

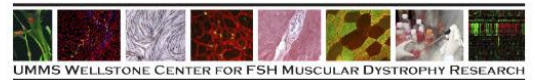


29TH ANNUAL FSHD SOCIETY
**INTERNATIONAL
RESEARCH
CONGRESS**

Orlando, Florida
June 16–17, 2022

ABSTRACT BOOK

THANK YOU TO OUR SPONSORS



JAMES CHIN, SR.,
SCHOLARSHIP FUND



SLIDE PRESENTATIONS

DAY 1 – THURSDAY, JUNE 16, 202

- 8:00 a.m. **Welcoming Remarks**
- 8:15 a.m. [Special Session: Patient Perspective on Living with FSHD](#)
Lexi Pappas
- 8:30 a.m. [Keynote: Facts and Fiction: How DUX4 Came on Stage, Could Play Good or Bad Guy, and Might Still Surprise Us](#)
Alexandra Belayew, MSc, PhD, University of Mons
- 9:30 a.m. **Session 1: Discovery Research**
Session Chairs: Yi-Wen Chen, DVM PhD & Julie Dumonceaux, PhD
- 9:35 a.m. [S1.101](#) **Transcriptional and Post-transcriptional Mechanisms Induced by DUX4 Reshape Protein Synthesis**
Danielle Hamm
- 9:55 a.m. **Morning Networking Break**
- 10:30 a.m. [S1.102](#) **DUX4 Expression Activates JNK and p38 MAP Kinases in Myoblasts**
Nicolas Christoforou, **Christopher Brennan**, Abby Hill, Vijaya Madeti, Susanne Breikopf, Seth Garren, Liang Xue, Tamara Gilbert, Angela Hadjipanayis, Mara Monetti, Charles P. Emerson Jr., Rob Moccia, Jane Owens
- 10:50 a.m. [S1.103](#) **Structure-Function Characterization of a DUX4 Inhibitor for a Drug-Like Approach to Treat FSHD Muscular Dystrophy**
Paola Ghezzi, Andrea Berardi, Valeria Runfola, Maria Pannese, Giovanna Musco, Davide Gabellini
- 11:10 a.m. **Session 2: Genetics & Epigenetics**
Session Chairs: Frédérique Magdinier, PhD & Russell Butterfield, MD PhD
- 11:15 a.m. [S2.201](#) **A Novel Epigenetic Activator of DUX4 for the Therapy of FSHD Muscular Dystrophy**
Emanuele Mociaro, Roberto Giambruno, Stefano Micheloni, Maria Pannese, Valeria Runfola, Giulia Ferri, Davide Gabellini
- 11:35 a.m. [S2.202](#) **dCAS-CTCF Modifies 3D Genome Organization and DUX4 Expression in FSHD Myoblasts**
Anna Karpukhina, Evgenia Tuikacheva, Zhenrui Pan, Sergey Ulyanov, Yegor Vassetzky
- 11:55 a.m. [S2.203](#) **Nanopore Sequencing Reveals Size-Dependent Methylation Gradients in D4Z4 Repeat Arrays**
Russell Butterfield, Diane M Dunn, Brett O Duvall, Sarah Moldt, Brith Otterud, Kristen Wong, Robert B Weiss
- 12:15 p.m. **Lunch**
-

- 1:00 p.m. **Poster Viewing & Networking**
- 3:00 p.m. **Session 3: Pathology & Disease Mechanisms**
Session Chairs: Amy Campbell, PhD & Yegor Vassetzky, PhD
- 3:05 p.m. **[S3.301](#) DUX4 RNA G-quadruplexes as Drivers of Cellular RNA-Protein Granule Formation in FSHD**
Prakash Kharel, Paul Anderson, Pavel Ivanov
- 3:25 p.m. **[S3.302](#) DUX4 Activation of Human Pericentric Satellite Repeats Impairs DNA Damage Signaling**
Tessa Arends
- 3:45 p.m. **[S3.303](#) Interplay Between Mitochondrial Reactive Oxygen Species, Oxidative Stress and Hypoxic Adaptation in FSHD: Metabolic Stress as Potential Therapeutic Target**
Philipp Heher, Massimo Ganassi, Adelheid Weidinger, Elise Engquist, Johanna Pruller, Thuy Hang Nguyen, Alexandra Tassin, Anne-Emilie Decleves, Kamel Mamchaoui, Christopher Banerji, Johannes Grillari, Andrey Kozlov, Peter Zammit
- 4:05 p.m. **[S3.304](#) Antiapoptotic Protein FAIM2 is a Regulatory Node, Downstream of DUX4-TRIM21 and miR-3202**
Michael Kyba, Hossam Soliman, Erik Toso, Inas Darwish, Samia Ali
- 4:25 p.m. **Day 1 Closing Remarks**
Jamshid Arjomand, PhD, FSHD Society
- 6:00 p.m. **Reception**
- 7:00 p.m. **Banquet**

DAY 2 – FRIDAY, JUNE 17, 2022

- 9:00 a.m. **Welcoming Remarks**
- 9:05 a.m. **[Keynote: SOLVE FSHD: Catalyst for a Cure for FSHD](#)**
Eva Chin, PhD, Solve FSHD
- 9:25 a.m. **Session 4: Interventional Strategies**
Session Chairs: Lindsay Wallace, PhD & Valeria Sansone, MD PhD
- 9:30 a.m. **[S4.401](#) Investigation of Human Bone Marrow Mesenchymal Stem Cell-Derived Extracellular Vesicles as Therapeutic Agents for Facioscapulohumeral Muscular Dystrophy (FSHD)**
Lindsay Wallace, Scott Harper, **Nizar Saad**
- 9:50 a.m. **[S4.402](#) AOC 1020: An Antibody Oligonucleotide Conjugate (AOC) in Development for the Treatment of FSHD**
-

Barbora Malecova, David Sala, Garineh Mary Melikian, Gulin Erdogan, Rachel Johns, Joanne Young, Erwann Ventre, Sole Gatto, Matthew Onorato, Orsolya Kiraly, Martin Koegler, Philipp Hadwiger, Lukas Perkams, Arthur Levin, Michael Flanagan

- 10:10 a.m. **[S4.403](#) AAV-CRISPR-Cas13 Gene Therapy for FSHD: DUX4 Gene Silencing Efficacy and Immune Responses to Cas13b Protein**
Afroz Rashnonejad, Gholamhossein Amini Chermahini, Noah Taylor, Allison Fowler, Emma Kraus, Oliver King, Scott Harper
- 10:30 a.m. **Morning Networking Break**
- 11:00 a.m. **[S4.404](#) Cell therapy Counteracts Disease Phenotypes in a Mouse Model of FSHD**
Karim Azzag, Darko Bosnakovski, Sudheer Tungtur, Peter Salama, Michael Kyba, Rita Perlingeiro
- 11:20 a.m. **[Keynote: Data Aggregation and Disease Modeling to Accelerate Rare Disease Drug Development](#)**
Jane Larkindale, PhD, PepGen
- 12:00 p.m. **Lunch**
- 1:00 p.m. **Poster Viewing & Networking**
- 3:00 p.m. **Session 5: Clinical Studies & Outcome Measures**
Session Chairs: Doris Leung, MD PhD & Giorgio Tasca, MD PhD
- 3:05 p.m. **[S5.501](#) Clinical Trial Readiness to Solve Barriers to Drug Development in FSHD (ReSolve): Baseline Characteristics**
Jeffrey Statland, **Kate Eichinger**, Michael McDermott, Kiley Higgs, Michaela Walker, Doris Leung, Sabrina Sacconi, Karlien Mul, Valeria Sansone, Elena Carraro, Leo Wang, Perry Shieh, Bakri Elsheikh, Samantha LoRusso, Russell Butterfield, Nicholas Johnson, Rabi Tawil, the ReSolve Investigators of the FSHD CTRN
- 3:25 p.m. **[S5.502](#) Muscle Ultrasound as Imaging Biomarker in Facioscapulohumeral Muscular Dystrophy: Possibilities and Pitfalls**
Sjan Teeselink, Sanne Vincenten, Nicol Voermans, Baziel van Engelen, Karlien Mul, Nens van Alfen
- 3:45 p.m. **[S5.503](#) Diagnostic MRI Biomarkers for FSHD Identified by Machine Learning**
Mauro Monforte, Sara Bortolani, Eleonora Torchia, Lara Cristiano, Francesco Laschena, Tommaso Tartaglione, Enzo Ricci, Giorgio Tasca
- 4:05 p.m. **[S5.504](#) Reachable Workspace to Evaluate Efficacy of Losmapimod in FSHD in Two Phase 2 Studies**
Christopher Morabito, Jay Han, Anthony Accorsi, Jordi Diaz, Miriam Freimer, Angela Genge, Summer Gibson, Nuria Gomez, Namita Goyal, Johanna Hamel, Lawrence Hayward, John Jiang, Nicholas Johnson, Joost Kools, David Reyes Leiva, Doris Leung, Hanns Lochmuller, Samantha Lorusso, Michelle L. Mellion, Alan Pestronak, L. Alejandro Rojas, Sabrina Sacconi, Perry Shieh, Jennifer Shoskes, **Jeffrey Statland**, S.H. Subramony, Rabi Tawil, Baziel van Engelen, Juan Vilchez, Kathryn Wagner, Leo Wang
- 4:25 p.m. **Best Poster Prize & Young Investigator Award**
Moderators: Julie Dumonceaux, PhD & Doris Leung, MD PhD
-

4:45 p.m. **2023 IRC Announcement & Final Remarks**
Jamshid Arjomand, PhD, FSHD Society

4:50 p.m. **Adjourn**

POSTER PRESENTATIONS

Discovery Research

[P1.01](#)

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

Adam Bittel, Surajit Bhattacharya, **Yi-Wen Chen**

[P1.02](#)

Single-nucleus RNA-Seq reveals cellular heterogeneity in facioscapulohumeral muscular dystrophy in late myogenic stage

Dongxu Zheng, Anita van den Heuvel, Ahmed Mahfouz, Annelot Wondergem, Susan Kloet, Judit Balog, Baziel van Engelen, Rabi Tawil, Stephen Tapscott, Silvère Van der Maarel

[P1.03](#)

Generation of a craniofacial muscle model of FSHD from iPSCs

Dongsheng Guo, Oliver King, **Lawrence Hayward**, Charles P. Emerson Jr.

[P1.04](#)

The inflammatory muscle phenotype of uninduced ACTA1-MCM;FLEXD mice

Jessica de Greef, Linde Bouwman, Bianca den Hamer, Silvère Van der Maarel

[P1.05](#)

Towards a muscle-targeted delivery of antisense agents against DUX4

Maëlle Limpens, Aline Derenne, Carmen Burtea, Alexandre Legrand, Anne-Emilie Decleves, Frédérique Coppée, Alexandra Tassin

P1.06 WITHDRAWN

[P1.07](#)

p38-independent DUX4 regulatory mechanisms in FSHD myotubes

Rajanikanth Vangipurapu, Francis M. Sverdrup

[P1.08](#)

SLC34A2 as a protein biomarker of FSHD

Maria Traficante, Andrea O'Neill, Ujwala Pimparkar, Rabi Tawil, Jeffrey Statland, Robert Bloch

[P1.09](#)

Dynamic proteome profiling of myoblasts from FSHD patients and their unaffected siblings

Jatin Burniston, Radoš Stefanović, Adam Bittel, Yi-Wen Chen

[P1.10](#)

Promoting clinical trial readiness to Brazil

Fabio Eliezer Figueiredo, Cristiane A Martins Moreno

[P1.11](#)

Transient DUX4 expression leads to muscle degeneration

David Oyler, Ana Mitanoska, Ahmed Shams, Natasha Santos, Natalie Xu, Jasmine Gulik, MacKenzie Molina, Erik Toso, Michael Kyba, and **Darko Bosnakovski**

Genetics & Epigenetics

[P2.12](#)

Generation of human skeletal myocyte models harboring SMCHD1 and/or D4Z4 mutations reveals critical roles of epigenetic modifiers for stable FSHD phenotype

Nam Nguyen, Xiangduo Kong

[P2.13](#)

Methylation analysis of proximal region on D4Z4 repeats in FSHD1 patients compared to healthy individuals

Ceren Hangul, Öznur Tokta, Sibel Berker Karauzum, Hilmi Uysal, Filiz Koc

[P2.14](#)

Cis D4Z4 repeat duplications in FSHD

Richard Lemmers, Patrick van der Vliet, Silvère van der Maarel, Jan de Bleecker, Ludo van der Pol, Corrie van Erasmus, Marc D'Hooghe, Peter van den Bergh, Baziel van Engelen, Jeffrey Statland, Rabi Tawil, Nicol Voermans, Sabrina Sacconi, John Vissing, Silvère M van der Maarel

[P2.15](#)

Maternal SMCHD1/LRIF1 haploinsufficiency triggers homeotic transformations in genetically wild-type offspring

Shifeng Xue, Frederique Magdinier, Bruno Reversade

Pathology & Disease Mechanisms

[P3.16](#)

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

Yosuke Hiramuki, Ichizo Nishino, Hiroyuki Kugoh, Yasuhiro Kazuki

[P3.17](#)

Transcriptomic analysis of inflamed and non-inflamed FSHD muscle, together with peripheral blood mononucleated cells, reveals a circulating biomarker of clinical severity in FSHD

Christopher Banerji, Anna Greco, Leo Joosten, Baziel van Engelen, Peter Zammit

[P3.18](#)

Relationship between DUX4 and Hypoxia-Inducible Factor (HIF1 α) in human and murine muscle cells in vitro and in vivo

Thuy Hang Nguyen, Alexandre Legrand, Anne-Emilie Declèves, Philipp Heher, Alexandra Belayew, Christopher Banerji, Peter Zammit, Alexandra Tassin

[P3.19](#)

Fibro-adipogenic progenitors and the progression of the FSHD myopathy

Carlo Serra, Kathryn Wagner, Thomas Lloyd

[P3.20](#)

DUX4, nucleolar stress, and FSHD myopathy

Carlo Serra, Kathryn Wagner, Thomas Lloyd

[P3.21](#)

Innate immunity model of FSHD muscle pathology activates complement genes

Katelyn Daman, Jing Yan, Oliver King, Michael Brehm, Charles P. Emerson Jr.

[P3.22](#)

Intramuscular fibrosis correlates with disease activity and progression in Facioscapulohumeral muscular dystrophy patients

Elvira Ragozzino, Sara Bortolani, Lorena Di Pietro, Ornella Parolini, Mauro Monforte, Giorgio Tasca, Enzo Ricci

[P3.23](#)

Non-myogenic mesenchymal cells contribute to muscle degeneration in facioscapulohumeral muscular dystrophy patients

Lorena Di Pietro, Flavia Giacalone, Elvira Ragozzino, Valentina Saccone, Marco De Bardi, Mario Picozza, Wanda Lattanzi, Enrico Guadagni, Sara Bortolani, Giorgio Tasca, Enzo Ricci, Ornella Parolini

[P3.24](#)

Interaction between mesenchymal stem cells and myoblasts contributes to the FSHD phenotype

Yegor Vassetzky, Ekaterina Kiseleva, Olesya Serbina, Anna Karpukhina

Interventional Strategies

P4.25 - WITHDRAWN

[P4.26](#)

Hit-and-run silencing of endogenous DUX4 by targeting DNA hypomethylation on D4Z4 repeats in in vitro FSHD-iPSC model

Mitsuru Sasaki-Honda, Junjie He, Hidetoshi Sakurai

[P4.27](#)

Improving FSHD RNAi gene therapy using myotropic MyoAAVs

Lindsay Wallace, Tessa Riley, Matthew Guggenbiller, Gholamhossein Amini Chermahini, Scott Harper

[P4.28](#)

Identification and targeting of hypoxia signaling for translational potential in facioscapulohumeral muscular dystrophy

Justin Cohen, Vincent Ho, Alec Desimone, Monkol Lek, Angela Lek

[P4.29](#)

An AAV-shRNA DUX4-based therapy to treat facioscapulohumeral muscular dystrophy (FSHD)

Virginie Mariot, Eva Sidlauskaitė, Laura Le Gall, Emilio Corbex, Julie Dumonceaux

[P4.30](#)

Development of safe and efficacious RNA therapeutics for FSHD

Christian Kinney, Anthony Saleh

Clinical Studies & Outcome Measures

[P5.31](#)

Motor outcomes to validate evaluations in facioscapulohumeral muscular dystrophy (MOVE FSHD): protocol for an observational study.

