

Research conference report

26th Annual Facioscapulohumeral Dystrophy International Research Congress Marseille, France, 19–20 June 2019

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1. Introduction

Research in Facioscapulohumeral Muscular Dystrophy (FSHD) has reached the stage where we are seeing the first drug trials aiming at reduction of its pathological gene product DUX4. Driven by this development and by increased international collaboration on translational research and trial preparedness, the FSHD Society has decided to hold its annual International Research Conference (IRC) alternating between the USA and in Europe, beginning this year with the 26th IRC held in Marseille, France. The meeting occurred on 19–20 June, 2019, at the Palais du Pharo, a historical palace built in the second half of the 19th century by Napoleon III for his wife, the empress Eugenie.

In a full program of two days, 52 podium talks and 35 posters were presented, offering inspiration and collaboration among the 180 participants. The program committee made special efforts to attract clinical researchers, as advancement of drug development brings greater urgency to improving our understanding of the clinical features of FSHD, genotype-phenotype correlations, natural history, and optimal ways to measure disease progression. With the recent advances in FSHD studies now including large sets of data, greater availability of biomaterials from multiple large research initiatives, and the rapid approach of more clinical trials, the FSHD IRC is more relevant than ever to ensure dissemination of the latest research findings, set needs and priorities, and encourage collaboration to speed up progress toward delivering effective treatments to patients and families.

In the welcome session, Mark Stone, CEO of the FSHD Society, summarized the international patient advocacy group meeting that was held on the previous day (June 18), which gathered representatives of patients associations from six European countries as well as Brazil, China, Israel, Japan, and the United States.

2. Plenary and keynote lectures

The meeting opened with a plenary session aimed at presenting the condition from the point of view of affected individuals. A 10-min video gave an intimate look into the life of Pierre Laurian. Afterward, Pierre Laurian and Marie Martine Fleck discussed the impact of FSHD on their daily lives.

Jamshid Arjomand, CSO of the FSHD Society, presented an overview of a recent Industry Collaborative meeting the Society hosted in March of 2019 (available at www.fshdsociety.org) aimed at identifying gaps in FSHD clinical trial readiness. These gaps included issues with genetic testing, lack of molecular or validated imaging biomarkers and a need for a more comprehensive understating of the natural history of FSHD that might be obtained by the integration of data from the existing registries. To address these gaps, he highlighted the FSHD Society's launch of the Therapeutic Accelerator Initiative and a set of prioritized activities, involving all stakeholders, to help expedite their resolution.

Three keynote lectures opened the first and second day scientific sessions. The first keynote lecture was given by Michel Fardeau, founder of the Institut de Myologie in Paris and curator of the archives of Professor Dejerine. He presented the first description and illustration of FSHD by

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Duchenne in his famous book on the “electrification localisée,” and dwelled on the lives of the two authors who gave FSHD its eponym – Landouzy and Dejerine – and on their relationships with various famous neurologists active in the medical circles of Paris at the end of the 19th century [1]. Professor Fardeau also reviewed postmortem muscle samples of Dejerine’s patients proving that FSHD is a myopathy. Pathology slides of the facial muscles showed in one case fatty degeneration and in another normal muscle, leaving open the discussion whether the facial muscle involvement in FSHD is more a hypoplastic or a dystrophic process.

Next, Nicolas Levy, head of the Department of Medical Genetics at Aix-Marseille University, summarized the genetic features of FSHD, from the early identification of the locus in 1990 to the latest improvements [2,3]. He presented an overview of the different regions of the 4q35 locus associated with the disease including the D4Z4 repeat array, the DUX4 gene with its polyA addition signal on the permissive type A telomeric allele and the proximal SSLP of still unknown significance in the pathogenesis [4,5]. He then summarized the recent findings related to the discovery of mutations in the *SMCHD1* gene. This gene is mutated in FSHD2 patients presenting with clinical FSHD but in the absence of D4Z4 array shortening, but also in patients with Bosma arhinia microphthalmia syndrome, an unrelated disease without any clear sign of muscular dystrophy [6,7]. Dr. Levy also presented the molecular combing technique allowing a direct visualization of D4Z4-associated alleles (4q35 and 10q26) and the recent identification of recurrent *cis* duplication of the 4q35 region in a number of patients and other complex rearrangements of the FSHD locus [8]. Dr. Levy concluded his talk by underlining the remaining difficulties for genotype-phenotype correlations and prediction of the course or onset of the disease in the majority of patients.

