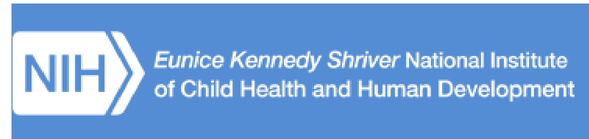
FSH Society Facioscapulohumeral Muscular Dystrophy [FSHD]
2013 International Research Consortium & Research Planning Meetings

Sponsored by:



FSH Society Facioscapulohumeral Muscular Dystrophy [FSHD]
2013 International Research Consortium & Research Planning Meetings

Monday, October 21, 2013
9:00 a.m. – 6:00 p.m.
[Registration and breakfast begins 8:30 a.m.-]

Tuesday, October 22, 2013
9:00 a.m. – 2:00 p.m.
[Registration and breakfast begins 8:30 a.m.-]

Massachusetts Institute of Technology
David H. Koch Institute for Integrative Cancer Research
Building 76 Room 156
500 Main Street
Cambridge, Massachusetts 02139 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

Organizers:

Daniel Paul Perez
FSH Society
David E. Housman, PhD
Stephen J. Tapscott, MD, PhD
Silvère van der Maarel, PhD

Sponsored By:

Association Française Contre les Myopathies (AFM)
Cytokinetics
FSH Society
FSHD Global Research Foundation
Genzyme, a Sanofi Company
Muscular Dystrophy Association United States (MDAUSA)
NIH Eunice Kennedy Shriver NICHD Senator Paul D. Wellstone MDCRC for FSHD at University of Massachusetts Medical School
Quest Diagnostics / Athena Diagnostics
Regeneron

PREFACE

October 21, 2013
Cambridge, Massachusetts

Dear Colleagues,

Welcome to the FSH Society 2013 International Research Consortium & Research Planning Meetings. This workshop brings together clinicians, scientists, patient representatives and policy makers to discuss the latest developments in facioscapulohumeral muscular dystrophy (FSHD). For almost 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 over the past three years and even in the past few 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 new and existing therapies for the disorder.

Over the two days, we will revisit the five priority areas identified at last year's meeting, and discuss what we have achieved, what are the critical gaps that remain, and where we need to focus. 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 if new areas should be considered.

This meeting is organized by the FSH Society and sponsored by Association Française Contre les Myopathies (AFM), Quest Athena Diagnostics, Cytokinetics, FSH Society, FSHD Global Research Foundation, Genzyme, a Sanofi Company, Muscular Dystrophy Association (MDAUSA), the U.S. DHHS NIH Eunice Kennedy Shriver NICHD Sen. Paul D. Wellstone UMMS FSHD Muscular Dystrophy Cooperative Research Center and Regeneron. We thank our sponsors for their generous financial support.

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

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

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

Daniel Paul Perez
FSH Society

Monday, October 21, 2013

Session	Time	Details
Registration & Breakfast	8:30 a.m.-9:00	
Welcome	9:00-9:05	Welcome **Daniel Perez, David Housman, Silvere van der Maarel, Stephen Tapscott**
Review of 2012	9:05-9:20	**Review of 2013 priorities** as stated by FSHD workshop in 2012 Moderators: **David Housman, Silvere van der Maarel, Michael Altherr**
Platform Session 1	9:20-10:10	**Clinical Studies** (2x15 minutes; 2x10 minutes) **Moderators: Stephen Tapscott, Rabi Tawil**
	10:10-10:40	Discussion
Platform Session 2	10:40-11:55	**Genetics and epigenetics** (3x15 minutes; 3x10 minutes) **Moderators: Rune Frants, Louis Kunkel**
	11:55-12:25	Discussion
Poster Introductions	12:25-1:40	Lunch and **Poster Viewing** [collect and have **Lunch**]
Platform Session 3	1:40-2:30	**Models** (2x15 minutes; 2x10 minutes) **Moderators: Davide Gabellini, Scott Harper**
	2:30-3:00	Discussion
Platform Session 4	3:00-4:05	**Molecular mechanisms** (3x15 minutes; 2x10 minutes) **Moderators: Stephen Tapscott, Alexandra Belayew**
	4:05-4:35	Discussion
Platform Session 5	4:35-5:20	**Therapeutic studies** (3x15 minutes) **Moderators: David Glass, Rabi Tawil**
	5:20-5:50	Discussion
Assembly Session	5:50-6:00	Discuss charge for **Tuesday's meeting to define 2013/2014 research priorities**, future directions, etc. **Moderators: David Housman, Rune Frants, Louis Kunkel**
Adjourn	6:00 p.m.	

Tuesday, October 22, 2013

Registration & Breakfast	8:30 a.m.-9:00
Welcome	9:00-9:10
Discussion and planning	9:10-12:50

Break out sessions

Five discussion sessions with entire group of attendees based on data presented at day 1

9:10-9:50
Genetics/Networks
Moderators: Silvere van der Maarel, Louis Kunkel, Alexandra Belayew

9:50-10:30
Clinical Trials/Readiness
Moderators: Rabi Tawil, Sabrina Sacconi, Baziel van Engelen

10:30-11:10
Model Systems/Sharing
Moderators: Michael Altherr, David Housman

11:10-12:00
Is there anything we don't agree on?
Moderators: Silvere van der Maarel, Stephen Tapscott, Charles Emerson, Jr.

12:00-12-50
Are there critical gaps in our knowledge?
Moderators: David Housman, Louis Kunkel, Rune Frants

Lunch	12:50 -2:00 p.m.
Adjourn	2:00 p.m.

The FSH Society, Inc. (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, Inc., Daniel Paul Perez, phones: (781) 301-6650 and (781) 275-7781, fax: (781) 862-1116, e-mail: daniel.perez@fshsociety.org, website: http://www.fshsociety.org

NOTES ON TALKS AND POSTERS

	First Author	Presenting Author	Topic	
Platform Session 1				
Clinical Studies				
Chairs: Stephen Tapscott, Rabi Tawil				
9:20-9:35 a.m.	Voet	van Engelen	Effectiveness of aerobic	
9:35 -9:50 a.m.	Tasca	Ricci	Muscle MRI	
9:50-10:00 a.m.	*Winston	Upadhyaya	Identification of two novel SMCHD1	
10:00-10:10 a.m.	*Sacconi	Sacconi	Inflammation in FSHD1	
Platform Session 2				
Genetics and epigenetics				
Chairs: Rune Frants, Louis Kunkel				
10:40 -10:55 a.m.	Gabellini	Gabellini	Epigenetic regulation of FSHD	
10:55-11:10 a.m.	Lemmers	Lemmers	SMCHD1 mutations in FSHD	
11:10-11:25 a.m.	Robin	Wright	Length dependent telomere looping	
11:25 -11:35 a.m.	*Casa	Casa	D4Z4 as a mammalian polycomb response element	
11:35 -11:45 a.m.	*Gaillard	Magdinier	FSHD, D4Z4 hypomethylation and DUX4	
11:45-11:55 a.m.	*Ansseau	Ansseau	Interplay between SP1, YYI and MyoD	
Platform Session 3				
Models				
Chairs: Davide Gabellini, Scott Harper				
1:40:1:55 p.m.	Zavaljevski	Chamberlain	AAV delivery of the D4Z4 L42	
1:55-2:10 p.m.	Wallace	Wallace	The DUX4 promoter mouse	
2:10-2:20 p.m.	*Caron	Schmidt	Towards a human stem cell model	
2:20-2:30 p.m.	* Das	Das	Developing resources to alleviate muscle	
Platform Session 4				
Molecular mechanisms				
Chairs: Stephen Tapscott, Alexandra Belayew				
3:00-3:15 p.m.	Rahimov	Rahimov	Expression analysis of DUX4 targets	
3:15-3:30p.m.	Banerji	Banerji	Unraveling FSHD patho-mechanisms	
3:30-3:45p.m.	Pandey	Chen	Lower autophagy activities in FSHD	
3:45-3:55 p.m.	*Ferreboeuf	Dumonceaux	Nuclear protein spreading	
3:55 -4:05 p.m.	*Moyle	Moyle	Ret contributes to DUX4-mediated	
Platform Session 5				
Therapeutic studies				
Chairs: David Glass, Rabi Tawil				
4:35-4:50p.m.	Gatica	Rosa	DUX4 negatively regulates	
4:50-5:05p.m.	Sharma	Chen	Poly(ADP-ribose) polymerase 1	
5:05-5:20 p.m.	Sverdrup	Sverdrup	Chemical genetics identifies	
Posters [	Eichinger	Tawil	Outcome Measures	Poster
	Domire	Harper	DUX4 p63	Poster
	Jones	Jones	Epigenetic variability is a modifier	Poster

NOTES ON TALKS AND POSTERS (Continued)

Posters (Continued)

First Author	Presenting Author	Topic	
Kazakov	Kazakov	FSHD clinical heterogeneity	Poster
Lahaut	LaHaut	Self reported outcome	Poster
Li	Rutkove	Electrical impedance myography	Poster
Lim	Lim	CTCF binding at the D4Z4	Poster
Mariot	Dumonceaux	Fetal FSHD / dux4	Poster
Ousterout	Gersbach	ZFN, TALEN,CRISPR Cas9 systems in DMD	Poster
Sakellariou	Bloch	model – xenograft	Poster
Statland	Tawil	Serum biomarkers	Poster
Windelborn	Emerson	model – xenograft	Poster]

Talks that are asterisked (*) indicate a shorter 10 minute presentation

Priorities as Stated by FSHD Research Community for FSHD Research at 2012 IRC: 2013 and Beyond

The summary and recommendations of the group state that given the recent developments in our definition of FSHD1A and FSHD1B [FSHD2] and there is a need to ramp up the preclinical enterprise and build/organize infrastructure needed to conduct clinical trials on FSHD1A and FSHD1B. Our immediate priorities should be to confirm the DUX4-fl hypothesis, if valid then understand normal DUX4 function, and finally, understanding the naturally occurring variability should allow us to manipulate the disease in our favor. We need to be prepared for this new era in the science of FSHD, by accelerating efforts in the following five areas:

1. Genetics / epigenetics. There is general acceptance that transcriptional deregulation of D4Z4 is central to FSHD1 and FSHD2. The FSHD2 gene SMCHD1 explains approximately 80% of FSHD2. There is a need for better understanding of the factors that modulate DUX4 activity and disease penetrance.

2. FSHD molecular networks. D4Z4 chromatin relaxation on FSHD-permissive chromosome-4 haplotypes leads to activation of downstream molecular networks. In addition to considering DUX4 as the "target" and downstream targets, the upstream processes and targets – triggering of activation – are equally important. Hence, understanding what DUX4lf does as a target and targets up- and down-stream of it are priorities. Detailed studies on these processes are crucial for insight in the molecular mechanisms of FSHD pathogenesis and may contribute to explaining the large intra- and interfamily clinical variability. Importantly such work may lead to intervention (possibly also prevention) targets. Additional FSHD genes and modifiers are still likely to exist. Apart from chromatin modifiers, these include, but are not limited to, CAPN3 and the FAT1 gene that was recently suggested to be involved in FSHD.

3. Clinical trial readiness. It is now broadly accepted that deregulation of the expression of D4Z4 / DUX4 is at the heart of FSHD1 and FSHD2. This finding opens perspectives for intervention along different avenues. Intervention trials are envisaged within the next several years. The FSHD field needs to be prepared for this crucial step. There is an increasing need to improve the translational process. This includes, but is not limited to, the need for consensus on data capture and storage, overcoming national and international barriers, definition of natural history, identification of (meaningful) and sensitive outcome measures, biomarkers, and meaningful functional measures. There is a need to work more closely with FDA to help define acceptable measures for trials.

4. Model systems. There was already a good set of cellular and models, based on different pathogenic (candidate gene) hypotheses. This was further expanded during the last year. The phenotypes are very diverse and often difficult to compare with the human FSHD phenotype. Many basic questions remain unanswered and dearly need to be answered for further translational studies: when and where is DUX4 expressed in skeletal muscle and what regulates DUX4 activity. It was recognized that there still exists a gap in our knowledge linking the basic genetic and molecular findings with the observed muscle pathology. The BBRI NIH Sen. Wellstone center and the Fields Center continue to generate human cellular resources. These resources continuously deserve attention and need to be replenished. Recent progress in ES-cell technology, including iPS lines, allows for inter-group distribution and dedicated molecular (epi)genetic studies.