Michaela Walker, Russell Butterfield, John Day, Kate Eichinger, Bakri Elsheikh, Anna Faino, Seth Friedman, Kiley Higgs, Nicholas Johnson, Peter Jones, Doris Leung, Leann Lewis, Bill Martens, Dennis Shaw, Perry Shieh, Subramony Subramony, Jaya Trivedi, Leo Wang, Mathew Wicklund, Rabi Tawil, Jeffrey Statland

[P5.32](#)

Muscle imaging in facioscapulohumeral muscular dystrophy (FSHD): relevance for clinical trials. Report from the 265th ENMC Workshop

Giorgio Tasca, Shahram Attarian, John Vissing, Jordi Diaz-Manera, Nicol Voermans

[P5.33](#)

The face of facioscapulohumeral muscular dystrophy: exploring facial muscle involvement using ultrasound

Sanne Vincenten, Karlien Mul, Nicol Voermans, Nens van Alfen, Baziel van Engelen

[P5.34](#)

Analyzing the impact of FSHD on patient outcomes

Elan Schonfeld, Charulatha Nagar

[P5.35](#)

A world-wide survey of standardised outcome measure use in FSHD clinical care

Katy de Valle, Jenny McGinley, Fiona Dobson, Monique Ryan

[P5.36](#)

Longitudinal assessment of facial weakness in facioscapulohumeral muscular dystrophy by physicians and patients

Karlien Mul, Tom Loonen, Sanne Vincenten, Sjan Teeselink, Nicol Voermans, Thomas Maal, Baziel van Engelen

[P5.37](#)

Muscle ultrasound in an open-label study of losmapimod in subjects with FSHD1

Joost Kools, Nicol Voermans, Karlien Mul, John Jiang, Jennifer Shoskes, Kelly Marshall, Michelle L. Mellion, Baziel van Engelen, Markus Karlsson

[P5.38](#)

Feasibility of measuring functional performance of FSHD patients using wearable sensors to quantify physical activity

Joost Kools, Nicol Voermans, Karlien Mul, Michelle L. Mellion, John Jiang, Jennifer Shoskes, Kelly Marshall, David Jackson, Yuxi Zhao, Anil Tarachandani, Joanita Figueredo, Damien Eggenpieler, Baziel van Engelen

[P5.39](#)

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

Joost Kools, Johanna Deenen, Anna Greco, Renée Thewissen, Wiecke Van de Put, Anke Lanser, Nicol Voermans, Mara Tihaya, André Verbeek, Silvère Van der Maarel, Leo Joosten, Baziel van Engelen

[P5.40](#)

The UK FSHD Patient Registry: a key tool linking patients with national and international research projects

Helen Walker, Richard Orrell, Chiara Marini-Bettolo, Andrew Graham, Kate Adcock, Suzanne Watt, Peter Lunt, Fiona Norwood, Mark Roberts, Tracey Willis, Emma Matthews, Robert Muni-Lofra

[P5.41](#)

Prevalence and impact on quality of life of gastrointestinal and genitourinary symptoms in facioscapulohumeral muscular dystrophy

Michael Cole, June Kinoshita, Angelena Edwards, Christopher Cooper, Eyad Hanna, M. Bridget Zimmerman, Katherine Mathews

[P5.42](#)

Inpatient admissions and emergency department visits for patients with facioscapulohumeral muscular dystrophy (FSHD): a real-world retrospective data analysis of pre- and post-diagnosis events

Chamindra Konersman, **Kathryn Munoz**, Richard Brook, Nathan Kleinman, Kelly DiTrapani, Bradley McEvoy, Alissa Peters, Chao-Yin Chen, Teresa Brandt, Mark Stahl

[P5.43](#)

Design of REACH: Phase 3 randomized, double-blind, placebo-controlled, 48-week study of the efficacy and safety of losmapimod in FSHD

Christopher Morabito, Rabi Tawil, Jay Han, Leo Wang, John Vissing, Baziel van Engelen, Jeffrey Statland, Michelle L. Mellion, **Jennifer Shoskes**, John Jiang, Jennifer Webster

[P5.44](#)

Annualized rates of change from a Phase 2, randomized, double-blind, placebo-controlled, 48-week study of losmapimod in subjects with FSHD: ReDUX4

Christopher Morabito, Sabrina Sacconi, Jordi Diaz, David Reyes Leiva, Doris Leung, Kathryn Wagner, Angela Genge, Juan Vilchez, Nuria Gomez, Miriam Freimer, Samantha Lorusso, Hanns Lochmuller, Joost Kools, Baziel van Engelen, Namita Goyal, Perry Shieh, S.H. Subramony, Jeffrey Statland, Lawrence Hayward, Johanna Hamel, Rabi Tawil, Summer Gibson, Leo Wang, Nicholas Johnson, Alan Pestronak, Michelle L. Mellion, Anthony Accorsi, **Jennifer Shoskes**, John Jiang, L. Alejandro Rojas

[P5.45](#)

Understanding falls in FSHD

Enrico Bugiardini, Kate Eichinger, Michael Hanna, Gita Ramdharry, Michael McDermott, Kiley Higgs, Michaela Walker, Leann Lewis, Bill Martens, Doris Leung, Sabrina Sacconi, Karlien Mul, Valeri Sansone, Leo Wang, Perry Shieh, Bakri Elsheikh, Russell Butterfield, Nicholas Johnson, Rabi Tawil, Jeffrey Statland, the ReSolve Investigators of the FSHD CTRN

[P5.46](#)

TREAT-NMD FSHD Global Registry Network: a collaboration of neuromuscular and FSHD patient registries

Ben Porter, Neil Bennett, David Allison, Craig Campbell, Michela Guglieri, Anna Ambrosini, Rossella Tupler

[P5.47](#)

A case story: supervised FSHD patient self-analysis of its respiratory data

Patrick Valentin, Frederic Lofaso

[P5.48](#)

Longitudinal whole-body MRI and muscle function in FSHD1

Doris Leung, Sharanya Suresh, Shivani Ahlawat, Alex Bocchieri, Vishwa Parekh, Vladimir Braverman, Michael Jacobs

[P5.49](#)

The FSHD Composite Outcome Measure (FSHD-COM) is reliable, valid, and measures disease progression

Katy Eichinger, Michael McDermott, Kiley Higgs, Michaela Walker, Leann Lewis, Bill Martens, Doris Leung, Nikia Stinson, Megan McNerney, Sabrina Sacconi, Jeremy Garcia, Victor De Paz Benito, Karlien Mul, Valeria Sansone, Elena Carraro, Stefano Becchiati, Maria Frisoni, Leo Wang, Catherine Kieu, Perry Shieh, Christ Skura, Bakri Elsheikh, Kristina Kelly, Andrea Jaworek, Samantha LoRusso, Russell Butterfield, Amelia Wilson, Melissa McIntyre, Nicholas Johnson, Amanda Butler, Aileen Jones, Melissa Hayes, Sandhya Sasidharan, Lindsay Baker, Rabi Tawil, Jeffrey Statland, the ReSolve Investigators of the FSHD CTRN

[P5.50](#)

Understanding the perseverance of the muscular dystrophy community one year into the COVID-19 pandemic

Leann Lewis, Katy Eichinger, Nuran Dilek, Kiley Higgs, Michaela Walker, David Palmer, John Cooley, Nicholas Johnson, Rabi Tawil, Jeffrey Statland

[P5.51](#)

Clinical and molecular evaluation of FSHD patients in Turkey

Serpil Eraslan, Sahin Avci, Ilker Eren, Gulshan Yunisova, Piraye Oflazer, Mehmet Demirhan, Hulya Kayserili

[P5.52](#)

Prevalence and disease progression of genetically confirmed facioscapulohumeral muscular dystrophy type 1 (FSHD1) in China between 2001 and 2020: a nationwide population-based study

Zhiqiang Wang, Liangliang Qiu, Minting Lin, Long Chen, Fuze Zheng

[P5.53](#)

Association between D4Z4 hypomethylation and disease severity: a retrospective cohort study in China

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

SPEAKER ABSTRACTS

Special Session: Patient Perspective on Living with FSHD

Lexi Pappas¹

¹*Dell Technologies*

Lexi was diagnosed with FSHD at around 12 years old. Her mother, uncle, and grandfather (passed away in 2016) all have/had FSHD, so when she started showing symptoms, her family knew what that meant. With each generation, symptoms have developed earlier. Lexi disclosed her condition publicly for the first time at the end of college, and since then has become an advocate for FSHD and disability rights. Now 28, she is a video producer and editor at Dell Technologies. She volunteers her time as the Walk and Roll leader for the New England chapter of the FSHD Society.

Keynote: Facts and Fiction: How DUX4 Came on Stage, Could Play Good or Bad Guy, and Might Still Surprise Us
Alexandra Belayew¹

¹*University of Mons*

Today, it is common knowledge that DUX4 expression causes FSHD. However, more than 20 years ago, at a time when textbooks referred to repeated elements as "junk DNA," it was unexpected to identify a functional gene located within the D4Z4 repeats, and controversial to hypothesize that its expression caused FSHD! We shall present this scientific background, and how the startling DUX4 concept progressively intrigued diverse scientists who performed key experiments that all together showed it was not a pseudogene; it was expressed in FSHD muscle cells and produced a highly toxic transcription factor. Although the mechanism of DUX4 toxicity still has to be fully clarified, researchers in academia and industry are developing strategies to prevent its expression by altering its DNA sequence or chromatin structure, suppressing co-activators, targeting its mRNAs by antisense agents, preventing its transcriptional activity, or normalizing DUX4-mediated toxic pathways. Once DUX4 was listed in DNA/RNA databases, it could be identified in transcriptomic studies by researchers in other fields. This revealed key roles of DUX4 both in early embryo development and in the field of cancer, either in escape from the immune system or part of neo-oncogenes generated by chromosome translocation. However, open questions remain about the role of DUX4 in healthy cells, which is an important and timely issue since inhibitory agents are to be administered systemically to patients with FSHD.

S1.101

Transcriptional and post-transcriptional mechanisms induced by DUX4 reshape protein synthesis

Danielle Hamm¹

¹*Fred Hutchinson Cancer Center*

The Double Homeobox Protein 4 (DUX4) transcription factor is normally expressed in the testis, thymus, and early embryo, and is epigenetically silenced in healthy somatic tissues. Aberrant DUX4 expression in skeletal muscle is the causal factor of facioscapulohumeral muscular dystrophy (FSHD). The gene-regulatory network induced by DUX4 has been well characterized, but the functional significance of this rests upon the assumption that changes in mRNA levels accurately reflect protein expression. It is becoming increasingly clear that post-transcriptional mechanisms play a pivotal role in regulating gene expression, and a more complete analysis of the transcriptome and proteome are needed to understand the complexity of DUX4 biology. My research investigates post-transcriptional regulation by DUX4, in which we discovered that transient DUX4 expression disrupts several key regulators of translation. Using high-throughput ribosome footprinting and polysome profiling, we have implicated the DUX4 transcriptional program as a driver of broad translational suppression that alters de novo protein synthesis. We propose that coordinated regulation of transcription and translation is employed by DUX4 to reshape the cellular transcriptome in FSHD.

S1.102**DUX4 expression activates JNK and p38 MAP kinases in myoblasts**

Nicolas Christoforou¹, **Christopher Brennan**², Abby Hill¹, Vijaya Madeti¹, Susanne Breitkopf³, Seth Garren¹, Liang Xue¹, Tamara Gilbert¹, Angela Hadjipanayis⁴, Mara Monetti¹, Charles P. Emerson Jr.⁵, Rob Moccia¹, Jane Owens¹

¹*Pfizer Inc.*

²*Entrada Therapeutics*

³*Kymera Therapeutics*

⁴*Sanofi*

⁵*UMass Chan Medical School*

Facioscapulohumeral muscular dystrophy (FSHD) is a progressive muscle wasting disease caused by misexpression of the DUX4 transcription factor. DUX4 expression in skeletal muscle cells results in dramatic transcriptional alterations, causing a range of abnormal phenotypes that lead to cell death. To gain insight into the kinetics of DUX4-induced stresses, we activated DUX4 expression in myoblasts and performed longitudinal RNA sequencing paired with proteomics and phosphoproteomics. This analysis revealed changes in cellular physiology including DNA damage and altered mRNA splicing before detectable changes in protein levels. Phosphoproteomic analysis uncovered widespread changes in protein phosphorylation that preceded changes in protein levels, indicating that alterations in kinase signaling may play a role in DUX4-mediated stress and cell death. Here we demonstrate that two stress-responsive MAP kinase pathways, JNK and p38, are activated in response to DUX4 expression. Pharmacological inhibition of each of these pathways ameliorated DUX4-mediated cell death in myoblasts. These findings uncover JNK as a novel pathway involved in DUX4-mediated cell death, as well as provide additional insights into the role of the p38 pathway, a clinical target for the treatment of FSHD.

S1.103**Structure-function characterization of a DUX4 inhibitor for a drug-like approach to treat FSHD muscular dystrophy**Paola Ghezzi¹, Andrea Berardi¹, Valeria Runfola¹, Maria Pannese¹, Giovanna Musco¹, Davide Gabellini¹¹*San Raffaele Scientific Institute*

Facioscapulohumeral muscular dystrophy (FSHD) is the most prevalent neuromuscular disease affecting children and adults of all ages and both sexes. Unfortunately, no treatment is currently available. FSHD is caused by gain of expression of the Double Homeobox 4 (DUX4) gene, encoding for a transcription factor largely silent in somatic tissues. In FSHD, DUX4 activates a pro-apoptotic program resulting in muscle wasting. While blocking DUX4 activity is a plausible therapeutic option for FSHD, the mechanism underlying DUX4-induced toxicity is poorly understood. Previous analyses from our group identified MATRIN 3 (MATR3) as the first endogenous protein able to inhibit both DUX4 expression and activity. MATR3 directly binds to DUX4 DNA-binding domain, reducing its expression and its ability to activate gene expression. As a result, MATR3 administration rescues cell viability and myogenic differentiation of FSHD muscle cells while it is safe to healthy muscle cells. We are currently combining protein engineering with ultrastructural studies to characterize the interaction between DUX4 and MATR3 at the atomic level. These results will be key to designing a MATR3-based drug-like DUX4 inhibitory molecule that, in perspective, might be applied to a spectrum of diseases associated with aberrant DUX4 expression or activity.

S2.201**A novel epigenetic activator of DUX4 for the therapy of FSHD muscular dystrophy**

Emanuele Mocchiaro¹, Roberto Giambruno¹, Stefano Micheloni¹, Maria Pannese¹, Valeria Runfola¹, Giulia Ferri¹, Davide Gabellini¹

¹*San Raffaele Scientific Institute*

Facioscapulohumeral muscular dystrophy (FSHD) is an inherited progressive neuromuscular disorder that afflicts both children and adults regardless of gender. FSHD is caused by aberrant gain of expression of the transcription factor DUX4, which triggers a pro-apoptotic transcriptional program leading to muscle wasting. As of today, no cure or therapeutic option is available to FSHD patients. Given DUX4's centrality in FSHD, blocking its expression with small molecule drugs is an attractive option. We previously showed that the long non-coding RNA DBE-T is required for aberrant DUX4 expression in FSHD. Using affinity purification followed by proteomics, we identified a chromatin remodeling protein as a novel DBE-T interactor and a key player required for the biological activity of the lncRNA. We found that the novel DBE-T binding protein is required for the expression of DUX4 and its target genes in FSHD muscle cells. Moreover, targeting the novel DBE-T binding protein rescues both cell viability and myogenic differentiation of FSHD muscle cells. Remarkably, we obtained comparable results by pharmacological inhibition of the novel DBE-T binding protein. Importantly, the treatment is safe to healthy muscle cells. These results not only support a pivotal role of the DBE-T binding protein in the regulation of DUX4 expression, but also identify a druggable target, opening the possibility of an innovative therapeutic approach for FSHD.

S2.202**dCAS-CTCF modifies 3D genome organization and DUX4 expression in FSHD myoblasts****Anna Karpukhina**^{1,2}, Evgenia Tuikacheva^{1,2}, Zhenrui Pan¹, Sergey Ulyanov², Yegor Vassetzky^{1,2}¹*CNRS UMR9018, Villejuif, France*²*Koltzov Institute of Developmental Biology, Moscow, Russia*

The human genome is organized inside cells into an intricate 3D structure: chromosomes are organized into compartments that are subdivided into a set of topologically associating domains (TADs) and chromatin loops that often include co-regulated and co-transcribed genes. In mammals, TADs are physically separated by CCCTC-binding factor (CTCF) and cohesin complex binding sites. The 3D genome architecture is perturbed in FSHD, and this affects gene expression and epigenetic pattern. In FSHD, two loops located in the 4q35 locus are fused together, allowing the pathological interaction between transcription control elements and DUX4, DUX4C, and FRG2. We used the dCas9-CTCF fusion protein to re-establish transcriptional silencing in the 4q35 locus. First, as a proof of principle, we deleted the TAD/loop anchorage element containing the CTCF binding site in the RUNX1 gene locus. We next targeted the dCas9-CTCF to the TAD border using gRNAs specific for sequences adjacent to the deleted element. Hi-C analysis demonstrated the reconstitution of the TAD border. We next targeted dCas9-CTCF to the loop anchorage region located upstream of the D4Z4 array in the AB1080 immortalized myoblasts from the FSHD patients. This significantly reduced expression of the DUX4 target genes ZSCAN and TRIM43, but not FRG2. Thus, dCAS-CTCF can be used to re-establish the correct 3D genome architecture and gene expression pattern in myoblasts from the FSHD patients.

S2.203**Nanopore sequencing reveals size-dependent methylation gradients in D4Z4 repeat arrays****Russell Butterfield¹**, Diane M Dunn¹, Brett O Duvall¹, Sarah Moldt¹, Brith Otterud¹, Kristen Wong¹, Robert B Weiss¹¹*University of Utah*

Toxic expression of DUX4 from contracted 4qA sub-telomeric D4Z4 arrays in FSHD1 is due to D4Z4 CpG hypomethylation and chromatin relaxation. FSHD2 pathogenicity is similar but arrives at 4qA epigenetic reactivation via mutations in modifier genes such as SMCHD. Diagnosis of FSHD is based on determination of the size of the array, its methylation state, and variants in modifier genes. Previous studies have shown that array length is proportional to methylation, DUX4 expression, and disease severity, but our understanding of this length-dependent mechanism is incomplete. To address this question, we used nanopore Cas9-targeted sequencing in FSHD1, FSHD2, and unaffected subjects to measure CpG methylation with long reads spanning the array, while distinguishing 4qA from 4qB and 10q by sequence in a single assay. We observed in both blood and iPSCs, a stepwise D4Z4 gradient of low (centromeric) to high (telomeric) methylation on both 4q and 10q D4Z4 arrays. The methylation gradient spans ~12 D4Z4 repeats, and FSHD1 contractions changed the gradient's intercept while FSHD2 SMCHD1 mutations changed its slope. Divergent D4Z4 repeats on other chromosomes did not show evidence of methylation gradients, suggesting this phenomenon is unique to chr4q and 10q arrays. These observations suggest a DUX4 reactivation model whereby the D4Z4 contraction moves the distal pathogenic 4qA repeat down a CpG methylation gradient whose properties may contribute to length-dependent disease severity.

S3.301**DUX4 RNA G-quadruplexes as drivers of cellular RNA-protein granule formation in FSHD**Prakash Kharel^{1,2}, Paul Anderson^{1,2}, Pavel Ivanov^{1,2}¹*Brigham and Women's Hospital*²*Harvard Medical School*

Aberrant DUX4 expression in skeletal muscle cells is the primary known genetic cause of facioscapulohumeral muscular dystrophy (FSHD). One of the potential approaches in FSHD treatment involves direct targeting of DUX4 transcript using different approaches such as antisense oligos and siRNAs. A recent discovery suggests guanine-rich regions of DUX4 transcript can fold into a four-stranded secondary structure known as G-quadruplexes (G4s), and modulation of stabilities of such structures may contribute to the downregulation of DUX4. Nevertheless, the existence of such structures in vivo and their putative functional roles in DUX4 misexpression have not been determined. Here, we have demonstrated that several G-rich regions in DUX4 full-length RNA (DUX4-fl) can fold into G4s. Only DUX4 G4s but not their mutants can induce the formation of G3BP1-positive membrane-less RNA-protein complexes in differentiated skeletal muscle cells. In addition, our data show a significant variation in the binding of DUX4 RNA with several RNA metabolic proteins, such as G3BP1, HNRNPH1, and EIF4G1, in G4 dependent manner. We will present different possible mechanisms of DUX4 G4-mediated toxicity in the FSHD model system and the potential application of such structures as therapeutic targets.

