pathology.

Supported by Friends of FSHD Research, NINDS and NICHD UMMS Wellstone MDCRC

P26 Elucidating the Role of Metabolic Stress and Mitochondrial Dysfunction in FSHD.

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A poor response to oxidative stress and mitochondrial dysfunction contributes to FSHD pathology. FSHD myoblasts are more vulnerable to oxidative challenge, resulting in oxidative damage-induced apoptosis and muscle fiber atrophy. The oxidative stress response is tightly linked to cell metabolism and thus, to mitochondrial function.

We have performed a dynamic transcriptomic analysis in patient-derived isogenic FSHD myogenic cell lines. Multivariate regression revealed perturbation of pathways that control mitochondrial biogenesis, in particular of the PGC1α-ERRα-NRF1/2 signaling axis (Banerji et al., 2018, doi:10.1093/hmg/ddy405). Indeed, patient-derived FSHD myogenic cells have lower mitochondrial content than controls, and differentiate into hypotrophic myotubes. Notably, treatment with ERRα agonists rescues this phenotype in vitro. Furthermore, DUX4 expression in human myoblasts drastically reduces the capacity of the mitochondrial electron transport chain, leading to a deficiency in oxidative phosphorylation. Therefore, a thorough characterisation of mitochondrial (dys)function in FSHD may reveal novel therapeutic entry points.

P27 Intramuscular pattern of fat infiltration measured by MRI to identify disease initiation in FSHD.

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Introduction: Discovering the location of DUX4 initiation and the subsequent muscular fat infiltration could guide therapy development in FSHD.

Objective: To study the intramuscular fat infiltration pattern in 396 lower extremity muscles of 9 FSHD patients from tendon to tendon using quantitative MRI and semi-automatic muscle segmentation.

Methods: Per muscle, on average 44 slices (thickness =[5] mm) were semi-automatically segmented on 2pt-Dixon fat fraction (FF) maps to determine the FF per slice. Subsequently, each muscle was divided in five proximo-distal segments.

Results: FF depended on the segments position along the muscle ($p < 0.001$), being highest distally. The muscles exhibited a fat infiltration front, being most evident in intermediately fat infiltrated muscles (10%<FF<50%). The position of the fat infiltration front was more proximally when the muscle's average FF was higher.

Conclusion: Muscles in FSHD patients exhibit a fat infiltration front that appears to start distally, indicating that DUX4 expression starts here as well.

P28 The French National Registry of patients with Facioscapulohumeral muscular dystrophy.

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Background: Facioscapulohumeral muscular dystrophy is a rare inherited neuromuscular disease with an estimated prevalence of 1/20,000 and France therefore harbors about 3,000 FSHD patients.

Objectives: With research progresses and the development of targeted therapies, patients' identification through registries can facilitate the description of disease natural history and improve recruitment in clinical trials and studies. The French National Registry of FSHD patients was designed as a mixed model registry to collect molecular and clinical data. It involves both patients and physicians, through self-report and clinical evaluation questionnaires respectively. As for all rare diseases, because of the limited number of patients, data quality is a major goal of the registry and various automatic data control features have been implemented in the bioinformatics system. In addition, data are manually validated by molecular and clinical curators.

Results: Since its creation in 2013, data from 752 FSHD patients have been collected, representing about 25% of the French FSHD population. The mixed model strategy allowed to collect 62.4% of data from both patients and clinicians; 23.1% from patients only and 14.5% from clinicians. With the identification of the FSHD2 form, specific questionnaires have been designed. Though FSHD2 patients are progressively included, FSHD1 patients still account for the majority (94.5%). The registry is compatible with the FAIR principles as data are Findable, Accessible and Interoperable. To do so, we used the molecular international nomenclature and standardized clinical terms used by the FILNEMUS French network of reference centers for the diagnosis and follow-up of patients suffering from a rare neuromuscular disease. The implemented clinical terms were shown to mostly map to dictionaries and terminology systems such as SNOMED-CT (75% of terms), CTV3 (61.7%) and NCIt (53.3%). Because of the sensitive nature of data, they are Reusable as aggregated data after evaluation and approval by the registry oversight committee.