5. Sharing. Timely sharing of information and resources remains a critical contributor to the progress in the field. Wellstone and Fields Center continue to share their resources to the scientific community. The Fields Center website also continues to share other information (e.g. protocols, guide to FSHD muscle pathology, etc.).

	first author	presenter	topic	preference
1.	Voet	van Engelen	trials - exercise / cognitive	oral
2.	Tasca	Ricci	MRI	oral
3.	Winston	Upadhyaya	SCHMD1 mutations	oral
4.	Sacconi	Sacconi	trials -- anti-inflammatory	oral
5.	Gabellini	Gabellini	epigenetic reg DBE-T / FSHD genes	oral
6.	Lemmers	Lemmers	SCHMD1 mutations	oral
7.	Robin	Wright	telomere position effect	oral
8.	Casa	Casa	D4Z4 polycomb	oral
9.	Gaillard	Magdinier	DUX4 isoforms / hypomethylation	oral
10.	Ansseau	Ansseau	Sp1, YY1, MYOD regulation DUX4	oral
11.	Zavaljevski	Chamberlain	AAV DUX4 L42	oral
12.	Wallace	Wallace	model - DUX4 promoter	oral
13.	Caron	Schmidt	model – human stem cell	oral
14.	Das	Das	TALEN DUX4 PolyA removal	oral
15.	Rahimov	Rahimov	DUX4 expression analysis	oral
16.	Banerji	Banerji	Unraveling FSHD pathomechanisms	oral
17.	Pandey	Pandey	autophagy	oral
18.	Ferreboeuf	Dumonceaux	nuclear protein spreading DUX4	oral
19.	Moyle	Moyle	DUX4	oral
20.	Gatica	Rosa	DUX4 progesterone regulator	oral
21.	Sharma	Chen	PARP1 DUX4 promoter	oral
22.	Sverdrup	Sverdrup	DUX4/BEti screen and inhibition	oral
23.	Domire	Harper	DUX4 p63	poster
24.	Eichinger	Tawil	outcome measures	poster
25.	Jones	Jones	epigenetic variability	poster
26.	Kazakov	Kazakov	FSHD clinical heterogeneity	poster
27.	Lahaut	LaHaut	self reported outcome	poster
23.	Li	Rutkove	electrical impedance myography	poster
29.	Lim	Lim	CTCF binding at the D4Z4	poster
30.	Mariot	Dumonceaux	fetal FSHD / DUX4	poster
31.	Ousterout	Gersbach	ZFN, TALEN,CRISPR Cas9 systems	poster
32.	Sakellariou	Sakellariou	model - xenograft	poster
33.	Statland	Tawil	serum biomarkers	poster
34.	Windelborn	Emerson	model – xenograft	poster

1. **Effectiveness of aerobic exercise training and cognitive behavior therapy in facioscapulohumeral muscular dystrophy: a randomized controlled trial**

N.B.M. Voet, MD[1], G. Bleijenberg, PhD[2], I.J.M. de Groot MD, PhD[1,] G.W. Padberg MD., PhD[3], B.G.M. van Engelen, MD, PhD[3], A.C.H. Geurts, MD, PhD[1]

[1] Radboud University Medical Centre, Centre for Evidence Based Practice, Department of Rehabilitation, Nijmegen, The Netherlands
[2] Expert Centre Chronic Fatigue, Radboud University Medical Centre, Nijmegen, The Netherlands
[3] Radboud University Medical Centre, Donders Centre for Neuroscience, Department of Neurology, Nijmegen, The Netherlands

Abstract
Background: We have previously reported that 61% of the patients with facioscapulohumeral dystrophy (FSHD) are severely fatigued and that loss of muscle strength, physical inactivity, sleep disturbances and pain contribute to chronic fatigue. The primary aim of the present trial was to study the effects of aerobic exercise training (AET) and cognitive behavior therapy (CBT) on chronic fatigue in patients with FSHD as assessed with the subscale fatigue of the Checklist Individual Strength.

Methods: A multi-centre, assessor-blinded, randomized controlled trial (RCT) was conducted, including 57 FSHD patients with severe chronic fatigue who were randomly allocated to either (1) AET, (2) CBT, or (3) a waiting list, usual care (UC) group. Outcomes were assessed at baseline, immediately post intervention (after 16 weeks) and after 12 weeks follow-up. After a 28-weeks waiting period the participants in the UC group were randomized to either AET or CBT.

Findings: Both interventions were well tolerated. Post treatment, both intervention groups showed a significant difference in fatigue compared to the UC group: -9•1 for AET (95%CI -12•4 to-5•8) and -13•3 for CBT (95%CI -16•5 to-10•2). These positive effects were maintained at follow-up: -8•2 for AET (95%CI -12•4 to -5•8) and -10•2 for CBT (95%CI -16•5 to -10•1). After CBT, physical and experienced activity, sleep, and social participation improved, whereas after AET only physical activity increased. The increase in physical activity in both groups and the improvement in social participation after CBT were maintained at follow-up.

Interpretation: This is the first RCT indicating that chronic fatigue can be ameliorated in patients with muscular dystrophy. Both AET and CBT could be part of an evidence-based treatment program for reducing fatigue in patients with FSHD.

2. **Muscle MRI of scapular girdle in facioscapulohumeral muscular dystrophy**

Giorgio Tasca, Mauro Monforte, Elisabetta Iannaccone, Marcella Masciullo, Francesco Laschena, Pierfrancesco Ottaviani, Marco Pelliccioni, Giuliana Galluzzi, Giancarlo Deidda, Enzo Ricci

Institute of Neurology, Università Cattolica, Rome, Italy
Institute of Cell Biology and Neurobiology, CNR, Rome, Italy
Department of Radiology, Istituto Dermopatico dell'Immacolata, IRCCS, Rome, Italy
Molecular Genetics Laboratory, Unione Italiana Lotta Distrofia Muscolare (UILDM) Rome Section, Santa Lucia Foundation, Rome, Italy

Objectives: to assess the pattern and degree of involvement of scapular girdle muscles in Facioscapulohumeral muscular dystrophy (FSHD).

Methods: we standardized an MRI protocol that allowed the identification and evaluation of 14 muscle groups of the neck and upper girdle. 108 FSHD patients were included in the study. The involvement in FSHD was compared with that of limb girdle muscular dystrophies and other myopathies with upper girdle weakness.

Results: The trapezius and serratus anterior were the most affected muscles in FSHD, followed by the latissimus dorsi and pectoralis major, while spinati and subscapularis were consistently spared. Asymmetry was a consistent feature, similarly to what is found in lower limb muscles, and STIR hyperintensities could be detected as well. The pattern of involvement significantly differed from that encountered in other myopathies.

Conclusion: upper girdle imaging is a helpful tool in guiding the clinical diagnosis of FSHD. Moreover, the detailed knowledge of single muscle involvement provides information useful for understanding and following the natural history of the disease and developing rehabilitation protocols.

3. **Identification of two novel SMCHD1 sequence variants in families with FSHD-like muscular dystrophy**

Jincy Winston, Laura Duerden, Matthew Mort, Ian Frayling, Mark T. Rogers, Meena Upadhyaya

Institute of Medical Genetics, Cardiff University, Heath Park, Cardiff CF14 4XN United Kingdom

Abstract

FSHD1 is caused by a contraction in the number of D4Z4 repeats on chromosome 4, resulting in relaxation of D4Z4 chromatin causing inappropriate expression of *DUX4* in skeletal muscle. Clinical severity is inversely related to the number of repeats. In contrast, FSHD2 patients also have inappropriate expression of *DUX4* in skeletal muscle, but due to constitutional mutations in *SMCHD1* (structural maintenance of chromosomes flexible hinge domain containing 1), which cause global hypomethylation and hence general relaxation of chromatin. Thirty patients originally referred for FSHD testing were screened for *SMCHD1* mutations. Twenty-nine had >11 D4Z4 repeats. Two sequence variants were detected in *SMCHD1* . A splice-site mutation in *SMCHD1* c.1040+1G>A, identified in a FSHD1 family with a borderline number of D4Z4 repeats (10) and a variable phenotype (in which a *LMNA1* mutation was previously described), and *SMCHD1* c.2606 G>A, a putative missense mutation (p.Gly869Val) with strong *in vitro* indications of pathogenicity, was identified in a family with an unusual muscular dystrophy with some FSHD-like features. The two families described here emphasise the genetic complexity of muscular dystrophies**.** As *SMCHD1* has a wider role in global genomic methylation the possibility exists that it could be involved in other complex undiagnosed muscle disorders Thus far, only 12 constitutional mutations have been published in *SMCHD1*, and these mutations add to the molecular and phenotypic spectrum associated with FSHD2.

4. **Inflammation in FSHD1: to treat or not to treat?**

Sabrina SACCONI [1,2], Pauline LAHAUT [1], Leonardo SALVIATI [3], Elena PEGORARO[4], Françoise BOUHOUR[5], Christophe VIAL[5], Claude DESNUELLE[1, 2]

[1] Neuromuscular diseases specialized center, CHU de Nice, France
[2] CNRS UMR7277/ INSERM , Faculté de Médicine de Nice, Nice, France
[3] Department of Genetics, University of Padua, Padua, Italy
[4] Department of Neurosciences, NPSRR, University of Padua, Italy
[5] Neuromuscular disease specialized center, Bron Hospital, Lyon, France

Corresponding author: sacconi@unice.fr

OBJECTIVES: To analyze the possible effect of classical anti-inflammatory therapy in patients with FSHD1.

BACKGROUND: Autosomal dominant FSHD1 may be associated to a certain degree of T-cell inflammation in skeletal muscles. As in dysferlinopathies, the inflammatory infiltrate may be very similar to the one encountered in polymyositis and may lead to misdiagnosis and treatment.

DESIGN/METHODS: We retrospectively studied 12 unrelated FSHD1 patients, (7 man an 5 women, mean age: 45 (IQR 34-65), D4Z4 repeated units ranging from 5 to 8) followed in several Neuromuscular Disease Specialized Centers, misdiagnosed of polymyositis and treated with high dose corticosteroids (CS) 1mg/kg/day (6 patients) in association or not with Immunoglobulines (IGGIV 0.4-1gr/kg every 4-6weeks, 4 patients) or cyclosphorine (CY) (2gr/kg/day, 2 patients).

Only patients that have had ≥ [ge] 6-months of treatment were included in this study. We compare the Clinical Severity Score and a Manual Muscle Testing Score before the treatment and at 12 and 24, 48 months and, if available, at 5 years after the beginning of the treatment.

RESULTS: At the baseline, no statistical difference was noted in term of muscular involvement between the three groups of FSHD1 patients (CS, CS+IGGIV, CS+CY). After at least 6 months of treatment, they all display a worsening of their CSS and MMT score. The rate of worsening was significantly higher at 12 months from the beginning of the treatment compare to 12, 24 months and 5 years ($p< 0.01$, $p<0.05$ and $p<0.05$). The patients receiving longer CS therapy seems to have the higher worsening rate. Quadriceps and iliopsoas muscles were the most affected by disease progression. No differences were evident when comparing sex, disease duration and importance of inflammatory infiltrate.

CONCLUSION: Taking into account the limited number of patients included in this study, this study suggest that classical anti-inflammatory therapies are not useful in stopping FSHD1 disease progression.

5. **Epigenetic regulation of the FSHD locus**

Davide Gabellini, PhD

Dulbecco Telethon Institute and Division of Regenerative Medicine, San Raffaele Scientific Institute, Milano, Italy

Only about 1% of the genome encodes for the 20000 human proteins, which are similar in number and largely orthologous to those found in organisms of significant lower complexity. On the contrary, the proportion of non protein-coding DNA has increased with developmental complexity reaching 98.5% in humans. Interestingly, up to two thirds of the human genome is composed of non protein-coding repetitive sequences. Furthermore, a significant portion of the epigenetic modifications is present in these regions and DNA repeats are dynamically transcribed in different cells and developmental stages producing a vast pool of non protein-coding RNA (ncRNA) molecules. Thus, ncRNAs produced by DNA repeats may hold the key to understanding the regulatory complexity inherent in advanced biological networks.