S3.302

DUX4 activation of human pericentric satellite repeats impairs DNA damage signaling

Tessa Arends¹

¹*Fred Hutchinson Cancer Center*

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common form of muscular dystrophy and is currently incurable. FSHD is caused by de-repression of the D4Z4 microsatellite repeat array that leads to transient expression of the human double-homeodomain retrogene DUX4 in muscle cells. We have shown that DUX4 robustly activates bi-directional transcription of pericentric human satellite II (HSATII) repeats, and nuclear HSATII RNA aggregation contributes to DUX4-mediated cell toxicity. Here we show that de-repression of HSATII regions sequesters epigenetic regulators, resulting in impaired DNA damage response signaling. Enrichment of epigenetic modifiers at HSATII loci results in global redistribution of histone post-translational modifications. Our data implicate HSATII de-repression and sequestration of protein complexes as mechanisms of regulating or dysregulating epigenetic and DNA repair pathways.

S3.303**Interplay between mitochondrial reactive oxygen species, oxidative stress, and hypoxic adaptation in FSHD: metabolic stress as a potential therapeutic target**

Philipp Heher¹, Massimo Ganassi¹, Adelheid Weidinger², Elise Engquist¹, Johanna Pruller¹, Thuy Hang Nguyen³, Alexandra Tassin³, Anne-Emilie Decleves³, Kamel Mamchaoui⁴, Christopher Banerji¹, Johannes Grillari², Andrey Kozlov², Peter Zammit¹

¹*Randall Centre for Cellular and Molecular Biophysics, King's College London*

²*Ludwig Boltzmann Institute for Traumatology*

³*Research Institute for Health Sciences and Technology, University of Mons*

⁴*Institut de Myologie, Sorbonne University*

Oxidative damage characterizes FSHD, and recent evidence suggests metabolic dysfunction and perturbed hypoxia signalling as potential pathomechanisms. Here, we pinpoint the kinetic involvement of altered mitochondrial ROS metabolism and impaired mitochondrial function in the aetiology of oxidative stress in FSHD. Transcriptomic analysis in FSHD muscle biopsies reveals pathway enrichment for mitochondrial complex I assembly, oxidative stress response, and hypoxia signalling. Elevated mitochondrial ROS levels correlate with increased steady-state mitochondrial membrane potential in FSHD myogenic cells. DUX4 triggers mitochondrial membrane polarisation prior to oxidative stress generation and apoptosis, and affects mitochondrial health through lipid peroxidation. We identify complex I as the primary target for DUX4-induced mitochondrial dysfunction, with strong correlation between complex I-linked respiration and cellular oxygenation/hypoxia signalling in environmental hypoxia. Thus, FSHD myogenesis is uniquely susceptible to hypoxia-induced oxidative stress as a consequence of metabolic mis-adaptation. Mitochondria-targeted antioxidants rescue FSHD pathology more effectively than conventional antioxidants, highlighting the central involvement of disturbed mitochondrial ROS metabolism. Summarizing, this work provides a pathomechanistic model where DUX4-induced changes in oxidative metabolism impair muscle function, amplified when metabolic adaptation to varying O₂ tension is required.

S3.304**Antiapoptotic protein FAIM2 is a regulatory node, downstream of DUX4-TRIM21 and miR-3202****Michael Kyba**¹, Hossam Soliman¹, Erik Toso¹, Inas Darwish², Samia Ali²¹*University of Minnesota*²*University of Alexandria*

Inappropriate expression of DUX4 induces cell death at high levels of expression, impairs myoblast differentiation at low levels of expression, and leads to the development of facioscapulohumeral muscular dystrophy (FSHD); however, the pathological mechanisms downstream of DUX4 responsible for muscle loss are poorly defined. We performed a screen of 1,972 miR inhibitors for their ability to interfere with DUX4-induced cell death of human immortalized myoblasts. The most potent hit identified by the screen, miR-3202, is known to target the antiapoptotic protein FAIM2. Inhibition of miR-3202 led to the upregulation of FAIM2, and, remarkably, expression of DUX4 led to reduced cellular levels of FAIM2. We show that the E3 ubiquitin ligase and DUX4 target gene, TRIM21, is responsible for FAIM2 degradation downstream of DUX4. Human myoblasts overexpressing FAIM2 showed increased resistance to DUX4-induced cell death, whereas in wild-type cells FAIM2 knockdown resulted in increased apoptosis and failure to differentiate into myotubes. The necessity of FAIM2 for myogenic differentiation of WT cells led us to test the effect of FAIM2 overexpression on the impairment of myogenesis by DUX4. Strikingly, FAIM2 overexpression rescued the myogenic differentiation defect caused by low-level expression of DUX4. These data implicate FAIM2 levels, modulated by DUX4 through TRIM21, as an important factor mediating the pathogenicity of DUX4, both in terms of cell viability and myogenic differentiation, and thereby open a new avenue of investigation towards drug targets in FSHD.

Keynote: SOLVE FSHD: Catalyst for a Cure for FSHD

Eva Chin¹

¹*SOLVE FSHD*

Eva is the inaugural Executive Director of SOLVE FSHD, a recently formed Canadian organization focused on accelerating the development of effective new treatments for FSHD. SOLVE FSHD's focus is to be a catalyst for transforming the innovative science that has evolved over the past decade into effective therapies. Eva will discuss the overall strategy of SOLVE FSHD and its six year plan to support key initiatives in the global FSHD community. She will also address how SOLVE FSHD, as a venture philanthropic organization, will balance support for innovative new ideas in academic institutions with investments in the biopharma/biotech industries. This will enable the organization to continuously support growth of the FSHD therapeutics pipeline over the next decade. Eva will utilize her expertise in muscle biology, her global experience in pharmaceutical R&D and her passion for developing new treatments for patients with rare neuromuscular diseases to accelerate a cure for FSHD. Together with the relentless drive and support of founders Chip Wilson and Neil Camarta, this team will Solve FSHD!

S4.401**Investigation of human bone marrow mesenchymal stem cell-derived extracellular vesicles as therapeutic agents for facioscapulohumeral muscular dystrophy (FSHD)**Lindsay Wallace¹, Scott Harper^{1,2}, **Nizar Saad**^{1,2}¹*Nationwide Children's Hospital*²*The Ohio State University*

The DUX4 transcription factor triggers aberrant gene expression in FSHD skeletal muscle, leading to muscle differentiation defects, oxidative stress, and chronic inflammation. Mesenchymal stem cell-derived extracellular vesicles (MSC EVs) are known for their regenerative, antioxidant, and anti-inflammatory properties. We therefore hypothesized that MSC EVs might help address these defects associated with DUX4 expression in FSHD muscle. Accordingly, we tested the regenerative properties of human bone marrow MSC EVs using our previously published AAV.DUX4 mouse model. When co-injected with AAV.DUX4 in the tibialis anterior (TA) of C57BL/6 mice for two weeks, which is an acute model of DUX4-induced muscle damage, MSC EVs efficiently reduced DUX4-induced muscle toxicity. Surprisingly, DUX4 expression and the DUX4-responsive mouse biomarkers, *Wfdc3* and *Trim36*, were decreased. In ongoing studies, we are testing the long-term impacts of MSC EVs on chronic pathology in our uninduced FSHD TIC-DUX4 mouse model and analyzing the molecular content of MSC EVs to uncover prospective mechanisms to explain the beneficial effects of MSCs on DUX4-associated pathology. Our preliminary results support MSC EVs as prospective therapeutic agents for FSHD.

S4.402**AOC 1020: an antibody oligonucleotide conjugate (AOC) in development for the treatment of FSHD**

Barbora Malecova¹, David Sala¹, Garineh Mary Melikian¹, Gulin Erdogan¹, Rachel Johns¹, Joanne Young², Erwann Ventre², Sole Gatto³, Matthew Onorato³, Orsolya Kiraly⁴, Martin Koezler⁵, Philipp Hadwiger⁵, Lukas Perkams⁵, Arthur Levin¹, Michael Flanagan¹

¹*Avidity Biosciences*

²*CYTOO SA, Minatec*

³*Monoceros Biosystems*

⁴*The Jackson Laboratory*

⁵*LGC Axolabs GmbH*

Strategies aimed at reducing DUX4 expression in skeletal muscle of FSHD patients are promising therapeutic approaches. The main challenge limiting clinical development of oligonucleotides for muscular diseases is difficulty delivering oligonucleotides into muscle cells. Avidity's AOC platform combines the specificity of monoclonal antibodies with the precision of oligonucleotides. The lead DUX4-targeting siRNA siDUX4.6 was selected based on comprehensive in vitro screening of a DUX4 siRNA library in 11 FSHD patient-derived muscle cell lines to maximize activity and minimize off-target effects. The AOC 1020 therapeutic candidate comprises siDUX4.6 conjugated to the human transferrin receptor 1 (TfR1) mAb AV01mAb to facilitate delivery to muscle. In cynomolgus monkey skeletal muscle, AOC 1020 produced a dose-dependent increase in siRNA tissue exposure following single systemic IV dose. At therapeutically relevant doses, the muscle tissue concentration observed for siDUX4.6 exceeded the IC50 values determined for other TfR1-based AOCs. siDUX4.6 was conjugated to the murine TfR1 mAb (DUX4 AOC) to assess pharmacology in the ACTA1-MCM;FLEXDUX4 mouse model of FSHD. A robust dose response activity was observed 8 weeks after single IV dose of DUX4 AOC, with more than 75% downregulation of DUX4-regulated genes in skeletal muscle at 6 mg/kg of siRNA within AOC dose. Data presented provide support for AOC 1020 to enter the clinic for the treatment of FSHD by end of 2022.

S4.403**AAV-CRISPR-Cas13 gene therapy for FSHD: DUX4 gene silencing efficacy and immune responses to Cas13b protein**

Afroz Rashnonejad¹, Gholamhossein Amini Chermahini¹, Noah Taylor¹, Allison Fowler¹, Emma Kraus¹, Oliver King², Scott Harper^{1,3}

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

²*Department of Neurology, University of Massachusetts Medical School*

³*Armatus Bio*

We developed an AAV6-CRISPR-Cas13 strategy to silence DUX4 mRNA. Cas13 targets and cleaves RNA instead of DNA, and avoids potential risks of permanent off-target genome editing that could arise with DNA-targeting systems. Intramuscular delivery of an AAV6 vector encoding a PspCas13b enzyme and DUX4-targeting guide RNAs reduced DUX4 mRNA by >50%, and improved histopathological outcomes in FSHD mice. To investigate possible off-target effects, we performed RNA-seq of treated versus control or untreated human myoblasts, and also examined potential collateral RNA cleavage activity using a dual reporter system. Although we did not detect collateral cleavage, our RNA-sequencing results suggested some guide RNAs could induce potential off-target gene expression changes. We are currently exploring mechanisms to explain these differential off-target effects. To address whether PspCas13b can activate a mammalian host immune response, we injected wild-type mice with AAV-Cas13b and investigated immune cell infiltration and pro-inflammatory cytokine profiles. We find evidence of an immune response against PspCas13b in mice, including CD8+, CD3+, and NK T-cell, but not B cell, infiltration in injected muscles. Importantly, transient immunosuppression reduced immune responses to Cas13b in treated animals. In conclusion, our data support that Cas13b can target and reduce DUX4 expression in FSHD muscles, but minimizing cellular immune response may be necessary to translate AAV-Cas13b therapy.

Keywords: facioscapulohumeral muscular dystrophy, FSHD, DUX4, D4Z4, CRISPR-Cas13, gene silencing, RNA targeting therapy, adeno-associated virus vectors, AAV, muscle

S4.404**Cell therapy counteracts disease phenotypes in a mouse model of FSHD****Karim Azzag**¹, Darko Bosnakovski¹, Sudheer Tungtur¹, Peter Salama¹, Michael Kyba^{1,2}, Rita Perlingeiro^{1,2}¹*University of Minnesota, Lillehei Heart Institute*²*Stem Cell Institute*

Facioscapulohumeral muscular dystrophy (FSHD) is a genetically dominant progressive myopathy caused by improper silencing of the DUX4 gene, leading to fibrosis, muscle atrophy, and fatty replacement. Approaches focused on muscle regeneration through the delivery of stem cells represent an attractive therapeutic option for muscular dystrophies. To investigate the potential for cell transplantation in FSHD, we have used the doxycycline-regulated iDUX4pA-HSA mouse model in which low-level DUX4 can be induced in skeletal muscle. We find that mouse pluripotent stem cell (PSC)-derived myogenic progenitors engraft in muscle actively undergoing DUX4-mediated degeneration. In the best cases, we obtained more than 600 donor-derived fibers, corresponding to 25% of the muscle area. Donor-derived muscle tissue displayed reduced fibrosis, and, importantly, engrafted muscles showed improved contractile specific force compared to non-transplanted controls. These data demonstrate for the first time the feasibility of replacement of diseased muscle with PSC-derived myogenic progenitors in a mouse model for FSHD, and highlight the potential for the clinical benefit of such a cell therapy approach.

Keynote: Data Aggregation and Disease Modeling to Accelerate Rare Disease Drug Development
Jane Larkindale¹

¹*PepGen*

Despite promising new technologies to treat rare diseases such as oligonucleotide and gene therapies, 90% of rare diseases remain without an approved treatment. Progress toward the development of therapies to treat these diseases is hampered by a limited understanding of the course of each rare disease, how changes in disease progression occur and can be effectively measured over time, and challenges in designing and running clinical trials in diseases where the natural history is poorly characterized. Data that could be used to characterize the natural history of each disease are frequently in different places and have been collected in various ways, making use of data to inform future studies challenging. The Rare Disease Cures Accelerator – Data and Analytics Platform (RDCA-DAP) is an FDA-funded effort to aggregate data across all rare diseases and make that data available to the community to support understanding of rare disease natural history and inform drug development. RDCA-DAP curates, standardizes, and tags data across rare disease datasets to make them findable within the database, and contains a built-in analytics platform to help visualize, interpret, and use data to support drug development. Examples of use of such aggregated data to develop disease progression models of Duchenne muscular dystrophy, and how such models can be used in drug development, will be discussed.

S5.501**Clinical trial readiness to solve barriers to drug development in FSHD (ReSolve): baseline characteristics**

Jeffrey Statland¹, Kate Eichinger², Michael McDermott², Kiley Higgs¹, Michaela Walker¹, Doris Leung³, Sabrina Sacconi⁴, Karlien Mul⁵, Valeria Sansone⁶, Elena Carraro⁶, Leo Wang⁷, Perry Shieh⁸, Bakri Elsheikh⁹, Samantha LoRusso⁹, Russell Butterfield¹⁰, Nicholas Johnson¹¹, Rabi Tawil², the ReSolve Investigators of the FSHD CTRN¹

¹University of Kansas Medical Center

²University of Rochester Medical Center

³Kennedy Krieger Institute

⁴Nice University

⁵Radboud University

⁶The NEMO Clinical Center

⁷University of Washington

⁸University of California, Los Angeles

⁹The Ohio State University

¹⁰University of Utah

¹¹Virginia Commonwealth University

Background: As targeted drugs move into human trials for FSHD, validating clinical trial tools is essential to facilitate the drug development process.

Methods: A 24-month longitudinal study in genetically confirmed and clinically affected FSHD participants in the FSHD Clinical Trial Research Network (11 sites). Visits occur at baseline and months 3, 12, 18, and 24. At each visit we collected: a novel FSHD functional composite (FSHD-COM) made up of 18 evaluator-administered motor tasks; Domain 1 of the Motor Function Measure (MFMD1); clinical severity (0=unaffected, 10=non ambulatory); and strength measured by quantitative myometry (14 muscles) and manual muscle testing (32 muscles). Here we present baseline characteristics and test-retest reliability of the functional composites using intraclass correlation coefficients (ICC).

Results: A total of 240 FSHD participants were enrolled: 45% female, spanned the adult age (median 53 years, range 19-75 years), severity range (median 6, range 1-9), and the genetic spectrum (median D4Z4 repeats 6, range 2-10). The median age at symptom onset was 20 years, with a mean diagnostic delay of 10 years, and women became symptomatic 4.9 years later than men (P=0.02). The median forced vital capacity (FVC) was 90% predicted (range 34-143), with 26% having an FVC < 80% predicted. The test-retest reliability of the functional composites was excellent, with the FSHD-COM having an ICC of 0.98 (95% lower confidence bound [LCB] 0.97) and the MFMD1 0.97 (LCB 0.96).

Conclusion: ReSolve will allow evaluation of inclusion criteria and power and sample size calculations by determining FSHD progression rates as measured by standard COAs.

S5.502

Muscle ultrasound as imaging biomarker in facioscapulohumeral muscular dystrophy: possibilities and pitfalls

Sjan Teeselink¹, Sanne Vincenten¹, Nicol Voermans², Baziel van Engelen², Karlien Mul², Nens van Alfen¹

¹*Radboud University Medical Center*

²*Radboud University*

Objective: To assess the potential of muscle ultrasound as imaging biomarker in patients with FSHD.

Methods: Genetically confirmed FSHD patients of 18 years or older were included. Muscle ultrasound was conducted bilaterally using a standardized protocol of the following muscles: trapezius, biceps brachii, rectus abdominis, rectus femoris, vastus lateralis, tibialis anterior, and the medial head of the gastrocnemius muscle. Echogenicity z-scores and muscle thickness z-scores were calculated, and for every image the Heckmatt score was determined. Muscle ultrasound outcomes were correlated with multiple clinical outcome measures.

Results: A total of 115 patients were included (52% male, age 22-80 years). The trapezius muscle was most often affected, followed by the rectus femoris. Both the compound z-score and the compound Heckmatt score correlated strongly with the FSHD-CS, Ricci score, MRC sum score and MFM (CC between 0.71-0.85, $p < 0.001$). Separate MRC scores correlated moderately to highly with the echogenicity z-scores or Heckmatt scores of the corresponding muscles (CC between -0.36 - -0.76, $p < 0.001$). In asymptomatic patients ($n=7$) 50% of the trapezius muscles had an echogenicity z-score > 2.0 . In non-penetrant patients ($n=5$) we found an echogenicity z-score > 2.0 in 40% of the vastus lateralis muscles.

Discussion: Muscle ultrasound outcomes correlate strongly with clinical outcome measures, and can detect changes in echogenicity in asymptomatic and non-penetrant patients.

