30th Annual FSHD Society
International
Research Congress

Where the world's leading clinicians, scientists, companies, and advocates gather to accelerate research toward treatments and a cure

June 15-16, 2023

Radisson Blu Hotel

Milan, Italy

Wi-Fi network:
RADISSON-GUEST

#FSHDConnect

#CureFSHD

Tag @FSHDSociety
in your posts

View the Abstracts

FSHD
SOCIETY
LIGHTING THE WAY TO A CURE

FSHD SOCIETY
LIGHTING THE WAY TO A CURE

WE THANK OUR GENEROUS SPONSORS:

AVIDITY BIOSCIENCES

Fulcrum Therapeutics

Dyne THERAPEUTICS

Roche

VITA Therapeutics

miRecule RNA Therapeutics

JAMES CHIN, SR., SCHOLARSHIP FUND

ARMATUSBIO

KATE THERAPEUTICS

The Chris Carrino Foundation for FSHD

AFMTÉLÉTHON CURE THROUGH INNOVATION

SANOFI

MDA Muscular Dystrophy Association

NIH National Institutes of Health

FSHD STICHTING

UMMS Wellstone Center for FSHD

FSHD

ultragenyx

FSHD Global Research Foundation Ltd

SPEAKER ABSTRACTS

Session 1: Discovery Research & Genetics

S1.01

FSHD-like muscle pathology in a mouse *Dux* inducible transgenic mouse model

Darko Bosnakovski[1], Ana Mitanoska[1], Natasha Santosh[1], Jasmine Gulik[1], Ahmed S. Shams[1], Michael Kyba[1], David Oyler[1]

[1] *University of Minnesota*

FSHD is caused by ectopic expression of the *DUX4* in muscles. The *DUX* gene family comprises 3 clades: A, B, C, of which *DUX4* is the human and *Dux* is the mouse representative of the *DUXC* clade. Despite the tremendous progress achieved in understanding the genetic components of FSHD, we know very little about cellular events that contribute to the dystrophic muscle phenotype. Therefore, animal models are the most suitable models to study complex tissue pathology in this disease. Several transgenic mouse models, including iDUX4pA, based on *DUX4* expression in myofibers, have previously been developed to study FSHD. Despite the similarities between these animal models and FSHD pathologies, there is a degree of uncertainty regarding the relevance of studying FSHD by expressing a human gene (*DUX4*) in a mouse. The concern is that divergence between Dux and *DUX4* might lead to induction of different molecular and cellular responses by each gene when expressed in the other species. To address this issue, we generated the iDux;HSA mouse designed to tunably express the Dux gene in skeletal muscles. Initial studies revealed that Dux expression in skeletal muscles provokes similar pathological alterations as seen with *DUX4*. High levels of Dux induce rapid myofiber damage followed by muscle infiltration with fibroadipogenic progenitors and inflammatory cells, leading to muscle fibrosis and impaired muscle function. These data show that in spite of differences in some of the target genes induced by *Dux* and *DUX4*, the two proteins drive the same fundamental pathological pathway in vivo. We will present current phenotyping results and perspectives on utilizing mouse models to study FSHD.

S1.02

Activation of the germline transcription factor *DUX4* is essential for herpesvirus replication

Florian Full[1], Eva Neugebauer[1], Jiang Tan[1], Emanuel Wyler[2], Vedran Franke[2], Stephanie Walter[3], Bettina Henzi[4], Armin Ensser[3]

[1] *Institute of Virology, University Medical Center, Freiburg, Germany*
[2] *Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine, Helmholtz*
[3] *Institute for Clinical and Molecular Virology, University Hospital Erlangen, Erlangen, Germany*
[4] *Department of Neuropediatrics and Muscle Disorders, Medical Center - University of Freiburg, Faculty*

The pioneer factor *DUX4* is a master regulator of zygotic genome activation (ZGA) and the aberrant expression of *DUX4* in muscle cells is the cause of FSHD. During early embryogenesis, ZGA is crucial for maternal to zygotic transition in order to overcome silencing of genes and enable transcription from the zygotic genome. Afterwards, *DUX4* expression is normally silenced in adult somatic cells. We found *DUX4* expression upon lytic replication of all subfamilies of herpesviruses, but not of adenoviruses, negative strand RNA viruses or positive strand RNA viruses. Herpesviruses cause a variety of diseases in humans and can be especially dangerous for immunocompromised patients. RNA-Seq analysis showed that *DUX4* expression in herpesviral infection leads to the activation of hundreds of *DUX4* target genes as well as endogenous retroelements. The analysis of single cell sequencing datasets from herpesvirus-infected individuals confirmed that herpesviral activation of *DUX4* is also of relevance in vivo. CUT & Tag experiments showed that *DUX4* directly binds to the virus genome upon infection and thereby supports viral gene expression. Accordingly, CRISPR/Cas9 mediated knockout of *DUX4* abrogates viral gene expression and prevents viral replication. Our data show that herpesviruses induce an embryonic-like state in infected cells, mediated by the germline transcription factor *DUX4*. This prevents silencing of the viral genome and supports viral gene expression.

S1.03

SIX transcription factors promote differentiation-dependent activation of *DUX4* expression in FSHD

Amelia Fox[1], Fran Sverdrup[1]

[1] *Saint Louis University School of Medicine*

Facioscapulohumeral muscular dystrophy (FSHD) is a progressive muscle degenerative disease caused by the epigenetic de-repression of D4Z4 repeat units, leading to the aberrant reactivation of *DUX4*. *DUX4* reactivation leads to disruption of signaling pathways and toxicity in skeletal muscle. Identification of key epigenetic and transcriptional regulators that control the reactivation of *DUX4* transcription is imperative to understanding the disease mechanism and to better direct potential FSHD therapeutic strategies. Using an siRNA screening approach, we discovered a family of transcription factors that promote *DUX4* transcription in FSHD: SIX1, SIX2, and SIX4. Knocking down all three SIX proteins in combination prior to differentiation resulted in suppression of *DUX4* and *DUX4* target mRNA levels by ~98% in myotubes, with SIX2 having the greatest effect individually. The siRNA knockdowns of SIX1, SIX2, and SIX4 did not inhibit myotube differentiation, in vitro. In proliferating myoblasts, we were not able to suppress *DUX4* with siRNA-mediated SIX1,2,4 knockdown, suggesting that SIX proteins control *DUX4* transcription in a differentiation-dependent manner. Interestingly, exogenous *DUX4* expression resulted in decreased SIX RNA levels, suggesting the possibility of negative feedback regulation. In summary, our results suggest that SIX transcription factors are key regulators of *DUX4* expression in muscle and potentially other tissues in FSHD.

S1.04

Long-read sequencing reveals novel transcripts induced by misexpression of *DUX4* in FSHD muscle

Dongxu Zheng[1], Anita van den Heuvel[2], Ahmed Mahfouz[1], Sean Bennett[3], Stephen Tapscott[4], Silvère van der Maarel[1]

[1] *Leiden University Medical Center*
[2] *University Utrecht*
[3] *Fred Hutchinson Cancer Center*
[4] *University of Washington/Fred Hutchinson Cancer Center*

Misexpression of *DUX4* is considered the major cause of Facioscapulohumeral muscular dystrophy (FSHD) as it activates a cascade of transcriptional events leading to muscle wasting. *DUX4* is known to regulate not only protein-coding genes, but also several classes of repetitive elements, and is known to affect RNA processing. Only using data from short-read sequencers limits our understanding of the transcriptional events provoked by *DUX4*. We combined Pacbio long-read isoform sequencing with short-read RNA-seq of *DUX4*-inducible (*DUX4i*) myocytes to investigate the full-length transcriptome landscape inflicted by DUX4. We observed a more intricate transcriptional landscape after overexpressing *DUX4*, which was distinguished by the emergence of novel isoforms for known genes, and a considerable number of extensive alternative splicing events. Additionally, *DUX4*-dependent transcriptional activation of 652 intergenic loci was identified, which was verified by bulk RNA-seq data of primary myotubes, and embryonic scRNA-seq data. Analysis of public *DUX4* ChIP-seq data and ATAC-seq data of *DUX4*i myoblasts and human embryonic stem cells, respectively, revealed 361 intergenic loci to be directly regulated by *DUX4*. Intergenic loci with predicted coding transcripts could be confirmed in Ribo-seq data of *DUX4*i myoblasts, indicating that these novel intergenic transcripts are translated into novel polypeptides. Taken together, our study elaborates on the transcriptional features induced by *DUX4* and reveals unannotated transcripts at transcriptome and translatome levels. These can be considered potential biomarkers for disease diagnosis, progression, and therapeutic intervention in FSHD.

S1.05
Candidate Circulating Biomarkers for FSHD
Joel Chamberlain[1]

[1] University of Washington School of Medicine

Identification of disease-associated biomarkers is typically approached in 2 different ways, either discovery-based or hypothesis-driven discovery. We have chosen a hypothesis-driven route focused on the involvement of innate immunity in the pathogenesis of FSHD. The association of innate immunity with FSHD was first identified in FSHD myoblasts grown in the laboratory and further supported by an innate immunity expression signature observed in transgenic mouse models. In a complementary mouse model of disease, that we developed using adeno-associated virus (AAV) expressed DUX4, distinct aspects of the immune system response are present. AAV-DUX4 local injection of wild type mouse muscle produces dose-dependent expression of DUX4 for either extended low-level focal expression or more widespread expression leading to larger areas of muscle damage. We hypothesized that early DUX4 muscle damage could be propagated by immune system signaling. From RNAseq studies of AAV-DUX4 injected muscle we identified the GO biological process of 'neutrophil chemotaxis' and proceeded to examine circulating by-products of neutrophil activation in FSHD blood-derived plasma. ELISA detection of neutrophil cellular contents in plasma showed almost complete separation of 34 FSHD Wellstone Study samples from controls for one candidate biomarker and minimal overlap of a second candidate. There were 3-fold and 2-fold difference in the means, respectively, for the 2 candidate biomarkers relative to controls ($p<0.0001$). Further longitudinal studies of FSHD samples are in progress to determine whether these biomarkers can track disease severity, predict progression, and monitor response to therapy.

S1.06

Molecular Diagnosis of FSHD1 in India suggests a lower clinical susceptibility compared to patients with a European background

Richard J. F. L. Lemmers[1], William Macken[2], Stephanie Efthymiou[2], Michael Hanna[2], Lindsay Wilson[2], Perrone Benedetta[2], Enrico Bugiardini[3], Patrick J. van der Vliet[1], Silvère van der Maarel[1], Marcelina Kretkiewicz[1], VY Vishnu[4], Madakasira V. Padma Srivastava[4], Rinkle Mishra[4]

[1] *Leiden University Medical Center, Leiden, The Netherlands*
[2] *University College London, UK*
[3] *National Hospital For Neurology and Neurosurgery, UCL Queen Square Institute of Neurology, London, UK*
[4] *All India Institutes of Medical Sciences*

Genetically, FSHD is mainly studied in patients with a European or Northeast (NE) Asian background. These studies showed a difference in the repeat size distribution of the FSHD1 allele, which ranges from 1-10 units in Europeans to 1-7 units in NE Asian patients. Despite this difference, the global threshold for FSHD1 for genetic counsellors is still 1-10 units and the patient's genetic background is generally not taken into account. Until now, the diagnosis of FSHD in India, with over 1.4 billion people one of the biggest populations in the world, is based predominantly on clinical diagnosis only. The International Centre for Genomic Medicine in Neuromuscular Diseases (ICGNMD) aims to perform clinical and genetic testing for Neuromuscular Diseases patients in low and middle-income countries (LMIC) such as India. Here, we analyzed 57 Indian FSHD families collected via the ICGNMD consortium. Genetic testing in the Indian cohort revealed 27/57 (47%) probands with FSHD1 and two with FSHD2. We also identified two probands that carried a dominant duplication allele. Based on the repeat size distribution of the FSHD1 allele, the Indian population is more comparable to the NE Asian than the European population. This possible reduced penetrance of FSHD in NE Asian individuals is strengthened by the substantial proportion of asymptomatic carriers with a rather short FSHD1 allele in India. Our study reinforces that there is a difference in FSHD susceptibility between individuals from different populations. Therefore, in genetic counseling, it is important to know the genetic background of a patient when discussing the possible consequences of a genetic FSHD test.

Session 2: Outcome Assessments

S2.01

Disease progression in FSHD1 patients with different D4Z4 methylation levels: a Chinese follow-up study

Zhiqiang Wang[1], Fuze Zheng[1], Ying Zheng[1], Liangliang Qiu[1], Long Chen[1], Minting Lin[1], Ying Fu[1], Ning Wang[1]

[1] *The First Affiliated Hospital of Fujian Medical University*

NOTE: This presentation was not given at the IRC due to technical difficulties.

Objective: To examine whether the regional methylation levels at the most distal D4Z4 repeat units (RU) in the 4qA-permissive haplotype were associated with rapid disease progression in facioscapulohumeral muscular dystrophy type 1 (FSHD1). Methods: This nationwide, single-center, prospective cohort study was conducted from a Chinese FSHD1 cohort, enrolled FSHD1 patients with hypermethylation and hypomethylation of D4Z4 region. Disease progression was assessed by FSHD clinical score (CS), clinical severity scale (CSS), and age-corrected CSS (ACSS) over median 5 years of follow-up. Results: After a median follow-up period of 5 years (range 3–9), FSHD1 patients with low D4Z4 methylation levels displayed higher CS increases of (3 points; range 1–4) and higher age-corrected CSS increases (34.2 points; range -46.6–176.5) than FSHD1 patients with high D4Z4 methylation levels (CS: 1 point; range 0–22, age-corrected CSS: 4.8 points; range -54.9–105.3) (all p <0.001) (eFigure 3). 8 of 20 patients with low D4Z4 methylation levels developed lower extremity involvement, of which 1 patient progressed to independent ambulation loss, while only 2 of the 23 patients with high D4Z4 methylation levels developed lower extremity involvement. Conclusion: 4q35 distal D4Z4 hypomethylation promotes more rapid disease progression in FSHD1.

S2.02

Identification of pharmacodynamic and monitoring biomarkers for facioscapulohumeral muscular dystrophy

Yi-Wen Chen[1], Aiping Zhang[1], Settas Nikolaos[1], Ze Chen[1], Heather Gordish-Dressman[1]

[1] *Children's National Research Institute*

Background: Using plasma samples from individuals affected by facioscapulohumeral muscular dystrophy (FSHD), we identified circulating proteins and miRNAs that are different in abundances in the FSHD samples when compared to individuals who are not affected by FSHD. The goal of this study is to further study the proteins and miRNAs to identify suitable circulating biomarkers for upcoming drug trials targeting *DUX4*. Approaches: Immortalized FSHD myoblasts and myoblasts from unaffected siblings were cultured. Cell lysates and exosomes were collected for proteomics and miRNA profiling (n=4). The myoblasts were either treated with antisense oligonucleotide gapmers targeting *DUX4* or PBS as control. To examine the proteins in the circulation, serum samples from mice carrying human FSHD and control xenografts were collected. Proteomics profiling was conducted to identify human proteins released to the circulation. Results & Conclusions: Using proteomics profiling, we identified 13 proteins that are consistently different in abundance in one of the conditions (cell lysate, exosome, xenograft). When comparing to the published human studies, 7 proteins were validated by the exosome data and 6 proteins were validated by the cell lysate data. The differences in protein abundances were partially corrected by the antisense treatment. miRNA profiling validated two of the published miRNAs by the exosome data. The proteins and miRNAs that were validated in the study are good biomarker candidates. Those responded to antisense treatment are good candidates for pharmacodynamics biomarkers for FSHD.

S2.03

Muscle imaging in natural history of FSHD: Quantitative MRI and ultrasound results compared head-to-head

Sanne Vincenten[1], Nicol Voermans[2], Baziel van Engelen[2], Karlien Mul[1], Nens van Alfen[1]

[1] *Radboud University Medical Center*

[2] *Neuromuscular Centre Nijmegen, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands*

Introduction: Though in FSHD research muscle MRI is the most frequently used imaging technique to quantify fatty replacement, muscle ultrasound may provide an alternative or complimentary technique. Few cross-sectional studies that describe the relationship between muscle MRI and ultrasound found that ultrasound has a wider dynamic range than MRI and it detected muscle changes in muscles that appeared normal on MR images. In this study, we provide follow-up data from one of these studies, comparing longitudinal muscle MRI and ultrasound data characterizing structural muscle changes head-to-head, aiming to assess the overlap and additional value of both techniques. Methods: All patients were assessed twice: at baseline and at 5-year follow-up. Clinical outcome measures included the FSHD-Clinical Score (CS) and Ricci Score. Muscle MRI and muscle ultrasound images of five leg muscles were assessed bilaterally. Fatty replacement was quantified using MRI as fat fraction (FF) and using ultrasound as echogenicity z-scores. Compound scores for MRI and ultrasound were calculated per patient. MRI and ultrasound results were correlated to each other and to clinical outcome measures. Results: We included 20 genetically confirmed FSHD patients (male=55%, mean age 54±11, mean FSHD-CS 6±4). Median compound FF change over 5 years was 2.7 [0.7-4.4] ($p<0.001$) and median compound z-score change was -0.2 [-0.9-0.4] ($p=0.227$). Conclusion: In the meeting we will present further results of this study on muscle MRI and muscle ultrasound data in FSHD, to provide insight in the optimal use of both imaging biomarkers for clinical trials.

S2.04

Evaluation of disease progression in facioscapulohumeral muscular dystrophy using multiparametric MRI

Mauro Monforte[1], Matteo Paoletti[2], Leonardo Barzaghi[3], Sara Bortolani[1], Eleonora Torchia[1], Beatrice Ravera[1], Chiara Bonizzoni[2], Niels Bergsland[4], Francesco Santini[5], Xeni Deligianni[5], Giancarlo Germani[6], Giorgio Tasca[7], Enzo Ricci[1], Anna Pichiecchio[8]

[1] *Fondazione Policlinico Universitario A. Gemelli IRCCS*
[2] *Advanced Imaging and Radiomics Center, Neuroradiology Department, IRCCS Mondino Foundation, Pavia*
[3] *Department of Mathematics, University of Pavia, Pavia, Italy*
[4] *IRCCS, Fondazione Don Carlo Gnocchi ONLUS, Milan, Italy*
[5] *Department of Radiology, University Hospital Basel, Basel, Switzerland*
[6] *Neuroradiology Department, IRCCS Mondino Foundation, Pavia, Italy*
[7] *John Walton Muscular Dystrophy Research Centre*
[8] *Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy*

Objective: To evaluate disease progression in patients affected by facioscapulohumeral muscular dystrophy (FSHD), identify possible predictors of worsening and explore correlations between radiological and clinical assessments. Methods: We designed a prospective longitudinal study applying yearly repeated clinical and instrumental evaluations (CSS, 6MWT, FSHD-Score, MRC, hand-held dynamometry), qualitative (T1-weighted and short-tau inversion recovery STIR sequences) and quantitative (6-point Dixon and multi-echo T2 sequences) lower limb muscle MRI assessments over a period of 24 months. Results: One-hundred and ten patients (57% males) were enrolled and underwent clinical and qualitative MRI assessments at baseline, 12 months and 24 months. The first 30 consecutive patients who had at least one STIR+ muscle at baseline were also enrolled for the quantitative MRI study. Several clinical and radiological indexes showed changes over the study period, with different responsiveness. Significative correlations between both qualitative and quantitative MRI metrics and clinical scores were found. The average global yearly delta fat-fraction resulted in +2% (± 0.65). STIR positive muscles, those intermediately fatty-replaced (15-30% at baseline) and muscles with higher wT2 values (> 41ms) had the greatest increase in fat-fraction. Discussion: Data derived from this natural history study provide a starting point to evaluate different metrics as possible endpoints of disease progression and give hints to prioritize enrollment of patients in interventional clinical trials.

S2.05

Motor Outcomes to Validate Evaluations in Facioscapulohumeral muscular dystrophy (MOVE FSHD): Preliminary Baseline Characteristics

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

[1] *University of Kansas*
[2] *University of Utah*
[3] *Stanford University*
[4] *University of Rochester Medical Center*
[5] *The Ohio State University*
[6] *Seattle Children's Hospital*
[7] *McGill University*
[8] *Virginia Commonwealth University*
[9] *University of Nevada Reno*
[10] *Kennedy Krieger Institute*
[11] *Ottawa Hospital Research Institute & Children's Hospital of Eastern Ontario*
[12] *University of California, Los Angeles*
[13] *University of Florida Gainesville*
[14] *University of Texas Southwestern*
[15] *University of Washington*
[16] *University of Colorado*

The MOVE FSHD study aims to determine the predictive value of clinical and motor assessments, patient-reported outcomes, imaging, and tissue biomarkers on disease progression in FSHD. This comprehensive study will be important not only for improving patient care, but to understand what kind of change would be meaningful for clinical trials. Study will evaluate 450 FSHD participants over three years with 200 participating in an MRI and muscle biopsy sub-study to validate FSHD evaluations. Annual visits collect FSHD history, physical examination, patient reported outcomes, strength, timed functional tasks, and respiratory parameters. Sub-study participants will have additional biomarkers collected, including reachable workspace at every visit, whole body MRI at Baseline and 12-Month visits, muscle biopsy at Baseline and at 4-months (n=40). The MOVE FSHD study has over 240 participants across 12 US sites who have completed their Baseline visit, more than 120 have returned for annual follow-up visits and sites have also begun enrolling MOVE+ sub-study participants. Our cohort is predominantly non-Hispanic white with 58% being male, 88% FSHD Type 1, and 92% are ambulatory. We currently have 12 individuals enrolled under the age of 18. Lastly, more than 50 of our previous 161 US participants from the ReSolve FSHD study have enrolled in the MOVE study with the remainder expected to roll-over within the next 1-2 years. MOVE FSHD addresses barriers to clinical trials by validating motor, clinical, and patient reported outcomes, as well as potential biomarkers. The data from MOVE FSHD can also improve our understanding of FSHD and directly impact patient care.

