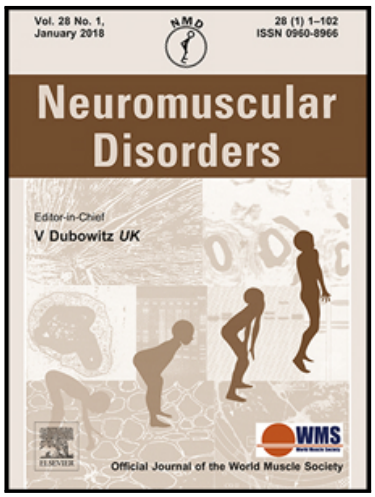


# MEETING REPORT: THE 2022 FSHD INTERNATIONAL RESEARCH CONGRESS

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# MEETING REPORT: THE 2022 FSHD INTERNATIONAL RESEARCH CONGRESS

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## Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common genetic myopathies affecting ~1 in 8,000 to 15,000 individuals. FSHD is caused by a toxic-gain-of-function due to de-repression of a pioneer developmental gene, Double homeobox protein 4 (*DUX4*). Although highly heterogeneous and asymmetric in its presentation, FSHD typically affects the muscles of the face, upper arms, scapula, abdomen and legs. The 2022 FSHD International Research Congress (IRC) was organized as a hybrid event on June 16-17 in Orlando, Florida, USA. The meeting convened approximately 220 attendees to discuss and present the latest developments in the understanding of the pathophysiology and interventional strategies for FSHD.

## Keynotes and Guest Speakes

The 2022 FSHD IRC opened with a keynote presentation by Lexi Pappas, a patient advocate in her late twenties. She presented a personal and moving account of the progressive toll this disease has taken on her life, highlighting how the disease can result in significant functional losses over just a few years. Alexandra Belayew (University of Mons, Belgium) followed with a historical perspective on the discovery of DUX4 as the toxic agent in FSHD and the many challenges she and her collaborators faced in deconvoluting the complex genetics of repetitive DNA elements surrounding the DUX4 gene. Eva Chin, executive director of Solve FSHD (Canada), introduced their venture philanthropic organization that has committed $100M towards the development of treatments for FSHD. Solve FSHD's strategic plan includes providing seed funding for novel therapeutic strategies, creating preclinical solutions to accelerate IND-enabling studies, and promoting collaborative networks. Jane Larkindale (PepGen, USA), former

Executive Director at Critical Path Institute, provided an overview of the development of the Rare Disease Cures Accelerator-Data and Analytics Platform (RDCA-DAP) which has now expanded to include datasets from the placebo arms of several historical clinical trials in FSHD.

**Session 1: Discovery Research**

Studies presented in the discovery research session provided new insights regarding molecular pathways regulated by DUX4 and potential therapeutic targets. Danielle Hamm (Fred Hutchinson Cancer Center, USA) presented evidence that transient DUX4 expression disrupts several key regulators of translation, which leads to broad translational suppression and reduction of de novo protein synthesis. Christopher Brennan (Entrada Therapeutics, USA) investigated regulatory events activated by DUX4 in myoblasts using longitudinal RNA sequencing paired with proteomics and phosphoproteomics indicating that DUX4 expression activates JNK and p38 MAP kinases in myoblasts [1]. Paola Ghezzi (San Raffaele Scientific Institute, Italy) presented data regarding a candidate therapeutic for FSHD. The details are not included in this report at the request of the authors.

Session 1 posters can be found in the supplementary material.

**Session 2: Genetics & Epigenetics**

Studies in this session reported the latest findings related to the exploration of the 4q35 locus using long read sequencing approaches together with findings on the epigenetic regulation of the region.

Russell Butterfield (University of Utah, USA) described a Cas9-targeted enrichment for Nanopore-based DNA sequencing and methylation profiling of D4Z4 in FSHD patients. Differential DNA methylation between healthy individuals and FSHD patients can be quantified using this approach. Overall, DNA methylation is lower in FSHD patients compared to controls, and methylation increases in a gradient from the most proximal to the most distal D4Z4 unit in all samples. These methylation patterns are stable over generations of affected individuals. Each D4Z4 repeat has regions of very low methylation overlapping with the previously identified CTCF binding site and insulator region but a higher methylation starting at the DUX4 transcription start site.

Emanuele Mocciaro (St. Raffaele Scientific Institute, Italy) described a strategy to inhibit *DUX4* expression by targeting factors able to regulate the DBE-T, a long non-coding RNA that acts as a regulator of DUX4. Using a proteomic approach, he identified WDR5, a component of the MLL/SETDB1 complex as a key factor required for DBE-T activity and regulation of *DUX4*, that when knocked-down decreased *DUX4* expression and ameliorated FSHD muscle differentiation. He then described results on a small molecule inhibitor of WDR5 as a possible therapeutic approach for FSHD.