On the second day, Bradley R. Cairns of the Huntsman Cancer Institute at the University of Utah gave a keynote address on the role of DUX4 in development. He summarized his published work showing that human DUX4 and mouse Dux are transiently expressed at the onset of gene expression in the early cleavage stage embryo, where DUX4/Dux drive expression of a large component of the initial wave of zygotic gene activation [9]. He then discussed new work from his lab led by postdoctoral fellow Edward Grow on the signaling pathways and transcription factors that activate Dux in mouse embryos and DUX4 in human embryos. Both Dux and DUX4 display a brief burst of expression in embryos, limited to less than one cell cycle and coincident with zygotic genome activation (ZGA). Cairns described his unpublished work in embryonic stem cell lines showing that both Dux and DUX4 are activated by DNA damage, utilizing components of the canonical signaling pathway. Prior work has shown that DNA damage signaling is present in virtually all early mammalian embryos, due to the repackaging of the paternal genome into nucleosomes (from protamine) following fertilization, which temporally precedes ZGA. Next steps, currently in progress, include determining the mechanisms linking DNA

damage to DUX4 regulation and whether DNA damage activates DUX4 in FSHD patient-derived iPS cells and muscle cells.

Session 1: The FSHD clinical phenotype. Co-chairs, George Padberg and Peter Lunt.

The first talk, by Nicol Voermans (Radboud UMC, Nijmegen, The Netherlands), discussed the clinical characteristics of childhood FSHD and the implications for trial-readiness [10]. She presented the data on the natural history in 32 children with FSHD. FSHD in childhood consisted of facial weakness with normal or only mildly affected motor performance, decreased functional exercise capacity, pain and fatigue, decreased quality of life, lumbar hyperlordosis, and abnormalities on muscle ultrasound. Systemic features such as hearing loss, retinal, and cardiac abnormalities were infrequent and often subclinical. Patients had a mean D4Z4 repeat array of 5 units (range 2–9 units) and 14% of the mutations were *de novo*. FSHD in childhood is more prevalent than previously known and has a broader phenotype than early-onset FSHD. It resembles classic FSHD both genetically and clinically.

In the second presentation, Jos IJspeert (Radboud UMC, Nijmegen, The Netherlands), showed videos of FSHD patients with various degrees of scapular dyskinesia and demonstrated how to discriminate dyskinesia due to loss of muscle strength from dyskinesia due to loss of motor control and suggested that FSHD patients with the latter condition might benefit from scapular coordination training.

Ceren Hangül (Akdeniz University, Antalya, Turkey) discussed endocrinological parameters in FSHD. She tested the effects of estradiol and other hormones on severity, by using facial 3D morphology scan (f3D) as an indicator of facial severity and the clinical severity score (CSS) as an indicator of total severity. Neutral (fN), eyebrow elevation (fEE), maximal closing of the eyes (fCE), maximal showing of the teeth (fST), whistling (fW) and maximal compression of teeth (fCT) were measured. fCE and fW were significantly different in FSHD compared to the control group. fW correlated with free-testosterone, total-testosterone, estradiol, progesterone, estradiol/free-testosterone ratio, estradiol/total-testosterone ratio in 11 FSHD patients. CSS correlated with estradiol, progesterone, testosterone, estradiol/free-testosterone ratio, estradiol/total-testosterone ratio in 38 FSHD patients. The correlation was significant after menopause in 15 females as well as in 23 males before the age of thirty. These results suggest that there is a correlation between hormone levels and disease severity and that fW could be used as a specific indicator of facial severity in FSHD.

The last presentation in this session was by Sabrina Sacconi (University Côte d’Azur, Nice, France). She reported on an increased frequency of a wide range of keratinocyte-related skin diseases in FSHD, particularly in patients with 7–10 D4Z4 repeats and with FSHD Type 2. No correlation with disease severity or disease duration was found.

Session 2: Genetics and molecular findings for genotype-phenotype correlations and genetic diagnostics. Co-chairs, Rossella Tupler and Meena Upadhyaya.

