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ABSTRACT BOOK

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SPEAKER ABSTRACTS

S1.101 Active skeletal muscle regeneration in facioscapulohumeral muscular dystrophy

Peter Zammit[1], Christopher Banerji[1], Rabi Tawil[2], Don Henderson[2]

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[2]*Department of Neurology, University of Rochester Medical Center*

Facioscapulohumeral muscular dystrophy (FSHD) is a prevalent, incurable, autosomal-dominant myopathy, characterized by a slowly progressive descending skeletal muscle weakness and wasting. In many muscular dystrophies, myofiber damage provokes a regenerative response to compensate for loss of muscle fibers. However, little is known about whether a regenerative response is regularly elicited in the slowly progressing FSHD, and if so, how common regenerating myofibers in muscle biopsies are. To address this, we first used the 200 human gene Myogenesis biomarker to determine if FSHD muscle biopsies have a transcriptomic signature characteristic of muscle regeneration. The Myogenesis score was elevated on meta-analysis and in most independent FSHD studies. To determine how often muscle regeneration is found in needle biopsies from FSHD patients, we also immunolabeled for Developmental Myosin Heavy Chain. Immunolabeling revealed regenerating myofibers in 77% (26/34) of muscle biopsies from quadriceps and 91% (10/11) from tibialis anterior in FSHD. More regenerating myofibers per biopsy were also found in the tibialis anterior compared to the quadriceps. Muscle biopsies were scored for pathologic changes, which showed that muscle regeneration in FSHD was correlated with central nucleation, fibrosis, or necrosis. Our data reveal that muscle mounts a regenerative response in the vast majority of FSHD patients.

S1.102 Single-nucleus RNA-seq identifies divergent populations of FSHD2 myotube nuclei

Katherine Williams[1], Shan Shan Jiang[1], Xiangduo Kong[1], Weihua Zeng[1], Nam Viet Nguyen[1], Xinyi Ma[1], Rabi Tawil[2], Kyoko Yokomori[1], Ali Mortazavi[1]

[1]*University of California, Irvine*
[2]*Department of Neurology, University of Rochester Medical Center*

DUX4 upregulation in skeletal muscle is thought to be the cause of FSHD, but DUX4 is lowly expressed in patient samples. To understand the native expression profile of DUX4 and its targets, we performed bulk RNA-seq on a six-day differentiation time-course in primary FSHD2 patient myoblasts. We identified a set of 54 genes upregulated in FSHD2 cells, termed FSHD-induced genes. Using single-cell and single-nucleus RNA-seq on myoblasts and differentiated myotubes, respectively, we captured DUX4 expressed at the single-nucleus level in a native state. We identified two populations of FSHD myotube nuclei based on low or high enrichment of DUX4 and FSHD-induced genes ("FSHD-Lo" and "FSHD Hi," respectively). FSHD-Hi myotube nuclei coexpress multiple DUX4 target genes including DUXA, LEUTX, and ZSCAN4, and upregulate cell cycle-related genes with enrichment of E2F target genes and p53 signaling activation. We found more FSHD-Hi nuclei than DUX4-positive nuclei, and confirmed that DUX4 transcribed in only a few nuclei is sufficient for DUX4 protein to activate target genes across nuclei within the same myotube. A DUX4 paralog, DUXA, is more widely expressed than DUX4, and depletion of DUXA suppressed the expression of LEUTX and ZSCAN4 in late differentiation. The results suggest that the DUXA can take over the role of DUX4 to maintain target gene expression. This raises the possibility of a self-sustaining network of gene dysregulation triggered by limited DUX4 expression.

S1.103 SLC34A2 as a Potential Biomarker for FSHD

Maria Traficante[1], Andrea O'Neill[1], Amber Mueller[2], Robert Bloch[1]

[1]*University of Maryland, Baltimore*
[2]*Harvard Medical School*

Despite exciting developments of FSHD models and a growing understanding of the disease state, the pathophysiology underlying the loss of FSHD muscle fibers is still poorly understood. In addition, researchers have yet to find a reliable biomarker that can be used as a diagnostic. We have identified the potential biomarker SLC34A2, a pH-sensitive Na^+-dependent PO_4^{2-}-cotransporter and upregulated DUX4 target, both in human biopsies and in our model of human muscle xenografts (Mueller et al., Exp. Neurol. 320: 113011, 2019). In healthy individuals, the cotransporter is expressed in lung, kidney, and gut epithelia, but not in mature muscles. Our previous work has found that SLC34A2 is present in about 1%-2% of FSHD-affected fibers, which correlates with the relatively low level of DUX4 expression we observe in the xenografts. Based on these promising results, we hypothesize that SLC34A2 is a surrogate biomarker for the DUX4 program. Our preliminary studies detected intact or nearly intact SLC34A2 in lysates of cultured FSHD muscle cells as well as in FSHD xenografts. In addition, immunoblots of sera from mice carrying FSHD xenografts, but not control grafts, show a band for SLC34A2 at close to the predicted molecular weight. Future work will investigate the relationship between disease severity and SLC34A2 levels, and whether therapies that suppress DUX4 expression reduce SLC34A2 levels in muscle and serum. Funded by the FSHD Society, Friends of FSH Research (USA and Canada), and the NIH.

S1.104 FSHD zebrafish models for drug discovery

Yuanfan Zhang[1], Angela Lek[2], Anna Pakula[1], James Conner[1], Jeffrey Widrick[1], Matthias Lambert[1], Louis Kunkel[1]

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[2]*Department of Genetics, Yale School of Medicine*

The zebrafish has become a prominent vertebrate model for disease and contributed to several successful phenotype-based drug discoveries. Previous studies have shown that FSHD disease pathogenesis can be driven by very low misexpression of the germ line transcription factor DUX4-fl. We have created a platform to perform therapeutic evaluation using two DUX4-fl-based zebrafish models with different severity of disease phenotype. Specifically, DUX4-fl mRNA-injected zebrafish recapitulated the severe phenotypes, including asymmetric abnormalities of ears, eyes, and fin muscle; disorganization of facial musculature; and degeneration of trunk muscle. Our inducible DUX4-fl transgenic zebrafish model with quantifiable DUX4 expression showed that a burst of DUX4 expression during the developmental stage is sufficient to induce later muscle pathologies such as fat infiltration and fibrosis. We have developed an integrated platform using both models to evaluate the efficacy of small molecule drugs in alleviating DUX4-induced pathology in zebrafish.

S2.105 High-throughput analysis of tandem repeat contraction associated with facioscapulohumeral muscular dystrophy (FSHD) by optical mapping

Ernest Lam[1], **Sven Bocklandt[1]**

[1]Bionano Genomics

Molecular diagnosis of facioscapulohumeral muscular dystrophy (FSHD) type 1 requires the detection of a contracted D4Z4 array, a tandem repeat on chromosome 4, on a permissive 4qA haplotype. The repeat is polymorphic and can span hundreds of kilobase pairs. There is a homologous array on chromosome 10, and rearrangements between the two regions have been reported. Due to these challenges, Southern blotting, while specialized and laborious, remains the gold standard for diagnosis.

We developed a workflow based on the Bionano Genome Imaging platform, which offers several advantages. Based on specific labeling and mapping of ultra-long molecules in nanochannels, optical mapping provides a high-resolution analysis of D4Z4 and other large tandem repeats. Because of the single-molecule nature of the platform, it is possible to detect mosaic alleles. We showed that the analysis workflow had high sensitivity, specificity, and reproducibility. We detected repeat contractions on the 4qA haplotype in FSHD-positive samples. We also analyzed 58 FSHD-negative samples; none had repeat counts in the clear pathogenic range.

Bionano offers sample preparation, DNA imaging, and genomic data analysis technologies combined into one streamlined workflow that enables high-throughput analysis of tandem repeat regions of interest. Together, these components allow for efficient analysis of diseases associated with repeat expansion and contraction.

S2.106 A long-read sequencing approach for investigating repeat number and DNA methylation of the D4Z4 region

Alexander E Liu[1], Timothy Gilpatrick[2], Adam Bittel[1], Brianna Banting[1], Winston Timp[2], Yi-Wen Chen[1,3]

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[2]*Department of Biomedical Engineering, Johns Hopkins University*
[3]*Department of Genomics and Precision Medicine, School of Medicine and Health Science, George Washington University*

Standard genetic testing for FSHD1 is to detect contracted D4Z4 array using pulsed-field gel electrophoresis (PFGE) in combination with Southern blotting, which is labor intensive and expensive. In addition, FSHD2 cannot be diagnosed using this method. The goal of this study is to develop a cost-effective long-read sequencing-based assay that can determine repeat number and DNA methylation of the D4Z4 region. In this study, a CRISPR/Cas9-based enrichment protocol in combination with the Nanopore long-read sequencing was used to specifically target the D4Z4 region. In the study, we targeted regions upstream and downstream of the D4Z4 array and successfully obtained complete D4Z4 arrays spanning from the p13e11 region to the pLAM region. Additional guide RNAs were designed to target relevant regions to improve the assay.

S2.107 Two families with chromosome 10q-linked FSHD identify DUX4 as principal disease gene

Richard J.L.F. Lemmers[1], Patrick J. van der Vliet[1], Ana Blatnik[2], Judit Balog[1], Rabi Tawil[3], Janez Zidar[4], Rianne Goselink[5], Stephen Tapscott[6], Nicol Voermans[7], George Padberg[5], Baziel van Engelen[8], Silvère van der Maarel[1]

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The prevailing disease model postulates that FSHD is caused by a partial failure in somatic repression of the transcription factor DUX4 embedded in the D4Z4 repeat on the 4q subtelomere. A D4Z4-like repeat can be found on chromosome 10q, but here, according to the model, repeat derepression does not cause FSHD due to a DUX4 polyadenylation signal disrupting DNA variant. However, because of the position of the FSHD locus close to the telomere and the complex (epi)genetic etiology of FSHD, there is ongoing dispute about the transcriptional deregulation of other 4q genes and their contribution to FSHD muscle pathology. In this study, we identified two FSHD families with a de novo exchange between chromosomes 4 and 10, resulting in derepression of the FSHD-sized D4Z4 repeat on chromosome 10. The patients in these families present with a classical FSHD phenotype, and their muscle cell cultures exhibit molecular features characteristic of FSHD, including DUX4 expression. We did not find evidence for transcriptional deregulation of other 4qter candidate genes in these muscle cell cultures. This study shows that D4Z4 repeat rearrangements on chromosome 10, in the appropriate genetic context, can cause DUX4 expression and FSHD disease presentation. It supports the model that DUX4 derepression is the dominant FSHD disease pathway and justifies efforts to identify therapeutic compounds that suppress DUX4.