S2.06

Facioscapulohumeral Muscular Dystrophy (FSHD) Surgeries, Cardiovascular Testing, Mobility Aids and Healthcare Utilization after Diagnosis from a Real-World Data Analysis

Elizabeth Ackermann[1], Kathryn Munoz[1], Chamindra Laverty[2], Richard Brook[3], Nathan L. Kleinman[3], Chao-Yin Chen[1], Teresa Brandt[1] Mark C. Stahl[7], Amy Halseth[8]

[1] *Avidity Biosciences, Inc.*
[2] *UCSD, Rady Children's Hospital, and VA San Diego Healthcare System*
[3] *Better Health Worldwide*

Objective: Describe the management of patients with FSHD compared with matched controls (MCs) after diagnosis (Dx). Design/Methods: We conducted a retrospective claims analysis of patients with FSHD (≥2 FSHD ICD-10 codes ≥30 days apart, N=79) vs MCs (N=395); Jan 2015-Mar 2021. Subjects had continuous data 2 years pre-/post-index date. Cost data were adjusted to 2020 US dollars. Non-percentage data are per-member-per-year. All reported findings were significant ($p<0.05$). Results: Post-index date, patients with FSHD had higher mean costs [$17,322 vs $5231 for MCs], more services [75.2 vs 31.0], and more days of care [27.6 vs 9.5]. Patients with FSHD were more likely to use walking aids and wheelchairs (20.3% vs 2.3%) and undergo electrocardiograms (54.4% vs 24.3%) and echocardiograms (35.4% vs 9.4%). Patients with FSHD underwent more integumentary system (39.2% vs 22.0%), musculoskeletal (30.4% vs 14.2%), respiratory (10.1% vs 2.5%), ocular (10.1% vs 3.5%), cardiovascular (8.9% vs 3.3%), and urinary (7.6% vs 2.5%) surgeries. Conclusions: Health care utilization was significantly higher in patients with FSHD post-diagnosis compared with MCs. With the lack of an approved therapy, the increased utilization likely reflects manifestations of managing FSHD. The data reflect the burden on patients with FSHD and their families.

Session 3: Disease Mechanisms & Interventional Strategies

S3.01
Immunopathogenesis of Facioscapulohumeral Muscular Dystrophy (FSHD)
Beatrice Biferali[1], Mara Salomè[1], Davide Gabellini[1]

[1] *San Raffaele Scientific Institute*

ABSTRACT WITHDRAWN AT AUTHORS' REQUEST

S3.02

An innate immune cell/FSHD muscle xenograft model to investigate the role of complement pathway activation in FSHD muscle pathology

Katelyn Daman[1], Jing Yan[1], Leonard Shultz[2], Michael Brehm[1], Charles Emerson[1]

[1] *UMass Chan Medical School*
[2] *The Jackson Laboratory*

To investigate a role for innate immunity in FSHD muscle pathology, we have developed a novel humanized blood-FSHD muscle xenograft mouse model. The NSG-SGM3-W41 mouse strain has been engineered to selectively expand human innate immune cell lineages following engraftment of umbilical cord blood (UCB) derived hematopoietic stem cells (HSCs) and co-engraftment of patient derived FSHD (or unaffected control) muscle stem cells into the mouse tibialis anterior (TA) muscle. *DUX4* expressing FSHD muscle xenografts in HSC engrafted NSG-SGM3-W41 mice preferentially accumulate human macrophages and early B cells and express early RNAs encoding activators of both the classical and alternative complement pathways and complement factor C3, which is the mediator of early complement response. Notably, FSHD xenografts undergo muscle turnover dependent on specific HSC immune donors, supporting a role for early complement activation in FSHD muscle pathology and the concept that innate immunity contributes to clinical variability of FSHD disease progression. Experiments in progress are using our innate immune cell/FSHD muscle xenograft model to investigate the efficacy of complement targeting therapeutics for the treatment of FSHD.

S3.03

Regulation of muscle regeneration through FSHD disease progression

Elise Engquist[1], Anna Greco[2], Leo Joosten[2], Baziel van Engelen[3], Christopher Banerji[4], Peter Zammit[1]

[1] *King's College London, UK*

[2] *Radboud University Medical Center, Nijmegan, The Netherlands*

[3] *Neuromuscular Centre Nijmegen, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands*

[4] *The Alan Turing Institute, London, UK*

Facioscapulohumeral muscular dystrophy (FSHD) is a prevalent, incurable myopathy with heterogenous clinical trajectories. Inflammation can accelerate fibrosis and fatty replacement in FSHD muscle, further compounding heterogeneity both within and between patients. Well-controlled studies are thus essential to further understand pathomechanisms and disease progression. Here, we investigate FSHD through transcriptomic and histological analysis of FSHD muscle biopsies, using MRI-guidance to compare both inflamed and non-inflamed muscles from the same patient, alongside unaffected control individuals [1]. Transcriptomic analyses show that processes related to mitochondria and fibro-adipogenic progenitor cells (FAPs) are mis-regulated in FSHD muscle prior to the onset of overt inflammation. Muscle regeneration is evident in FSHD muscle biopsies [2]. We generated novel gene signatures to investigate multiple muscle-resident cell populations in bulk RNA-sequencing data. This suggested elevated muscle stem (satellite) cell activity in FSHD muscle, that correlates with clinical severity. Histological analysis of these same biopsies is underway to further examine fibrosis in disease progression as well as quantify satellite cells and regenerating muscle fibres. These findings enable better understanding of disease progression and identify consistent and novel pathomechanisms for this highly heterogeneous condition.

[1] Banerji, C.R.S., et al. (2022). bioRxiv (doi: 10.1101/2022.08.31.506017).

[2] Banerji, C.R.S., et al. (2020). Hum Mol Genet, 2746–2760 (doi: 10.1093/hmg/ddaa164).

S3.04

DUX4 and *DUX4c* directly interact with C1qBP in FSHD regenerating myofibers

Clothilde Claus[1], Moriya Slavin[2], Karimatou Bah[3], Céline Lancelot[3], Saskia Lassche[4], Manon Fiévet[3], Anna Greco[4], Benno Kusters[4], Anne-Emilie Declèves[3], Baziel van Engelen[5], Alexandra Belayew[3], Nir Kalisman[6], Frédérique Coppée[3]

[1] *Laboratory of Metabolic and Molecular Biochemistry, University of Mons, Belgium*
[2] *Department of Biological Chemistry, Hebrew University of Jerusalem, Israel*
[3] *University of Mons, Belgium*
[4] *Radboud University Medical Center*
[5] *Neuromuscular Centre Nijmegen, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands*
[6] *Hebrew University of Jerusalem*

Pathological *DUX4* expression in skeletal muscle cells interferes with various pathways and ultimately leads to their death. *DUX4c* shares identical homeodomains with *DUX4* and is normally expressed in healthy muscle cells. In order to better understand the FSHD pathophysiology, we previously determined that C1qBP is one of the strongest DUX4 protein partner that directly interacts with its second homeodomain. We now determined in FSHD muscle sections, using in situ proximity ligation assays that *DUX4*/4c-C1qBP interactions occurred in myocytes/myofibers showing regeneration features. We then confirmed that both *DUX4* and *DUX4c* are co-expressed with regeneration markers (dMHC or MYOD) in such myofibers. These findings are in agreement with our previous *DUX4c* gain- and loss-of-function studies and suggest that C1qBP also has a role during human muscle cell differentiation. Little is known about the C1qBP function in muscle cells and how *DUX4* could impact it, however, we observed atypical morphology of FSHD myofibers suggesting a fusion defect of the muscle progenitors. Our results imply that *DUX4* may compete with normal *DUX4c* functions and its interaction with C1qBP in muscle cells during their regeneration. As several therapeutic strategies have been developed to target C1qBP dysfunctions in cancer and mitochondrial disorders, they could be useful for the rational design of FSHD polytherapy, as well as molecules that will improve a healthy muscle regeneration.

S3.05

The Interactome of *DUX4* Reveals an Inherent Feedback Mechanism by RFPL4

Moriya Slavin[1], Keren Zohar[2], Clothilde Claus[3], Michal Linial[2], Anne-Emilie Declèves[4], Frédérique Coppée[4], Nir Kalisman[7]

[1] *Department of Biological Chemistry, Hebrew University of Jerusalem, Israel*
[2] *The Hebrew University, Jerusalem, Israel*
[3] *Laboratory of Metabolic and Molecular Biochemistry, University of Mons, Belgium*
[4] *University of Mons, Belgium*
[5] *Department of Biological Chemistry, Hebrew University of Jerusalem, Israel*

The ectopic expression of *DUX4* in skeletal muscle cells results in extensive gene induction that is harmful to the cells. In order to trace those early toxicity events, several studies have strived to characterize the proteins that directly interact with *DUX4*. However, these studies reported large sets of putative protein partners, from which it is difficult to identify the few most functionally relevant interactions. We believe that those unspecific interactions are caused by the highly polarized sequence of the *DUX4*. Here, we have taken several steps to reduce these electrostatic artifacts in AP-MS measurements, and thus provide a rigorous analysis of the *DUX4* interactome. Surprisingly, we find that the strongest interaction of *DUX4* is with members of the *RFPL4* family, a set of genes strongly induced by *DUX4*. We have localized the *DUX4*-*RFPL4* interaction using cross-linking mass-spectrometry (XL-MS) and deletion studies, and found that *RFPL4A* binds to the disordered region of *DUX4*. Although the *RFPL4* family is poorly-characterized functionally, its sequence homology strongly suggests it to belong to the E3-ubiquitin ligase class, and thus it may be involved in *DUX4* ubiquitin-dependent degradation. Hence, these results suggest that *DUX4* may be inhibited by its own activation products, explaining its pulse-like expression profiles. Our findings reveal a novel regulatory pathway of *DUX4* that may be employed in the future to inhibit the toxicity of *DUX4*.

S3.06

Developing Cas13-ADAR-mediated *DUX4* mRNA editing as a prospective therapy for FSHD

Scott Harper[1], Noushin Saljoughian[2], Genesis Snyder[2], Lara Rizzotto[3], Colin Maguire[2], Yasemin Sezgin[2], Lindsay Wallace[2], Meisam Naeimi Kararoudi[2], Dario Palmieri[3]

[1] *Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio*
[2] *Nationwide Children's Hospital*
[3] *The Ohio State University*

There are currently no approved treatments exists that alter the course of FSHD, and therapy development remains an unmet need in the field. We propose that the most direct route to FSHD therapy will involve *DUX4* inhibition. Here we describe an approach to silence *DUX4* mRNA using new CRISPR/Cas13-based RNA editing strategy. Cas13 was originally developed as a RNA-guided CRISPR enzyme that cleaves RNA and not DNA. In addition, modified Cas13 can also be employed to edit single bases on mRNAs. To do this, Cas13 is fused to a modified ADAR2 sequence (Adenosine Deaminase Acting on RNA) to direct cytidine-to-uridine editing on target mRNAs (C-to-U). Using this approach, glutamine and arginine codons can be edited to stop codons, thereby producing truncated, potentially non-toxic *DUX4* open reading frames. Editing requires the use of guide RNAs. We developed reporter cells to test Cas13/ADAR-mediated editing of *DUX4* mRNA. These cells express doxycycline-inducible Cas13/ADAR; a transcriptionally active but non-toxic *DUX4* ORF; and a NanoLuc reporter gene driven by a *DUX4*-induced promoter. NanoLuc is expressed in the presence of *DUX4* and reduced or absent with *DUX4* knockdown. We designed 117 different guide RNAs targeting 34 *DUX4* glutamine and 5 arginine codons, with the goal of editing to premature STOP codons. We identified *DUX4*-modifying gRNAs that significantly reduced NanoLuc expression, suggesting successful editing of *DUX4* mRNA. Our results may provide another treatment option for FSHD, and has implications for using this system to edit other transcripts.

Session 4: Clinical Studies & Trial Designs

S4.01

Results from 96-Week Open-Label Extension of a Phase 2 Trial of Losmapimod in Subjects with FSHD: ReDUX4

Leo Wang[1], **Marie-Helene Jouvin**[3], Jay Han[2], John Jiang[3], Rabi Tawil[4]

[1] *University of Washington*
[2] *University of California, Irvine*
[3] *Fulcrum Therapeutics*
[4] *University of Rochester*

Facioscapulohumeral muscular dystrophy (FSHD) is a relentless, variably progressive disease leading to accumulation of disability. ReDUX4 is a 48-week, placebo-controlled study assessing 80 subjects, 18-65 years old with genetically confirmed FSHD1, Ricci score 2-4, randomized 1:1 to receive 15 mg losmapimod, a small molecule p38 α/β MAPK inhibitor, or placebo (PO BID), followed by an Open-Label Extension (OLE) with all participants receiving losmapimod. Seventy-six of 77 (99%) participants entered the OLE after 48 weeks, and 74 (97%) were enrolled at Week 96. For participants continuing to receive losmapimod (LOS/LOS), durability of treatment response was observed at 96 weeks, by assessing upper extremity function with Reachable Workspace (RWS). Placebo participants who converted to losmapimod (PBO/LOS) at Week 48 demonstrated trends of slowing or stopping disease progression based on RWS. Annualized total (Q1-5) relative surface area (RSA) in the dominant arm with weights demonstrated stability in the LOS/LOS group during the 2nd year of dosing compared to the first (0.31%/yr vs -0.62%/yr, respectively) and improvement in PBO/LOS (0.98% vs -7.49%). Mean change in RSA from Week 48 to Week 96 was 0.00 for LOS/LOS and 0.00 for PBO/LOS with similar findings in the non-dominant arm in both groups and without weight. No related serious adverse events or discontinuations due to adverse events were reported in 96 weeks of dosing. Most commonly reported AEs were fall (22.4%), headache (14.5%), arthralgia (7.9%), and back pain (7.9%). Losmapimod slowed disease progression and demonstrated maintenance of effect through a 96-week period and continues to be well tolerated.

S4.02

Phase 1/2 Trial Evaluating AOC 1020 in Adults with FSHD: FORTITUDE Trial Design

Amy Halseth[1], Elizabeth Ackermann[1], Teresa Brandt[1], Chao-Yin Chen[1], Mark C. Stahl[1], Kelly DiTrapani[1], Steve Hughes[1], Rabi Tawil[2], Jeff Statland[3]

[1] *Avidity Biosciences, Inc.*
[2] *University of Rochester*
[3] *University of Kansas*

AOC 1020 is an antibody-oligonucleotide conjugate (AOC) comprised of a *DUX4*-targeting siRNA conjugated to a humanized anti-transferrin receptor 1 (TfR1) antibody to facilitate delivery to muscle. Methods: This phase 1/2 study (NCT05747924) is a randomized, placebo-controlled, double-blind trial. The study will enroll 72 adults aged 18 to 65 years with a genetic diagnosis of FSHD1 or FSHD2. All participants will receive 5 doses of study medication administered quarterly with 1 booster at 6 weeks. Part A utilizes a dose-titration design to evaluate the safety of AOC 1020 at 2 low doses. Part B is a nested single/multiple-ascending dose design evaluating 2 higher doses. Staggered cohorts will be initiated based on a safety data review of the preceding cohorts. Part C is a parallel, placebo-controlled design to be conducted with 2 selected doses to evaluate exploratory clinical outcomes. After their final dose, participants enter a 3-month follow-up period. The total duration is 12 months. Eligible participants may enroll in an open-label extension study. The primary objective of the study is to evaluate safety and tolerability. Secondary objectives include PK of AOC 1020. Exploratory measures of efficacy will be evaluated.

S4.03

MANOEUVRE study design: A study of GYM329 (RO7204239) in patients with facioscapulohumeral muscular dystrophy (FSHD)

Giorgio Tasca[1], Katy Eichinger[2], Jasper M. Morrow[3], Jeff Statland[4], John Vissing[5], Angela Dodman[6], Birgit Jaber[7], Heidemarie Kletzl[8], Tammy McIver[9], Renata S. Scalco[6], Wai Yin Yeung[9], Eleni Gaki[9], Kathryn R Wagner[6]

[1] *JWMDRC, Newcastle University and Newcastle Hospitals NHS Foundation Trusts, Newcastle upon Tyne, UK*

[2] *University of Rochester Medical Center*

[3] *Queen Square Centre for Neuromuscular Diseases, UCL Institute of Neurology, London, UK*

[4] *University of Kansas*

[5] *University of Copenhagen, Denmark*

[6] *Pharma Development Neurology, F. Hoffmann-La Roche Ltd, Basel, Switzerland*

[7] *Pharma Development, Safety, F. Hoffmann-La Roche Ltd, Basel, Switzerland*

[8] *Roche Pharmaceutical Research and Early Development, Roche Innovation Center Basel, Switzerland*

[9] *Roche Products Ltd, Welwyn Garden City, UK*

There is no approved therapy for facioscapulohumeral muscular dystrophy (FSHD); thus, there is a high unmet medical need as the disease can cause significant morbidity and reduce the quality of life of affected patients. GYM329 (RO7204239) is an investigational, recycling and antigen-sweeping monoclonal anti-myostatin antibody. MANOEUVRE (NCT05548556) is a Phase 2, multicentre, randomised, placebo-controlled, double-blind study that will assess the pharmacodynamics (PD), safety, tolerability, pharmacokinetics (PK) and efficacy of GYM329 in adult ambulant patients with a genetically confirmed diagnosis of FSHD1 or FSHD2. Participants (target enrolment N~48) will complete a pre-treatment period to collect baseline movement data via a wearable device before randomisation (1:1, GYM329: placebo) for the 52-week double-blind treatment period. GYM329 or placebo will be administered every 4 weeks by subcutaneous injection. The primary endpoints are the percentage change from baseline in the contractile muscle volume of the quadriceps femoris muscle, as assessed by magnetic resonance imaging (MRI) at Week 52, and the safety of GYM329. Secondary endpoints include the change from baseline in the volumes of various muscles and groups of muscles, as assessed by MRI. Exploratory efficacy endpoints include assessment of motor function and strength. Here we present the study design of MANOEUVRE, which aims to provide valuable information about the PD, safety, tolerability, PK and efficacy of GYM329 in ambulant adult patients with FSHD.

S4.04

PERSPECTYV FSHD: PERsonalized Medicine and SPECialized TherapY for better LiVing with FSHD

Dalila Laoudj-Chenivesse[1], Florence Portet[2], Christine Fedou[2], Eric Raynaud de Mauverger[1], Gerald Hugon[1], Fares Gouzi[1], Marie Christine Picot[3], Jean-Paul Cristol[1], Jacques Mercier[1], Maurice Hayot[1], Sandrine Arbogast[1]

[1] *PhyMedExp, Université de Montpellier, INSERM, CNRS, CHU de Montpellier, Montpellier, France*
[2] *Department of Clinical Physiology, CHU of Montpellier, Montpellier, France*
[3] *Department of Biostatistics and Epidemiology, University Hospital, CIC 1001, Montpellier, France*

Compelling evidence has shown that free radicals play major roles in FSHD. Evaluation of the impact on physical muscle performance of an oral administration of vitamins and minerals (specifically designed from the oxidative stress profile of FSHD blood samples) in a randomized double blinded, placebo-controlled pilot trial (NCT01596803) involving 54 patients with FSHD suggested that the antioxidant response could be improved by this antioxidant supplementation. It reduced oxidative stress and increased the antioxidant defenses associated with an improvement of quadriceps muscle strength. Each supplementation component was qualified for orphan drug designation for the treatment of FSHD by the European Medicine Agency Committee. Moreover, as our study demonstrated the need to adjust the dose of each component based on its specific systemic level in individual patients, we are further investigating the long-term effects of the adjustment of antioxidant supplementation doses (NCT02622438). Our preliminary data showed a significant increase in quadriceps muscle strength. Based on these results, a medical decision support algorithm (patent pending) has been designed allowing the adjustment of antioxidants supplementation doses based on individual patient blood profile. The software solution is developed by AxLR SATT Montpellier (an innovation accelerator for academic research) and KONDREE start-up (Claire Lefranc, CEO, 2021 Winner of “La French Tech Austin and Houston” Business Excellence Award).

Session 5: Pediatric FSHD

S5.01
Dutch pediatric study in childhood FSHD
Corrie Erasmus[1], Jildou Dijkstra[2], Nicol Voermans[1], Baziel van Engelen[1]

[1] *Neuromuscular Centre Nijmegen, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands*
[2] *Radboud University Medical Center*

Rationale: There is a knowledge gap in childhood FSHD. Aims: to increase knowledge on the natural history, outcome measures, and to optimize the participation of children in future FSHD trials. Design: prospective nationwide natural history study. Population: Dutch children with genetically proven FSHD. Measurements: FSHD clinical score, 6-MWT, MFM, PUL, QoL questionnaires, muscle ultrasound, retinal tests. Results: A 5-year follow-up is available. We performed measurements at baseline, 2 and 5 years. These data will be presented. Highlights: broad spectrum of childhood FSHD; mild progression; diminished quality of life and fatiguability seem to be the typical features. Clinical aspects of childhood FSHD might differ from early onset FSHD as there is a low prevalence of systemic features. What is relevant? FSHD clinical scores and muscle ultrasound are promising outcomes. Future perspectives: The 8-year follow-up is planned. We are preparing a clinical study on the muscle fatigability in this population.

S5.02

Measuring function in childhood FSHD - Does the FSHD-COM Peds measure up?

Katy de Valle[1], Fiona Dobson[2], Ian Woodcock[1], Kate Carroll[1], Jenny McGinley[2]

[1] *The Royal Children's Hospital Melbourne*
[2] *University of Melbourne*

Despite being the third most common muscular dystrophy, childhood onset facioscapulohumeral dystrophy (FSHD) is rare. Low incidence, diagnostic challenges, variable severity, and slow progression have impacted development and validation of functional measures, especially for children. The novel FSHD-COM developed in adults, was adjusted and its reliability, validity and longitudinal utility explored in a group of children and adolescents with FSHD. Measurement properties of the FSHD-COM Peds were evaluated in 18 participants (7-18yrs, mean 13.3yrs, 10 males). Intra-rater test re-test reliability (n=15) was excellent ICC1,2>0.99 (CI95% 0.98-0.99), standard error measurement (SEM) low 1.31 (CI95% 0.96-2.1) and minimal detectable change (MDC95) score calculated at 3.6 points. Convergent validity was supported by moderate to very good correlations with FSHD-specific severity scales, PUL 2.0, and self-reported measures of disease burden. The ability to discriminate between FSHD and age matched typically developing individuals (n=18) was excellent (p=0.0005). Longitudinal utility (n=16) indicates the FSHD-COM Peds can identify change in function related to typical development and disease progression. Agreement between change in function measured with FSHD-COM Peds and participant-reported global rating of change was strong in 50% and moderate in 33% of participants. The FSHD-COM Peds shows potential when measuring function in childhood FSHD and would benefit from scoring refinement through the incorporation of pediatric normative reference data and larger scale studies with increased numbers to explore responsiveness and inter-rater reliability properties.

