**FSH Society Facioscapulohumeral Muscular Dystrophy [FSHD]**
**2016 International Research Consortium & Research Planning Meetings**

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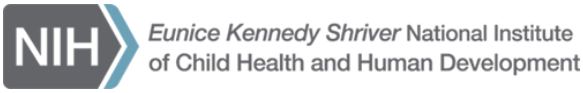

# FSH Society Facioscapulohumeral Muscular Dystrophy [FSHD]
# 2016 International Research Consortium & Research Planning Meetings

**Thursday, November 10, 2016**
**8:30 a.m. – 6:00 p.m.**
[Registration and breakfast begins 7:30 a.m.-]

**Friday, November 11, 2016**
**8:30 a.m. – 12:45 p.m.**
[Registration and breakfast begins 7:30 a.m.-]

**The Westin Copley Place Hotel, Staffordshire & Essex Rooms**
**10 Huntington Ave, Boston, MA 02116 USA**

Co-Chairs: **David E. Housman**, PhD
Massachusetts Institute of Technology, Cambridge, Massachusetts
**Stephen J. Tapscott**, MD, PhD
Fred Hutchinson Cancer Research Center, Seattle, Washington
**Silvère van der Maarel**, PhD
Leiden University Medical Center, Leiden, the Netherlands
**Kathryn Wagner**, MD, PhD
Kennedy Krieger Institute & Johns Hopkins SOM, Baltimore, Maryland

Organizer: **Daniel Paul Perez**
FSH Society, Lexington, Massachusetts

## Sponsored By:

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## PREFACE

November 10, 2016
Boston, Massachusetts

Dear Colleagues,

Welcome to the FSH Society 2016 International Research Consortium & Research Planning Meetings!

This workshop brings together clinicians, scientists, industry, patient representatives and policy makers to discuss the latest developments in facioscapulohumeral muscular dystrophy (FSHD). For twenty years, this gathering has provided the FSHD community with a forum to present and discuss new findings, reinforce collaborative efforts, facilitate new initiatives, and coordinate research and clinical activities.

Impressive scientific progress has been made in recent years and months in our understanding of the disease. This is a critically important time for the community to convene and discuss new data and advances in FSHD; discuss strategies to verify and independently corroborate the findings; discuss focusing efforts and resources in the preclinical gap and translational phase of research; improve diagnostic techniques and criteria for FSHD; and consider and evaluate with industry how to move forward with new and existing therapies for the disorder.

Over the two days, we will revisit the priority areas identified at last year's meeting, and discuss what we have achieved, evaluate the gaps that need addressing, and where we need to focus and invest intellectual, scientific and financial resources. **By the end of day two we should be able to identify whether any of last year's priority areas should change or be modified, and outline a new list/set of priority areas to be considered**.

This meeting is organized by the FSH Society and sponsored by Acceleron, Association Française contre les Myopathies (AFM), aTyr Pharma, BioMarin, Cytokinetics, Facio Therapies BV, FSH Society, Fulcrum Therapeutics, Genea Biocells, Genomic Vision, Genzyme / Sanofi, Idera Pharma, Mouse Specifics, Muscular Dystrophy Association, Muscular Dystrophy Campaign (UK), NIH NICHD UMass Senator Paul Wellstone MD Cooperative Research for FSHD, Quintiles, Sarepta, Ultragenyx. We thank our sponsors for their generous financial support.

**Thursday platform and poster presentations are considered confidential scientific presentations that contain unpublished data and should not be photographed or incorporated in news letters or used in any other manner without the permission of the reporting scientists. There is an assumption of confidentiality for Friday's discussions as well like other scientific meetings.**

It is truly a pleasure to come together to accelerate solutions for FSHD. Thank you for your extraordinary efforts and hard work on behalf of patients and their families.

Sincerely,

David E. Housman, PhD
Massachusetts Institute of Technology, Cambridge, Massachusetts & FSH Society Scientific Advisory Board

Stephen J. Tapscott, MD, PhD
Fred Hutchinson Cancer Research Center, Seattle, Washington

Silvère van der Maarel, PhD
Leiden University Medical Center, Leiden, the Netherlands

Kathryn Wagner, MD, PhD
Kennedy Krieger Institute & Johns Hopkins SOM, Baltimore, Maryland

Daniel Paul Perez
FSH Society, Lexington, Massachusetts

**Thursday, November 10, 2016**
(Meets in Staffordshire Room, 3rd Floor)

| | | |
|---|---|---|
| **Registration & Breakfast** | 7:30 a.m.-8:30 (Essex Ballroom Foyer) | |
| **Welcome** | 8:30-8:40 | **Welcome**<br>David Housman, Daniel Perez, Stephen Tapscott, Silvere van der Maarel, Kathryn Wagner |
| **Review of 2015** | 8:40-9:00 | **Review of 2015/2016 priorities** as stated by FSHD workshop in 2015<br>Moderators: Michael Altherr, Stephen Tapscott |
| **Platform Session 1** | 9:00-10:25 | **Clinical Studies; Genetics & epigenetics** (3x15 minutes & 4x10 minutes)<br>Moderators: Rabi Tawil, Kathryn Wagner |
| | 10:25-10:55 | Discussion |
| Break | 10:55-11:10 | |
| **Platform Session 2** | 11:10-12:15 | **Molecular mechanisms** (3x15 minutes & 2x10 minutes)<br>Moderators: Scott Harper, Michael Kyba |
| | 12:15-12:45 | Discussion |
| **Poster Introductions & Lunch** | 12:45-2:00 | Lunch and **Poster Viewing** [collect and have **Lunch**]<br>(lunch located in Essex Ballroom Foyer) |
| **Platform Session 3** | 2:00-3:15 | **Models** (5x15 minutes)<br>Moderators: Yi-Wen Chen, Louis Kunkel |
| | 3:15-3:45 | Discussion |
| Break | 3:45-4:00 | |
| **Platform Session 4** | 4:00-5:15 | **Therapeutic studies (**3x15 minutes & 3x10 minutes)<br>Moderators: Charles Emerson, Jr., Davide Gabellini |
| | 5:15-5:45 | Discussion |
| **Assembly Session** | 5:45-6:00 | **Discussion and Review of Friday's (Day 2) Agenda**<br>Moderators: David Housman, Stephen Tapscott, Silvere van der Maarel, Kathryn Wagner, Michael Altherr, and Daniel Perez |
| **Adjourn** | 6:00 p.m. | |

*The FSH Society (Facioscapulohumeral Muscular Dystrophy) is an independent, non-profit 501(c)(3) and tax-exempt U.S. corporation organized to address issues and needs specifically related to facioscapulohumeral muscular dystrophy (FSHD). Contributions and financial donations are acknowledged for tax purposes. All inquiries should be addressed to: FSH Society, Daniel Paul Perez, 450 Bedford Street, Lexington, MA 02420. Phones: (781) 301-6650 and (781) 275-7781, fax: (781) 862-1116, e-mail: daniel.perez@fshsociety.org, website: http://www.fshsociety.org*

**Friday, November 11, 2016**
(Meets in Essex Ballroom, 3rd Floor)

| | | |
|---|---|---|
| **Registration & Breakfast** | 7:30 a.m.-8:30 | |
| **Welcome** | 8:30-8:35 | **Welcome**<br>David Housman, Daniel Perez, Stephen Tapscott, Silvere van der Maarel. and Kathryn Wagner |
| Discussion/Planning | 8:35-12:45 | **International "lab meeting"** |

**Planning and problem solving session(s)**

Moderated discussion sessions with entire group of attendees based on data presented at day 1. Co-chairs and organizers will meet end of day Thursday and/or Friday morning before the session to help identify specific topics of interest to lead the discussion around. The goals are to 1.) help identify and troubleshoot bottlenecks; and, 2.) define the research/clinical priorities for the next year 2016/2017.

| | |
|---|---|
| 8:35-11:15 | **Discussion to identify and troubleshoot bottlenecks; and, define the research/clinical priorities going forward**<br>Moderators: Michael Altherr, David Housman and others TBD |
| 11:15-11:45 | **Finalizing listing of items, areas and priorities** |

| | |
|---|---|
| **Lunch** | 11:45 -12:45 p.m.<br>(lunch located in Staffordshire Room, 3rd Floor) |
| **Adjourn** | 12:45 p.m. |

**NOTES ON TALKS AND POSTERS**

| | First/Last Author | Presenting Author | Topic |
|---|---|---|---|
| * = 10 minute talk; others 15 minute | | | |
| Platform Session 1 | | | |
| **Clinical Studies & Genetics and Epigenetics** | | Moderators: Rabi Tawil, Kathryn Wagner | |
| 9:00 – 9:15 a.m. | Capet/Sacconi | Sacconi | 18p deletion |
| 9:15 – 9:30 a.m. | Eichinger/Statland | Eichinger | composite outcome: 1 year find |
| 9:30 – 9:45 a.m. | Gershman/Ashlock | Shukla | ATYR1940 |
| *9:45 – 9:55 a.m. | Gordon/Reversade | Xue | SMCHD1 congenital arhinia |
| *9:55 – 10:05 a.m. | Lassche/van Englen | Lassche | Muscle weakness in FSHD |
| *10:05 – 10:15 a.m. | Shaw/Talkowski | Shaw | SMCHD1 congenital arrhinia |
| *10:15 – 10:25 a.m. | Vercelli/ Tupler | Tupler | Disease progression and natural history |
| 10:25 – 10:55 a.m. | **Discussion** | | |
| 10:55 – 11:10 a.m. | **Break** | | |
| Platform Session 2 | | | |
| **Molecular mechanisms** | Moderators: Scott Harper, Michael Kyba | | |
| 11:10 – 11:25 a.m. | Casa/Gabellini | Gabellini | PRC1 |
| *11:25 – 11:35 a.m. | Eidahl/Harper | Eidahl | DUX4 modifications and interactors |
| *11:35 – 11:45 a.m. | Jagannathan/Bradley | Jagannathan | RNA and Protein Toxicity |
| 11:45 – noon | Lemmers/van der Maarel | Lemmers | bi-allelic expression of DUX4 |
| noon – 12:15 p.m. | Whiddon/Tapscott | Whiddon | DUX4 network |
| 12:15 – 12:45 p.m. | **Discussion** | | |
| 12:45 – 2:00 p.m. | **Lunch and Posters** (lunch located in Essex Ballroom Foyer) | | |
| Platform Session 3 | | | |
| **Models** | Moderators: Yi-Wen Chen, Louis Kunkel | | |
| 2:00 – 2:15 p.m. | Bloch/Jones | Bloch | Mouse xenograft model |
| 2:15 – 2:30 p.m. | Chal/Pourquie | Chal | iPS modeling |
| 2:30 – 2:45 p.m. | Giesige/Harper | Giesige | inducible mouse model |
| 2:45 – 3:00 p.m. | Kyba | Kyba | Cell and animal models |
| 3:00 – 3:15 p.m. | Shi/Emerson | Emerson | iPS models |
| 3:15 – 3:45 p.m. | **Discussion** | | |
| 3:45 – 4:00 p.m. | **Break** | | |
| Platform Session 4 | | | |
| **Therapeutic Studies** | Moderators: Charles Emerson, Jr., Davide Gabellini | | |
| 4:00– 4:15 p.m. | Jubert/Dumonceaux | Dumonceaux | Talen mutation of DUX4 pA |
| 4:15 – 4:30 p.m. | Murphy/Chen | Chen, YW | 3rd generation antisense |
| *4:30 – 4:40 p.m. | Rickard/Schmidt | Rickard | hESC chemical screen |
| *4:40 – 4:50 p.m. | Saad/Harper | Saad | mir-675 and FSHD |
| 4:50 – 5:05 p.m. | Teveroni/Moretti | Moretti | estrogens suppress DUX4 activity |
| *5:05 – 5:15 p.m. | Wallace/Harper | Wallace | siRNA therapies for FSHD |
| 5:15 – 5:45 p.m. | **Discussion** | | |
| **Posters** | [ Ansseau | Ansseau | Transcription DUX4 and DUX4c |
| | Chagarlamudi | Chagarlamudi | Bone Health Cross-sectional Study |
| | Chen | Chen, J | Third Generation Oligonucleotide |
| | Choi | Choi | FSHD myogenic cell model and transcriptome |
| | DeSimone | DeSimone | C1QBP Inhibits DUX4 targeted with 4MU |
| | Gershman | Mendlein/Shukla | ATYR1940 |
| | Glasser | Glasser | ACE-083 |

**NOTES ON TALKS AND POSTERS (continued)**

| | First/Last Author | Presenting Author | Topic |
|---|---|---|---|
| **Posters** | Goselink | Goselink | Characterizing early onset FSHD |
| | Goselink | Goselink | FSHD biomarker: focus on the face |
| | Hall | Chamberlain | Mouse model of FSHD |
| | Hamanaka | Hamanaka | FSHD1 carrying 5-10 D4Z4 repeats and FSHD2 |
| | Homma | Homma | Nuclear bodies |
| | Jones | Jones | Large family cohorts of LCLs |
| | Kazakov | Kazakov | Erb and Landouzy–Dejerine concerning |
| | Lek | Lek | Genome-wide gain- and loss-of-function |
| | Lemmers | Lemmers | SSLP-converter tool to enable |
| | Llach-Martinez | Llach-Martinez | Quality of Xenografts in Mice |
| | Mueller | Mueller | Xenografting Human Muscle Stem into Mice |
| | Moore | Moore | FSHD Diagnostic Testing at Iowa |
| | Pakula | Pakula | transgenic zebrafish model |
| | Statland | Statland | The FSHD Clinical Trial Research Network |
| | Sverdrup | Sverdrup | A High Throughput Xenograft Model |
| | Tasca | Tasca | Muscle microdialysis |
| | Tidwell | Tidwell | FSHD Tissue Donation Registry |
| | Udaka | Udaka | Physiological charact. Early Stage Disease |
| | Wang | Wang | Smchd1 regulating gene expression ] |

**Priorities as stated by FSHD Research Community for FSHD Research in 2016 at the 2015 FSH Society FSHD International Research Consortium, held October 5-6, 2015 in Boston. Massachusetts**

**Priorities Part I. Identifying and Troubleshooting Bottlenecks for FSHD 2015-2016**
As defined by the FSHD clinical and research community

The 2015-2016 IRC for FSHD brought together active members of the research community as well as members of the drug development industry. This was a powerful assemblage that is helping to define the parameters for success moving forward. A number of specific issues were called out that need support both in terms of dollars, as well as consensus of the research community and potential mandates from funding organizations.

The meeting represented a catalyst to move from fundamental studies of genetics and molecular mechanisms (while remaining important) to defining parameters necessary for successful clinical trials. Toward that end an often-cited refrain by the industrial participants was the requirement for the development of therapeutics that have measurable biological outcomes.

A significant priority should, therefore, be the establishment of standards across the community in a variety of areas partially described below. These standards could be required, if demanded, by the organizations, both private and governmental, supporting FSHD research.

I.A. In establishing these standards, the following considerations should be weighed:

1. What is being measured?
    a. How might the measure be used?
    b. Can it be measured with accuracy and reliably in different locations / facilities?
2. How is the measure related to FSHD?
3. How does the measure impact those afflicted?
4. How would modifying the measured phenomena look to the patient

Examples of measurements that might be included are:

1. Quantitative measures of muscle strength
2. Definitive measure of DUX4
3. Other biomarkers

I.B. What's needed:

1. The community should agree on a "robust" model that involves multiple facets of disease for which reliable assays are in hand.
    a. DUX4/Dux4 readouts
    b. Other Biomarkers?
    c. Genetic characterization
    d. Epigenetic characterization
    e. Phenotypic measures

        i. muscle strength
        ii. quantitative walk test
        iii. ophthalmology
        iv. hearing
    f. Cell models
    g. Xenografts
    h. Animal models
        i. Sufficient animal models may already be in hand to support safety studies and phase I trials
        ii. A primate model would be an expensive endeavor. Marmosets are small, known to contain structurally relevant copies of Dux4, and 'relatively' inexpensive primates. This is a recurring theme and should be seriously evaluated

2. Natural history studies are accumulating, but support for longitudinal assessments are lacking. These studies should be linked to and similar imaging analyses should be supported in a longitudinal analysis.
    a. These measurements need to be moved from the laboratory to the clinic
    b. Certainly, a collection of routine measurements can be identified on routine visits
    c. If necessary legislation needs to require payers to support these measurements as part of primary care
    d. These features will likely be critical to long range studies of therapeutic impact

3. Unifying features need to be established between registries so that all groups are including the same or clearly translatable measurements
    a. Sustaining support for these repositories is critical!
    b. Phenotypic measures
    c. 4q genetic description
    d. Epigenetic profile
    e. Cell lines from individuals with measured phenotypic data

It is imperative that the FSHD research community move to a better position in the approach to the problem, and into a highly organized assessment of therapeutic leads. To do so, will require better coordination of widely distribute and often distinct efforts. This could be accomplished through the establishment of defined processes associated with the 'robust model', and used by all members of the research community. These processes could be established by the sharing of protocols and defined substrates made available to the community from laboratories across the world. A defined process could be validated by the sharing of data produced by distributed groups within the boundaries of experimental norms and agreed on by FSH IRC and supporting organizations. Once established new and improved processes could be benchmarked to established processes, and subsequently agreed to replace or add to the processes defining the model. Funding organizations, both public and private,

could and should demand, through their support mechanisms, that established processes be used as benchmarks in all of their supported studies.

