



FSHD research priorities stated by the FSHD research community at the  
**2018 International Research Congress IRC Workshop June 8, 9 in LVNV**

More than 135 scientists, patients, advocates, biotech and pharmaceutical company representatives, and clinicians from throughout the world gathered at the 2018 FSHD International Research Congress (IRC) and Research Planning meetings in Las Vegas, Nevada on June 8-9, 2018 to share and discuss their latest progress and ideas on facioscapulohumeral muscular dystrophy (FSHD) research since the last IRC meeting in 2016. The meeting was co-chaired by Michael Altherr PhD (FSH Society Scientific Advisory Board & Los Alamos National Laboratory, Los Alamos, New Mexico), Marnie Blewitt PhD (Walter and Eliza Hall Institute, Melbourne, AUS), Peter Jones PhD (University of Nevada, Reno), Michael Kyba PhD (Lillehei Heart Institute, University of Minnesota), Jeffrey Statland MD (University of Kansas, Kansas City, Kansas), Stephen Tapscott MD, PhD (Fred Hutchinson Cancer Research Center, Seattle, Washington), Silvère van der Maarel, PhD (Leiden University Medical Center, Leiden, Netherlands), Baziel van Engelen MD, PhD (Radboud University Nijmegen Medical Center, Nijmegen, Netherlands) and Peter Zammit, PhD (King's College London, London, United Kingdom). Daniel Paul Perez (FSH Society, Lexington, Massachusetts) served as the organizational chair.

The goal of this meeting was to integrate clinical and basic FSHD research, explore and verify the complex disease mechanism and various features of FSHD, and to follow up on considerations to move into the development of potential treatments for FSHD. All volunteer agencies working on FSHD were invited by the organizers and encouraged to attend. After a brief welcome by the organizers an overview was presented of previous IRC's priorities and their follow up as defined by the FSHD community in the calendar year 2016. Based on the publications that have appeared in the past year, it was again clear that there had been an exceptionally impressive response to the priorities formulated during the 2016 meeting.

The overview was followed by a full day of 29 platform presentations, combined with poster sessions, reviewing the latest advancements in 1.) genetics, epigenetics and related syndromes and diseases, 2.) the role of DUX4 in development and disease 3.) preclinical studies, including cellular and animal models, 4.) clinical studies and clinical trials, and 5.) Industry aspects and therapy development. Each platform session included presentations selected from pre-submitted abstracts. Group discussion followed each platform session, each moderated by two distinguished scientists, whose role was to facilitate discussion and active and candid debate of the topic. There was time to review and further discuss the latest developments at the posters. The IRC is a working meeting with experts, developing future plans in the context of what we know now and what we need to know. It was a very successful workshop with a positive, constructive and collaborative atmosphere where new and unpublished findings were communicated to the audience, and with excellent interaction between all participants. For the first time, we had presentations from industry with insightful and deep details about their drug development programs.

On Saturday, June 9, after being informed about the funding opportunities at the NIH by Glen Nuckolls (NINDS), a discussion and planning session ensued that included four sessions chaired and moderated by the scientific organizers. The following research priorities were selected for further discussion: (1) Therapeutics, (2) Pathophysiology, (3) Molecular Mechanisms and (4) Genetics and Epigenetics. From these discussions and analysis of transcripts of the meeting the following conclusions were made.

Priorities for FSHD research and funding in the coming year:

## **1. Therapeutics**

Trial Tool Kit. While drug development pipelines are beginning to produce candidate drugs, the trial tool kit needs to be completed. Evidence was presented that MRI STIR positivity correlates well with DUX4 target gene expression but this needs further refinement and to be reproduced. MRI directs to active disease processes but longitudinal studies are needed. Uniform definitions for (interpretation of) MRI imaging

need to be established by the community. In the cardiac field stress tests are informative, which may also be explored in FSHD. Ultrasound is not a priority as it is hard to standardize between users, although does have the advantage of being lower cost and higher throughput, and may be useful earlier in the disease process. Electrical impedance myography may correlate to changes in muscle structure but its sensitivity to changes in muscle over time still needs to be determined. The tool kit comprises biological and non-invasive biomarkers and clinical outcome assessments which will be important for drug approvals.

Biological Biomarkers. The search for biological biomarkers need to be continued. While DUX4 target genes in the muscle may be better targets than DUX4 itself, several efforts are ongoing including RNAseq and proteomics studies, which also point towards suppression of PAX7 target genes as a potential FSHD biomarker. Peripheral serum markers for FSHD are preferred due to their easy assessment from blood samples. Caution should be taken to avoid as many potential confounders as possible.

Clinical Outcome Assessments. Strength, function and functional tests are relevant outcome measures, likely for later phase clinical trials. . Non-invasive or peripheral serum markers may also facilitate trial recruitment. Studies into these COAs preferably need to be done in multi-center context such as the FSHD clinical trial research network, or through international partnerships. Video measures are experimental and need further studies. Home devices, such as activity monitors, allow to follow patients longitudinally, for example in training trials. Patient reported outcomes are in development and will be important ultimately for clinical trials.