Posters: Discovery Research & Genetics

P1.01

Dominant pathogenic cis D4Z4 repeat duplications in FSHD

Richard Lemmers[1], Russell Butterfield[2], Patrick J. van der Vliet[1], Robert B. Weiss[2], Silvère van der Maarel[1], Jan De Bleecker[3], Ludo van der Pol[4], Corrie Erasmus[5], Baziel van Engelen[5], Nicol Voermans[5], Marc D'Hooghe[6], Kristof Verhoeven[6], **Jeff Statland**[7], Enrico Bugiardini[8], Nienke van der Stoep[9], Teresinha Evangelista[10], Chiara Marini-Bettolo[11], Peter van den Bergh[12], Rabi Tawil[13], John Vissing[14], Judit Balog[15]

[1] *Leiden University Medical Centre, Netherlands*
[2] *University of Utah*
[3] *Universitair Ziekenhuis, Gent, Belgium*
[4] *University Medical Center Utrecht, Netherlands*
[5] *Neuromuscular Centre Nijmegen, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands*
[6] *Algemeen Ziekenhuis Sint-Jan, Brugge, Belgium*
[7] *University of Kansas*
[8] *National Hospital For Neurology and Neurosurgery, UCL Queen Square Institute of Neurology, London, UK*
[9] *Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands*
[10] *Unité de Morphologie Neuromusculaire, Institut de Myologie, AP-HP, Paris, France*
[11] *The John Walton Muscular Dystrophy Research Centre, Newcastle upon Tyne, UK*
[12] *Saint-Luc UCL, Bruxelles, Belgium*
[13] *University of Rochester*
[14] *University of Copenhagen, Denmark*
[15] *Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands*

FSHD is caused by the depression of the *DUX4* gene within the D4Z4 macrosatellite repeat on 4qter. The D4Z4 repeat is usually organized in a single repeat and ranges between 8-100 units (U) in the European population. *DUX4* depression in FSHD is most often caused by a repeat contraction to 1-10U (FSHD1), or by a digenic mechanism requiring pathogenic variants in a D4Z4 chromatin repressor like SMCHD1, combined with a D4Z4 repeat of 8-20U (FSHD2). Recently, in cis duplications of the D4Z4 repeat have been reported, where two adjacent D4Z4 repeats are interrupted by a spacer sequence. Cis duplication alleles were shown to be pathogenic in FSHD2 patients. However, there is inconsistent evidence for the necessity of a SMCHD1 mutation as two patients have been identified with a possible dominant cis duplication allele. To explore the pathogenic nature of these alleles we compared cis duplication alleles that are dominantly pathogenic (n=9) with those that become only pathogenic in combination with an SMCHD1 mutation (n=9). For both groups we showed duplication-allele-specific *DUX4* expression. We studied these alleles in detail using Southern blotting, molecular combing and optical mapping, emphasizing the challenges in the characterization of these rearrangements. Nanopore sequencing was instrumental to study the methylation of the duplicated D4Z4 repeats and to identify the breakpoints and the spacer sequence between the repeats. By comparing the repeat composition of cis duplication alleles in both groups we revealed the criteria that determine their pathogenicity.

P1.02

Whole Exome Sequencing of 126 patients provides evidence for novel candidate genes in FSHD

Claudia Strafella[1], Domenica Megalizzi[1], Valerio Caputo[1], Giulia Trastulli[1], Luca Colantoni[1], Sara Bortolani[2], Eleonora Torchia[2], Mauro Monforte[2], Carlo Caltagirone[3], Enzo Ricci[2], Giorgio Tasca[4], Emiliano Giardina[5], Raffaella Cascella[1]

[1] *Genomic Medicine Laboratory-UILDM, Santa Lucia Foundation IRCCS*
[2] *Fondazione Policlinico Universitario A. Gemelli IRCCS*
[3] *Department of Clinical and Behavorial Neurology, Santa Lucia Foundation IRCCS*
[4] *UOC di Neurologia, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy*
[5] *Santa Lucia Foundation IRCCS; Tor Vergata University of Rome*

The work employed Whole Exome Sequencing (WES) to investigate known and unknown genetic contributors that may be involved in FSHD and represent potential disease modifiers, even in presence of a D4Z4 Reduced Allele (DRA). On this subject, 126 patients with clinical signs of FSHD were included in the study and were characterized by D4Z4 sizing, methylation analysis and WES. In-house protocols were employed for D4Z4 sizing and methylation analysis, whereas the Illumina® Next-Seq 550 system was utilized for WES. In presence of variants of interest, the molecular analysis was extended to the family members. The analysis identified 20 relevant variants in 19 patients. In particular, 14 variants were located in known genes (SMCHD1, DNMT3B and LRIF1), whereas 6 variants were found in novel genes implicated in the *DUX4*-repressive machinery. Most of them were found together with a permissive short (4-7 RU) or intermediate/long DRA (8-20RU), suggesting that different genes can contribute to disease heterogeneity in presence of a FSHD permissive background. Our results support FSHD as a genetically complex disease, in which variations in known (SMCHD1, DNMT3B, LRIF1) and candidate novel genes could influence the phenotype, penetrance and severity of disease among patients as well as within the same family. Moreover, the study further emphasizes the importance of extending the analysis of molecular findings within the proband's family, with the purpose of providing a broader framework for understanding single cases and allowing finer genotype-phenotype correlations in FSHD-affected families.

P1.03

Do the novel mutations in FSHD point to polygenic disease? Co-segregated *MYH2* and *GP1BA* gene mutations in a FSHD family with hereditary thrombocytopenia

Ceren Hangul[1], Haldun Dogan[2], Sibel Berker Karauzum[1], Hilmi Uysal[3], Serdar Ceylaner[2]

[1] *Akdeniz University Faculty of Medicine Department of Medical Biology and Genetics*
[2] *Intergen Genetic and Rare Diseases Diagnosis and Research Center*
[3] *Akdeniz University Faculty of Medicine Department of Neurology*

We still encounter newly found genetic changes and inheritance patterns that cause FSHD. An observation of co-incidental FSHD with familial thrombocytopenia in pedigree lead to the investigation of co-segregated novel mutation. In order to capture all genetic changes that may affect the clinical findings, whole exome sequencing (WES) was performed as a trio. Trio was formed as father, son and mother. Father and son had typical FSHD clinical features and D4Z4 deletion with 6 RU on 4qter in addition to macrothrombocytopenia. Mother was healthy and didn' t have FSHD or macrothrombocytopenia. As a result, frameshift mutation of GP1BA gene on 17p13.2 and non-synonymous nucleotide change of MYH2 gene on 17p13.1 had been detected. Additional changes detected in this family can be evaluated as co-incidental mutations which segregated together and modified the clinical findings. This novel data emphasize the necessity of comprehensive polygenic investigation especially in FSHD patients exhibiting clinical variability.

P1.04

Optical genome mapping for the molecular diagnosis of facioscapulohumeral muscular dystrophy: Advancements and challenges

Stephanie Efthymiou[1], VY Vishnu[2], Richard J. F. L. Lemmers[3], Lindsay Wilson[1], Patrick J. van der Vliet[3], Natalia Dominik[1], Perrone Benedetta[1], Stefano Facchini[1], Andrea Cortese[1], Silvère van der Maarel[3], Michael Hanna[1], Enrico Bugiardini[4]

[1] *University College London*
[2] *All India Institutes of Medical Sciences*
[3] *Leiden University Medical Center, Leiden, The Netherlands*
[4] *National Hospital For Neurology and Neurosurgery, UCL Queen Square Institute of Neurology, London, UK*

Facioscapulohumeral muscular dystrophy (FSHD) is the second most common muscular dystrophy in adults. FSHD is associated with a contraction of the D4Z4 microsatellite repeat on chromosome 4. In most diagnostic centers, FSHD1 diagnosis is Southern blot (SB)-based, however SB protocol in FSHD is technically challenging, time consuming and requires of staff trained in results interpretation. Currently only a few laboratories worldwide offer a full FSHD genotyping work-up and access to FSHD genetic diagnosis of populations in low and middle-income countries (LMIC) is limited. Optical genome mapping (OGM) is a new promising technology to assess structural variants in the genome. We aimed to investigate the use of OGM as diagnostic tool in testing FSHD cases from UK and India. We also aimed to compare the results with traditional techniques such as linear gel (LGE) and Pulsed-field gel electrophoresis (PFGE) SB. Samples were processed with the Saphyr Genome Imaging Instrument (1-color) established at the UCL Queen Square Institute of Neurology following manufacturer's guidelines and data were analysed using the custom EnFocus FSHD analysis. 31 probands with suspected or confirmed diagnosis of FSHD were analysed. OGM was able to confirm diagnosis of FSHD1 in 22 out of 31 cases and D4Z4 sizing highly correlates with SB ($p<0.001$). Two cases were identified as mosaic on the permissive 4qA chromosome by OGM and one case was found with an homozygous 10U D4Z4 contraction. The latter was not identified by standard LGE. Eight cases were found to be negative with both OGM and SB. OGM is a promising new technology able to unravel structural variants and seems a valid tool.

P1.05

D4Z4 methylation analysis combined with machine learning pipelines: a novel tool for identifying FSHD subjects

Raffaella Cascella[1], Valerio Caputo[1], Claudia Strafella[1], Giulia Trastulli[1], Domenica Megalizzi[2], Carlo Fabrizio[3], Andrea Termine[3], Luca Colantoni[1], Juliette Gimenez[4], Mauro Monforte[5], Carlo Caltagirone[6], Enzo Ricci[5], Giorgio Tasca[7], Emiliano Giardina[8]

[1] *Genomic Medicine Laboratory-UILDM, Santa Lucia Foundation IRCCS*
[2] *Genomic Medicine Laboratory-UILDM, Santa Lucia Foundation IRCCS; Tor Vergata University*
[3] *Data Science Unit, Santa Lucia Foundation IRCCS*
[4] *Epigenetics and Genome Reprogramming Laboratory, Santa Lucia Foundation IRCCS*
[5] *Fondazione Policlinico Universitario A. Gemelli IRCCS*
[6] *Department of Clinical and Behavorial Neurology, Santa Lucia Foundation IRCCS*
[7] *Unità Operativa Complessa di Neurologia, Fondazione Policlinico Universitario A. Gemelli IRCSS, Rome*
[8] *Santa Lucia Foundation IRCCS; Tor Vergata University of Rome*

The study describes a protocol for identifying FSHD subjects by means of D4Z4 methylation assessment and Machine Learning (ML). Two independent cohorts were included in the study, namely a training group of 133 patients with clinical signs of FSHD and 150 control subjects (CTRL) and a testing set of 27 FSHD patients and 25 CTRL. The DNA methylation levels of two D4Z4 regions (DR1 and DUX4-PAS) were assessed by an in-house protocol based on bisulfite sequencing (BSS) and capillary electrophoresis with the Amplification Fragment Length Polymorphisms (AFLP) module, followed by statistical and ML analysis. As expected, FSHD patients showed significantly reduced methylation levels compared to CTRL (FDR $p<0.001$). Of note, single CpG sites were utilized to develop a ML pipeline able to discriminate FSHD subjects. The model identified four CpG sites (namely, DUX-PAS_CpG6, DUX-PAS_CpG3, DR1_CpG1, DR1_CpG22) as the most relevant for the identification of FSHD patients, reporting high metrics values (accuracy: 0.94, sensitivity: 0.93, specificity: 0.96). Overall, the developed tool enables an accurate classification of FSHD patients, providing additional evidence for DNA methylation as a powerful biomarker for prioritizing subjects to be tested for FSHD. Moreover, the test proved to rapid (~ 72 h) and accessible (€15/sample), further supporting its application into the clinical practice.

P1.06

MethylSeq-based assay to assess the epigenetic setting of D4Z4 repetitive elements in facioscapulohumeral muscular dystrophy

Valentina Salsi[1], **Sara Pini[1]**, Matteo Chiara[2], Lucia Ruggiero[3], Filippo Santorelli[4], Stefano Previtali[5], Diego Lopergolo[6], Maria Grazia D'Angelo[7], Carmelo Rodolico[8], Silvia Bonanno[9], Lorenzo Maggi[9], Rossella Tupler[10]

[1] Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena

[2] Dipartimento di Bioscienze, Università degli Studi di Milano, Milan, Italy

[3] Department of Neurosciences, Reproductive and Odontostomatological Sciences, University Federico

[4] IRCCS Fondazione Stella Maris, Calambrone, Pisa, Italy

[5] INSPE and Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy

[6] U.O.C. Neurologia e Malattia Neurometaboliche Policlinico "S.Maria alle Scotte", Siena, Italy

[7] Department of Neurorehabilitation, IRCCS Eugenio Medea, Bosisio Parini, Italy

[8] Department of Neurosciences, Policlinico "G. Martino", University of Messina, Messina Italy

[9] IRCCS Foundation, C. Besta Neurological Institute, Milan, Italy

[10] Università degli Studi di Modena e Reggio Emilia

Facioscapulohumeral muscular dystrophy type 2 comprehends 5% of FSHD clinical cases carrying D4Z4 alleles with 11 repeat units or more. In these cases, mutations in chromatin-remodeling factors SMCHD1, DNMT3B, LRIF1 have been described. It has thus been hypothesized that FSHD results from chromatin changes determining the anomalous expression of 4q35 genes and D4Z4 reduced methylation. We designed a high-depth methylation assay testing 126 CpGs spanning the 3.3 kb D4Z4 repeat. Bisulfite treated DNAs from 40 subjects (26 FSHD2 index cases and 14 relatives) were tested and mapped. Tailored bioinformatic analysis uncovered the great variability of repetitive elements. Low, intermediate and high D4Z4 methylation subdivide samples in three clusters. Of the 40 samples 16 (40%) presented reduced D4Z4 methylation at 4q and 10q. The majority, 11 samples, were from SMCHD1 mutated cases. Our analysis revealed that reduced D4Z4 methylation at 4q and 10q has no strict correlation with the clinical phenotype. Indeed, the FSHD classical phenotype (category A) was reported in 22 subjects. In 10 of them we observed reduced D4Z4 methylation. Of the 16 cases carrying SMCHD1 pathogenic variants 11 displayed the classic FSHD phenotype, 1 incomplete phenotype, 2 atypical. A new pipeline is proposed for the interpretation of D4Z4 methylation results in FSHD diagnosis that considers the complexity of the epigenetic setting of repetitive elements.

P1.07

DUX4 protein interactors are involved in the DNA damage response

Karimatou Bah[1], Moriya Slavin[2], Clothilde Claus[1], Anne-Emilie Declèves[3], Nir Kalisman[4], Frédérique Coppée[3]

[1] *Laboratory of Metabolic and Molecular Biochemistry, University of Mons, Belgium*
[2] *Department of Biological Chemistry, Hebrew University of Jerusalem, Israel*
[3] *University of Mons*
[4] *Department of Biological Chemistry, Hebrew University of Jerusalem, Israel*

Abnormal expression of DUX4 in skeletal muscle cells is accompanied by increased oxidative stress, accumulation of DNA damages and ultimately cell death. The DUX4 transcriptomic impact is not sufficient to explain all the FSHD features and how muscle cell death occurs. In collaboration with Professor Kalisman lab, we determined the DUX4 interactors by affinity purification followed by mass spectrometry. We also investigated the protein partners of DUX4c, a DUX4 homologue with a high sequence identity and that is normally expressed in muscle cells. We identified that the major interactors of both DUX4 and DUX4c are C1qBP, PARP1, XRCC5 and XRCC6. Interactions might therefore most probably occur by the DUX4/4c identical homeodomains. We were surprised to find that all these 4 proteins are involved in the repair of DNA double-strand breaks which are considered to be the most toxic genomic lesions. Moreover, our preliminary data suggest that DUX4 impact the level/localization of several DNA damage response (DDR) proteins during muscle cell differentiation. Adequate DDR is essential for muscle regeneration. We speculate that DUX4c role during muscle regeneration is linked to DDR and that DUX4 competes with this function allowing for the accumulation of unrepaired DNA lesions leading to the death of regenerating muscle cells.

P1.08

SMCHD1 regulates biological pathways relevant for Bosma syndrome and facioscapulohumeral dystrophy phenotype

Frédérique Magdinier[1], Camille Laberthonniere[1]

[1] *Aix-Marseille University*

Many genetic syndromes are linked to mutations in genes encoding factors that guide chromatin organization. Among them, distinct rare genetic diseases are linked to mutations in SMCHD1 that encodes the Structural Maintenance of Chromosomes flexible Hinge Domain containing 1 chromatin-associated factor. In Humans, its function as well as the impact of its mutations remain poorly defined. To fill this gap, we determined the epi-signature associated to heterozygous SMCHD1 variants in primary cells and cell lineages derived from induced pluripotent stem cells for Bosma Arhinia and Microphthalmia Syndrome (BAMS) and type 2 Facio Scapulo Humeral Dystrophy (FSHD2). In human tissues, SMCHD1 regulates the distribution of methylated CpGs, H3K27 trimethylation and CTCF at repressed chromatin but also at euchromatin. Based on the exploration of tissues affected either in FSHD or BAMS, i.e. skeletal muscle fibers and neural crest stem cells respectively, our results emphasize multiple functions for SMCHD1, in chromatin compaction, chromatin insulation and gene regulation with variable targets or phenotypical outcomes. We concluded here that in rare genetic diseases, SMCHD1 variants impact gene expression in two ways, (i) by changing the chromatin context at a number of euchromatin loci or (ii) by directly regulating loci, in particular some loci encoding master transcription factors required for cell fate determination and tissue differentiation.

P1.09

A study of *DUX4* expression pattern with FSHD patient-derived iPSC model

Mitsuru Sasaki-Honda[1], Álvaro Rada-Iglesias[2], Hidetoshi Sakurai[3]

[1] *IBBTEC, University of Cantabria; CiRA, Kyoto University*
[2] *IBBTEC, University of Cantabria*
[3] *CiRA, Kyoto University*

How *DUX4* gene expression is regulated in skeletal muscle cells in FSHD remains unclear. It is well known that a limited percentage of cells become *DUX4* positive even in FSHD patient-derived muscle cells, indicating multiple genetic and epigenetic factors behind this sporadic and cell type-specific expression pattern rather than a straightforward gene activation mechanism. We successfully modeled the *DUX4* expression pattern using our FSHD patient iPSC models, including gene-repaired clones in FSHD2, by which we can more precisely compare epigenetic features without clonal genetic variations. For example, open chromatin analysis identified a limited number of statistically significant patient-specific regions in the 4q35 and others, while *DUX4* negative patient muscle cells are comparable to gene repaired clones. Moreover, non-amplification long-read sequencing showed D4Z4 hypomethylation in 4qA allele in FSHD2 iPSCs and fibroblasts, supporting the idea of unknown random epigenetic factors behind sporadic expression pattern. These results fit previous studies and motivated us to further explore their functional significance in *DUX4* regulation (or mis-regulation). In this presentation, we will share our recent understanding of the unique gene expression in the context of functional genomics approach.

P1.10

Fibro-adipogenic progenitors and FSHD myopathy

Carlo Serra[1], Kathryn Wagner[2], Andrew Wilson[1], Thomas Lloyd[1]

[1] *Department of Neurology and Neuroscience, Johns Hopkins University School of Medicine, Baltimore*
[2] *Kennedy Krieger Institute*

Intramuscular fat and fibrosis characterize the FSHD muscle. Finding how adipose and fibrotic tissues are accumulated during FSHD progression will allow setting new treatments for FSHD. Fibro-adipogenic progenitors (FAPs) are muscle resident mesenchymal stem cells able to differentiate into fibroblasts and adipocytes during chronic muscle regeneration. Exosomes are extracellular vesicles released to establish cell-to-cell communications in the body and regulate many aspects of muscle physiology. Our hypothesis is that *DUX4* expression inside FSHD muscle fibers reduces the muscle release of FAPs-specific anti-fibrogenic and -adipogenic exosomes, which results in higher FAPs adipogenic and fibrogenic differentiation rates. We developed the triple transgenic FLExDUX4-/+; ACTA-Cre-/+; hCD63-6xHis-GFP-/+ (TTG) mice, in which *DUX4* and green fluorescent protein (GFP)- tagged exosomes are expressed upon tamoxifen (TMX)-induction in muscle fibers. Preliminary results show that TTG mice plus TMX have a significant increment of the number of regenerating muscle fibers when compared to the TTG mice treated with vehicle alone. Female TTG mice plus TMX showed an increased number of FAPs when compared with ACTA-Cre-/+; hCD63-6xHis-GFP-/+ mice treated with TMX, but similar numbers of FAPs vs. female TTG mice treated with vehicle alone. Female TTG mice treated with TMX showed a wider extension of perilipin A+/B+ area (adipocytes) when compared with ACTA-Cre-/+; hCD63-6xHis-GFP-/+ mice treated with TMX, but not vs. TTG mice treated with vehicle alone. We are currently expanding the analysis for FAPs, fibroblasts, and intramuscular fat distribution in different muscles of the same mice.

P1.11

DUX4, nucleolar stress, apoptosis, and FSHD myopathy

Carlo Serra[1], Kathryn Wagner[2], Andrew Wilson[1], Thomas Lloyd[1]

[1] *Department of Neurology and Neuroscience, Johns Hopkins University School of Medicine, Baltimore*
[2] *Kennedy Krieger Institute*

A fundamental obstacle to develop efficacious FSHD therapies is the partial knowledge of FSHD etiology and progression. Using confocal microscopy, we found the presence of nucleolar *DUX4* in myotubes generated by MB135-iDUX4CA (iDUX4) cells, a human muscle cell line that expresses *DUX4* upon doxycycline (Doxy) treatment. Human primary FSHD myotubes showed nucleolar *DUX4* only in a small number of the nucleoli of *DUX4* expressing nuclei. iDUX4 myotubes, and human primary FSHD and control myotubes showed a wide nucleolar distribution of DUX4C, indicating a potential antagonistic action of the two proteins inside the nucleolus. iDUX4 myotubes plus Doxy expressed specific non-coding RNAs (ncRNAs) transcribed from the long intergenic spacer (IGS) region of the ribosomal DNA, and the upregulation of transcripts for nucleolar associated proteins and regulatory RNAs. Human primary FSHD myotubes did not show the upregulation of the IGS ncRNAs, probably due to the very low *DUX4* expression. IGS-derived ncRNAs are associated with the activation of both nucleolar stress response and nucleolar detention pathway (NoDP). In this regard, we found the presence of amyloid-like bodies, a sign of NoDP, inside the nucleoli of *DUX4*-expressing iDUX4 myotubes. Since nucleolar stress regulates cellular apoptosis, the *DUX4*-dependent nucleolar change we found may be one of the early etiological steps of the muscle fiber death in FSHD. We are conducting an RNA-Seq on iDUX4 myotubes treated with and without Doxy, with a focus on transcripts derived from the nucleolar DNA, and on nuclear transcripts associated with nucleolar function.