**Priorities Part II. Research/Clinical Priorities Defined 2015-2016**
As defined by the FSHD clinical and research community

**II.A Genetics and epigenetics.**

Priority 1: Continued identification of the parameters that determine disease severity and progression, including identification of additional modifier and disease loci. FSHD1 and FSHD2 were initially identified as two distinct genetic pathways that cause FSHD. Work published over the last year and presented at this year's meeting showed that FSHD was 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. Although a subtle change in how we think of the disease, it is possibly profound in understanding disease penetrance and developing models that predict disease progression, the latter being critical for future clinical studies. Additional efforts in identifying disease genes in unexplained FSHD2 cases may further strengthen this process.

Priority 2: Improved diagnostic tests and tests to better predict onset and severity. The advances described in Priority 1 gives guidance on how to develop assays of D4Z4 repression that can be tested for correlation with disease onset and severity. The most obvious is DNA methylation at certain sites or particular D4Z4 repeats, but additional approaches, either allele-specific or based on chromosome or genome averaging, can be envisioned.

**II.B. Mechanisms and targets.**

Priority 3: Determine the major mechanism(s) of muscle damage caused by DUX4 expression. DUX4 in muscle activates a diverse panel of pathways and mechanisms, which individually, or combined lead to muscle pathology. However, our understanding of the primary mechanism and the resulting pathological processes is still limited. A better understanding between cause and consequence will aid biomarker discoveries and therapy development.

Priority 4: Determine the relationship between DUX4 expression and disease onset and progression. The asymmetric onset of disease could be due to different propensities to express DUX4, or DUX4 could be expressed in all muscles and a second event is necessary for the disease pathology. Distinguishing these two models is critical for guiding therapeutic development.

Priority 5: Determine how the expression of DUX4 in one muscle cell nucleus results in the spread of the pathology throughout the muscle. DUX4 protein to spread to adjacent

nuclei in a muscle fiber, but a major goal will be to determine how regional expression of DUX4 spreads both through the entire length of the fiber and to adjacent muscle fibers.

**II.C. Models.**

Priority 6: Continued development and validation of pre-clinical models to test specific pre-clinical goals. A healthy number of pre-clinical models have been developed over the last few years. Further development should focus on validating existing models and developing new models that are needed to test specific interventions and determine successful targeting of specific aspects of the molecular pathogenesis.

**II.D. Clinical and therapeutic studies.**

Priority 7: Validation of subjective and objective measurements of disease onset and progression. Quality of life, muscle function measurements and other physical biomarkers, molecular biomarkers, and imaging biomarkers all show tremendous promise for monitoring disease onset and progression. Individual and cooperative studies to identify, validate, and determine the best standard measurements are critical for trial preparedness in FSHD.

**Part III. "FSHD Champions" Initiative Considerations**

Additional considerations from discussion session on the introduction (to industry, researchers and clinicians) of "FSHD Champions Initiative of FSHD Funding Agencies."

**III.A. FSHD Champions can help with Clinical Trial Preparedness**

The Tuesday, October 6 discussion session on Industry Relations underscored the need for FSHD foundations and groups such as the Champions collective of funding stakeholders to lead in the area of FSHD clinical trials preparedness. Each company will commit resources necessary for its clinical program, but is not likely to commit significant resources prior to having a treatment to move forward, which will be later than ideal and might delay clinical testing. However, each company is interested in "risk management" and might be willing to contribute to a foundation-sponsored effort in return for access to the process and results. The Champions have already taken a lead in this area by sponsoring the second international workshop on FSHD Clinical Trials Preparedness held in Rochester, New York in May 2016. The discussion during the October 6 session on Industry Relations suggested that the Champions leverage their demonstrated ability to make progress in this area by soliciting support from interested companies in exchange for participation in the process.

**III.B. FSHD Champions: might help accelerate efforts in increased targeted approach based on overall assessment of research**

There has been a huge change in FSHD research over the last several years. Several years ago there was little agreement on the genetic mechanism and pathophysiology of FSHD

and no consensus on the most important drug targets or areas of investigation. Now, there is general consensus on the pathophysiology of FSHD and general agreement on the path forward. This "sea change" alters the landscape of FSHD research and has several important implications for the role of FSHD foundations.

1. Achieving a consensus on the genetics and pathophysiology of FSHD increased the participation of major funding agencies. Previously, the fundamental disagreements among the reviewers on NIH or MDA panels regarding the pathophysiology of FSHD prevented the consensus needed for any individual grant to be funded, and might have resulted in low funding success rates for FSHD grants. Funding from these agencies has dramatically increased following a general agreement in the field on the cause of FSHD. Therefore, while the past objective of FSHD foundations was to broadly support the FSHD research community, this role is rapidly being fulfilled by NIH and other major funding groups, providing the opportunity for foundations to begin a re-assessment to determine their most valuable role.

2. Achieving consensus on the pathophysiology of FSHD focuses the field on a common path. The near-term opportunities in FSHD research are relatively clear and many different research groups will be seeking support for similar studies. FSHD foundations might be quickly overwhelmed by trying to support all qualified studies, or might have significant difficulties, or conflicts of interest, in deciding which of the competing studies or approaches to fund. In this regard, the success of the prior FSHD foundation support that led to the consensus model of FSHD is also a major new opportunity for the foundations because it requires a shift in the funding goals to ensure that the foundations continue to drive FSHD research and therapeutic development faster than would be achieved by conventional funding mechanisms. Because the peer-review process of conventional funding mechanisms is now appropriate for the majority of research proposals on FSHD, the FSHD foundations might need to consider mechanisms to identify and fund efforts that have high impact or that make major advances to the fields of FSHD pathophysiology and FSHD therapeutics.

3. Foundations need to support clinical trials preparedness. This is a critical area for therapeutic development that will not be pro-actively addressed by industry nor adequately funded by NIH. Foundations can lead in this area and gain financial support from industry by offering a mechanism of risk-management for industry participants.

4. Foundations can reward research that accomplishes goals critical for FSHD therapeutic development. Previously foundations needed to provide seed money to keep investigators working on FSHD because it was nearly impossible to get grants from the major funding sources. Now major funding sources are supporting FSHD research and the opportunity for foundations to have a major impact on therapeutic development is shifting from funding discovery-oriented research toward rewarding research that solves major obstacles to developing successful FSHD therapies. Although premature to implement, it is not too soon to discuss FSHD PRIZES based on overcoming specific barriers toward therapeutic development. This could be as simple as a small monetary prize (designated for

future FSHD research, such as the further development or implementation of the advance) based on improving diagnostic procedures, finding new modifier loci, prioritizing mechanisms of pathogenesis, advances in pre-clinical models, or identifying new targets for drug development. It might also still be a bit premature to consider a “moon-shot” prize for a cure, but intermediate small prizes might further motivate current FSHD researchers and might recruit new researchers to the field.

| first author | presenter | topic | assign |
|---|---|---|---|
| 1. Bloch | Bloch | Mice by Xenografting Control and FSHD Myogenic Cells | spoken |
| 2. Capet | Sacconi | FSHD type 2 associated with 18p deletion syndrome | spoken |
| 3. Casa | GabeLlini | Polycomb Repressive Complex 1 repeat copy number | spoken |
| 4. Chal | Chal | Modeling Skeletal Myogenesis Pluripotent Stem cells | spoken |
| 5. Eichinger | Eichinger | Composite Outcome Measure: 1 Year Findings | spoken |
| 6. Eidahl | Eidahl | Protein chemistry of DUX4 | spoken |
| 7. Gershman | Shukla | Randomized Double-blinded Placebo study ATYR1940 | spoken |
| 8. Giesige | Giesige | New Inducible DUX4 Knock-in Mouse Model | spoken |
| 9. Gordon | Xue | Gain-of-function mutations SMCHD1 congenital arhinia | spoken |
| 10. Jagannathan | Jagannathan | DUX4 Expression Induces RNA and Protein Toxicity | spoken |
| 11. Joubert | Dumonceaux | Gene surgery using TALEN technology: a therapy FSHD | spoken |
| 12. Kyba | Kyba | Cell and animal models for studying DUX4 in FSHD | spoken |
| 13. Lassche | Lassche | Muscle weakness FSHD loss of contractile mass and | spoken |
| 14. Lemmers | Lemmers | Biallelic DUX4 expression in FSHD2 aggravates severity | spoken |
| 15. Murphy | Chen, YW | Reduction of DUX4 by third generation antisense | spoken |
| 16. Rickard | Rickard | epigenetic modulatory small molecules for DUX4 hESC | spoken |
| 17. Saad | Saad | miR-675 reduces DUX4 expression / confers resistance | spoken |
| 18. Shaw | Shaw | Mutations in SMCHD1 associated with Isolated Arhinia | spoken |
| 19. Shi | Emerson, Jr. | iPSC Modeling of Human Skeletal Myogenesis Disease | spoken |
| 20. Teveroni | Moretti | Estrogens improve differentiation FSHD myoblasts | spoken |
| 21. Vercelli | Tupler | Disease progression and natural history in 246 subjects | spoken |
| 22. Wallace | Wallace | Translating DUX4-targeted RNAi therapy for FSHD | spoken |
| 23. Whiddon | Whiddon | Conservation innovation DUX4-family gene network | spoken |
| 24. Ansseau | Ansseau | Transcription factors DUX4 and DUX4c mRNP granules | poster |
| 25. Chagarlamudi | Chagarlamudi | Bone Health in FSHD: A Cross-sectional Study | poster |
| 26. Chen | Chen, J. | 3RD Generation Gene Silencing Oligonucleotide Thera | poster |
| 27. Choi | Choi | novel human FSHD myogenic cell model transcriptome | poster |
| 28. DeSimone | DeSimone | C1QBP Inhibits DUX4-Dependent Gene Activation 4MU | poster |
| 29. Gershman | Mendlein | Randomized Double-blinded Placebo study ATYR1940 | poster |
| 30. Glasser | Glasser | A Phase 2 study to evaluate ACE-083, a local muscle | poster |
| 31. Goselink | Goselink | Characterizing early onset FSHD | poster |
| 32. Goselink | Goselink | FSHD biomarker: focus on the face | poster |
| 33. Hall | Chamberlain | Analysis DUX4 protein expression biopsies and mouse | poster |
| 34. Hamanaka | Hamanaka | FSHD1 carrying 5-10 D4Z4 repeats FSHD2 are spectrum | poster |
| 35. Homma | Homma | Nuclear bodies reorganize myogenesis differentially | poster |
| 36. Jones | Jones | Large family cohorts LCL a new cellular model for FSHD | poster |
| 37. Kazakov | Kazakov | Erb and Landouzy–Dejerine concerning define FSHD | poster |
| 38. Lek | Lek | Genome-wide gain- and loss-of-function screens | poster |
| 39. Lemmers | Lemmers | SSLP-converter: a new software | poster |
| 40. Llach-Martinez | Llach-Martinez | Methods for Improving Quality of Xenografts in Mice | poster |
| 41. Moore | Moore | FSHD Diagnostic Testing at Iowa from 1998 to 2016 | poster |
| 42. Mueller | Mueller | Xenografting Human Muscle Stem Cells into Mice | poster |
| 43. Pakula | Pakula | A transgenic zebrafish model for FSHD | poster |
| 44. Statland | Statland | The FSHD Clinical Trial Research Network | poster |
| 45. Sverdrup | Sverdrup | High Throughput Xenograft Model to Eval Potential | poster |
| 46. Tasca | Tasca | Muscle microdialysis in FSHD | poster |

| | | | |
|---|---|---|---|
| 47. Tidwell | Tidwell | FSHD Tissue Donation Registry | poster |
| 48. Udaka | Udaka | Physiological char. muscles FSHD Early Stage Disease | poster |
| 49. Wang | Wang | Role of Smchd1 in regulating gene expression | poster |

1.
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**Characterization of muscles generated in mice by xenografting control and FSHD myogenic cells**

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**Abstract**

One in 8,000 to 1 in 20,000 individuals worldwide have Facioscapulohumeral Muscular Dystrophy (FSHD). As manipulations of the mouse genome have not generated a murine model that reproduces the pathophysiology of FSHD, we have developed methods to generate mature human muscle tissue in mice starting with immortalized human muscle precursor cells (hMPCs) from individuals with FSHD and closely related, unaffected controls. We X-irradiate the hindlimbs of NOD-*Rag1nullIL2rγnull* immune-deficient mice, then treat with a myotoxin to destroy the mouse tibialis anterior (TA) muscle. We then inject the hMPCs and electrically stimulate the injected hindlimb periodically to promote engraftment. The grafts that form contain mature human muscle fibers that are innervated and fully differentiated, and that are minimally contaminated by murine myonuclei (see Sakellariou et al., Skelet. Muscle, 2016, 6:4). Grafts formed from FSHD and control hMPCs can be composed of as many as 1,000 human muscle fibers per TA muscle, and some have no detectable murine TA fibers. FSHD grafts are similar to controls, but they express significantly more DUX4 and several downstream targets of DUX4, including TRIM43, ZSCAN4 and MBD3L2. The levels of expression of downstream targets increase linearly with increased DUX4 levels. Consistent with the characteristics of the hMPCs used, the chromosome 4 terminal D4Z4 region in the FSHD grafts is hypomethylated (25% mCpG). These observations indicate that the xenografts reproduce key genetic and epigenetic features of mature FSHD and control muscle tissue. We continue to explore methods to improve the size and quality of the grafts, and have begun studies to examine the factors that promote pathogenesis in FSHD in our xenograft model.

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**Facioscapulohumeral muscular dystrophy type 2 associated with 18p deletion syndrome**

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by genetic and epigenetic derepression of *DUX4*, a gene located within a repeat array of D4Z4 sequences of polymorphic length and located in the subtelomeric region of chromosome 4. FSHD type 1 (FSHD1) is associated with the pathogenic contraction of the D4Z4 repeat array. In contrast, FSHD type 2 (FSHD2) is linked to mutations in *SMCHD1*, a chromatin modifier gene located in chromosome 18. Both types of FSHD require the presence of a permissive polyadenylation signal (4qA) downstream of the D4Z4 array. Recently, 18p microdeletion encompassing the *SMCHD1* gene and causing haploinsufficiency have been associated with an FSHD clinical phenotype in individuals carrying a permissive 4qA allele with a borderline number of D4Z4 repeats, raising the possibility that patients with 18p deletion (18p-) syndrome may also develop FSHD when carrying a relatively short D4Z4 array and a 4qA allele. Here we report clinical, radiological, genetic and epigenetic data on three families confirming this hypothesis. We conclude that clinical signs of FSHD have to be searched in patients with 18p– syndrome and, when present, FSHD2 diagnosis should be suspected.

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**Polycomb Repressive Complex 1 provides a molecular explanation for repeat copy number dependency in FSHD muscular dystrophy**

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**ABSTRACT**

DNA repeats play key roles in the regulation of gene expression at multiple levels. For example, mammalian genomic repeats have been shown to harbor the greatest proportion of histone modifications and to provide binding sites for regulatory factors modulating the activity of tissue-specific promoters or enhancers. Tandem repeats represent more than 20% of the human genome and account for a significant source of genomic variation, since their copynumber is usually highly polymorphic among individuals. Accordingly, to preserve genome integrity, tandem repeats can be targeted by multiple repressive pathways leading to the formation of constitutive or facultative heterochromatin.