Studies in this session reported the complexity of genotype-phenotype correlation in FSHD. Cinzia Bettio (University of Modena and Reggio Emilia, Italy) presented data on the clinical evaluation of 1703 individuals carrying one contracted allele with 1–10 D4Z4 repeats from the Italian National Registry for FSHD using the FSHD Comprehensive Clinical Evaluation revealed different clinical phenotypes among 842 probands ranging from the classical phenotype, to facial sparing or atypical myopathic phenotypes. Among 862 relatives belonging to 413 unrelated families and bearing the D4Z4 contracted allele segregating in the family, 45.1% have no muscle weakness; however, 4q haplotyping was not reported. These results highlight the importance of building a precise phenotypic classification of probands and families for a correct stratification of patients.

Muriel Kuipers (Leiden University Medical Center, The Netherlands) presented a study on the nonpenetrance of the 4A166 haplotype as shown in multiple unaffected carriers of FSHD1-sized D4Z4 repeats associated with 4A166 allele. Detailed genetic studies were presented of the 4A166 allele alongside with the presentation of a novel family with multiple unaffected carriers of an FSHD-sized D4Z4 repeat on a 4A166 background. The genetic studies did not reach a definitive conclusion to understand the non- or reduced permissiveness of this haplotype.

Variants in the chromatin modifier SMCHD1 are associated with two clinically unrelated genetic disorders: FSHD2 and the nasal developmental syndrome (Bosma arhinia microphthalmia syndrome, or BAMS). In both diseases, quantifiable hypomethylation of the DUX4 locus is found. Richard J. L. F. Lemmers (Leiden University Medical Center, The Netherlands) reported that sequence analysis and *in silico* prediction is often not sufficient to predict the pathogenicity of identified SMCHD1 variants, but that D4Z4 methylation analysis is essential to establish the pathogenicity of the SMCHD1 mutated alleles [11].

In a study by Peter Lunt (Bristol University, UK), 59 individuals from 9 three-generation families in which alleles with 5–6 D4Z4 repeats segregate with disease, were analysed for sex of transmitting grandparent, parent, and grandchild. Similar analysis was made on published cell-line D4Z4 methylation data in members of 9 multi-generation US pedigrees with 5–6 D4Z4 repeats. D4Z4 methylation results suggested that imprinting may occur in FSHD1 with earlier onset and/or more hypomethylation from maternal inheritance than paternal, and an enhanced or cumulative effect with same sex of grandparent and parent.

Well-designed genotype-phenotype studies merit great attention to define the molecular basis of FSHD.

Session 3: Molecular mechanisms, DUX4, downstream targets, other players. Co-chairs, Peter Zammit and Michael Kyba.

Stephen J. Tapscott (Fred Hutchinson Cancer Research Center, USA) updated us on a recent study analysing MRI-

guided muscle needle biopsies from clinically-assessed FSHD patients. The biopsied samples were graded for pathology with histology and for gene expression via RNA-Seq. This showed gene expression consistent with inflammation, extracellular matrix, and immunoglobulins [12]. A power of this study is that it is also longitudinal, with the same analysis on the same patients then being performed a year after the initial biopsies. Preliminary follow up data indicated that MRI was largely unchanged and DUX4 target gene and ECM/inflammatory markers were overall stable, although some variability in pathology score was observed.

Lawrence Hayward and Dongshend Guo (University of Massachusetts medical school, USA) are modeling primary and secondary developmental myogenesis *in vitro*, using induced pluripotent stem cells from early-onset or late-onset FSHD patients to generate both PAX3-expressing primary myogenic lineages and also ‘induced’ secondary myoblasts. Transcriptomic analysis on these revealed insights into disease mechanism, such as a higher frequency of DUX4 biomarker expression in early-onset- compared to late-onset-derived primary myogenic lineages. Mitsuru Sasaki-Honda (Kyoto University, Japan) has established iPSCs from FSHD1 and FSHD2 patients and used the model to understand how environmental factors including oxidative and genotoxic stress influence DUX4 expression, reporting that both oxidative stress and DNA damage act to increase DUX4 levels.