Conclusions: The French National Registry of FSHD patients belongs to a national effort to develop databases, which should now interact with other initiatives to build a European and/or an international FSHD virtual registry for the benefits of patients. It is accessible at https://fshd.fr and various useful information, links, and documents, including videos, are available for patients and professionals.

P29 An Update on the UK FSHD Patient Registry in 2019 and Future Considerations.

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Background: The UK Facioscapulohumeral Muscular Dystrophy (FSHD) Patient Registry is a patient driven, clinician verified tool designed to support clinical research. The Registry is used to capture longitudinal, self-reported data through an online portal available to patients and clinicians.

Objectives: The registry aims to facilitate and accelerate clinical research and act as the most comprehensive distributor of information relating to upcoming academic and non-clinical studies in FSHD.

Results: Between May 2012 and May 2019, 912 patients participated in the Registry, of which 88% had a patient-reported diagnosis of FSHD or FSHD type 1 (FSHD1), 3% with FSHD type 2 (FSHD2) and 9% with an unspecified or unconfirmed diagnosis. Genetic confirmation of FSHD1 was provided in 51% of patients who had a patient-reported diagnosis of FSHD or FSHD1, and genetic confirmation of FSHD2 was provided in 59% of patients who had a patient-reported diagnosis of FSHD2.

Conclusions: A wide range of patients have participated in the Registry helping provide new insights into clinical research and standards of care. By sharing a common dataset with an increasing number of FSHD registries, the registry will be positioned to continue to contribute to future clinical research.

P30 Safety and tolerability of losmapimod, a selective p38alpha/beta MAPK inhibitor, for treatment of FSHD at its root cause.

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Background: Fulcrum Therapeutics is developing losmapimod, a selective p38α/β MAPK inhibitor that selectively reduces double homeobox 4 (DUX4) protein expression in FSHD myotubes, for treatment of FSHD at its root cause.

Objectives: To report the known safety and tolerability of losmapimod.

Results: There has been extensive clinical testing of losmapimod for its anti-inflammatory potential in over 3,500 healthy subjects and patients across at least 10 different adult indications (including COPD, rheumatoid arthritis, neuropathic pain, and atherosclerosis) but not FSHD. Although losmapimod was not effective in any indication previously tested and never filed for approval, it demonstrated favorable pharmacokinetic, pharmacodynamic, safety, and tolerability profile.

Conclusions: Losmapimod was safe and tolerable in all previous indications including for chronic oral administration.

P31 Antisense therapy for facioscapulohumeral muscular dystrophy.

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Background: Antisense oligonucleotides (AONs) therapy has shown promise for treating an array of disorders and can be used to reduce DUX4 in FSHD.

Objectives: The goal of this study is to develop antisense strategies to reduce the pathogenic DUX4 mRNA in affected muscles.

Results: We examined three different types of AONs, including third-generation antisense (3GA), LNA gapmers and 2'MOE gapmers. The AONs were delivered by either intramuscular injections (i.m.) or subcutaneous injections (s.c.) to the DUX4-expressing FLExDUX4 FSHD-like mouse model. Our results showed that the AONs were present in skeletal muscles 24 hours after either one i.m. or one s.c. injection. The AONs delivered by either routes effectively reduced the DUX4. In addition to DUX4 reduction, mice received longer treatments showed pathological improvement and muscle functional improvement measured by grip strength testing.

Conclusions: The data support the use of AONs, delivered either locally or systemically, as therapeutic approaches for FSHD.

P32 Phase 1 Clinical Trial of Losmapimod in FSHD.

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Background: Fulcrum Therapeutics is developing losmapimod to treat FSHD at its root cause by inhibiting DUX4 expression. Fulcrum developed the losmapimod capsule for initial testing in FSHD; the losmapimod tablet formulation was previously tested, but not in FSHD.

Objectives: The objective of this study is to investigate the initial safety, tolerability, pharmacokinetic (PK) profile, and target engagement (TE) in healthy volunteers (HV) and FSHD patients as well as drug concentrations in muscle of FSHD patients.

Results: In part A 10 HV were randomized to losmapimod 7.5 mg in the first period (n=8) and 15 mg in the second period or to single oral dose placebo in both dosing periods (n=2). Part B is a parallel study design randomizing 15 FSHD patients to placebo (n=3), losmapimod 7.5 mg (n=6) or losmapimod 15 mg (n=6) po BID for 14 days.