FacioScapuloHumeral muscular Dystrophy (FSHD) is one of the most prevalent neuromuscular disorders. In its major form, accounting for 95% of cases, the disease is linked to deletions reducing the copy-number of a macrosatellite repeat called D4Z4, located in 4q35. While healthy subjects display 11-100 D4Z4 units, FSHD patients usually present with only 1-10 D4Z4 units. Interestingly, the residual D4Z4 copy number at the deleted 4q35 allele correlates with disease onset and progression. FSHD patients carrying 1-3 residual D4Z4 units tend to develop the disease earlier and with a more severe outcome than patients displaying 9-10 units. Nevertheless, the molecular mechanism underlying FSHD dependency on residual D4Z4 copy-number is poorly understood.

Here we show that the proximal promoter of the D4Z4-embedded DUX4 gene (DUX4p) is sufficient to nucleate the enrichment of both constitutive and facultative heterochromatin, and to mediate a copy-number dependent gene silencing. We found that both the CpG/GC dense DNA content and the repetitive nature of DUX4p are required for their repressive ability. We showed that DUX4p mediates a copy number-dependent Polycomb Repressive Complex 1 (PRC1) recruitment, which is responsible for the copynumber dependent gene repression. Overall, we directly link genetic and epigenetic defects in FSHD by proposing a novel molecular explanation for the copy number-dependency in FSHD pathogenesis, and offer insight into the molecular functions of repeats in chromatin regulation.

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**Modeling skeletal myogenesis *in vitro* with pluripotent stem cells**

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Skeletal muscles constitute the most abundant tissue in our body and are affected by a number of pathologies, ranging from inheritable progressive muscle dystrophies to cachexia. Progresses to find cures have been slow due to the absence of relevant cellular models of the diseases for drug screening. During development, myogenic progenitors originate essentially from the paraxial somitic mesoderm. Using pluripotent stem cells (PSCs), we developed an efficient, stepwise directed differentiation methods to generate skeletal muscles *in vitro*. This PSC-based system recapitulates several key aspects of skeletal myogenesis including foetal myogenesis, formation of large striated fibers and specification of Pax7+ progenitors exhibiting satellite cells properties (Chal et al, 2015). This allows to design novel in-a-dish models to study and manipulate myogenesis and pathological conditions which are difficult to access *in vivo.* With this in mind, we have recently optimized further human PSC-derived myogenic cultures to enable better phenotypical characterizations and developed novel bioengineering approaches to address the unique challenges associated with the modeling of muscle diseases (Chal, Al Tanoury et al, 2016). Human genotype-specific PSC myogenic cultures offer an unprecedented platform to dissect the molecular mechanisms of muscle pathologies and to explore possible therapeutic avenues.

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**The facioscapulohumeral dystrophy composite outcome measure: 1 year findings**

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**Background**: Developments in the understanding of the pathophysiology of FSHD has escalated the need for reliable, sensitive, and clinically meaningful outcome measures for clinical trials. The facioscapulohumeral dystrophy composite outcome measure (FSHD-COM) is a recently developed, evaluator administered functional measure designed to assess patient identified areas of burden.

**Methods:** We conducted a prospective 12-month observational study of genetically confirmed FSHD participants. As part of this study, assessments of strength and function were made at baseline, <3 weeks, 6 months and 12 months. Strength was assessed using standard manual muscle testing on 14 bilateral muscle groups and fixed quantitative myometry system on 8 bilateral muscle groups. The FSHD-COM was used to evaluate the function of areas and systems typically affected by FSHD including the legs, shoulders and arms, trunk, hands and balance/mobility. Paired t-tests were used to calculate the one-year change for the total FSHD-COM score as well as for individual sub-scale scores and individual items.

**Results:** Thirty-six (67% male) individuals with FSHD completed the study. The mean age was 52.7 years (range 22.2-67.7; sd-11.6). They had a disease duration (age-age of diagnosis) of 13.3 years (range 1-29; sd= 9.5) In terms of disease severity, they had a mean FSHD Clinical Score of 7.0 (range 2-12; sd=2.8). The intraclass correlation coefficient for the FSHD-COM was 0.97 with the subscale components ranging from 0.90 to 0.98). We will present the data for 1-year change at the time of the meeting.

**Conclusions:** The FSHD-COM is an evaluator administered composite of standard functional motor tasks representing a range of body functions from gait and mobility to arm and shoulder function.

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**Protein chemistry of DUX4**

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The *DUX4* gene associated with FSHD encodes a transcription factor protein that is toxic when expressed in cells and muscles of numerous organisms *in vitro* and *in vivo*. We hypothesized that one avenue for developing an FSHD treatment could involve inhibiting DUX4 protein activity. However, little is known about the mechanisms that may confer toxic properties to the DUX4 protein. We hypothesized that characterizing the biochemical properties of the DUX4 protein would provide fundamental information required to ultimately design FSHD therapies aimed at inhibiting DUX4 toxicity at the protein level. Our objectives in this study were to identify DUX4 cofactor proteins and protein modifications that may contribute to DUX4 toxicity in cells and FSHD muscle.

- Identification of DUX4 co-factor proteins: We previously used yeast two-hybrid and co-immunoprecipitation methods to identify DUX4 interacting proteins. However, these assays only provided information about proteins with strong and direct interaction with DUX4. To identify proteins that may interact with DUX4 transiently (e.g. kinases) or indirectly (e.g. transcriptional complex) we performed Rapid Immunoprecipitation Mass Spectrometry of Endogenous Proteins (RIME), which involves chromatin immunoprecipitation of cross-linked proteins. Using this method, we identified and are characterizing putative members of a large DUX4 transcription factor complex in human myoblasts.

- DUX4 post-translational modifications: We performed mass spectrometry to identify DUX4 post-translational modifications (PTMs), including phosphorylated, methylated, and acetylated residues, followed by mutagenesis to determine the impact of modified residues on DUX4 toxicity and transactivation function. We found modification events occurring in both the DUX4 DNA-binding and transactivation domains, some of which impact DUX4-associated toxicity. Finally, we performed a high-throughput kinase assay to identify protein kinases capable of phosphorylating DUX4. We began screening compounds targeting DUX4-specific kinases with high activity, to determine whether we can alter DUX4 toxicity or transactivation function *in vitro*.

Defining the DUX4 interactome, functionally significant residues, and PTMs will help us understand the factors regulating DUX4 protein activity, such as ligand binding affinity, protein stability or subcellular localization. We ultimately hope that this work will provide greater understanding of DUX4 protein biochemistry, which can be exploited to design FSHD therapies that inhibit the toxic effects of DUX4 protein expression

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**A randomized, double-blinded, placebo-controlled, multiple ascending dose study to evaluate the safety, tolerability, pharmacokinetics, immunogenicity, and biological activity of ATYR1940 in adult patients with facioscapulohumeral muscular dystrophy (FSHD)**

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**Rationale**: FSHD, a rare, debilitating, genetic skeletal myopathy is associated with inflammation and dystrophic changes in muscle tissue. ATYR1940, a Physiocrine based protein that modulates immune responses in skeletal muscles preclinically, is identical to substantially all of human histidyl tRNA synthetase, a protein that is released from skeletal muscle.

**Methods**: This double-blinded, phase 1b/2a study evaluated the safety, tolerability, pharmacokinetics (PK), immunogenicity, and biological activity of multiple ascending doses of intravenous ATYR1940 in adult FSHD. Exploratory pharmacodynamic (PD) measures included the individualized neuromuscular quality of life (INQoL) questionnaire, lower extremity muscle targeted MRI and circulating PD markers. Weekly doses of 0.3 (n=4), 1.0 (n=8), and 3.0 (n=8) mg/kg were tested over 4, 4, and 12 weeks, respectively. Patients (n=20) were randomized 3:1 (ATYR1940: placebo) across all dose groups and followed for 4 & 12 weeks after the last study drug dose.

**Results:** A dose-related improvement was detected by INQoL from baseline in all dose cohorts and at 3.0 mg/kg at 12 weeks an improvement compared to placebo of 25.5% was observed (p=0.03). Measured circulating PD markers and MRI did not record substantial differences between placebo and ATYR1940. Manual muscle testing indicated no reportable disease progression. All reported adverse events were assessed as mild or moderate in intensity. One moderate adverse event reported in a test article treated patient (a reversible generalized infusion related reaction in the 3.0 mg/kg dose cohort) was reclassified to a serious adverse event by the sponsor. PK was consistent throughout the study course and across all dose cohorts.

**Conclusion:** The potential signals of activity of ATYR1940 in the INQoL and the safety profile support advancement of ATYR1940 in FSHD and potentially other rare diseases.

Acknowledgments: S Shukla, aTyr consultant and CTI, Inc.

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**Characterization of a new inducible DUX4 knock-in mouse model**

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**Background:** Mouse models are important tools for translational research, if they display phenotypes that can be used for outcomes measures of therapy. Prior efforts to produce mouse models with heritable DUX4 transgenes yielded animals that recapitulate epigenetic and transcriptional phenotypes associated with FSHD but lack overt muscle damage phenotypes. The absence of a model with robust DUX4-associated myopathy is at least partly explained by the extreme toxicity of DUX4, which can impact mouse development and lead to embryonic or perinatal lethality. For several years, we have worked to develop an animal model in which a DUX4 transgene could be (1) passed through the germline without causing developmental arrest, and (2) induced specifically in skeletal muscle at any desired time point. Here, we report the successful generation of such an animal.

**Objective:** To generate an FSHD mouse model that is viable, contains a heritable DUX4 gene and recapitulates overt muscle damage phenotypes useful as outcome measures for therapy development.

**Results:** We generated a new mouse model in which DUX4 can be turned on in skeletal muscle using Tamoxifen-inducible Cre recombinase (called iDUX4-ROSA). In this system, Cre recombinase expression is tightly controlled in a temporal and spatial manner, which allows these mice to develop normally without toxic DUX4 expression. Upon Tamoxifen induction, iDUX4-ROSA mice develop DUX4 dose-dependent myopathic phenotypes, and recapitulate molecular, cellular, and functional deficits associated with muscular dystrophy.

**Conclusions:** We have generated a new FSHD mouse model that expresses a heritable DUX4 transgene and displays overt myopathy including muscle weakness. These animals will be useful for developing of DUX4-targeted therapies of FSHD

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**De novo gain-of-function mutations in the epigenetic regulator SMCHD1 cause congenital arhinia**

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Congenital arhinia is an extremely rare and striking condition characterized by complete absence of the nose with or without ocular defects. Here we report that *de novo* missense mutations in the extended ATPase domain of the epigenetic regulator *SMCHD1* cause congential arhinia. Biochemical tests and *in vivo* assays in *Xenopus* embryos suggest that these mutations behave as gain-of-function alleles. This is in contrast to loss-of-function mutations in *SMCHD1* that have been associated with facioscapulohumeral muscular dystrophy (FSHD) type 2. Our results establish SMCHD1 as a key player in nasal development and provide biochemical insight into its enzymatic function that may be exploited for development of therapeutics for FSHD.

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**DUX4 expression induces RNA and protein toxicity in human myoblasts**

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DUX4 is a transcription factor of the double homeodomain family that binds and activates the expression of hundreds of genes. The characterization of gene expression signature of DUX4 and FSHD by several studies shows diverse genes and biological pathways that are affected in FSHD and by DUX4 expression, including cancer testis antigens, retroelements and repetitive elements, genes involved in RNA processing and splicing, inflammatory response, oxidative stress response, protein degradation and RNA quality control, among others. It is unclear which of these pathways are misregulated directly by DUX4 and which are side effects of the toxicity experienced by the cell. One way to disentangle the various interconnected pathways that are misregulated by DUX4 is to use temporal relationships between such events to generate testable hypotheses on the causal relationship between them. To facilitate such a study, we recently developed a model of stable, inducible DUX4 expression in a clonal myoblast cell line. Through a systematic comparison of RNA-seq datasets, we showed that the transcriptome induced by DUX4 in control skeletal muscle cells accurately recapitulates DUX4-associated differences in gene expression observed in FSHD muscle cells that endogenously express DUX4. Next, we conducted time course studies which show that NMD inhibition is an early event that follows DUX4 expression and that proteolytic degradation of key NMD factors – especially UPF1 - could be at the nexus of DUX4-induced NMD inhibition. Moreover, significant upregulation of various protein folding stress response pathways is observed following DUX4-induced NMD inhibition, supporting the hypothesis that aberrant RNA and protein production may underlie the cytotoxicity seen in DUX4-expressing cells

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**Gene surgery using TALEN technology: a therapy for facioscapulohumeral dystrophy?**

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FSHD is one of the most common muscular dystrophies and so far there is no curative or preventive treatment. FSHD is characterized by a loss of repressive epigenetic marks within the D4Z4 macrosatellite located in the sub-telomeric region of chromosome 4 associated with chromatin relaxation. This loss of epigenetic marks leads to the aberrant transcription of the DUX4 double homeobox transcription factor whose ORF is present in each D4Z4 repeat, resulting in poison protein through induction of multiple downstream genes. However, this chromatin relaxation alone is not sufficient to trigger the disease and must be associated with a permissive chromosome 4 including the presence of a 4qA region containing polyadenylation site distal to the last D4Z4 repeat allowing the stabilization of the DUX4 mRNA by the poly(A) tail.

Because DUX4 is the common pathogenic target between FSHD1 and 2 patients, our goal was to perform gene editing using transcription activator-like effector nuclease (TALEN) and/or CRISPR/Cas9 technology to modify the FSHD locus and permanently inhibit DUX4 expression. We have chosen to mutate the DUX4 poly(A) signal since it has been shown that a single point mutation in this poly(A) sequence is sufficient to inhibit DUX4 mRNA expression by modifying its stability. Several nucleases were designed and tested in control human cells to select the most efficient and specific nucleases to perform the D4Z4 sequence modifications. The most promising nuclease was transfected into FSHD cells with a single strand oligonucleotide donor in which the polyA sequence has been replaced. Clones carrying genomic mutation within the polyA sequence were isolated. DUX4 mRNA expression was analyzed as well as the levels of several genes downstream of DUX4. Results will be presented during the meeting.

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**Cell and animal models for studying DUX4 in FSHD**

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Using conditional inducible systems, we have developed various cellular and animal model systems to study DUX4. We present recent progress on using these models to study the functional activities of the DUX4 protein, physiological effects of DUX4, and efforts at genetic correction using sequence-specific nucleases.

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**Muscle weakness and disability in facioscapulohumeral muscular dystrophy are caused by loss of contractile
mass and intrinsic weakness of remaining tissue.**

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common hereditary muscle disorders. Despite increasing knowledge about the molecular mechanisms underlying FSHD, exactly how these changes translate to muscle weakness in patients with FSHD remains unknown. This study combines *in vivo* and *ex vivo* methods to investigate the causes of muscle weakness in FSHD.

We included 14 FSHD patients with variable disease severity and 12 healthy controls. All participants underwent functional assessment and quantitative muscle testing. MRI imaging of the lower limb was performed to quantitatively assess muscle cross-sectional area, muscle fraction and fat fraction. Muscle contractile crosssectional area was calculated by multiplying the total cross-sectional area by the muscle fraction. Participants contributed muscle biopsies of the tibialis anterior and vastus lateralis to study fiber size and type distribution, and single fiber contractile function. All investigations were performed in all participants.

FSHD disease severity is associated with progressive loss of lower limb contractile cross-sectional area (CCSA). Furthermore, CCSA accurately predicts loss of functional performance in patients with FSHD. This process is mediated not only by fatty replacement of muscle tissue, but also by muscle-dependent atrophy of residual contractile tissue. In addition to loss of muscle contractile mass due to atrophy and fatty infiltration, FSHD patients also suffer from inherent weakness of residual muscle tissue. Inherent weakness was observed in all participants independent of disease severity, which suggests that this is an early feature of disease pathology.