P1.12

Deciphering the role of SMCHD1 in disease and development

Rachel Eiges[1], Uria Aviel[2], Silvina Epsztein-Litman[2], Yotam Drier[3]

[1] *Shaare Zedek Medical Center, Jerusalem, Israel*
[2] *Stem Cell Laboratory, Shaare Zedek Medical Center*
[3] *The Hebrew University School of Medicine, Jerusalem*

SMCHD1 modifies chromatin and affects DNA methylation during early embryo development, playing a role in X inactivation and gene cluster silencing. It regulates de novo DNA methylation and chromatin interactions, most likely by antagonizing CTCF binding. Mutations in SMCHD1 can cause two dominant diseases: FSHD2, which leads to DNA hypomethylation and chromatin relaxation, and BAM syndrome, which results in midline defects. It's unclear how SMCHD1 mutations lead to different conditions, and how it mediates chromatin interactions. The goal of our research is to explore the potential involvement of SMCHD1 in mediating de novo methylation at repetitive elements that are associated with various pathologies other than FSHD. Thus far, we established a dozen SMCHD1 KO hESC clones by gene editing on the background of wild type versus mutant hypermethylated alleles underlying fragile X (CGG expansion in FMR1) and myotonic dystrophy type 1 (CTG expansion in DMPK) and monitored for a change in DNA methylation. Using bisulfite DNA colony sequencing, we find no evidence for a change in abnormal methylation at either loci. Strikingly though, methylation levels remained unchanged also at the D4Z4 repeats. Nevertheless, we identified several clusters of CpG islands that consistently become hypomethylated. Some correspond with ectopic CTCF binding, supporting the view that SMCHD1 antagonizes CTCF occupancy by hypermethylation in hESCs. Altogether, we conclude that the activity of SMCHD1 in de novo methylation at long repetitive elements are most likely limited to a critical developmental window before blastocyst formation or take place only upon cell differentiation.

P1.13

Generation of mouse artificial chromosome carrying FSHD1-derived chromosome 4q35 for a novel FSHD1 mouse model

Yosuke Hiramuki, Ichizo Nishino[2], Hiroyuki Kugoh[1], Yasuhiro Kazuki[1]

[1] *Chromosome Engineering Research Center, Tottori University*
[2] *National Center of Neurology and Psychiatry*

DUX4 is conserved in primates and located in the 3.3 kb D4Z4 macrosatellite repeat array containing 11-100 units in the subtelomeric regions of human chromosome 4q35. *DUX4* is normally expressed in testis and cleavage stage embryo, while it is repressed in somatic tissues including skeletal muscles. The contraction of the D4Z4 units combined with the presence of a polymorphic *DUX4* polyadenylation signal results in aberrant *DUX4* expression in skeletal muscles leading to FSHD. However, it remains unclear how the *DUX4* expression is genetically and epigenetically regulated in vivo. Here, we aim to generate a novel FSHD1 mouse model that carries a total of 5 Mb of the FSHD1-derived chromosome 4q35 extending from the SLC25A4 gene to telomeric regions. To this end, we use mouse artificial chromosome (MAC), which can be independently maintained and carry megabase size of genomic DNA. First, we inserted a loxp site into the 4q35 region using CRISPR/Cas9 genome editing in FSHD1 cells. Second, we transferred it from FSHD1 cells to CHO cells carrying MAC which has a loxp site by microcell-mediated chromosome transfer (MMCT). Third, we performed reciprocal translocation between chromosome 4 and MAC in the CHO cells using Cre/loxp system, and generated MAC carrying the 5 Mb 4q35 region (4q35-MAC). As a final step, we are now transferring the 4q35-MAC from CHO cells into mouse embryonic stem cells by MMCT. Successful completion of this study will result in the characterization of transchromosomic mice carrying the 4q35-MAC for understanding the molecular mechanisms regulating human *DUX4* locus in germ cells and somatic cells.

P1.14

Development of a new *DUX4*-responsive reporter mouse

Lindsay Wallace[1], Jessica Camp[1], Noah Taylor[1], Scott Q. Harper[2]

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

Several FSHD mouse models are available, including our lab's TIC-DUX4 model. Both male and female animals develop molecular, histopathological, and functional myopathic phenotypes in a Tamoxifen- and *DUX4*-dose dependent fashion. Due to low-level, stochastic leakiness in the Tamoxifen control system, older TIC-DUX4 mice also develop histopathology and molecular phenotypes without Tamoxifen induction, but do not show significant functional deficits. We propose that the leaky, stochastic *DUX4* expression system more accurately models low level *DUX4* expression in humans, compared to Tamoxifen-induced mice that express higher levels of *DUX4* in virtually all myonuclei. Unfortunately, at these low *DUX4* levels, individual TIC-DUX4 mice show phenotypic variability, necessitating relatively large N's in therapeutic *DUX4*-inhibiting studies. To track DUX4 expression and phenotypes in vivo, we generated a *DUX4*-responsive reporter mouse. This model contains a single copy transgene expressing Luciferase-P2A-mScarlet. In preliminary assessments, we show the model produces *DUX4*-responsive luciferase, which can be imaged in live mice over time, and mScarlet fluorescence, for tagging individual myonuclei after sacrifice. This system can provide a quantitative tool to measure *DUX4* inhibiting therapies, and potentially reduce animal numbers needed for long-term experiments due to variable phenotypes and unpredictable progression seen in low-expressing *DUX4* models.

P1.15

FRG1-mouse as an effective model of muscle wasting to test novel therapeutic options for FSHD

Sebastian Fantini[1], Grazia Bisceglia[1], Beatrice Bertarini[1], Rui Li[2], Marcello Manfredi[3], Mathieu Michel[4], Marco Spinazzi[4], Lihua Zhu[2], Pascal Reynier[4], Giuseppe D'Antona[5], Rossella Tupler[1]

[1] *Università degli Studi di Modena e Reggio Emilia*
[2] *UMass Chan Medical School*
[3] *Università del Piemonte Orientale "Amedeo Avogadro"*
[4] *CHU ANGERS Centre Hospitalier Universitaire (CHU) d'Angers*
[5] *Università degli Studi di Pavia*

The pathogenesis of facioscapulohumeral dystrophy (FSHD) has been so far only partially elucidated. Because of the complexity of the genetic and molecular factors underlying the disease, no effective therapeutic interventions are currently available. Therefore, the exploitation of an effective animal model that can mimic aspects of disease pathophysiology is still needed. FRG1-mice, overexpressing the RNA-binding protein FRG1, represent an effective model of progressive myopathy displaying features of human disease. Longitudinal multi-omics analyses of FRG1-mice allowed monitoring the onset and progression of the dystrophic process. An impairment of muscle maturation and metabolism characterizes the pre-dystrophic phase and translates in metabolomics changes that can be detected both in plasma and muscle. At older age a sustained activation of proinflammatory molecules characterizes the dystrophic phase with changes that correlate with the degree of muscle wasting. The identification of molecules characterizing the different phases of muscle dystrophy lays the bases for the selection of plasma and muscle biomarkers that can be used in clinical practice for FSHD follow up and highlights the importance of FRG1-mouse as a preclinical model to evaluate effective therapeutics for FSHD.

P1.16

FSHD 3D-modeling through different bioprinting approaches

Stefano Testa[1], Lucas Duvert[1], Patricia Alloncle[1], Adrien Casanova[1], Frédérique Magdinier[1]

[1] *Aix-Marseille University*

In the last two decades, 3D printing technology has been increasingly used in bio-medical research as it turned out to be an optimal tool for Tissue Engineering (TE) approaches. Among the different 3D bio-printing systems, the extrusion and laser based ones are the most appropriate for skeletal muscle TE. The first one is the most practical in reproducing the skeletal muscle tissue architecture, through the organized deposition of printing fibers in parallel lines, layer by layer. Moreover, the extrusion mechanism forces the cells in orienting along the printing axis, allowing the differentiation of muscle progenitors in properly organized bundles of myotubes. The laser-based approach allows to achieve an incredible level of resolution, making possible to design any pattern of cell deposition. Further, several cellular populations can be printed on the same sample in a spatially organized and highly reproducible manner, a key step for studying cellular interactions, such as those that occur at the neuromuscular and myotendinous junctions. The aim of our research is to exploit the potentialities of these techniques for modeling normal and pathological human skeletal muscle tissue. Combining primary and IPS cells, we want to setup a healthy vs FSHD muscle model for a deeper comprehension of the pathology mechanisms, together with functional analyses to evaluate muscle fiber contraction and strength. This “human muscle platform” will be a useful tool for drug testing.

P1.17

WDR5 is required for *DUX4* expression and its pathological effects in FSHD muscular dystrophy

Emanuele Mocciaro[1], Roberto Giambruno[1], Stefano Micheloni[1], Filippo Cernilogar[2], Annapaola Andolfo[1], Cristina Consonni[1], Maria Pannese[1], Giulia Ferri[1], Valeria Runfola[1], Gunnar Shotta[2], Davide Gabellini[1]

[1] *San Raffaele Scientific Institute*
[2] *Ludwig-Maximilians-University (LMU)*

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most prevalent neuromuscular disorder with no cure or therapeutic option currently available to patients. FSHD is caused by aberrant mis-expression of the transcription factor *DUX4*. In FSHD, *DUX4* initiates a cascade of processes triggering the activation of a pro-apoptotic transcriptional program leading to muscle wasting. Given its pivotal role in FSHD, blocking *DUX4* expression with small molecule drugs is an attractive solution. We previously demonstrated that the long non-coding RNA DBE-T is essential for aberrant *DUX4* expression in FSHD. Using affinity purification followed by proteomics we identified the chromatin remodeling protein WDR5 as a novel DBE-T interactor and a key player required for the biological activity of the lncRNA. We found that, in FSHD muscle cells, WDR5 is necessary for the expression of both *DUX4* and its target genes. Moreover, WDR5 silencing rescues both cell viability and myogenic differentiation of FSHD muscle cells as efficiently as silencing directly *DUX4*. Remarkably, we obtained analogous result by WDR5 pharmacological inhibition with a small molecule. Intriguingly, RNA-seq indicates that the WDR5 inhibitor globally blocks the *DUX4*-induced gene expression, which is the major molecular signature in FSHD. These results not only support a crucial role of WDR5 in the regulation of *DUX4* expression but also provide a novel druggable target opening a promising therapeutic path to the treatment of FSHD.

P1.18

Discordant monozygotic twins with reduced-length D4Z4 allele and FSHD-like phenotype

Giulio Gadaleta[1], Liliana Vercelli[1], Guido Urbano[1], Enrica Rolle1, Valentina Salsi[2], Rossella Tupler[2], Tiziana Enrica Mongini[1]

[1] *Neuromuscular Unit, Department of Neurosciences "Rita Levi Montalcini", University of Turin*

Introduction. Discordant monozygotic twins carrying reduced D4Z4 alleles (DRA) are reported in the FSHD literature, with various pathogenetic hypotheses (postzygotic mutations, somatic mosaicism, epigenetic and nongenetic influences such as inflammatory triggers). We report the case of discordant female monozygotic twins, one asymptomatic and one with severe infantile-onset FSHD showing Coats syndrome and binaural neurosensorial hearing impairment. Case presentation. The girls were delivered prematurely at 32 weeks due to reduced intrauterine growth of one fetus. At the age of 7, one twin disclosed orbicularis oris and orbicularis oculi weakness with no other muscle weakness. Both girls carry a 4qA de novo DRA with 1 repeat. At the age of 20, one twin remained asymptomatic, whereas the other displayed severe hyperlordotic waddling gait, winged scapulae with restricted upper-limb abduction, complete loss of orbicularis oris and oculi function, and positive poly-hill and Beevor signs. Brachial biceps biopsies performed at 16 revealed chronic myopathic and inflammatory features in both twins, more enhanced in the affected girl. Conclusion. Besides known epigenetic modifications, FSHD discordances may be underpinned by somatic mosaicism, and environmental factors, such as physical activity, diet and exposure to toxins, potentially playing a role during gestation. In our case, the reduced intra-uterine growth of the affected twin, might have contributed to FSHD pathogenesis.

Posters: Outcomes Assessments

P2.01

Assessment of the burden of outpatient clinic and MRI-guided needle muscle biopsies as reported by patients with facioscapulohumeral muscular dystrophy

Joost Kools[1], Willem Aerts[1], Erik Niks[2], Karlien Mul[1], Lisa Pagan[3], Jake Maurits[1], Renée Thewissen[1], Baziel van Engelen[4], Nicol Voermans[4]

[1] *Radboud University Medical Center*
[2] *Leiden University Medical Center*
[3] *Centre for Human Drug Research*
[4] *Neuromuscular Centre Nijmegen, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands*

Muscle biopsies are used in clinical trials to measure target engagement of the investigational product. With many upcoming therapies for patients with facioscapulohumeral dystrophy (FSHD), the frequency of biopsies in FSHD patients is expected to increase. Muscle biopsies were performed either in the outpatient clinic using a Bergström needle (BN-biopsy) or in a Magnetic Resonance Imaging machine (MRI-biopsy). This study assessed the FSHD patients' experience of biopsies using a customized questionnaire. The questionnaire was sent to all FSHD patients who had undergone a needle muscle biopsy for research purposes, inquiring about biopsy characteristics and burden, and willingness to undergo a subsequent biopsy. Forty-nine of 56 invited patients (88%) completed the questionnaire, reporting on 91 biopsies. The median pain score (scale 0-10) during the procedure was 5 [2-8], reducing to 3 [1-5] and 2 [1-3] after one and 24 hours respectively. Twelve biopsies (13.2%) resulted in complications, eleven resolved within 30 days. BN-biopsies were less painful compared to MRI-biopsies (median NRS: 4 [2-6] vs. 7 [3-9], p=0.001). The burden of needle muscle biopsies in a research setting is considerate and should not be underestimated. MRI-biopsies have a higher burden compared to BN-biopsies.

P2.02

Development and validation of the facioscapulohumeral muscular dystrophy-health index (FSHD-HI), a disease-specific patient-reported outcome measure to facilitate clinical trials

Anika Varma[1], Jennifer Weinstein[1], Jamison Seabury[1], Spencer Rosero[1], Charlotte Engebrecht[1], Ellen Wagner[1], Christine Zizzi[2], Elizabeth Luebbe[3], Nuran Dilek[3], Michael McDermott[4], John Kissel[5], Valeria Sansone[6], Chad Heatwole[2]

[1] *Center for Health + Technology, University of Rochester*
[2] *Center for Health + Technology & Department of Neurology, University of Rochester*
[3] *Department of Neurology, University of Rochester*
[4] *Department of Neurology & Department of Biostatistics, University of Rochester*
[5] *Department of Neurology, The Ohio State University Wexner Medical Center*
[6] *Department of Biomedical Sciences for Health, University of Milan & The NEMO Clinical Center*

Objectives: In response to the need for therapeutic advances in FSHD, it is imperative to have a comprehensive, sensitive, and reliable patient-reported outcome measure (PRO) to accurately track changes in FSHD disease burden. Using a patient-centric research approach, we developed a disease-specific PRO, titled the Facioscapulohumeral Muscular Dystrophy-Health Index (FSHD-HI). Methods: We conducted qualitative interviews and a cross-sectional study involving 328 individuals to determine what symptoms are most important in FSHD. The most relevant symptoms were included in the FSHD-HI. We used patient interviews, test-retest reliability evaluation, known groups validity testing, and factor analysis to further evaluate and optimize the FSHD-HI. Results: The FSHD-HI contains 14 subscales that measure FSHD disease burden from the patient's perspective. Fourteen adults with FSHD participated in semi-structured beta interviews and found the FSHD-HI to be clear, usable, and relevant to them. Thirty-two adults with FSHD participated in test-retest reliability assessments, which demonstrated the high reliability of the FSHD-HI total (intraclass correlation coefficient = 0.924). The final FSHD-HI and its subscales also demonstrated a high internal consistency (Cronbach's alpha = 0.988). Conclusions: Our research demonstrates the validity of the FSHD-HI as a marker of disease burden for use in FSHD therapeutic trials, clinical monitoring, and the support of drug labeling claims.

P2.03

A systematic literature review to assess the level of evidence in facioscapulohumeral muscular dystrophy

Lianne Barnieh[1], Rachel Beckerman[1], Helena Emich[1], Katy Eichinger[2], Adi Eldar-Lissai[3]

[1] *Maple Health Group*
[2] *University of Rochester Medical Center*
[3] *Fulcrum Therapeutics*

Objectives: Facioscapulohumeral muscular dystrophy (FSHD) is a rare, debilitating disease for which there are no approved pharmacological interventions. With disease progression, patients lose function, mobility, independence and often live with chronic pain. This systematic literature review (SLR) assessed the evidence base in FSHD. Methods: PubMed, EMBASE and Cochrane Reviews databases were searched for studies on FSHD, with no limits on interventions, comparators, outcomes, study design or date of publication. Three conferences not indexed in EMBASE were also hand searched (limit of last three years). Screening was performed two independent researchers and articles were categorized as: pharmacological and non-pharmacological treatments; outcome measures and validation; humanistic burden and patient reported outcomes; economic burden; disease classification; diagnosis; and guidelines. Results: A total of 2,211 full texts and 754 conference abstracts were identified for review with 153 full texts and 101 conference abstracts included. The most reported topic among full texts was pharmacological and non-pharmacological treatments (n=52), with outcome measures and validation being the second most reported topic (n=32). Among conference abstracts, the most reported study topic was outcome measures and validation (n=43), with disease classification second (n=20). Conclusion: FSHD research interests mainly include pharmacological and non-pharmacological treatments, followed by the validation of outcome measures for use in research. Research efforts on minimizing the impact of the debilitating and progressive nature of FSHD should continue until a treatment is approved.

P2.04

Developing a therapist-led FSHD clinic at the Atkinson-Morley Neuromuscular Centre

Sherryl Chatfield[1], Pamela Appleton[1], Niranjanan Nirmalananthan[1], Emma Matthews[2]

[1] St George's University Hospitals NHS Foundation Trust
[2] St George's University of London

Facioscapulohumeral dystrophy (FSHD) is a neuromuscular condition with an incidence of between 3 and 5 in 100,000. St George's Hospital in London, UK is a tertiary neurosciences centre and sees adults with FSHD from South West London, Surrey and surrounding areas in the South East. In October 2022 we established a therapy-led FSHD clinic at St George's Atkinson-Morley Neuromuscular Centre. The clinic is run by a specialist neuromuscular physiotherapist and a neuromuscular care advisor with an occupational therapy background. The aim of the clinic is to optimise the holistic management of care for our adults with FSHD by considering physical, environmental and psychosocial factors that impact on wellbeing. From this clinic we can link patients with the orthotics service and orthopaedic team. Yearly clinical assessments of function and quality of life enable comparison from year to year. Three clinics have been run to date with a total of 10 patients seen. Outcomes of the clinic have included exercise advice (n=10), referral to orthotics for lower limb orthoses (n=2) and shoulder supports (n=3), fatigue management advice and group referral (n=3), signposting to the patient support group (n=10), advice on local resources and daily living aids (n=4), support regarding work (n=2) and referral to orthopaedics for scapular stabilisation (n=1).

P2.05

Muscle diffusion tensor imaging in facioscapulohumeral muscular dystrophy

Leonardo Barzaghi[1], Matteo Paoletti[1], Mauro Monforte[2], Sara Bortolani[2], Chiara Bonizzoni[1], Thorsten Feiweier[3], Niels Bergsland[4], Francesco Santini[5], Xeni Deligianni[5], Giorgio Tasca[6], Silvia Figini[7], Enzo Ricci[2], Anna Pichiecchio[8]

[1] *Advanced Imaging and Radiomics Center, Neuroradiology Department, IRCCS Mondino Foundation, Pavia*
[2] *Fondazione Policlinico Universitario A. Gemelli IRCCS*
[3] *Siemens Healthcare GmbH, Erlangen, Germany*
[4] *IRCCS, Fondazione Don Carlo Gnocchi ONLUS, Milan, Italy*
[5] *Department of Radiology, University Hospital Basel, Basel, Switzerland*
[6] *UOC di Neurologia, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy*
[7] *Department of Political and Social Sciences, University of Pavia, Italy*
[8] *Department of Brain and Behavioral Sciences, University of Pavia, Italy*

We explored for the first time the application of muscle Diffusion Tensor Imaging (mDTI) in Facioscapulohumeral muscular dystrophy (FSHD). We assessed diffusivity parameters in FSHD subjects compared to healthy controls (HCs) and their ability to precede any fat substitution in muscles. From a cohort of ten FSHD subjects and fifteen age-matched HCs we calculated Fat Fraction (FF), water T2 (wT2), Mean, Radial, Axial Diffusivity (MD,RD,AD) and Fractional Anisotropy (FA) of thigh muscles. All parameters were compared between FSHD subjects and controls, exploring also their variation from proximal to distal thigh levels. We investigated whether diffusivity parameters are able to predict disease involvement in muscle compartments with no significant fat substitution and muscle edema. Whole-thigh mDTI values were correlated to clinical severity scores. FF and wT2 were significantly higher in FSHD than controls whereas MD, RD and AD were significantly lower than controls ($p < .05$). No difference with controls was shown for FA. FF positively correlated with FA and negatively with MD, RD and AD. FF and FA showed significantly higher values distally than proximally whereas wT2, MD, RD, AD showed lower values distally than proximally ($p < .05$). Muscles with no significant fat replacement or edema showed a significantly lower AD and FA than controls. FA was the only mDTI parameter to positively correlate with the 6-minute Walking Test (6-MWT). Our results suggest that mDTI parameters appear to predominantly reflect fat replacement in FSHD and might be able to show disease involvement in muscles even before significant fat substitution. (Funding: Ministry of Health RC 2022-2024).