S5.503

Diagnostic MRI biomarkers for FSHD identified by machine learning

Mauro Monforte¹, Sara Bortolani¹, Eleonora Torchia¹, Lara Cristiano², Francesco Laschena², Tommaso Tartaglione², Enzo Ricci³, Giorgio Tasca¹

¹Fondazione Policlinico Universitario A. Gemelli IRCCS

²Dipartimento di Radiologia, IDI IRCCS

³Università Cattolica del Sacro Cuore

Background: The diagnosis of FSHD can be challenging in patients not displaying the classical phenotype or with atypical clinical features. Despite the identification by MRI of selective patterns of muscle involvement, their specificity and added diagnostic value are unknown

Methods: We aimed to identify the radiological features more useful to distinguish FSHD from other myopathies and test the diagnostic accuracy of MRI. A retrospective cohort of 295 patients (187 FSHD, 108 non-FSHD) studied by upper- and lower-limb muscle MRI was analyzed. Scans were evaluated for the presence of 15 radiological features. A random forest machine learning algorithm was used to identify the most relevant features for FSHD diagnosis, combine them in patterns, and test their diagnostic accuracy

Results: Trapezius involvement and bilateral subscapularis muscle sparing achieved the best diagnostic accuracy (0.89, 95% CI [0.85–0.92]) with 0.90 (0.85–0.94) sensitivity and 0.88 (0.80–0.93) specificity. This pattern correctly identified 91% atypical FSHD patients of our cohort. The combination of trapezius involvement, bilateral subscapularis and iliopsoas sparing, and asymmetric involvement of upper- and lower-limb muscles was pathognomonic for FSHD, yielding a specificity of 0.99 (0.95–1.00).

Conclusions: We identified MRI patterns that showed a high diagnostic power in promptly discriminating FSHD from other muscle disorders, with comparable performance irrespective of typical or atypical clinical features.

S5.504**Reachable workspace to evaluate efficacy of losmapimod in FSHD in two Phase 2 studies**

Christopher Morabito¹, Jay Han², Anthony Accorsi¹, Jordi Diaz³, Miriam Freimer⁴, Angela Genge⁵, Summer Gibson⁶, Nuria Gomez⁷, Namita Goyal², Johanna Hamel⁸, Lawrence Hayward⁹, John Jiang¹, Nicholas Johnson¹⁰, Joost Kools¹¹, David Reyes Leiva³, Doris Leung¹², Hanns Lochmuller¹³, Samantha Lorusso⁴, Michelle L. Mellion¹, Alan Pestronak¹⁴, L. Alejandro Rojas¹, Sabrina Sacconi¹⁵, Perry Shieh¹⁶, Jennifer Shoskes¹, Jeffrey Statland¹⁷, S.H. Subramony¹⁸, Rabi Tawil⁸, Baziel van Engelen¹⁹, Juan Vilchez²⁰, Kathryn Wagner¹², Leo Wang²¹

¹*Fulcrum Therapeutics, Inc.*

²*University of California, Irvine*

³*Hospital de la Santa Cruz, Barcelona, Spain*

⁴*Ohio State University Medical Center*

⁵*McGill University*

⁶*University of Utah*

⁷*Neuromuscular Diseases Unit, Neurology Department, Hospital Universitari I Politècnic La Fe*

⁸*University of Rochester Medical Center*

⁹*University of Massachusetts Medical School*

¹⁰*Virginia Commonwealth University*

¹¹*Radboud University Medical Center*

¹²*Kennedy Krieger Institute*

¹³*Ottawa Hospital Research Institute*

¹⁴*Washington University*

¹⁵*Nice University*

¹⁶*University of California, Los Angeles*

¹⁷*University of Kansas Medical Center*

¹⁸*University of Florida*

¹⁹*Radboud University*

²⁰*Neuromuscular and Ataxias Research Group, Instituto de Investigación Sanitaria La Fe*

²¹*University of Washington*

Aim: Assess efficacy of losmapimod to slow or stop disease progression with Reachable Workspace (RWS).

Background: RWS is a 3D assessment of function that measures upper extremity mobility. Fulcrum is developing losmapimod (a small molecule inhibitor of p38 α/β MAPK) to treat FSHD.

Methods: Eighty subjects (N=40/group) age 18-65 yrs with FSHD1, CSS 2-4, were randomized 1:1 losmapimod 15 mg BID: placebo in ReDUX4. Single-site open-label study (OLS) evaluated efficacy in 14 subjects on losmapimod for 52 weeks with same inclusion. RWS was performed with and without 500g wrist weights in dominant (D) and non-dominant (ND) arm. Assessment includes total relative surface area (RSA) of 5 domains measured on a 0-1.25 scale representing frontal and posterior inferior reachable area, each domain measuring .25.

Results: In ReDUX4, losmapimod resulted in significant clinical improvements in total RSA, losmapimod vs. placebo D: .019 vs. -.048; dif.067 p=.01; ND: .021 vs. -.024, dif.045 p<.05. Placebo subjects lost 2.6%-3.6% total RSA without weights and 1.9%-3.8% with weights. In OLS, improvements were measured bilaterally; range .03-.04. In ReDUX4, total RSA annualized rate of change (%) in losmapimod vs. placebo D: -0.44 vs. -8.42; p=.07; ND: 4.88 vs. -4.02; p=.01 and OLS range 3.28-5.68. RSA by domain showed improvements/no worsening in losmapimod, particularly in Q1 and Q3 (above shoulder) and Q5 (posterior inferior).

Conclusion: RWS is a clinically meaningful upper extremity assessment of function that can be used to assess disease progression and treatment efficacy accurately. RWS demonstrated that losmapimod significantly preserves or improves function.

POSTER ABSTRACTS

P1.01

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

Adam Bittel¹, Surajit Bhattacharya¹, **Yi-Wen Chen**^{1,2}

¹*Children's National Hospital*

²*George Washington University*

Background: FSHD myoblasts have significant delays in plasmalemmal repair, which may negatively alter the skeletal muscle transcriptome and exacerbate FSHD pathology.

Approach: Immortalized FSHD and healthy myoblasts were collected before (baseline), and at 6-hr, and 24-hr after scrape injury for single cell sequencing. Early (6-hr) and late (24-hr) "injury response" genes (IRGs) were defined as the genes that are differentially expressed in healthy cells at 6-hr vs. baseline, and 24-hr vs. baseline, respectively. We compared the expression of IRGs (log₂ Fold Change (log₂FC) relative to baseline), and their associated molecular pathways (Gene Ontologies), in FSHD and healthy myoblasts to identify similar and dissimilar transcriptional responses to injury.

Results & Conclusions: We found >2,000 early and >1,700 late IRGs in healthy cells (FDR p<0.05). Also, 1/4 of IRGs were differentially expressed in FSHD myoblasts, with ~80% showing consistent directional log₂FC with healthy myoblasts (p<0.001). We also identified groups of mis-regulated IRGs post-injury in FSHD cells, defined as those IRGs with opposite log₂FC in FSHD vs. healthy myoblasts at the same timepoint. At 6-hrs, these mis-expressed IRGs were associated with cell signaling regulation and cell death. At 24-hrs, they were linked to reductions in cell cycle, regulation of gene expression, and DNA damage signaling. Additionally, 25% of early and 15% of late mis-expressed IRGs in FSHD myoblasts are known downstream targets of DUX4. These data provide molecular insights on the differential responses to membrane injury in FSHD myoblasts, which may contribute to disease progression downstream.

P1.02**Single-nucleus RNA-Seq reveals cellular heterogeneity in facioscapulohumeral muscular dystrophy in late myogenic stage**

Dongxu Zheng¹, Anita van den Heuvel¹, Ahmed Mahfouz^{1,2}, Annelot Wondergem¹, Susan Kloet¹, Judit Balog¹, Baziel van Engelen³, Rabi Tawil⁴, Stephen Tapscott⁵, Silvère Van der Maarel¹

¹*Leiden University Medical Center*

²*Delft University of Technology*

³*Radboud University*

⁴*University of Rochester Medical Center*

⁵*Fred Hutchinson Cancer Research Center*

Misexpression of the transcription factor DUX4 is the critical disease-initiating event in facioscapulohumeral dystrophy (FSHD). Yet, DUX4 expression in FSHD muscle is sporadic: Only ~1/200 patient-derived myonuclei have detectable levels of DUX4 protein. Consistent with a sporadic expression pattern, bulk RNA sequencing analyses detect DUX4 or DUX4-target gene expression in only ~60% of FSHD muscle biopsies. Until now, it remained unclear why only a few nuclei start to express DUX4 and how this sporadic expression resulted in severe muscle wasting in patients. We applied single-nucleus RNA-sequencing on patient-derived differentiated muscle cells to study FSHD disease etiology and cellular progression. Focusing specifically on late-myogenic nuclei allowed us to capture more DUX4-(target)-positive nuclei in FSHD samples than previously reported, providing a better resolution of the transcriptional changes associated with DUX4 expression. Systemic comparison of FSHD and control transcriptional profiles revealed both global and DUX4-specific changes, and combined with pseudotime trajectory modeling enabled us to identify two distinct FSHD-associated transcriptional profiles, each showing partially overlapping but distinct DUX4-target activation and distinct downstream FSHD-associated responses. Identifying two separate DUX4-induced responses may guide future therapeutic strategy design by improving our understanding and predictions of the therapeutic potential of treatment.

P1.03**Generation of a craniofacial muscle model of FSHD from iPSCs**Dongsheng Guo¹, Oliver King¹, **Lawrence Hayward¹**, Charles P. Emerson Jr.¹¹*UMass Chan Medical School*

FSHD pathology includes weakness of pharyngeal arch-derived facial muscles and somite-derived muscles. Facial and somitic muscle development are controlled by different transcription factors: PITX2 for facial muscles, and PAX3 and PAX7 for somitic muscles, raising the possibility that the DUX4 is regulated and functions differently in facial and somitic muscles, and might be responsive to different therapeutics. Our goal is to develop a craniofacial muscle model of FSHD for investigations of disease mechanisms and therapeutic development. Our approach is to differentiate FSHD iPSCs into craniofacial muscles by mimicking in vivo craniofacial muscle development. qPCR assays show that craniofacial muscles differentiated from iPSCs express PITX2, TCF21, and MYOR, the hallmarks of craniofacial muscles, but the expression of DUX4 and its target genes were repressed, as we have reported for iPSC differentiated somitic muscles, but not for iPSC iMyoblasts isolated by reserve cell selection (Guo et al., 2022). We are utilizing reserve cell selection approaches to isolate craniofacial iMyoblasts for comparative investigations of the epigenetic and differentiation-specific regulation of DUX4 and its transcriptional target genes in craniofacial and somitic iMyoblast lineages. The craniofacial myogenesis iPSC protocol provides a novel model for cell and molecular investigations of FSHD pathology in craniofacial muscle.

Supported by the UMass Wellstone Center and Friends of FSH Research.

P1.04**The inflammatory muscle phenotype of uninduced ACTA1-MCM;FLEXD mice**Jessica de Greef¹, Linde Bouwman¹, Bianca den Hamer¹, Silvère Van der Maarel¹¹*Leiden University Medical Center*

Several studies have reported on the presence of inflammatory cells as a common histological feature in skeletal muscles of individuals with facioscapulohumeral dystrophy (FSHD). Additionally, inflammation seems an important factor in FSHD disease progression. We performed a natural history study of the tamoxifen-inducible bi-transgenic ACTA1-MCM;FLEXD mouse model to determine whether this model can be used for therapeutic studies targeting inflammation in FSHD. We performed extensive histopathology and RT-qPCR analyses on different muscles of 10-week, 20-week, and 40-week uninduced ACTA1-MCM;FLEXD mice. We also performed flow cytometry to establish which immune cell populations infiltrate the muscles of these mice. Even without tamoxifen treatment, ACTA1-MCM;FLEXD mice present with DUX4 expression and develop a progressive muscle phenotype characterized by fibrosis, immune cell infiltration, myofibers with central nuclei, and smaller myofibers. The muscles of uninduced ACTA1-MCM;FLEXD mice express complement, cytokine, and chemokine genes, and the expression of many of these genes increases with age. The immune cell populations in muscles of uninduced ACTA1-MCM;FLEXD mice mainly consist of macrophages with a pro-inflammatory phenotype and T cells. Collectively, we show that the inflammatory muscle phenotype of uninduced ACTA1-MCM;FLEXD mice matches what has been found in patient biopsies and we conclude that this model is suitable for anti-inflammatory drug studies for FSHD.

P1.05**Towards a muscle-targeted delivery of antisense agents against DUX4**

Maëlle Limpens¹, Aline Derenne¹, Carmen Burtea¹, Alexandre Legrand¹, Anne-Emilie Decleves¹, Frédérique Coppée¹, Alexandra Tassin¹

¹*University of Mons*

In a therapeutic goal, antisense oligonucleotides (ASOs) directed against DUX4 mRNAs have been developed at UMONS. However, in the field of muscle disorders, the use of ASOs is currently limited because of their restricted tissue delivery, lack of tissue selectivity, and rapid clearance after systemic delivery. Using two phage-display library screenings (against either #1 myotubes or #2 a Muscle-Membrane Protein [MMP]), we selected peptides able to specifically bind to human and mouse muscle surface proteins (MSPep). The 4 most promising phage clones were sequenced, and the encoded peptides were synthesized with Rhodamine conjugation for in vitro testing.

Our results showed a more efficient endocytosis of MSPep selected against myotubes in muscle cells compared to hepatocytes and renal cells. MSPep were also partially co-localized with lysosomes and early endosomes. We also observed an efficient endocytosis of MSPep in myotubes and in non-muscle cells transfected with a plasmid encoding MMP, but not in non-transfected cells (negative controls). Finally, we observed a strong co-localization of MSPep with the targeted MMP.

In conclusion, to develop a muscle-targeted therapeutic approach for FSHD, we defined 4 promising peptides (MSPep) selected for their ability to specifically bind to skeletal muscle surface proteins. Next experiments will aim to evaluate MSPep-ASO ability to target skeletal muscle and deliver ASO efficiently both in vitro, ex vivo, and in vivo.

P1.07**p38-independent DUX4 regulatory mechanisms in FSHD myotubes****Rajanikanth Vangipurapu¹**, Francis M. Sverdrup¹¹*Saint Louis University*

Facioscapulohumeral muscular dystrophy (FSHD) is a skeletal muscle disorder caused by the de-repression of D4Z4 subunits at 4q35 leading to aberrant DUX4 expression. DUX4 expression has been associated with activation of pathways toxic to muscle tissue leading to muscle cell death. In the current study, we attempted to dissect the molecular basis of DUX4 suppression mediated by p38 MAPK inhibition. To probe the influence of p38 MAPK on DUX4 regulation during early and late myogenic events, we used genetic and pharmacological approaches. Genetic knockout p38 α and p38 β using CRISPR-Cas9 or treatment with p38 α/β inhibitor losmapimod significantly suppressed DUX4 and its target gene mRNA levels during the early myogenesis in vitro, confirming previous observations. However, at later time points, neither genetic nor pharmacological inhibition of p38 α/β showed significant inhibition of DUX4 expression, as there is persistent low-level DUX4 expression in knockout/drug treated cells. In xenograft studies, FSHD cells with p38 α/β double knockout showed persistent low-level DUX4 target gene expression throughout myogenesis. In summary, our results suggest that while p38 α/β MAPK signaling promotes robust DUX4 expression induced early during myogenesis, at later time points there is persistent lower-level expression that is p38-independent. To fully understand the therapeutic potential of p38 inhibition in FSHD, it is necessary to determine the mechanism of p38-independent DUX4 expression and its effect on myogenic differentiation and muscle health.

P1.08

SLC34A2 as a protein biomarker of FSHD

Maria Traficante¹, Andrea O'Neill¹, Ujwala Pimparkar¹, Rabi Tawil², Jeffrey Statland³, Robert Bloch¹

¹*University of Maryland Baltimore*

²*University of Rochester Medical Center*

³*University of Kansas Medical Center*

Protein biomarkers linked to FSHD have the potential to be used to monitor disease progression and the efficacy of treatments currently in development. Our laboratory is investigating the protein SLC34A2 as a potential FSHD biomarker. SLC34A2 is a DUX4 target gene, and in healthy epithelia it functions in sodium-dependent phosphate uptake in cells. 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). We show here that intact, glycosylated SLC34A2 protein is significantly increased in serum from FSHD patients, and can be detected by several different antibodies made in mice and in rabbits. These results suggest that SLC34A2 may be useful as an FSHD serum biomarker.

P1.09**Dynamic proteome profiling of myoblasts from FSHD patients and their unaffected siblings**Jatin Burniston¹, Radoš Stefanović¹, Adam Bittel², Yi-Wen Chen²¹*Liverpool John Moores University*²*Children's National Hospital*

We used stable isotope (deuterium oxide; D2O) labelling and peptide mass spectrometry to investigate the abundance and turnover rates of proteins in cultured muscle cells from 2 FSHD patients and their unaffected siblings (UASb). Our analysis encompassed 4,485 proteins and highlighted 41 significant ($P < 0.01$) differences in abundance between FSHD and UASb groups. FSHD cells were enriched in inhibitors of muscle development including LIM and cysteine rich domains protein 1 (2.4-fold) and TGF- β receptor type 2 (2.2-fold), whereas heterogeneous and small nuclear ribonucleoproteins were significantly less abundant in FSHD myoblasts. Average protein turnover was not different between groups, but there were differences in protein-specific turnover rates. In growth media, 12 proteins exhibited differences ($P < 0.01$); e.g., the turnover of protein kinase MRCKA was 13-fold less in FSHD, whereas the turnover of laminin β -1 was 2.9-fold greater compared to UASb cells. Under differentiation conditions, 13 proteins exhibited differences ($P < 0.01$) in turnover rate. FSHD myoblasts had a 14-fold greater turnover of FK506-binding protein 15 and a 2.7-fold greater turnover of ATP-dependent RNA helicase A, whereas the turnover of COP9 signalosome complex subunit 4 was 4.9-fold less, and defender against cell death 1 (DAD1) was 14-fold less in FSHD myoblasts. These data represent the first large-scale study of proteostasis in FSHD, and provide new insight and a resource for exploring new therapeutic targets.

P1.10

Promoting clinical trial readiness to Brazil

Fabio Eliezer Figueiredo¹, Cristiane A Martins Moreno²

¹*Abrafeu Assoc Bras Facio Escapulo Umeral*

²*University of São Paulo*

The lack of genetic testing and a patient registry in Brazil has been a major obstacle to improving care and trial readiness. In response, ABRAFEU has established a nationwide network of 18 neuromuscular clinicians, who are providing genetic testing to Brazilian patients in collaboration with the Peter Jones laboratory at the University of Nevada. In addition, ABRAFEU is establishing a mobile phone-based national registry database. We have also initiated a collaboration between Radboud University and Institute Vita to train physical therapists in Brazil on FSHD clinical assessments. As of July 2019, we have engaged 300 active patients of an average age of 42, 56% female and 44% male.