Long ncRNAs (lncRNAs) represent the most numerous and functionally diverse class of RNA produced by mammalian cells. Despite the growing interest on lncRNAs, they still remain poorly explored in terms of biological relevance, cellular function, mechanism of action and involvement in disease. We have recently contributed to this field through the identification of the first activating lncRNA involved in a human genetic disease: facioscapulohumeral muscular dystrophy (FSHD).

FSHD is the third most prevalent myopathy and is characterized by progressive wasting of facial, upper arm, and shoulder girdle muscles. The classical form of FSHD (FSHD1, MIM 158900) is not caused by mutation in a protein-coding gene. Instead, the disease is associated with a reduced copy number of the D4Z4 macrosatellite repeat mapping to 4q35. Several FSHD clinical features, such as the variability in severity and rate of progression, the gender bias in penetrance, the asymmetric muscle wasting, and the discordance of the disease in monozygotic twins, strongly suggest the involvement of epigenetic factors. Accordingly, a number of epigenetic alterations have been reported in FSHD patients. While these features of the disease have been established for over a decade, the molecular mechanism through which D4Z4 repeats regulate chromatin structure and gene expression at 4q35 has remained elusive. We recently identified DBE-T, a chromatin-associated lncRNA produced preferentially in FSHD patients. We showed that DBE-T regulates the chromatin structure of the FSHD locus and the expression of FSHD candidate protein-coding genes.

Here, I will discuss how newly discovered epigenetic modifications regulate the expression of DBE-T and FSHD candidate genes. I will also present new results regarding the molecular mechanism through which DBE-T activates 4q35 genes.

6. **SMCHD1 mutations in FSHD2**

Richard JLF Lemmers[1], Merlijn P Nieuwenhuizen[1], Patrick van der Vliet[1], Marianne Vos-Versteeg[1], Rabi Tawil[2], Jelle J Goeman, Daniel G Miller[3], Stephen J Tapscott[4], Sabrina Saconni[5], Bert Bakker[6] and Silvère M van der Maarel[1]

[1] Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands
[2] Neuromuscular Disease Unit, Department of Neurology, University of Rochester Medical Center, Rochester, New York, USA
[3] Department of Pediatrics, University of Washington, Seattle, Washington, USA and Seattle Children's Hospital, Seattle, Washington, USA
[4] Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA
[5] Centre de référence des Maladies neuromusculaires and CNRS UMR6543, Nice University Hospital, Nice, France
[6] Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands

In FSHD2 patients, chromatin relaxation-associated DUX4 expression occurs largely independent of D4Z4 repeat array size and is in most cases caused by mutations in the chromatin modifier structural maintenance of chromosomes flexible hinge domain containing 1 (SMCHD1) on chromosome 18. The chromatin modifier SMCHD1 binds directly to the D4Z4 repeat where it is involved in establishment and/or maintenance of a repressive chromatin structure. In FSHD2 patients there is reduced binding of SMCHD1 to D4Z4, resulting in opening of the D4Z4 chromatin structure and causing disease when combined with an allele that is permissive for DUX4 expression. This finding was supported by knockdown experiments of SMCHD1 in normal myoblasts containing a permissive haplotype, which lead to DUX4 expression.

We performed a SMCHD1 mutation screen in 61 unrelated FSHD2 families with the objectives to define the mutation spectrum of SMCHD1. In 52 families (85%) we identified heterozygous SMCHD1 mutations that were inferred to be disease causing. These mutations were distributed over the entire gene. The mutation spectrum is unique and strongly suggests a selection bias in FSHD2. Our findings suggest a balanced interplay between SMCHD1 activity and D4Z4 repeat size in the somatic repression of DUX4. Further genotype-phenotype analyses, combined with functional studies might facilitate our understanding of repeat mediated epigenetic silencing mechanisms in mammalian cells.

7. **Length dependent telomere looping affects long-distant gene expression (5Mb) in FSHD**

Jerome D Robin[1,2], Andrew T Ludlow[1], Guido Stadler[1], Frederique Magdinier[2], Jerry W Shay[1], Woodring E Wright[1]

[1] UT Southwestern Medical Center, Department of Cell Biology, Dallas, Texas 75390-9039 USA
[2] Faculte de Medecine de La Timone, UMRS910 , Marseille, 13008 France

The genetic locus of Facioscapulohumeral Muscular Dystrophy (FSHD) is only ~25-50 kb from the telomere of Chr.4q, and shows an age-related onset of symptoms. This suggested that Telomere Position Effects (TPE) might regulate its poorly understood pathogenetic mechanism. FSHD patients show a reduction in the number of 3.3kb D4Z4 repeats on at least one copy of chromosome 4. We first showed that continuous spreading of telomeric heterochromatin (classic TPE) could extend at least 100kb into the subtelomeric region and increased Dux4-fl expression >10-fold when telomeres were short.

We then examined whether telomere looping existed/influenced gene expression over much larger distances. We adapted the Hi-C technique to study the 4q region in an unbiased fashion. We established clonal myoblast cell lines containing a floxable hTERT that we removed at different times to initiate telomere shortening, producing isogenic subclones with different telomere lengths. We identified multiple novel long-range interactions at 4q associated with telomere shortening. We validated one interaction using 3C and 3D co-FISH experiments. A 4.8Mb DNA loop present in FSHD myoblasts with long telomeres (20Kb) and in cells obtained from unaffected age matched siblings (regardless of telomere length) was lost on the FSHD contracted allele when telomeres became shorter (9Kb,long before telomeres became short enough to induce replicative senescence). Intriguingly, interactions involved several putative FSHD genes and a gene supposedly expressed only in cardiac muscle. Droplet-digital PCR (ddPCR) demonstrated changes in the abundance of different exon-pairs for a variety of candidate genes affecting FSHD in cells with short vs long telomeres, indicating telomere looping was at least altering splicing patterns if not overall transcription.

These results suggest a role for telomere looping in FSHD pathology that could explain much of the age-related onset and variability in this disease.

8. **D4Z4 as a mammalian Polycomb response element**

Valentina Casa[1,2] and Davide Gabellini[1]

[1] Dulbecco Telethon Institute at Division of Regenerative Medicine, San Raffaele Scientific Institute, Milano, Italy
[2] Universita' Vita-Salute San Raffaele, Milano Italy

FSHD is an autosomal dominant myopathy characterized by a complex interplay of genetic and epigenetic events. FSHD is not due to a mutation in a protein-coding gene, instead it is linked to a contraction of a macrosatellite repeat called D4Z4 on 4q35. By an unknown mechanism, D4Z4 contraction is associated with loss of gene silencing of 4q35 genes.

Polycomb group (PcG) proteins are essential epigenetic regulators of development and differentiation. In Drosophila, PcG proteins are recruited to genetic elements called Polycomb Response Elements (PREs). Much less is known about PcG recruitment to target genes in vertebrates.

The FSHD locus displays similarities with Polycomb group proteins (PcG) targets. In particular, a sequence inside D4Z4 called DBE (D4Z4 binding element) is identical to a consensus motif shared by Drosophila PREs.

By Chromatin Immunoprecipitation (ChIP) we found a specific enrichment on D4Z4 for the core components of the two main PcG complexes, PRC1 and PRC2, and for their specific histone marks, H2AK119Ub1 and H3K27me3, hallmarks of PcG silencing. We found that muscle cells from patients display a reduced binding of Polycomb to the FSHD locus. RNAi experiments indicate that Polycomb is required for repression of FSHD candidate genes.

One of the cardinal features of Drosophila PREs is their ability to recruit PcG complexes when inserted in ectopic sites. By inserting in ectopic locations 4q35 DNA sequences containing or not D4Z4 repeats, we found robust recruitment for PcG only when D4Z4 repeats are present. This indicates that D4Z4 repeats are able to initiate de novo recruitment of PcG complexes, and we are now performing functional experiments to associate this to a repressive PcG function, as required to define a DNA element as a PRE.

Overall, these results indicate that loss of PcG silencing is involved in the de-repression of 4q35 genes in FSHD and suggest a possible role for D4Z4 as a PRE.

9. **FSHD, D4Z4 hypomethylation and DUX4**

Marie-Cécile Gaillard, Natacha Broucqsault, Stéphane Roche, Emmanuelle Salort-Campana, Marc Bartoli, Nicolas Levy, Rafaëlle Bernard, Shahram Attarian, Karine Nguyen, Frédérique Magdinier

Aix Marseille Universite, INSERM UMRS_910, 27, Boulevard J. Moulin, 13385 Marseille, France
APHM, Centre de Référence des maladies Neuromusculaire et de la SLA, Hôpital de la Timone, 264 Rue Saint-Pierre, 13385, Marseille, France
APHM, Laboratoire de génétique médicale, Hôpital de la Timone, 264 Rue Saint-Pierre, 13385, Marseille, France

DNA methylation, a covalent post-synthetic modification of cytosines engaged in CpG dinucleotides, is a heritable epigenetic mark. In particular, repetitive DNA sequences are usually packed as condensed by dense CpG methylation while hypomethylation of repetitive DNA sequences is observed in a number of pathologies including FSHD, the third most common hereditary myopathy.

The disease, linked in most cases to the deletion of an integral number of repetitive macrosatellite DNA sequence (D4Z4) in the terminal 4q35 region has a variable course with a high inter-individual and intra-familial heterogeneity. Meanwhile, the pathogenesis remains partially understood. In particular the link between the severity of phenotypic features and DNA methylation remains to be elucidated.

To this aim, we investigated the correlation between DNA methylation and disease penetrance in a cohort of 95 samples including unaffected controls, symptomatic FSHD1 carriers of a shortened D4Z4 array, asymptomatic carriers and phenotypic FSHD (FSHD2) patients. Furthermore, we explored the link between DNA methylation and DUX4 expression.

We first confirmed that symptomatic FSHD2 patients display less methylation than FSHD1 and a performed a detailed mapping of DNA methylation across the D4Z4 repeat which reveals differences between asymptomatic carriers and FSHD1 and FSHD2 patients. We also show that DUX4 isoforms are differentially induced by DNA hypomethylation.

In conclusion, we suggest that local hypomethylation within D4Z4 might serve as a biomarker for FSHD penetrance and developed new tools which could be applied systematically to patient's samples in routine diagnosis.

10. **Interplay between Sp1, YY1 and MYOD in transcription regulation of the DUX4 gene that causes facioscapulohumeral muscular dystrophy (FSHD)**

Eugénie Ansseau[1], Sébastien Charron[1], Dalila Laoudj-Chenivesse[2], Frédérique Coppée[1] and Alexandra Belayew[1,#]

[1] Laboratory of Molecular Biology, Research Institute for Health Sciences and Technology, University of Mons, 7000 Mons, Belgium
[2] INSERM U1046, CHU A. de Villeneuve, 34295 Montpellier, France

FSHD is linked to DNA hypomethylation of the D4Z4 repeat array in 4q35 resulting in a gain of function of the DUX4 gene we have identified within the D4Z4 unit. The DUX4 gene functionality was questioned for a decade because of its location in repeated DNA elements and the characterization by Gabellini et al (2002) of a transcription inhibitory element (DBE) that precisely mapped on its promoter. The DBE bound a multi-protein complex including the YY1 transcription factor. The Sp1-binding GC box that mediates basal DUX4 promoter activity maps close to the DBE suggesting a steric hindrance upon YY1 binding. We confirmed YY1 could bind to the DBE. However co-transfection of a human rhabdomyosarcoma cell line with a YY1 expression vector activated a reporter gene fused to the proximal DUX4 promoter (DUX4-Luc). This lack of repression was confirmed by the observed co-localisation of the endogenous YY1 and DUX4 proteins in nuclei of FSHD myoblasts/myotubes. We characterized an E-box on the major DUX4 transcription start site, and showed its interaction with MYOD repressed DUX4-Luc expression. During FSHD myoblast differentiation endogenous DUX4 expression was increased when MYOD levels were the lowest i.e. during fast proliferation or in myotubes, but not at myoblast fusion time. In another experiment, we tranfected mouse myoblast C2C12 cells with the DUX4-Luc reporter gene and measured luciferase activity after 0, 1, 3, 4 or 6 days of differentiation. The luciferase activity was decreased at day 1 and reactivated at days 3, 4 and 6. Those data are in keeping with what we have observed here in primary myoblast cultures. In conclusion the interplay between Sp1, YY1 and MYOD could explain why DUX4 expression is observed in proliferating FSHD myoblasts and differentiated myotubes.