S3.108 Hypoxia signaling is a key driver of DUX4-induced pathology

Angela Lek[1]

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DUX4 misexpression results in cell death and is a key feature of FSHD pathology. This study aims to identify the genes and pathways involved in enacting DUX4's toxic effect, reasoning that it may provide targets for therapeutic intervention. To achieve this, we performed a genome-wide CRISPR loss-of-function screen in a DUX4 inducible cell model of FSHD, and identified genes that are necessary for DUX4-induced cell death, due to their ability to confer survival in the presence of DUX4 when knocked out. Among our top hits were several regulators of the cellular hypoxia response, suggesting a role for hypoxia in FSHD pathology that contributes to DUX4-induced cell death. Further investigation revealed that DUX4 expression causes myoblasts to become hypoxic and that pharmacological inhibition of the hypoxia response was able to prevent cell death. These hypoxia signaling inhibitors also proved effective in reducing FSHD disease biomarkers and pathology in patient cells, a xenograft mouse model, and a zebrafish model of FSHD.

S3.109 Role for aberrant protein synthesis in facioscapulohumeral muscular dystrophy

Amy Campbell[1], Mike Dyle[1], Lorenzo Calviello[2], Austin Gillen[1], Stephen Floor[2], Sujatha Jagannathan[1]

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Misexpression of DUX4 in skeletal muscle causes facioscapulohumeral dystrophy (FSHD), a progressive muscle disease for which there are no therapeutics. DUX4 misexpression disrupts diverse cell processes, including the essential quality control pathway nonsense-mediated RNA decay (NMD), and causes cell death in a poorly understood sequence of molecular events. NMD detects and degrades aberrant RNAs containing a premature translation stop codon, preventing expression of truncated proteins that could be harmful to the cell. To test the hypothesis that DUX4-mediated inhibition of NMD contributes to FSHD pathology through the accumulation of truncated proteins, we carried out RNA-seq and ribosome footprint sequencing in an engineered muscle cell line that expresses DUX4 synchronously upon addition of a small molecule inducer. We identified hundreds of aberrant RNAs stabilized by NMD inhibition that are robustly translated to produce truncated proteins unique to DUX4-expressing cells. Overexpression of a single truncated protein, SRSF3-TR, induced protein aggregation and led to cell death. SRSF3-TR contains a unique neoepitope that might be harnessed as an FSHD biomarker, and we have generated a custom antibody to investigate this possibility. Overall, these results confirm the presence of aberrant proteins in DUX4-expressing cells and lead us to conclude that aberrant RNAs and/or their protein products impact DUX4-expressing cells via toxic gain of function.

S3.110 Regulated necrosis is involved in DUX4-mediated toxicity

Virginie Mariot[1], Romain Joubert[2], Laura Le Gall[1], Eva Sidlauskaite[1], Thomas Voit[1], Maximilien Bencze[3], **Julie Dumonceaux[1]**

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[3]INSERM

In FSHD, DUX4 expression is thought to induce cell death, but the mechanisms leading to these dysfunctions are poorly understood. The concept of cell death has considerably changed over the last 10 years, and similar to apoptosis, necrosis may also occur in a programmed/regulated fashion and cannot be considered only as an environmental stress response.

We focused on the role of regulated necrosis in DUX4-mediated cell death. Indeed, whereas muscle fiber death is usually linked to necrosis as defined by histological criteria, the necrotic death pathway has never been investigated. Here we show the contribution of regulated necrosis to DUX4-mediated toxicity both in vitro and in vivo. In vitro we used the iC2C12-DUX4 cells that carry a doxycycline-inducible DUX4 transgene. We observed that DUX4 expression causes a regulated cell death in both myoblasts and myotubes. In vivo, we used the cre-inducible FLeX DUX4 transgenic mouse model that conditionally expresses human DUX4. When this mouse model was crossed with a regulated necrosis-deficient mouse, littermates showed a weight loss 8%-10% lower compared to regulated necrosis-competent animals. The same observations were made when the muscle weights were analyzed. We also measured the levels of genes downstream of DUX4 and observed a two- to threefold lower expression in the regulated necrosis-deficient animals.

These results highlight the role of regulated necrosis in DUX4-mediated cell death and open a new avenue of research.

S3.111 An AAV-DUX4 mouse model of FSHD reflects disease pathology based on the level of DUX4 protein expression

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FSHD is caused by expression of DUX4. To identify therapeutic disease targets we are focused on the relationship between DUX4 protein expression and progressive muscle damage. With fine-tuned gene dosing of our AAV-DUX4 vector, we can induce incremental DUX4 protein expression in adult mouse muscle driven by the endogenous DUX4 promoter. AAV-DUX4-mediated protein expression leads to isolated areas of focal damage of myofibers in muscle that become much larger without increased protein expression. Modeling FSHD in mouse muscle has allowed us to profile DUX4 protein and mRNA expression in both phases of pathology, where this has been difficult in FSHD muscle biopsy studies. RNA-seq gene expression profiling from treated muscle revealed changes in cellular pathways overlapping cell and transgenic mouse DUX4 studies, including innate immune responses (STING, Rig-I, Ticam, interferons, Trim proteins, Wdfc3, RNasel), complement induction (C1qa, Has1), cell cycle control (Myc, p21, Rb1, Aurka), polycomb protein expression (Eed, Phc2, Ezh2), DNA damage response (Wrap53, Trp53, Brca2, Gadd45a, Bax, Fancu), Nfkb/p38 induction (Relb, Nfkb2, Dad1, Map3k14, Dusp5, Stk39), lipid biosynthesis (Anax2, Insig1, Pltp, Pik3r6, Atp11a, Crot), and mTOR regulation (Rsp6ka1, Ifi215, Ppp1r9b, Dapk1). We identified several new pathways that will be presented. In summary, the AAV-DUX4 model displays disease features and serves as a platform for further investigation of FSHD pathology and therapy testing.

S3.112 Mechanisms of pathology in FSHD

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DUX4 causes FSHD, but it is not understood how, neither at the cellular level, nor at the tissue level. I will present new insights on both the molecular mechanisms underlying the protein function of the DUX4 transcription factor, as well as new insights into effects of DUX4 on skeletal muscle in vivo.

S4.201 LNA and 2'MOE gapmers for treating facioscapulohumeral muscular dystrophy

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Antisense oligonucleotide (AON) therapy has shown promise for treating an array of disorders and can be used to reduce DUX4 in FSHD. Based on our in vitro data generated using human FSHD myoblasts, we selected an LNA gapmer AON and a 2'MOE gapmer AON for in vivo efficacy studies using the FLExDUX4 mouse model. The AONs were delivered by subcutaneous injections (20 mg/kg, s.c.) twice a week for 10 weeks. Muscle strength (grip strength testing) and pathologies (fibrosis, fat infiltration, inflammation, fiber size) were evaluated. In addition, we determined the serum levels of creatine kinase (CK), alanine transaminase (ALT), alkaline phosphatase (ALP), r-glutamyl transferase (GGT), bilirubin, urea nitrogen, and creatine for signs of muscle, hepatic, and renal damages. Our results showed that both AONs effectively reduced the DUX4 mRNA. In addition to DUX4 reduction, 10 weeks of treatments improved muscle pathology and function. Serum enzyme assays showed elevated liver enzymes in mice receiving the treatments of LNA gapmer AON. This elevation was not seen in serum samples collected from mice treated with the 2'MOE gapmer AON. The data showed that both LNA and 2'MOE gapmer AONs are highly effective. However, prolonged treatment of the LNA gapmer AON might cause liver damage. Our results suggest that 2'MOE gapmer AON shows greater promise for therapeutic development for FSHD.

S4.202 DUX4 inhibition by AAV.CRISPR-Cas13b in FSHD mouse models

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The CRISPR-Cas13 system does not cleave DNA, but can be specifically directed to an RNA transcript of interest using a sequence-specific guide RNA (gRNA), thereby reducing the risk of permanent DNA damage. The goal here is to develop an AAV6.CRISPR-Cas13 therapy for silencing toxic DUX4 expression in muscles of our FSHD mouse model. To do this, Cas13 and our lead-candidate gRNA, which markedly decreased DUX4 expression in FSHD myoblasts/myotubes in vitro, were packaged into separate AAV6 particles. After performing safety and dose escalation studies in C57BL/6 mouse muscles, AAV6.Cas13 and scAAV6.U6-gRNA were co-injected into adult and neonatal FSHD mice via intramuscular injection. RNAscope and qRT-PCR results to detect DUX4 mRNA demonstrated reduction in DUX4 expression in treated mice, corresponding with improved histopathological outcomes. To investigate possible off-target effects of our CRISPR-Cas13 system, we performed RNA-seq analysis of treated versus untreated human myoblasts. This study provides proof of principle for using CRISPR-Cas13 gene therapy as a novel strategy to treat FSHD through DUX4 mRNA inhibition.

S4.203 DUX4 transcript knockdown with antisense gapmers for the treatment of facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD), characterized by progressive muscle weakness and deterioration, is genetically linked to aberrant expression of DUX4 in muscle. DUX4, in its full-length form, is cytotoxic in non-germline tissues. Here, we designed locked nucleic acid (LNA)/2'-O-methoxyethyl (2'MOE) gapmer antisense oligonucleotides (AOs) to knock down DUX4 in immortalized FSHD muscle cells and the FLExDUX4 FSHD mouse model. Gapmers are short DNA oligonucleotides with LNA/2'MOE modification at both ends. They hybridize to target mRNAs and lead to cleavage of the RNA/DNA hybrid, which induces RNase H-mediated mRNA degradation. Using a screening method capable of reliably evaluating the knockdown efficiency of LNA/2'MOE gapmers against endogenous DUX4 mRNA in vitro, we demonstrated that several designed LNA/2'MOE gapmers selectively and effectively reduced DUX4 expression with nearly complete knockdown. We also found, for the first time, potential functional benefits of AOs on muscle fusion and structure in vitro. Finally, we showed that LNA/2'MOE gapmers were taken up and induced effective silencing of DUX4 upon local treatment in vivo. The antisense gapmers developed here will help facilitate the development of FSHD therapies.

S4.204 A combined ex vivo and xenograft pipeline for FSHD drug development

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Facioscapulohumeral muscular dystrophy (FSHD) is a commonly diagnosed form of muscular dystrophy that is inherited in an autosomal-dominant manner and characterized by the frequently asymmetric, progressive weakening of muscles in the face, back, and shoulder girdle. Misexpression of the skeletal muscle gene DUX4 has been implicated as the cause of muscle pathology in FSHD, providing a therapeutic target. In order to advance potential FSHD therapeutics to clinical trials, we have developed and optimized a robust FSHD drug development pipeline combining ex vivo cell culture and in vivo cell xenograft models using primary, patient-derived myogenic cells from the UMMS Wellstone Center Biorepository. In collaboration with industry partners and the RNA Therapeutics Institute at UMMS, we have identified promising lead RNA and small molecule FSHD therapeutics.