In conclusion, muscle weakness and disability in FSHD patients are caused by 1) loss of muscle contractile mass due to atrophy and fatty infiltration, and 2) intrinsic weakness of remaining muscle tissue. Our findings identify muscledependent mechanisms that may contribute to the specific pattern of muscle involvement in FSHD, and are important to consider when designing therapeutic trials or collecting tissue for research

14.
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**Biallelic DUX4 expression in FSHD2 aggravates disease severity**

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FSHD is caused by either contraction of the D4Z4 array to a size of 1-10 units (FSHD1), or by heterozygous mutations in genes encoding D4Z4 chromatin repressors in somatic tissue such as SMCHD1 and DNMT3B (FSHD2). Either event results in somatic de-repression of the *DUX4* gene of which a full copy is localized in the most distal D4Z4 unit of the array and adjacent sequences that contain an additional exon with a stabilizing *DUX4* polyadenylation signal (PAS). The *DUX4*-PAS is polymorphic in nature, which explains the chromosome- and haplotype-specificity of FSHD: a *DUX4*-PAS is present on 4qA chromosomes but absent from 4qB chromosomes and from chromosome 10. Hence, all FSHD patients have at least one chromosome 4qA that expresses *DUX4* in their muscles.

Several 4qA haplotypes have been described that can be divided based on a simple sequence length polymorphism (SSLP) proximal to the repeat and the length of the most distal incomplete repeat unit, between the DUX4 ORF and the pLAM sequence, differing approximately 1.5kb in size between 4qA and 4qA-L (long). Previously, we showed that 1-10 units D4Z4 repeat on a 4qA-L haplotype causes FSHD, but the specific DUX4 transcript was not yet identified.

We studied the frequency and distribution of 4qA and 4qA-L in the control population and in FSHD1 and FSHD2 patients. The 4qA-L variant seems to be a specific variant of the most common 4qA haplotype 4A161, which are indicated as 4A161fl (full length) and 4A161fle (full-length extended). Our preliminary results show that 4A161fle alleles have a different size distribution than 4A161fl and might be more prone to D4Z4 rearrangements. We identified the 4A161fle-associated DUX4 transcript, which enabled the analysis of DUX4 transcription in myoblasts of FSHD2 patients carrying both a 4A161fl and a 4A161fle allele. We showed biallelic expression for most of these samples and found that these individuals were in general more severity affected than FSHD2 individuals with one 4qA allele.

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**Reduction of DUX4 expression and improvement of myoblast differentiation by third generation antisense**

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The goal of this study is to evaluate selected Third Generation Antisense compounds (3GAs) targeted to DUX4 for their effectiveness in knocking-down DUX4 in FSHD myoblasts. In addition, we examined the effects of the treatments on myogenic differentiation of FSHD myoblasts. Five 3GA compounds (3GA1-5) were examined and the results showed significant knock-down of DUX4 in the FSHD myoblasts, as examined by quantitative RT-PCR. We evaluated two 3GA compounds (3GA3 and 3GA5) at 4 different concentrations, 2.5 nM, 5nM, 25nM, and 50nM, and observed dose-dependent responses. Genes that have been reported to be regulated by DUX4 were examined by quantitative RT-PCR and also showed dose-dependent responses. The effects of 3GAs at 1.25 nM and 10nM on cell differentiation were determined by fusion index and number of atrophic myotubes after 7 days of muscle differentiation in culture. Our results showed that the treatments increased fusion index and reduced the number of atrophic myotubes. The studies showed that 3GAs successfully knocked-down DUX4, which improved differentiation of FSHD myoblasts. The findings demonstrated that the 3GAs are promising therapeutic molecules to be further developed for FSHD treatment.

16.
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**Identification of epigenetic modulatory small molecules for DUX4 regulation in FSHD hESC-derived myoblasts**

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by a contraction of the D4Z4 macrosatellite repeat on the sub-telomere of chromosome 4. This genetic lesion results in epigenetic de-repression and aberrant transcription of the pathogenic Double Homeobox Domain-Containing Protein 4 (DUX4) gene. Understanding the pathogenesis of FSHD and development of a DUX4-silencing therapy will require characterization of the molecular players that direct transcriptional activation of the D4Z4 locus. Genea Biocells has leveraged our bank of disease-affected human pluripotent stem cells (hPSCs), skeletal muscle differentiation protocol, DUX4-activated GFP reporter system, and high-throughput live cell imaging capabilities to establish a quantifiable FSHD disease-in-a-dish assay. This system provides a virtually limitless source of FSHD-affected myogenic cells for testing of DUX4 modulatory cell treatments. Low levels of DUX4-GFP can be detected in FSHD-affected hPSC-derived myoblasts and peak reporter expression occurs in differentiated myotubes. As a proof of concept, FSHD hPSC-myoblasts were exposed to the published DUX4-activating histone methyltransferase inhibitor Chaetocin and the number of GFP positive cells increased by 12x, as expected. Next, a small proprietary compound library of epigenetic modifiers was compiled and applied to FSHD hPSC-myoblasts at two concentrations, and live cell imaging captured DUX4-GFP positive cells through myotube formation. Comparison of maximal DUX4-GFP expression after compound treatment identified a group of novel DUX4 modulatory epigenetic effector small molecules, establishing a chemically-induced model of FSHD. Next steps include confirmation of the mechanism of action of these DUX4-activating compounds and combinatorial screens to identify DUX4-inhibitory drugs as potential FSHD therapeutics.

17.
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**_miR-675_ reduces _DUX4_ expression and confers resistance to DUX4 toxicity in FSHD myoblasts: a framework to define the _DUX4_-targeted miRNome**

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Facioscapulohumeral dystrophy (FSHD) is an autosomal dominant muscle disease recognized as one of the most commonly inherited muscular dystrophies. FSHD arises from a complicated pathogenic mechanism that ultimately leads to over-expression of the transcription factor DUX4, causing muscle damage *in vivo*. FSHD is associated with progressive weakness in muscles of the face, shoulder-girdle and arms. However, asymmetrical weakness is common, and there may be extreme variability in severity of symptoms, rate of progression and age at onset, even in families with several affected relatives, which suggests that modifier genes may influence *DUX4* expression and toxicity. We hypothesize that endogenous microRNAs may suppress *DUX4* expression, and protect asymptomatic FSHD patients from DUX4-induced toxicity.

Objective: Identify new modifiers of DUX4 toxicity that inhibit its expression and attenuate its activity. This work would broaden our understanding of FSHD pathogenesis and pave the way to develop new therapies.

Methods: *DUX4* expression was measured using dual-luciferase assays, western blotting and qPCR, and DUX4 cytotoxicity was measured with caspase 3/7 assay.

Results: We show that *miR-675* reduces DUX4 protein levels and thus confers resistance to DUX4 toxicity *in vitro*. We found that myoblasts (MB) isolated from an FSHD patient biopsy (15Abic), with low *DUX4* expression, have high levels of *miR-675* compared to controls. Moreover, these MB were more resistant to cell death. Importantly, inhibition of *miR-675* increased cell death and DUX4 protein expression. Interestingly, the FSHD patient from which 15Abic was isolated does not show severe muscle weakness.

Conclusions: *miR-675* reduces DUX4 expression and toxicity in human myoblasts. *miR-675* is a good modifier candidate of FSHD since its high expression in 15Abic FSHD MB confers resistance to DUX4 toxicity and may be responsible in maintaining biceps strength in the 15A FSHD patient. Our work led to the identification of the first miRNA modifier of DUX4 toxicity. Subsequently, we are defining the DUX4-targeted miRNome to identify additional miRNA modifiers of FSHD.

18.
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**Mutations in *SMCHD1* are associated with isolated arhinia, bosma arhinia microphthalmia syndrome, and facioscapulohumeral muscular dystrophy type 2**

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**Abstract**

Arhinia, or the complete absence of an external nose, is a rare congenital malformation of unknown etiology that is often accompanied by ocular and reproductive defects. To identify the genetic cause of arhinia, we assembled an international cohort of 38 arhinia patients (24% of all reported cases in the medical literature and 19 new cases) and performed whole-genome, whole-exome (WES), and gene targeted sequencing. These analyses identified rare missense variants (7 recurrent) in *SMCDH1* in 86% of independent probands, none of which were present in the Exome Aggregation Consortium (ExAC) database of 60,706 healthy individuals with WES data. Gene-based burden testing of rare variants confirmed that *SMCHD1* was the only gene to achieve genome-wide significance ($p=2.9x10^{-17}$).

SMCHD1 is an epigenetic repressor of both autosomal and X-linked genes that has previously been implicated in type 2 fasciculohumeral dystrophy (FSHD2). Whereas *SMCHD1* variants observed in FSHD2 patients include both missense and truncating variants that span the entire gene, all variants observed in arhinia subjects were missense variants in exons 3-13, tightly clustered around the 5' GHKL-type ATPase domain, the only region of the gene that is under strong evolutionary constraint ($p = 1.26x10^{-8}$). Several FSHD2-specific missense variants have been identified in this 5' region, and two of these variants (L107P and G137E) were also detected in our arhinia cohort. Neither subject has features of both disorders but they are well below the average age of FSHD disease onset.

We explored the functional mechanisms by which *SMCHD1* mutations cause arhinia through CRISPR/Cas9 studies in mouse and zebrafish embryos and methylation analyses of the D4Z4 repeat array. These analyses suggest that loss of SMCHD1 repressive activity recapitulates the nasal, ocular, and reproductive defects observed in arhinia subjects. We also found hypomethylation patterns at the 4q35 D4Z4 repeat region in arhinia subjects that are comparable to FSHD2 patients. This suggests that loss of this particular SMCHD1 function (gene silencing by methylation) also occurs in arhinia, and thus does not by itself explain the difference in phenotypic outcomes. Additional factors must be involved in producing this distinction, such as interactions at the genetic or protein level. Studies are in progress to understand the pleiotropic effects of *SMCHD1* dysfunction.

19.
[O]

**iPSC modeling of human skeletal myogenesis and muscle diseases**

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Our lab, in collaboration with Genea Biocells, has developed iPSC myogenesis technologies to investigate developmental and epigenetic mechanisms controlling human myogenesis and pathologies associated with muscular dystrophy. Utilizing Genea's step-wise, gene-free myogenesis induction protocol, our findings show that human iPSCs derived from control subjects or dystrophic patients with facioscapulohumeral muscular dystrophy (FSHD) or LGMD2i undergo programmed activation of the PAX3 and MYOD genes, and associated epigenetic changes in DNA methylation, leading to specification of myogenic "reserve" cell lineages. iPSC-derived myogenic reserve cells can be maintained as a proliferative population that can be induced to undergo myotube differentiation in response to environmental signals, as well as xenografted into regenerating TA muscles of immuno-deficient mice. Myotubes derived from FSHD and LGMD2i reserve cells express disease phenotypes, including misexpression of DUX4-fl target genes and deficiencies in laminin binding, respectively. These iPSC myogenesis technologies are being utilized to develop disease models for therapeutic development.

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20.
[O]

**Estrogens improve differentiation of facioscapulohumeral muscular dystrophy myoblasts by antagonizing DUX4 activity**

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**Abstract**

Facioscapulohumeral muscular dystrophy (FSHD) is characterized by extreme variability in symptoms with females being less severely affected than males and presenting a higher proportion of asymptomatic carriers. Gender factors involved in the disease have not been completely defined. Using myoblasts derived from FSHD patients, both primary and immortalized cell cultures, we investigated the effect of estrogens on muscle differentiation features. Our results demonstrate that estrogens improve the differentiation of isolated FSHD myoblasts without affecting cell proliferation or survival. Particularly, fusion index and positivity to the differentiation marker myosin heavy chain are significantly reduced in FSHD myoblasts grown in estrogen-deprived medium compared to CTR whereas estrogen supplementation recover this reduction. This effect is mediated by estrogen receptor beta (ERβ) which antagonizes the function of the homeobox protein DUX4. Estrogen decreases DUX4 residency on the promoter of its targets whose transcription is accordingly reduced. Interestingly, during myoblast differentiation the levels and activity of DUX4 increase progressively associated with its enhanced recruitment in the nucleus; ERβ interferes with this recruitment by re-localizing DUX4 in the cytoplasm. This work identifies estrogens as a potential factor underlying FSHD gender difference with protective function against this disease.

21.
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**Disease progression and natural history in 246 subjects from the FSHD Italian registry**

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A prominent feature of facioscapulohumeral muscular dystrophy (FSHD) is its wide clinical variability, ranging from asymptomatic carriers to people who become wheelchair-bound by age 30 or need Non-Invasive Ventilation. Overall the natural history of FSHD is obscure.

A relevant diagnostic significance is attributed to the detection of D4Z4 alleles associated with the 4qA polymorphism regardless the phenotypic features. An atypical phenotype is considered FSHD on the basis of molecular findings (short D4Z4 repeat and 4qA polymorphism). However our current knowledge shows that (1) alleles with reduced numbers of D4Z4 repeats at 4q35 combined with permissive 4A sequence have a frequency of 2% among healthy subjects from the general population, (2) that 25% of first degree relatives and 47% of more distantly related family members are healthy, (3) several cases with atypical phenotypes are reported, including the so called "double trouble". Thus there are overlapping conditions between affected and healthy people as well as with people presenting possible co-morbidities. Based on these premises clinical trials conducted for FSHD treatments may include people with very different phenotypes, which would interfere with the interpretation of results.

We performed a longitudinal study on people carrying D4Z4 reduced alleles to assess disease progression on a long-term period and define natural history of disease also considering the clinical variability of FSHD.

Data were accrued in the Italian FSHD Registry by Italian Network for FSHD on the basis of a nationwide collaboration. We selected 246 consecutive subjects accrued in the period 2007-2011 with a follow up at least 5-years long (second evaluation in the period 2013-2016). We used MRC scale, FSHD clinical score (range 1-15) in both examinations and applied, in the second evaluation, the newly developed Comprehensive Clinical Evaluation Form (CCEF). The CCEF classifies: (1) subjects presenting facial and scapular girdle muscle weakness typical of FSHD (category A, subcategories A1–A3), (2) subjects with muscle weakness limited to scapular girdle or facial muscles (category B subcategories B1, B2), (3) asymptomatic/healthy subjects (category C, subcategories C1, C2), (4) subjects with myopathic

phenotype presenting clinical features not consistent with FSHD canonical phenotype (D, subcategories D1, D2).

We examined 246 subjects (136 males, aged 12-84 years, with a D4Z4 allele ≤41kb, ≤10 RU). Subjects were subdivided in nine clinical categories on the basis of the CCEF. On average we detected an increase of 1 point of the FSHD clinical score over an average period of 72 months. We observed a different disease trajectory in index cases in comparison with their relatives. Remarkably incomplete phenotypes (B categories) or asymptomatic or healthy carriers with a FSHD score equal to 0 (C categories) did not progress in 5-years.

Out of 246 subjects 10 (4%) lost ambulation and 7 (3%) became NIMV-dependent. Affected people who received an FSHD between 3 and 6 score at the first evaluation worsened more than the other groups. Our study also considered muscle groups affected at disease onset and age at onset. Moreover, we evaluated disease progression in separate muscle groups affecting different functions. Finally, we evaluated the role of co-morbidities on disease progression.

Overall our study on this large cohort of subjects enabled us to describe the history of disease in different clinical categories and to display the slow progression of muscle impairment. Our longitudinal study shows the predictive value of clinical categories and provides crucial information for the recruitment of patients in potential therapeutic studies.

Importantly our analysis stresses the importance of relying on more sensitive outcome measures, specifically designed to monitor disease progression in view of future clinical trials.

22.
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**Translating DUX4-targeted RNAi therapy for FSHD**

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The emergence of DUX4 enabled the development of FSHD therapies. Previously our lab demonstrated proof-of-principle for the use of a DUX4-targeted RNA interference (RNAi)-based gene therapy as a putative treatment for FSHD. To accomplish this we pursued two lead engineered microRNAs as target products for DUX4 inhibition. Both sequences showed strong therapeutic efficacy determined by their ability to suppress pathogenic levels of DUX4, and prevent myopathy in DUX4-expressing mice. The goal of this study was to pursue the next steps toward translation by assessing safety and off-target effects of our lead miRNA target products following dose-escalation. To do this, we delivered adeno-associated viral (AAV) vectors expressing U6 promoter-driven, DUX4-targeted miRNAs to mice, either by local intramuscular or intravascular injections. Over-expression related toxicity (or lack thereof) in multiple organ systems was assessed at acute (3 week) and long term (5 month) time points by an independent veterinary pathologist using a blinded experimental design. One sequence showed time- and dose-dependent toxicity in the skeletal muscle (at high doses), while the other produced no overt deleterious effects in any organ system. To assess sequence-specific off-target effects, we performed RNA-seq using RNA harvested from human myoblasts transfected with our lead therapeutic miRNAs. Surprisingly, only 4 transcripts were reduced upon miRNA expression, and of these, only 1 contained seed match binding sites for our lead miRNA target product sequences suggesting the promiscuity of the DUX4-targeted miRNAs is not a major concern. As a secondary safety measure we have also developed a next generation of vectors in which the ubiquitous U6 promoter was replaced with the muscle specific promoter tMCK promoter, to restrict our therapeutic miRNAs to skeletal muscle. Following optimization, we showed that these new targeted systems protect muscles from DUX4-induced damage as efficiently as the first-generation U6 system. Our goal is to translate this work toward a clinical trial, and we are now performing additional safety and efficacy measures in anticipation of pre-IND discussions with FDA in the near future.