Upon injury in healthy muscle, the immune system helps regulate the repair program by using cytokines and other signaling molecules to control muscle stem cell function. Maryna Panamarova (King’s College London, UK) presented data showing suppression of a transcription factor in FSHD patient-derived muscle cells. Indeed, restoring its levels in FSHD myoblasts rescued proliferation and improved myogenic differentiation, indicating the potential use of regenerative therapies to augment the endogenous repair processes in skeletal muscle. In contrast, FSHD muscle often exhibits signs of abnormal immune response, with both perivascular and endomysial inflammation. Anna Greco (Radboud University Medical Center, The Netherlands) measured circulating inflammatory markers in blood and found differences including increased IL-6 and TNF α in FSHD patients compared to healthy individuals. To investigate the contribution of trained immunity to these changes, both FSHD and healthy control blood-derived monocytes were trained ex-vivo, but then showed no differences in the cytokine profile produced on stimulation. Such observations imply that changes in circulating inflammatory markers are a response to muscle damage, rather than a result of trained innate immunity.

Another prominent feature of FSHD is the sensitivity of cells to oxidative stress, and Anna Karpukhina (Lomonosov Moscow State University, Russia) reported that inducing DUX4 expression in human M136-iDUX4 myoblasts led to higher levels of reactive oxygen species being generated, and impaired myogenesis. The mitochondria-targeted antioxidant SkQ1 was able to rescue myogenic differentiation in

MB136-DUX4 myoblasts and restored expression of many genes, including *Pitx1*, to control levels. Alec DeSimone (University of Massachusetts Medical School, US) found that another effect of DUX4 was to cause mitochondrial mislocalization. DUX4 caused accumulation of hyaluronic acid, which correlated with delocalization of mitochondria. Inhibiting hyaluronic acid synthesis with 4MU re-established correct mitochondrial localization and reduced DUX4-induced toxicity.

Finally in the session, Nizar Saad (The Abigail Wexner Research Institute at Nationwide Children's Hospital, US) is investigating how to suppress DUX4 levels using miRNAs and demonstrated that miR-675 could directly inhibit DUX4 expression and counteract DUX4 pathogenicity in skeletal muscle and non-muscle cells. Deletion of miRNA-675 in the TIC-DUX4 mouse model of FSHD improved the DUX4-induced pathological phenotype. The authors speculate that varying levels of miRNA-675 and other miRNA could act as gene modifiers for FSHD, contributing to differences in patient pathology. They are also screening miRNA libraries to identify further DUX4-targeting miRNA candidates.

Session 4: Molecular mechanisms and animal models. Co-chairs, Alexandra Belayew and Scott Harper.

Peter Jones (University of Nevada Reno, USA) crossed his FLExDUX4 [13] and the previously published ACTA1-MerCreMer mice [14] to allow for muscle-specific DUX4 expression upon recombination by Tamoxifen-inducible Cre recombinase. The Jones lab previously showed these mice had chronic low DUX4 levels and myopathic phenotypes before Tamoxifen induction and presented new data that muscle pathology could be increased upon Tamoxifen induction, similar to other inducible DUX4 models [15–17].

Robert Bloch (University of Maryland Medical School, USA) grafted immortalized human myoblasts in immunodeficient mice. The engrafted cells formed mature “human” muscles with innervation and satellite cell niche. DUX4 and its target genes were activated in the FSHD xenografts and inhibited by systemic p38 MAPK inhibitors [18].

Francis Sverdrup (St. Louis University, USA) also showed inhibitors of alpha or beta p38 MAPK suppressed DUX4 and its target genes in muscle cell cultures. Clinically advanced p38 inhibitors developed for inflammatory condition did not interfere with muscle differentiation and might be repurposed for FSHD.

Paul Gregorevic (University of Melbourne, Australia) injected mouse muscles with AAV-expressing inducible Dux and studied early RNA alterations before local myopathy onset. He presented a model of organoid muscles grown from human myoblasts *in vitro* showing a contractile capacity that was weaker for FSHD than in healthy donor cells.

Joel Chamberlain (University of Washington, USA) injected mouse muscles with AAV6 expressing low DUX4 levels from its natural promoter. By confocal microscopy on muscle sections she detected DUX4 immunofluorescence in rare myonuclei and found angular or split fibers suggesting

regeneration defects. RNA seq data largely overlapped with P. Jones's FLExDUX4 mouse data.