P1.11

Transient DUX4 expression leads to muscle degeneration

David Oyler¹, Ana Mitanoska¹, Ahmed Shams¹, Natasha Santos¹, Natalie Xu¹, Jasmine Gulik¹, MacKenzie Molina¹, Erik Toso¹, Michael Kyba¹, **Darko Bosnakovski**¹

¹*Department of Pediatrics, Lillehei Heart Institute, University of Minnesota*

DUX4 transient expression is one of the possible explanations for the lack of DUX4 protein in FSHD muscle biopsies. We tested the effect of DUX4 transient expression on muscle homeostasis in the iDUX4pA-HSA mouse. We found that muscle histology does substantially recover after DUX4 expression, with recovery correlating inversely with the duration of prior DUX4 expression. However, despite fairly normal muscle histology post-recovery, the levels of fibroadipogenic cells in the muscle do not return to their normal levels even after five months post-transient DUX4 induction. Furthermore, muscles that experience a burst of DUX4 have an impaired regeneration ability and are prone to fibrosis. Here we will present our findings of profibrotic perturbation in the muscle caused by transient DUX4 expression in the FSHD mouse model.

P2.12**Generation of human skeletal myocyte models harboring SMCHD1 and/or D4Z4 mutations reveals critical roles of epigenetic modifiers for stable FSHD phenotype**Nam Nguyen¹, Xiangduo Kong¹¹*University of California Irvine*

FSHD is primarily linked to mono-allelic deletion of D4Z4 repeat sequences at the subtelomeric region of chromosome 4q (FSHD1), while ~5% of cases exhibit no D4Z4 repeat contraction (FSHD2). Mutations in the SMCHD1 gene were linked to FSHD2, and also exacerbate the phenotype of FSHD1. Because D4Z4 repeats are primate-specific and not all DUX4 target genes are present in the mouse genome, it has not been straightforward to study the mechanism of the disease in genetically defined model organisms. Thus, we generated isogenic human skeletal myoblast clones carrying single or double D4Z4 and SMCHD1 mutations using CRISPR-Cas9. D4Z4 mutations were further characterized using nanopore long-read sequencing. We found highly synergistic effects of double mutations on DUX4 target gene induction. Despite prominent D4Z4 DNA hypomethylation associated with FSHD2, SMCHD1 mutation affected H3K9me3 rather than DNA methylation at D4Z4, and additional forced DNA hypomethylation significantly stimulated DUX4 target gene expression, suggesting the possible involvement of additional epigenetic modifier(s) in FSHD2. We also found that H3.X/H3.Y encoding related histone variants are differentiation-independent early DUX4 target genes necessary for the expression of LEUTX, a differentiation-dependent late DUX4 target transcription factor, which in turn stimulates H3.X/Y expression, indicative of two-step and feedback loop mechanisms of the DUX4-activated gene program. Taken together, our results indicate the critical roles of epigenetic modifiers in the establishment of the FSHD phenotype, and that these mutant cells provide important insights into the mechanism of FSHD pathogenesis.

P2.13**Methylation analysis of proximal region on D4Z4 repeats in FSHD1 patients compared to healthy individuals**Ceren Hangul¹, Öznur Tokta¹, Sibel Berker Karauzum¹, Hilmi Uysal¹, Filiz Koc²¹Akdeniz University²Cukurova University

Molecular infrastructure of FSHD consists of genetic and epigenetic components. The main genetic mutation is the shortening of the D4Z4 macrosatellite repeat sequences located on the 4q35 region. Epigenetic changes associated with the disease can occur in many different layers. For example, epigenetic components may include changes related to non-coding RNAs such as miRNA and lncRNA, as well as histone modification and DNA methylation changes. Some of the methylation changes in the D4Z4 region have been associated with mutations in proteins involved in methylation such as SMCHD1, DNMT3B, and LRIF1.

With the studies examining methylation changes in detail, it had been revealed that FSHD patients have more hypomethylation at the D4Z4 region compared to healthy individuals. When D4Z4 divided into three main parts as 5' - mid - 3', it had been shown that 5' region was the most significantly hypomethylated part of D4Z4 sequence compared to control individuals. To be able to test the same epigenetic change, we analysed 5' region of D4Z4 repeats in 31 FSHD patients that had been followed by our study group. All of these 31 FSHD1 patients had molecular diagnosis with less than 10 D4Z4 repeat units. Twenty-nine control individuals were also investigated for the methylation status of 5' region. The bisulfite sequencing method had been used to discriminate methylated and unmethylated CpGs. As a result, we revealed that 5' region was significantly hypomethylated ($p=0.016$) in FSHD patients compared to healthy individuals. These results are compatible with hypomethylation of 5' region in literature and support the epigenetic component in the molecular pathogenesis.

P2.14**Cis D4Z4 repeat duplications in FSHD**

Richard Lemmers¹, Patrick van der Vliet¹, Jan de Bleecker², Ludo van der Pol³, Corrie van Erasmus⁴, Marc D'Hooghe⁵, Peter van den Bergh⁶, Baziel van Engelen¹¹, Jeffrey Statland⁷, Rabi Tawil⁸, Nicol Voermans¹¹, Sabrina Sacconi⁹, John Vissing¹⁰, Silvère van der Maarel¹

¹*Leiden University Medical Center*

²*UZ Gent*

³*WKZ Utrecht*

⁴*Amalia kindziekenhuis Radboudumc*

⁵*Algemeen Ziekenhuis*

⁶*Saint-Luc UCL*

⁷*University of Kansas Medical Center*

⁸*University of Rochester Medical Center*

⁹*Nice University*

¹⁰*University of Copenhagen, Denmark*

¹¹*Radboud University*

FSHD1 and FSHD2 are associated with somatic D4Z4 repeat chromatin relaxation and DUX4 de-repression on 4qA chromosomes. The D4Z4 repeat is usually organized in a single repeat and ranges between 9-100 units in control individuals. In FSHD1, the D4Z4 repeat varies between 1-10 units, and in FSHD2 it usually is between 8-20 units. In some patients with a clinical diagnosis of FSHD, the genetic cause has not yet been identified.

About 2% of individuals in the population carry a cis duplication of the D4Z4 repeat, a situation in which a long and short D4Z4 repeat are found adjacent to each other with a spacer sequence in between. Recently we reported that such duplication alleles can result in FSHD, but only in combination with D4Z4 hypomethylation caused by pathogenic variants in SMCHD1 (FSHD2). A French study, however, identified a duplication allele in two FSHD patients without pathogenic variants in SMCHD1, warranting further evaluation of duplication alleles.

Here we studied phenotypic FSHD patients with unknown genetic etiology and identified 10 families in which FSHD was associated with a cis duplication allele without D4Z4 hypomethylation. In most cases the size of the distal D4Z4 repeat was between 1-6 units. We also identified a patient in which FSHD was caused by a de novo contraction of the distal D4Z4 repeat. By comparing the repeat composition of duplication alleles in this new group of FSHD patients with those identified in controls and in FSHD2 patients, we found evidence for a specific sizing pattern which likely explains why some duplication alleles are immediately susceptible to FSHD, while others require hypomethylation to become pathogenic.

P2.15**Maternal SMCHD1/LRIF1 haploinsufficiency triggers homeotic transformations in genetically wild-type offspring**Shifeng Xue¹, Frederique Magdinier², Bruno Reversade³¹*National University of Singapore*²*Aix-Marseille University*³*Genome Institute of Singapore, A*STAR*

Loss-of-function mutations in SMCHD1 and LRIF1 cause facioscapulohumeral dystrophy type 2 (FSHD2). SMCHD1 is an epigenetic repressor that is maternally deposited into the oocyte. As it is required for X-inactivation in mammals, Smchd1 KO female mice are embryonic lethal, and the role of maternal SMCHD1 is unknown. Here we report that SMCHD1 is maternally required for timely HOX expression. Using zebrafish and mouse Smchd1 knockout animals, we demonstrate that Smchd1 haploinsufficiency brings about precocious hox transcription during oogenesis and embryogenesis. Unexpectedly, wild-type offspring born to SMCHD1^{+/-} zebrafish mothers also exhibited vertebrate patterning defects. The loss of Smchd1 was accompanied by aberrant DNA methylation on hox genes. We further show that this regulation is mediated by Lrif1, an interacting partner of Smchd1, whose knockout in zebrafish phenocopies that of Smchd1. Rather than being a short-lived maternal effect, HOX mis-regulation is stably inherited through cell divisions and persists in cultured fibroblasts derived from FSHD2 patients haploinsufficient for SMCHD1. We conclude that maternal Smchd1/Lrif1 sets up an epigenetic state in HOX loci that can only be reset in the germline. This has potential implications for the genetically normal children of FSHD2 women. This unusual inter-generational inheritance, whereby a phenotype can be a generation away from its genotype, casts a new light on how unresolved Mendelian diseases may be interpreted.

P3.16

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

Yosuke Hiramuki¹, Ichizo Nishino², Hiroyuki Kugoh¹, Yasuhiro Kazuki¹

¹*Chromosome Engineering Research Center, Tottori University*

²*National Center of Neurology and Psychiatry*

DUX4 is abnormally expressed in skeletal muscle of facioscapulohumeral muscular dystrophy (FSHD). The DUX4 gene is conserved in primates and located in D4Z4 macrosatellite repeats on chromosome 4q35. Currently, there is no FSHD mouse model that exhibits the pathology observed in FSHD patients using DUX4 expression under the control of the regulatory elements. Here, we aim to generate a novel FSHD1 mouse model that carries a total of 5 Mb of the human chromosome 4q35 region from an FSHD1 patient, extending from upstream of the ANT1 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, and transfer the MAC containing the 5 Mb chromosome 4q35 (chr4q35-MAC) into mouse embryonic stem cells through microcell mediated chromosome transfer. For isolation of the genomic regions, we selected one FSHD1 patient with 1 D4Z4 repeat unit who showed the expression of DUX4 and severe pathological phenotypes such as infiltration of inflammatory cells, heterogeneous myofiber size, and fibrosis in skeletal muscle. We will present our current progress regarding the construction of the chr4q35-MAC.

P3.17**Transcriptomic analysis of inflamed and non-inflamed FSHD muscle, together with peripheral blood mononucleated cells, reveals a circulating biomarker of clinical severity in FSHD****Christopher Banerji**¹, Anna Greco², Leo Joosten², Baziel van Engelen³, Peter Zammit¹¹*Randall Centre for Cellular and Molecular Biophysics, King's College London*²*Radboud University Medical Center*³*Radboud University*

Clinical trials for FSHD are hindered by heterogenous biomarkers, which show poor association with clinical severity and require invasive muscle biopsy. FSHD presents as a slow fatty replacement of muscle, accelerated by inflammation. Mis-expression of DUX4 underlies pathogenesis, but parallel mechanisms such as PAX7 target repression have been proposed. We perform RNA-seq on biopsies from MRI guided inflamed (TIRM+) and non-inflamed (TIRM-) muscle from the same clinically characterised FSHD patients, alongside paired peripheral blood mononucleated cells (PBMCs). PAX7 target gene repression discriminates control, inflamed, and non-inflamed FSHD muscle, while discriminatory power of DUX4 target genes is limited to distinguishing control from inflamed FSHD muscle. Importantly, PAX7 target gene repression in TIRM- muscle associates with Ricci and Lamperti clinical assessments of patients, and with disease duration in TIRM+ muscle. DUX4 target gene biomarkers associate with lower limb fat fraction and D4Z4 repeat length, but not clinical assessment. Lastly, PAX7 target gene repression in muscle correlates with the level in matched PBMCs, and a refined PAX7 target gene biomarker (FSHD muscle-blood biomarker) computed in PBMCs associates with Ricci and Lamperti assessments of FSHD patients. Our circulating biomarker validates as a classifier of clinical severity in an independent data set of 54 FSHD patient blood samples, with improved power in older patients.

P3.18**Relationship between DUX4 and Hypoxia-Inducible Factor (HIF1 α) in human and murine muscle cells in vitro and in vivo**

Thuy Hang Nguyen¹, Alexandre Legrand¹, Anne-Emilie Decleves¹, Philipp Heher², Alexandra Belayew¹, Christopher Banerji², Peter Zammit², Alexandra Tassin¹

¹*University of Mons*

²*Randall Centre for Cellular and Molecular Biophysics, King's College London*

Studies examining FSHD pathogenesis revealed pathways involved in hypoxic response and oxidative stress to be disturbed (e.g., Heher et al., 2022), with HIF1 α being of particular interest (reviewed in Nguyen et al., 2021). To decipher mechanisms underlying aberrant DUX4-HIF1 α crosstalk, we used DUX4-inducible myoblasts (MB). In proliferating human and murine MB expressing DUX4, HIF1 α and its direct target PDK1 are downregulated. In human myocytes, HIF1 α and target gene expression are not modified by DUX4, but PDK1 protein level is decreased. In human myotubes (MT), DUX4 induces the HIF1 α pathway. Electroporation of a DUX4 expression vector into mouse muscle (Derenne et al., 2020) shows a transient increase of Hif1 α expression at day 1. Hif1 α was no longer upregulated at day 7, but muscle lesions are present. Since DUX4 (i) alters myogenesis in vitro leading to hypotrophic MT and (ii) induces the HIF1 pathway in human MT/mouse muscles, we investigated the effects of sustained HIF1 α activation on myogenic differentiation. Hypoxia enhanced myogenic differentiation in human MB. Consistent results were obtained in HIF1 α gain/loss-of-function studies showing that this effect is HIF1 α -dependent. Hypoxia enhanced MB fusion into multinucleated MT, but our results suggest that this is HIF1 α -independent. In conclusion, the HIF1 α pathway is modulated by DUX4, but changes depend on the differentiation stage of muscle cells. As well, HIF1 α sustained activation influences early myogenic differentiation.

P3.19**Fibro-adipogenic progenitors and the progression of the FSHD myopathy**Carlo Serra¹, Kathryn Wagner², Thomas Lloyd¹¹*Department of Neurology and Neuroscience, Johns Hopkins University*²*Center for Genetic Muscle Disorders, The Kennedy Krieger Institute*

Understanding the mechanism of the accumulation of fat and fibrotic tissue in the FSHD muscle will be beneficial to set up new therapies for this disease. Fibro-adipogenic progenitors (FAPs) can differentiate into fibroblasts and adipocytes upon chronic muscle regeneration. Exosomes are extracellular vesicles important in cell-to-cell communication and play a major role in muscle physiology. We hypothesized that the presence of DUX4 reduces the release from the muscle fibers of FAPs-targeted anti-adipogenic and anti-fibrogenic exosomes, a change that may compromise the physiologic equilibrium governing FAPs fate. We have developed the triple transgenic FLExDUX4-/+;ACTA-Cre-/+;hCD63-6xHis-GFP-/+ (TTG) mice, a mouse model in which the expression of DUX4 is coupled to the production of exosomes tagged with the human CD63 protein plus a histidine tag and a green fluorescent protein (GFP), in a tamoxifen (TMX)-inducible and muscle fiber-specific manner. A preliminary evaluation conducted in TTG mice treated with TMX at long term showed that the continuous expression of DUX4 results in a considerable reduction of the myofibers' content of GFP+ endosomal vesicles and exosomes vs. that shown by the ACTA-Cre-/+;hCD63-6xHis-GFP-/+ control mice treated with TMX. We are currently investigating the tissue distribution of the GFP+ exosomes, as well as their content, in TTG and control mice upon TMX treatment.

P3.20**DUX4, nucleolar stress, and FSHD myopathy**Carlo Serra¹, Kathryn Wagner², Thomas Lloyd¹¹*Department of Neurology and Neuroscience, Johns Hopkins University*²*Center for Genetic Muscle Disorders, The Kennedy Krieger Institute*

A fundamental obstacle to develop therapies for facioscapulohumeral muscular dystrophy (FSHD) is the incomplete knowledge of the molecular mechanism underlying the muscle fiber death induced by DUX4. Using myotubes generated by the MB135-iDUX4CA cells, a human muscle cell line expressing DUX4 upon doxycycline (Doxy) treatment, and human primary FSHD myoblasts, we found evidence of the presence of DUX4 inside the nucleolus. Moreover, myotubes generated by control human primary myoblasts, and by untreated MB135-iDUX4CA myoblasts, showed the nucleolar presence of DUX4C, the non-pathological form of DUX4. We also identified an almost fully conserved DUX4 DNA binding site in the intergenic spacer (IGS) sequence of the ribosomal DNA. MB135-iDUX4CA myotubes treated with Doxy showed changes in the 3D nucleolar structure, and the generation of several specific IGS-derived non-coding RNAs associated with the activation of the nucleolar stress response and of the nucleolar detention pathway (NoDP). In this regard, we found the presence of amyloid-like bodies inside the nucleoli of DUX4-expressing MB135-iDUX4CA myotubes. Since NoDP and nucleolar stress regulate the balance between cell survival and apoptosis, the DUX4-dependent induction of the NoDP may be one of the initial molecular drivers of the muscle fiber apoptosis we observe in FSHD. We are currently evaluating the composition of the nucleolar transcriptome and the presence of nucleolar epigenetic changes in myotubes expressing DUX4, as well as the efficacy of new potential therapeutic treatments.

P3.21**Innate immunity model of FSHD muscle pathology activates complement genes**Katelyn Daman¹, Jing Yan¹, Oliver King², Michael Brehm¹, Charles P. Emerson Jr.¹¹*UMass Chan Medical School*²*Department of Neurology, University of Massachusetts Medical School*

Facioscapulohumeral muscular dystrophy (FSHD) is the third most diagnosed muscular dystrophy presenting with asymmetric progressive weakening of muscles in the face, upper body, and shoulder girdle, progressing to loss of ambulation and profound physical disabilities. Misexpression of the transcription factor DUX4 is associated with FSHD muscle pathology, but disease onset and severity are highly variable. We hypothesize that this clinical variability is associated with DUX4 transcriptional signature that triggers an FSHD patient-specific innate immune response. To investigate the role of innate immune response in FSHD muscle pathology, we developed a human immune-muscle double xenograft model in an immune deficient mouse strain that is genetically engineered for the selective expansion of innate immune cells in conjunction with engraftment of primary FSHD or control myoblasts into the tibialis anterior (TA) muscle. Our findings show that human CD45+ cells preferentially infiltrate FSHD engrafted muscle relative to control muscle. Further, NanoString analysis reveals a selective expression of complement genes in FSHD xenograft muscle, also expressed in FSHD muscle biopsies. These findings support our hypothesis that innate immunity plays a central role in FSHD muscle pathology and establishes an FSHD muscle-immune xenograft model to investigate the mechanisms underlying FSHD innate immunity and development of targeted immune therapeutics for FSHD muscle pathology.