P2.06

Exploring the use of facial muscle ultrasound in facioscapulohumeral muscular dystrophy

Sjan Teeselink[1], David Lamers[1], Sanne Vincenten[1], Nens van Alfen[1], Baziel van Engelen[2], Nicol Voermans[2], Karlien Mul[1]

[1] *Radboud University Medical Center*
[2] *Neuromuscular Centre Nijmegen, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands*

Objective: To investigate correlations between quantitative muscle ultrasound (QMUS) outcomes and clinical outcome measures as a means to describe facial muscle involvement in FSHD, and to assess changes in muscle composition and thickness over time. Methods: This exploratory longitudinal cohort study assessed muscle echogenicity (ME) and muscle thickness (MT) of 5 facial and 3 masticatory muscles in genetically confirmed FSHD patients twice over a period of approximately 18 months. The following muscles were examined: temporalis, masseter, depressor anguli oris, orbicularis oris, zygomaticus major and minor. In the digastric muscles an geniohyoid muscle only ME was measured. Clinical severity was assessed using the Ricci score, FSHD clinical score facial subscale and facial weakness score. Results: For now we included 61 patients (46% male, age 23-78 years), covering a broad spectrum of facial muscle weakness and overall disease severity. At baseline the mean ME of all muscles showed a weak to moderate correlation with all clinical outcome measures (Spearman's rho 0.3-0.4, $p < 0.05$). Discussion: Preliminary longitudinal results of this study will be presented.

P2.07

Facioscapulohumeral muscular dystrophy (FSHD) age-related differences in management among patients over and under 40 years

Kathryn Munoz[1], Chamindra Laverty[2], Richard Brook[3], Nathan L. Kleinman[3], Chao-Yin Chen[1], Teresa Brandt[1], Mark C. Stahl[1], **Amy Halseth**[1]

[1] *Avidity Biosciences, Inc.*
[2] *University of California, San Diego, Rady Children's Hospital, and VA San Diego Healthcare System*
[3] *Better Health Worldwide*

Objective: Describe the differences between patients with FSHD and matched controls (MCs) based on age. Design/Methods: Retrospective claims analysis (Jan 2015—Mar 2021) of patients with FSHD (≥2 ICD-10 codes >30 days apart) age <40 years (N=81) vs MCs (N=397) age ≥40 years (FSHD, N=199: MCs, N=1006). All subjects had >1 year of continuous data. Utilization, comorbidities, and costs (adjusted to 2020 USD) at 1-year post-diagnosis. Reported FSHD vs MC comparisons were significant (p≤0.001). Results: Compared with MCs, patients with FSHD <40 had $10,499 higher mean medical costs, utilized 51 more services, and received 20 more care days, and patients with FSHD ≥40 had $7123 higher mean medical costs, utilized 33.8 more services, and received 12.9 more care days. Most prevalent conditions (FSHD vs MC) included nervous system disorders, connective tissue diseases, lower respiratory diseases, malaise/fatigue, cardiac dysrhythmias, and non-traumatic joint disorders. Blindness/vision defects were elevated only in the young patient group. Nutritional deficiencies, gastrointestinal disorders, acquired foot deformities, spondylosis, hypertension, and non-traumatic joint disorders were elevated only in older patients. Conclusions: FSHD management changes as patients age. Overall utilization remains significantly higher compared to MCs, but differences may be less pronounced and driven by different conditions. The data reflect the difference in burden on patients with FSHD based on their age.

P2.08

FSHD European Trial Network

Nicol Voermans[1], Valeria Sansone[2], Giorgio Tasca[3], Federica Montagnese[4], Karlien Mul[5], Richard J. F. L. Lemmers[6], Emiliano Giardina[7], Pascal Laforet[8], María Vriens-Munoz Bravo[9], Alexandre Mejat[9], Julie Dumonceaux[10], Teresinha Evangelista[11]

[1] *Neuromuscular Centre Nijmegen, Radboud University Nijmegen Medical Centre, Netherlands*
[2] *Department of Biomedical Sciences for Health, University of Milan & The NEMO Clinical Center*
[3] *UOC di Neurologia, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy*
[4] *University of Munchen*
[5] *Radboud University Medical Center, Nijmegen*
[6] *Leiden University Medical Center, The Netherlands*
[7] *Santa Lucia Foundation IRCCS; Tor Vergata University of Rome*
[8] *Garches hospital, Paris*
[9] *FSHD Europe*
[10] *University College London, UK*
[11] *Unité de Morphologie Neuromusculaire, Institut de Myologie, AP-HP, Paris, France*

The FSHD European Trial Network was founded in 2021. In 2022 two ENMC workshops have taken place, on muscle imaging (working group 4; April 2022) and on clinical and genetic diagnosis, clinical outcome measures and biomarkers (working group 1, 2 and 3; September 2022). This has enabled the strengthening of the network. Major steps have been taken:

- A consensus on a number of topics have been reached, which was included in the workshop reports;
- WG1 is working on an update of the 2012 Best practice guidelines on genetic diagnostics of Facioscapulohumeral dystrophy;
- The WG 2, 3 and 4 have established a strong collaboration with the parallel working groups of FSHD CTRN;
- Many centers are actively involved in the Phase 3 Trial (REACH) of Fulcrum;
- Various members of the European Trial Network are involved in the update of the international standard of care;
- The Communication team of FSHD Europe / FSHD ETN is continuously working to improve the quality of the website;
- We aim to involve experts from all European countries, and experts of Israel and Turkey have joined;
- We collaborated with the FSHD Taskforce in TREAT NMD for harmonization of registries worldwide and training for professionals;
- We will start a 5th working group on health economics to prepare for the process of drug approval and reimbursement in different European countries.

The next steps to be taken are identifying the FSHD clinical and trial centres in Europe, and assessing the available facilities. This information will be made available on the FSHD Europe website. We have recently submitted a grant proposal to Solve FSHD for a project manager for FSHD Europe and the FSHD ETN.

P2.09

Respiratory tests and models in FSHD

Patrick Valentin[1]

[1] *Independent researcher*

We presented previously the FRT (Fast Respiratory Test) as a way for a FSHD patient to self monitor the evolution of its respiratory problems. Here we show that evolution may be positive provided exercize is appropriate or a cough assist is used to slightly expand thorax. A parallel gazometric test showed that the wristwatch used for FRT differs by only 0.3% from the gazometric saturation. Thus FRT proves to be both precise and sensitive to respiratory changes in terms of SpO2 and pulse. However on the long range, daily FRT yields a rather large dispersion, typical range of SpO2 is 5%, pulse is 15 bpm. Although of unknown (and multifactorial) nature, such a variability turns out to be an advantage because it shows a set of different respiration modes: a least-squares analysis of the scatter plot shows a linear relation with a fixed point at about 60 bpm at 100% SpO2 and a pulse decrease of 2.3 bpm for every 1% increase in saturation. Such a negative slope holds true for FSH and non-FSH. Obviously, this is linked with the fact that a higher arterial SpO2 needs a lower blood flow-rate to be transported. Quantitative steady state mass balance formulae link FRT data with metabolic O2 consumption and CO2 production rates at rest. Deduced mixed veinous blood saturation and partial pressures compare well with the classical values, such as given by the OSA model (Siggaard Andersen et al). Therefore FRT data can be combined with gazometric data, to get a complete evaluation of the respiratory state at a given time. Its potential applications are multiple, including home following of new therapeutic tests.

P2.10

Standard of care and management of facioscapulohumeral muscular dystrophy

Ronne Pater[1], Sarah el Markhous[2], June Kinoshita[3]

[1] *Radboud University Medical Center, Nijmegen*
[2] *None*
[3] *FSHD Society*

Background: Since disease-modifying therapies are entering clinical trials for FSHD, there is a pressing need for a unified and multidisciplinary international standard of diagnostics and care. Aim: This project aims to update and expend the current FSHD standard of care (Tawil et al., 2015) to address the knowledge gaps and present the current evidence base for diagnosis and management of FSHD patients. This international guideline is intended for physicians and healthcare professionals who are involved in the diagnosis and care of people with FSHD. Method: An evidence-based approach is used, including a systemic literature search, assessment of methodological quality of included articles using evidence tables, and assessment of the strength of evidence for each outcome measure using GRADE. For smaller topics a consensus-based approach is more suitable, due to limited evidence in the field. Together with the clinical expertise of twelve working groups, recommendations will be provided on important topics such as (genetic) diagnosis, pain and fatigue, functional impairments, pregnancy, managing pulmonary and sleep impairment, cardiac abnormalities, surgery, retinal vascular disease, hearing loss, communication, speech and swallow impairments and mental health. Finally, the prospects for therapeutic interventions and current research gaps will be addressed. The updated standard of care is expected to be finished by the end of 2023.

Posters: Disease Mechanisms & Interventional Strategies

P3.01

Characterization of the D4Z4 subtelomeric region of a human derived isogenic iPSC line and identification of a CRISPR/Cas9 strategy for *DUX4* inactivation in facioscapulohumeral muscular dystrophy type 1 (FSHD1)

Edoardo Malfatti[1], Chéryane Lama[2], Nicolas de Graaf[3], Reem Bou Akar[2], Prisca Danaus[4], Laurence Suel-Petat[5], Juliette Nectoux[4], François-Jérôme Authier[1], Frédéric Relaix[2], Isabelle Richard[5]

[1] *Reference Center for Neuromuscular Disorders - APHP Henri Mondor Hospital - INSERM IMRB – UPEC*
[2] *INSERM IMRB – UPEC*
[3] *Radboud University Medical Center, Nijmegen*
[4] *APHP Cochin Hospital*
[5] *Généthon - INSERM*

FSHD1 is the second most common muscular dystrophy in adulthood. The defect involves a contraction and a relaxation of the D4Z4 macrosatellite of chromosome 4q35 to less than 11 Repeated Units (RU) associated with the permissive haplotype 4qA161 leading to the myotoxic *DUX4* primate specific gene expression. The absence of the D4Z4 region is not associated with the disease. hiPSCs are a good model to comprehend the mechanisms leading to muscular degeneration in FSHD1. The CRISPR/Cas9 system is a promising tool to modify the D4Z4 macrosatellite region altered in FSHD1 patients. The aim of this study is to characterize the D4Z4 macrosatellite region of an isogenic hiPSC line to model FSHD1 in vitro and to design a CRISPR/Cas9 gene editing strategy to reduce the D4Z4 RU number and completely ablate the D4Z4 locus. Haplotype determination was performed by a PCR-sequencing experiment on the 4q35 alleles of interest. A double restriction enzyme digestion coupled to a Southern Blot was performed and validated by Molecular Combing to discriminate and quantify the D4Z4 RU in the 4q35/10q26 alleles. Subsequent sequencing in 15 DNA samples allowed the determination of conserved sequences located upstream and downstream of the D4Z4 RU in the alleles of interest and the identification of undesired SNPs, thus permitting the design of specific SgRNAs for CRISPR/Cas9 experiments. In conclusion, we identified a permissive 4qA161 allele containing 30 D4Z4 RU allowing a FSHD1 modeling thanks to CRISPR/Cas9 approaches. Furthermore, we designed specific SgRNAs suitable for all the tested patients, a significant step to a promising gene therapy.

P3.02

EPI-321: A promising gene therapy for facioscapulohumeral muscular dystrophy (FSHD) targeting D4Z4 epigenome

Abhinav Adhikari[1], Alexandra Collin de l'Hortet[1]

[1] *Epic Bio*

Facioscapulohumeral muscular dystrophy (FSHD) affects 800,000 globally with no cure available, current therapies only manage symptoms. Disease-causing DUX4 protein expression in muscle leads to progressive muscle wasting through activation of apoptotic and other pathways. DUX4 is encoded in the distal region of 4q35 chromosome from D4Z4 microsatellite array, which is hypomethylated in FSHD leading to DUX4 expression. At Epic Bio, we leveraged our proprietary GEMS platform to develop EPI-321, a treatment for FSHD that targets the D4Z4 epigenome and suppresses DUX4 expression permanently. EPI-321 is an AAV serotype rh74 vector with a catalytically inactive Cas protein fused to gene-suppressing modulators and a gRNA targeting D4Z4. EPI-321 showed no off-target to any known human protein coding gene in silico. We showed that EPI-321 robustly suppress DUX4 and downstream genes in patient derived myoblasts in vitro, irrespective D4Z4 repeat length. Functionally, in vitro treatment of FSHD myoblasts with EPI-321 decreased rate of apoptotic nuclei to the level of normal sibling control. Also, we showed robust delivery and expression of EPI-321 in the humanized muscle tissue in vivo following a single intravenous dose in mice. In addition to decreasing the DUX4 pathway, EPI-321 was able to rescue FSHD muscle cell survival by 55% after 4 weeks of treatment. Importantly, EPI-321 in mice and NHP demonstrated no signs of toxicity, with no abnormal clinical, histopathological, or blood chemistry responses, indicating the safety of the treatment. Our findings support EPI-321 as a potential gene therapy for FSHD, with IND submission planned for 2023 and first-in-human trials in 2024.

P3.03

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

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

[1] *San Raffaele Scientific Institute*

Facioscapulohumeral muscular dystrophy (FSHD) is the most prevalent neuromuscular disease affecting children and adults of all ages and both sexes. Unfortunately, no treatment is currently available. FSHD is caused by gain of expression of the double homeobox 4 (*DUX4*) gene, encoding for a transcription factor normally silent in most adult somatic tissues. In FSHD, *DUX4* triggers a pro-apoptotic program resulting in muscle wasting. Due to unknown molecular mechanisms, FSHD displays clinical and pathological manifestations overlapping with amyotrophic lateral sclerosis (ALS). While blocking *DUX4* activity is a plausible therapeutic option for FSHD, the mechanism underlying *DUX4*-induced toxicity is poorly understood. We identified MATR3 as the first direct inhibitor of *DUX4*. MATR3 is a nuclear protein mutated in ALS and dominant distal myopathy. We found that MATR3 directly binds to *DUX4* DNA-binding domain and blocks *DUX4*-mediated gene expression. As a result, MATR3 administration rescues cell viability and myogenic differentiation of FSHD muscle cells, while it does not affect healthy muscle cells. Notably, we characterized a shorter MATR3 fragment that is necessary and sufficient to directly block *DUX4*-induced toxicity to the same extent of the full-length protein. Genome-wide experiments confirmed MATR3 proficiency in decreasing *DUX4* bona fide target genes. Our data promote MATR3 as therapeutic molecule to develop a rational treatment for FSHD, that in perspective might be applied to a spectrum of related and currently incurable diseases. We are currently working on the generation of a small drug-like MATR3-based peptide inhibiting *DUX4*.

P3.04

ANT1 overexpression models: Phenotype similarities with FSHD

Sandrine Arbogast[1], Heinrich Kotzur[2], Corinna Frank[2], Nathalie Compagnone[3], Thibault Sutra[1], Fabien Pillard[4], Sylvia Pietri[5], Nisrine Hmada[1], Daouda Moustapha Abba Moussa[6], Jacques Mercier[1], Jean-Paul Cristol[1], Marie-Christine Dabauvalle[2], Dalila Laoudj-Chenivesse[1]

[1] *PhyMedExp, Université de Montpellier, INSERM, CNRS, Montpellier, France*
[2] *Image Core Facility, Biocenter, Julius Maximilian University of Würzburg, Würzburg, Germany*
[3] *Innovative Concepts in Drug Development (ICDD), Gemenos, France, Current address: mtBIOLABS, Auriol*
[4] *Sport Medicine Department, University Sport Clinic, Pierre Paul Riquet University Hospital Toulouse*
[5] *Aix Marseille Univ, CNRS, ICR, UMR 7273, Marseille, France*
[6] *IRMB, Univ Montpellier, INSERM (METAMONTP), Montpellier, France*

Despite years of controversy, Adenine nucleotide translocator 1 (ANT1 or SLC25A4) is now recognized as strongly expressed in FSHD skeletal muscle biopsies. ANT1 is the only 4q35 gene involved in mitochondrial function, however, its role in FSHD is unclear. We developed two models of ANT1 overexpression (in primary myoblasts from healthy controls and in Xenopus laevis during organogenesis) and compared them with the phenotype of FSHD primary muscle cells and biopsies. ANT1 overexpression in human muscle cells induced a phenotype presenting similarities with FSHD muscle cells and biopsies. ANT1-overexpressing muscle cells showed disorganized morphology, altered cytoskeletal organization, enhanced mitochondrial respiration and glycolysis, ROS production, oxidative stress and mitochondrial alterations as observed in FSHD muscle cells. In Xenopus laevis embryos, ANT1 overexpression affected skeletal muscle development, impaired skeletal muscle structure, altered mitochondrial ultrastructure and led to oxidative stress as observed in FSHD muscle biopsies. In aggregate, our data suggest that ANT1 is a modifier gene in FSHD. It contributes to mitochondrial dysfunction and oxidative stress in FSHD muscle cells by altering their bioenergetic profile associated with ROS production. Further investigations are necessary to better understand the interplay between *ANT1* and *DUX4* in energy metabolism, ROS production and cytotoxicity in FSHD muscles.
Reference: Arbogast et al Redox Biol. 2022, Vol 56, 102450.

P3.05

Apabetalone, a clinical-stage, selective BET inhibitor, opposes *DUX4* expression in primary human FSHD muscle cells

Ewelina Kulikowski[1], Christopher Sarsons[1], Dean Gilham[1], Laura Tsujikawa[1], Li Fu[1], Sylwia Wasiak[1], Brooke Rakai[1], Stephanie Stotz[1], Agostina Carestia[1], Michael Sweeney[1], Jan Johansson[1], Norman Wong[1]

[1] *Resverlogix Corp.*

Background: FSHD is a degenerative muscle disease caused by epigenetic *DUX4* de-repression. Bromo- and extraterminal domain (BET) proteins regulate *DUX4* transcription in FSHD. Apabetalone, a BET inhibitor (BETi) in late-stage development for cardiovascular disease, has selectivity for bromodomain 2 (BD2) of BET proteins and an established clinical safety record. Objectives: In primary human skeletal muscle cells (pHSMC) from FSHD patients, we evaluated apabetalone's effect on *DUX4*-driven mediators of FSHD. Methods: We compared apabetalone's BD2-selective inhibition with the pan BETi JQ1, and losmapimod – a p38 MAPK inhibitor and *DUX4* repressor. RNA-seq and bioinformatics categorized effects on the transcriptome. Differentiation was assessed by PCR; viability by colorimetric assays. Results: BETi downregulated *DUX4* target gene expression, including ZSCAN4, MBD3L2, and TRIM43. JQ1 suppressed pHSMC differentiation and induced apoptosis, but apabetalone preserved differentiation and viability. BETis normalized expression in *DUX4*-altered pathways of metabolism and cell death, while losmapimod affected different pathways including transcription and intracellular adhesion. Conclusions: Apabetalone inhibited *DUX4* target genes and reversed pathologic transcriptional activity in pHSMC. No impact on differentiation or apoptosis illustrated the preferential safety profile of apabetalone versus JQ1. This, coupled with its clinical safety, makes apabetalone a promising FSHD therapeutic.

P3.06

Sustained efficacy of CRISPR-Cas13b gene therapy for FSHD is challenged by immune response to AAV.Cas13b vectors

Scott Q. Harper[1], Afrooz Rashnonejad[2], Gerald Coulis[3], Noah Taylor[2], Gholamhossein Amini-Chermahini[2], Armando Villalta[3], Oliver King[4]

[1] *Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio*
[2] *Nationwide Children's Hospital*
[3] *University of California, Irvine*
[4] *UMass Chan Medical School*

Facioscapulohumeral muscular dystrophy (FSHD) is potentially devastating muscle disease caused by de-repression of the toxic *DUX4* gene in skeletal muscle. FSHD patients may benefit from *DUX4* inhibition therapies, and although several experimental strategies to reduce *DUX4* levels in skeletal muscle are being developed, no approved disease modifying therapies currently exist. We developed a CRISPR-Cas13b system that cleaves and eliminates *DUX4* mRNA and protein, protects cells from *DUX4*-mediated death, and reduces FSHD-associated biomarkers in vitro. We found little to no evidence of collateral transcript cleavage or significant off-target effects in human myoblasts in vitro. In vivo delivery of the CRISPR-Cas13b system with adeno-associated viral vectors (AAV6) reduced acute damage caused by high *DUX4* levels in a mouse model of severe FSHD. However, protection was not sustained over time, with reductions in Cas13b and guide RNA levels between 8 weeks and 6 months after injection. In addition, wild-type mice injected with AAV.Cas13b showed muscle inflammation with infiltrates containing AAV.Cas13b-responsive CD8+ cytotoxic T cells. Our findings suggest successful in vivo implementation of CRISPR/Cas13-based gene therapies may require strategies to mitigate immune responses.

P3.07

A modular system to convert therapeutic miRNAs from ubiquitous RNA pol III-based promoters to RNA pol II-driven muscle-specific promoters while maintaining fidelity of processing and efficacy

Noah Taylor[1], Matthew Guggenbiller[1], Lindsay Wallace[1], Scott Q. Harper[2]

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

RNAi-based gene therapy systems often employ ubiquitous RNA polymerase III (pol III)-driven promoters to express shRNA or miRNA. We are translating a U6-promoter-driven system to express a therapeutic miRNA targeting *DUX4*, named mi405. Commonly used tissue-specific promoters utilize RNA polymerase II (pol II) promoters, which often have multiple transcription start sites and require different termination signals. As a result of these sequence differences, converting a pol III-driven miRNA to a pol II-driven system can change the secondary structure of the primary miRNA transcript, thereby altering the maturation process by the RNAse enzymes Drosha and Dicer. This is important because even a single nucleotide change in a mature miRNA sequence can impact specificity and efficiency. To address this, we generated several new mi405 designs, for which we incorporated novel secondary structure elements in the primary transcript regions flanking Drosha and Dicer processing sites, to convert our existing U6/Pol III system to a muscle-specific, pol II-based system. We then confirmed comparable *DUX4*-targeting efficacy and expression of the mature sequences in vitro. Our system provides a new approach for restricting the expression of miRNAs to a desired tissue while maintaining both normal miRNA biogenesis and therapeutic efficacy of the mature product.

P3.08

Safety and efficacy of a possible gene therapy approach for FSHD muscular dystrophy

Maria Pannese[1], Beatrice Biferali[1], Ellen Wiedtke[2], Valeria Runfola[1], Paola Ghezzi[1], Dirk Grimm[2], Davide Gabellini[1]

[1] *San Raffaele Scientific Institute*
[2] *University of Heidelberg, Medical Faculty BioQuant*

THIS ABSTRACT HAS BEEN REMOVED AT THE REQUEST OF THE AUTHORS.