Fabiola Moretti (National Research Council of Italy) studied the regeneration capacity of human pericytes implanted in injured mouse muscle. DUX4 expression from a lentiviral vector interfered with the process, while estrogen treatment improved it. These data confirm the protective role of estrogens against DUX4 toxicity previously described by this group in myoblasts [19].

Michelle Percharde (MRC London Institute of Medical Sciences and Imperial College, London, UK) and her group study Dux normal function and its narrow activation peak in 2-cell stage embryos leading to zygotic genome activation. She found Dux activated expression of a LINE1 retrotransposon RNA that bound to D4Z4 repeats and associated with nucleolin and KAP1, resulting in inhibitory chromatin and suppression of Dux expression [20].

Session 5: DNA methylation and epigenetics. Co-chairs, Frédérique Magdinier and Marnie Blewitt.

D4Z4 methylation changes have been observed in FSHD patients upon shortening of the array in FSHD1 or in association with *SMCHD1* mutations in FSHD2, putting epigenetics as one of the main molecular features associated with the condition.

In this session, Marnie Blewitt (The Walter and Eliza Hall Institute of Medical Research, Australia) presented the crystal structure and functional analyses of *SMCHD1*. Critical residues for *SMCHD1* DNA binding and chromatin localization were identified, including the single residue where a missense mutation has been reported in FSHD. However, these residues exhibited a significant degree of functional redundancy in chromatin localization. Taken together with cellular studies that show *SMCHD1* localization is dependent on H2AK119ub, presented data suggest that nucleic acid interactions are required to stabilize *SMCHD1* chromatin binding.

Giancarlo Deidda's lab (Institute of Cell Biology and Neurobiology, National Research Council of Italy) has analyzed a large cohort of FSHD1 subjects for correlation between the length of the D4Z4 array on chromosome 4 and methylation at CpGs in close proximity to the DUX4 polyadenylation sequence (PAS). They showed that DNA methylation is lower than predicted on the basis of the size of the pathogenic alleles suggesting additional factors affecting methylation of the pathogenic allele.

By analyzing the 3D organization of the 4q35 locus, Jérôme D. Robin (Aix Marseille University, France) correlated shortening of the D4Z4 array and the presence of *SMCHD1* mutations with changes in the long-distance interactions with a number of genes along the 4q35 region. Together with previous results, these data highlight the role of D4Z4 as a topological element involved in long-distance interactions of the 4q35 locus [21].

Christopher D. Sarsons from Resverlogix corporation (Calgary, Canada) presented on the efficacy of bromo- and extraterminal domain protein inhibitors (BETi), including apabetalone, in epigenetically mediated DUX4 inhibition.

Davide Gabellini (IRCCS San Raffaele Scientific Institute, Milano, Italy) revealed the identification of a novel interactor of the non-long coding RNA, DBE-T. Silencing or pharmacological inhibition of this factor reduced *DUX4* expression in FSHD muscle cells, suggesting that the factor might be targeted to impede *DUX4* activation in the disease.

Session 6: muscle pathology and imaging. Co-chairs, Rabi Tawil and Giorgio Tasca.

In this session, Yi-Wen Chen (Children's National Hospital, Washington DC, USA) presented elegant data showing plasma membrane repair deficits in FSHD patient-derived myoblasts that is reversed using ASOs targeting *DUX4* mRNA. Additionally, she showed higher levels in cytoplasmic and membrane associated reactive oxygen species (ROS) in FSHD myoblasts. Follow up investigations will test whether ASO treatment will reduce ROS in the FSHD myoblasts and improve muscle membrane repair. Baziel van Engelen (Radboud UMC, The Netherlands) presented pilot study data of muscle ultrasound in 22 FSHD patients followed over one year. The data showed good cross-sectional correlation between muscle ultrasound in five muscles, and in clinical measures of disease severity. One year follow-up evaluations showed no progression in clinical parameters but significant changes in muscle echo intensity in three of the five muscles tested; these changes were observed using both qualitative and quantitative measures of echo intensity. More definitive results of the utility of muscle ultrasound as a disease biomarker awaits the completion of the ongoing large FOCUS 2 study.