P3.22

Intramuscular fibrosis correlates with disease activity and progression in facioscapulohumeral muscular dystrophy patients

Elvira Ragozzino¹, Sara Bortolani², Lorena Di Pietro¹, Ornella Parolini¹, Mauro Monforte², Giorgio Tasca¹, Enzo Ricci¹

¹*Università Cattolica del Sacro Cuore*

²*Fondazione Policlinico Universitario A. Gemelli IRCCS*

Introduction: Facioscapulohumeral muscular dystrophy (FSHD) displays an extreme variability in muscle involvement, with atrophic and apparently spared muscles coexisting in the same patient. Muscle magnetic resonance imaging (MRI) is a non-invasive technique able to detect both terminal muscle degeneration (with T1-weighted sequences) and the active phase of the disease (on STIR sequences). In this study, we evaluated the presence of intramuscular fibrosis, a feature of muscle degeneration, to determine its possible correlation with disease activity and progression.

Methods: Muscle biopsies from 33 FSHD STIR-, 25 FSHD STIR+, and 6 healthy subjects were processed for picosirius red staining. MRI scans of the FSHD muscles were assessed for the presence of fatty replacement on T1-weighted sequences at the time of the biopsy and until 3 years after the sample collection.

Results: Fibrosis was found to be increased in all STIR+ muscles, independently from the presence of fat accumulation on T1-sequences, compared to healthy controls. Milder fibrosis was also found in several STIR- muscles. The extent of fibrosis in STIR+ muscles positively correlated with progression of fat accumulation as observed in the 3-year follow-up MRI examination.

Conclusions: Fibrosis is a sign of muscle degeneration undetectable with the MRI. Our data show that all STIR+ muscles have a collagen deposition which precedes disease progression. Some STIR- muscles are also characterized by excessive fibrosis.

P3.23**Non-myogenic mesenchymal cells contribute to muscle degeneration in facioscapulohumeral muscular dystrophy patients**

Lorena Di Pietro¹, Flavia Giacalone¹, Elvira Ragozzino¹, Valentina Saccone¹, Marco De Bardi², Mario Picozza², Wanda Lattanzi¹, Enrico Guadagni¹, Sara Bortolani³, Giorgio Tasca³, Enzo Ricci³, Ornella Parolini¹

¹*Università Cattolica del Sacro Cuore*

²*IRCCS Santa Lucia Foundation*

³*Fondazione Policlinico Universitario A. Gemelli IRCCS*

In the skeletal muscle, different cell types cooperate to determine tissue maintenance, but also to contribute to muscle regeneration in disease. Non-myogenic mesenchymal cells (i.e., fibroadipogenic progenitors) have recently emerged as remarkable therapeutic targets for neuromuscular disorders, but are poorly investigated in FSHD. We thus aim to characterize non-myogenic mesenchymal cells in affected muscles of FSHD patients and to elucidate their possible implication in the pathophysiology of the disease.

Muscle specimens were collected through needle muscle biopsies after muscle magnetic resonance imaging examination to identify early affected and apparently unaffected muscles in FSHD patients. Non-myogenic mesenchymal cells isolated from muscle specimens were analyzed *in vitro*, and comparisons between patient and control cultures were performed. Qualitative and quantitative analyses of patient and control skeletal muscle sections were also carried out.

Cells isolated from affected muscles of patients displayed a peculiar expression pattern of selected mesenchymal markers and an altered response to an *in vitro* adipogenic induction. FSHD muscles showed a significant expansion of non-myogenic mesenchymal cells that positively correlated with the extent of intramuscular fibrosis.

Our results prompt for a direct association between an accumulation as well as an altered differentiation potential of non-myogenic mesenchymal cells with muscle degeneration in FSHD patients' muscles.

P3.24**Interaction between mesenchymal stem cells and myoblasts contributes to the FSHD phenotype****Yegor Vassetzky**¹, Ekaterina Kiseleva^{1,2}, Olesya Serbina^{1,2}, Anna Karpukhina^{1,2}¹*Gustave Roussy*²*Koltzov Institute of Developmental Biology*

Muscle degeneration in FSHD is accompanied by muscle tissue replacement with fat and connective tissue. Expression of DUX4 in myoblasts stimulates MSC migration via the CXCR4-CXCL12 axis. Mesenchymal stem cells (MSCs) participate in adipose and connective tissue formation, and can contribute to fibrosis. Here we studied the interaction between myoblasts and MSCs, and the consequences of this interaction in the FSHD context. The growth medium conditioned by FSHD myoblasts stimulated MSCs migration as compared to non-conditioned medium. Blocking the CXCL12-CXCR4 axis with the CXCR4 inhibitor (AMD3100) or neutralizing antibodies to CXCL12 abolished this effect. FSHD myoblasts stimulated MSC proliferation 1.5-2 times compared to the control myoblasts, while the presence of MSCs impaired myoblast differentiation. Under inflammatory conditions, medium conditioned by FSHD myoblasts stimulated collagen secretion by MSCs 2.2 fol. Thus, FSHD myoblasts attract MSCs via the CXCL12-CXCR4 axis, and stimulate MSC proliferation and collagen secretion by MSCs. Interaction between MSCs and FSHD myoblasts accounts for several important aspects of FSHD pathophysiology. The CXCL12-CXCR4 axis may serve as a potential target to improve the state of the diseased muscles.

P4.26**Hit-and-run silencing of endogenous DUX4 by targeting DNA hypomethylation on D4Z4 repeats in in vitro FSHD-iPSC model****Mitsuru Sasaki-Honda¹**, Junjie He¹, Hidetoshi Sakurai¹¹*CIRA, Kyoto University*

Facioscapulohumeral muscular dystrophy (FSHD), a progressive skeletal muscle disorder, is epigenetically characterized with DNA hypomethylation of D4Z4 repeats in the 4q35 region, allowing aberrant DUX4 expression. Sustainable DUX4 suppression is a promising therapeutic clue to prevent disease progression, but most of the currently proposed methods depend on expression of their mediator biochemical entity, potentially narrowing QoL of individuals with FSHD in the clinical context. In this presentation, we will report that by applying hit-and-run silencing with dCas9-mediated epigenetic editing targeting DNA hypomethylation on D4Z4 repeats, we could achieve suppression of endogenous DUX4 in our FSHD patient-derived iPSC model including FSHD2 with SMCHD1 mutation and FSHD1. Notably, DNA methylation was significantly upregulated in FSHD2 cells, and suppression effect was observed after at least two weeks of expansion followed by 10 days of myogenic differentiation, which was not the case by transient induction of typical dCas9-KRAB alone. Off-target analysis showed that despite the potential genome-wide risk in DNA methylation level, the impact on the transcriptome was limited. Even though our results were still obtained limitedly in an iPSC model, hit-and-run silencing can be a promising option to prevent disease progression with minimum intervention for individuals with FSHD.

P4.27**Improving FSHD RNAi gene therapy using myotropic MyoAAVs****Lindsay Wallace**¹, Tessa Riley¹, Matthew Guggenbiller¹, Gholamhossein Amini Chermahini¹, Scott Harper^{1,2}¹*Nationwide Children's Hospital*²*The Ohio State University*

We previously published pre-clinical efficacy and safety studies for a DUX4-targeted RNAi-based gene therapy for FSHD using a product called mi405. We also performed dose finding studies in FSHD animal models, demonstrated inhibition of human DUX4-associated biomarkers in xenografts, confirmed long-term expression in vivo, and characterized the biodistribution of two lead AAV vector serotypes (AAV6 and AAV9). In line with muscular dystrophy gene therapy studies now in clinical trials (e.g., micro-dystrophin), our dose-finding work demonstrated that sufficient muscle transduction required high-dose systemic delivery of AAV9 (1×10^{14} vg/kg), and our mouse toxicology studies showed no significant pathology, even up to 6×10^{14} vg/kg. Though several muscle-directed systemic gene therapy studies used doses that exceed our target range, serious adverse events and deaths have raised concerns about the safety of first-generation AAV vectors delivered at such high doses. Here we seek to reduce potential perceived or real safety issues. We aim to boost muscle transduction and reduce vector doses required to provide therapeutic benefit. In this study, we compare our first-generation AAV9 vector with MyoAAVs carrying the same mi405 genome using intramuscular and systemic delivery. We expect that the new muscle-targeted vectors combined with the highly efficacious mi405 product could enable lower AAV doses while preserving the therapeutic efficacy of high-dose first-generation vectors.

P4.28

Identification and targeting of hypoxia signaling for translational potential in facioscapulohumeral muscular dystrophy

Justin Cohen¹, Vincent Ho¹, Alec Desimone¹, Monkol Lek¹, Angela Lek¹

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. Furthermore, everolimus reduced DUX4 biomarkers in a xenograft mouse model. Results were more mixed in the DUX4-inducible ACTA1-MCM/FLExDUX4 mouse models with effect on muscle function but trends towards improvement in molecular assays. While mixed, these results demonstrate the utility of using CRISPR screening to identify novel therapeutic targets for FSHD. Importantly, emphasis on FDA-approved compounds would be a major boon for patients, as any successful candidate would have reduced time in the clinical trial pipeline.

P4.29

An AAV-shRNA DUX4-based therapy to treat facioscapulohumeral muscular dystrophy (FSHD)

Virginie Mariot¹, Eva Sidlauskaitė¹, Laura Le Gall¹, Emilio Corbex¹, Julie Dumonceaux¹

¹*NIHR Biomedical Research Centre, University College London, Great Ormond Street Institute of Child Health and Great Ormond Street Hospital NHS Trust, London, UK*

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common muscular dystrophies in adults, and so far there is no curative or preventive treatment. FSHD is characterized by the aberrant expression of the DUX4 transcription factor in muscle. DUX4 is a toxic protein, which has been implicated in increased sensitivity in oxidative stress, defects in myogenesis, muscle atrophy, etc., ultimately leading to myofiber death. Here, we designed an AAV vector carrying an shRNA directed against DUX4 (AAV-shDUX4) to knock down DUX4 expression in FSHD muscle cells in the Cre-inducible DUX4 bi-transgenic mouse model (ACTA1-MCM/FLEXDUX4).

In this model, DUX4 is expressed upon Cre-mediated translocation in the nucleus, which occurs after tamoxifen injection. ACTA1-MCM/FLEXDUX4 mice were injected with either an AAV-shScrambled or an AAV-shDUX4. After 4 weeks, we observed that all pathological signs of the DUX4 expression were reduced in the AAV-shDUX4 animals compared to the AAV-shScrambled animals, including: a dramatic decrease in the expression of the genes downstream of DUX4, a decrease in the number of fibers with centrally located nuclei, reduced inflammation, etc.

Our data suggest that AAV-shDUX4-based therapy is a promising therapeutic strategy for FSHD.

P4.30**Development of safe and efficacious RNA therapeutics for FSHD****Christian Kinney¹**, Anthony Saleh¹¹*miRecule, Inc.*

As a transcription factor, DUX4 is not directly “druggable” by traditional small molecules or biologic therapeutics. Several studies have displayed that ASO therapy has the potential to directly repress DUX4, reversing muscle pathology in preclinical models. Originating from the repetitive D4Z4 repeats which occur on multiple chromosomes, development of an anti-DUX4 ASO presents several challenges that we have overcome. miRecule’s DREAMiR™ analytics affords accurate assessment of conservation, splice isoform and pseudogene overlap, patient expression patterns, ASO and binding site structure, affinity, and potential toxicity. These tools have enabled miRecule to develop a best-in-class ASO that can simultaneously repress DUX4 with pM activity, and also related targets that participate in FSHD pathology. To deliver our ASO, miRecule has developed the NAVigator™ technology for antibody and formulation of RNA therapeutics. To identify a muscle-specific target for antibody targeting to enable delivery of an effective quantity of our DUX4 ASO, we have analyzed expression patterns from hundreds of patients with 20 different neuromuscular disorders, low expression in non-muscle tissues, and that are internalized to enable uptake. This analysis identified 5 high-priority receptors that we are testing to enable selective delivery of our conjugates to skeletal muscles in FSHD. With a preliminary antibody to one of these receptors, we have demonstrated a proof-of-concept muscle-specific delivery of our ASO and DUX4 knockdown in mouse models of FSHD. In this presentation, we will discuss the recent progress in developing an ASO-Ab conjugate, the MC-DX4, to treat FSHD.

P5.31**Motor outcomes to validate evaluations in facioscapulohumeral muscular dystrophy (MOVE FSHD): protocol for an observational study**

Michaela Walker¹, Russell Butterfield², John Day³, Kate Eichinger⁴, Bakri Elsheikh⁵, Anna Faino⁶, Seth Friedman⁶, Kiley Higgs¹, Nicholas Johnson⁷, Peter Jones⁸, Doris Leung⁹, Leann Lewis⁴, Bill Martens⁴, Dennis Shaw⁶, Perry Shieh¹⁰, Subramony Subramony¹¹, Jaya Trivedi¹², Leo Wang¹³, Mathew Wicklund¹⁴, Rabi Tawil⁴, Jeffrey Statland¹

¹University of Kansas Medical Center

²University of Utah

³Stanford University

⁴University of Rochester Medical Center

⁵The Ohio State University

⁶Seattle Children's Hospital

⁷Virginia Commonwealth University

⁸University of Nevada

⁹Kennedy Krieger Institute

¹⁰University of California, Los Angeles

¹¹University of Florida

¹²University of Texas Southwestern

¹³University of Washington

¹⁴University of Colorado

Aim: To determine the predictive value of motor assessments, neuroimaging, and tissue biomarkers on milestones of disease progression in FSHD. Most studies evaluating risk of functional outcomes or relationship between genetics and age at onset have been cross-sectional – few evaluated longitudinal risk of functional outcomes or risk factors for FSHD. A more comprehensive study tying motor functional performance, biomarkers, or changes in performance to life-modifying outcomes would be important not only for improving patient care, but to understand what kind of change would be meaningful for clinical trials. Ours will be a prospective observational study of 450 clinically affected and genetically confirmed FSHD participants over 3 years with 200 participating in an MRI and muscle biopsy sub-study. Visits will occur annually and collect FSHD history, physical exam, severity scores, patient-reported outcomes, and functional performance (timed functional tasks, strength, and respiratory parameters); sub-study participants will have whole body MRI at baseline and 12-month visits, muscle biopsy at baseline, and at 4-months (n=40). The MOVE FSHD study has more than 160 participants who have completed their baseline visit across 12 United States sites. MOVE FSHD addresses the barriers to clinical trials in FSHD by helping to validate motor, clinical and patient-reported outcomes, and potential biomarkers. The results can have a direct impact on patient care, our understanding of FSHD, and future clinical trials.

P5.32

Muscle imaging in facioscapulohumeral muscular dystrophy (FSHD): relevance for clinical trials. Report from the 265th ENMC Workshop

Giorgio Tasca¹, Shahram Attarian², John Vissing³, Jordi Diaz-Manera⁴, Nicol Voermans⁵

¹*Fondazione Policlinico Universitario A. Gemelli IRCCS*

²*CHU de Marseille*

³*University of Copenhagen, Denmark*

⁴*Newcastle University*

⁵*Radboud University*

In the context of the FSHD European Trial Network, several experts in different areas related to muscle imaging (neurologists, neuromuscular specialists, radiologists, physicists, and engineers) as well as patient representatives will gather in April 2022 to discuss and agree on relevant outcomes derived from muscle imaging techniques with impact on FSHD clinical trial readiness. The topics of the discussion will span from qualitative MRI, with both diagnostic and prognostic implications, to quantitative MRI, with feedback coming from the experience derived from previous trials, to the emerging applications of muscle ultrasound. The final aim is to reach consensus on the use of imaging in diagnosis, follow-up, and future clinical studies in FSHD.

P5.33**The face of facioscapulohumeral muscular dystrophy: exploring facial muscle involvement using ultrasound**Sanne Vincenten¹, Karlien Mul², Nicol Voermans¹, Nens van Alfen², Baziel van Engelen¹¹Radboud University Medical Center²Radboud University

One of the most striking clinical features of facioscapulohumeral muscular dystrophy (FSHD) is facial weakness, leading to functional impairments as well as diminished facial expression. Despite its clinical relevance, little is known about the exact pattern of involvement of the facial muscles, variability in involvement, and progression over time. In this study, we explored the use of muscle ultrasound to structurally assess changes in the facial muscles.

Muscle ultrasound images of the following facial muscles of genetically confirmed FSHD patients were assessed: depressor anguli oris, orbicularis oris, buccinator, temporalis, masseter, digastricus, zygomaticus major and minor bilaterally, and the geniohyoid. Ultrasound images were scored both quantitatively and semi-quantitatively. Ultrasound results were correlated to clinical outcome measures on facial weakness (FSHD clinical score facial subscale, physician-reported Facial Weakness Score and patient-reported Facial Function Scale). We included 107 FSHD patients (male=54.2%, mean age 52±14.6) covering the entire severity spectrum of facial weakness from minimal asymmetry to myopathic facies. In all patients our facial muscle ultrasound protocol was performed successfully. A total of 1,561 muscle ultrasound images were assessed. In conclusion, we used muscle ultrasound to provide insight in the pattern of involvement of facial muscles in FSHD. Results of this study will be presented.

P5.34

Analyzing the impact of FSHD on patient outcomes

Elan Schonfeld¹, Charulatha Nagar²

¹*Glenbrook North High School*

²*Northwestern Medicine*

Facioscapulohumeral muscular dystrophy (FSHD) is a genetic muscular dystrophy affecting the facial, scapular, and humeral musculature predominantly. FSHD is the third most common type of muscular dystrophy and has an estimated prevalence of about 4 out of 100,000 individuals. Patients with FSHD are generally young and employed; however, through the development of their disease, they slowly lose the ability to predominantly use their arms, and slowly lower extremities. FSHD needs early recognition; yet there are limited other treatment options as of now. However, current research has been looking to further care and treatment for FSHD. Scapular winging is a debilitating condition of impaired functionality of the upper extremity. Through a census of FSHD patients in a neuromuscular clinic, it was determined that nearly all FSHD patients have scapular winging. This presentation aims to educate about the impact of FSHD on clinical outcomes, specifically regarding the impact of FSHD-induced scapular winging.