23.
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**Conservation and innovation in the DUX4-family gene network**

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**Abstract**

Facioscapulohumeral dystrophy (FSHD) is caused by the mis-expression of the DUX4 transcription factor in skeletal muscle. Animal models of FSHD have been hampered by incomplete knowledge of the conservation of the DUX4 transcriptional program in other species. Several studies have characterized the human DUX4 (hDUX4) transcriptome in human muscle cells; however, until now, hDUX4's transcriptome in mouse muscle cells had not been assayed with genome-wide high-throughput sequencing methods. Genome-wide transcriptome data is critical because the hDUX4 transcriptome in human muscle cells has many features that are not captured via microarray (e.g. transcription of repetitive elements, splicing between repetitive elements and annotated genes). Furthermore, we assessed non-hDUX4-based strategies for modeling FSHD. Although hDUX4 only has established orthologs in the primate lineage, other model organisms have double homeodomain transcription factors that share an evolutionary history with hDUX4. For example, mice have a gene called mouse Dux (mDux), which was derived by retroposition of DUXC mRNA. Although hDUX4 was also created via retroposition of DUXC mRNA, there is not yet a consensus on whether hDUX4 and mDUX are true orthologs. Regardless, since mDux and hDUX4 have significantly different sequences, particularly in the DNA-binding homeodomain regions, empirical data comparing the mDUX and hDUX4 transcriptomes is required to determine the extent to which mDux could provide a meaningful model of FSHD. In conclusion, our comparative genome-wide studies of hDUX4 and mDUX improve our understanding of their evolutionary relationship and critically inform improvements to murine models of FSHD.

24.
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**Transcription factors DUX4 and DUX4c associate with mRNP granules in the cytoplasm of fusing myoblasts: a new function in translation regulation?**

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DoUble HomeoboX 4 (DUX4) is a transcription activator and induces a large gene deregulation cascade causing the major pathological features of FSHD. Its homologue, DUX4c expressed in healthy muscles is also induced in FSHD and contributes to the pathology. In a recently published search for DUX4/4c protein partners we have unexpectedly identified many cytoplasmic or nucleo-cytoplasmic proteins involved in myofibril organization and mRNA translation control. The functionality of such interactions is suggested by the cytoplasmic location of otherwise nuclear DUX4/4c when myoblasts fuse. Moreover, we have already validated a number of partners by co-immunoprecipitation, co-immunofluorescence or in situ Proximal Ligation Assay.

Terminal muscle differentiation is associated with a general transcription inhibition and the required structure proteins (actin, tubulin…) are synthesized by increased translation of pre-existing mRNAs associated with ribonucleoparticles (RNPs). An interactome built with validated or identified DUX4/4c partners showed 12 of these belonged to mRNP-granules associated with IGF2 mRNA binding proteins (IGF2BPs, also named IMPs or ZBP). These mRNPs contain untranslated mRNAs, transport them along microtubules to subcellular areas (like elongating cell tips) where their translation is required at specific times.

Our previous studies mostly involved overexpressed DUX4/4c proteins. By the use of new specific antibodies, we could observe endogenous DUX4 and DUX4c and validate their association with multiple IGF2BP1-associated mRNP granules components (such as IGF2BP1, ILF3, NF90, FUS, SFPQ). We also checked if some of those partners were differently expressed between FSHD and healthy muscle cells.

We also, observed in elongating myoblasts the co-localization of endogenous DUX4c with newly synthesized Troponin T stacks near clusters of myonuclei and of endogenous DUX4 with alpha-crystallin B chain, a chaperone required for newly synthesized desmin folding.

In conclusion, besides transcription, DUX4/4c appear involved in translational control of mRNAs producing structure proteins required for muscle terminal differentiation.

25.
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**Bone health in facioscapulohumeral muscular dytrophy: a cross-sectional study**

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***Background:*** Poor bone health is a major complication of some muscular dystrophies. This study, provides a comprehensive overview of bone health in facioscapulohumeral muscular dystrophy.

***Methods***: Ninety-four individuals with FSHD1 from two sites were included in this cross-sectional study. Clinical characteristics and determinants of bone health were examined. Relationships between bone mineral density (BMD), strength and functionality were explored.

***Results***: The mean FSHD severity score was 6.7, indicating a moderate level of weakness. Nearly a third of subjects were deficient in vitamin D3. Mean whole body BMD z-score was -0.7; 11% had greater than age-related reductions in whole body BMD (z-score < -2.0). Whole body and regional BMD were associated with strength and functionality. Five percent were non-ambulatory and 36% had a history of fractures, including vertebral and non-vertebral fractures. Likelihood for fractures was reduced for those with a whole body BMD within the expected normal range for age (OR=0.25, 95% CI: 0.04-0.78). Overall, we found significant variability in BMD, strength and functionality.

***Discussion***: A diagnosis of FSHD is not necessarily predictive of reduced BMD or increased fracture rate. Given the considerable variability of bone health in the FSHD population, strength and function can serve as predictors of BMD. Overall, effective management of bone health in FSHD must rely on serum biomarkers and periodic DXA scans to create patient-specific treatment plans.

This work was supported by FSHD Global and NIH (U54DH060848)

26.
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**Third generation gene silencing oligonucleotide therapeutics for FSHD**

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[2] Idera Pharamaceuticals, Cambridge, MA
[3] Kennedy Krieger Institute and Johns Hopkins School of Medicine, Baltimore, MD

DUX4 is misexpressed in skeletal muscles of patients with facioscapulohumeral muscular dystrophy (FSHD), and is a strong candidate for antisense oligo (ASO) therapeutics. Importantly, Idera has developed proprietary third generation gene silencing oligo (GSO) technology that is stable and efficiently delivered without carrier molecules, and exhibits reduced immunotoxicity and hepatotoxicity compared to earlier ASO chemistries. We here present our results examining the effects of GSOs targeting the DUX4 transcript in primary FSHD myotubes. Cells were treated with GSOs for four days and analyzed by RT-qPCR for DUX4 and its downstream target genes, as well as by DUX4 immunohistochemistry. Expression of FSHD biomarkers exhibited dose-dependent decreases following treatment with GSO-5. DUX4 protein expression was also reduced. In conclusion, we have demonstrated successful reduction of DUX4 expression and function in FSHD myotubes using GSOs.

Supported by the UMMS Wellstone Muscular Dystrophy CRC and the FSH Society.

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**Development of a novel human FSHD myogenic cell model and transcriptome analysis**

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**Abstract**

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common muscular dystrophies, and has a progressive symptom of muscle weakness, particularly in face and shoulder region. FSHD pathogenesis is highly complex and not yet fully understood. One of the mostly discussed culprit genes is *DUX4* that is highly expressed in germ-line cells of the testis and epigenetically repressed in somatic tissues. Whereas during normal development, *DUX4* expression disappeared rapidly, in FSHD, *DUX4* is thought to be dys-regulated particularly in myogenic lineages. We hypothesize that the very early myogenic stage, such as *MESOGENIN1+* somite cells, can be the best biological window to study the aberrant transcription changes between pluripotent stage and lineage-committed skeletal muscle cells. Human induced pluripotent stem cells (iPSCs) provide a potentially unlimited source of specialized cell types for regenerative medicine. We recently developed a novel skeletal muscle specification system from hiPSCs in defined condition and built a FACS purification strategy to isolate myoblasts. To isolate patient-specific somite and myoblasts, we successfully generated hiPSCs of FSHD patient (03ABIC) and genetically paired samples from healthy donor (03UBIC). Furthermore, to isolate somite cells, we generated genetic reporter lines by CRISPR/Cas9 system, of *MESOGENIN1::eGFP* FSHD-hiPSCs and control-hiPSCs. The samples of primary FSHD myoblasts, undifferentiated FSHD-hiPSCs, *MESOGENIN1::eGFP+* FSHD-specific somite cells, FACS-purified FSHD-hiPSC-derived myoblasts as well as their controls were submitted to RNA sequencing. Currently these data are being analyzed, and the result will be presented in the FSH Society Conference. We hope that our approach can provide a previously unknown aspects of transcriptional dys-regulation in the somite stage of FSHD development.

28.
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**C1QBP inhibits DUX4-dependent gene activation and can be targeted with 4MU**

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Development of FSHD is linked to the de-repression and stabilization of the DUX4 gene contained within the D4Z4 repeats on chromosome 4. However, individuals have been identified who express DUX4 in their muscle biopsies, but who do not show any clinical symptoms of the disease, suggesting that there are other factors that modify FSHD penetrance or severity. We hypothesized that an FSHD-modifying factor would physically interact with and modify the function of DUX4, and we therefore took a proteomic approach to screen for DUX4-interacting proteins that may act as disease modifiers. We identified the multifunctional C1QBP protein as one such candidate. C1QBP is known to regulate several of the processes that DUX4 affects, including gene expression, oxidative stress, apoptosis, and pre-mRNA splicing. We used siC1QBP knockdown assays and quantitative RT-PCR experiments to determine if C1QBP affects DUX4 activity. We found that C1QBP has little effect on the expression of DUX4-target genes in myotubes, but that it inhibits the kinetics of DUX4-target gene activation during myogenic differentiation. This identifies C1QBP as a regulator of DUX4 activity and raises the possibility that it could be a target for FSHD therapeutics. Importantly, C1QBP is known to bind to the intra- and extra-cellular signaling molecule hyaluronic acid (HA), which can regulate its phosphorylation state. We have found that that decreasing intracellular HA by treating cells with 4-methylumbelliferone (4MU), an inhibitor of HA synthesis, results in a sharp decline in DUX4-target gene expression, possibly due to its disrupting the normal subcellular localization of C1QBP. These results potentially identify 4MU as an FSHD therapeutic compound.

29.
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**A randomized, double-blinded, placebo-controlled, multiple ascending dose study to evaluate the safety, tolerability, pharmacokinetics, immunogenicity, and biological activity of ATYR1940 (resolaris™) in adult patients with facioscapulohumeral muscular dystrophy**

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**Rationale**: FSHD, a rare, debilitating, genetic skeletal myopathy is associated with inflammation and dystrophic changes in muscle tissue. ATYR1940, a Physiocrine based protein that modulates immune responses in skeletal muscles preclinically, is identical to substantially all of human histidyl tRNA synthetase, a protein that is released from skeletal muscle.

**Methods**: This double-blinded, phase 1b/2a study evaluated the safety, tolerability, pharmacokinetics (PK), immunogenicity, and biological activity of multiple ascending doses of intravenous ATYR1940 in adult FSHD. Exploratory pharmacodynamic (PD) measures included the individualized neuromuscular quality of life (INQoL) questionnaire, lower extremity muscle targeted MRI and circulating PD markers. Weekly doses of 0.3 (n=4), 1.0 (n=8), and 3.0 (n=8) mg/kg were tested over 4, 4, and 12 weeks, respectively. Patients (n=20) were randomized 3:1 (ATYR1940: placebo) across all dose groups and followed for 4 & 12 weeks after the last study drug dose.

**Results:** A dose-related improvement was detected by INQoL from baseline in all dose cohorts and at 3.0 mg/kg at 12 weeks an improvement compared to placebo of 25.5% was observed (p=0.03). Measured circulating PD markers and MRI did not record substantial differences between placebo and ATYR1940. Manual muscle testing indicated no reportable disease progression. All reported adverse events were assessed as mild or moderate in intensity. One moderate adverse event reported in a test article treated patient (a reversible generalized infusion related reaction in the 3.0 mg/kg dose cohort) was reclassified to a serious adverse event by the sponsor. PK was consistent throughout the study course and across all dose cohorts.

**Conclusion:** The potential signals of activity of ATYR1940 in the INQoL and the safety profile support advancement of ATYR1940 in FSHD and potentially other rare diseases.

Acknowledgments: S Shukla, aTyr consultant and CTI, Inc.

# A Randomized, Double-blinded, Placebo-controlled, Multiple Ascending Dose Study to Evaluate the Safety, Tolerability, Pharmacokinetics, Immunogenicity, and Biological Activity of ATYR1940 (Resolaris™) in Adult Patients With Facioscapulohumeral Muscular Dystrophy

Gershman A,[1] Chiang K,[1] Do M-H,[1] Abbink E,[2] Harbers V,[2] Audebert C,[3] Campana-Salort E,[4] Monforte M,[5] Iyadurai S,[6] Carey L,[1] Heskamp L,[2] Kan HE,[7] Heerschap A,[2] Kissel J,[6] Ricci E,[5] Attarian S,[4] Blackburn K,[1] Mendlein JD,[1] and Ashlock MA[1]

[1]aTyr Pharma Inc., San Diego, CA, USA; [2]Radboud University Medical Center, Nijmegen, Netherlands; [3]CIC-CPCET, Service de Pharmacologie Clinique et Pharmacovigilance, AP-HM-Aix-Marseille University Institut Neurosciences Timone, Marseille, France; [4]Reference Center of Neuromuscular Disorders and ALS, AP-HM-Aix-Marseille University Institut Neurosciences Timone, Marseille, France; [5]Institute of Neurology, Catholic University School of Medicine, Rome, Italy; [6]Ohio State University Medical Center, Columbus, OH, USA; [7]Leiden University Medical Center, Leiden, Netherlands

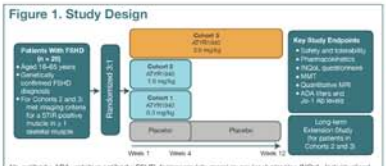
## Introduction

- Facioscapulohumeral muscular dystrophy (FSHD) is a rare genetic myopathy in which immune cells invade diseased skeletal muscle and for which there are no approved treatments. The primary clinical phenotype of FSHD is debilitating skeletal muscle deterioration and weakness:
  - Estimates of FSHD prevalence vary between 1:14,000 and 1:20,000.[1]
- ATYR1940 (Resolaris™) is a slightly truncated form of human histidyl tRNA synthetase (HARS), which is 100% identical to the naturally occurring wild type molecule over the common protein sequence:
  - Aside from its established intracellular role in protein synthesis, we believe HARS also plays extracellular roles, including modulating immune responses in skeletal muscle.
  - Because the immune component may play a central role in FSHD pathophysiology,[2] immunomodulators, including ATYR1940, are being investigated as potential therapies for this disease.
- In preclinical experiments using a rat model of statin-induced myopathy, ATYR1940 reduced skeletal muscle degeneration and necrosis, reduced the numbers of immune cells in muscle, and downregulated immune regulatory proteins in diseased tissue in a dose-dependent manner.
- This first-in-patient study evaluated the safety, tolerability, pharmacokinetic (PK) properties, immunogenicity, and biological activity of ATYR1940 in adult patients with FSHD (ClinicalTrials.gov: NCT02239224).

## Methods

- This Phase 1b/2, double-blinded, placebo-controlled, multiple ascending dose study randomly assigned eligible patients 3:1 to receive weekly I.V. ATYR1940 (Cohort 1: 0.3 mg/kg; Cohort 2: 1 mg/kg; Cohort 3: 3 mg/kg) or placebo (**Figure 1**).

**Figure 1. Study Design**




Ab, antibody; ADA, antidrug antibody; FSHD, facioscapulohumeral muscular dystrophy; INQoL, Individualized Neuromuscular Quality of Life; MMT, manual muscle testing; MRI, magnetic resonance imaging; STIR, short tau inversion recovery.