Anna Kapurkina (CNRS, France) presented her work on the 3D organization of the 4q35 region and implication in disease using a dCas9-CTCF fusion to reestablish the loop that is lost in patients carrying a short D4Z4 array. In this reporter system, the dCas9-CTCF construct exhibits insulator activity on the D4Z4 enhancer *in vitro*. Furthermore, the dCas9-CTCF fusion decreased expression of *DUX4* and of DUX4 target genes confirming a role of the 4q35 3D folding in the regulation of D4Z4.

Session 2 posters can be found in the supplementary material.

**Session 3: Pathology & Disease Mechanisms**

Studies in this session reported novel insights into DUX4-regulated RNA-protein complexes, DNA damage signaling, mitochondrial metabolism, and antiapoptotic proteins. The session began with a presentation from Prakash Kharel (Brigham and Women's Hospital and Harvard Medical School, USA) investigating guanine-rich regions of the *DUX4* mRNA transcript that are predicted to fold into a four-stranded secondary structure known as a G-quadruplex (G4). Kharel showed that regions of both the *DUX4* open reading frame and the *DUX4* 3'UTR formed G4s in vitro. These RNA G4s contributed to DUX4 toxicity in skeletal muscle cell cultures, possibly via their formation of G3BP1-positive stress granules. This work provides mechanistic insights into the potential role of G4s in FSHD pathogenesis.

This was followed by Tessa Arends (Fred Hutchinson Cancer Center, USA) who showed that DUX4-expressing cells have increased *HSATII* mRNA levels and elevated DNA damage as measured by phosphorylated H2AX, although HSATII knockdown did not diminish DNA damage. Interestingly, cells with elevated DNA damage displayed a global loss of ubiquitylated H2A and a redistribution of the H2A E3 ligase, RNF2, into aggregates. Arends further demonstrated that *HSATII* RNA and RNF2 protein co-localize, with RNF2 occupying HSATII genomic loci via ChIP analysis. Myoblasts with aberrant RNF2 foci had impaired 53BP1 recruitment, decreased DNA damage signaling via ATM / CHK2 / p53, and unrepaired DNA breaks. Several of these results were confirmed using FSHD patient-derived muscle cells. Overall, Arends' model suggests that activation of *HSATII* RNA by DUX4 sequesters PRC1 components, which disrupts DNA damage repair signaling pathways.

The last 2 presenters Philipp Heher (King's College London, UK) and Michael Kyba (University of Minnesota, USA) reported published studies on the role of DUX4 in oxidative stress and a new DUX4-mediated apoptosis regulatory mechanism respectively [2; 3].

Session 3 posters can be found in the supplementary material.

**Session 4: Interventional Strategies**

This session highlighted a diverse array of therapeutic strategies being developed by both academia and industry. In the first presentation, Nizar Saad (Nationwide Children's Hospital, USA) described his techniques for isolating and characterizing various types of extracellular vesicles with a focus on mesenchymal stem cell-derived extracellular vesicles (MSC EVs) and showed promising data on how MSC EVs can provide protection against DUX4-induced muscle damage. Next, Karim Azzag's (University of Minnesota, USA) therapeutic focus was targeting fibrosis, muscle regeneration, and strength, through the delivery of pluripotent stem cell derived myogenic progenitors. Using the iDUX mouse model they showed improvements in all the targeted readouts and even enhanced engraftment in the presence of DUX4; thus, suggesting a potential therapeutic benefit for patients. Afrooz Rashnonejad (Nationwide Children's Hospital, USA) presented an update on her AAV-based CRISPR-Cas13 gene therapy and showed promising *in vitro* data including strong DUX4 silencing and reduction of FSHD biomarkers. However, while early timepoint in vivo treatments of the AAV.DUX4 co-injection mouse model showed promising results, pronounced immune cell infiltration was detected at later timepoints highlighting that minimizing the cellular immune response may be necessary to translate AAV-Cas13b therapy.

The final presentation was from Barbora Malecova (Avidity Biosciences, Inc., USA) describing the company's transferrin receptor 1 antibody conjugated antisense oligonucleotide therapy: AOC1020. Malecova described how AOC1020 reduced DUX4 biomarkers in FSHD patient-derived cell lines, as well as dosing studies using a mouse equivalent molecule in the ACTA1-MCM FLExDUX4 mouse and PK results in nonhuman primates. Since the 2022 FSHD IRC congress, Avidity Biosciences announced a Phase 1/2 FORTITUDE™ trial of AOC1020 in adults with FSHD.

Session 4 posters can be found in the supplementary material.