S4.205 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. Extensive studies have been completed to determine the top therapeutic candidate including miRNA screens, AAV serotype comparisons, promoter screens, and toxicology studies in wild-type mice.

Objectives: If an animal model for a disease exists, the FDA requires a gene therapy to be tested in said model using the same route of delivery proposed for a clinical trial. Here we have performed studies in TIC-DUX4 mice to satisfy this requirement for both intramuscular (IM) and vascular (IV) delivery.

Results: We compared AAV6 and AAV9 serotypes expressing mi405 from the ubiquitous (U6) promoter in adult TIC-DUX4 mice. IV injections were performed followed by a 10-week induction of DUX4 via oral gavage of tamoxifen. During these 10 weeks, open field and rearing data were collected weekly. Treated TIC-DUX4 mice performed significantly better than untreated mice, further supporting the therapeutic benefit of mi405. IM-injected TIC-DUX4 mice are currently being assessed along with biomarker analysis for both studies.

Conclusions: In all systems tested, mi405 has shown conclusive therapeutic effects. We believe AAV.mi405 is a strong therapeutic candidate to treat FSHD.

S5.206 Facioscapulohumeral muscular dystrophy 1 patients participating in the UK FSHD registry can be subdivided into four patterns of self-reported symptoms

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Facioscapulohumeral muscular dystrophy (FSHD) classically comprises a descending skeletal myopathy, beginning in the facial muscles and progressing to the shoulder girdle and lower limb; however, presentation is highly heterogeneous. We analyzed 643 FSHD1 patients in the UK FSHD patient registry, investigating factors affecting rate of onset of five major FSHD symptoms: facial, periscapular, foot dorsiflexor, and hip girdle weakness, and hearing loss. Shorter D4Z4 repeat length associated with accelerated onset of each symptom. Furthermore, paternal inheritance of the pathogenic allele associated with accelerated onset of foot dorsiflexor weakness, while pregnancy and carrying multiple children to term associated with slower onset of all muscle symptoms. Lastly, we performed clustering analysis on age of onset of the four muscle symptoms across 222 patients. We identified four clinical presentations of FSHD1: a classical presentation (74%) describing a descending myopathy, and three facial sparing phenotypes – a mild presentation (5%) with later facial and periscapular involvement, an early shoulder presentation (10%) with accelerated periscapular weakness, and an early foot presentation (9%) with accelerated foot dorsiflexor weakness. The mild presentation associated with longer D4Z4 repeat lengths, while the early foot presentation had a female bias. Symptom progression differs across our four clinical presentations independently of D4Z4 length and sex, indicating further FSHD1 modifiers.

S5.207 Interaction of factors in (epi)genotype-phenotype relationship in FSHD1 explored in scatter plots created from our own and published data

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Background: Factors affecting a person's presentation/penetrance of FSHD include 4q35-genotype and methylation, modifier genes, coincidental second NM condition, age, sex, probably ethnicity, and possibly transmission sex combination. In cohort or family studies, ascertainment mode is also key (proband vs. family, and cascade study). Understanding the contribution and interaction of each factor is important for prognostic prediction, design, and interpretation of therapeutic trials, and for therapeutic targets.

Method: Assessing the relative contribution of each factor to variance in penetrance/phenotype requires a multivariate analysis approach. To guide this, visual display of raw data in cohort or family studies can help spot overall trends and exceptions. We present scatter plots created from our own and others' published data to provide a visual illustration of the interrelationships among three or more factors simultaneously.

Results: The plots clearly illustrate reduced methylation and earlier onset age following grandmaternal-maternal versus grandpaternal-paternal transmission in US cell line data and our UK cohort, respectively. Other scatter plots illustrate how having few families, and mode of ascertainment, can skew data, and the potential role of ethnicity.

Conclusion: Use of scatter plots created from raw data can provide pointers to relative importance and interaction of the different factors contributing to variance in phenotype in FSHD to guide multivariate analyses.

S5.208 Design and baseline characteristics of a Phase 2, randomized, placebo-controlled, 24-week study of the efficacy and safety of losmapimod in treating subjects with FSHD: ReDUX4

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Objective: Evaluate efficacy of losmapimod inhibiting aberrant expression of DUX4 in skeletal muscle. Secondary objectives were to evaluate safety, tolerability, PK, and target engagement (TE) in blood and muscle.

Background: FSHD is caused by aberrant expression of DUX4 in skeletal muscle. DUX4 activates a transcriptional program causing skeletal muscle loss and disability. Losmapimod is a small molecule inhibitor of p38α/β. Preclinical studies with losmapimod resulted in dose-dependent reduction of DUX4 protein, transcriptional program, and skeletal muscle cell death in FSHD myotubes.

Design/Methods: Approximately 76 subjects age 18 to 65 with genetically confirmed FSHD1, clinical severity score of 2 to 4 (Ricci scale 0-5), and MRI-identified skeletal muscle for biopsy were randomized 1:1 to 15 mg losmapimod or placebo PO BID for 24 weeks. Muscle biopsies were performed pretreatment and at week 16 to measure treatment effect on DUX4 activity. PK and TE were measured in blood and muscle. Musculoskeletal MRIs were performed at screening, week 12, and week 24 for assessment of change. Clinical outcome assessments included reachable workspace, FSHD-timed up and go, dynamometry, and motor function measure domain 1. Patient-reported outcomes included FSHD-Health Index and patient global impression of change.

Conclusion: Based on supportive preclinical and preliminary clinical data, we have designed and launched a Phase 2b clinical trial to assess the efficacy of losmapimod to treat FSHD.

S5.299 ReDUX4: addressing the challenges of clinical trial conduct during the COVID-19 pandemic

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Objective: Adapt ReDUX4 protocol and trial management procedures to monitor safety of subjects, preserve underlying objectives, and ensure continuation of the study during the COVID-19 pandemic.

Background: Clinical trial conduct changed significantly due to the COVID-19 pandemic. Regulatory agencies, institutional review boards (IRBs), and ethics committees (ECs) moved quickly to provide guidance regarding the conduct of clinical trials. Fulcrum and the ReDUX4 study sites worked together to ensure the safety of subjects while preserving the underlying objectives of the ReDUX4 study.

Results: A ReDUX4 Emergency Guidance Document was provided to all sites in March 2020 to enable safety monitoring through virtual visits and mobile phlebotomy. ReDUX4 protocol was amended to reflect the guidance and minimize treatment gaps through direct-to-patient (DTP) shipment of investigational treatment. Duration of the randomized controlled portion was extended from 24 to 48 weeks to enable collection of key efficacy data from missed visits and to support more robust assessment of safety and efficacy of losmapimod in FSHD. The optionality to participate in the open-label extension (OLE) after week 48 is preserved.

Conclusions: The ReDUX4 protocol was promptly amended in response to the COVID-19 pandemic to manage patient safety and preserve the ability for robust assessment of efficacy while maintaining the option to participate in the OLE.

S5.209 A biomarker of DUX4 activity to evaluate losmapimod treatment effect in FSHD Phase 2 trials

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Objective: To identify a panel of DUX4-regulated gene transcripts expressed in affected FSHD muscle biopsies.

Background: Pathogenic expression of DUX4 results in transcriptional dysregulation and activity of a program that causes myofiber death in FSHD. While pharmacodynamic (PD) detection of DUX4 protein and mRNA is challenging, DUX4-regulated gene transcripts are readily detected in affected muscles. Fulcrum is developing losmapimod, a small molecule inhibitor of p38α/β, to reduce aberrant DUX4 expression in FSHD. A preparatory biomarker study was performed to identify a set of stable DUX4-regulated gene transcripts that will provide a PD biomarker endpoint to measure treatment effect on the root cause of FSHD.

Design/Methods: Patients age 18 to 65 with genetically confirmed diagnosis of FSHD1, CSS 2 to 4, and a muscle that met criteria for biopsy were included. Eligible muscles were identified by MRI. Muscle biopsies were performed twice approximately six weeks apart on the same muscle. Biopsies were analyzed by RNA sequencing and assessment by differential expression profiling and machine learning algorithms.

Results: Sixteen subjects completed the study. Using published RNA-seq data (Wang, 2019, and Wong, 2020) and this study, a subset of DUX4-regulated gene transcripts was identified.

Conclusion: A set of stable DUX4-regulated gene transcripts was identified that provides a PD biomarker endpoint to measure treatment effect on the root cause of FSHD in losmapimod FSHD clinical trials.

S5.210 Results from a Phase 2 study of ACE-083 in patients with facioscapulohumeral muscular dystrophy (FSHD) – implications for future clinical trials

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Background: ACE-083 is a locally acting muscle therapeutic based on follistatin that binds myostatin and other muscle regulators. ACE-083 has been shown to increase muscle mass of injected muscles in animal models, healthy subjects, and patients with FSHD or Charcot-Marie-Tooth (CMT) disease.

Objectives/Methods: The primary objectives were to determine if ACE-083 could safely increase total muscle volume (TMV) of the tibialis anterior (TA) or biceps brachii (BB) in patients with FSHD1 or 2. Part 1 was dose-ranging (N=37, previously reported); Part 2 was double-blind, placebo-controlled for six months followed by a six-month open-label period. Patients were treated with ACE-083 240 mg/muscle or placebo (1:1) injected into the TA or BB bilaterally q3 weeks. Secondary endpoints included fat fraction (FF), strength, timed function tests, and quality of life (FSHD-Health Index, HI).

Results: We enrolled 58 patients (safety set) in Part 2, and 55 were evaluable for efficacy/PD endpoints. Median (range) baseline age (yr) was 46.0 (18-70) in TA cohorts, 46.0 (21-80) in BB cohorts. We saw mean (SEM) increases in TMV of 13.8% (2.9) for ACE-083 versus 4.3% (2.7) for placebo ($p = 0.01$) in TA, and increases of 19.1% (2.8) for ACE-083 versus 2.7% (2.8) ($p<0.0001$) for placebo in BB. There were no statistically significant differences between ACE-083 and placebo groups for percentage change in six-minute walk distance, 10 m walk/run, four-stair climb (TA), or Performance of Upper Limb (BB), nor for raw change in FSHD-HI scores. ACE-083 was generally well tolerated; common adverse events included injection site reactions and myalgia.