- Key study endpoints were the evaluation of safety and tolerability as assessed by incidence of adverse events (AEs), antidrug antibody (ADA) titers, Jo-1 antibody (Ab) levels, ATYR1940 PK properties, and measures of clinical activity:
  - **Manual Muscle Testing (MMT):**
    - Motor function was assessed in 14 muscle groups and graded based on a 5-point scale.
    - For patients in Cohorts 1 and 2, MMT was assessed during screening; at Week 3; Week 4, Day 4; and Week 6; for patients in Cohort 3, MMT was assessed during screening; at Week 6; Week 10; and Week 14.
  - **Individualized Neuromuscular Quality of Life (INQoL) Questionnaire:**
    - A validated muscle disease-specific quality of life measure, with 45 questions within 10 sections. The questionnaire focuses on 4 dimensions: Symptoms, Life Domains, Treatment Effects, and Overall Quality of Life. Overall INQoL score is derived from Life Domains and comprises 5 subsections: Activities, Independence, Social Relationships, Emotions, and Body Image.
    - The self-administered questionnaire was completed during screening and at Week 6 in all cohorts as well as at Week 14 for patients in Cohort 3.
  - **Muscle Surveillance and Targeted Magnetic Resonance Imaging (MRI):**
    - Lower extremity surveillance MRI was performed during screening to identify active immune responses (as evidenced by short tau inversion recovery [STIR] positive signal).
    - The presence of ≥ 1 STIR-positive muscle as assessed by a central reviewer was an inclusion criterion for this study (Cohorts 2 and 3).
    - STIR-positive muscles were monitored using targeted quantitative MRI for the analysis of fatty infiltration, inflammation, and muscle volume.

## Results

- All patients completed the study; however, 1 patient did not receive all doses of study drug due to an AE of infusion-related reaction (IRR).
- All patients had FSHD Type 1; patient demographics and characteristics are shown in **Table 1.**

**Table 1. Patient Demographics and Baseline Characteristics**

| Characteristic | Placebo (n = 5) | COHORT 1 ATYR1940 0.3 mg/kg (n = 3) | COHORT 2 ATYR1940 1.0 mg/kg (n = 6) | COHORT 3 ATYR1940 3.0 mg/kg (n = 6) |
|---|---|---|---|---|
| Median age, years (range) | 52.0 (39–66) | 53.0 (25–55) | 46.0 (33–52) | 39.5 (25–72) |
| Male, % | 60.0 | 66.7 | 33.3 | 66.7 |
| White, % | 100 | 100 | 100 | 100 |
| Mean BMI, kg/m² (SD) | 29.9 (3.2) | 28.6 (1.8) | 25.3 (4.6) | 22.5 (5.0) |
| Median disease duration, years (range) | 18.6 (4.4–24.8) | 6.2 (2.8–21.8) | 22.6 (10.2–41.1) | 23.0 (9.3–56.4) |
| Mean FSHD clinical severity score (SD) | 2.7 (0.7) | 2.5 (0.9) | 3.7 (0.5) | 3.0 (0.8) |

BMI, body mass index; FSHD, facioscapulohumeral muscular dystrophy; SD, standard deviation.

### Safety and Tolerability Profile

- ATYR1940 was generally well tolerated across all dose groups (**Table 2**).
- All treatment-emergent AEs (TEAEs) were Grades 1 or 2 (mild or moderate in intensity):
  - No dose-response relationship was suggested in the intensity and incidence of events.
- One patient experienced 3 successive IRR events; this was assessed as an AE by the investigator, but was considered a medically important event by the sponsor and was upgraded to a serious AE.
- No trends in hematology or serum chemistries were observed.
- No signals or trends in electrocardiograms or pulmonary function tests were observed.
- TEAEs reported for ≥ 2 patients treated with ATYR1940 are shown in **Table 2.**

**Table 2. Summary of Safety**

| Parameter, n* (%) | Placebo (n = 5) | COHORT 1 ATYR1940 0.3 mg/kg (n = 3) | COHORT 2 ATYR1940 1.0 mg/kg (n = 6) | COHORT 3 ATYR1940 3.0 mg/kg (n = 6) |
|---|---|---|---|---|
| TEAEs | 5 (100) | 3 (100) | 6 (100) | 6 (100) |
| Treatment-related | 0 | 0 | 0 | 0 |
| Grade ≥ 3 TEAEs | 0 | 0 | 0 | 0 |
| Treatment-related | 0 | 0 | 0 | 0 |
| SAEs | 0 | 0 | 0 | 1† |
| Deaths | 0 | 0 | 0 | 0 |
| *Most common TEAEs* | | | | |
| Cough | 0 | 1 (33.3) | 0 | 2 (33.3) |
| Headache | 2 (40.0) | 0 | 1 (16.7) | 2 (33.3) |
| Presyncope | 0 | 0 | 0 | 2 (33.3) |
| Arthralgia | 0 | 2 (66.7) | 0 | 1 (16.7) |
| Flushing | 0 | 0 | 1 (16.7) | 1 (16.7) |
| Nausea | 0 | 0 | 2 (33.3) | 0 |
| Back pain | 1 (20.0) | 1 (33.3) | 3 (50.0) | 0 |
| Myalgia | 0 | 1 (33.3) | 1 (16.7) | 0 |

*Number of patients.
†Designated as SAE by sponsor.
SAE, serious adverse event; TEAE, treatment-emergent adverse event.

### PK Properties

- PK was generally consistent throughout the study, with dose-proportional increases in exposure.
- The mean clearance was low, and mean volume of distribution at steady state was small, with a mean terminal half-life of 3–5 hours.

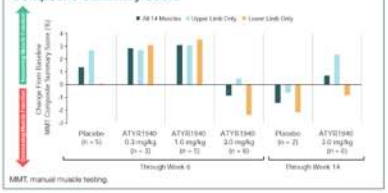
### Immunogenicity Profile

- Of the 15 patients treated with ATYR1940, 6 were confirmed positive for ADA, but Ab levels fell upon cessation of therapy. No patient had titers high enough to trigger testing in a neutralizing Ab assay.
- No patients had Jo-1 Ab levels considered positive or equivocal for the assay.
- No impact of Ab levels on ATYR1940 PK was detected.

### Clinical Activity

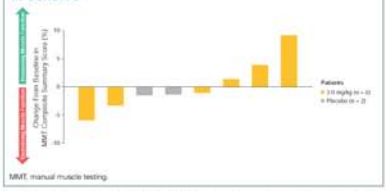
- A trend for improvement in MMT results with ATYR1940 treatment was observed compared with placebo, especially in the upper limb (**Figure 2 and 3**).

**Figure 2. Percentage Change From Baseline in MMT Composite Summary Score**




MMT, manual muscle testing.

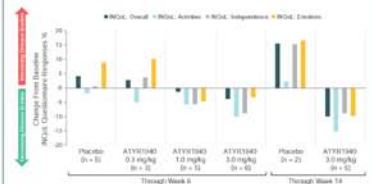
**Figure 3. Percentage Change From Baseline to Week 14 in MMT Composite Summary Score for Individual Patients in Cohort 3**




MMT, manual muscle testing.

- Patients treated with ATYR1940 were generally improved compared with placebo as assessed by INQoL, with patients in Cohort 3 (12 weeks) showing the greatest improvement compared with Cohort 1 and Cohort 2 (4 weeks) (**Figure 4**):
  - Patients in Cohort 3 reported ~ 10% improvement in INQoL overall responses compared with ~15% worsening in the placebo group.
  - The largest differences between Cohort 3 and placebo were seen in the Activities, Independence, and Emotions domains.
- In general, there was a good correlation between changes in INQoL and MMT, whereby all patients who experienced an improvement in muscle function also showed improvement in INQoL scores.

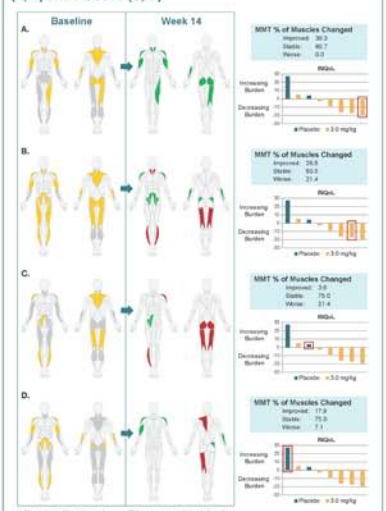
**Figure 4. Percentage Change From Baseline in INQoL Questionnaire Response**




INQoL, Individual Neuromuscular Quality of Life.

- In surveillance and targeted quantitative MRI scans of patients treated with placebo or ATYR1940, we observed:
  - Significant measurement variability was seen, driven in part by alignment, motion artifact, and muscle heterogeneity.
  - Little change in STIR, Dixon fat fraction percentage, or quantitative T2 (indicative of the presence of inflammation) in targeted muscles in either the ATYR1940 or placebo groups.
- No elevation from baseline in circulating human biomarker levels in plasma was observed.
- Patient profiles are shown in **Figure 5.**

**Figure 5. Efficacy Results: Change From Baseline to Week 14 in Representative Patients Treated With ATYR1940 (A, B) and Placebo (C, D)**




MMT, manual muscle testing; INQoL, Individual Neuromuscular Quality of Life.

## Conclusions

- ATYR1940 (Resolaris™) is generally safe in adult patients with FSHD, and was well tolerated with the exception of 1 patient who experienced 3 IRR events.
- ATYR1940 PK properties were dose-proportional and consistent throughout the study, with no measureable impact from ADA.
- Clinical activity of ATYR1940 was supported by signals of improvement in INQoL questionnaire responses and MMT measures.
- Other exploratory measures, including lower extremity targeted MRI, did not demonstrate activity:
  - Variability in image acquisition may have diminished the opportunity to demonstrate a treatment effect at 3 months.
- Patients in Cohorts 2 and 3 were allowed to enroll in the ongoing, long-term extension study investigating the safety and efficacy of ATYR1940.

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### Acknowledgements

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**A Phase 2 study to evaluate ACE-083, a local muscle therapeutic, in patients with FSHD**

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**Background**: ACE-083 is an investigational protein therapeutic that acts as a localized ligand trap for myostatin and other negative regulators of muscle growth. In wild-type mice, *mdx* model of Duchenne muscular dystrophy, and SOD1 model of amyotrophic lateral sclerosis, local injection of ACE-083 into a target muscle produced dose-dependent increases in muscle mass and force without systemic pharmacodynamic (PD) effects.

In a recently completed phase 1, double-blind, placebo-controlled, dose-escalation study in healthy post-menopausal women, unilateral injection of ACE-083 into the rectus femoris (RF) or tibialis anterior (TA) muscle was generally safe and well tolerated. Mean percent change from baseline in muscle volume of the injected muscle was +14.5% in the RF and +8.9% in the TA at the highest dose administered (200 and 150 mg per muscle, respectively, for 2 doses 3 weeks apart). Minimal changes were observed in the uninjected, contralateral side and in placebo-treated subjects. All AEs were grade 1-2 and reversible. Common related AEs (≥15%) included injection site pain, muscle twitching, myalgia, and injection site reaction, with similar incidences in ACE-083 and placebo-treated groups.

Together, these preclinical and clinical results support further studies of ACE-083 in diseases with focal loss of muscle strength and function, such as FSHD.

**Study Design**: Study A083-02 is a multicenter, phase 2 study to evaluate the safety, tolerability, efficacy, PD, and pharmacokinetics (PK) of ACE-083 in adult patients with FSHD, to be conducted in two parts. Eligible patients must have genetically confirmed FSHD1 or FSHD2 and mild to moderate weakness in ankle dorsiflexion or elbow flexion. ACE-083 will be administered once every 3 weeks for up to 5 doses.

Part 1 is open-label with up to 36 patients enrolled, evaluating ascending dose levels of ACE-083 in either the TA or the biceps brachii (BB) muscle (up to 3 dose cohorts per muscle). A Safety Review Team (SRT) will review safety data and make recommendations regarding dose escalation and study conduct. Prior to the initiation of Part 2, the SRT will review safety and efficacy data from Part 1 to determine whether one or both muscles will be pursued in Part 2, as well as the recommended dose for each muscle. The primary objective for Part 1 is safety and tolerability.

Part 2 is randomized, double-blind, and placebo-controlled, with up to 40 new patients (20 patients per muscle) enrolled and randomized (3:2) to receive either ACE-083 or placebo, to either the TA or BB muscle. The efficacy outcomes for Part 2 will include muscle volume by MRI, strength by quantitative muscle testing, function by motor function tests, and quality of life using the FSHD-Health Index questionnaire.

**Summary:** ACE-083 is a locally-acting agent for the growth and regeneration of muscle. Its mode of action is well-suited for FSHD due to the focal involvement of specific muscles. Study A083-02 (NCT02927080) is a phase 2 study of ACE-083 in patients with FSHD.

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**Characterizing early onset facioscapulohumeral dystrophy – a systematic review of literature cases**

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Infantile or early onset occurs in 5-10% of patients with facioscapulohumeral dystrophy (FSHD) and is considered to have a severe course with multisystem involvement. Systematic data on the severity and systemic complications of this early onset FSHD subgroup is scarce, thereby hampering adequate clinical management.

We systematically reviewed articles on patients with early onset FSHD defined as first sign of facial weakness before the age of 5 years and first sign of scapular weakness before the age of 10 years. This resulted in 227 individual case descriptions. With a mean age of 18.8 years at the time of reporting, 40% is wheelchair dependent and systemic complications include hearing loss (40%), retinal abnormalities (17%) and mental retardation (8%). Predictors for disease severity are age at onset, repeat length and systemic complications**.** Univariate analysis showed a strong correlation between retinal abnormalities and hearing loss (OR 3.79, $p<0.001$) and between respiratory abnormalities and spinal deformities (OR 3.2, $p=0.005$).

This systematic review in 227 patients with early onset FSHD shows an evident clinical heterogeneity, similarly as in the classical form of FSHD. 60% of patients are still ambulant at investigation (average age 18 years) and only half of patients has systemic features. As such, it confirms that early onset FSHD is a severely affected subgroup with wide variation. Future research should focus on natural history and new (epi)genetic predictors to improve prognostication and clinical management

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**FSHD biomarker: focus on the face**
**A research protocol on muscle ultrasonography in FSHD**

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## Introduction

Objective, relevant and patient friendly biomarkers are highly needed in FSHD(1). Muscle ultrasonography is a perfect candidate: muscle ultrasonography is a patient-friendly, non-invasive method to screen for dystrophic changes in skeletal muscle (2). It can detect myopathy and inflammation and has been well-validated for screening and follow-up in several childhood and adult neuromuscular disorders (3). In addition, with this technique we can focus on the facial muscles as well. Muscle ultrasonography has never been investigated as a follow-up tool in FSHD.

## Methods

A prospective, longitudinal study is performed within the Radboud university medical centre, a national referral centre for FSHD. All patients with FSHD who visit the Radboudumc (≥1 consult/year) are asked to participate with a baseline measure and one (12 months) or two (six and 12 months) follow-up measures. Six muscles are screened on both sides and the echo intensity will be compared to muscle specific reference values. A composite sum score will be calculated. The sum scores will be compared to clinical functioning (MRC grading, the FSHD evaluation score and a short version of the motor function measure). The first results are expected at the end of 2017.

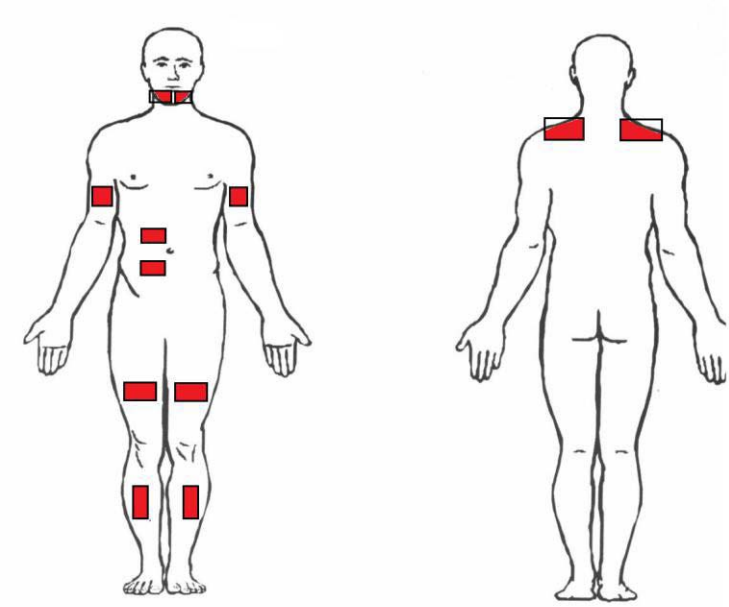
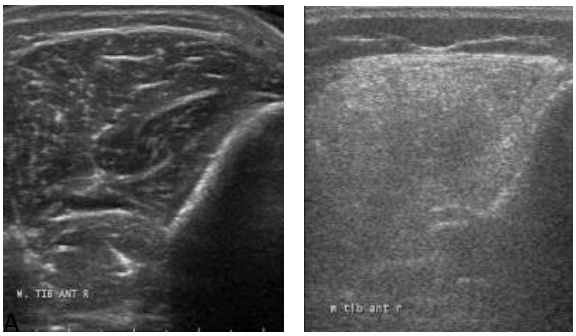


Figure 1. The muscle ultrasound screening protocol.