P3.09

DUX4-mediated hypoxia signaling and impairment of oxygen metabolism in facioscapulohumeral muscular dystrophy

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

[1] *Yale School of Medicine*

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common myopathies, affecting an estimated 1 in 8,000 individuals. Despite major progress in understanding the underlying genetics behind the pathology, no treatment or cure currently exists. We performed CRISPR screening in order to identify genes and pathways of which modulation leads to apoptosis resistance from DUX4, the toxic protein associated with FSHD's pathology. Hypoxia signaling was identified as one of the most promising targets and so we explored the potential of compounds that target this pathway as a therapeutic strategy. The mTOR inhibitor everolimus, which acts upstream of hypoxia signaling, successfully reduced DUX4 toxicity in vitro. We further explored the impact of this signaling and its inhibition by everolimus on cellular metabolism. DUX4 induction inhibited oxygen consumption rates (OCR), which was attenuated by everolimus, suggesting a shift away from oxidative metabolism. This was further explored in vivo using the DUX4-inducible ACTA1-MCM/FLExDUX4 mouse model. The highly oxidative soleus showed a fiber type shift towards glycolytic IIB fibers and had reduced NADH staining, confirming a reduction in oxidative metabolism. Promisingly, NADH staining on FSHD patient biopsy sections showed the same pattern, demonstrating physiological relevance. These results demonstrate a shift in oxidative metabolism as a component of FSHD pathology and provides a pathway to identify novel therapeutic targets for FSHD.

P3.10

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

Maëlle Limpens[1], Aline Derenne[1], Carmen Burtea[1], Sophie Laurent[11], Alexandre Legrand, Steve Wilton[2], Alexandra Belayew[1], Frédérique Coppée[1], Anne-Emilie Declèves[1], Alexandra Tassin[1]

[1] *University of Mons*
[2] *Murdoch University*

The first antisense oligonucleotides (ASOs) directed against DUX4 mRNAs have been developed at UMONS. Our strategy consists in coupling the ASOs with peptides that will ensure the muscle delivery and avoid/or minimize their unspecific clearance after systemic delivery. Therefore, by screening a phage-display library of linear peptides against either myotubes or a muscle-membrane protein, we selected phage clones that specifically bound to human and mouse muscle surface proteins (MSPep). The peptides encoded by the 4 most promising clones were then synthesized with Rhodamine conjugation for testing in cell cultures in vitro. Endocytosis of the MSPeps was first investigated in muscle cells compared to hepatocytes and renal cells. We then optimized the cellular assay and validated a quantification method to compare MSPep uptake into muscle or non-muscle cells. Moreover, the efficiency of various ASOs targeting *DUX4* mRNAs is also tested in vivo in the IMEP-DUX4 mouse model in which a *DUX4* expression plasmid is electroporated into the tibialis anterior and induces the development of an easily quantifiable muscle lesion. Finally, MSPep-ASO complexes were also designated in silico based on literature data and collaborators' expertise. Based on our in vitro and in vivo results, the most efficient MSPeps will be complexed with the most efficient ASOs. Further experiments will aim to evaluate the ability of MSPep-ASOs to target skeletal muscle in the IMEP mouse and deliver efficient ASO against *DUX4* mRNA.

P3.11

Estrogen interferes with *DUX4* nuclear import

Giada Mele[1], Fabiola Moretti[1], Giancarlo Deidda[1], Patrizia Calandra[1], Emanuela Teveroni[2], Silvia Maiullari[1], Marco Crescenzi[3], Maria Luisa Casella[3]

[1] *Consiglio Nazionale delle Ricerche (CNR-IBBC)*
[2] *Università Cattolica di Roma*
[3] *Istituto Superiore di Sanità*

DUX4 (Double Homeobox Protein 4) pathogenic activity is developed through transcriptional function and is associated with its nuclear localization. Three monopartite nuclear localization signals (NLS) are present in *DUX4*, but their function has not been completely defined in myoblasts (Corona et al., 2013). Recently, we demonstrated that in vitro differentiation of myoblasts from FSHD patients is improved by estrogen treatments. Estrogen through estrogen receptor β (ERβ) reduce *DUX4* transcriptional activity and impair its nuclear localization (Teveroni et al., 2017). To understand how estrogen interferes with *DUX4* activity, we analysed the interactome of *DUX4* in myoblasts grown for three days in differentiation medium in presence or absence of estrogen. Interactome analysis revealed that *DUX4* binds some Nuclear Pore Complex (NPC) proteins. Estrogen treatment decreases such binding without altering these NPC protein levels. Preliminary immunofluorescence experiments confirmed increased co-localization of *DUX4* with these proteins during myoblast differentiation. Overexpression of these proteins increases *DUX4* transcriptional activity, supporting the relevance of such interaction for the development of *DUX4* activity. Finally, a loss of function mutant in one of the three NLS showed decreased transcriptional function compared to wild-type *DUX4*. Overall these data suggest that the nuclear import of *DUX4* is an active process and that estrogen can impair it.

P3.12

THIS POSTER HAS BEEN WITHDRAWN

P3.13

Unravelling the contribution of non-myogenic mesenchymal cells in the pathogenesis of facioscapulohumeral muscular dystrophy

Lorena Di Pietro[1], Andrea Papait[1], Elvira Ragozzino[1], Antonietta Silini[2], Pietro Romele[2], Wanda Lattanzi[3], Enzo Ricci[3], Ornella Parolini[1]

[1] *Università Cattolica del Sacro Cuore*
[2]*Centro di Ricerca E. Menni, Fondazione Poliambulanza-Istituto Ospedaliero*
[3]*Università Cattolica del Sacro Cuore/Fondazione Policlinico Universitario A. Gemelli IRCSS*

Maintaining tissue homeostasis in skeletal muscle involves cooperation between different cell types, including non-myogenic mesenchymal cells that play key roles in tissue homeostasis but also contribute to muscle degeneration. Our recent study demonstrated that altered accumulation and differentiation of non-myogenic mesenchymal cells are associated with muscle degeneration in FSHD patients. We currently aimed to define the specific mechanisms through which non-myogenic mesenchymal cells contribute to muscle degeneration in FSHD by examining their crosstalk with myoblasts. We isolated non-myogenic mesenchymal cells and myoblasts from muscle specimens of FSHD patients and controls. We evaluated the expression of *DUX4* and its targets in non-myogenic mesenchymal cells. Our data showed that *DUX4* signalling is modulated in FSHD cells also during in vitro adipogenic induction. We also assessed the effects of non-myogenic mesenchymal cells on the proliferative and differentiation properties of FSHD myoblasts through co-culture experiments. Our results indicated that the presence of non-myogenic mesenchymal cells affected both myoblast proliferation and differentiation. The role of non-myogenic mesenchymal cells in tissue damage is an emerging topic in current research on muscular dystrophies, so elucidating their role in FSHD could help to clarify disease pathogenesis and to identify specific pathways eligible as novel therapeutic targets in patients.

P3.14

Reversing the altered behavior of non-myogenic mesenchymal cells from FSHD patients: Potential of human amniotic cell conditioned medium

Flavia Giacalone[1], Lorena Di Pietro[1], Andrea Papait[1], Elvira Ragozzino[1], Antonietta Silini[2], Pietro Romele[2], Wanda Lattanzi[3], Enzo Ricci[3], Ornella Parolini[1]

[1] *Università Cattolica del Sacro Cuore*
[2]*Centro di Ricerca E. Menni, Fondazione Poliambulanza-Istituto Ospedaliero*
[3]*Università Cattolica del Sacro Cuore/Fondazione Policlinico Universitario A. Gemelli IRCSS*

Facioscapulohumeral muscular dystrophy (FSHD) is associated with an expansion of the non-myogenic mesenchymal cell compartment, which may contribute to the disease pathogenesis. We have previously shown that patient-derived non-myogenic mesenchymal cells have an increased proliferation rate and an altered ability to differentiate into adipocytes. Here, we investigated the effects of conditioned medium from human amniotic cells (CM-hAMSC) on the proliferation and differentiation of non-myogenic mesenchymal cells isolated from FSHD and control muscles. We evaluated the dose-response effect of 3 different CM-hAMSC concentrations on cell proliferation and differentiation. Proliferation was monitored for 72 hours using the Incucyte Live-Cell Analysis System and by gene expression analysis. The effects on differentiation capabilities were assessed by gene expression and immunofluorescence analysis after 10 days of induction. We found that CM-hAMSC significantly decreased the proliferation of patient-derived non-myogenic mesenchymal cells, restoring their growth rate to control levels. Furthermore, the adipogenic differentiation of both patient and control cells was hindered in the presence of CM-hAMSC. These findings suggest that CM-hAMSC may affect the altered behaviour of FSHD non-myogenic mesenchymal cells. Further research is needed to determine the precise mechanisms by which CM-hAMSC modulates proliferation and differentiation.

P3.15

A retrospective cohort study identifies fibrosis as candidate biomarker for muscle degeneration in facioscapulohumeral muscular dystrophy patients

Elvira Ragozzino[1], Sara Bortolani[2], Lorena Di Pietro[1], Andrea Papait[1], Ornella Parolini[1], Mauro Monforte[2], Giorgio Tasca[3], Enzo Ricci[2]

[1] *Università Cattolica del Sacro Cuore*
[2] *Fondazione Policlinico Universitario A. Gemelli IRCCS*
[3] *John Walton Muscular Dystrophy Research Centre*

Ongoing clinical trials, aimed to counteract muscle degeneration in facioscapulohumeral muscular dystrophy (FSHD), increase the need for reliable biomarkers. Muscle magnetic resonance imaging (MRI) studies show that the appearance of STIR-positive (STIR+) lesions represents an initial stage of muscle damage, preceding irreversible adipose changes. Herein we studied fibrosis, a parameter of muscle degeneration undetectable by MRI, in FSHD patients' muscles. Fibrosis was evaluated on 27 STIR+, 28 STIR- and 12 healthy muscles by picrosirius red staining. Oedema (STIR) and fat content (T1) of FSHD muscles (n=55) were evaluated at baseline MRI scan before biopsy and, when available, at 1- (n=45) and 2-year MRI follow-up (n=36). Fibrosis was significantly higher in FSHD muscles, compared to healthy muscles, and STIR+ muscles showed the highest amount of intramuscular collagen. FSHD muscles with a worsening in fatty infiltration during 1- and 2-year MRI follow-up showed 3.6- and 3.7-fold higher fibrosis compared to FSHD muscles with no progression. After 2 years, STIR+ muscles with increased fat content showed significantly higher fibrosis compared to STIR+ muscles with no changes. To study muscle degeneration in FSHD, we evaluated fibrosis in relation to the radiological features of muscles. Our data showed that 23/27 STIR+ and 12/28 STIR- FSHD muscles had higher fibrosis compared to healthy muscles. At 1- and 2- year MRI follow-up, fibrosis was higher in FSHD muscles with a worsening in fatty infiltration, suggesting that the evaluation of fibrosis could be a candidate prognostic biomarker for FSHD, useful to stratify patients and to evaluate the efficacy of treatments.

P3.16

Retrospective analysis of muscle biopsy findings in a cohort of patients with facioscapulohumeral dystrophy type 1

Lucia Ruggiero[1], Marianna Pezzella[1], Ruggiero Di Leo[1], Floriana Vitale[1], Dario Zoppi[1], Roberta Bencivenga[1], Rosa Iodice[1], Rossella Tupler[2] Fiore Manganelli[1]

[1] *Department of Neurosciences, Reproductive and Odontostomatological Sciences, University Federico II*
[2] *Università degli Studi di Modena e Reggio Emilia*

Background: muscle biopsy does not play a definite role in the diagnostic flowchart and the management of Facioscapulohumeral Muscular Dystrophy (FSHD). Therefore, there are no studies that have systematically carried out a correlation of the phenotype and genotype under the light of histopathological findings. Aim: a standardized analysis of histopathological changes in muscle biopsies of FSHD1 patients. Materials and methods: the muscle biopsies of 20 FSHD1 subjects were analyzed. In order to standardize the severity of muscle damage, a score ranged from 0 (normal) to 36 was assigned, taking into account several parameters (fibro-adipose tissue, necrosis, nuclear alterations). The biopsy score was then correlated with the degree of disability of the subjects through the FSHD clinical score and the clinical category identified by the comprehensive clinical evaluation form (CCEF). Results: severe changes are present in one third of patients; there is a linear correlation between biopsy score and FSHD score. Subjects with the classic phenotype have worse biopsy scores than subjects with incomplete or atypical phenotypes. Furthermore, a low frequency of inflammatory signs and mitochondrial and oxidative alterations metabolism was observed. Conclusion: our data suggest that muscle biopsy could be an additional tool for stratification of FSHD patients for future clinical trials as well. In particular, using a standardized biopsy score can be considered a valid tool for improving the phenotypic characterization and making it easier to compare patients. Finally, the muscle biopsy data could provide useful information for a better understanding of the pathogenetic mechanisms.

P3.17

Symptom onset and cellular pathology in facioscapulohumeral muscular dystrophy is accelerated by cigarette smoking

Christopher Banerji[1], Philipp Heher[2], Husain Bin Haidar[3], Ketan Patel[3], Peter Zammit[2], **Jeff Statland**[4]

[1] *The Alan Turing Institute, London, UK*
[2] *King's College London*
[3] *University of Reading*
[4] *University of Kansas*

FSHD is currently incurable. In the absence of therapy, any lifestyle factor that impacts disease progression is important for clinical management. Monozygotic twins with FSHD often exhibit dramatically different disease progression, indicating the existence of environmental disease modifiers. We analysed the USA National Registry for Myotonic Dystrophy & Facioscapulohumeral Dystrophy, comprising 511 FSHD1 patients followed up annually for an average of 8 years. This complex, multimodal, longitudinal dataset comprises 189 baseline and 37 annually assessed features. We developed a workflow for prospective cohort analysis and identify cigarette smoking as associated with a two-fold increase in risk of facial and lower limb involvement in FSHD1 patients. Our definition of lower limb involvement includes inability to run and climb steps unaided, important functional outcomes for FSHD patients. We then employed an assay to test the effects of cigarette smoke extract on human myoblasts in vitro. Cigarette smoke drove disproportionate defects in proliferation and myogenic differentiation of FSHD1 patient-derived myoblasts, compared to matched controls. Mitochondrial function was also inordinately affected in FSHD1 myoblasts exposed to cigarette smoke extract, with increased mitochondrial membrane potential and mitochondrial radical oxygen species (mitoROS) generation. Overall, we identify cigarette smoking as associated with doubling the risk of facial and lower limb involvement in FSHD1 patients, and reveal that cigarette smoke inhibits myoblast function, in a manner linked to mitochondrial dysfunction. Our findings support smoking cessation in clinical management of FSHD.

P3.18

Single cell proportion analysis identifies unique transcriptional responses to plasma membrane injury in FSHD

Adam Bittel[1], Yi-Wen Chen[1], Surajit Bhattacharya[1]

[1] *Children's National Research Institute*

Background: FSHD myoblasts have significant delays in plasmalemmal repair compared to healthy myoblasts. We hypothesize repair deficits may lead to aberrant transcriptional responses post-injury, which could potentiate muscle pathology downstream. Approach: Two sibling pairs of FSHD and Healthy primary myoblasts were collected prior to, 6-hrs, and 24-hrs after cell scrape injury for single cell RNA sequencing. Datasets from each pair were integrated and clustered at each timepoint using Seurat. To identify unique FSHD transcriptional responses to plasma membrane injury, single cell proportion tests were used to identify clusters with consistent under-/over-representation of FSHD myoblasts in both pairs (FDR $p<0.05$, $log2FC>0.58$). Ingenuity Pathway Analysis and Gene Set Enrichment analyses were used for functional annotation of cluster marker genes. Results & Conclusions: Overall, the majority of the FSHD myoblasts share similar transcriptional expression profiles with myoblasts from their unaffected siblings. However, at the pre-injury time point, we identified subpopulations of myoblasts that consist predominantly of FSHD myoblasts (FSHD clusters), which showed expression signatures of well-known hallmarks of FSHD. At 6-hrs post-injury, the transcriptional signatures of FSHD clusters progressed toward increased pro-inflammatory and fibrosis pathway activation, as well mitochondrial dysfunction. At 24-hrs post-injury, the transcriptional signatures were defined by reduced cell growth and cell cycle activation. These results identify molecular signatures in unique subpopulations of FSHD myoblasts, as well as disease-specific responses to plasma membrane injury.

P3.19

Facioscapulohumeral disease as a myodevelopmental disease: Applying Ockham's razor to its various features

Nicol Voermans[1], George Padberg[2], Baziel van Engelen[1]

[1] *Neuromuscular Centre Nijmegen, Radboud University Nijmegen Medical Centre, Netherlands*
[2] *Radboud University Medical Center*

Facioscapulohumeral muscular dystrophy (FSHD) is caused by the loss of epigenetic repression of the D4Z4 repeat on chromosome 4q35 resulting in inappropriate transcription of *DUX4*. Muscles become involved in a rostro-caudally order with an extremely variable progression rate. Mild disease and non-penetrance in families with affected individuals is common. In order to explain the various features of FSHD we applied Ockham's Razor to all possible scenarios and removed unnecessary complexities. We postulate that early in embryogenesis a few cells escape epigenetic silencing of the D4Z4 repeat. Their number is assumed to be roughly inversely related to the residual D4Z4 repeat size. By asymmetric cell division, they produce a rostro-caudal and medio-lateral decreasing gradient of weakly D4Z4-repressed mesenchymal stem cells. The gradient tapers towards an end as each cell-division allows renewed epigenetic silencing. Over time, this spatial gradient translates into a temporal gradient based on a decreasing number of weakly silenced stem cells. These cells contribute to a mildly abnormal myofibrillar structure of the fetal muscles. They also form a downward tapering gradient of epigenetically weakly repressed satellite cells. When activated by mechanical trauma, these satellite cells de-differentiate and express *DUX4*. When fused to myofibrils they contribute to muscle cell death in various ways. Over time and dependent on how far the gradient reaches the FSHD phenotype becomes progressively manifest. We thus hypothesize FSHD to be a myodevelopmental disease with a lifelong attempt to restore *DUX4* repression.

P3.20

Developmental repression/derepression of *DUX4* 4qA during FSHD embryonic and adult myogenesis

Dongsheng Guo[1], Katelyn Daman[1], Jing Yan[1], Lawrence Hayward[1], Oliver King[1], **Charles Emerson**[1]

[1] *UMass Chan Medical School*

FSHD is an epigenetic disease caused by hypomethylation of the chr4 D4Z4 repeat locus, enabling misexpression of the germline *DUX4* transcription factor in skeletal muscle, and leading to muscle toxicity. Ch4 D4Z4 hypomethylation is a consequence of germline contractions to less than 10 4qA D4Z4 repeats (FSHD1), or by loss of function mutations in the chromatin modifying enzyme, SMCHD1 or DNMT3B (FSHD2). Our investigations of FSHD1 ESCs and FSHD1 and FSHD2 iPSCs have revealed a developmental mechanism controlling *DUX4* expression in embryonic and adult skeletal muscle. RNA expression studies show that *DUX4* 4qA expression is repressed in pluripotent FSHD ESC and IPSCs during gene-free induction and embryonic skeletal muscle differentiation, even though *DUX4* 4qA remains hypomethylated (Guo, Daman, et al., eLife, 2022). However, *DUX4* 4qA expression is upregulated in myotubes differentiated from iPSC-derived "adult-like" FSHD myogenic progenitors (iMyoblasts), adult biopsy Myoblasts (bMyoblasts), as well as differentiating embryonic myocytes (iMyocytes) following a pulse pre-treatment of proliferating progenitors with the DNA hypomethylating agent, 5-AzaC. These findings suggest that *DUX4* 4qA expression leading to muscle toxicity is controlled by a muscle gene regulatory program that is repressed by a DNA methylation mechanism in the germline and embryonic muscle and becomes operative in adult muscle. Further understanding of the developmental epigenetic mechanisms controlling *DUX4* transcription using FSHD iPSC myogenesis technologies may provide novel drug targets to modulate *DUX4* misexpression as a treatment for FSHD muscle toxicity.

Supported by MDA & UMass Wellstone Center.

P3.21

An in silico FSHD muscle fibre for modelling *DUX4* dynamics and predicting the impacts of therapy

Matthew Cowley[1], Johanna Pruller[2], Massimo Ganassi[2], Peter Zammit[3], Christopher Banerji[4]

[1] *Centre for Sustainable and Circular Technologies, Department of Chemistry, University of Bath*
[2] *King's College London, Randall Centre for Cell and Molecular Biophysics*
[3] *King's College London, UK*
[4] *The Alan Turing Institute, London, UK*

Targeting *DUX4* is the leading therapeutic approach for facioscapulohumeral dystrophy (FSHD), however it is only detectable in 0.1-3.8% of FSHD myonuclei [1, 2]. How rare *DUX4* expression drives FSHD and the optimal anti-*DUX4* strategy is unclear. We combine stochastic gene expression with compartment models of cell states, building a simulation of *DUX4* expression and consequences in FSHD myoblasts and multi-nucleated myotubes. Using iDUX4 myoblast, scRNA-Seq and snRNA-Seq data from differentiated FSHD muscle cell cultures, we estimated parameters including *DUX4* mRNA degradation, transcription and translation rates, and *DUX4* target gene activation rates. Our model accurately recreates the distribution of *DUX4* and *DUX4* target gene positive cells seen in scRNAseq of FSHD myocytes. Importantly, we show *DUX4* drives significant cell death despite expression in only 0.8% of live cells at any point. Comparing scRNAseq of unfused FSHD myocytes to snRNAseq of fused FSHD myonuclei, we find evidence of DUX4 protein syncytial diffusion and estimate its rate via genetic algorithms. We package our model into freely available, user-friendly online tools, to rapidly investigate consequences of anti-DUX4 therapy [3].

- Compartment Models: https://crsbanerji.shinyapps.io/compartment_models/
- Cellular Automaton: https://crsbanerji.shinyapps.io/ca_shiny/
- Survival Analysis: https://crsbanerji.shinyapps.io/survival_sim/.

1. A. van den Heuvel et al., Hum. Mol. Genet., 2019, 28, 1064–1075.
2. S. Jiang et al., PLOS Genet., 2020, 16, e1008754.
3. M. V Cowley, J. Pruller, M. Ganassi, P. S. Zammit and C. R. S. Banerji, bioRxiv, 2022, 2022.12.12.520053.