Acknowledgments: This study was partly funded by the ABMM (Belgium), the AFM (France) and the FSH Society (USA). EA held a post-doctoral position from the FNRS (Belgium) and SC held a PhD fellowship from the FRIA (Belgium).

11. **AAV delivery of the D4Z4 L42 terminal repeat induces muscle histological changes similar to FSHD**

Maja Zavaljevski, Darren R. Bisset, Carrie A. Stoltzman, John K. Hall, Stephen J. Tapscott, and Joel R. Chamberlain

University of Washington, Seattle, Washington USA
Fred Hutchinson Cancer Research Center, Seattle, Washington USA

Our focus is developing a treatment for FSHD with delivery of RNAi expression cassettes targeting DUX4 region RNAs and we are seeking in vivo models to test this approach. Reproducing FSHD in mice as a model system has been challenging and has yielded little, despite repeated efforts to engineer transgenic mice expressing DUX4. Recently, a transgenic mouse was characterized, the D4Z4-2.5 mouse, that recapitulates the high germline and reduced somatic expression of DUX4 in muscles with hypomethylation of the transgene, as observed in patients. However, this mouse does not display a dystrophic muscle phenotype expected from ectopic expression of DUX4 in other systems. We have attempted to mimic the low level of expression of DUX4 detected in patients in mouse muscle through ectopic expression of DUX4 from the last D4Z4 repeat of the pathogenic allele from the L42 human clone. The last D4Z4 repeat was packaged into a vector derived from adeno-associated virus 6 (AAV6) and delivered to the TA muscle of wild-type mice by intramuscular injection. One week post injection of a high dose of the AAV6-DUX4 vector we observed pathological changes consistent with FSHD biopsies, including central nuclei, fiber size variation, split fibers with nuclei at the periphery, angular fibers, mononuclear cell infiltrate, and clusters of inflammatory cells surrounding single fibers. Muscle cell turnover appears to continue at longer timepoints and may be an indication of a threshold of DUX4 associated tissue destruction. At a 10-fold lower dose we observe occasional groups of fibers with central nuclei, small fibers, split fibers, and inflammatory cell clusters around single fibers. We have detected expression of DUX4 with RT qPCR and are focusing our efforts on characterizing DUX4 protein expression and mononuclear cell infiltrates. We hope this model will provide further insights into the human cellular pathways stimulated by DUX4 that overlap with the mouse and prove useful for testing promising therapies.

12. **The DUX4 promoter mouse: an updated characterization**

Lindsay M. Wallace[1], Jian Liu[1], Sara E. Garwick-Coppens[1], Susan M. Guckes[1], Catherine Smith[2], Yvonne Krom[3], Silvere van der Maarel[3], Jodi McBride[2] and Scott Q. Harper[1]

[1] The Center for Gene Therapy, Nationwide Children's Hospital, Columbus Ohio, USA
[2] Division of Neuroscience, Oregon National Primate Research Center, Beaverton, Oregon, USA
[3] Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands

Difficulties in developing animal models for FSHD have proved to be a limiting factor in deciphering molecular mechanisms and developing therapeutic interventions for the pathogenic overexpression of DUX4. At last year's FSH Society meeting, we first reported our development of transgenic reporter mice containing a putative DUX4 promoter cloned upstream of GFP. We generated three separate lines of DUX4 promoter-GFP mice to identify DUX4 expression patterns and the involvement of selected muscles (and ear and retinal pathologies) in FSHD. In short, we found the DUX4 promoter directed GFP expression in the face and limbs of newborn and adult mice, as well as multiple cell types in the retina. Essentially all other organs were GFP negative with a few exceptions including the pancreas and brain. Closer analysis of GFP positive tissues has revealed extensive expression in both myogenic and neuronal cell types. Strikingly, all lines also showed variable penetrance and asymmetrical expression in all GFP-positive tissues, even within individual litters. We are currently determining whether epigenetic factors could be playing a role in these expression patterns and very preliminary data suggests a trend toward reduced methylation in high expressing animals. We will provide an update on the progress we have made characterizing these mice, as well as discuss a parallel set of mice designed to express DUX4 using a similar strategy.

13. **Towards a human stem cell model of facioscapulohumeral muscular dystrophy (FSHD)**

Leslie Caron and Uli Schmidt
Genea Biocells, Level 3, 321 Kent Street, Sydney NSW 2000 Australia

FSHD presents a major unmet clinical need with no specific treatments being available, and the disease mechanisms and etiology are still poorly understood at a cellular level. Research efforts are hampered by shortcomings of existing model systems: the complex genetics of the disease makes the establishment of animal models mirroring human aberrations difficult, while human biopsy material is too limited and variable. In order to develop an accurate human, scalable and consistent cellular disease model we created 4 human embryonic stem cell lines from donated pre-implantation genetic diagnosis (PGD) embryos carrying the FSH chromosomal deletion. We then used several rounds of high-content screening to establish a protocol for the differentiation of human pluripotent stem cells to skeletal muscle. In a unique 3-step process, we initially produced satellite cells, similar to resident adult muscle stem cells which were then differentiated to myoblasts before maturing to myotubes which showed typical multinucleated morphology, stained positive for muscle-specific markers and could be induced to contract. To our knowledge, this is the world's first process that allows simple and robust industrial-scale cell production achieving final yields typically >80% without any cell sorting, selection or genetic manipulation. We further developed an efficient freeze/thaw protocol for myoblasts that can be matured into functional myotubes within 7-10 days of plating using our stage 3 medium. We are now investigating disease-related phenotypes by comparing myotubes from a range of normal and FSHD-affected cell lines. Our stem cell model should be a valuable resource for future FSHD research and phenotypic drug screening.

Dr Uli Schmidt
General Manager, Genea Biocells
t +61 2 9229 6453
f +61 2 9229 6478
m +61 2 404 043 452
e uli.schmidt@geneabiocells.com
w www.geneabiocells.com
in /company/genea-biocells

Level 3, 321 Kent Street, Sydney NSW 2000 Australia

14. **Developing resources to alleviate muscle atrophy in FSHD by genome engineering**

Sunny Das and Brian P. Chadwick

Department of Biological Science, Florida State University, 319 Stadium Drive, King 3076
Tallahassee, Florida 32306-4295 USA
phone: (850) 645-9279, e-mail: chadwick@bio.fsu.edu
phone: (850) 591-9502, e-mail: sdas@bio.fsu.edu
fax: (850) 645-8447

Facioscapulohumeral muscular dystrophy (FSHD), a debilitating disease with currently no cure or effective therapy, is the third most common inherited form of muscular dystrophy. FSHD is primarily characterized by progressive weakness and atrophy of skeletal muscle of the face, shoulders and upper arms. Changes to the transcriptional state of the macrosatellite array D4Z4 on chromosome 4q35 is believed to be responsible for FSHD, with each unit of this array containing the open reading frame for the double homeobox gene DUX4. Both forms of this autosomal dominant disorder (FSHD1 and FSHD2) are likely caused by the reactivation of DUX4, which is epigenetically repressed in somatic cells, but inhibits muscle differentiation and replenishment, on reactivation. Importantly, both forms of FSHD have a strict requirement for specific allelic variants of chromosome 4q and at least one unit of D4Z4, as complete loss of the repeat does not result in disease. Taking advantage of this, we are focused on development of an effective therapeutic strategy for FSHD using genome engineering in human cells through custom-built Transcription Activator Like Effector Nucleases (TALENs). We have developed TALENs to target regions immediately proximal and distal to the D4Z4 array that can be used to remove the array or disrupt the polyadenylation signal on a permissive chromosome, in order to alleviate toxic gain of function. These nucleases have been successful in cutting desired regions of the genome in model cell lines and we are currently testing the feasibility of generating a truncated, yet functional chromosome 4 lacking the entire D4Z4 array. This strategy, if successful, will permit use of our custom-built nucleases to modify patient specific induced pluripotent stem cells (iPS), with the ultimate goal of using modified cells as a possible therapeutic strategy for muscle restoration. Our approach could be used in FSHD patient-specific cell transplantation therapy, in an attempt to alleviate this debilitating condition, and improve quality of life.

15. **Expression analysis of DUX4 target genes in FSHD muscles and cells**

Fedik Rahimov[1,2], Oliver D. King[2,3], Jennifer C.J. Chen[2,3], Charles P. Emerson Jr.[2,3], Kathryn R. Wagner[2,4], Louis M. Kunkel[1,2]

[1] Program in Genomics, Division of Genetics, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts USA
[2] DHHS NIH NICHD Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD
[3] Department of Cell and Developmental Biology, University of Massachusetts Medical School, Worcester, Massachusetts USA
[4] The Hugo W. Moser Research Institute, Kennedy Krieger Institute, Baltimore, Maryland USA

Facioscapulohumeral muscular dystrophy (FSHD) is a progressive neuromuscular disorder caused by contractions of repetitive units within the macrosatellite D4Z4 on chromosome 4q35. Contraction of these repetitive elements induces the expression of the transcription factor double homeobox 4 (DUX4) in skeletal muscle, which in turn leads to muscle cell death through a yet unknown mechanism. Previous studies have shown that DUX4 induces a large number of genes expressed in germ-line and stem cells in FSHD muscles. We analyzed the expression levels of its target genes in muscles and cells obtained from a large cohort of FSHD families recruited for our studies within the Wellstone Center for FSHD Research. Biopsy samples were obtained from two distinct muscles from 52 FSHD subjects and 40 unaffected first-degree family members. Biceps typically shows an early and severe disease involvement, whereas deltoid is relatively uninvolved. We analyzed the expression levels of ten DUX4 target genes and fifteen biomarker candidate genes in a large number of FSHD families, using high-throughput microfluidic qPCR approach. Consistent with previous findings, the expression levels of DUX4 target genes (PRAMEF6, TRIM49, MBD3L5, PRAMEF1, SLC34A2, RFPL1, ZSCAN4, SPRYD5, DEFB103B and TRIM43) were elevated in both affected muscle types and cells compared with unaffected controls.

16. **Unraveling FSHD patho-mechanisms: taking InSpiRation from network rewiring**

Christopher Banerji, Louise Moyle, Simone Severini and Peter Zammit

Centre of Mathematics and Physics in the Life Sciences and Experimental Biology, University College London, England

Facioscapulohumeral muscular dystrophy (FSHD) is the most prevalent inherited myopathy and is caused by aberrant expression of the transcription factor DUX4. FSHD is a highly complex and heterogeneous condition, for which there is no cure, manifesting clinically as a tissue specific skeletal muscle atrophy, but also associated with retinal vascular abnormalities and sensorineural hearing loss. Elucidating in detail the molecular mechanisms of FSHD is essential to the development of treatments and consequentially this problem has been set as a main aim for the community at the most recent FSH Society International Research Consortiums.

Many gene expression studies have been performed on FSHD, and other muscular dystrophies, in an attempt to elucidate pathomechansisms, but these have not been thoroughly analysed by the latest bioinformatic techniques. To address this, we developed a novel differential network methodology, InSpiRe (Interactome Sparsification and Rewiring), which detects network rewiring between phenotypes by combining gene expression data with known protein interactions. Using this novel mathematical methodology, we performed the first meta-analysis of multiple publicly available gene expression data sets from FSHD muscle biopsies. We then removed changes associated with muscle wasting, aging, atrophy and inflammation following meta-analysis of appropriate data sets. This integrated output is a high-confidence unified network of pathway changes explaining in detail FSHD pathomechanisms.

By performing a microarray to detect transcriptional changes elicited by DUX4, we also showed that DUX4 significantly modifies expression of the genes in our network. Furthermore, interrogation of our network of rewired interactions identified β- catenin as central to dysregulated signaling in FSHD. We therefore provide the first unified molecular map of FSHD pathomechanisms, capable of guiding therapeutic development.