Conclusions: Although ACE-083 demonstrated significant increases in mean TMV, there were no statistically significant improvements in other clinical outcome measures (COMs). Learning and placebo effects identified in some COMs support consideration of a run-in period and emphasize the importance of an appropriate comparator arm in neuromuscular studies.

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POSTER ABSTRACTS

P501 Baseline MRI data and correlations with functional measures from the creatine in FSHD clinical trial

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Facioscapulohumeral muscular dystrophy (FSHD) is a clinically heterogeneous condition with marked phenotypic variability. Reliable outcome measures are essential for disease staging biomarkers and for clinical trials. Previous studies in adults have shown MRI to have good correlation between the visual Mercuri scale with physical functioning. This has not yet been shown in the paediatric population.

Eleven patients were recruited to a randomized, double-blind, placebo-controlled crossover clinical trial, Effect of Creatine Monohydrate on Functional Muscle Strength in Children with FSHD (ClinicalTrials.gov Identifier: NCT02948244). Each participant had serial MR scans and their functional strength measured using a number of outcome measures. The data show excellent correlation between the cumulative Mercuri Score and the FSHD Clinical Severity Scale (r 0.95), FSH-COM (r 0.92) and FSH-Clinical Score (r 0.93), with a good inverse relationship between the Mercuri score and the total six-minute walk distance (r -0.72) and the PUL2.0 (r -0.85). The MRI score correlates with the Motor Function Measure for Neuromuscular Disease (r -0.83) but the trend line suggests that this scale may not be sensitive to changes in the muscles of patients with less severe FSHD. These data add to the growing evidence to support the use of muscle MRI as a biomarker in patients with FSHD. These data show that MRI is a safe, useful, and well-tolerated FSHD biomarker in the paediatric population.

P502 Paediatric FSHD: Cross-sectional description of patient demographics and physical functioning from an Australian cohort

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Facioscapulohumeral muscular dystrophy (FSHD) is amongst the most prevalent of the adult muscular dystrophies (1 in 8,000) but is somewhat rarer in childhood, with an estimated paediatric prevalence of 0.7 to 1 per 100,000 of population. With the exception of an ongoing Dutch natural history study, the majority of natural history data focuses on the adult population. We present baseline demographic data from potential participants approached for a randomized, double-blind, placebo-controlled crossover clinical trial, Effect of Creatine Monohydrate on Functional Muscle Strength in Children with FSHD. Participants approached for this study were given the option of participating in the full RCT or a parallel study assessing the clinometric properties of a FSHD specific outcome measure (discussed elsewhere). All participants underwent the same functional outcome measures including: Motor Function Measure (MFM), Performance of the Upper Limb2.0 (PUL), FSH-Composite Outcome Measure (FSH-COM), FSH-Clinical Score, and FSH-Clinical Severity Scale. Seventeen paediatric patients with a clinical diagnosis of FSHD were included. Fifty-nine percent of patients were male with a population mean age of 13.2 years. There was genetic confirmation in 89% with average D4Z4 repeat number of 4.8%. A single patient had FSHD2. Beevor's sign was positive in 65%. The mean FSH-CS was 5.6 and average FSH-CSS 2.0. Mean functional scores included: FSH-COM 24.3, MFM 90%, and PUL 34.3. These data add to the limited descriptive data of FSHD in the paediatric population.

P503 Analysis of the facioscapulohumeral muscular dystrophy clinical score (FSHD CS) using item response theory

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We aim to describe the test characteristics of the facioscapulohumeral muscular dystrophy clinical score (FSHD CS) using item response theory (IRT) for testing items with varying levels of ordinal response.

We collected FSHD CS data from 130 subjects with genetically confirmed FSHD from four sites. Forty-two subjects from two sites underwent further clinical evaluation, including forced vital capacity (FVC) and Iowa Oral Performance Instrument (IOPI) testing. Dimensionality of the items was tested using a confirmatory factory analysis (CFA), and the properties of the items using the graded response model.

Mean FSHD CS score was 7.12 ± 3.57. CFA confirmed that a one-factor model was appropriate for the data. Facial weakness and abdominal muscle involvement contributed the least to total FSHD CS score. The information provided by the scale was nearly unchanged when the model was run without these items. The FSHD CS standard error of measurement was <1 over approximately ±2 S.D. of the mean, which includes >95% of participants. FVC and IOPI performance were correlated with FSHD CS total score in exploratory evaluations.

The FSHD CS is capable of detecting change of as little as one point in 95% of participants, though it is unknown how the scale captures meaningful disease progression. The FSHD CS is least informative in those with either extremely mild or severe symptoms. Further development of a scale that includes improved proxies for facial and abdominal weakness is warranted.

P504 The UK FSHD Patient Registry: an important tool in the facilitation of translational and clinical research

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

The registry has adopted the TREAT-NMD core dataset (https://treat-nmd.org/downloads/file/registries_toolkit/FSH_core_dataset_May2011.pdf). It captures longitudinal, self-reported, and clinician-reported data through an online portal.

Between May 2013 and February 2020, 977 participants registered with the UK registry. On average, nine new participants register each month. For those who have a clinical diagnosis, 97% reported FSHD/FSHD1, and 3% reported FSHD2. Overall, 48% have genetic confirmation of their condition.

The registry has previously supported 18 research enquiries including the ACTMuS clinical trial and a natural history study of infantile-onset FSHD. Since 2019, the registry has facilitated six enquiries, including a national clinical trial readiness survey, and an industry-derived patient survey to assess a proposed clinical trial protocol.

As one of the largest national FSHD patient registries, the UK FSHD Patient Registry is an example of a versatile, cost-effective research tool.

P506 Results of machine learning analysis of the National Registry for FSHD

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This session will describe the data contained in the National Registry for FSHD and the results of an AI-based analysis of the data. Description of the data includes visualization of the demographics, average number of follow-ups, genetic data, and various outcomes such as progression to assistive device usage. The AI-based analysis used multiple types of machine learning (ML) algorithms to discover patterns in the data that could have predictive power of disease severity. Once discovered, ML used these patterns to model disease severity in patients and predict eventual outcomes of individual patients. This predictive power could be used clinically to manage new and existing patient expectations as well as in research to assist in understanding the efficacy of a therapy by measuring the deviation of a patient under therapy from the ML model.

P507 Development and evaluation of a whole-body MRI imaging protocol and analysis algorithms to measure changes in skeletal muscle in FSHD

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Objective: Establish a whole-body MRI (WB-MSK-MRI) imaging protocol and analysis algorithms to measure replacement of skeletal muscle tissue by fat in FSHD.

Background: FSHD is caused by aberrant expression of DUX4 resulting in skeletal muscle loss and disability. Clinical severity is strongly correlated with MRI assessments. Fulcrum Therapeutics is developing losmapimod, a selective small molecule inhibitor of p38α/β, to reduce DUX4 expression. An effective treatment for FSHD is expected to reduce or arrest progression of muscle replaced by fat. This biomarker study was performed to evaluate and standardize a WB-MSK-MRI protocol.

Methods: Seventeen adults with FSHD1 age 18 to 65 were enrolled at six sites. Sixteen patients completed the study and were imaged twice, 4 to 12 weeks apart. Volumetric analyses of 18 different individual muscles or muscle groups from shoulder, proximal arm, trunk, and legs were performed bilaterally. Outcomes included muscle fat fraction (MFF), lean muscle volume (LMV), and muscle fat infiltration (MFI).

Results: All but one patient tolerated the repeated MRI protocol well. Measurement of total MFF (r = 0.992), LMV (Pearson's r = 0.998), and MFI (r = 0.985) showed strong correlations between the two time points.

Conclusions: An optimized WB-MSK-MRI protocol feasible for repeated quantification of MFF, LMV, and MFI in most muscles and muscle regions in FSHD has been developed. These MRI measures are currently being used in losmapimod clinical trials.

P508 Establishment of gene-corrected iPSC clones derived from FSHD2 patients with different mutations

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Patient-derived iPSCs can be a useful research resource for investigation of human genetic diseases due to their capacity for unlimited proliferation and controllable differentiation into various kinds of tissues including skeletal muscle, which allows biotechnical processes, such as gene editing, that require long-term and stable cell culture. Mutations in SMCHD1 gene combined with relatively short D4Z4 repeats cause FSHD2, and genetic correction of those mutations would be theoretically sufficient to meet the condition for non-FSHD genetic backgrounds. We applied distinct gene editing strategies to two FSHD2 patients with two different mutations, and successfully obtained transgene-free gene-corrected FSHD2 patient-derived iPSC clones. These clones for both donors, compared to each parental disease clone, showed DUX4 suppression after myogenic differentiation by MyoD overexpression, which models the relationship between genetic backgrounds and DUX4 gene expression in FSHD2, and indicates that these iPSC clones provide a genetically rigid platform for further comparative studies of FSHD pathology.

P509 In vivo detection of DUX4 mRNA using RNAscope in situ hybridization

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Objectives: Our objective is to develop RNAscope to detect DUX4 mRNA in skeletal muscle sections from the TIC-DUX4 mouse model and human FSHD patient biopsies expressing endogenous DUX4.

Results: In mouse muscle sections: TIC-DUX4 mice express Tamoxifen-inducible DUX4 in skeletal muscles in a dose-dependent manner. We found that RNAscope probes can detect low levels of human DUX4 mRNA in muscle sections from TIC-DUX4 animals induced at low Tamoxifen doses.

In human FSHD muscle biopsy sections: In a blinded pilot study, we used DUX4-specific RNAscope probes to stain skeletal muscle cross-sections from three FSHD patients and three unaffected controls. In some sections stained with the DUX4 probe, we found rare myofibers containing RNAscope signal, and also found serial sections from the same biopsies with co-localized TRIM43 probe signal. As of this writing, we are continuing to stain additional sections and have not yet broken the blind, so we do not yet know if the DUX4- and TRIM43-positive signals are present in FSHD affected sections.

Conclusions: We found that RNAscope is a highly sensitive method for detecting low levels of DUX4 mRNA in induced TIC-DUX4 mouse muscle sections. Our preliminary data demonstrate that DUX4 and TRIM43 probes can detect signals in human skeletal muscle sections, but at this point in this blinded study we cannot definitively report whether or not these signals represent DUX4 and TRIM43 expression in vivo.