Conclusions: In Part A of the study, the losmapimod capsule showed similar safety and PK profile to published data of the losmapimod tablet. Part B of the study is ongoing.

P33 Losmapimod reduces DUX4 expression across FSHD patient-derived myotube cells.

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Background: FSHD is caused by the loss of repression at the D4Z4 locus leading to DUX4 expression in skeletal muscle, activation of its early embryo transcriptional program and muscle fiber death. While some progress toward understanding the signals driving DUX4 expression has been made, the factors and pathways involved in the transcriptional activation of this gene remain largely unknown.

Objectives: To characterize the in vitro efficacy of losmapimod in reducing DUX4 and its downstream consequences across genotypes in FSHD.

Results: Using optimized myotube culture conditions, we investigated the preclinical efficacy of losmapimod in FSHD1 and FSHD2. Results showed a robust reduction of DUX4 expression, activity and cell death with losmapimod in both FSHD1 and FSHD2 patient-derived myotubes. RNA-seq studies revealed that only a small number of genes were differentially expressed after treatment, ~75% targets of DUX4 without impacting cellular health pathways such as those involved in myogenesis.

Conclusions: We have discovered that p38 inhibition reduces the expression of DUX4 across all patient-derived myotubes tested including FSHD1 and FSHD2 genotypes. Fulcrum has nominated losmapimod, a specific p38α/β inhibitor, for clinical development.

P34 MyoScreen™, a drug discovery platform for FSH muscular dystrophy.

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Background: Effective treatments are missing for degenerative muscle disorders such as FSHD because the current in vitro muscle pathological models lack physiology relevance and pharmacology predictivity. We recently reported MyoScreen™, human patient-derived skeletal myotubes that demonstrate sarcomeric organization, acetylcholine receptor expression, response to chemical and electrical stimulation and pharmacologically relevant drug responses (SLAS Discov. 2018 23(8):790-806).

Objectives: The objective was to develop a physio-pathological FSHD MyoScreen platform compatible with high-throughput screening.

Results: To do so, primary myoblasts from two FSHD patients and healthy donors were sourced, amplified and characterized. Differentiation into mature myotubes was performed using MyoScreen™ and characterization was run using a panel of assays.

Conclusions: Although healthy and FSHD myoblasts and myotubes present similar differentiation and morphological features, some promising phenotypic differences were detected.

P35 Single-nucleus RNA-seq identifies divergent populations of FSHD2 myotube nuclei.

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FSHD is characterized by the misexpression of DUX4 in skeletal muscle. Although DUX4 upregulation is thought to be the pathogenic cause of FSHD, DUX4 is lowly expressed in patient samples, and analysis of the consequences of DUX4 expression has largely relied on artificial overexpression. To better understand the native expression profile of DUX4 and its targets, we first performed pooled RNA-seq on a 6-day differentiation time-course in FSHD2 patient-derived primary myoblasts and identified early-and late-induced sets of FSHD-associated genes during differentiation. Using single-cell and single-nucleus RNA-seq on FSHD2 myoblasts and 3-day differentiated myotubes respectively, we captured, for the first time, DUX4 expressed at the single-nucleus level and found that DUX4 and its targets expression do not always occur within the same nucleus. We identified two populations of FSHD myotube nuclei with distinct transcriptional profiles. One population is highly enriched with DUX4 and FSHD related genes, including the DUX4 paralogous gene DUXA (“FSHD-Hi”). The other population has no expression of DUX4 and expresses low amounts of FSHD related genes (“FSHD-Lo”), but it is marked by the expression of CYTL1 and CHI3L1, distinct from normal control nuclei. Thus, “FSHD-Lo” nuclei may represent those patient myocytes that may have entered a different cell state. In contrast, “FSHD-Hi” myotube nuclei upregulated a set of transcription factors (TFs) in addition to the known DUX4 target transcription factors, such as ZSCAN4 and LEUTX, that are actively involved in germ cell and early development as well as muscle atrophy. These TFs may form a self-sustaining network of gene dysregulation that perpetuates this disease after DUX4 is no longer expressed.