17. **Lower autophagy activities in FSHD**

Sachchida N. Pandey[1], Woodring Wright[2], Charles P. Emerson[3], Yi-Wen Chen[1, 4]

[1] Center for Genetic Medicine Research, Children's National Medical Center, Washington DC
[2] Departments of Cell Biology and Internal Medicine, UT Southwestern Medical Center, Texas
[3] Departments of Cell and Developmental Biology and Neurology, University of Massachusetts Medical School, Worcester, Massachusetts
[4] Department of Integrative Systems Biology and Department of Pediatrics, George Washington University, Washington DC

Autophagy is a cellular process responsible for maintaining cell homeostasis by removal of damaged organelles, malformed proteins, and nonfunctional long-lived proteins. Both excessive and insufficient autophagy activities have been reported to cause muscle disorders. To investigate whether the autophagy activities are affected in FSHD, we studied protein expression of genes involved in autophagosome formation and autophagy function in muscle biopsies of patients with FSHD. In addition, immortalized FSHD myoblasts were used to study autophagy responses to serum starvation. Our results showed a higher expression of p62 (2.2 fold, $p<0.05$) and total ubiquitinated proteins (1.4 fold, $p<0.05$) in biceps of patients with FSHD suggesting lower autophagy activities, but not in the less affected deltoid muscles from the same patients (n=5). In the immortalized FSHD myoblasts, we observed a 1.3 fold higher expression of p62 ($p<0.05$). In addition, we observed lower expression of ATG5 (1.6 fold; $p<0.01$) and ATG4B (1.9 fold; $p<0.01$), which are key regulators involved in LC3B-I to LC3B-II conversion. We also observed lower expression of LAMP1 (8 fold; $p<0.01$) in the immortalized FSHD cells. The serum starvation increased autophagy activities in both FSHD and control myoblasts. However, this did not correct the differences between the FSHD and control cells. In addition, serum starvation did not change expression of ATG4B in the FSHD myoblasts. Our studies showed that autophagy activities are lower in FSHD myoblasts. The higher expression of p62 associated with suppression of autophagy was also observed in the more affected biceps muscles in patients with FSHD. Additional studies are needed to determine how the change of autophagy activities is involved in FSHD and how it contributes to the disease.

18. **Nuclear protein spreading in myotubes: consequences for pathophysiology of muscular diseases**

Maxime Ferreboeuf[1], Virginie Mariot[1], Denis Furling[1,] Gillian Butler-Browne[1], Vincent Mouly[1], Julie Dumonceaux[1, *]

[1] INSERM U974, UMR 7215 CNRS, Institut de Myologie, UM 76 Université Pierre et Marie Curie, 75013, Paris, France

FSHD is one of the most prevalent dystrophy with an incidence of 4/100,000 births and FSHD patients present a selective and asymmetric atrophy of facial, shoulder and arm muscles. Two loci of the disease have been characterized, both leading to chromatin de-repression allowing the expression of a transcription factor called DUX4. Interestingly, whereas DUX4 is expressed in not more than 1/1,000 nuclei, DUX4 protein was detected in 0.5-4% nuclei. Moreover, the detection of DUX4 protein exhibits a particular pattern characterized by a progressive decrease in the signal intensity in consecutive nuclei suggesting that DUX4 might not be transcribed in all nuclei.

In order to determine if, while DUX4 is transcribed in only very few nuclei within a fibre, the resulting DUX4 protein is spread in the neighbouring nuclei, we used RNA-FISH against DUX4 mRNA on differentiated FSHD cultures. We observed that in myotubes, only one nucleus was DUX4-positive while DUX4 protein is always detected in several neighbouring nuclei. We then performed co-cultures between murine C2C12 cells and either immortalized FSHD or control human cells. We observed that DUX4 protein is present in both human and murine nuclei. Since DUX4 is not expressed in C2C12 nuclei, this demonstrates the spreading of DUX4 into the murine nuclei. We next investigated whether or not the spreaded DUX4 proteins were functional and activated murine genes described as targets of DUX4. We observed an increase of the expression level of both murine and human DUX4 downstream genes in the co-cultures with the FSHD cells, while no change in expression was observed with control cells. This result shows that, in murine nuclei, human DUX4 is indeed active and can activate some of its target genes, We propose a new “spreading model” to explain the effects of DUX4 expression in myotubes: in human cells, only few nuclei express DUX4 but the resulting protein can be directed to the same nucleus, but also to neighbouring ones, resulting in the spreading of the pathological phenotype.

Finally, we investigated if this spreading of nuclear proteins could have consequences for other neuromuscular diseases and demonstrated that it is indeed the case. These results will be presented and discussed during the meeting.

19. **The receptor tyrosine kinase Ret affects myoblast proliferation and contributes to DUX4-mediated inhibition of myoblast fusion**

Louise Moyle, Paul Knopp, Robert Knight and Peter Zammit

King's College London, Guy's Campus, London, SE1 1UL, United Kingdom

Facioscapulohumeral muscular dystrophy (FSHD) is the third commonest inherited myopathy, linked to deletion of tandem 3.3kb repeats (D4Z4 units) in chromosome 4q35. Between 1-10 D4Z4 repeats on a specific chromosomal haplotype leads to expression of the double homeodomain protein DUX4 from the last D4Z4 unit. Expression of DUX4 inhibits myogenic differentiation and is pro-apoptotic.

To understand the molecular mechanisms of FSHD, we performed a microarray on murine satellite cell -derived myoblasts expressing DUX4, or its non-apoptotic ortholog DUX4c. The receptor tyrosine kinase Ret (rearranged during transfection) was significantly upregulated by DUX4 but not DUX4c, suggesting a potential role in FSHD pathology. We found that Ret is dynamically expressed during satellite cell differentiation and that retroviral-mediated constitutive expression of either Ret9 or Ret51 isoforms increased myoblast proliferation. Neither isoform affected myotube formation or expression of the myogenic regulatory factors Myod, Myf5 and Myogenin. However, siRNA-mediated knockdown of Ret reduced proliferation and Pax7 expression and increased expression of Myogenin in satellite cell-derived myoblasts, suggesting that Ret keeps myoblasts in a proliferative state. Finally, we used siRNA against Ret to block DUX4-mediated Ret signaling and were able to rescue the suppression of myogenic differentiation. Preliminary data shows that RET expression is also increased in myoblasts isolated from an FSHD patient.

This research suggests that Ret acts to either prevent early myogenic differentiation or maintain satellite cells in a proliferative state. DUX4-mediated activation of Ret could contribute to the FSHD phenotype, and highlights Ret as a potential drug target for FSHD pathobiology.

20. **DUX4 negatively regulates the activity of the progesterone receptor while gonadal steroids protects cells from DUX4-mediated toxicity**

Laura Virginia Gatica[1], Cecilia Proietti[2], Patricia Elizalde[2], Edgardo Daniel Corona[1,3], Daniela Jacquelin[1], Claudia Pellizas[4], Alberto Luis Rosa[1,5]

[1] Laboratorio de Biología Celular y Molecular, Fundación Allende, Córdoba, Argentina
[2] IBYME-CONICET, Buenos Aires, Argentina
[3] CIQUIBIC-CONICET, Universidad Nacional de Córdoba, Córdoba, Argentina
[4] CIBICI-CONICET, Universidad Nacional de Córdoba, Córdoba, Argentina
[5] Servicio de Genética Médica, Sanatorio Allende, Córdoba, Argentina

FSHD seemed to run a milder clinical course in females than males. Females, however, appear to be more affected following menopause. No explanation is available on this gender difference in severity and progression of FSHD. In this work we present preliminary evidence suggesting a possible connection between this clinical observation and a potential physiological role we propose here for DUX4, the main candidate pathogenic protein in FSHD [(1,2)]. In previous studies on molecular determinants for nuclear import and pathogenicity of DUX4 [(3)], we performed a detailed in silico analyses of the C-terminal domain of DUX4 and DUX4c proteins. We found that DUX4, but no DUX4c, contains a functional motif (LXXLL or NR box) previously recognized in co-regulators of nuclear receptors [(4)]. This domain is highly conserved among DUX4-homologous proteins during evolution. We hypothesized and propose here that DUX4 is a potential hormone receptor co-regulator and its cell toxicity may be connected with its potential role at the endocrine pathway. Interestingly, DUX4 is highly expressed in gonadal tissues [(4)]. In our first set of experiments we used a gene reporter strategy to study if DUX4 regulates the activity of the progesterone receptor (PR). In these studies, T47D cells (breast cancer) were co-transfected, in culture media supplemented with physiological doses of progesterone, with a MMTV-luciferase reporter gene, pRL-CMV Renilla (internal control), and different amounts of a vector expressing DUX4. In these studies we observed that ligand activated- PR transcriptional activation was inhibited in a DUX4-dose dependent manner. These preliminary results highlight a potential novel function for DUX4 as a negative co-regulator of the progesterone receptor. Additional studies concerning DUX4 as a potential regulator of the androgen receptor (AR) are currently being performed. In our second set of experiments we studied if treatment of cultured cells with different doses of sex steroids (i.e. progesterone, estrogen and testosterone) modifies DUX4-mediated cytotoxicity. In these studies, DUX4 toxicity was measured using a DUX4/GFP co-transfection assay developed in our laboratory [(1,3)]. Cells were pre-treated with various amounts of the analyzed hormones (or vehicle) and then co-transfected with plasmid vectors expressing GFP and DUX4 (or empty vector). A 2-fold increase in GFP-positive cells (i.e. diminished toxicity of DUX4) was observed when cells were pre-treated with physiologically doses of progesterone or supra-physiological doses of estrogen or testosterone. These results indicate that sex hormones protect cells from DUX4 toxicity. Our studies show for the first time that DUX4 modulates the activity of a nuclear hormone receptor and that gonadal steroids modulate DUX4-mediated cytotoxicity. These

findings may have physiological relevance concerning the normal/pathological role of DUX4 contributing to explain the gender difference observed in FSHD clinical manifestations.

[1] Kowaljow,V., Marcowytz, A., Ansseau, E., Conde, C., Sauvage, S., Mattéotti, C., Arias, C., Corona, E.D., Nuñez, N.G., Leo, O. et al. (2007) The DUX4 gene at FSHD1A locus encodes a pro-apoptotic protein. Neuromuscul. Disord., 17, 611-623.
[2] Lemmers, R.J., van der Vliet, P.J., Klooster, R., Sacconi, S., Camaño, P., Dauwerse, J.G., Snider, L., Straasheijm, K.R., van Ommen, G.J., Padberg, G.W. et al (2010) A unifying genetic model for facioscapulohumeral muscular dystrophy. Science, 329, 1650-1653.
[3] Corona, ED, Jacquelin, D, Gatica, L. and Rosa, A.L. (2013) Multiple protein domains contribute to nuclear import and cell toxicity of DUX4, a candidate pathogenic protein for facioscapulohumeral muscular dystrophy. PLoS ONE (in press)
[4] Leo, C. and Chen, J.D. (2000) The SRC family of nuclear receptor coactivators. Gene., 245, 1-11.
[5] Snider, L., Geng, L.N., Lemmers, R.J., Kyba, M., Ware, C.B., Nelson, A.M., Tawil, R., Filippova, G.N., van der Maarel, S.M., Tapscott, S.J. et al. (2010) Facioscapulohumeral dystrophy: incomplete suppression of a retrotransposed gene. PLoS. Genet., 6, e1001181.

21. **Poly (ADP-ribose) polymerase 1 differentially interacts with the promoter region of DUX4 gene in FSHD myoblasts**

Vishakha Sharma[1], Kristy J. Brown[2], Yetrib Hathout[2,3], Yi-Wen Chen[2,3]

[1] Department of Molecular Medicine, George Washington University, Washington DC, USA
[2] Research Center for Genetic Medicine, Children's National Medical Center, Washington DC, USA
[3] Department of Integrative Systems Biology, George Washington University, Washington DC, USA

Double homeobox protein 4 (DUX4) has been shown to be de-repressed by epigenetic changes in facioscapulohumeral muscular dystrophy (FSHD). However, gene regulatory factors interacting with the DUX4 promoter is unknown. Here we performed a DNA pull down assay using a 282 b.p. biotin labeled DUX4 promoter probe, followed by mass spectrometry analysis to identify potential gene regulatory factors that interact with the DUX4 promoter. Our results showed that PARP1 was ranked the first by the number of peptide hits among a total of 13 proteins identified. We validated the interaction between the PARP1 and the DUX4 promoter probe by repeating the DNA pull-down assay followed by immunoblotting, which demonstrated a 2-fold enrichment of PARP1 ($p<0.05$), as compared with the control. Since PARP1 has been shown to interact with and inhibit the catalytic activity of DNMT1, an enzyme involved in regulation of DNA methylation, we further explored whether DNMT1 also interacted with the DUX4 promoter. Our data showed a 2-fold enrichment of DNMT1 bound to the DUX4 promoter probe ($p<0.05$). To further validate the interaction of the PARP1 protein with the DUX4 promoter in FSHD myoblasts, we performed ChIP assay targeting the DUX4 promoter region in immortalized FSHD myoblasts of a patient and an unaffected sibling. Real-time quantitative PCR showed a 65-fold enrichment ($p<0.01$) of PARP1 at the DUX4 promoter in the FSHD myoblasts, while no enrichment was observed in the myoblasts of unaffected sibling. The results suggest that PARP1 interacts with the promoter region of the DUX4 gene differentially between the patient and control myoblasts, which may potentially affect the expression of the DUX4 gene in FSHD. To determine whether a PARP1 inhibitor can suppress DUX4 expression in FSHD cells, we culture the immortalized FSHD cells with 3-aminobenzamide for 24 hours and showed a 4-fold suppression of ZSCAN4 ($p<0.05$), a downstream target of DUX4. Our results demonstrated that PARP1 and DNMT1 interact with DUX4 promoter and may be involved in regulating DUX4 expression.