**Session 5: Clinical Studies & Outcome Measures**

The final session of the 2022 FSHD IRC provided an update on the latest results from ongoing clinical studies. Starting the session, Katy Eichinger (University of Rochester Medical Center, USA) presented baseline data from 247 enrollees in the Clinical Trial Readiness to Solve Barriers to Drug Development in FSHD (ReSolve FSHD) study, which is being conducted across 11 sites in the United States and Europe. The overall goal of the study is to validate new clinical outcome assessments (COA) and refine trial-planning strategies in the FSHD population. The study enrolled genetically-confirmed ambulatory adults and collected data at 6 visits over 24 months, with the first two visits taking place on consecutive days to perform repeatability assessments of functional outcomes. COAs included dual-energy X-ray absorptiometry, pulmonary function testing, reachable workspace, electrical impedance myography, and patient-reported outcomes. The median forced vital capacity was 90% of predicted across the cohort, with approximately 25% of enrollees having a forced vital capacity of <80%. The study examined a new composite assessment (the FSHD-COM), which combines 13 motor

assessments to reflect whole-body function. A stronger linear correlation was observed among more mildly affected enrollees, with more severely affected participants showing greater day-to-day variability. As data collection is completed (in spring of 2023), the investigators will examine the responsiveness of these COAs and assess the minimal clinically important change in performance. They will also evaluate the impact of age, gender, D4Z4 repeat number, and baseline functional status on participant performance. The data from the ReSolve-FSHD study will be made available to potential collaborators for the purpose of designing clinical trials and studies in FSHD.

Sjan Teeselink (Radboud University Medical Center, the Netherlands) presented data from a cross-sectional single-center study aimed at evaluating muscle ultrasound (US)-derived biomarkers in a large cohort (n=115) of FSHD patients with differing levels of disease severity and compared with clinical outcome measures. The trapezius was found to be the most frequently affected muscle, followed by the rectus femoris, while the rectus abdominis was the least affected. The latter finding differs from prior observations made using magnetic resonance imaging (MRI). Strong correlations between composite US and clinical measures, both global and muscle specific, were found. There were similarly strong correlations between qualitative and quantitative scores, with some discrepancies in specific muscles such as the tibialis anterior and the medial gastrocnemius. Longitudinal studies to evaluate the responsiveness of these US biomarkers are ongoing.

Mauro Monforte (Fondazione Policlinico Universitario A. Gemilli IRCCS, Italy) presented a study aimed at identifying the muscle MRI features that can distinguish FSHD from disorders with significant clinical overlap. A large sampling of MRI scans (n=295) was

analyzed using a machine learning approach to identify the important features and combinations of muscle involvement that were most specific to FSHD, which have now been published [4].

Jeffrey Statland (University of Kansas Medical Center, USA) presented an analysis of reachable workspace (RWS) data collected as part of two clinical trials of losmapimod in FSHD. The first trial was an open-label, single-site trial in which 14 enrollees received oral losmapimod for 52 weeks. The second trial was a phase 2 trial that randomized 80 participants to either losmapimod or placebo. Over the duration of the dosing period, the RWS volume of the placebo group appeared to decrease while the RWS volume of the losmapimod group remained stable. The separation between groups was more visible in the weighted trials. The RWS data was analyzed as an annualized rate of change to allow comparisons with natural history studies, which have shown a loss in RWS volume of 1.6-1.8% over a year. In contrast, the placebo group of the losmapimod trial lost 4-8% of the RWS volume, while the losmapimod group did not show a measurable change in RWS volume. Based on the results of these early-stage trials, the RWS has been selected as the primary outcome measure for an upcoming phase 3 trial of losmapimod.

Session 5 posters can be found in the supplementary material.

**Conclusions & Future Directions**

The 2022 FSHD IRC concluded with the presentation of two awards in recognition of exemplary work in the field of FSHD. Dr. Mitsuru Sasaki-Honda (Kyoto University, Japan) was the recipient of the best poster award for his work on epigenetic silencing in

FSHD1 and FSHD2 iPSC-based FSHD disease models. Dr. Mauro Monforte (Fondazione Policlinico Universitario A. Gemilli IRCCS, Italy) was awarded the young investigator prize for his seminal clinical work using magnetic resonance imaging aimed at characterizing changes associated with disease progression.

With an increasing understanding of the complex regulatory networks influencing DUX4 expression, as well as the ensuing pathophysiological signaling leading to skeletal muscle wasting in FSHD, innovative interventional therapeutic strategies are being developed to address this devastating indication. These efforts, combined with the development of sensitive measures for tracking disease progression and clinically meaningful endpoints, allow for the design of more accurate clinical trials, are reflected by a concomitant increase in participation from the pharmaceutical sector and should be seen as a promising sign that treatment(s) for the FSHD patient population are being realized. The 2023 FSHD International Research Congress is planned for June 15-16 as a hybrid event to be held in Milan, Italy.



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Giorgio Tasca, Rome, Italy

Yegor Vassetzky, Paris, France

Lindsay M. Wallace, Columbus, USA

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