P510 Investigating HA-dependent mechanisms of DUX4-induced toxicity

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De-repression of the transcription factor DUX4 in skeletal muscle results in the misregulation of multiple cellular pathways leading to cell death, and likely underlies FSHD pathology. While the effects of DUX4 expression have been well documented, how DUX4 protein causes these pathologies remains unclear. Recently, we demonstrated that DUX4 physically interacts with the hyaluronic acid (HA)-binding protein C1QBP, raising the possibility that DUX4-induced pathology may be mediated by an HA-dependent mechanism. Using 4MU, a competitive inhibitor of HA synthesis, we demonstrated that HA is necessary for the pathological effects of DUX4, including DUX4-induced cell death, thereby identifying HA-dependent pathways as potential therapeutic targets for FSHD. We tested this hypothesis by treating DUX4-expressing cells with several inhibitors that target HA-dependent signaling pathways, and surprisingly found that several compounds prevent pathology by reducing the concentration of DUX4 in the cells via a post-transcriptional mechanism. To learn more about the role of HA in pathology, we used RNA-seq to investigate the most altered molecular pathways in DUX4-expressing myoblasts. Strikingly, we found that the spliceosome was the most DUX4-induced molecular pathway and was also the pathway most downregulated in DUX4-expressing, 4MU-treated cells, suggesting an HA-dependent role for the spliceosome in pathology.

P511 Exploring muscle inflammation in FSHD with a multidisciplinary-based approach

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To investigate the presence of muscle inflammation in FSHD, we carried out a case-control study aimed at characterizing the presence of active muscular inflammatory markers integrating clinical, radiologic, and immunological outcomes. FSHD patients underwent a full leg magnetic resonance imaging (MRI) to assess the degree of muscle inflammation, qualitatively evaluated as turbo inversion recovery magnitude (TIRM) hyperintensity. Patients displaying TIRM-positive muscles were selected and gave their permission for both a TIRM-positive MRI guided muscle biopsy and a TIRM-negative MRI guided muscle biopsy. TIRM-positive and TIRM-negative muscle samples, as well as healthy control (HC) muscle samples, were ex vivo challenged for 24 hours with either cell culture medium or with the Toll Like Receptors (TLRs) ligands LPS and Pam3Cys. Forty-three FSHD patients (48 ± 12 years, 50% men, CSS = 5 ± 3) and eight sex- and age-matched HC (43 ± 12 years, 50% men) were included in the study. Twenty-four of 43 FSHD patients displayed at least one TIRM-positive muscle and were selected for an MRI guided muscle biopsy, whereas eight HCs were asked for a vastus lateralis Bergström needle muscle biopsy. FSHD TIRM-positive whole muscle specimens ex vivo stimulated significantly produced higher levels of IL-6 compared to both FSHD TIRM-negative whole muscle specimens and HC whole muscle specimens. Conversely, FSHD TIRM-negative whole muscle specimens did not differ from HC whole muscle specimens when ex vivo stimulated, confirming an inactive disease phase. Our results provide an immunological validation of TIRM hyperintensity as a marker of active disease. This suggests an active involvement of the immune system in FSHD pathology whose understanding, in view to new therapeutic targets, requires a multidisciplinary approach, merging clinicians' and immunologists' expertise, to fully grasp the complexity of FSHD pathogenesis.

P512 Characterization of FSHD patient-derived cells

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Introduction: Expression of DUX4 in skeletal muscle cells results in dramatic alterations in the transcriptome of the cell. This causes a variety of phenotypes and ultimately leads to cell death. There is little understanding of how specific changes in gene expression result in cell stress, death, and the disease process. Understanding how DUX4 expression in a single nucleus can alter the physiology of an entire myofiber is critical to defining the mechanism of DUX4-induced cytotoxicity in FSHD muscle. We propose to perform single nucleus RNA sequencing on nuclei isolated from FSHD patient-derived cells to gain insight into the changes in cellular physiology brought on by sporadic, low-level DUX4 expression.

Methods: Expansion, immortalization, and characterization of FSHD patient-derived muscle cells.

Results: We have characterized DUX4 expression, DUX4 activity, and differentiation capacity of myoblasts isolated from FSHD patients and healthy volunteers. In differentiated FSHD patient-derived cells, DUX4 expression was detected in individual nuclei by immunofluorescence, and DUX4 activity was observed as measured by increased RNA levels of target genes. In cells derived from healthy volunteers, no DUX4 activity was detected.

Conclusion: Our well-characterized cellular system will be a valuable resource for understanding the molecular mechanism underlying the pathophysiology of FSHD.

P513 Utilization of dual-label optical mapping with Bionano genome imaging for FSHD diagnosis

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Short-read exome/genome sequencing (SRS) and chromosomal microarrays (CMA) have helped increase diagnostic rates across many genetic disorders. However, despite this success, disorders such as facioscapulohumeral muscular dystrophy (FSHD) are still challenging to diagnose due to the methodological limitations of SRS and CMA. Both fail to provide the underlying structural context and epigenetic profiles in the repetitive region of the human genome.

These limitations are alleviated with a novel dual-label optical genome mapping (DL-OGM) technology for detection of both genetic and epigenetic changes in one assay. The method relies on differential labeling of high molecular weight DNA. First, long DNA molecules are nicked with BspQI endonuclease and labeled with red fluorescent nucleotides. Second, the same DNA molecules undergo treatment with M.TaqI methyltransferase, which attaches green fluorescent cofactor onto non-methylated CpGs in TCGA sequences throughout the genome. Third, the pattern of fluorescent labels is captured in nanochannel arrays for de novo genome assembly, variant calling, and quantification of epigenetic marks.

Here, we show the ability of DL-OGM to detect large copy number variants and methylation levels for FSHD diagnosis. DL-OGM technology offers substantial advantages over the current clinical diagnostic practice of FSHD diagnosis, which relies on Southern blotting, as it provides both the genetic- and epigenetic-level information in a single assay.

P515 A roadmap to patient engagement in FSHD

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Patient engagement, defined as an active collaboration between patients and the clinicians and scientists involved in research, is a central tenet of the FSHD Clinical Trial Research Network. It can increase recruitment and retention, and also contributes to the relevance of the research. Relatively few resources exist describing the unique engagement features necessary for a rare disease group. Our engagement efforts in FSHD serve as an example, which can apply to other rare disorders.

A multipronged approach to patient engagement was utilized in the FSHD ReSolve trial: 1) a Patient Advisory Council (PAC) provided feedback on clinical trial processes; 2) patient focus groups advised on the study protocol; 3) collaboration with FSHD advocacy organizations was sought; and 4) patient and family days were held.

The PAC made recommendations regarding recruitment and retention such as increasing peer-to-peer sharing about research opportunities and creating a webinar about the study. Patient focus groups provided feedback on patient outcome measures in the study protocol. In response, some outcome measures were added or changed.

Patient engagement in research adds to its value and can increase recruitment and retention. Our engagement efforts give an example of how collaboration with patients and families can be accomplished in FSHD.

P516 Phase 1 clinical trial of losmapimod in facioscapulohumeral muscular dystrophy (FSHD): safety, tolerability, and target engagement

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Objective: Investigate safety, tolerability, pharmacokinetics (PK), and target engagement (TE) of losmapimod in healthy volunteers (HV) and FSHD1 patients.

Background: FSHD is caused by aberrant expression of DUX4 in skeletal muscle. DUX4 activates a transcriptional program resulting in muscle loss and disability. Losmapimod is a selective small molecule inhibitor of p38α/β shown to reduce DUX4 expression.

Design/Methods: Three-part study. Part A: 10 HV randomized to single oral doses of losmapimod (7.5 mg, then 15 mg; n = 8) or placebo (both dosing periods; n = 2). Part B: Parallel-group study of 15 FSHD1 patients randomized to placebo (n = 3), losmapimod 7.5 mg (n = 6), or 15 mg (n = 6) twice daily for 14 days. Part C: Open-label losmapimod 15 mg (n = 5) twice daily for 14 days. Muscle biopsies performed baseline and during treatment, targeting MRI normal-appearing (Part B) and STIR-positive (Part C) muscle tissue. PK and TE assessed in blood and muscle.

Results: Adverse events were mild. PK profiles were similar between HV and FSHD patients: mean Cmax 36.6 (HV) and 40.9 ng/mL (FSHD) for 7.5 mg, and 74.6 (HV) and 85.0 ng/mL (FSHD1) for 15 mg. Dose-dependent concentrations in muscle (42.1 and 63.6 ng/g) observed, with a plasma-to-muscle ratio of approximately 1:1. Dose-dependent TE was measured by HSP27 in blood.

Conclusions: Losmapimod was well tolerated and achieved dose-dependent exposure in plasma and muscle. Target engagement was confirmed. Results support advancing 15 mg dose into Phase 2.

P518 Functional modeling of FSHD1 in hiPSC-derived tissue-engineered skeletal muscles

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While muscle weakness and wasting are hallmarks of FSHD, it is challenging to study these parameters in traditional 2D tissue culture models. For this reason, we modified a transgene-free myogenic differentiation procedure to robustly generate myogenic progenitor cells (MPCs) from human induced pluripotent stem cells (hiPSCs). We obtained fibroblasts from mosaic FSHD1 patients with similar genotypes. Genetically matched controls and FSHD1 hiPSCs were generated and differentiated into multiple MPC lines. The purified MPCs can be expanded up to 5 x 10^11-fold, have sustained differentiation capacity, and form contractile myotubes in 2D cultures. This demonstrated that MPCs are a suitable cell source for the generation of tissue-engineered skeletal muscles (TESMs). With 3D printing we next engineered a silicon chip that, with optimized culture conditions, supported the formation of functional TESMs. To validate our model, we then tested contractile force in TESMs of genetically matched controls and FSHD1 MPCs. We observed a correlation between clinical severity and reduction in force which coincided with DUX4 expression. In conclusion, with this TESM model we can study the muscle function of FSHD patients in vitro as well as the consequences of DUX4 expression on contractile force.

P519 Single-cell transcriptomes in facioscapulohumeral muscular dystrophy

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Objectives: We are using single-cell analyses to characterize 1) infrequent cells expressing DUX4 during distinct stages of myogenic reprogramming, and 2) cellular heterogeneity among FSHD models and acutely isolated cells from affected muscle. We aim to define gene co-expression networks that may suggest novel FSHD therapeutic targets or biomarkers.

Approach: We induced iPSC-derived myogenic lineages from eight individuals (two with early-onset FSHD, three with late-onset FSHD, and three controls) using a gene-free directed differentiation protocol. Single-cell RNA-seq reads were aligned to the reference genome GRCh38, and downstream expression analyses were performed using Seurat v3.1.

Results: Proliferating myogenic progenitors (S1 cells), primary myoblasts (S2 cells), and induced secondary myoblasts (iSM cells) showed distinct single-cell gene expression patterns. S1 cells with two to four D4Z4 repeats (74I and 85I, early-onset phenotype) showed a higher frequency of DUX4 biomarker expression compared to S1 cells harboring five to eight D4Z4 repeats (15A and 17A, adult-onset phenotype). Further studies are in progress to characterize the phenotypes of mononuclear cells acutely isolated from FSHD tissues.