22. **Chemical genetics identifies BET proteins as potential therapeutic targets in FSHD**

Francis M. Sverdrup[1], Lauren Snider[2], Matthew P. Yates, Sean Shadle, Silvere M. van der Maarel[3], Rabi Tawil[4], Stephen J. Tapscott[2]

[1] Center for World Health & Medicine, Saint Louis University, Saint Louis, Missouri USA
[2] Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, Washington USA
[3] Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands
[4] Neuromuscular Disease Unit, Department of Neurology, University of Rochester Medical Center, Rochester, New York USA

Facioscapulohumeral Musclular Dystrophy (FSHD) is characterized by epigenetic changes resulting in the aberrant expression of the DUX4 gene in muscle. The long term objectives of this research are to identify druggable targets that regulate DUX4 expression and to evaluate the therapeutic potential of the corresponding inhibitory compounds. We have used a unique cell based assay that detects DUX4 expression in FSHD muscle cells to screen a library of compounds that target epigenetic modifier proteins. This "chemical genetics" approach identified the bromodomain and extra terminal (BET) family of proteins as key targets involved in DUX4 expression. Selective inhibitors of BET proteins (BETi) blocked the induction of DUX4 and its downstream targets ZSCAN4 and TRIM43 during differentiation of FSHD1 and FSHD2 muscle cells in vitro. BETi also blocked DUX4 expression in undifferentiated FSHD myoblasts as evidenced by decreased ZSCAN4 and TRIM43 mRNA levels 48 to 72 hours after compound addition. Cells grown continuously in the presence of low dose BETi JQ1exhibited a greater than 95% reduction is steady state levels of ZSCAN4 mRNA and this effect was sustained, requiring longer than 6 days to recover after removal of JQ1 from the cultures. These data suggest that BETi may have therapeutic potential in FSHD and that further investigation into the role of BET proteins in FSHD is warranted.

23 [p]. **DUX4 regulates expression of the pro-apoptotic gene, p63**

Jacqueline Domire, Lindsay Wallace, Susan Guckes, Jian Liu, Scott Q. Harper

The Center for Gene Therapy, Nationwide Children's Hospital, Columbus Ohio, USA

DUX4 expression has emerged as a primary factor in FSHD pathogenesis. Although it is clear DUX4 functions as a transcription factor, we know little about the pathways it controls both normally and in FSHD. The objective of this study was to investigate prospective DUX4-controlled pathways. We hypothesized that this approach would help further define the pathogenic events involved in FSHD and identify new therapeutic targets. Toward this end, we used microarray data from DUX4-overexpressing muscle to identify potential transcriptionally regulated targets of DUX4. We found that several genes involved in apoptosis pathways were up-regulated, including p53-pathway members. Since DUX4 is a transcription factor, we therefore hypothesized that it could directly activate p53-pathway genes. In this study, we focused on one such gene, p63, because it was increased in DUX4-expressing muscles, transactivates p53 target genes, and sensitizes cells to apoptosis. Using multiple approaches, we found that DUX4 binds to the p63 promoter and is capable of regulating its expression. Moreover, we show that p63 co-localizes with DUX4 in myofiber nuclei of DUX4-overexpressing mouse muscle. We further show that p63 inhibition mitigates the pro-apoptotic activity of over-expressed DUX4, suggesting at least one mechanism for DUX4-induced cell death. Nevertheless, in the work just described, we took advantage of an over-expression system that overcomes the difficulties in working with normal levels of DUX4, which are typically extremely low. To confirm that this DUX4-p63 axis also occurs in cells/tissues containing "physiological" levels of DUX4, we are now examining DUX4 and p63 expression in cell lines from FSHD patients and unaffected controls. Importantly, in our preliminary work using cells obtained from the Boston Wellstone group, we find evidence of a unique p63 expression profile present only in FSHD myotubes but not controls

Together, these results suggest that p63 is a downstream target of DUX4, and may play an important role in DUX4-mediated cell death. As such, p63 could be a therapeutic target for FSHD, and we are currently investigating the effects of p63 ablation on DUX4-induced muscle pathology by expressing DUX4 in muscles of conditional p63-null mice.

24 [p]. **Clinically relevant outcome measures for facioscapulohumeral muscular dystrophy: preliminary results from baseline testing**

Katy Eichinger, PT, Chad Heatwole, MD, Colleen Donlin-Smith, MA, Rabi Tawil, MD, and Jeffrey Statland, MD

Department of Neurology, University of Rochester Medical Center, Rochester, New York

Background: Recent molecular pathological advances in our understanding of Facioscapulohumeral muscular dystrophy (FSHD) have identified potential therapeutic targets. Clinically relevant outcome measures will be vital for future clinical trials.

Methods: A prospective 12 month observational study of 40 FSHD participants to evaluate the reliability and responsiveness of 1) a disease specific functional rating scale; 2) a disease specific FSHD Health Inventory; and 3) electrical impedance myography as a quantitative measure of muscle structure. The functional rating scale is a 72 point scale made up of standardized evaluator-administered functional instruments designed to assess key elements of gait, shoulder function, axial strength, and balance. Here we report preliminary baseline results on the functional rating scale, including the 6 minute walk test.

Results: Baseline and 2 week follow up data was analyzed for 10 FSHD participants. The six minute walk test shows excellent preliminary test-retest reliability (Intraclass Correlation Coefficient [ICC] 0.98, 95% Confidence Interval [CI] 0.90, 0.99), with a moderate to strong correlation to average lower extremity manual muscle testing composite scores (r=0.69, P=0.03). Interestingly almost all participants enrolled so far have had distances on the six minute walk test outside the range of normal, including participants with minimal to no lower extremity weakness. For participants with average lower extremity manual muscle testing scores > 4.8 the average six minute walk test was 459.2 feet (standard deviation 87.4). The functional rating scale also shows excellent initial test-retest reliability estimates (ICC 0.99, 95% CI 0.93, 0.998), with strong correlations to measures of disease severity (r=0.88-0.93 for published disease severity scales, r=0.90, P=0.0003 for average manual muscle testing composite score).

Discussion: Initial data suggests the 6 minute walk test and the FSHD-specific functional rating scale are reliable measures for FSHD, with moderate to strong cross sectional correlations to other measures of disease severity. However caution is needed in interpreting these initial study results due to the small sample size. Our ongoing study will further elucidate the range of responses, reliability, and responsiveness of these novel disease-relevant outcomes and their relationship to traditional measures of disease progression.

Study support provided by the FSH Society and the National Institutes of Health.

25 [p]. **Epigenetic variability is a modifier of facioscapulohumeral muscular dystrophy**

Takako Jones[1,2], Celine Debonot[2,3], Charis Himeda[1,2], Chia-Yun Sun[2,5], Chi Yan[1,4] and Peter L. Jones[1,2]

[1] University of Massachusetts Medical School, The Wellstone Program & Department of Cell and Developmental Biology, Worcester, Massachusetts, USA
[2] Boston Biomedical Research Institute, Watertown, Massachusetts, USA
[3] European School of Biotechnology of Strasbourg, France
[4] Huanzhong Agricultural University, Wuhan, P.R. China
[5] Current address: EMD Millipore, USA

Facioscapulohumeral muscular dystrophy (FSHD) is strongly associated with epigenetic changes of the 4q35 D4Z4 macro satellite, FSHD pathology requires the misexpression and missplicing of the DUX4 (double homeobox 4) gene encoded within the repeat array resulting in production of a pathogenic protein, DUX4-FL. We have analyzed DUX4 mRNA and protein expression in a large collection of myogenic cells and muscle biopsies derived from muscles of FSHD1 affected subjects and their unaffected first-degree relatives. We confirmed that stable DUX4-fl mRNA and protein were expressed in myogenic cells and muscle tissues derived from FSHD affected subjects, including several genetically diagnosed adults yet to show clinical manifestations of the disease; however, there was great individual and familial variation in the levels of DUX4-FL. In addition, we found DUX4-fl mRNA and protein expression in muscle biopsies and myogenic cells from genetically unaffected relatives of the FSHD subjects, although at a significantly lower frequency. These results establish that DUX4-fl expression per se is not sufficient for FSHD muscle pathology. To investigate if subtle differences in the epigenetic status of the 4q35 region could account for the wide variation in DUX4-fl expression among FSHD1 subjects and potentially the spurious expression in certain unaffected controls, family cohorts of myogenic cells from FSHD1 subjects were tested for their sensitivity to small molecules that can alter the chromatin state. We find that myogenic cells from FSHD1 subjects are overall epigenetically poised to express DUX4 compared with unaffected subjects; however, FSHD1 subjects show individual differences in their capacity to express DUX4-fl in response to DNA demethylation and blocking histone deacetylation. Therefore, individual differences in the epigenetic status likely impacts progression of disease pathology, variability in age of onset, disease severity, and asymmetry of affected muscles.

26 [p]. **About clinical heterogeneity of FSHD with some historical remarks**

Kazakov V. M.
Department of Neurology, Pavlov State Medical University, St. Petersburg, Russia

In the present time there is an opinion that FSHD is genetically a heterogeneous disease, but clinically a homogeneous one. "… in clinical, genetic and epigenetic features of facioscapulohumeral muscular dystrophy (FSHD) allowed the identification of the two forms of FSHD, the classical autosomal dominant FSHD type 1, and FSHD type 2 characterized by identical clinical phenotype but associated with a different (epi-) genetic defect" and then… "… FSHD1 and FSHD2 share a common pathophysiological pathway since they present an identical clinical phenotype and FSHD2 genetic defect, and that the FSHD2 gene may act as a modified for disease in FSHD1 families (Sacconi S. et al. 2012).

During 1969-1971 I analyzed the patterns of muscle involvement (67 isolated muscles and their parts bilaterally) at different stages of the disease of 200 patients with FSHD which I called as a FSLD (a facioscapulolimb dystrophy). 145 cases were taken from the World literature and 55 cases were under my personal observation. 78 of these 200 patients (31 personal cases and 47 from literature, 59 hereditary and 19 sporadic) had developed FSLD2 a descending with a jump type, with the FSP or FSP(H) phenotypes (38 patients) and with the final FSPFGH (facio- scapulo-peroneo-femoro (posterior thigh muscles)-gluteo (gluteus maximus)-humeral (biceps brachial) or FSPHFG phenotypes (40 patients) among them. In most own patients the FSP phenotype existed in clinical picture on the average for 11-16 years. Diagnosis of FSHD (or FSLD2 a descending type with "a jump") in own patients was confirm by molecular genetic analysis in 1996 (Kazakov et al. 2000, 2008, 2012).

Besides this, among these 200 analyzed FSLD (FSHD) cases in 60 of them (47 hereditary and 11 sporadic cases, including Duchenne's cases hereditary 8 and sporadic 3, taken from the World literature and only 2 (of 44) my personal cases) had developed FSLD1 a gradually descending type. Among these 60 cases 31 were presented with the FSHGF (facio-scapulo-humero – gluteo- femoral) phenotypes and 29 cases were presented with the final FSHGFP (facio-scapulo-humero-gluteo-femoro-peroneal) phenotypes. In the last group of patients the pelvic girdle and thigh muscles were more severely affected in comparison with peroneal muscle group excluding some cases in which the peroneal group of muscles had the same severe degree of affection.