P3.22

AOC 1020: An antibody oligonucleotide conjugate (AOC) in development for the treatment of FSHD

Barbora Malecova[1], David Sala[1], Garineh Mary Melikian[1], Nathan Delos Santos[1], Gulin Erdogan[1], Rachel Johns[1], Maryam Jordan[1], Marc Hartmann[1], Danny Arias[1], Arvind Battacharya[1], Ramana Doppalapudi[1], Hanhua Huang[1], Michael Flanagan[1], Arthur Levin[1]

[1] *Avidity Biosciences, Inc.*

Strategies to reduce *DUX4* expression in FSHD patient skeletal muscle via oligonucleotides are promising therapeutic approaches. The main challenge has been the productive delivery of oligonucleotides into muscle cells. The AOC 1020 therapeutic candidate for FSHD consists of *DUX4*-targeted siRNA (siDUX4.6) conjugated to a humanized anti-transferrin receptor 1 (TfR1) antibody AV01mAb to facilitate delivery to muscle. SiDUX4.6 was selected via in vitro screening of a *DUX4* siRNA library in 11 FSHD patient-derived muscle cell lines to maximize potency and specificity. In cynomolgus monkey skeletal muscle, AOC 1020 produced a dose-dependent increase in siRNA tissue exposure following a single IV dose. The pharmacology of siDUX4.6 was characterized in ACTA1-MCM; FLExDUX4 mouse model of FSHD expressing human *DUX4*. Robust activity in muscle was observed 8 weeks after single IV dose of murine TfR1 antibody-based DUX4 AOC, with over 75% reduction of DUX4-regulated genes at 6 mg/kg of siRNA dose. Transcriptome analysis revealed that many genes and pathways are dysregulated upon DUX4 induction in muscle of this mouse model. This was largely prevented with single systemic DUX4 AOC treatment. These data support the evaluation of AOC 1020 in the Phase 1/2 FORTITUDE trial in adults with FSHD.

Posters: Clinical Studies & Trial Designs

P4.01

Safety and tolerability of losmapimod for the treatment of FSHD

Marie-Helene Jouvin[1], Vivekananda Ramana[1], John Jiang[1]

[1] *Fulcrum Therapeutics*

FSHD is a relentless, variably progressive disease leading to accumulation of disability over decades. Fulcrum has assessed losmapimod, a small molecule p38 α/β MAPK inhibitor, in FSHD in 1 completed Phase 1 study (FIS-001-2018) and 2 ongoing Phase 2 studies in the open-label extension period (FIS-001-2019 and FIS 002-2019). Subjects aged 18-65 years with genetically confirmed FSHD1, Clinical Severity Score 2-4, and MRI-eligible muscles for biopsy were exposed to losmapimod 7.5 or 15 mg BID PO for 14 days and up to 76 weeks. In study FIS 001-2018, 6 subjects were exposed to 7.5 mg and 11 subjects to 15 mg BID dosing for 14 days. In studies FIS-001-2019 and FIS-002-2019, 14 and 77 subjects respectively, received at least 1 dose of losmapimod 15 mg BID for up to 76 weeks. A total of 108 subjects with FSHD1 have been exposed to losmapimod, with ~131 patient-years of exposure. Fifty-seven subjects were exposed to losmapimod for 12 to 18 months, and 30 were exposed for over 18 months. Most AEs observed were considered mild to moderate in severity. Most common AEs were ALT increase, headache, dizziness, dry skin, eczema and gastrointestinal disorders; majority of AEs resolved with continued dosing. Dosing was paused for 14 days in 4 subjects (3 in FIS 001-2019, 1 in FIS-002-2019) due to COVID-19 infection. No drug-related SAEs, deaths, discontinuations due to AEs, or clinically significant changes in vital signs, clinical laboratory results, or ECG parameters were reported. Losmapimod administered up to 15 mg BID in >100 subjects with FSHD1 for up to 76 weeks has been generally well-tolerated; the benefit-risk profile of losmapimod remains positive and favorable.

P4.02

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

Ian Woodcock[1], Katy de Valle[1], Anita Cairns[2], Nisha Varma[1], Martin Delatycki[3], Eppie Yiu[1], Zoe Davidson[4], Michael Kean[1], Aneke Grobler[5]

[1] *The Royal Children's Hospital*
[2] *Queensland Children's Hospital*
[3] *Victorian Clinical Genetics Service*
[4] *Monash University*
[5] *Murdoch Children's Research Institute*

Paediatric facioscapulohumeral muscular dystrophy (FSHD) is an ultra-rare, progressive muscle wasting disease presenting with facial and shoulder weakness and progressing to involve lower limbs, typically with an asymmetric pattern. There is no treatment proven to improve symptoms in FSHD. Creatine monohydrate is an endogenously produced protein involved in muscle energy production through ATP formation. Synthetically manufactured creatine monohydrate (CM) supplement has been shown to improve muscle strength in adults with muscular dystrophies, but it has not been tested in young people with FSHD. In a randomised placebo-controlled double-blind crossover study, powdered CM at a dose of 100mg/kg (max. 10g daily) was compared to maltodextrin placebo in thirteen paediatric participants (mean age 13.2 years) in three-month treatment periods with a six-week washout between crossover arms. Primary efficacy outcome measure was the MFM with secondary outcomes including FSHD-COM, FSHD-HI, FSHD-CS, PUL and MRI. Mean difference between treatment groups was not significantly different in the MFM (0.19 95%CI -0.71 to 1.08 p=0.68), FSHD-COM (-0.99 -3.53 to 1.53), FSHD-CS (-0.04 -0.76 to 0.69), 6MWT (3.2 -48.08 to 54.48), 10mw/r (-0.17 -0.39 to 0.04) or PUL (-0.03 -0.41 to 0.36). Although individual outcomes had large CIs, when taken as a whole, the majority of outcomes favoured treatment with CM. There were no SAEs. Serum creatinine increased by mean 15 umol/L post-CM treatment but no patients were symptomatic or discontinued CM due to reported intolerance. CM is a generally safe and well tolerated treatment option which may improve endurance in ambulant young people with FSHD.

P4.03

Building an integrated machine learning-based platform to study FSHD: From deep phenotyping to predictive biomarkers

Giulia Ricci[1], Stefano Cotti Piccinelli[2], Francesca Torri[1], Giulio Gadaleta[3], Roberto Gatta[4], Emanuele Frontoni[5], Federica Decorato[2], Stefano Regondi[2], Alessandro Tonacci[6], Francesco Sansone[6], Raffaele Conte[6], Alessandro Padovani[4], Tiziana Mongini[3], Gabriele Siciliano[1], Massimiliano Filosto[2]

[1] *Department of Clinical and Experimental Medicine, University of Pisa, Italy*
[2] *Nemo-Brescia Clinical Center for Neuromuscular Diseases, Brescia, Italy*
[3] *Neuromuscular Unit, Department of Neurosciences "Rita Levi Montalcini", University of Turin*
[4] *Department of Clinical and Experimental Sciences, University of Brescia, Italy*
[5] *Nemo Lab, ASST GOM Niguarda Cà Granda Hospital, Milan, Italy*
[6] *Institute of Clinical Physiology, National Research Council of Italy (IFC-CNR), Pisa, Italy*

FSHD represents a complex disease with high clinical variability and an intricate genetic landscape. Diagnostic criteria are based on the detection of the molecular signature; however, this paradigm does not completely fulfill the understanding of disease natural history and often complicates familial genetic counseling. Many molecules are at study both in pre-clinical and clinical trials, further prompting the need for prognostic indicators and biomarkers describing disease progression. A diagnostic flow-chart based only on genetic signature may not highlight the differences among patients and possibly bias the interpretation of response to a certain treatment. On the other hand, the clinical complexity hides further molecular mechanisms to be investigated. Starting from these considerations, we aim at using FSHD as a disease model and develop a dedicated digital platform that can comprehensively collect all the variables involved, enabling a deep phenotyping towards a personalized plan of diagnosis and care. This will be achieved through an integrated and multiparametric approach, converging on a single support cloud-based, GDPR-compliant platform. The study will involve a multicenter collaboration between neurologists and IT-professionals. The platform will be developed starting from the structure of the Clinical Comprehensive Evaluation Form, a tool shared by the Italian Clinical Network for FSHD, and will comprise modules for phenotype and pedigree, genetics and MRI. A machine-learning approach will inform automatic algorithms for recognition of clinical, genetic and imaging patterns and provide clinicians with correlation analysis among variables.

P4.04

Prescription of pain medication for people with facioscapulohumeral muscular dystrophy in the Muscular Dystrophy Surveillance, Tracking, and Research Network

Kristin Conway[1], Jonathan Suhl[1], Katherine Mathews[1], Sonja Rasmussen[2], James Howard, Jr.[3], Jennifer Andrews[4], Shiny Thomas[5], Nicholas Johnson[6], Joyce Alese[7], Paul Romitti[1]

[1] *University of Iowa*
[2] *The Johns Hopkins School of Medicine*
[3] *University of North Carolina School of Medicine*
[4] *The University of Arizona*
[5] *New York State Department of Health*
[6] *Virginia Commonwealth University*
[7] *Centers for Disease Control and Prevention*

Background: Pain is a recognized complaint in muscular dystrophy (MD), but little is known about management. Using population-based surveillance data, we examined prescribed opioid and nonopioid medications among people with facioscapulohumeral MD (FSHD) as identified by the MD Surveillance, Tracking, and Research Network (MD STARnet). Methods: Prescription data were abstracted from medical records for people diagnosed and had health encounters during 2008-2016 in Colorado, Iowa, western New York, North Carolina (Piedmont Region), South Carolina, and Utah. Percentages of people prescribed pain medications for ≥6 weeks (any pain medication, any opioid medication, and medication class) during follow-up were estimated and classified by sex (male/female), MD STARnet site, and age at final visit. Results: Approximately 31% of 170 people with FSHD were prescribed any pain medication; opioids were most common (50% of any pain medication). No differences by sex were found for any pain medication (30% and 31%) nor opioids (16% and 15%). Observed MD STARnet site differences (any: 10%-29%; opioids: 8-31%) may be due to individual characteristics between sites (e.g. FSHD subtype, age). Pain medications were prescribed at a lower rate in children than adults: 2% of those aged <20yr and 29% of those ≥20yr at last visit were prescribed pain medications (opioids, 1% and 15%, respectively). Discussion: Pain medications were documented for over 25% of people with FSHD, of which 50% were opioids, suggesting prescription medication is a frequent component of FSHD care. Understanding modifiable factors associated with MD-related pain and effective interventions may help improve care.

P4.05

Diaphragmatic ultrasound: A promising technique for respiratory assessment of patients with facioscapulohumeral muscular dystrophy (FSHD)

Eleonora Torchia[1], Sara Bortolani[2], Riccardo Inchingolo[3], Andrea Smargiassi[3], Mauro Monforte[2], Giorgio Tasca[2], Matteo Bonini[3], Luca Richeldi[3], Enzo Ricci[1]

[1] *Fondazione Policlinico Universitario A. Gemelli IRCSS*
[2] *Unità Operativa Complessa di Neurologia, Fondazione Policlinico Universitario A. Gemelli IRCSS, Rome*
[3] *Unità Operativa Complessa di Pneumologia, Fondazione Policlinico Universitario A. Gemelli IRCSS, Rome*

Introduction: A restrictive respiratory impairment has been described in up to 40% of patients affected by Facio-scapulo-humeral muscular dystrophy (FSHD). Spirometry may underestimate early respiratory alterations in these patients, as inspiratory muscle impairment may occur before Forced vital capacity (FVC) variation. Ultrasonography has recently emerged as a non-invasive tool to assess the main inspiratory muscle, the diaphragm. The aim of this study was to thoroughly characterize the respiratory function of a cohort of FSHD comparing spirometric and ultrasonographic data. Methods: Twenty-three genetically confirmed adult FSHD patients (15 male and 8 female) were enrolled. US diaphragmatic thickness at the end of a normal expiration (basal-DT), after a maximal inspiration (max-DT) and diaphragmatic excursion were calculated. The difference between max-DT and basal-DT represented “diaphragmatic thickening”. FVC, forced expiratory volume in first second (FEV1), total lung capacity (TLC) and residual volume (RV) were also assessed by spirometry. Values were compared to normative data. Results: US evaluation was able to detect abnormalities in 12 patients (52%), especially in diaphragmatic kinetic: median “diaphragmatic thickening” was 1,55 mm (range 0-4,6). Ten patients showed an asymmetric US pattern. A smaller portion of patients showed alterations on spirometric indexes. Inspiratory dysfunction with low TLC was detected in 6 (26%) patients, three of whom also displayed a restrictive pattern with a low FVC. Conclusions: This pilot study suggests that diaphragmatic US could be a promising technique to identify early inspiratory dysfunction in FSHD patients.

P4.06

Quality of life and support needs in children and adolescents with facioscapulohumeral dystrophy: A qualitative study

Jildou Dijkstra, Nicol Voermans[2], Corrie Erasmus[2], Baziel van Engelen[2], Edith Cup[1], Sandra Altena[1], Eline Boon[1], Nathaniël Rasing[1], Anke Lanser[3], Anouska Ramaker[3]

[1] *Radboud University Medical Center*
[2] *Neuromuscular Centre Nijmegen, Radboud University Nijmegen Medical Centre, Netherlands*
[3] *Dutch FSHD Patient Organization*

Objective: Quality of life (QoL) in children with FSHD is decreased. Little is known about factors influencing QoL in children with FSHD. Our objective is to describe which factors contribute to the QoL of children and adolescents with FSHD and to explore their support needs. Methods: We performed a qualitative study with individual age-appropriate semi-structured interviews assessing QoL in children and adolescents with FSHD and their parents. To characterize the cohort, quantitative data on QoL, pain, fatigue and participation were collected. Interview data was analyzed using the constant comparison approach based on the Straussian Grounded Theory. Results: 14 patients participated (age between 9-26 years old). QoL was not depended on FSHD severity. Aging was perceived to decrease QoL. FSHD affected different aspects of life. Children and adolescents strived for normality regardless of physical discomfort. Phenotypical features of FSHD led to social insecurity aggravated by hurtful comments of others. The unpredictability of disease progression and its implications for career and parenthood choices led to a generalized feeling of uncertainty about the future. Support was found within family and friends. Peer support and psychological support was recommended by the participants. Discussion: QoL in childhood FSHD is diminished caused by their physical limitations, altered appearance, fear social rejection and uncertainty of the disease progression in the future. A fear of social rejection most likely contributes to striving for normality regardless of physical dyscomfort. Support should be focused on acceptance and dealing with hurtful comments.

P4.07

Clinically Relevant Outcome Measures in FSHD (CROMFiSH): Results of a twelve-month longitudinal natural history study

Anika Varma[1], Katy Eichinger[2], Michael Todinca[3], Susanne Heininger[4], Nuran Dilek[4], Bill Martens[2], John Kissel[5], Rabi Tawil[6], **Jeff Statland[7],** Michael McDermott[8], Chad Heatwole[9]

[1] *Center for Health + Technology, University of Rochester*
[2] *University of Rochester Medical Center*
[3] *Baptist Neurology Group, Baptist Health*
[4] *Department of Neurology, University of Rochester*
[5] *Department of Neurology, The Ohio State University Wexner Medical Center*
[6] *University of Rochester*
[7] *University of Kansas*
[8] *Department of Neurology & Department of Biostatistics, University of Rochester*
[9] *Center for Health + Technology & Department of Neurology, University of Rochester*

Objectives: In preparation for clinical trials in FSHD, it is useful to understand how disease burden changes over time and to assess the capability of specific outcome measures to detect these changes. In this research, we conducted a 12-month observational study with individuals with FSHD. Methods: Forty-one adults with FSHD participated in our 12-month study. These individuals were evaluated at baseline, 6 months, and 12 months with serial assessments of their strength, clinical function, sleep and fatigue, lean body mass, respiratory status, and patient-reported disease burden. We assessed changes in these outcomes over the 12-month period, and we determined the associations between changes in outcomes and age and sex. Results: Over a 12-month period, individuals with FSHD, on average, demonstrated a mild decline in upper body and total strength, as measured by quantitative muscle testing. Participant function otherwise remained relatively stable over the 12-month study period. Conclusions: The functional status of adults with FSHD is largely static over a 12-month period with participants demonstrating a mild average reduction in some measures of strength.

P4.08

FSHD European Patient Survey: Assessing patient preferences in clinical trial participation

Magan McNiff[1], Sheila Hawkins[2], Bine Haase[2], Joanne Bullivant[1], Tammy McIver[3], Olga Mitelman[4], Nicholas Emery[5], Giorgio Tasca[1], Nicol Voermans[6], Jordi Diaz-Manera[1]

[1] *John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Institute, Newcastle*
[2] *FSHD Europe*
[3] *Roche Products Ltd, Welwyn Garden City, UK*
[4] *Fulcrum Therapeutics*
[5] *The Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, UK*
[6] *Neuromuscular Centre Nijmegen, Radboud University Nijmegen Medical Centre, Netherlands*

Facioscapulohumeral muscular dystrophy (FSHD) is a genetic disorder characterized by progressive muscle weakness leading to permanent disability. There are no curative treatments, however, there are several upcoming clinical trials testing new therapies in FSHD. This study aimed to explore the patient preferences of people with FSHD across Europe to ensure that clinical trials can be designed to include outcome measures that are relevant and important to patients. A total of 1147 participants responded to an online survey, representing 26 countries across Europe and a range of disease severities. The study highlighted the key symptoms causing concern for FSHD patients - muscle weakness and mobility issues - reflecting what participants want targeted for future therapies. The need for clear information and communication throughout clinical trials was emphasised. Factors most encouraging trial participation included access to new investigational therapies, access to trial results and benefits for the FSHD community. Factors most discouraging trial participation included travel related issues and fear of side effects. The results from this study identified the unmet healthcare needs of FSHD patients and should provide researchers and industry with areas of therapeutic research that would be meaningful to patients, as well as supporting the development of patient centric outcome measures in clinical trials.

P4.09

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

Helen Walker[1], Chiara Marini-Bettolo[1], Richard Orrell[2], Andrew Graham[3], Fiona Norwood[4], Mark Roberts[5], Tracey Willis[6], Emma Matthews[7], Mark Mencias[7], Kate Adcock[8], Robert Muni-Lofra[9]

[1] *John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Institute, Newcastle*
[2] *University College London, Queen Square Institute of Neurology, UK*
[3] *Patient Representative, UK*
[4] *Department of Neurology, King's College Hospital, London, UK*
[5] *Department of Neurology, Salford Royal NHS Foundation Trust, Salford, UK*
[6] *Neuromuscular Service, The Robert Jones and Agnes Hunt Orthopaedic Hospital NHS Foundation Trust, UK*
[7] *Atkinson Morley Regional Neurosciences Centre, St George's University Hospital NHS Foundation Trust*
[8] *Muscular Dystrophy UK*
[9] *JWMDRC, Newcastle University, and Newcastle upon Tyne Hospitals NHS Foundation Trust, UK*

The UK FSHD Patient Registry is a self-enrolling, patient-entered, clinician verified online database established in May 2013 by Newcastle University with support from MDUK. The registry facilitates academic and clinical research, and aims to better characterise and understand FSHD and to disseminate information about upcoming studies and research advancements. The registry collects longitudinal real-world evidence and genetic information and supports data and trial recruitment enquiries from industry. The registry has supported 32 enquiries to date, including a Health Economics and Outcomes project, patient preference studies, and dysphagia, sleep, and pregnancy surveys. The registry also contributes data to TREAT-NMD registry network global enquiries. As of March 2023, there were 906 active, UK based patient registrations; 95.4% have a clinical diagnosis FSHD or FSHD1, 3.3% have FSHD2, and 58% of patients diagnoses are genetic confirmed. In addition to collecting specific genetic data inputted by clinicians, the registry can also now receive patient's genetic reports directly via a secure upload portal. The registry is one of the largest national FSHD patient registries and is an example of a versatile, cost-effective research tool, helping facilitate and advance a wide range of FSHD research. Additional work continues to be done to improve reporting of genetic information on the registry, and to facilitate the collection of patient reported outcome measures and trial preferences. There are also future data linkage plans between the registry and the Newcastle Research Biobank for Rare and Neuromuscular Diseases, to support real world evidence data collection.

P4.10

FSHD UK: Creating a strong multi-stakeholder group to strategically drive clinical trial readiness and co-ordination of FSHD activities in the UK. On behalf of FSHD UK

Rajeshri Badiani[1]

[1] *FSHD UK*

The UK has an excellent reputation for high quality FSHD clinical services, research teams and an established Patient Registry. However, these accomplished UK centres were mainly working in their own areas without an overarching strategy or central co-ordination. In July 2021 FSHD UK was formed with a strong representation from key stakeholder groups; clinicians, clinical services, patients, UK FSHD Registry, research scientists, MDUK and the FSHD Society. Our mission is to ready the UK for clinical trials and to become the recognised coordinating group for FSHD in the UK. Now with six clinical sites and the Muscular Dystrophy Support Centre Midlands (MDSC), we have connections to c.800 patients. We have started standardized in-clinic assessments across 6 sites, launched the HEOR (cost of FSHD) study, initiated patients@site engagement, two sites are running ReSolve and MOVE, and most importantly clinical trials have started in two sites with two novel FSHD therapies. We work closely with many FSHD International groups: CTRN, FSHD Europe, FSHD World Alliance. Our future plans include establishing an FSHD UK Network Database, expanding the patients@site program, launching a UK Research Strategy and establishing fundraising opportunities. With a core team of 16 and an additional c.17 extended members we have only just begun.

Posters: Late-Breaking Abstracts

P5.01

Best practice guidelines on genetic diagnostics of facioscapulohumeral muscular dystrophy (FSHD): Update of the 2021 guidelines

Emiliano Giardina[1], Pilar Camaño[2], Nienke van der Stoep[3], Valerie Race[4], Sara Burton-Jones[5], Victoria Williams[6], Silvère M. van der Maarel[7], Nicol Voermans[8]

[1] *Santa Lucia Foundation IRCCS; Tor Vergata University of Rome*
[2] *BioDonostia Health Research Institute, Hospital Donostia, San Sebastián, Spain*
[3] *Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands*
[4] *Department of Neurology University Hospitals Leuven, Belgium*
[5] *South West Genomics Laboratory Hub, Severn Pathology, Southmead Hospital, Bristol, UK*
[6] *NHS Wales*
[7] *Leiden University Medical Center, Leiden, The Netherlands*
[8] *Neuromuscular Centre Nijmegen, Radboud University Nijmegen Medical Centre, Netherlands*

The complex genetic picture and clinical heterogeneity of FSHD makes the diagnosis challenging and dependent on a close correlation between genetics and the clinical data. These aspects complicate the FSHD diagnosis causing a lack of standardization of testing procedures. These guidelines aim to update previous approaches with the last scientific achievements and propose standardized diagnostic workflows to be shared among all laboratories. These guidelines consider the continuum of clinical spectrum between FSHD1 and FSHD2 and suggest minimum criteria for the FSHD confirmation. FSHD1 is diagnosed in the presence of permissive haplotype and D4Z4 array size between 1-7 units (U) or alternatively in patients with 8-10U fragment size, permissive haplotype, together with methylation levels assessment. FSHD2 is confirmed in presence of permissive haplotype, usually 8-20U fragment size, D4Z4 hypomethylation and SMCHD1 pathogenic variants. In case of D4Z4 hypomethylation and absence of SMCHD1 mutations, it is useful to consider SMCHD1 deletions or rare variants in other FSHD2 genes (DNMT3B, LRIF1). FSHD phenotype without specific molecular evidence can be addressed for differential diagnosis to rule out other forms of muscular dystrophy. These guidelines provide a recommended order for genetic investigation consisting of (epi)genetic analysis of the D4Z4 repeats (sizing, haplotyping and methylation), sequencing of SMCHD1 and eventually additional FSHD2 genes.