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.

P521 Selection and validation of a p38α/β target engagement assay for measurement of phospho- and total HSP27 in human skeletal muscle

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Objectives: Evaluate p38α/β target engagement (TE) biomarkers and validate an assay for assessment in skeletal muscle in trials of losmapimod in FSHD.

Background: Expression of DUX4 in FSHD skeletal muscle activates a transcriptional program causing skeletal muscle loss and physical disability. Preclinical studies with losmapimod, a small molecule inhibitor of p38α/β, showed reduction of DUX4 in FSHD myotubes. Changes in phosphorylated (p) HSP27, a substrate of p38, were used to detect p38α/β inhibition in clinical trials of losmapimod. Phosphorylation of p38α/β and MK2 were also tested for inhibition of p38α/β activity.

Methods: Assays for the relative quantification of p and total (t) p38α/β, MK2, and HSP27 were developed and evaluated for performance in human myotubes and skeletal muscle biopsy tissue. Formal validation was done for p and t HSP27.

Results: Phosphorylation of p38α/β, MK2, and HSP27 showed responsiveness to losmapimod in FSHD myotubes. However, only the HSP27 assay showed sensitivity in FSHD muscle tissue. The range of detection for the validated assay was 86.8 to 3,318 ng/ml for the pHSP27, and 569 to 5,098 ng/ml for the tHSP27. The pHSP27/tHSP27 ratio was evaluated in eight FSHD subjects with repeated muscle needle biopsies under natural history. The mean change from baseline in this ratio was -0.06 (SD 0.11).

Conclusion: An assay to measure TE in skeletal muscle using pHSP27/tHSP27 was developed and validated, and is being employed in losmapimod FSHD trials.

P522 DUX4 expression in synovial tissue of patients with axial spondyloarthritis

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Axial spondyloarthritis (axSpA) is a rheumatic disease characterized by chronic inflammation of joints of the axial skeleton. A subset of axSpA patients also presents with psoriasis, enthesitis, and sarcopenia. Recently, we found autoantibodies reactive against a fragment corresponding to the DUX4 C-terminal domain in a subset of axSpA patients (18/250).

To study the biological relevance of DUX4 and antibody reactivity in axSpA pathology, we aimed to investigate DUX4 expression in axSpA synovial tissue.

DUX4 mRNA expression in axSpA synovial tissue was shown by RT-PCR and sequencing of the resulting transcripts. Interestingly, different DUX4 transcripts cloned from synovial mRNA of a single axSpA patient contained multiple single nucleotide polymorphisms. Additionally, DUX4 protein was detected in synovial extracts from axSpA patients via Western blot with a mouse monoclonal antibody against the C-terminal domain of human DUX4. Finally, immunohistochemistry showed clear DUX4 signal in cells of the synovial lining layer (cells with a fibroblast morphology) in synovial tissue of three investigated axSpA patients.

So far, presence of the DUX4 protein had not yet been described in synovial tissue, or elsewhere in the context of rheumatic diseases. Further research is necessary to investigate whether synovial expression of DUX4 is involved in axSpA pathology, and whether this might be extended to other tissues involved both in axSpA and FSHD such as skin and muscle.

P523 Using a CRISPR loss-of-function screen to identify therapeutic compounds for facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common myopathies, with an estimated 1 in 8,000 affected. Despite major progress in understanding the underlying genetics behind the pathology, no treatment or cure currently exists. We sought to use a CRISPR-Cas9 loss-of-function screen to identify genes and pathways whose inhibition leads to apoptosis resistance from DUX4, the toxic protein associated with FSHD's pathology. One of the most promising pathways from this screen was the hypoxia signaling pathway, so we explored the potential of compounds that target this pathway as a therapeutic strategy. These compounds, with an emphasis on the mTOR inhibitor everolimus, successfully reduced expression of DUX4 and FSHD biomarkers using in vitro models. Biomarkers were also reduced in vivo during a pilot xenograft study. These results demonstrate the utility of using CRISPR screening to identify novel therapeutic targets for FSHD. Our emphasis on FDA-approved compounds would be a major boon for patients, as any successful candidate would have reduced time in the clinical trial pipeline due to their status.

P524 Is the FSH-COM a reliable measure of physical functioning in children diagnosed with FSHD?

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The complex genetics and recently elucidated pathophysiology of FSHD is paving the way for development of pharmaceutical interventions. To evaluate efficacy of treatments and optimize clinical care, we require reliable outcome measures. Current evidence of the clinometric properties of physical function measures in children is sparse and of low quality. The FSH-Composite Outcome Measure (FSH-COM) is a performance-based measure of physical function with evidence supporting its use in adults (22-70 years). This cross-sectional prospective study investigates test-retest reliability of the FSH-COM in a group of children (7-18 years) with genetically confirmed FSHD. Data were collected from 15 children, mean age 12.5 ± 3.17 (7-18), males n = 10 (67%), mean FSHD clinical score 5.3 ± 3.49 (2-13). Measurements were collected by a single physiotherapist on two occasions, average test interval 14.9 (range 7-22) days. Intraclass correlation coefficients (ICC2,1) were estimated as 0.995 (95%CI 0.985-0.998) with standard error of measurement (SEM) 1.25 points (95%CI 0.91-0.97) and minimal detectable change (MDC90) 2.92 points (95%CI 2.1-4.6). Results indicate excellent intra-rater reliability, low error, and suggest a >3 point change in total score represents real change in function. These findings provide evidence to support the reliable use of the FSH-COM in children diagnosed with mild to severe FSHD. Research to evaluate inter-rater reliability, validity, and responsiveness in a larger sample is required.

P525 Safety, stability, and efficacy validation of oligonucleotides for an FSHD therapeutic

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There are currently no FDA-approved treatments for facioscapulohumeral muscular dystrophy (FSHD) despite a validated target in DUX4. As a transcription factor, blocking DUX4's activity has proven to be particularly difficult with standard small molecule or protein-based inhibitors. RNA therapeutics offer a viable strategy for direct repression of transcription factor expression; however, delivery remains a significant challenge. miRecule's technology combines two innovative elements for RNA therapeutics that can address and overcome this obstacle. First, our patented chemistry allows us to create highly stable RNA therapeutics, as well as improve safety by reducing non-specific activation of the innate immune response. Second, our Muscle-NAVTM platform is designed to deliver RNA therapeutics via antibody targeting to a muscle-expressed receptor. Here, we describe development of an anti-DUX4 RNA formulated into Muscle-NAV (MC-DX4) for the treatment of FSHD. In order to screen a library of RNAs targeting DUX4, we developed a fluorescent cell reporter system that detects DUX4 expression for high-throughput screening in myotubes. Effective RNAs were tested for stability in human serum and inflammatory response via PBMC cytokine release. Finally, we confirmed lead RNA candidates' ability to knock down DUX4 and downstream genes via qPCR in patient myotubes. Through this screen, we have selected three lead compounds for testing of efficacy and safety in animal models.

P526 Searching for genetic modifiers of FSHD severity in a large Utah kindred

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In 1950, Frank Tyler and Fayette Stephens described the segregation and disease severity of FSHD in a pedigree of 1,249 individuals descended from a pioneer family who emigrated to Utah in the 1850s. It was clear from this early study that severity and onset were highly variable among parent, offspring, and affected siblings. In the 1990s, Mark Leppert and Kevin Flanigan demonstrated that a 6 repeat unit in the D4Z4 locus was the causal mutation, and the 6 repeat contraction was stably transmitted to distantly related branches of this kindred.

Here, we are re-ascertaining members of this kindred to test the hypothesis that severity and onset are modified by common variants discoverable by genome-wide association. High-density genotyping and whole genome sequencing from distantly related individuals in the kindred have identified a tightly linked set of 4qter SNPs that tag the founder mutation haplotype. To determine penetrance, severity, and onset of disease in members of this kindred, we are digitizing original notes from encounters over the last 70 years and using targeted outreach to recruit new family members into this study for updated phenotypic measures. New and updated phenotype assessment, combined with genome-wide SNP genotyping from archival and newly obtained samples, will be used to identify genetic modifiers of penetrance, onset, or clinical severity. Identification of genetic modifiers may provide targets for development of new therapies.

P527 Molecular responses to aerobic exercise training in a murine model of FSHD

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Background: Exercise is known to improve skeletal muscle health and function in animals and humans. However, the role of exercise as a treatment for FSHD is poorly understood. We aimed to characterize the effects of aerobic exercise on grip strength, oxidative stress, and muscle architecture in a mouse model of FSHD (FLExDUX4) and wild-type (Wt) mice.

Approach: Five-month-old FLExDUX4 (16 male, 16 female) and Wt (seven male, eight female) mice were randomly assigned to voluntary wheel running or no-wheel-running control for six weeks. Outcomes included: daily running distance (VitalView Software), grip strength (isometric force transducer), and muscle fibrosis (Masson's trichrome staining).

Results: FLExDUX4 mice demonstrate significantly reduced grip strength ($p < .005$), and elevated fibrosis ($p < .001$) relative to Wt mice at baseline. Both FLExDUX4 and Wt mice increased their running distance ($p < .001$) and running velocity ($p < .001$) during training, with female FLExDUX4 mice running significantly farther and faster than FLExDUX4 male mice ($p < .001$, $p < .01$, respectively). Forelimb grip strength significantly increased in FLExDUX4 mice with training ($p < .001$). Exercise had no effect on fibrosis.

Conclusion: Aerobic exercise improves muscle function and running performance in FLExDUX4 mice independent of changes in muscle pathology. Our data show beneficial effects of aerobic exercise in the FSHD-like mouse model.

P528 Clinical and genetic features of patients with facial-sparing facioscapulohumeral muscular dystrophy

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Facial-sparing scapular myopathy (SHD) is the most common atypical form of facioscapulohumeral muscular dystrophy (FSHD), clinically defined as without apparent facial muscle weakness on neurological examination. The clinical profiles and genetic features of SHD are limited. A cohort of 21 Chinese patients with SHD were confirmed by molecular genetic analysis based on pulsed-field gel electrophoresis. The clinical assessments and methylation analysis were noted. The patients had FSHD-related EcoRI fragments with 4qA haplotype ranging from 18 kb to 33 kb (mean 26.3 ± 4.6 kb). The mean onset age was 25.52 ± 8.3 years. More than half of the patients had scapular winging and asymmetry weakness consistent with FSHD, without facial symptoms during their visit. Their facial electromyogram results showed almost normal or mild myogenic damage, as well as the myopathology and serum creatine kinase. However, a conflict was unexpectedly found in intergenerational DR1 methylation analysis. In summary, facial-sparing scapular myopathy is characterized as mild myopathic symptoms and chronic progression of weakness. The diagnosis should be accurately confirmed through FSHD-sized fragment detection and 4qA/B variant determination. Although the next generations of SHD had more severe muscular symptoms, local hypomethylation within D4Z4 was not found as a modifier for clinical heterogeneity.