P5.35

A world-wide survey of standardised outcome measure use in FSHD clinical care

Katy de Valle¹, Jenny McGinley², Fiona Dobson², Monique Ryan²

¹*The Royal Children's Hospital*

²*University of Melbourne*

Evidence- and consensus-based care standards for FSHD recommend annual assessment of symptoms and their progression without mention of which outcome measures should be used. Inconsistency exists in outcome measure selection for clinical trials. Anecdotal evidence suggests this is similar in the clinical environment. Unlike other neuromuscular diseases, the variability in presentation and unpredictable disease progression of FSHD make understanding and measuring change in symptoms more challenging. Without a standardised method of measuring outcomes, our knowledge of how FSHD progresses and the ability to identify signs of deterioration which contribute to anticipatory care are challenging. A survey to evaluate clinician reported use of outcome measures in the care of FSHD was formulated and distributed worldwide. The survey aimed to identify which measures are used and to understand any barriers/facilitators to their implementation.

To maximise participation, a "snowballing" strategy using an anonymous link in the QualtricsXM platform was used. Known neuromuscular experts were identified and asked to complete/forward survey to others within their network. Established global FSHD-specific and neuromuscular generic networks were approached to assist with survey distribution.

Demographics, response numbers, barriers to and use of measures evaluating severity, function, strength, balance, QoL, pain, and fatigue will be presented.

P5.36

Longitudinal assessment of facial weakness in facioscapulohumeral muscular dystrophy by physicians and patients

Karliën Mul¹, Tom Loonen², Sanne Vincenten², Sjan Teeselink², Nicol Voermans¹, Thomas Maal², Baziël van Engelen¹

¹*Radboud University*

²*Radboud University Medical Center*

Objective: This longitudinal study explores the rate of progression of facial weakness in FSHD from a physician's perspective, and (progression in) its consequences from a patients' perspective.

Methods: Genetically confirmed FSHD patients of 18 years or older were included. At baseline, and after 5 and 6.5 years, their degree of facial weakness was scored by physicians using the FSHD-specific "Facial Weakness Score." During the final 1.5 years of the study, longitudinal patient-reported data were collected on functional consequences of facial weakness using the "FSHD Facial Function Scale" and the "Communicative Participation Item Bank" questionnaire.

Results: A total of 162 patients (84 males, age at baseline 18-77 years) were assessed after 5 years, and 67 of them underwent an additional follow-up visit after 6.5 years. Facial weakness at baseline ranged from subtle asymmetry to severe weakness with myopathic facies.

Discussion: Preliminary longitudinal results of this study will be presented.

P5.37

Muscle ultrasound in an open-label study of losmapimod in subjects with FSHD1

Joost Kools¹, Nicol Voermans², Karlien Mul², John Jiang³, Jennifer Shoskes³, Kelly Marshall³, Michelle L. Mellion³, Baziel van Engelen², Markus Karlsson⁴

¹*Radboud University Medical Center*

²*Radboud University*

³*Fulcrum Therapeutics, Inc.*

⁴*AMRA Medical AB*

Aim: Evaluate muscle ultrasound (US) in an open-label trial of losmapimod in FSHD1 patients.

Background: Natural history studies have identified US as a viable imaging biomarker for FSHD muscle progression, complementary to MRI. Losmapimod is an orally active, selective, small molecule inhibitor of p38 α / β .

Methods: Fourteen subjects ages 18 to 65 years with genetically confirmed FSHD1, clinical severity score 2 to 4 (range 0-5) and MRI-eligible skeletal muscles for needle biopsy received open-label 15 mg twice daily losmapimod for 52 weeks with the primary objective of safety. Assessments included safety, MRI, US, clinical outcomes, and patient-reported outcomes. US was performed on 7 muscles bilaterally using a standardized protocol. US echointensity was expressed as a z-score relative to matched healthy controls, with abnormal being defined as >2.

Results: The mean (SD) change from baseline in echogenicity of all muscles was -0.17 (0.9), in the upper extremity muscles -0.32 (0.9), and lower extremities -0.13 (1.0), representing a trend towards improvement. The distribution of muscle z-scores (<2, 2-4, 4-6, and >6) at baseline and after 52 weeks of treatment remained the same. Echointensity correlated strongly with MFI for the biceps brachii ($r=0.84$, $p<0.01$), tibialis anterior ($r=0.76$, $p<0.01$), and gastrocnemius medialis ($r=0.50$, $p<0.01$). Correlations between US and clinical outcomes will be presented.

Conclusions: Losmapimod demonstrated stability in muscle US over 52 weeks

P5.38

Feasibility of measuring functional performance of FSHD patients using wearable sensors to quantify physical activity

Joost Kools¹, Nicol Voermans², Karlien Mul¹, Michelle L. Mellion³, John Jiang³, Jennifer Shoskes³, Kelly Marshall³, David Jackson³, Yuxi Zhao³, Anil Tarachandani⁴, Joanita Figueredo⁴, Damien Eggenspieler⁵, Baziel van Engelen²

¹*Radboud University Medical Center*

²*Radboud University*

³*Fulcrum Therapeutics, Inc.*

⁴*UsinLife LLC*

⁵*Sysnav SAS*

Objective: Evaluate feasibility to monitor daily activity and assess functional outcomes using wearable sensor devices in an open label study (OLS) of losmapimod in facioscapulohumeral muscular dystrophy (FSHD).

Background: Fulcrum is evaluating the safety and efficacy of losmapimod for the treatment of FSHD in the OLS. Assessment with wearables may be a sensitive measure of activities of daily living (ADL) and mobility in FSHD.

Methods: Actimyo wearable activity monitoring devices (1 wrist and 1 ankle) were used to monitor activity during the day intermittently (2 weeks on/off) throughout the study. An 8-week baselining period was collected prior to starting treatment. Subjects performed prescribed movements of upper and lower extremities twice daily.

Results: Fourteen subjects age 18 to 65 years with genetically confirmed FSHD1, clinical severity score 2 to 4 (range 0-5), receiving 15 mg losmapimod twice daily completed the study over 60 weeks. Compliance for wearing the devices was 99%. All 14 subjects were monitored for a total of 2,941 days or 36,758 hrs., for an average of 2,626 hours per subject. Multiple upper and lower extremity parameters were found to be reliable (ICC > .90). Analysis of clinical assessment correlations will be reported.

Conclusion: Measurement of activity in FSHD patients using wearable sensors is feasible, reliable, and correlates with multiple clinical assessments, ADLs, and mobility.

P5.39**Living with FSHD during the pandemic corona outbreak in the Netherlands: pitfalls and challenges of COVID-19 in FSHD**

Joost Kools¹, Johanna Deenen¹, Anna Greco¹, Renée Thewissen¹, Wiecke Van de Put¹, Anke Lanser², Nicol Voermans³, Mara Tihaya⁴, André Verbeek¹, Silvère Van der Maarel⁴, Leo Joosten¹, Baziel van Engelen³

¹*Radboud University Medical Center*

²*Spierziekten Nederland, Baarn, the Netherlands*

³*Radboud University*

⁴*Leiden University Medical Center*

The SARS-Cov-2 virus has heavily affected many aspects of our lives. While the impact of the COVID-19 pandemic on patients with neuromuscular disorders (NMD) in general has been investigated, discussing the impact on physical activity, loneliness, anxiety, and the organization of care, detailed information on individuals with facioscapulohumeral muscular dystrophy (FSHD) is still lacking. Therefore, we conducted a survey-based study, which longitudinally monitored the incidence of COVID-19 infections and COVID-19-related stress, using questionnaires taken at three timepoints. In addition to COVID-19-related stress and COVID-related symptoms, which have also been reported by previous studies in NMD patients, we looked at COVID-19 preventive measures and household living arrangement of the patients. A total of 347 FSHD patients from the Dutch National FSHD Registry were reached to fill out the questionnaires, providing answers on themselves and their housemates. Two hundred ten patients completed round 1 of the questionnaires, 186 patients completed round 2, and 205 patients completed round 3. A total of 134 patients completed all three questionnaires. Preliminary results of the questionnaire data of round 1, presented at the FSHD IRC of 2020, did not show a higher nor lower risk for FSHD patients at getting COVID-19. At the FSHD IRC of 2022, we will present the results of the full study.

P5.40**The UK FSHD Patient Registry: a key tool linking patients with national and international research projects**

Helen Walker¹, Richard Orrell², Chiara Marini-Bettolo^{1,3}, Andrew Graham⁴, Kate Adcock⁴, Suzanne Watt⁴, Peter Lunt⁵, Fiona Norwood⁶, Mark Roberts⁷, Tracey Willis⁸, Emma Matthews⁹, Robert Muni-Lofra^{1,3}

¹*John Walton Muscular Dystrophy Research Centre, Newcastle University*

²*UCL Queen Square Institute of Neurology*

³*Newcastle upon Tyne Hospitals*

⁴*Muscular Dystrophy UK*

⁵*National Genetics Education and Development Centre, University of Bristol*

⁶*Department of Neurology, King's College Hospital*

⁷*Greater Manchester Neurosciences Centre, Salford Royal NHS Foundation Trust*

⁸*Neuromuscular Service, The Robert Jones and Agnes Hunt Orthopaedic Hospital*

⁹*The Atkinson Morley Regional Neurosciences Centre, St George's University Hospital*

The UK FSHD Patient Registry is a self-enrolling online database collecting clinical and genetic information. The registry aims to facilitate academic and clinical research, better understand the condition, and disseminate information relating to upcoming studies and research advancements. It was established in May 2013 supported by MDUK, and is coordinated at Newcastle University. The registry captures longitudinal, self-reported, and clinician-reported data through a secure online portal. The registry is recognised as a core member of the TREAT-NMD Global Registry network and collects the TREAT-NMD core dataset for myotonic dystrophy, including questionnaires on pain, quality of life, and scapular fixation.

Between May 2013 and March 2022, 1,079 participants were enrolled with the registry. The registry accepts non-UK patients where there is no national registry available, but 83% of participants are UK based, and on average, nine new participants register each month. Overall, 52% of UK-based patients have genetic confirmation of their condition, 96% reported FSHD/FSHD1, and 4% reported FSHD2. The most commonly reported symptom was periscapular shoulder weakness (93%), followed by facial weakness and hip girdle weakness (both 70%), then foot dorsiflexor weakness (67%).

The registry supports the FSHD community both in the UK and globally, and has supported more than 30 research enquiries and surveys, including de-identified data reports to facilitate and advance research.

P5.41**Prevalence and impact on quality of life of gastrointestinal and genitourinary symptoms in facioscapulohumeral muscular dystrophy**

Michael Cole¹, June Kinoshita², Angelena Edwards³, Christopher Cooper³, Eyad Hanna¹, M. Bridget Zimmerman⁴, Katherine Mathews¹

¹*University of Iowa Hospitals and Clinics Department of Pediatrics*

²*FSHD Society*

³*University of Iowa Hospitals and Clinics Department of Urology*

⁴*University of Iowa, College of Public Health (Biostatistics)*

Anecdotally, patients with FSHD describe gastrointestinal (GI) and genitourinary (GU) symptoms, but the prevalence is unknown. We used a questionnaire to explore the frequency and severity of these symptoms and their impact on quality of life in people with FSHD compared to healthy household controls. The survey was distributed by email to all FSHD Society patient contacts. A total of 702 responses were included in the analysis (650 with FSHD, 52 controls). All were >18 years old, with median age 56 years in both groups. Examining only symptoms with frequency of >1x/week, the FSHD group had increased prevalence of difficulty swallowing food ($p=0.026$), feeling that food “got caught” on the way down ($p=0.016$), abdominal pain ($p=0.026$), and constipation ($p=0.026$). Examining overall symptom prevalence, the FSHD group also had increased daytime enuresis ($p=0.027$), double voiding ($p=0.002$), and inability to hold bowel movements until reaching the restroom ($p=0.0002$). Symptoms in each of the discrete categories of swallowing, bowel, and bladder symptoms were reported to decrease quality of life. Of respondents who reported more symptoms per category, a higher proportion agreed or strongly agreed that quality of life was decreased. There was no difference between groups in the type or frequency of medications used for these symptoms. These results indicate that further investigation into etiology and treatment of these underappreciated symptoms of FSHD may improve patients’ quality of life.

P5.42

Inpatient admissions and emergency department visits for patients with facioscapulohumeral muscular dystrophy (FSHD): a real-world retrospective data analysis of pre- and post-diagnosis events

Chamindra Konersman¹, **Kathryn Munoz**², Richard Brook³, Nathan Kleinman³, Kelly DiTrapani², Bradley McEvoy², Alissa Peters², Chao-Yin Chen², Teresa Brandt², Mark Stahl²

¹University of California, San Diego

²Avidity Biosciences

³Better Health Worldwide

Background: FSHD is a rare, slowly progressive, genetic skeletal muscle disease causing significant physical limitations, pain, fatigue, and an overall negative impact on well-being. Real-world data on the FSHD-patient journey are limited.

Objective: To understand the causes of FSHD inpatient admissions and ED visits.

Design/Methods: We conducted a retrospective de-identified claim analysis of FSHD patients (N=79) vs. matched controls (MCs; N=395); January 2015-March 2021. We compared changes two years post-diagnosis (post-Dx) minus two years pre-diagnosis (pre-Dx) in the number of emergency department (ED) and inpatient admissions (INPT) and associated diagnosis using ICD10 codes.

Results: A greater increase in post-Dx ED visits was observed in the FSHD cohort vs. the MC cohort ($\uparrow 16.5\%$ vs. $\uparrow 2.8\%$; $p=0.0302$), with the most common diagnosis of pneumonia. A non-statistically significant increase was seen in INPT visits (FSHD $\uparrow 7.6\%$ vs. MC $\downarrow 1.0\%$), with the most common diagnoses of shortness of breath and acute respiratory failure with hypoxia. Overall, the greatest post-Dx differences between cohorts for ED and INPT included respiratory, kidney, and cardiovascular diagnoses.

Conclusions: The FSHD diagnostic journey includes higher INPT admissions and ED visits pre- and post-Dx. Cardiovascular and respiratory conditions were most commonly associated with healthcare utilization in this study.

Conclusion: Annualized rates of change in clinical outcomes support disease slowing or improvement in losmapimod treated subjects compared to placebo.

P5.43

Design of REACH: Phase 3 randomized, double-blind, placebo-controlled, 48-week study of the efficacy and safety of losmapimod in FSHD

Christopher Morabito¹, Rabi Tawil², Jay Han³, Leo Wang⁴, John Vissing⁵, Baziel van Engelen⁶, Jeffrey Statland⁷, Michelle L. Mellion¹, **Jennifer Shoskes¹**, John Jiang¹, Jennifer Webster¹

¹*Fulcrum Therapeutics, Inc.*

²*University of Rochester Medical Center*

³*University of California, Irvine*

⁴*University of Washington*

⁵*University of Copenhagen, Denmark*

⁶*Radboud University Medical Centre*

⁷*University of Kansas Medical Center*

Objective: Evaluate the efficacy and safety of losmapimod for the treatment of FSHD.

Background: FSHD is a chronic, variably progressive disease leading to accumulation of disability over decades. Nonclinical studies have shown that losmapimod (a small molecule p38 α/β MAPK inhibitor) reduces the aberrant expression of DUX4, the underlying cause of FSHD. Two Phase 2 clinical studies, a 48-week randomized controlled study (ReDUX4, FIS-002-2019), and a 52-week open-label study (OLS, FIS-001-2019) demonstrated evidence of benefit of losmapimod on muscle structure and function, as well as FSHD-relevant clinical endpoints recognized by patients and favorable safety and tolerability.

Fulcrum is initiating a Phase 3 double-blind, placebo-controlled trial to support the development of losmapimod in FSHD.

Methods: Approximately 230 people with FSHD, 210 with genetically confirmed FSHD1 and 20 with FSHD2, will be randomized 1:1 to receive losmapimod or placebo orally, twice daily for 48-weeks. The primary endpoint is reachable workspace quantification of total relative surface area (Q1-Q5) with 500 g wrist weight in the dominant arm, with secondary endpoints of Neuro-QoL Upper Extremity, patient global impression of change, and muscle fat infiltration using whole-body musculoskeletal MRI. Exploratory assessments include muscle fat fraction, muscle strength by hand-held dynamometry, and patient-reported outcomes.

Results and Conclusions: The design of this Phase 3 study will be presented.

P5.44**Annualized rates of change from a Phase 2, randomized, double-blind, placebo-controlled, 48-week study of losmapimod in subjects with FSHD: ReDUX4**

Christopher Morabito¹, Sabrina Sacconi², Jordi Diaz³, David Reyes Leiva³, Doris Leung⁴, Kathryn Wagner⁴, Angela Genge⁵, Juan Vilchez⁶, Nuria Gomez⁷, Miriam Freimer⁸, Samantha Lorusso⁸, Hanns Lochmuller⁹, Joost Kools¹⁰, Baziel van Engelen¹⁰, Namita Goyal¹¹, Perry Shieh¹², S.H. Subramony¹³, Jeffrey Statland¹⁴, Lawrence Hayward¹⁵, Johanna Hamel¹⁶, Rabi Tawil¹⁶, Summer Gibson¹⁷, Leo Wang¹⁸, Nicholas Johnson¹⁹, Alan Pestronak²⁰, Michelle L. Mellion¹, Anthony Accorsi¹, **Jennifer Shoskes**¹, John Jiang¹, L. Alejandro Rojas¹

¹*Fulcrum Therapeutics, Inc.*

²*Nice University*

³*Hospital de la Santa Cruz*

⁴*Kennedy Krieger Institute*

⁵*McGill University*

⁶*Neuromuscular and Ataxias Research Group, Instituto de Investigación Sanitaria La Fe*

⁷*Neuromuscular Diseases Unit, Neurology Department, Hospital Universitari I Politècnic*

⁸*Ohio State University Medical Center*

⁹*Ottawa Hospital Research Institute*

¹⁰*Radboud University Medical Center*

¹¹*University of California, Irvine*

¹²*University of California, Los Angeles*

¹³*University of Florida*

¹⁴*University of Kansas Medical Center*

¹⁵*University of Massachusetts Medical School*

¹⁶*University of Rochester Medical Center*

¹⁷*University of Utah*

¹⁸*University of Washington*

¹⁹*Virginia Commonwealth University*

²⁰*Washington University*

Objective: Evaluate annualized clinical outcome changes in subjects with FSHD treated with losmapimod compared with placebo.

Background: Fulcrum is developing losmapimod (a small molecule p38 α / β MAPK inhibitor) to treat FSHD. Annualized analysis provides data on the slope of disease progression and allows comparison of change in slope over time.

Methods: Eighty subjects (N=40/group) aged 18-65 years with FSHD1, CSS 2-4, and MRI-eligible muscles for biopsy were randomized 1:1; losmapimod (15 mg BID):placebo. Assessments included MRI, reachable workspace (RWS), dynamometry, and TUG. Annualized rate of change was calculated using a linear mixed-effects model to estimate slope and percent change per year. Annualized RWS analysis was prespecified; all others are post-hoc.