It was found that only one of the two types of the disease (the gradually descending type or the descending one with a jump) occurred in each family. The distribution of muscle's involvement among the members of the same family did not usually overstep the limits of the type of the development of the disease. Besides this, it was found that the kindreds who had either one or the other type of the disease belonged to the populations of different geographical regions.

Also the existence of FSLD1 is confirmed by the fact that in many Handbooks on Nervous Diseases and Handbooks on Muscle Diseases the FSHD was described as a gradually

descending muscular dystrophy with the affection of pelvic girdle and hip muscles during 15-30 years after the involvement of the face, scapular, humeral and trunk muscles (Gowers 1894, Roth 1895, Struempell 1911, Davidenkov 1932,Ford 1952, Elliott 1953,Turner 1953, Becker 1953, Wilson 1954, Merritt 1955, Adams, Denny-Brown & Pearson, 1953, 1962, Brain 1962, Baker 1962, Morton, Chung & Peters 1963, Bourne & Golarz 1963, Walton 1964, Emery & Walton 1967, Hausmanowa-Petrusewicz 1968, Adams & Victor 1985, Brooke 1986, Munsat & Serratrice 1992, Dubowitz 1995, Bushby 1997, Victor & Ropper 2002, Goetz 2007 and others).

In addition, we cannot ignore the publications of many famous authors who have described the gradually descending type of FSHD i.e. the FSLD1 (Duchenne 1848, 1855, 1861, 1862,1868, 1872, Friedreich 1873, Lichtheim 1878, Erb 1882-1891 (in some patients), Zimmerline 1883, Landouzy & Dejerine 1884-1886 (in some patients), Westphal 1886, Osler 1889, Peterson 1892, Gowers 1894, Roth 1895, Sabrazes & Brenques 1899, Bregmann 1899, Eulenburg & Cohn 1911,Davidenkov 1921,Weitz 1921, Riese 1922, Pearson K. 1933, Levison H. 1951,Becker 1952-1964,Stevenson 1953, Jequier & Hanhart 1954, Walton &Nattrass 1954 (in many patients), Walton 1955-1963,Chung & Morton 1959, Dowben 1964, Emery and Walton 1967, Hurwitz et al. 1967, Frezal & Feingolo 1968, Moser et al. 1968, Mertens et al. 1968 (in some patients), Beckmann 1972, Zellweger et al 1972, Carol 1979 (in some patients), Lunt & Harper 1991 (in many patients) and others.

Besides that, it's necessary to remark that the famous discussion between Erb and Landouzy-Dejerine dealt with the priority of recognition and description of the FSHD a descending type with a "jump" (i. e. the FSLD2) and both of them admitted the priority of Duchenne in describing of FSHD a gradually descending type (i. e. the FSLD1) (Kazakov, 1995).

Thus, in my opinion both FSLD1 and FSLD2 are clinically and historically well documented i.e. FSLD (or FSHD) is not only genetically but clinically as well a heterogeneous disease.

P. S. The FSLD2 a descending type with a "jump" with initial FSP phenotype which I distinguished in my thesis in 1971 may be corresponded by clinical picture to FSHD1 and FSHD2 [although the pattern of distribution of muscle's weakness and atrophy from lower leg to thigh and pelvic girdle muscles differs from Padberg's patients (1982)]. The FSLD2 as well as the FSHD1 are linked with chromosome 4q35. This can mean that the SMCHD1 gene located at chromosome 18p (Lemmers et al.2012) acts as an epigenetic modifier for the DUX4 gene in FSHD1 (the same as a FSLD2) and causes the development of FSHD2.

However the question arises: what is the FSLD1 a gradually descending type with initial FSH phenotype which I distinguished in my thesis in 1971 as well. The FSLD1 was first described by Duchenne in 1855 and later, under the name FSHD a descending type, it was described by many famous clinicians and was included in many old and modern Handbooks on Nervous Diseases and Handbooks on Muscle Diseases. May be the FSLD1 (or classical FSHD a descending type) do not exist as a nosological entity at the present time? However, it is very difficult to suppose that earlier descriptions of the clinical picture of FSHD as a descending type which were carried out by different clinicians were a great mistake?! I don't think so. To my opinion the FSLD1 occurs

very rarely and only inside definite geographical regions, similarly, for example, to distal types of muscular dystrophy described by Welander, Udd and Miyoshi. It is quite possible that FSLD1 and FSLD2 are connected with the same gene mutation but they are presented by different phenotypes due to the action of different modifier genes. However, one can propose as well that FSLD1 is connected with another basic gene than FSLD2 and it is not linked with chromosome 4q35.

Valery M. Kazakov, MD, PhD, Dr. med. Sc.,
Professor of Clinical Neurology,
Department of Neurology,
Pavlov State Medical University
L. Tolstoy Str. 6/8,
197022 St. Petersburg, Russia

27 [p]. **Self-reported form for FSHD patients: a fast and reliable tool to collect clinical and genetic data**

Pauline LAHAUT[1], Rafaelle BERNARD[2], Charlotte SAKAROVITCH[3], Gaëlle BLANDIN[4], Céline GUIEN[4], Katia NEHAL[2], Claude DESNUELLE[1], Christophe BEROUD[4], Sabrina SACCONI[1]

[1] Centre de Référence des Maladies Neuromusculaires du CHU de Nice & Hôpital Archet 1, 151 route de Saint Antoine de Ginestière CS 23079, 06202 Nice Cedex 3, France
[2] Laboratoire de Génétique Moléculaire, Département de Génétique Médicale, Hôpital d'Enfants de la Timone, 264 rue Saint-Pierre, 13385 Marseille cedex 05, France
[3] Département de la Recherche Clinique et de l'Innovation, Hôpital Cimiez, 4 Avenue Reine Victoria CS 91179, 06003 Nice Cedex 1, France
[4] INSERM UMR_S910, Faculté de Médecine La Timone, 4ème étage, 27 boulevard Jean Moulin 13385 Marseille Cedex 05, France

Corresponding Author:
Pauline LAHAUT
Centre de Référence des Maladies Neuromusculaires du CHU de Nice & Hôpital Archet 1, 151 route de Saint Antoine de Ginestière CS 23079, 06202 Nice Cedex 3, France
Tel : +33 (0) 492.03.57.53
Fax : +33 (0) 492.03.58.91
Mail :lahaut.p@chu-nice.fr

Background
In France, there are about 3250 patients affected by FSHD, but there is no specific database collecting clinical and genetic data. Most of the French rare disease registries are based on clinical evaluation forms filled by specialized physicians. Nevertheless, the number of inclusions is frequently limited by the fact that this activity is time-consuming for the physicians and/or patients cannot always have access to Specialized Centers. On the other hand, self reported forms can be filled directly by patients at home but they are thought to be less reliable, and likely to generate double entries in the registry.

Objective
The main objective of this study is to compare clinical and genetic data collected by a self reported form filled by the patients themselves with a clinical evaluation form containing the same item filled by a clinician, in order to establish the accuracy of patient's answers and to investigate the difficulty encountered by the patient in filling this form. The ultimate aim is to validate the self reported form in order to collect clinical and molecular data for the French National FSHD Registry.

Patients and Methods
42 genetically confirmed FSHD1 patients (24 women; 18 men; mean age: 57.95) carrying from 3 to 10 repeated units have been enrolled in this study. The self reported form consisted in 47 items including patient identity, diagnosis and genetics, muscle impairment, self functional and

pain assessment, other extra-muscular involvement, and medical care. The self-reported form was compared to clinical evaluation form filled by a physician. A matching rate were calculated, expressing the percentage of correspondence between the physician's and the patient's answers. Moreover, the patients rated the difficulty of each question using a score between 0 (question not understood) and 4 (question very easy).

Results
90.61% of the scores given by the 42 patients are 4/4, globally rating the self reported form as "very easy". The items receiving the lowest scores are related to the genetic diagnosis and lipid increase. Furthermore, 75% of the answers given by the patient are consistent with those given by the physician showing a matching rate above 90%. More than 95% of the answers show a matching rate above 75%. The highest frequency of inconsistent answers is about lipid increase and asymmetry of muscular involvement. An educational program has been set up to improve patient knowledge on these two issues, available at the French National FSHD Registry website: www.fshd.fr

Conclusion
The self reported form is a fast and reliable tool to collect clinical and genetic data on FSHD and has been integrated in French National FSHD Registry. A specific informatic tool has been developed to avoid double entries and to confirm genetic results. To improve patient's reliability in answering questions, an educational video will be soon available on the French National FSHD Registry website: www.fshd.fr.

28 [p]. **Electrical impedance myography for the in and ex vivo assessment of muscular dystrophy (mdx) mouse muscle**

Jia Li, PhD,[1] Tom R. Geisbush, BA,[1] Glenn D. Rosen, PhD,[1] Jennifer Lachey, PhD,[2] Aaron Mulivor, PhD,[2] and Seward B. Rutkove, MD[1]

Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts[1] and Acceleron Pharma, Inc, Cambridge, Massachusetts[2]

ABSTRACT

Introduction
Sensitive, non-invasive techniques that can provide biomarkers of disease status and the effects of therapy in muscular dystrophy are needed.

Methods
We evaluated electrical impedance myography (EIM) to serve in this role by studying 2-month-old and 18-month-old mdx and wild-type (WT) animals (10 animals in each of the four groups).

Results
Marked differences were observed in the EIM values between the mdx and WT animals, with those differences being more pronounced between the older age groups (e.g., reactance of 92.6±4.3 ohms for mdx animals vs. 130±4.1 ohms for the WT animals, $p < 0.001$). In addition, in vivo EIM parameters correlated significantly with the extent of connective tissue deposition in the mdx animals.

Discussion
These findings support that EIM has the potential to serve as a valuable non-invasive method for evaluating muscular dystrophy non-invasively, serving as a useful biomarker to assist with therapeutic testing in both pre-clinical and clinical studies.

Key Words: Duchenne muscular dystrophy, electrical impedance, biomarker, mdx mouse, connective tissue

29 [p]. **Role of CTCF in epigenetic regulation of the 4qD4Z4 macrosatellite repeat**

Jong Won Lim, James M. Moore, Natalia A. Rabaia, Yvonne D. Krom, Stephen J. Tapscott, Silvère van der Maarel, Galina N. Filippova

University of Washington, Seattle, Washington USA
Fred Hutchinson Cancer Research Center, Seattle, Washington USA
Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands

Facioscapulohumeral muscular dystrophy (FSHD) is caused by incomplete repression of the D4Z4 macrosatellite repeat array on the disease-permissive chromosome 4q that results in aberrant expression of DUX4, the candidate FSHD gene imbedded within the D4Z4 repeat. Loss of repressive chromatin modifications at the D4Z4 array in FSHD has been reported to be associated with increased binding of the chromatin insulator protein CTCF (Zeng, et al., 2009 and Ottaviani, et al., 2009). However due to repetitive nature of the D4Z4 array, there is very limited information available on CTCF binding at this region. Here we profiled CTCF binding to the 4q35 D4Z4 locus. We identified several CTCF binding sites within a D4Z4 reeat unit as well as both proximal and distal to the D4Z4 array. One of the proximal sites is located in the viscinity of the MAR/SSLP region that has been implicated in FSHD-specific chromatin looping. CTCF sites at the distal D4Z4/pLAM region flank the pLAM polyadenylation site, which is critical for generation of polyadenylated DUX4 transcripts, and show FHSD-related haplotype–specific binding. We confirmed that CTCF binds to the D4Z4 repeats in both FSHD1 and FSHD2 muscle cells but not in controls. Consistent with the role of CTCF in protection of genes from epigenetic silencing, we observed a significant loss of both histone H3K9 trimethylation and DNA methylation at CTCF binding sites at D4Z4 in FSHD cells in comparison to controls. Depletion of CTCF resulted in increase of histone H3K9 methylation at the D4Z4 and reduction of the DUX4 transcripts levels in FSHD myoblasts. Our findings suggest that CTCF binding at the D4Z4 locus in FSHD may affect chromatin structure of the locus and interfere with the setting and/or maintenance of repressive epigenetic marks resulting in inappropriate DUX4 transcription.