P529 Determination of the major DUX4/4c partner: the multifunctional C1qBP protein

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The DUX4 transcription factor encoded by the FSHD causal gene activates a gene deregulation cascade explaining most FSHD features. Its homologue DUX4c shares 342/374 amino acid residues. DUX4c is expressed at low levels in healthy muscle and is upregulated in FSHD muscle cells and biopsies. In order to unveil the DUX4/4c pathological role, we identified their protein partners. Most of them are RNA-binding proteins, suggesting a transcription-independent role of DUX4 that could potentially be involved in unexplained FSHD features.

Using an XL-MS approach, we found specific crosslinks between C1qBP and DUX4c in homeodomain 2 shared with DUX4. In addition, 71 MS measurements using MAX-Quant (six independent purifications) revealed 220 proteins as putative partners of DUX4/4c in significant quantities. Among them, we found that C1qBP was the main DUX4/4c partner. C1qBP is essential for cell metabolism and survival, while it is also implicated in several biological processes known to be altered in FSHD muscles, a.o. oxidative stress and RNA splicing. Despite its key role in human cells, its function in skeletal muscles remains unclear.

Overall, DUX4/4c-C1qBP interaction could be part (at the protein level) of molecular mechanisms leading to FSHD pathophysiology. Moreover, many therapeutic strategies have already been developed to target C1qBP dysfunctions (in cancer or mitochondrial disorders). This could be useful for the rational design of FSHD polytherapy.

P531 Design of an open-label pilot study of losmapimod to evaluate the safety, tolerability, and changes in biomarker and clinical outcome assessments in subjects with facioscapulohumeral muscular dystrophy 1 (FSHD1)

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by loss of repression at the D4Z4 locus, resulting in aberrant expression of the homeobox transcription factor DUX4. DUX4 expression activates its downstream transcriptional program, resulting in cell death, skeletal muscle loss, and progressive motor disability. Fulcrum Therapeutics is developing losmapimod to treat FSHD at its root cause. Losmapimod is a potent and highly selective small molecule inhibitor of p38α/β that reduces DUX4 activity and its downstream transcriptional program in FSHD myotubes, resulting in the prevention of cell death without impacting myogenesis. Losmapimod has been tested across many adult indications, resulting in more than 3,500 human exposures, and has shown satisfactory safety and tolerability. The hypothesis is that treatment of FSHD with losmapimod will slow or arrest disease progression by reducing aberrant DUX4 expression via inhibition of p38α/β MAP kinase. Fourteen genetically confirmed FSHD1 adult subjects were enrolled at Radboud University Medical Center. Subjects will participate for up to 64 weeks including an eight-week pre-treatment period followed by a 52-week treatment period. We will investigate the safety, tolerability, pharmacokinetics, and target engagement, and explore the treatment effects on select molecular and imaging biomarkers, various objective and subjective clinical outcomes, and real-world mobility assessments with wearables. We will present the design and baseline characteristics of this study.

P532 Muscle phenotype of the single transgenic FLExDUX4 mice

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The FLExDUX4 mice express DUX4 mRNA at a very low level (leaky transgene), before they are crossed to a Cre line. The purpose of this study is to examine muscles of the single transgenic FLExDUX4 at different ages to determine whether the muscle phenotype changes over time. We studied mice at two months, four months, eight months, and 12 months of age, and measured several phenotypic parameters, including body weight, muscle weights, muscle fiber size, pathology, and muscle strength. Our results showed that the body weight of the FLExDUX4 stopped increasing after four months of age, while the wild-type littermates continued to grow. The muscles of the FLExDUX4 mice were significantly smaller than their wild-type littermates. Male mice showed weight difference earlier than the female mice. Functional testing using grip strength measurements showed significant muscle weakness in both female and male mice. Pathological examinations showed age-dependent changes, including smaller type IIx fibers at two months and onward, and lesions in type IIB myofibers in the 12-month-old FLExDUX4 mice. The study shows progressive changes of muscle phenotypes in the FLExDUX4 mice, which should be considered when the mouse model is used for testing therapeutic agents.

P533 Novel coincidence: FSHD case with myeloproliferative disorder

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In addition to muscle involvement, extramuscular involvements also present in facioscapulohumeral muscular dystrophy (FSHD). We report a novel coincident FSHD case with essential thrombocythemia (ET).

A male FSHD case was diagnosed at age 17 with difficulty in elevating his arms. He had 4q35 D4Z4 repeat contraction. When the patient was 67 years old, he was admitted to the hematology clinic with facial redness and increased platelet count (1,200,000/mm 3) without hepatosplenomegaly.

Since myeloproliferative neoplasms (MPNs) are frequently related to JAK2, MPL, and CALR gene somatic mutations, the patient's blood sample was analyzed for the hot-spot mutations of these genes. There were no mutations in MPL and CALR target regions. In the exon 14 region of the JAK2 gene p.V617F (c.1849G> T), mutation was detected with 28% allele burden.

The presence of JAK2 p.V617F mutation confirmed the diagnosis of ET. There has been no report on the coincidence of FSHD with MPN, and this is the first case in the literature. Recently, a number of reports indicated that some of the germline DNA variants may predispose to MPN with JAK2 p.V617F mutation. Since JAK signaling has important functions in the development of both skeletal muscle and myeloid cells, it is possible that a common mechanism may be responsible for FSHD and MPN. Considering this association between JAK signaling and muscle tissue, we propose that this co-indication is an important focus in the role of the JAK pathway in FSHD pathophysiology.

P535 Living with FSHD during the pandemic coronavirus outbreak: pitfalls and challenges of COVID-19 in FSHD

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As the COVID-19 virus is rapidly spreading around the world, all aspects of our lives have been heavily influenced. While the impact of this crisis on patients facing chronic diseases such as hypertension, diabetes, and cardiovascular diseases seems considerable, the implications for such a complex and chronic disease like facioscapulohumeral muscular dystrophy (FSHD) are less evident. FSHD pathogenesis is only partially understood, as well as the possible role played by the immune system. Likewise, the influence of the COVID-19 pandemic on patients with FSHD is obviously unclear. This concerns the direct effect of the infection on the FSHD phenotype as well as the indirect effect of the COVID-19-related stress. Therefore, we designed a survey-based prospective case-control study which aims at longitudinally monitoring the incidence of COVID-19 infections and the effects of coronavirus-related stress in all patients enrolled in the Dutch National FSHD Registry. Patients' housemates are taken as the control group. The survey will be filled out every 12 weeks and contains partly standardized questions covering four main aspects: 1) household living arrangement; 2) COVID-19 preventive measures; 3) experienced COVID-19-related stress in multiple scenarios; and 4) COVID-19-related symptoms and related management. The study is currently ongoing and will provide new relevant unique data that will help clarify which consequences COVID-19 represents for FSHD patients.

P536 Relationship of DUX4 and target gene expression in FSHD myocytes

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Facioscapulohumeral dystrophy (FSHD) is the third most common muscular dystrophy and is associated with upregulation of DUX4, a transcription factor, and its target genes. Although target genes are easily detectable in FSHD, low-frequency DUX4 upregulation in patient myocytes is difficult to detect, and examining the relationship and dynamics of DUX4 and target gene expression without artificial overexpression of DUX4 has been challenging. Recently, single cell/nucleus RNA-sequencing was used to detect the native expression of DUX4, but spatial relationships with its target gene expression were missing. Using RNAscope® in situ hybridization with highly specific probes, we detect the endogenous DUX4 and target gene transcripts in situ in patient skeletal myotubes during differentiation in vitro. Our study reveals a unique DUX4 expression pattern and its relationship to the expression of target genes, and evidence for self-sustainability of the target gene network. The study provides important new insights into dynamics of the DUX4 transcriptional network in FSHD patient myocytes.

P537 qPCR-based approach for a FSHD1 diagnostic and its use for studying Russian population

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Introduction: Currently, the FSHD diagnostic is quiet, laborious, and expensive. We developed a new, simplified qPCR-based diagnostic approach and implemented it for studying the Russian population.

Materials and Methods: In total, 64 FSHD patients and 40 phenotypically healthy relatives were included in the study. The Southern blotting, molecular combing, and a newly developed qPCR-based approach were used for the FSHD diagnostic.

Results: The developed qPCR-based approach was validated using blotting and molecular combing results of 29 patients and 13 unaffected relatives. We observed 93% concordance of D4Z4 array sizing and complete concordance of 4qA haplotyping. FSHD1 was confirmed for 37 (57.81%) patients. We observed enrichment (64.9%) of the permissive allele with number of units between 3 and 6 in patients. In a group of 40 healthy relatives we diagnosed 13 (32.5%) carriers. Among carriers, 84.62% have the permissive allele with number of the D4Z4 units from 6 to 8.

Conclusions: We developed a qPCR-based FSHD1 diagnostic and validated it. To our knowledge, this is the first study of permissive 4qA alleles in a Russian population. Based on the results, the Russian population is similar to that previously reported in the distribution of permissive alleles. Observed difference of the permissive alleles between patients and carriers indicates the phenomenon of inverted correlation between permissive allele size and disease severity, penetrance, and age of onset.

P538 The socioeconomic burden of facioscapulohumeral muscular dystrophy

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Introduction: Several clinical trials in facioscapulohumeral muscular dystrophy (FSHD) are currently ongoing. Evaluation of cost-effectiveness of new treatments requires knowledge on the societal costs of a specific disease.

Objective: To determine the socioeconomic burden of FSHD.

Methods: FSHD patients from the Dutch FSHD registry were invited to complete a questionnaire on medical consumption, work productivity, and health-related quality of life (HR-QoL) using the EQ-5D-5L. A willingness to pay for a quality-adjusted life-year of €50,000 was used in the analysis.

Results: A total of 172 patients completed the questionnaire (response rate 65%). The per-patient annual direct costs of illness were estimated to be €14,282, which is three times higher than the mean per-person health expenditures in the Netherlands. Informal care and transport accounted for another €6,974. A considerable loss of productivity was observed in FSHD patients, accounting for €5,066 of indirect costs of illness. HR-QoL was significantly reduced in FSHD patients with a median health index of 0.625. The monetary value of the loss in QoL was estimated to be €14,528. Total socioeconomic burden was calculated to be €40,850 per FSHD patient per year.