Alternative/complementary therapies. Focus is currently on reducing DUX4 expression/function by various means, but therapies that also ameliorate some of the major pathological drivers in FSHD, and/or augment muscle repair, may be quicker to clinic and improve quality of life, while DUX4-targeted therapies are being developed.

## **2. Pathophysiology**

DUX4 With Regard to Fibrosis, Inflammatory Infiltrates and Fatty Infiltration. There is a gap between basic (DUX4) knowledge and clinical observations that need to be filled. It is unclear how assumingly sporadic, barely detectable expression of DUX4 results in generalized muscle weakness and wasting. Investigations in downstream consequences such as fibrosis, inflammatory infiltrates, oxidative stress, repair/regenerative response and fatty infiltration are warranted as these are often targets of therapy in other MDs as well. Studies in animal models may accelerate studies into these downstream pathways.

Temporal Analyses of Models. A number of animal models have been generated and there are more in development. All models have their utility and limitations. It is likely that these models may give us clues into what happens in patients and studies should focus on temporal analyses of these models to capture aspects of the disease over time. The models are also important for therapeutic studies. Imaging may aid temporal studies to define the order of events. Non-human primate models are not considered a priority due to cost and ethical issues?

Novel Models for Other Aspects. Novel models are likely necessary to capture other aspects of the disease. One example is to express mouse Dux in mice, as, although DUX4 and mDux have diverged considerably, there is large functional overlap between both proteins. Other examples are to continue investing in cellular models such as stem cells, iPS cells, organoids / organs-on-a-chip.

## **3. Molecular Mechanisms**

Mechanisms of DUX4 Pathology. While expression of DUX4 is considered the cause for FSHD, the exact molecular mechanisms of DUX4 pathology are only partly understood. Studies of DUX4 and mDux in cellular and animal models are also necessary to understand why cells die from DUX4. Temporal studies

are necessary to capture the heterogeneity and order of events. Caution should be taken with respect to endogenous versus overexpression, primary versus immortalized cells, and origin of the muscle biopsies need consideration.

DUX4 Functionality. Posttranslational modifications of DUX4 may affect its function, co-factors need to be identified and studied and its cellular localization and functionality should be further explored in the context of FSHD pathophysiology. Its role as transcription factor, also in relation to PAX3/PAX7, warrants further investigation. Most studies have focused on pathways that are activated by DUX4, and relatively few studies have addressed those genes and pathways that are suppressed. Roles of DUX4, in addition to being a transcription factor, are relatively understudied.

DUX4 Native and Conserved Function. The natural distribution and regulation of DUX4 expression should be studied. DUX4 is expressed at cleavage stage embryos but little is known about the regulation of DUX4 during development and which tissues express DUX4, as well as its (lack of) toxicity in those tissues. The distribution of DUX4 in FSHD muscle is not known, nor how it correlates with clinical and pathological aspects of FSHD muscle, nor at the preclinical level.

#### **4. Genetics and Epigenetics**

Harmonization of Genetic Testing. New diagnostic techniques are available, including those for prenatal or preimplantation diagnostics. While current DNA diagnostics is accurate, there remains a continuous need for harmonization and improvement, also in view of future trials. New technologies may aid this effort and allow for identification of new disease mechanisms, although it remains to be determined if new strategies are as cost- and time-efficient as current standards.

Disease Continuum and Genotype Classification. While for trial participation genetic confirmation is mandatory, the terms FSHD1 and FSHD2 need to be reconsidered as to prevent potential exclusion of FSHD2 patients from future therapies. The terms FSHD1 and FSHD2 have an historical basis but recent studies show that both are the result of the interaction of at least two genetic variables: the number of D4Z4 units on a 4qA permissive haplotype and variations in genes, such as SMCHD1, that modify the epigenetic repression of the D4Z4.

Understanding Drivers of Clinical Variability. Continuous efforts are necessary to increase our understanding of (the genetic and epigenetic basis) of clinical variability. Data presented at the workshop suggest that 50% of variance is familial, of which 10% comes from D4Z4 repeat size, but that this differs between muscle groups varying from facial muscles, upper and lower extremities approximately 30%, 15% and 3%, respectively. Suggests different vulnerability to DUX4 e.g. face is more sensitive to FSHD1 disease locus whereas leg depends on modifying factors. Genome wide studies in large patient cohorts may facilitate identification of modifiers, such as SMCHD1.

Role and Effects of Modifiers. The role of modifiers need to be better understood. We need to increase our ability to type the function of variants of these modifiers such as SMCHD1 and DNMT3B. Mutations in both disease genes can result in very discordant phenotypes (SMCHD1: FSHD and BAMS; DNMT3B: FSHD and ICF), but the cause for this discordance is not understood. Other modifiers are likely to exist and additional efforts are necessary to identify these genetic, epigenetic and non-genetic modifiers.