30 [p]. **FAT1 mRNA expression in FSHD and control biopsies and cells**

Virginie Mariot[1], Debora Portilho[1], Sabrina Sacconi[2, 3], Frederique Magdinier[4], Philippe Rameau[5], Anne-Lise Delezoide[6], Stephane Roche[4,] Claude Desnuelle[2, 3], Fabien Guimiot[7], Bettina Bessières[8], Sophie Collardeau[9], Françoise Helmbacher[10], Gillian Butler-Browne[1], Vincent Mouly[1], Julie Dumonceaux[1]

[1] INSERM U974, UMR 7215 CNRS, Institut de Myologie, UM 76 Université Pierre et Marie Curie, 75013, Paris, France. [2] Centre de Référence des Maladies Neuromusculaires, Nice University Hospital, Nice, France. [3] Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche (UMR) 7277, Nice University Hospital, Nice, France. [4] Aix-Marseille Université. Faculté de Médecine de la Timone, INSERM UMR 910. 27, Bd Jean Moulin, 13385 Marseille cedex 05, France. [5] Institut Gustave Roussy, Plate-Forme Imagerie et Cytométrie, 114 rue Edouard Vaillant, 94805, Villejuif, France. [6] Université Paris Diderot, Assistance Publique-Hôpitaux de Paris, Hôpital Robert Debré, Service de Biologie de Développement, Paris 75019, France. [7] Assistance Publique-Hôpitaux de Paris, Hôpital Robert Debré, Anatomie et Cytologie Pathologiques, Paris 75019, France. [8] INSERM U781 et Fondation IMAGINE, Assistance Publique-Hôpitaux de Paris, Hôpital Necker-Enfants Malades, Département de Génétique, Paris 75015, France. Université Paris Descartes, Paris 75006, France. [9] Centre de pathologie Est, CHU-Lyon, 59 bd Pinel, 69677, BRON. [10] Aix-Marseille Université, CNRS, IBDML UMR 7288, Parc Scientifique de Luminy, Case 907, Marseille, France

Recently, we have shown that mis-regulation of the Planar Cell Polarity (PCP) gene FAT1 in mice recapitulates the main FSHD symptoms including shoulder and face atrophy, asymmetry of the involved muscles and non-muscular defects (Caruso et al. Plos Genet 2013) Importantly, FAT1 mRNA was found to be specifically down regulated in several human FSHD1 fetal muscle biopsies compared to age matched controls. Since FAT1 has never been described to play a role in muscular physiology in mammals, our goal was to decipher the function of FAT1 in skeletal muscle in order to identify new potential therapeutic approaches for FSHD.

FAT1 gene (136 kb) is located in 4q35, in the same chromosomal region as D4Z4 repeats. Human FAT1 mRNA (14.7 kb) is composed of 27 exons. An alternative splicing between exon 26 and 27 has been described in kidney cells leading to different spliced isoforms but so far nothing is known in adult human biopsies and cells. Here, we investigated FAT1 expression in fetal and adult control and FSHD biopsies. Twenty eight control quadriceps biopsies isolated from control fetuses of 14 to 33 weeks of development were used to determine FAT1 mRNA expression during fetal muscle development. We observed that FAT1 expression level was progressively decreased by 90% during normal fetal development. We also show that FAT1 pre-mRNA generates at least 4 different isoforms. These variants include or exclude small portions of intron 26 and are in phase or not with exon 27 leading either to a longer or a truncated protein. The potential roles of these isoforms were analyzed by knocking-down each isoform using specific shRNA. We also compared FAT1 isoform expression in control and FSHD adult biopsies. Finally, the potential involvement of DUX4 in FAT1 regulation was investigated. All these results will be presented during the meeting.

31 [p]. **Genetic correction of Duchenne muscular dystrophy by genome editing with engineered nucleases**

David G. Ousterout, PhD and Charles A. Gersbach, PhD

Departments of Biomedical Engineering and Orthopaedic Surgery and
Institute for Genome Science and Policy, Duke University, Durham, North Carolina USA

Duchenne Muscular Dystrophy (DMD) is a relatively common degenerative disease that results from mutation of the gene encoding the dystrophin protein. The genetic nature of DMD has led to substantial interest in gene therapy-based approaches to this disease, including several clinical trials. However, these therapies typically require the random integration of exogenous DNA into the genome or the lifelong re-administration of transient gene therapy vectors, both of which have significant safety and practical concerns. Furthermore, these strategies have been limited by an inability to deliver the large and complex dystrophin gene sequence. An exciting alternative to these approaches is the targeted editing of the human genome to repair the endogenous mutant dystrophin gene. This concept represents a potential cure to DMD without the need for random integration of or repeated exposure to foreign biological material.

The focus of our work is to develop and implement strategies for directed modification of the genome for the treatment of genetic disorders. Engineered nucleases, including zinc finger nucleases (ZFNs), TALE nucleases (TALENs), and CRISPR/Cas9 constitute powerful tools for coordinating the site-specific manipulation of genomic DNA sequences. The ZFN and TALEN technologies have been developed by biomolecular engineering of novel enzymes comprised of synthetic DNA-binding domains fused to the catalytic domain of a restriction endonuclease. Engineering of the DNA-binding domain to target specific sites in the human genome can be used to direct nuclease activity and endogenous DNA repair machinery to any locus of interest. More recently, the RNA-guided nuclease Cas9, which has natural role in bacterial adaptive immunity, has been used in human cells as a method to direct nuclease activity to new targets without protein engineering. Using any of these systems, site-specific nuclease-mediated DNA cleavage can be used to frameshift or excise gene sequences via DNA re-ligation. Alternatively, DNA sequences can be added or exchanged at targeted loci via the nuclease-mediated enhancement of homologous recombination. Our goal is to use these genome editing technologies to repair mutated DNA sequences responsible for genetic diseases such as DMD.

We have engineered and optimized ZFNs, TALENs, and CRISPR/Cas9 systems that can mediate efficient manipulation of the dystrophin gene sequence in human cells. This includes the direct correction of mutations, the excision of exons, the site-specific addition of missing exons, and the introduction of dystrophin cDNA into the endogenous dystrophin locus under control of the natural promoter. We have used these approaches to restore dystrophin expression in human muscle progenitor cells derived from DMD patients as well as dermal fibroblasts that can be reprogrammed to the myogenic lineage. Substantial levels of dystrophin are expressed in the DMD cells following genetic correction. We further show that these nucleases are non-toxic with minimal off-target effects. Corrected cells have been transplanted into immunodeficient

mice. This work represents an exciting new avenue for DMD therapy that can permanently correct the underlying genetic mutations. Furthermore, these technologies and various genome editing approaches are generally applicable to correcting other genetic diseases affecting skeletal muscle, including Fascioscapulohumeral Dystrophy.

32 [p]. **Towards the development of a novel mouse model of FSHD**

Sakellariou P.[1], O'Neill A.[1], Roche JA.[1], Stadler, G.[2], Wright WE.[2], and Bloch RJ.[1]

[1] Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland USA
[2] Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, Texas USA

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common form of muscular dystrophy in adults, with a prevalence of 1 in 15,000 individuals worldwide. The lack of a valid animal model for FSHD has been a great obstacle in studying the molecular basis and pathophysiology of the disease, and in developing therapeutics. Here, we investigate the ability of human immortalized myogenic precursor cells to form mature human myofibers following implantation in irradiated and cardiotoxin (CTX)-injured tibialis anterior (TA) muscles of NOD-Rag1nullIL2rγnull immunodeficient mice. We used LHCN cells, immortalized human cells that express luciferase, and initially monitored their survival by luminometry. Survival at 4-7 weeks post-transplantation was ≤10%, whether 5x105 or 2x106 cells were injected, but more human myofibers, detected with antibodies to β-spectrin, formed under the latter conditions. These fibers were spatially segregated from the mouse myofibers in the TA region that survived intoxication with CTX. Neuromuscular electrical stimulation (NMES) of the engrafted TA via the peroneal nerve did not enhance survival but did improve the quality of the grafts, as measured by the increased number and size of the human myofibers formed and their closer packing in the engrafted region. After 4 weeks of NMES, the human myofibers were differentiated, as indicated by an intramyoplasmic reticulum of desmin, and innervated, as indicated by labeling with fluorescent α-bungarotoxin. Labeling with antibodies specific for human lamin A/C suggested that the engrafted fibers were >90% human in origin. Our results show that, over a 4 to 7 week period after transplantation, human myoblasts can successfully engraft into the mouse TA muscle to form mature myofibers with minimal contamination of murine myonuclei. We are now optimizing our methods to improve engraftment, with the aim of generating human FSHD muscles in mice.

Supported by contracts to R.J.B and W.E.W. from the Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center (U54 HD060848, Dr. C. Emerson, P.I.) and by a postdoctoral fellowship to P.S. from the FSH Society.

33 [p]. **Multi-analyte profile of potential serum biomarkers in facioscapulohumeral muscular dystrophy**

Jeffrey Statland, MD [1], Colleen Donlin-Smith, MA [1], Stephen J Tapscott, MD, PhD [2], Silvere van der Maarel, PhD [3], and Rabi Tawil, MD[1]

[1] Department of Neurology, University of Rochester Medical Center, Rochester, NY
[2] Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA
[3] Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands

Background: Recent studies have shown a large number of genes either up or down regulated by in vitro DUX4 expression, many of which are involved in the germline, immunity, RNA processing, ubiquitin pathways, or in cancer expressed genes. Downstream proteins regulated by these genes may be detectible in serum of Facioscapulohumeral muscular dystrophy (FSHD) patients. Reliable serum biomarkers related to either disease activity or progression will be important to help stratify participants and monitor response to therapy in future FSHD clinical trials.

Methods: We performed a cross sectional analysis of serum biomarkers in 22 FSHD patients (19 FSHD1, 3 FSHD2) and 22 age and gender matched controls using a commercial multiplexed, microsphere-based immune-fluorescent assay of 243 markers (Myriad, Human DiscoveryMAP 250, v1.0), including markers of inflammation, autoimmunity, and cancer. Markers were screened using 2 sample t-test and generalized linear model adjusted for age and gender. Correlations between physiologically related markers were performed.

Results: 21 markers had a p-value <0.05, including 7 with a p-value < 0.01. The majority of markers fell in the inflammatory category, including increases in epidermal growth factor (p=0.001) and monocyte chemotactic factor protein 1 (p=0.001), which recruits monocytes to sites of injury and infection. Cancer markers include decreases in mesothelin (p=0.009), a cell surface glycoprotein that is highly expressed in various cancers. Additional markers increased in FSHD include CK-MB fraction (p<0.0001), and tissue plasminogen activator (p<0.0001), a factor involved in fibrinolysis, but also in tumor angiogenesis. Many markers showed expected physiological correlations with factors involved in similar metabolic cascades.
Discussion: Markers involved in inflammation or angiogenesis show promise and will need a follow up validation study to confirm their relationship to disease. The MAP technology offers the opportunity to reliably and broadly screen large numbers of potential biomarkers on single serum samples in FSHD. Although commercially available as sets, disease-specific markers for proteins of interest can be created using the same technology.

Study support provided from the Muscular Dystrophy Association

34 [p]. **Dux4 target gene expression in mouse muscle transplanted with muscle cells from FSHD patients**

James A. Windelborn and Charles P. Emerson, Jr.

U.S. DHHS NIH NICHD Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD and Department of Cell and Developmental Biology, University of Massachusetts Medical School, Worcester, Massachusetts

james.windelborn@umassmed.edu and Charles.emersonjr@umassmed.edu

Abstract: Facioscapulohumeral Muscular Dystrophy (FSHD) is one of the most prevalent forms of muscular dystrophy. However, because of the unique nature of the genetic abnormality underlying the disease, there is currently no widely available laboratory model. In order to gain insights into FSHD molecular pathology, we developed a xenograft model by transplanting myogenic cells from patients with FSHD (4qA contractions) as well as from their unaffected relatives into the tibialis anterior muscles of immunodeficient mice. Our findings show that muscle xenografts derived from FSHD myogenic cells express Dux4 target genes, recapitulating the expression of these disease biomarkers in muscle biopsies of FSHD patients. FSHD muscle xenografts provide an animal model for investigations of the molecular pathogenesis of FSHD muscles and for drug development.