Conclusions: We show that FSHD is associated with considerable direct and indirect socioeconomic costs. These findings are important for health care decision makers, and aid in the evaluation of the cost-effectiveness of intervention programs and novel therapies.

P539 Analysis of genes regulated by DUX4 via oxidative stress reveals potential therapeutic targets for FSHD treatment

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Sporadic DUX4 expression and oxidative stress are the features of FSHD muscles, but clinical trials testing the effect of antioxidant treatment in FSHD had a limited impact on patients. The exact role of oxidative stress in the FSHD pathology and its interplay with DUX4 are not yet clear. Immortalized human myoblasts expressing DUX4 (MB135-DUX4) exhibit higher levels of reactive oxygen species (ROS) than normal MB135 myoblasts and do not differentiate properly, while antioxidant treatment efficiently restores their differentiation capacity. As direct antioxidant treatment is ineffective in clinics, we set up a screen for genes deregulated by DUX4 via oxidative stress with the aim to target these genes rather than the oxidative stress itself. The transcriptome analysis of antioxidant-treated MB135 and MB135-DUX4 myoblasts revealed 182 genes deregulated by DUX4 but normalized upon antioxidant treatment. Muscle tissue development was the most significant overrepresented term, with 10 genes falling into this category including PITX1, the gene previously linked to FSHD. Another enriched category was cellular response to inorganic substances (seven genes) represented by the genes encoding for metallothioneins (MTs). Here we explored the relevance of PITX1 and MTs to the oxidative stress-induced pathology in FSHD. We revealed that PITX1 is regulated by oxidative stress, and have shown that the silencing of this gene partially restored the differentiation capacity of MB135-DUX4 myoblasts.

P540 A homozygous nonsense variant in LRIF1 associated with facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) results from the attenuation of a repressive epigenetic landscape at the 4q D4Z4 repeat array. This is due to either a D4Z4 repeat contraction (FSHD1) or to mutations in D4Z4-chromatin associated proteins (FSHD2). While both genetic situations lead to misexpression of repeat-encoded DUX4 gene in skeletal muscle, a characteristic difference between FSHD1 and FSHD2 is the level of methylation at D4Z4. While D4Z4 hypomethylation in FSHD1 is restricted to the contracted allele, in FSHD2 both 4q D4Z4 alleles show reduced methylation. To date, only two genes, namely SMCHD1 and DNMT3B, have been identified as FSHD2 disease genes; however, some clinically suspected FSHD cases remain genetically unexplained. To identify new candidate genes, we combined bisulfite pyrosequencing of D4Z4 as an FSHD2 marker with whole exome sequencing in repeat contraction-negative FSHD cases and uncovered in one individual a homozygous loss-of-function variant in LRIF1. LRIF1 is a strong candidate gene for FSHD2, as it is known to interact with SMCHD1. This mutation resulted in the absence of the long isoform of LRIF1 and was associated with D4Z4 chromatin relaxation, and DUX4 and DUX4 target gene expression in proband cells. In concordance, we found that LRIF1 binds to the D4Z4 repeat and that knockdown of the LRIF1 long isoform in myoblasts results in DUX4 and DUX4 target gene expression. We conclude that LRIF1 is a bona fide disease gene for FSHD2.

P541 Interaction between mesenchymal stem cells and myoblasts in the context of FSHD

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We have previously identified the CXCR4 (C-X-C motif Receptor 4) and CXCL12 (C-X-C motif ligand 12 also known as SDF1) as DUX4 targets[1]. DUX4 overexpression in myoblasts induced migration of human bone marrow-derived mesenchymal stem cells (BMSCs)[1]. We have now studied this phenomenon and its consequences in the context of FSHD. We have shown that myoblasts from FSHD patients induced migration of BMSCs via the CXCL12-CXCR4 axis; they also stimulate BMSC proliferation. The presence of BMSCs in the myoblast culture inhibited myotube formation. FSHD myoblasts were also shown to stimulate expression of collagen, a factor provoking fibrosis in FSHD muscles, in BMSCs. These data explain several important aspects of FSHD pathophysiology and may potentially lead to development of new treatment approaches.

[1]Dmitriev et al. *Oncotarget* 2016 Oct 4;7(40):65090-65108.

P542 A validation of optical mapping for the molecular diagnosis of facioscapulohumeral muscular dystrophy (FSHD)

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The molecular diagnosis of FSHD relies on detecting contractions of the unique D4Z4 repeat array structure of the chromosome 4q35 locus in the presence of a permissive 4q35A haplotype. This unusual molecular mechanism begs for analysis of intact long DNA molecules for accurate sizing of D4Z4 repeat alleles. We validated an optical mapping assay to determine size and haplotype of D4Z4 alleles for FSHD analysis. The cohort included 40 unique DNA specimens from fresh blood samples or archived agarose plugs. High-molecular-weight DNA underwent sequence-specific labeling with DLE-1 enzyme followed by data collection and analysis on the Bionano Saphyr system. D4Z4 allele sizes were calculated and haplotypes determined from the DLE-1 label pattern. Additionally, each specimen underwent separate restriction enzyme digests with EcoRI, EcoRI/BlnI, and XapI, followed by pulse gel electrophoresis and Southern blot analysis with appropriate probes. Optical mapping detected alleles ranging from 1 to 79 D4Z4 repeats and showed strong correlation with Southern blot for allele sizing (R^2 = 0.95) and haplotyping (167/168, 99.4% haplotype match). Analysis of inter- and intra-assay runs showed extremely high reproducibility for optical mapping data (0.03-0.94 %CV). Optical mapping is an accurate and highly reproducible method for determining D4Z4 allele size and haplotype in FSHD analysis, with added benefits of smaller specimen requirements and faster turnaround time compared to Southern blot.

P543 Role of NR boxes 1 and 2 on the co-repressor activity of DUX4 on the human progesterone nuclear receptor

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Misexpression of DUX4 in muscle cells is associated with the pathogenesis of FSHD. Previous results from our laboratory demonstrate that DUX4 is a strong co-repressor of the progesterone (PG) nuclear receptor (NR). In this work we analyze the contribution of two NR box motifs (NR1 and NR2), in silico recognized in DUX4 and ubiquitously present in co-regulators of hormone NRs, on the NR co-repressor activity of DUX4. A PG responsive reconstituted experimental system, based on cultured HEK293 cells, was developed for these studies. As it was previously observed in T47D and HepG2 cells, the activity of the PG NR was strongly repressed by DUX4 in HEK293 cells. DUX4 mutants DUX4-ΔNR1 and DUX4-ΔNR2, as well as DUX4-NR1s and DUX4-NR2s, carrying either deletions (Δ) or amino acid substitutions (s) at NR box 1 (LDELL, amino acids 374 to 378) and/or NR box 2 (LLEEL, amino acids 420 to 424), were also studied in HEK293 cells. The mutants were independently co-transfected into HEK293 cells together with plasmids expressing the PG NR plus a standard luciferase-reporter system (MMTV-Luc/Renilla). A marked decrease in the co-repressor activity of DUX4, on the PR NR, was observed with mutants DUX4-ΔNR2 and DUX4-NR2s. In additional studies we observed that DUX4c, which conserves NR1 and lacks NR2, does not co-repress the PG NR. We concluded that NR box 2 is required for the strong co-repressor activity of DUX4 on the PG NR in HEK293 cells.

P544 Facioscapulohumeral muscular dystrophy type 1 (FSHD1) in Argentina: a molecular genetics-based epidemiological study

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Facioscapulohumeral muscular dystrophy (FSHD) develops following a complex interplay of genetic and epigenetic events. FSHD1 is associated with deletion of an integral number of 3.3 Kb (D4Z4) repeated elements at 4q35, a specific 4q subtelomeric haplotype (4qA), and decreased methylation of cytosines at the remaining D4Z4 units. In this work we used standard pulse field gel electrophoresis (PFGE) and a chemiluminescence method to analyze D4Z4 allele sizes as well as their associated pLAM 4qA and 4qB haplotypes. A total of 156 DNA samples, corresponding to individuals from Latin America having a clinical diagnosis of FSHD, were studied using digoxigenin-labeled probes p13E11, 4qA, and 4qB. Shortened D4Z4 alleles were observed only in 55% of the samples, suggesting that a large number of the patients received a wrong clinical diagnosis of FSHD. Characterization of the 4qA and 4qB haplotypes in short D4Z4 alleles (i.e., 1-10 units) showed that all of them carry the 4qA haplotype. A patient showing a normal D4Z4 allele plus a permissive 4qA haplotype was found to carry a c.3425G>C mutation on the SMCHD1 gene, thus confirming a FSHD2 diagnosis. The population of characterized D4Z4 alleles (n = 312) corresponds to 1-4 units (21%), 5-7 units (14%), 8-10 units (20%), and ≥11 units (45%). This work represents the first molecular characterization of D4Z4 alleles and 4q-pLAM (4qA and 4qB) haplotypes in Latin America.

P545 A retrospective analysis of CLIA laboratory testing for facioscapulohumeral dystrophy (FSHD)

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The results of FSHD1 and FSHD2 testing have not been reported in a large, clinical laboratory-derived patient sample. Here we summarize the University of Iowa Molecular Pathology Laboratory data from January 2015 to July 2019. Testing was by restriction enzyme digestion and Southern blot analysis with sequencing of SMCHD1, if indicated. Cases were classified as FSHD1 (4q35 EcoRI size ≤40kb; 1-10 D4Z4 repeats), FSHD2 (permissive 4q35A allele, D4Z4 hypomethylation, and pathogenic SMCHD1 variant), or non-FSHD1, 2. Of the 1,594 FSHD tests included in the analysis, 707 (44.3%) were diagnosed with FSHD. Among these positive tests, 664 (93.9%) met criteria for FSHD1, and 39 (5.5%) met criteria for FSHD2. Four additional cases (0.6%) met criteria for both FSHD1 and FSHD2. Twenty cases (1.3% of total cases) had a 4q35A allele of borderline size (11 D4Z4 repeats), 23 (1.5%) were somatic mosaics, and 328 (20.9%) had undergone translocation events. Among FSHD1 cases 16% had 1-3 D4Z4 repeats, 48% had 4-6 repeats, and 35% had 7-10 repeats. Fifty-one percent of FSHD2 cases had 11-15 repeats, 18% had 16-20 repeats, 18% had 21-25 repeats, while the remaining 13% were larger. The frequencies of specific FSHD subtypes and the complexities involved in genetic diagnosis in this large dataset will be useful in transitioning to new diagnostic testing platforms and in planning future clinical trials.