A B

Figure 2. Ultrasound images
A Normal tibialis anterior muscle. B Tibialis anterior muscle in patient with FSHD.

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33.
[P]

**Analysis of DUX4 protein expression in FSHD muscle biopsies and a mouse model of FSHD**

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Recapitulating phenotypic changes of FSHD muscle in an animal model of disease has been a fundamental roadblock to understanding disease pathogenesis. To overcome this obstacle, we have developed a mouse model that reproduces the slow, progressive muscle damage observed with FSHD. The mouse model muscle phenotype is dependent on the dose and time post administration of a recombinant AAV delivering the *DUX4* gene. In the process of characterizing our mouse model we developed conditions to detect expression of DUX4 protein by immunofluorescent staining of the DUX4-expressing myofibers. We adapted our methods to find DUX4 protein in human FSHD biopsies by coupling immunofluorescence with high magnification confocal microscopic imaging of muscle cryosections to identify potential DUX4-positive nuclei from non-specific background staining. Positive DUX4 nuclei were found in peripheral, central, and in non-peripheral positions in the mouse model. Human nuclei identified so far were peripheral or offset from a central position in myofibers. Comparisons between the mouse and human DUX4-positive nuclei highlight the damaging effect of DUX4 protein expression. Probing the cellular location of DUX4 protein in FSHD and DUX4 mouse myofibers provides potentially important insights into FSHD pathophysiology to guide the development of candidate therapies.

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**FSHD1 carrying 5-10 D4Z4 repeats and FSHD2 are a disease spectrum**

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FSHD1 is an autosomal dominant disease caused by contraction of D4Z4 to 1-10 repeats. FSHD2 is a digenic inheritance disease caused by D4Z4 of 11-16 repeats and *SMCHD1* mutations. Though the penetrance of FSHD1 is incomplete, the mechanism remains to be understood. We hypothesized that *SMCHD1* mutations might modify the penetrance of FSHD1. Therefore, we screened *SMCHD1* mutations in FSHD1.

We analyzed D4Z4 methylation in probands from 190 unrelated FSHD1 families carrying 1-10 D4Z4 repeats by bisulfite pyrosequence and identified16 probands with D4Z4 hypomethylation. Among them, we identified seven novel heterozygous *SMCHD1* mutations by Sanger sequence in seven probands carrying 5-10 D4Z4 repeats. Within their families, family members carrying both of contracted D4Z4 and *SMCHD1* mutations developed FSHD, while those carrying either of them did not.

In conclusion, we highlighted that FSHD1 carrying 5-10 D4Z4 repeats is caused by digenic inheritance of the contracted D4Z4 and *SMCHD1* mutations and shares the genetic causes with FSHD2. We suggest that FSHD1 carrying 5-10 D4Z4 repeats and FSHD2 are a disease spectrum.

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**Nuclear bodies reorganize during human myogenesis *in vitro* and are differentially disrupted by expression of FSHD-associated DUX4.**

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**Background.** Nuclear bodies, such as nucleoli, PML bodies, and SC35 speckles, are dynamic sub-nuclear structures that regulate multiple genetic and epigenetic processes. Additional regulation is provided by RNA/DNA handling proteins, notably TDP-43 and FUS, which have been linked to ALS pathology. Previous work showed that mouse cell line myotubes have fewer but larger nucleoli than myoblasts, and we had found that nuclear aggregation of TDP-43 in human myotubes was induced by expression of DUX4-FL, a transcription factor that is aberrantly expressed and causes pathology in facioscapulohumeral dystrophy (FSHD). However, questions remained about nuclear bodies in human myogenesis and in muscle disease.

**Methods.** We examined nucleoli, PML bodies, SC35 speckles, TDP-43, and FUS in myoblasts and myotubes derived from healthy donors and from patients with FSHD, laminin-alpha-2-deficiency (MDC1A), and alpha-sarcoglycan-deficiency (LGMD2D). We further examined how these nuclear bodies and proteins were affected by DUX4-FL expression.

**Results.** We found that nucleoli, PML bodies, and SC35 speckles reorganized during differentiation in vitro, with all three becoming less abundant in myotube vs. myoblast nuclei. In addition, though PML bodies did not change in size, both nucleoli and SC35 speckles were larger in myotube than myoblast nuclei. Similar patterns of nuclear body reorganization occurred in healthy control, MDC1A, and LGMD2D cultures, as well as in the large fraction of nuclei that did not show DUX4-FL expression in FSHD cultures. In contrast, nuclei that expressed endogenous or exogenous DUX4-FL, though retaining normal nucleoli, showed disrupted morphology of some PML bodies and most SC35 speckles and also co-aggregation of FUS with TDP-43.

**Conclusions.** Nucleoli, PML bodies, and SC35 speckles reorganize during human myotube formation in vitro. These nuclear body reorganizations are likely needed to carry out the distinct gene transcription and splicing patterns that are induced upon myotube formation. DUX4-FL-induced disruption of some PML bodies and most SC35 speckles, along with co-aggregation of TDP-43 and FUS, could contribute to pathogenesis in FSHD, perhaps by locally interfering with genetic and epigenetic regulation of gene expression in the small subset of nuclei that express high levels of DUX4-FL at any one time.

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**Large family cohorts of lymphoblastoid cells provide a new cellular model for investigating FSHD**

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**Abstract**

Facioscapulohumeral muscular dystrophy (FSHD) is associated with aberrant epigenetic regulation of the chromosome 4q35 D4Z4 macrosatellite repeat. The resulting DNA hypomethylation and relaxation of epigenetic repression leads to increased expression of the deleterious *DUX4-fl* mRNA encoded within the distal D4Z4 repeat. With the typical late onset of muscle weakness, prevalence of asymptomatic individuals, and an autosomal dominant mode of inheritance, FSHD is often passed on from one generation to the next and affects multiple individuals within a family. Here we have characterized unique collections of 114 lymphoblastoid cell lines (LCLs) generated from 12 multigenerational FSHD families, including 56 LCLs from large, genetically homogeneous families in Utah. We found robust expression of *DUX4-fl* in most FSHD LCLs and a good correlation between DNA hypomethylation and repeat length. In addition, *DUX4-fl* levels can be manipulated using epigenetic drugs as in myocytes, suggesting that some epigenetic pathways regulating *DUX4-fl* in myocytes are maintained in LCLs. Overall, these FSHD LCLs provide an alternative cellular model in which to study many aspects of D4Z4, DUX4, and FSHD gene regulation in a background of low genetic variation. Significantly, these non-adherent immortal LCLs are amenable for high-throughput screening of potential therapeutics targeting *DUX4-fl* mRNA or protein expression.

**Acknowledgements**

We are grateful to these families for participating in the study. We thank Dr. Stephen Jacobsen for clinically evaluating many of the subjects and collecting the clinical samples, and the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research repository for culturing, maintaining, and distributing the cell lines. We thank Dr. Yukiko K. Hayashi, Department of Neurophysiology, Tokyo Medical University, for sharing the protocol for linear southern blotting analysis.

**Funding**

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**Why did the heated discussion arise between Erb and Landouzy–Dejerine concerning the priority in describing the facio-scapulo-humeral muscular dystrophy and what is the main reason for this famous discussion?**
(About clinical heterogeneity of FSHD from a historical point of view)

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The famous historical discussion, which arose toward the end of the 19 century between Erb and Landouzy–Dejerine, has so far not received a correct interpretation. The main subject of the discussion has so far not been established. There has been an attempt to accept this famous discussion as a pointless argument between three physicians regarding the priority in describing the facio-scapulo-humeral muscular dystrophy (FSHD) because 'the earliest and the most detailed description of the disease was not by Landouzy–Dejerine, nor by Erb, but by Duchenne' (1) АгкглфFurukawa 1995x[1]Furukawa, T. Wilheim Erb's claim for priority on FSH dystrophy against Landouzy and Dejerine. *Muscle Nerve*. 1994; : S179

See all References[1]. This point of view raises some objections.

"Why did the heated discussion arise between Erb and Landouzy-Dejerine? " – Duchenne asked me.

From the right side to the left one: Erb, Duchenne, Kazakov, Dejerine and Landouzy. On the table there is the book called: Duchenne de Boulogne. De l'électrisation localisée. 1st ed. Paris, Baillière 1855. (I thank very much indeed for this picture to Valery Sergeev)

I replayed to Duchenne: These physicians admitted your priority in describing of the FSHD (a gradually descending type - author's note) in 1855 under the name "progressive hereditary fatty muscular atrophy beginning with a face in adults or in teenagers or in childhood" with subsequent involvement of shoulder girdle, then upper arms, abdomen, trunk, pelvic girdle and thighs muscles (2). As well as in new time the same authors supposed that FSHD usually begins initially by weakness of the facial and pectoral muscles and only 20 or 30 years does the pelvic girdle muscle become affected (3).
Erb and Landouzy-Dejerine in 1885-1891 discussed about the priority of describing of another variant the FSHD (a descending with a "jump" type - author's note) in which after affection of facial and shoulder girdle muscles the anterior tibial muscles were involved. Erb was the first who to describe a "hard" pattern of muscles that were isolately affected and those that were preserved in this variety and he also established the early and severe involvement of the anterior tibial muscles (4, 5). However, an autosomal dominant mode of inheritance of this variety was established by Landouzy and Dejerine (6)".

Thus, the famous discussion between Erb and Landouzy–Dejerine dealt with the priority of recognizing and describing this very special variety of FSHD characterized by a 'jump' of the myodystrophic process from the upper part of the body to the anterior tibial muscles (6-10) which I called as a facioscapulolimb muscular dystrophy type 2 (FSLD2), a descending with a "jump" with initial facioscapuloperoneal phenotype, autosomal dominant (7, 12, 13) which it is probably the same disease as a FSHD type 1 (FSHD1) linked with chromosome 4q35.

Erb and Landouzy-Dejerine admitted the priority of Duchenne in describing of a FSHD descending type (5,6) which I called as a facioscapulolimb muscular dystrophy type 1 (FSLD1), a gradually descending with initial facioscapulohumeral phenotype, autosomal dominant (7, 12, 13). Among Duchenne's casuistry (15 patients of FSHD) I could not find out the cases with early and severe affection of the anterior tibial muscles.

It is necessary to say about one more variant of FSHD type 2 characterized by an identical clinical phenotype and muscle pathology as FSHD1 (14, 15) (or with FSLD2) which I called as a facioscapulolimb muscular dystrophy type 3 (FSLD3), a descending with a "jump" with initial facioscapuloperoneal phenotype, autosomal dominant linked with chromosome 18p.

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**Genome-wide gain- and loss-of-function screens to identify genetic modifiers of FSHD**

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Facioscapulohumeral dystrophy (FSHD) is a complex autosomal dominant muscular dystrophy with an incomplete genetic story. The likely mechanism of disease is linked to epigenetic changes at the 4q35 locus, causing misexpression of the retro-transposon derived gene, DUX4. The DUX4 gene encodes a transcription factor whose expression is rare, but extremely toxic. Misexpression of DUX4 is hypothesized to induce inappropriate expression of downstream genes to cause disease pathogenesis. How DUX4 and its target genes cause disease remain unknown, but clinical evidence suggests that DUX4 misexpression is not an exclusive determinant of FSHD. Non-manifesting carriers of FSHD alleles who are found to express DUX4, point to the existence of genetic modifiers that may act to suppress DUX4 toxicity. Our aim is to perform genome-wide screens to identify all potential genetic modifiers of FSHD. Using CRISPR loss-of-function and gain-of-function libraries that target all genes in the human genome, we can systematically test every genetic on/off perturbation that can result in suppression of DUX4 toxicity. The identification of these naturally occurring DUX4 suppressors will not only shed light on the pathogenic pathways associated with FSHD, but will also help to identify potential targets for therapeutic intervention.

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**SSLP-converter: a new software program for the conversion of SSLP fragment information into D4Z4 haplotype configuration**

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We have developed a software program that allows to predict the D4Z4 haplotype composition on chromosomes 4 and 10 based on the SSLP sizing and stratified for ethnic background. This tool can be easily implemented in the diagnostic toolkit for FSHD and will be made freely available.

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**Methods for improving the quality of xenografts in mice by control and fshd myogenic cells**

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**Abstract**

Facioscapulohumeral Muscular Dystrophy (FSHD) is one of the most common muscular dystrophies in man, with a prevalence of 1 in ~8,000 individuals worldwide. Studies of the pathogenic mechanisms underlying human myopathies and muscular dystrophies often require animal models, but a model that recapitulates the signature pathophysiology of FSHD is not yet available. Our laboratory has developed a novel mouse model for FSHD. We optimized methods to xenograft immortalized human muscle precursor cells (hMPCs from biceps muscles from individuals with FSHD and unaffected controls) to promote the formation of mature human myofibers following implantation into the hindlimbs of NOD-*Rag1nullIL2rγnull* immune-deficient mice. We compare the effectiveness of some variations from our patented xenograft model: intermittent neuromuscular electrical stimulation (iNMES), use of cages with running wheels, xenografting both hindlimbs, and multiple injections of hMPCs. Specifically, iNMES increases the number and size of the engrafted human myofibers, and decreases the distances between the largest fibers and their neighbors. The human myofibers in the xenograft are innervated, their contractile apparatus is fully differentiated, and they are comprised of human myonuclei, with minimal contamination by murine myonuclei. Each of the other methods also improve engraftment compared to untreated controls, but none are as effective as iNMES alone. We are currently exploring the use of several of these manipulations to further improve engraftment, to enable biochemical and physiological studies of FSHD-derived human muscle under controlled conditions and for testing new therapeutic agents *in vivo*.

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**FSHD diagnostic testing at Iowa from 1998 to 2016**

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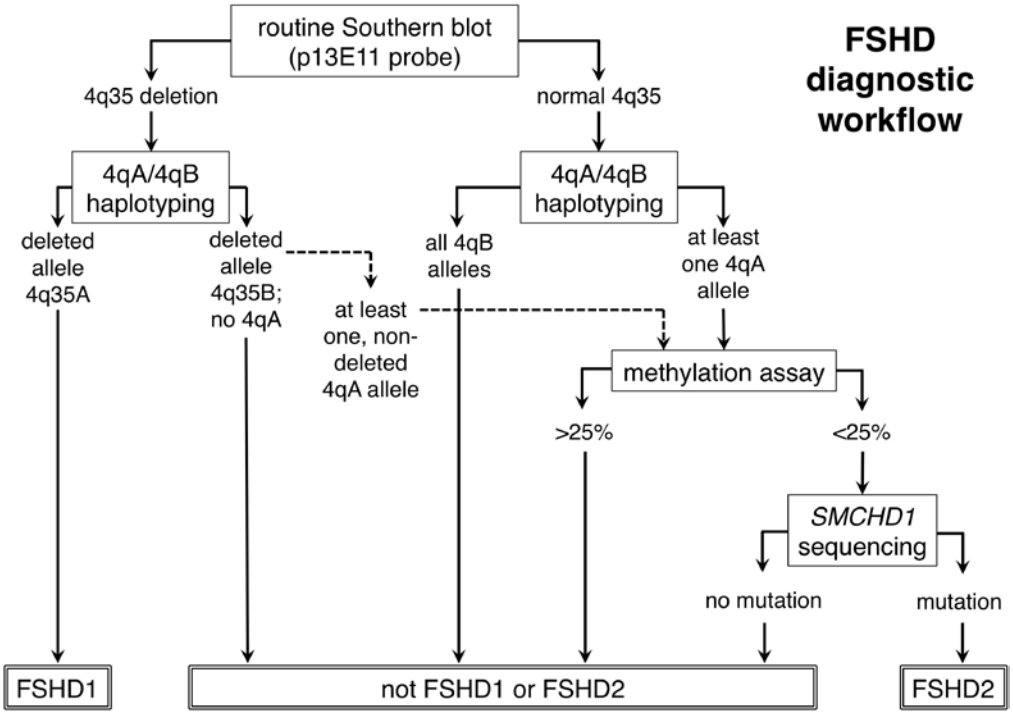
A molecular basis for FSHD (deletion within a 4q35 tandem repeat, D4Z4) was identified in 1993, providing the basis for diagnostic genetic testing. Over the past 20+ years, testing has evolved to now include FSHD1 and FSHD2 genotypes. In the beginning, clinical diagnostic testing services were quite limited. In the United States, genetic testing for the diagnosis of FSHD was first offered in a CLIA certified Molecular Pathology Laboratory in the summer of 1998 at the University of Iowa. Testing initially consisted of Southern blotting with a p13E-11 probe to detect contracted 4q35 alleles in EcoRI and EcoRI/BlnI restriction enzyme digests of isolated DNA, but it has progressively improved and expanded to include prenatal testing (2005), 4qA/4qB haplotyping (2007), embedding cells in agarose plugs to improve detection of large restriction fragments (2009), FseI methylation (2015) and *SMCHD1* sequencing (2015). Our current diagnostic testing workflow is depicted below. The annual sample volume over these 18 years has ranged from 130 to 350, with an average of 275 samples/year. Each year, between 32% and 47% of submitted cases were diagnosed with FSHD1; the overall average rate of positive samples is approximately 40%. By the end of 2016, our laboratory will have evaluated approximately 4900 individuals and diagnosed more than 1900 FSHD1 patients. Included in this total are approximately 60 prenatal evaluations of cultured chorionic villi (or amniocytes) with a very similar rate of positive testing for FSHD1. In the 18 months since beginning FseI methylation and *SMCHD1* sequencing, FSHD2 has been diagnosed in 7 patients. Applying a 40% positive rate for FSHD1 over the same 18-month interval, approximately 160 cases were diagnosed. Thus, 4% of the total FSHD patients diagnosed since implementing FSHD2 testing have had permissive 4q35A alleles, hypomethylation, and *SMCHD1* mutations.