Results: Annualized rate of change (%/yr) in total relative surface area for losmapimod (LOS) vs. placebo (PBO) dominant arm: -0.44 vs. -8.42; p=0.07; non-dominant arm: 4.88 vs. -4.02; p=0.01. MRI annualized rates of change for LOS vs. PBO were 0.31 vs. 3.82 for MFI, 4.42 vs. 5.96 for MFF, and -7.15 vs. -4.50 for LMV. Maximum dynamometry showed variability for LOS vs. PBO in the shoulder abductors (L -1.98 vs. 13.62; R -14.39 vs. -8.49) and handgrip (L -8.30 vs. -13.44; R -13.95 vs. -15.27). The annualized rate of change in TUG was -0.93 vs. 2.23 (LOS vs. PBO).

Conclusion: Annualized rates of change in clinical outcomes support disease slowing or improvement in losmapimod treated subjects compared to placebo

P5.45**Understanding falls in FSHD**

Enrico Bugiardini¹, Kate Eichinger², Michael Hanna¹, Gita Ramdharry¹, Michael McDermott², Kiley Higgs³, Michaela Walker³, Leann Lewis², Bill Martens², Doris Leung⁴, Sabrina Sacconi⁵, Karlien Mul⁶, Valeri Sansone⁷, Leo Wang⁸, Perry Shieh⁹, Bakri Elsheikh¹⁰, Russell Butterfield¹¹, Nicholas Johnson¹², Rabi Tawil², Jeffrey Statland³, the ReSolve Investigators of the FSHD CTRN³

¹*UCL Queen Square Institute of Neurology Department of Neuromuscular Diseases*

²*University of Rochester Medical Center*

³*University of Kansas Medical Center*

⁴*Kennedy Krieger Institute*

⁵*Nice University*

⁶*Radboud University*

⁷*The NEMO Clinical Center*

⁸*University of Washington*

⁹*University of California, Los Angeles*

¹⁰*The Ohio State University*

¹¹*University of Utah*

¹²*Virginia Commonwealth University*

Falls are frequently reported in neuromuscular diseases, impacting clinical care and potentially representing an important metric to follow for clinical trials. In this study we aimed to assess prevalence and consequences of falls in a large group of FSHD patients (ReSolve cohort) and determine clinical features associated with falls. A prospective 12-week weekly survey (Fsu) and a retrospective fall history questionnaire (Fhx) assessing falls in the previous 6 months were collected on 132 and 97 patients, respectively. Prevalence of falls was 36% and 52%, respectively. Fifty-two percent of cases are recurrent fallers, and 54% reported an injury as a consequence of falls. Based on survey falls count, patients were classified in non-fallers, infrequent (n=1), or recurrent fallers (n>1). Infrequent and recurrent fallers had a higher disease burden and motor impairments compared to non-fallers (FSHD-HI mean score 37.47, 41.15, 25.54 range 0-100; Motor Function Measurement (MFM) median 58.97, 53.85, 82.05, range 0-100; p <0.05). Recurrent fallers were weaker by manual muscle testing (MMT) in all muscle districts than non-fallers. Recurrent fallers had an earlier disease onset compared to infrequent fallers (mean 16.36 and 28.86, respectively; p <0.05). In conclusion, prevalence of falls and related injury are high in FSHD patients, and fall surveys are important to be included in trial design. Fallers are weaker and have lower quality of life compared to non-fallers.

P5.46**TREAT-NMD FSHD Global Registry Network: a collaboration of neuromuscular and FSHD patient registries****Ben Porter**¹, Neil Bennett¹, David Allison¹, Craig Campbell¹, Michela Guglieri¹, Anna Ambrosini¹, Rossella Tupler¹¹*On behalf of TREAT-NMD FSHD Subgroup & TREAT-NMD Global Registry Network*

TREAT-NMD is an international collaboration that aims to accelerate the development of new treatments for NMD. It operates a Global Registry Network, governed by an oversight committee, where member registries collect agreed disease specific datasets. The FSHD Global Registry Network collects data from 21 national (or regional) registries, representing four continents.

An electronic survey requesting demographic and diagnostic data was sent to all TREAT-NMD member registries, collecting FSHD data in 2022.

There were 13 (62%) registry survey responses, providing data on 3,372 FSHD patients (female: 1,528; male: 1,645; unknown: 199). Only 90 patients (3%) were aged ≤16 years old.

Most patients had FSHD1 (1,747/3,163, 55%) with fewer FSHD2 (82, 3%) cases. However, 42% of patients (1,334) were of unknown FSHD type. Overall, 43% of patients (1,463) received genetic confirmation of FSHD, with FSHD1 cases (1,262/1,747, 72%) expectedly higher than FSHD2 (32/82, 39%) or unknown FSHD type (171/1,334, 13%).

The TREAT-NMD FSHD Global Registry Network represents an international harmonised data resource, providing opportunities for researchers and industry to support clinical trial planning upon its interrogation. Despite most registries being clinician reported (62%), there were many patients without FSHD genetic confirmation or a specific FSHD type diagnosis. Understanding these aspects nationally will be important, as they represent clinical trial essential criteria.

P5.47

A case story: supervised FSHD patient self-analysis of its respiratory data

Patrick Valentin¹, Frederic Lofaso²

¹*Retired*

²*Hôpital Raymond Poincaré*

Nowadays, respiratory problems in FSHD, which often develop insidiously, are increasingly recognized. Due to progress in sensors and software technologies, home monitoring of its own respiratory data by a patient is possible today at a reasonable cost. In the present case, the patient (FSHD), retired, 80 years old, had a lifelong experience as an engineer and mathematical modeller. The needed clinical knowledge was provided by the co-author, a pneumologist specialized in NIV for NMD, as an able distant supervisor.

A wristwatch from Contec Medical (RS01) was the main source of data: SpO₂, pulse rate, flow rate, and photoplethysmogram. In addition, home data from the PPC ventilators, which accumulate at a daily rate of several megabytes, contain useful information on tidal volume, instantaneous pressure, and flow rate.

We present a new, Fast Respiratory Test (FRT) based on data collected during a 25 min, voice directed, yoga relaxation in supine position. After 10 min, data show an oscillating SpO₂ state without trend and post-treated in Maple and Matlab.

In 2020 and early 2022, two FRT sequences of respectively n=130 and n=50 tests were conducted. They showed a mean SpO₂ decrease from 94% to 92% and a respiration rate increase from 16 to 20 rpm. In 2022, 62% of the FRT were lower than 93%, compared with 18% in 2020. This provides a coherent picture of the evolution, in line with a Vital Capacity decrease from 88% to 80%.

Furthermore, using the FRT SpO₂ distribution a low FRT (<90.5%) relates with a lasting inner sensation of fatigue and discomfort after an overly strenuous treadmill session.

FRT reduces patient stress and maintains high motivation.

P5.48

Longitudinal whole-body MRI and muscle function in FSHD1

Doris Leung¹, Sharanya Suresh¹, Shivani Ahlawat², Alex Bocchieri³, Vishwa Parekh⁴, Vladimir Braverman⁴, Michael Jacobs⁴

¹*Kennedy Krieger Institute*

²*Johns Hopkins University School of Medicine*

³*University of Wisconsin, Madison*

⁴*Johns Hopkins University*

Introduction: There is a need for outcome measures that can assess disease progression in clinical trials in FSHD. Muscle imaging shows promise as a biomarker in muscular dystrophies; however, its use has been limited by the time needed to perform quantitative analysis of imaging features.

Objectives: 1) To evaluate a machine learning protocol to classify tissues across a broad spectrum of disease severity in FSHD1. 2) To evaluate associations between quantitative imaging with clinical measures of muscle function and strength over time.

Methods: Participants with FSHD1 completed 5 visits (baseline and 3, 9, 15, 21 months). At each visit, participants completed a whole-body MRI scan (T1, STIR, and Dixon imaging). Manual muscle testing, dynamometry, and timed function testing (TFT) were also performed. Machine learning algorithms were developed to identify and quantify muscle, subcutaneous fat, and intramuscular fat from Dixon images.

Results: Thirty participants (60% female, mean age 50.0±15.8 years, allele size 14-35kb, FSHD scores 1-14) completed 144 study visits. Muscle fat fractions (MFF) in the legs at baseline ranged from 6.9% to 55.7%. Correlation coefficients between MFF and TFTs ranged from 0.65-0.84 ($p<0.05$). Total MFF in the lower extremities did not change significantly over 21 months.

Conclusions: MFFs are associated with muscle function in FSHD1. Longer periods of observation may be needed to characterize changes in MFF across large regions of muscle.

P5.49**The FSHD Composite Outcome Measure (FSHD-COM) is reliable, valid, and measures disease progression**

Katy Eichinger¹, Michael McDermott¹, Kiley Higgs², Michaela Walker², Leann Lewis¹, Bill Martens¹, Doris Leung³, Nikia Stinson³, Megan McNeerney³, Sabrina Sacconi⁴, Jeremy Garcia⁴, Victor De Paz Benito⁴, Karlien Mul⁵, Valeria Sansone⁶, Elena Carraro⁶, Stefano Becchiati⁶, Maria Frisoni⁶, Leo Wang⁷, Catherine Kieu⁷, Perry Shieh⁸, Christ Skura⁸, Bakri Elsheikh⁹, Kristina Kelly⁹, Andrea Jaworek⁹, Samantha LoRusso⁹, Russell Butterfield¹⁰, Amelia Wilson¹⁰, Melissa McIntyre¹⁰, Nicholas Johnson¹¹, Amanda Butler¹¹, Aileen Jones¹¹, Melissa Hayes², Sandhya Sasidharan², Lindsay Baker¹, Rabi Tawil¹, Jeffrey Statland², the ReSolve Investigators of the FSHD CTRN²

¹University of Rochester Medical Center

²University of Kansas Medical Center

³Kennedy Krieger Institute

⁴Nice University

⁵Radboud University

⁶The NEMO Clinical Center

⁷University of Washington

⁸University of California, Los Angeles

⁹The Ohio State University

¹⁰University of Utah

¹¹Virginia Commonwealth University

Background: Standardized clinical outcome assessments may serve as endpoints for late-phase FSHD clinical trials. The FSHD-COM is a 13-item composite measure that assesses the functional impact of the disease by measuring areas that have been identified as meaningful to individuals with FSHD.

Methods: Individuals with FSHD participating in the multi-site, international ReSolve Study were assessed using the FSHD-COM at baseline, 12, 18, and 24 months. The FSHD-COM was performed twice over consecutive days at baseline to examine the test-retest reliability. Manual muscle testing (MMT) and the FSHD clinical score were also performed.

Results: A total of 237 participants (56% male) with a mean age of 50.3 (range 19-75) years completed baseline assessments. The interclass correlation coefficient for the total FSHD-COM score was 0.98, with individual items ranging from 0.85-0.99. The FSHD-COM score was correlated to overall MMT ($\rho=0.89$; $p=0.001$) and the FSHD clinical score ($\rho=0.82$; $p=0.001$). Changes in the FSHD-COM score were noted at 18 (mean change=1.41; 95% confidence interval [CI] 0.49-2.33; $n=133$) and 24 months (mean change=2.61; 95% CI 1.72-3.51; $n=95$).

Conclusions: The FSHD-COM score can be administered reliably in a multi-site study. Concurrent validity is supported by the correlations between the FSHD-COM score and measures of disease severity. In this ongoing study, changes in function, as measured by the FSHD-COM, can likely be detected at 18 months.

P5.50

Understanding the perseverance of the muscular dystrophy community one year into the COVID-19 pandemic

Leann Lewis¹, Katy Eichinger¹, Nuran Dilek¹, Kiley Higgs², Michaela Walker², David Palmer², John Cooley², Nicholas Johnson³, Rabi Tawil¹, Jeffrey Statland²

¹*University of Rochester Medical Center*

²*University of Kansas Medical Center*

³*Virginia Commonwealth University*

Objective: To assess the long-term impacts of the COVID-19 pandemic on people with muscular dystrophy (MD).

Background: As the COVID-19 pandemic persisted, it produced lasting impacts on daily life worldwide. People with MD are potentially at higher risk for COVID-19 complications, but little is known about the continued impact on the MD population.

Methods: We modified our prior COVID-19 Impact Survey using feedback from MD experts, patients, and advocacy group/registry representatives. The survey assessed COVID-19 medical history, and the effects of the pandemic on social aspects, muscle disease, and medical care. We included the Perceived Stress Scale, a validated 10-item scale. The de-identified, electronic survey was distributed to adults with MD via international patient registries or advocacy group websites from February 8, 2021, to March 22, 2021.

Results: Respondents (n=1,243: 49% FSHD; 43% DM, and 8% LGMD) were mostly women and middle-aged (range 18-90). COVID-19 infection rates were 8%. Reported recovery times were typically less than 2 weeks, and only 7% required hospitalization. Major challenges included stress management (27%) and wearing a mask (24%). The majority reported a slight worsening of their MD. Respondents reported moderate stress levels (average=16.4; range=0-39).

Conclusion: People with MD found ways to overcome obstacles during the COVID-19 pandemic. COVID-19 infection rates and medical complications were similar to a general population.

P5.51**Clinical and molecular evaluation of FSHD patients in Turkey****Serpil Eraslan**¹, Sahin Avci¹, Ilker Eren², Gulshan Yunisova³, Piraye Oflazer³, Mehmet Demirhan², Hulya Kayserili¹¹*Koc University, School of Medicine, Department of Medical Genetics*²*Koc University, School of Medicine, Department of Orthopaedics and Traumatology*³*Koc University, School of Medicine, Department of Neurology*

FSHD1 is generally associated with 1-10 D4Z4 repeat units (RU) and a permissive 4qA haplotype. Molecular diagnosis of the disease has always been challenging. Technical developments during the last decade have enabled a more comprehensive characterization of FSHD. This study aims to share our experience as Turkey's first FSHD Molecular Research & Diagnostics Unit. We have investigated D4Z4 RUs of 56 patients from 52 unrelated families using molecular combing method. FSHD evaluation scale was used for clinical examinations. The majority of patients, 32 (59.3%), were within the scores of 5-10. Familial cases were 52% of all cases, 30 females (54%) and 26 males (46%). Age of onset for 36 patients was ≤ 20 yo (64.3%) and 19 patients ≥ 20 yo (35.7%). Amongst 56 patients, diagnosis of FSHD1 was confirmed in 48 (86.5%), with a DRA between 1-10 RU, of which 39 (81.25%) had DRA between 1-7 RU. A patient with 10 RU was found to have a 421 kb deletion on chr18 by WES, resulting in the deletion of SMCHD1 gene along with 3 flanking genes, confirming an FSHD2 diagnosis. The analysis of his affected son revealed 8 RU for 4qA. The microarray analysis of both patients showed the extent of the deletion was actually 1.75 Mb. Seven patients without any reduced alleles, four of whom had reduced 10qA, will be further analysed for a possible 4q/10q recombination and FSHD2 in the scope of our studies. To the best of our knowledge, this is the largest molecular diagnosis cohort presented from Turkey.

P5.52**Prevalence and disease progression of genetically confirmed facioscapulohumeral muscular dystrophy type 1 (FSHD1) in China between 2001 and 2020: a nationwide population-based study****Zhiqiang Wang**¹, Liangliang Qiu¹, Minting Lin¹, Long Chen¹, Fuze Zheng¹¹*The First Affiliated Hospital of Fujian Medical University*

Facioscapulohumeral muscular dystrophy type 1 (FSHD1) is a rare disease, which is often underdiagnosed due to its heterogeneous presentations and complex molecular genetic basis, leading to a lack of population-based epidemiology data, especially of prevalence and disease progression. Fujian Neuromedical Centre (FNMC) is a diagnosis centre for clinical-genetic FSHD in China, and the only one employing pulsed-field gel electrophoresis-based Southern blotting for all FSHD1 genetic tests. Three sources distributed across China were used to obtain information regarding FSHD1 events, namely, FNMC, Genetic and Myopathy Group, and “FSHD-China” (an organization supported by FSHD patients). During 2001-2020, all genetically confirmed FSHD1 patients from China were registered in FNMC. Follow-up was conducted in the 20-year period to obtain data on disease progression. Of the 1,744 FSHD1 genetic tests (total test number 1,802) included in the analysis, 997 (57.2%) patients from 620 families were diagnosed with FSHD1. Our research captures the largest genetically confirmed FSHD1 population worldwide to calculate its prevalence of 0.75 per million in China from 2001 to 2020, with 0.78 in males and 0.71 in females. The estimated prevalence increased from 0.22 per million in 2001-2015 to 0.53 per million in 2016-2020 ($p < 0.001$). Approximately 12.0% of symptomatic plus asymptomatic patients of FSHD1 will lose independent ambulation in 40 years from onset of first-ever muscle weakness.

P5.53

Association between D4Z4 hypomethylation and disease severity: a retrospective cohort study in China

Zhiqiang Wang¹, Fuze Zheng¹, Long Chen¹, Liangliang Qiu¹, Lin Lin¹, Minting Lin¹, Ying Fu¹, Ning Wang¹

¹*The First Affiliated Hospital of Fujian Medical University*

Background: Facioscapulohumeral muscular dystrophy (FSHD) is a common benign muscular dystrophy with strong clinical heterogeneity. Hypomethylation of D4Z4 region at 4q35 is associated with FSHD. However, the relationship between methylation and the clinical phenotype of FSHD remains unclear.

Methods: This retrospective, observational, single-center cohort study was conducted from a Chinese FSHD1 cohort, enrolled 826 FSHD1 patients and 299 controls. The methylation level in the D4Z4-PAS region was detected by sodium bisulfite sequencing. We analyzed the relationship between methylation level (stratified by quartile: LM1, LM2, LM3, and HM) and disease severity.

Results: We found methylation has significant but relatively weak correlation with D4Z4 units, age of onset, CS, CSS, and ACSS. Hypomethylation groups (LM1, LM2) showed significantly higher clinical scores and earlier age of onset compared with a high methylation group (HM). Of individuals in LM1, 30 (14.5%) were more than twice as likely to suffer independent ambulation loss (HR 2.29, 95%CI 1.18-4.43, $p=0.014$) compared to 14 (6.8%) in HM. The same significant risk was also present in the LM2 group. After adjustment for sex, age of visit, and D4Z4 units, it still showed a significantly increased risk of disability (aHR 2.69, 95%CI 1.33-5.44, $p=0.006$).

Conclusion: The detection of hypomethylation has an indicative role in the prediction of disease progression and prognosis, and should be considered in FSHD1 clinical research.