P5.02

True cost of FSHD: Health economic study of facioscapulohumeral muscular dystrophy in the United States

June Kinoshita[1], **Amanda Hill**[1], Maryna Kolochavina[2], Man Hung[3], Eric Hon[4], Jamshid Arjomand[1]

[1] *FSHD Society*
[2] *Five Voices Consortium*
[3] *Roseman University of Health Sciences*
[4] *University of Utah*

Facioscapulohumeral muscular dystrophy (FSHD) is a progressive debilitating muscular dystrophy with heterogeneous and asymmetric manifestation. As the disease progresses, patients incur significant medical and nonmedical expenses. Most health economic studies to date have only looked at insurance claims data and healthcare usage in FSHD to measure the financial impact of the disease. These studies underestimate the full economic impact because during the course of the disease, patients often report requiring at-home assistance, extensive home modifications, lost educational and employment opportunities, unpaid care provided by family members, and other out-of-pocket costs not captured in claims data The purpose of this study was to conduct a cost analysis of direct medical costs, direct non-medical costs and indirect costs to arrive at a more comprehensive understanding of the economic burden of FSHD. A survey was administered to 354 patients, representing 312 households of patient population in the United States from October to November of 2022.

P5.03

TestFSHD: A fully sponsored direct-to-patient clinically approved genetic testing pilot program for US patients

June Kinoshita[1], Leigh Reynolds[1], **Jamshid Arjomand**[1]

[1] *FSHD Society*

Facioscapulohumeral muscular dystrophy (FSHD) is a genetically based muscular dystrophy caused by the misexpression of a toxic gene, *DUX4*, in skeletal muscle. FSHD can be classified into two subtypes, FSHD1, which comprises approximately 95% of known cases, and FSHD2. In both subtypes, expression of *DUX4* requires the presence of a stabilizing polyadenylation signal (PAS), as well as either the excision of 3,300 bp D4Z4 repeat units (RU) between 1 and 10 RUs (FSHD1) or mutations in genes involved in DNA methylation, such as *SMCHD1*, *DNMT3B* and *LIRF1* (FSHD2). Due to the complexity of the genetics, limited qualified testing facilities and denial of coverage by some insurance companies, most US patients do not have access to confirmatory genetic testing, which is often a requirement for enrollment in clinical trials. With an increase in the projected number of clinical trials, ensuring clinical trial eligibility with confirmatory clinically approved genetic tests for patient volunteers will be paramount for successful enrollment. To that end, the FSHD Society, along with support from the pharmaceutical industry, established a fully sponsored direct-to-patient genetic testing pilot program aimed at increasing the pool of eligible patients for enrollment in clinical studies. Implemented between April to November of 2022, the genetic testing program provided each applicant with certified genetic counseling, collected basic clinical status and family history and provided clinically approved testing for both FSHD1 and FSHD2. The successful outcomes and results of this pilot program, along with patient demographics, will be presented and discussed.

P5.04

Physical interaction between *DUX4* and hormone nuclear receptors

Sabrina Pagnoni[1]**,** Julieta Quintero[2], Nizar Y. Saad[3], Scott Q. Harper[3], Alberto L. Rosa[1]

[1] *Departamento de Farmacología Otto Orsingher, IFEC-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba*
[2] *IRNASUS-CONICET, FCQ, UCC- Argentina*
[3] *Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio*

DUX4 is a double homeobox transcription factor required during early embryonic development in placental mammals. Using model cultured cells (T47D, HepG2 and HEK293) we recently demonstrated (Quintero et al., 2022) that *DUX4* is a corepressor of the nuclear receptors (NRs) of progesterone (PR) and glucocorticoids (GR). Previously (2013 IRC FSHD Meeting), we showed that progesterone and estrogens protect cells from the toxic effect of *DUX4*, and synergize the lower toxicity observed in *DUX4* nuclear localization mutants. Studies concerning the potential coregulatory activity of *DUX4* on the α- and β-estrogen receptors are currently being performed, including the characterization of a short amino acid sequence (AQPLL388-392), at the *DUX4* C-ter, which matches the core ϕXXϕϕ proposed as a consensus sequence for repressor domain (CoRNR) present in well characterized corepressors of NRs. Using co-immunoprecipitation (Co-IP) assays we investigated the potential physical interaction between *DUX4* and PR and GR. Co-IP studies were performed using protein extracts from cells co-transfected with plasmids expressing tagged versions of *DUX4* and GR as well as cells that endogenously express PR transfected with a plasmid expressing DUX4-V5. Our results suggest that the corepressive effect of *DUX4* on the GR, but not PR, could be related to a direct protein-protein interaction. Studies performed using cultured human myocytes would be required to demonstrate if this potential endocrine role of *DUX4* has physiological significance in muscle and, eventually, clinical relevance on two remarkable FSHD phenotypes: gender differences and muscle inflammation.

P5.05

Using xenografts to identify protein biomarkers of FSHD in living muscle tissue: SLC34A2

Maria Traficante[1], Andrea O'Neill[1], Ujwala Pimparkar[1], Rabi Tawil[2], Jeff Statland[3], **Robert Bloch**[1]

[1] *University of Maryland School of Medicine*
[2] *University of Rochester Medical Center*
[3] *University of Kansas*

Clinical benefit can be gained by identifying biomarkers that correlate with FSHD disease progression. Our laboratory is investigating the protein SLC34A2 as a potential FSHD biomarker. *SLC34A2* is a *DUX4* target gene, and its protein functions normally in sodium-dependent phosphate uptake in cells. This cotransporter is expressed in lung, kidney, and gut epithelia, but not in mature muscles. Our previous work has detected SLC34A2 by immunofluorescence (IF) at ~10-fold higher levels in human FSHD biopsies as well as in our model of human muscle xenografts where SLC34A2 is present in about 1%-2% of FSHD-affected fibers (Mueller et al., Exp. Neurol. 320: 113011, 2019). This correlates with the relatively low prevalence of *DUX4* expression we observe in xenografts. Our research shows that SLC34A2 protein is significantly increased in FSHD matured myotube cultures compared to control, and is glycosylated. SLC34A2 protein levels decrease 2-fold when myotubes are exposed to losmapimod, as well as two other p38 kinase inhibitors. Additionally, SLC34A2 antibodies co-stain FSHD cultured myotubes and xenografts via IF. Recent experiments demonstrate the possibility of labeling FSHD xenograft fibers in situ, using an antibody specific to the exposed extracellular domain of SLC34A2 and tagged with IR-647. With further confirmation, this opens the prospect of tracking disease progression and the efficacy of therapeutics in the same individuals over time, without the need for muscle biopsies.

P5.06

Generation of new antibodies to specifically bind *DUX4* and not *DUX4c*

Laurence Quenault[1]

[1] King's College London

Facioscapulohumeral Muscular Dystrophy (FSHD) is linked to stochastic expression of the transcription factor *DUX4*. Once *DUX4* is expressed, it causes transcription of many genes that ultimately lead to muscle weakness and atrophy. However, how *DUX4* causes FSHD is still not well understood, partially due to its stochastic and low-level expression and lack of high affinity and specific antibodies to *DUX4*. To overcome this lack of reagents, I have been developing novel anti-*DUX4* antibodies that bind the C-terminal 76 amino acids of *DUX4*. Such antibodies will bind to *DUX4* and not *DUX4c*, which only significantly differs in sequence from *DUX4* at the C terminal. I generated poly Histidine and SUMO sequence-tagged full length and C-terminal fragments of *DUX4* via E. coli expression. These were purified by Nickel IMAC followed by SUMO protease treatment to remove the poly-Histidine tag and a reverse Nickel IMAC to remove this poly Histidine tag from the *DUX4* fragments. The *DUX4* fragments were then used to immunise large animal species. Blood, spleen and lymph nodes were obtained from the animals and anti-*DUX4* antibody producing cells were isolated by FACS. Antibodies that bind *DUX4* were then identified and *DUX4* binding was confirmed using Surface Plasmon Resonance to determine the affinity of the new anti-*DUX4* specific antibodies.

P5.07

Development of the HealthMeasures facioscapulohumeral muscular dystrophy-32 (HM FSHD-32) patient-reported outcome measure

Sara Shaunfield[1], Karen Kaiser[1], Courtney N. Hurt[1], George J. Greene[1], Devin Peipert[1], Adi Eldar-Lissai[2], David Cella[1]

[1] *Northwestern University*
[2] *Fulcrum Therapeutics*

Objective: Current FSHD-specific patient-reported outcome measures (PROMs) can be lengthy, burdensome, and insensitive to change. We aimed to develop a PROM of physical function (PF) and symptoms for FSHD that might be more responsive than available PROMs. Methods: We reviewed literature, data from a qualitative study on FSHD PF limitations, and longitudinal data from a prior clinical trial (item-level score correlations between the 116-item FSHD-Health Index and Patient Global Impression of Change) to identify priority limitations and symptoms that are sensitive to change over one-year. Evidence was reviewed to prioritize PF limitations and symptoms, which were mapped to existing items from PROMIS, Neuro-QoL, FACIT, and new items were drafted as needed. The item pool was reduced based on input from measure developers, clinicians, and FSHD researchers. Finally, persons with FSHD completed cognitive interviews to reduce items and revise the wording. Results: Findings informed identification of 26 priority limitations and 14 symptoms represented by an item pool of 150 items. The item pool was reduced to 40 items, which were assessed via 10 cognitive interviews; findings led to the removal of 10 and addition of 2 items prior to finalizing the 32-item HealthMeasures Facioscapulohumeral Muscular Dystrophy-32. Conclusions: The HM FSHD-32 reflects the most important PF limitations and symptoms that are responsive to change in a format less burdensome to FSHD patients.

P5.08

Using an artificial intelligence algorithm for serial fat fraction and fat volume analysis of whole-body muscle MRI in a paediatric patient with FSHD: A pilot analysis

Ian Woodcock[1], Olivia DuCharme[2], Lara Riem[2], Katy de Valle[1], Seth Friedman[3], Silvia Blemker[2]

[1] *The Royal Children's Hospital*
[2] *Springbok Analytics*
[3] *Seattle Children's Hospital*

Background: Whole-body muscle analysis has been evaluated as a potential biomarker for disease stage and clinical trial outcomes in FSHD. In the pediatric population, the effect of growth on the disease progression has not been established. Methods: A single paediatric patient's lower limb MRI data at two time points fourteen months apart were analysed using an artificial intelligence model to automatically segment individual muscles from axial DIXON MRIs to assess the fat fraction (FF), fat volume (FV) and muscle volume (MV) of the lower limb (LL) muscles. Results: There was a mean increase in FF of 3% across all LL muscles. However, this was not a uniform increase: changes in FF ranged from a 23% increase to a 12% decrease. Decreases in FF were explained by increases in total MV due to growth: in all cases FV either stayed approximately constant or increased. Patterns of change were heterogeneous, even contralaterally in the same muscle: the right gracilis exhibited greater increases in FF proximally, whilst on the left, greater increases in FF were seen distally. Conclusion: These results highlight the importance of whole, multi-muscle analyses as compared to focusing on specific muscles and/or a single slice as used in previous studies. This pilot case highlights the need for a larger pediatric study to establish FSHD's natural history progression and the impact growth has on parameters currently being assessed as potential clinical trial outcome measures.

P5.09

An integrative approach to FSHD molecular diagnosis: Tools for comprehensive genotype-phenotype correlation

Serpil Eraslan[1], Sahin Avci[1], Esra Borklu[1], Ilker Eren[2], Gulshan Yunisova[3], Gulleyla Kilic[1], Mehmet Demirhan[2], Piraye Oflazer[3]

[1] *Koç University Hospital, Genetic Diseases Evaluation Center, Istanbul, Turkey*
[2] *Koç University School of Medicine (KUSoM), Orthopedics and Traumatology Department, Istanbul, Turkey*
[3] *Koç University School of Medicine (KUSoM), Neurology Department, Istanbul, Turkey*

FSHD is primarily caused by genomic alterations resulting in epigenetic modifications on 4q35 enabling the toxic expression of *DUX4*. The majority of cases are FSHD1, associated with D4Z4 repeat unit contractions on 4q35 (~95%). However, nearly ~5% are FSHD2 patients with non-contracted D4Z4 region. The disease type, instead it has mostly been associated with pahtogenic variants in the SMCHD1 (~85%) and to a lesser extent in DNMT3B and LRIF1 genes. Recent scientific studies show that FSHD1 patients with borderline RUs (8-10 RU) might also have pathogenic variants in these associated genes. These detailed genotypic evaluations also shed light to our understanding of clinical variability. Sixty-seven patients were refered to FSHD Diagnosis & Research Unit after clinical evaluation. Molecular combing analysis revealed 1-10RUs in 59 individuals (n=59/67), whereas 8 had ≥11 RUs. Our aim was to further investigate the etiopathogenesis of the clinical condition in non-contracted D4Z4 patients (Group A) and also to screen a selected number of borderline patients (n=4; 9-10RUs) for a possible digenic condition (Group B). using whole exome sequencing. SMCHD1, DNMT3B and LRIF1 genes were prioritised during analysis. Our results of group A yielded one SMCHD1 (1/8) and 2 CAPN3 (2/8) previously identified pathogenic variants, whereas 5 patients did not yield any significant results (5/8). In group B we identified 2 pathogenic SMCHD1 variants (2/4) in combination with their borderline RU genotype. These results enabled us to confirm the FSHD2 diagnosis of one patient and allowed us to see that, inspite of clinical variabilty, there is a correlation between disease severity in borderline patients with SMCHD1 variations. Our short-term goal is to include methylation analyses for a comprehensive approach to clinical expression of the disease. Indeed, the technological and scientific advansement enabled a more wholistic approach to molecular diagnosis and prove to be crucial to direct us further into realms of disease mechanism.

P5.10

Patient-specific maps of muscles at risk: An improved biomarker

Seth Friedman[1], Leo Wang[2], Sarah Lowry[1], Michaela Walker[3], Leann Lewis[4], Aliya Shabbir[2], Olivia DuCharme[5], Lara Riem[5], Silvia Blemker[5], Doris Leung[6], Dennis Shaw[1], Tabi Rawil[4], Stephen Tapscott[7], **Jeff Statland**[3]

[1] *Seattle Children's Hospital*
[2] *University of Washington*
[3] *University of Kansas*
[4] *University of Rochester*
[5] *Springbok Analytics*
[6] *Kennedy Krieger Institute*
[7] *University of Washington/Fred Hutchinson Cancer Center*

Recent work by our group has further validated that aberrant expression of *DUX4*, as inferred by expression of *DUX4*-target genes, is present in muscles that exhibit fatty infiltration and elevated short-tau inversion recovery (STIR). An important finding was that measures of entire muscle fat infiltration correlated with *DUX4* expression and did not rely on fat or STIR measures exclusively in the region of the biopsy. If supported by additional studies, these new data suggest that measuring whole muscle MRI characteristic could create personalized maps that accurately reflect individual muscle disease activity and/or progression. These MRI measures might be used in clinical studies to assess response to therapeutic intervention. In our current report, a novel AI method was used to process baseline and 1 year follow up DIXON and STIR leg scans from 34 FSHD subjects, 16 female and 18 male, aged 21 to 69 (mean 47.1±14SD) recruited at three sites (University of Washington Medical Center, University of Rochester Medical Center, and Kansas University Medical Center). Our analyses identify patterns of whole muscle fatty replacement in different leg muscles over time that correlate with prior MRI measures of disease progression. Our studies provide a basis for creating patient-specific maps of muscles at-risk for progression based on MRI characteristics that may provide a valuable biomarker for assessing change over short-intervals and for assessing treatment response.

P5.11

Using ATP to trigger satellite cell activity for muscle regeneration therapy in patients with facioscapulohumeral muscular dystrophy

Michelle Kagramian[1]

[1] (MATES) Marine Academy of Technology and Environmental Science

For individuals diagnosed with FSHD, there is often no remedy for their muscular atrophy. Satellite cells, the stem cells within skeletal muscle, are triggered by damaged muscle and work with fibroblasts to repair and rebuild the muscle larger and stronger. Muscle damage has shown to be caused by strong enough muscle contraction. The factors which heavily influence the degree of muscle contraction were tested in this research by comparing the effects of various ratios of ATP and KCl-MgCl2 solutions had on a rabbit psoas muscle. A thin fiber of muscle was first measured for its initial length. Then, after its submergence under the solution being tested, the resulting length was measured once again and the degree of contraction was calculated. Statistically, there was a significant difference amongst the six different solutions tested; meaning, one ratio of ATP to salts created the largest contraction (ANOVA, $P<0.05$). ATP has shown to be a key factor in causing strong contractions, however only within the correct balance with its corresponding salts ($R2=0.2191$). Electrical muscle stimulation (E-stim) holds the potential to become a treatment for a variety of disabilities for thousands of people as a way to work against the progressive factor of muscular dystrophy. The method of using E-stim works by sending currents throughout the body to stimulate the release of Adenosine triphosphate (ATP) as well as increase the activity of satellite cells and fibroblasts.

P5.12

Quantitative mass spectrometric approach for the detection of DUX4-regulated proteins in FSHD patient serum

Lucienne Ronco[1], Sujatha Jagannathan[2], Oliver King[3], Yuehan Feng[4], Magdalena Bober[4], Rabi Tawil[5], Jamshid W Arjomand[6]

[1]*Independent consultant to FSHD Society*
[2]*University of Colorado Anschutz Medical Campus*
[3]*University of Massachusetts Chan Medical School*
[4]*Biognosys AG*
[5]*University of Rochester Medical Center*
[6]*FSHD Society*

Facioscapulohumeral dystrophy (FSHD) is a rare inherited muscular disease caused by the pathological overexpression of the homeobox transcription factor *DUX4* in affected skeletal muscle. Aberrant *DUX4* expression results in the expression of a well-characterized set of transcripts and proteins resulting in myofiber death, muscle weakness, and increasing disability.

Detection of DUX4 protein and mRNA is extremely challenging. While DUX4-regulated gene transcripts can be detected in affected muscles, they are expressed at low levels and from a limited set of muscle fibers making measurements from repeat biopsy challenging and variable. Therapeutic development in FSHD would be dramatically enhanced by the identification of circulating biomarkers of *DUX4* activity that would not require muscle biopsies.

Here, we report on the selection of a candidate panel of 23 proteins of interest, including 20 *DUX4*-induced proteins, and on the development of quantitative mass spectrometry-based assays to assess levels of peptides from these proteins in serum from subjects with FSHD and controls. Peptides from 5 out of 23 proteins of interest were detected in at least some of the samples. Several non-specific markers of muscle degradation (CKM, CA3), as well as several *DUX4*-induced proteins (SLC34A2, ZSCAN4 and CA2) were detected in a subset of samples, though often near the limits of confident detection. To improve the sensitivity of the assay, samples were processed through a depletion step to remove highly abundant blood proteins and re-analyzed. Although estimated concentrations for some of the proteins of interest increased 10-fold, only 3 candidates (CA2, CA3, CKM) were reliably detected and only one (CA3) was significantly different between FSHD and controls. From these data, we conclude that although protein markers can be detected in some FSHD subject serum, overall levels are extremely low. In addition, direct proteomic detection and quantification of *DUX4*-induced proteins in patient serum is not a reliable biomarker approach without additional assay development and optimization.

P5.13

Project Mercury: A Global Patient-Driven Platform for Accelerating Clinical Trials and Access in FSH Muscular Dystrophy

Ken Kahtava[1], Neil Camarta[2], Josie Godfrey[3], Amanda Hill[1], June Kinoshita[1], Beth Johnston[1], Mark Stone[1]

[1]FSHD Society
[2]FSHD Canada Foundation
[3]JZ Zebra Consulting

Companies developing drugs in rare disease face well-known challenges in clinical trial readiness and global access strategies. The research community must establish clinical trial sites and agree on outcome measures. The patient community must be actively engaged and well-characterized on a variety of demographic, clinical, and biological dimensions. While Health Technology Assessment (HTA) bodies and payors must be made aware of the burden of disease, unmet medical need, and emerging treatments and standards of care to make decisions about reimbursement and access. Often, companies tackle each of these challenges on their own, siloed by competition. Unfortunately, in rare diseases, resources are scarce – including data, researchers, clinicians, funding, patients, even hope – and the exhaustion of any one of these resources or the failure of one company can irrevocably harm all other future therapeutic development. We must, therefore, tackle these challenges collaboratively, breaking down siloes driven by competition, geography, or language.

To address these needs, we are developing a multi-stakeholder platform, which can be coordinated by FSHD patient advocacy organizations across different geographies. Named Project Mercury, this program establishes local working groups in countries where companies developing FSHD drugs plan to bring clinical trials and regulatory filings. Working groups are comprised of local patient advocates, key-opinion leaders, and subject-matter experts and are tasked with implementing three workstreams in their country:

1. Access – generating healthcare utilization and cost studies to satisfy requirements of local HTA bodies.
2. Clinical trial readiness – establishing clinical trial sites and genetic testing pipelines; engaging patients who are educated about clinical trials and willing to participate.
3. Sustainability – engaging patients and patient advocacy organizations in fundraising, advocacy, and general disease education and support.

Country working groups are supported and coordinated by a global task force, ensuring efforts are aligned and resources and learnings are shared across countries. The global task force is comprised of global leaders in each of the workstreams, representatives from each country working group, and representatives from companies developing FSHD drugs. Altogether, the Project Mercury framework will create alignment among multiple global stakeholders and speed the delivery of therapeutics for FSHD.