Emmanuelle Salort-Campana (Aix Marseille University, France) presented muscle MRI data from a cohort of 25 patients with FSHD1 followed for up to two years. MRI was limited to thigh muscles using T1-weighted images of 35–50 slices resulting in a mean pixel intensity (MPI) score, said to reflect both changes in fatty infiltration and muscle volume. MPI scores correlated well with cross-sectional clinical measures of disease severity. Whereas the clinical scores showed no significant change during the observation period, the MPI changed significantly at a rate of 2.3% per year. The investigators recognize that the MRI requiring manual segmentation of individual muscle is time-consuming, and that an established semi-automated process should facilitate application of this method across multiple muscle groups.

Wenhua Zhu (Fudan University, China) presented a cross-sectional study of fatty infiltration on whole body MRI in 30 FSHD1 patients in Shanghai. Eight body segments, scapular, humeral, pelvis, thigh, calf and axial (PATCHS) were separately measured resulting in better clinical correlation. Using this analysis, a subgroup of patients with greater lower than upper extremity weakness was identified; however, no significant demographic or repeat size differences between the two groups was identified.

Session 7: Natural History. Co-chairs, Sabrina Sacconi and Karlien Mul.

Genotypic/phenotypic correlations along the FSHD1–FSHD2 continuum are challenging. The Italian Clinical Network for FSHD, in a talk by Giulia Ricci (University of Modena and Reggio Emilia, Italy), proposes a classification of FSHD patients in clinical categories based on the use of the Comprehensive Clinical Evaluation Form (CCEF). This approach, which needs to be validated in longitudinal studies, may help in understanding the (epi) genetic complexity of this disease and the variability of its natural history, especially in patients without facial involvement and in patients carrying a borderline number of 4qA repeats (presented by Lucia Ruggiero, University Federico II of Naples, Italy).

Mauro Monforte (Fondazione Policlinico Gemelli IRCCS, Italy) discussed qualitative MRI as another promising sensitive tool, allowing FSHD1 patient stratification based on the presence of short-tau inversion recovery hyperintense muscles (STIR+) at single muscle level. Indeed, a longitudinal study conducted on 100 FSHD1 patients demonstrated that increased fat deposition at follow-up was significantly more present in muscles that were STIR+ at baseline. STIR hyperintense lesions represent promising prognostic biomarkers of an active phase of the disease with faster progression toward muscle destruction.

Session 8: Registries. Chair, Sabrina Sacconi.

In an invited talk, Karlien Mul (Radboud UMC, The Netherlands) noted that patient registries containing clinical and genetic information about FSHD patients are essential to achieve a state of 'clinical trial preparedness' for FSHD. Such registries can provide a valuable tool to quickly identify and contact eligible patients to prevent unnecessary delays in the testing of potential therapies as well as provide knowledge on many aspects of FSHD. Currently 13 national FSHD patient registries have been established and efforts have been made to harmonize data collection among these registries [22]. Over the last few years, the field has seen an increase in collaborative research projects based on data from the registries. Despite this increase in usage of registry data, there are still many opportunities to further expand the use of these data, optimize enrollment of patients into registries and add new ways of data collection.

Session 9: Therapeutic intervention. Co-chairs, Stephen J. Tapscott and Fran Sverdrup.

Therapy development in FSHD is finally reaching stride with talks summarizing new targets, ongoing and upcoming clinical trials, and advancement of antisense strategies toward the clinic. Categories for therapy development include targeting: (i) *DUX4* expression, (ii) *DUX4* mRNA, (iii) *DUX4* cofactors and downstream pathways, and (iv) muscle growth/function.

Screening platforms that measure expression of *DUX4* mRNA/protein during differentiation of FSHD muscle cells have enabled rapid identification of new drug targets. The obvious advantage of targeting *DUX4* expression is that all pathways downstream of *DUX4* are coordinately decreased. Owen Wallace (Fulcrum Therapeutics, USA) presented on the discovery that p38 inhibitors potently and efficiently

suppress the expression of DUX4 and prevent the induction of caspase activity and apoptosis in FSHD myotubes. Fulcrum Therapeutics plans to repurpose the clinically advanced p38alpha/beta inhibitor losmapimod, starting Phase II clinical trials for FSHD in the second half of 2019. Independent verification of p38 as a drug target was also presented in the *Molecular mechanisms and animal models* session.