42.
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**Xenografting Human Muscle Stem Cells into Mice, a novel model for FSHD**

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Facioscapulohumeral Muscular Dystrophy (FSHD) is the most common autosomal dominant muscular dystrophy in man with a prevalence of 1 in ~8,000 individuals worldwide. Studies of the pathogenic mechanisms underlying human myopathies and muscular dystrophies often require animal models, but a model that recapitulates the signature pathophysiology of FSHD is not yet available. Recently, our laboratory developed a novel mouse model for FSHD involving xeno-transplanting human muscle cell precursors into mice. We investigated the ability of immortalized human myogenic precursor cells (hMPCs) to form mature human myofibers following implantation into the hindlimbs of immune-deficient mice. We developed methods to promote the engraftment of immortalized hMPCs from bicep muscles from individuals with FSHD and unaffected controls. Specifically, intermittent Neuromuscular Electrical Stimulation (iNMES) increases the number and size of the engrafted human myofibers, and decreases the distances between the largest fibers and their neighbors. The human myofibers in the xenograft are innervated, their contractile apparatus is fully differentiated, and they are comprised of human myonuclei, with minimal contamination by mouse myonuclei. Most importantly, the xenografts replicate the pathogenic molecular signature of patients with FSHD. Our results indicate that iNMES is an effective tool in promoting the engraftment of hMPCs in mice and their development into mature human muscle fibers. Our methods provide an ideal model for studying FSHD-derived human muscle under controlled conditions within the mouse hindlimb and for testing new therapeutic agents in vivo.

43.
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**A transgenic zebrafish model for FSHD**

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common forms of muscular dystrophy, characterized by asymmetric weakness of the facial, shoulder and upper arm muscles, accompanied by hearing loss and retinal vasculopathy. Although the causative gene for FSHD remains controversial, the primate specific retrogene, DUX4, is a leading candidate. Using a tamoxifen-controlled CreERt2-loxP system, we have generated a transgenic zebrafish DUX4 line that successfully reproduces the mosaic, low-level expression of DUX4. We show that activating DUX4 expression during development results in a degenerative muscle phenotype. We found that DUX4 (+) nuclei are no longer present in muscles that have undergone degeneration and displayed inflammation, fibrosis and fatty replacement suggesting that FSHD is a developmental disease. This stable line will enable us to follow FSHD pathogenesis and perform functional studies in our aging zebrafish model.

44.
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**The FSHD Clinical Trial Research Network**

Jeffrey Statland, MD [(1)], Nicholas Johnson, MD [(2)], Leo Wang, MD, PhD [(3)], Perry Shieh, MD, PhD [(4)], John Kissel, MD [(5)], Kathryn Wagner, MD, PhD [(6)], Rabi Tawil, MD [(7)], and the FSHD CTRN Investigators

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[(2)] University of Utah, Salt Lake City, UT
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[(4)] University of California, Los Angeles
[(5)] The Ohio State University, Columbus, OH
[(6)] Kennedy Krieger Medical Center, Baltimore, MD
[(7)] University of Rochester Medical Center, Rochester, NY

**Background:** The discovery of a unifying model for the cause of FSHD means that, for the first time since the discovery of the genetic defect twenty years ago, *it is possible to develop targeted treatments for FSHD*. Laboratories are developing disease-directed therapies, and several pharmaceutical companies are actively investigating therapeutic approaches to treat FSHD. In parallel with these efforts, the clinical research community must consolidate efforts to prepare the tools and infrastructure to conduct future FSHD clinical trials.

**Objective:** To hasten drug development for FSHD by creating a network of FSHD specialty sites utilizing common protocols and infrastructure to refine trial planning strategies and validate new clinical outcome assessments.

**Methods:** We have assembled 7 sites with broad geographical representation across the United States (Kennedy Krieger, University of Rochester, The Ohio State, University of Kansas, University of Utah, University of Washington, and University of California – Los Angeles) and 3 back-up sites with a mandate to create a common infrastructure for regulatory, data and biostatistical support. The FSHD CTRN will use this infrastructure to: 1) support the development of reliable and sensitive outcome measures for FSHD; 2) create standard operating procedures which can be easily rolled out across multiple sites – creating a pathway for expanding the network as necessary; 3) define stratification strategies to handle the variability in the natural history of FSHD for efficient clinical trial planning; and 4) verify the ability to obtain similar results for our outcome measures across multiple sites – this is very important to gain FDA approval of any new drug. We will incorporate the patient voice into network activities by working in collaboration with patient advocacy groups, by including patients on the network advisory board, and utilizing a system of patient engagement circles to seek guidance on particular network projects, or issues related to recruitment and retention in studies. The data obtained from the FSHD CTRN will be made available to any company or researcher with a goal of therapeutic development for patients with FSHD.

**Results:** We convened a national conference of clinical evaluators to develop standard procedures for common FSHD strength and functional motor testing in July 2016. This meeting will result in the development of standard operating procedures and protocols for common outcome measures. We have started working on common data elements and electronic case report forms. We have assembled a network advisory board which include trialists, representatives from industry, and patient

representatives. We have started the process of streamlining regulatory oversight by leveraging existing national initiatives like the CTSA SMART initiative. We have submitted grant proposals to help refine clinical trial strategies, and validate new clinical outcomes assessments.

**Conclusion:** The FSHD CTRN is a new scalable research network with an overarching goals of hastening drug development for FSHD: which utilizes a collaborative model with oversight from trialists, industry, patient advocacy, and patients. The data from this network can help any researcher or company with a goal of improving the lives of people with FSHD.

*The FSHD CTRN is supported by a grant from the FSH Society and by donations from private philanthropy. JS work on this project is supported by a NCATS grant awarded to the University of Kansas Medical Center for Frontiers: The Heartland Institute for Clinical and Translational Research # KL2TR000119.*

45.
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**A high throughput xenograft model to evaluate potential therapeutic modulators of DUX4 expression in FSHD**

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Facioscapulohumeral dystrophy (FSHD) is characterized by epigenetic changes resulting in aberrant expression of the DUX4 retrogene in skeletal muscle. Therapeutic strategies aimed at epigenetic silencing of DUX4 are hampered by the lack of animal models to test the pharmacological modulation of DUX4 expression. Such strategies would benefit greatly from a robust animal model that faithfully recapitulates de-repression of DUX4 from its endogenous locus in human FSHD muscle cells. We have utilized a mouse xenotransplantation model in which human FSHD myoblasts suspended in a barium chloride solution are injected into the tibialis anterior muscles of immunodeficient mice, effectively combining muscle injury and human cell implantation into one step. DUX4, DUX4 target and normal human muscle gene expression were profiled over a 4 week time course after xenotransplantation. In this model, DUX4 expression is induced after FSHD1 or FSHD2 myoblasts are transplanted. DUX4 expression peaks approximately 4 days after transplantation, mimicking the differentiation-dependent increase of DUX4 that is seen in vitro. Markers of human muscle cell differentiation indicate that both FSHD and normal human myoblasts efficiently engraft and become part of mature muscle fibers present at weeks 3 and 4. This xenograft model of FSHD-specific gene dysregulation will enable evaluation of a broad range of potential therapeutics targets to promote the epigenetic repression and/or inhibit the activity of DUX4.

46.
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**Muscle microdialysis in facioscapulohumeral muscular dystrophy.**

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Recent progresses in the understanding of the genetic mechanisms underlying Facioscapulohumeral muscular dystrophy (FSHD) have opened the way to the development of targeted therapies. However, knowledge about the pathophysiology leading to muscle damage is still limited. Aim of our study was to collect and analyze interstitial fluid obtained from muscles with different MRI signal in FSHD patients, in particular comparing normal muscles ($STIR^-$) with muscles displaying early lesions ($STIR^+$), with a focus on inflammatory features.

We performed an exploratory study on a small cohort of FSHD patients and controls using prolonged muscle microdialysis with high cut-off membranes (100 kDa). Microdialysates were analyzed with xMAP technology (multi-analyte profiling beads) to compare the levels of a wide panel of inflammatory cytokines, chemokines and growth factors. The procedure was well tolerated by all the patients and healthy subjects. A small number of chemokines and inflammatory mediators appear to be dysregulated in $STIR^+$ versus $STIR^-$ and control muscles.

The results obtained with this innovative approach support the evidence of a selective inflammatory process taking place in $STIR^+$ FSHD muscles. The application of this technique could provide insights on novel mechanisms involved in muscle damage in FSHD as well as in other myopathies.

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**FSHD tissue donation registry**

Denee J. Tidwell and Honesto I. Nunez III

National Disease Research Interchange

The National Disease Research Interchange (NDRI) is a non-profit organization that serves as the link between individuals wishing to donate organs and tissues for research and the nation's leading investigators who are working to find new treatments or cures for a wide range of diseases. NDRI receives funding and oversight from the National Institutes of Health (NIH) to make human bio specimens available to researchers in academia and other non-profit organizations. NDRI's Donor Programs give patients and their family members an opportunity to make a significant contribution to research and development by providing a simple mechanism through which tissues and organs can be donated. In order to identify patients interested in donating bio specimens for research, NDRI works with numerous patient advocacy groups one being the Facioscapulohumeral Muscular Dystrophy Society. NDRI obtains consent for donation from patients or their next-of-kin and coordinates the collection, packaging and shipping of bio specimens to approved researchers at the time of surgery or death. NDRI's recovery partners include hospitals, Organ Procurement Organizations (OPOs), tissue banks, medical examiners and funeral homes; these relationships are critical to our success in sourcing FSHD specimens. NDRI's recovery process permits the collection of high-quality bio specimens. NDRI specializes in establishing researcher specific protocols for donor eligibility and organ and tissue collection procedures for optimal bio specimen sampling and preservation. These well annotated samples provided through our collaboration with our nationwide network of recovery partners are needed to continue the vital work to discover new therapies for FSHD treatment.

48.
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**Physiological characterization of muscles from FSHD patients with early stage disease**

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[2] University of Texas Health Science Center at Tyler, Tyler, TX
[3] Kennedy Krieger Institute, Baltimore, MD

The pathophysiology of Facioscapulohumeral Dystrophy (FSHD) is poorly understood and understudied. Lassche et al (2013, PMID:23365058) previously reported that active force in quadriceps muscles and tibia anterior (TA) muscle from FSHD subjects was reduced compared to control muscles, while passive force was elevated in FSHD muscles. To characterize muscle physiology at early clinical stages of FSHD, biopsies muscle from patients with mild weakness and first-degree relatives were subjected to single fiber analyses. We assayed physiological properties of muscles (specific force, Ca2+ sensitivity, passive force) from early stage FSHD patients. In contrast to Lasshe et al., (2013), the properties examined in early stage FSHD muscles were not different from controls. These results suggest that we need to analyze muscles from FSHD subjects at more advanced stages of disease to understand physiological changes in FSHD patients related to muscle weakness. Experiments are in progress to investigate passive force in muscle bundles to examine the relationship between extracellular matrix and muscle passive force in early disease and during later disease progression.

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49.
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**Investigating the role of Smchd1 in regulating gene expression**

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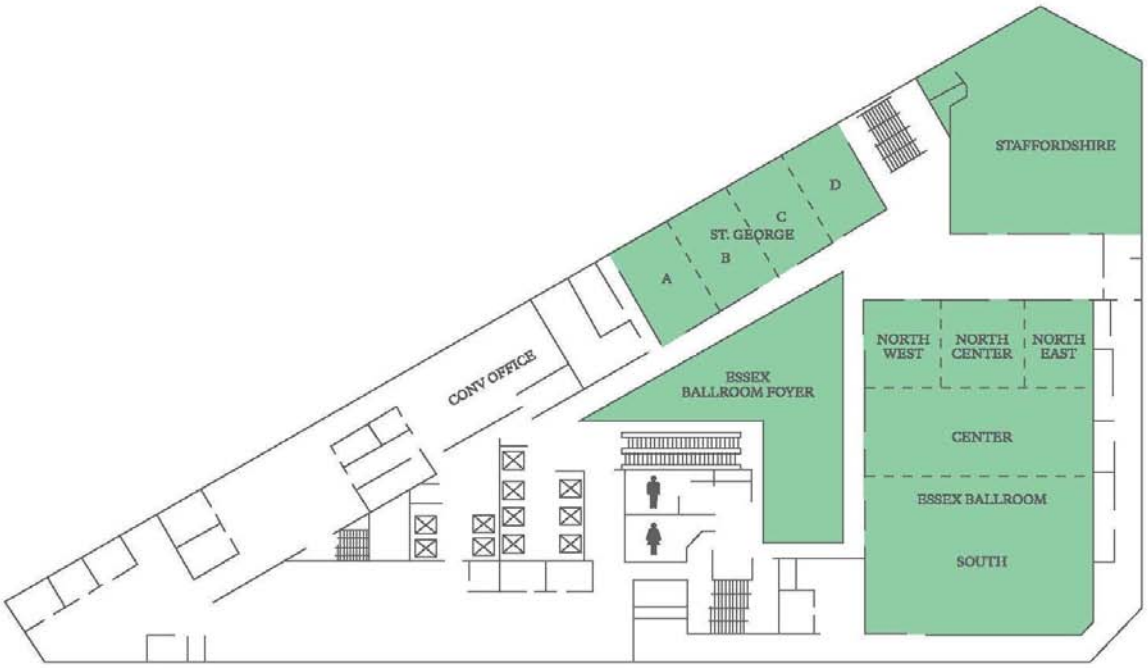
Mutations of the human *SMCHD1* gene cause FSHD2 and serve as genetic modifiers of disease severity of FSHD1. Recent studies on *Smchd1* knockout mice indicate that Smchd1 protein is an epigenetic repressor; however, the mechanism by which Smchd1 represses gene expression remains unknown. We used CRISPR-Cas9 technology to disrupt endogenous *Smchd1* loci in mouse cells and performed RNA-sequencing to investigate the role of Smchd1 in regulating gene expression. We detected de-repression of previously reported Smchd1 regulatory targets, such as protocadherin gene clusters and X-linked genes, indicating that our *Smchd1* knockout cells recapitulated the phenotype of *Smchd1* knockout mice. We plan to use this novel Smchd1 loss-of-function system to dissect the mechanism of Smchd1 function. This tractable system may also provide an opportunity to determine how FSHD-associated mutations affect the function of Smchd1 *in vivo*.

Hotel Map

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