Joris De Maeyer (Facio Therapies, The Netherlands) presented an elaborate high-content screening platform that simultaneously measured DUX4 protein expression and several phenotypic endpoints in primary muscle cells. Upon screening 90,000 compounds, casein kinase I (CK1) was identified as a drug target and a tool inhibitor of CK1 was demonstrated to suppress DUX4 expression in FSHD myotubes and in a xenograft model of FSHD. For both the p38 and CK1 pathways, the actual mechanism by which inhibition leads to suppression of DUX4 expression is currently unknown.

Lindsay Wallace (Nationwide Children's Hospital, USA) presented on efforts to translate an adeno-associated virus (AAV) RNAi-based therapy that targets DUX4 mRNA [23]. Optimization of AAV serotype, promoter and miRNA targeting sequence for efficacy and safety led to the selection of the development candidate AAV6.U6.mi405 for IND-enabling studies. Rika Maruyama of the University of Alberta reported on the ability of locked nucleic acid/2'-O-methoxyethyl gapmers to selectively and effectively reduce DUX4 mRNA levels in FSHD myotubes and in the FLEX-DUX4 mouse model.

Michael Kyba (University of Minnesota, USA) reported that DUX4 expression led to a massive increase in acetylated histone H3 and showed that a new small molecule inhibitor of p300/CBP acetyltransferase prevented this hyperacetylation, inhibited DUX4 target gene expression in FSHD myoblasts, and protected cells from DUX4-induced cell death. A selective inhibitor of the DUX4-p300 interaction may be a therapeutic strategy that avoids global inhibition of p300 activity.

Jeffrey Statland (University of Kansas Medical School, USA) presented data from the dose escalation part of an ongoing phase 2 study of ACE-083, a locally delivered trap for myostatin and other negative regulators of muscle growth, injected in the tibialis anterior or biceps, which showed dose-dependent increases in MRI muscle volume ~15% at the higher dose cohorts. The tibialis anterior showed concordant decreases in muscle fat fraction.

Session 10: Clinical evaluation, outcome measures and clinical trial readiness. Co-chairs, Jeffrey Statland and Baziel van Engelen.

The prospect of disease-targeted therapies creates a pressing need to advance clinical trial readiness, reflected in studies in this session to address each phase of drug development: molecular biomarkers, clinical outcome assessments (COAs), and patient reported outcomes (PROs). Studies used a variety of techniques to look for circulating or fluid biomarkers, including MRI guidance, mass spectrometry, and micro RNA assays [24], and found candidate biomarkers

which correlated with disease severity, and described pathways involved in inflammation associated with MRI STIR positive signal.

Significant progress has been made toward defining a set of downstream DUX4 targets in human muscle as potential proof-of-principle biomarkers for targeted therapies. The Seattle Wellstone demonstrated increased sensitivity for DUX4 targets by using MRI STIR positive informed muscle biopsies. Fulcrum Therapeutics plans to validate the approach in a separate cohort in preparation for their planned phase 2 study of losmapimod, a p38 inhibitor that blocks DUX4 expression as independently shown by F. Sverdrup's group [25].

Studies of standard ambulatory functional motor COAs revealed knee flexion strength was the primary driver of performance, consistent with prior MRI studies suggesting early hamstring muscle involvement in FSHD [26]. One study noted a need for higher quality psychometric testing in proposed FSHD-specific functional performance measures. The laboratory of Jay Han (University of California Irvine, USA) described a novel COA for upper extremity reachable workspace, which repurposes a Kinect stereographic camera to calculate the functional area of shoulder reach, and showed slow loss of functional area over 5 years depending on baseline shoulder range of motion [27]. The same team described a novel optimized timed up and go (TUG) which combines a supine to sit COA with a TUG.

Karlien Mul (UMC Radboud, The Netherlands) described the development of a Rasch-Built Overall Disability Scale (FSHD-RODS) for later-phase studies, which pared down 159 potential activity and participation items into a linearized 47 item clinimetrically sound PRO, using input from ~500 patients in 3 countries (Netherlands, France, and UK). Its responsiveness is currently being assessed and its cross-cultural validation extended to other countries.

3. Program committee and co-chairs

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