

# **FSH Society Facioscapulohumeral Muscular Dystrophy [FSHD] 2009 International Research Consortium & Research Planning Meetings**

Monday, November 9, 2009

7:30 a.m. – 6:00 p.m.

&

Tuesday, November 10, 2009

7:30 a.m. – 2:00 p.m.

## **Boston Biomedical Research Institute**

64 Grove Street, Watertown, Massachusetts 02472 USA

### **Co-Chairs: Kathryn R. Wagner, M.D., Ph.D.**

*Kennedy Krieger Institute, Baltimore, Maryland USA &*

*The Johns Hopkins University School of Medicine, Baltimore, Maryland USA &*

*NIH Eunice Kennedy Shriver NICHD Boston Biomedical Research Institute*

*Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center*

### **Silvère van der Maarel, Ph.D.**

*Leiden University Medical Center, Leiden, the Netherlands &*

*Fields Center for FSHD and Neuromuscular Research*

### **Organizers: Daniel Paul Perez**

*FSH Society, Inc.*

### **Silvère van der Maarel, Ph.D.**

### **Kathryn Wagner, M.D., Ph.D.**

### **Hosted By:**

FSH Society, Inc.

NIH Eunice Kennedy Shriver NICHD Boston Biomedical Research Institute Senator

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### **Sponsored By:**

Accelaron Pharma

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Institute Senator Paul D. Wellstone MDCRC

Muscular Dystrophy Association United States (MDAUSA)





**Boston Biomedical Research Institute**  
Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD Research



November 9-10, 2009

## PREFACE

Dear Colleagues,

Welcome to the FSHD International Research Consortium 2009. Thanks to you, we are seeing numerous developments in every aspect of FSHD basic and clinical research! We hope that this meeting will allow us to define the complex mechanism and various features of FSHD and enable us to move quickly to the development of potential treatments for FSH muscular dystrophy.

This past year has brought with it quite a significant increase in government, non-profit, and private funding for FSHD. It has also ushered in an international collaboration of volunteer health agencies and FSHD patients working side-by-side with research and clinical communities. It is essential for the entire community to work together at every level and to communicate clearly on programs, developments and needs.

This year's workshop participants include clinicians, scientists, biotechnology companies, pharmaceutical companies, government and non-profit funding agencies, along with patients – committed to solving, treating and curing FSHD at this workshop. More than 75 people have registered for this workshop making this the “place to be” for anyone with a keen interest in FSHD. At the second day, we will hold a round table discussion to discuss the future needs of FSHD. We hope for a thoughtful and productive morning in which all FSHD issues will be openly discussed to direct us towards a new and better future for patients suffering with FSHD.

This meeting is organized and sponsored by the FSH Society, the U.S. DHHS NIH Eunice Kennedy Shriver NICHD Sen. Paul D. Wellstone BBRI FSHD Muscular Dystrophy Cooperative Research Center, the Association Française Contre les Myopathies (AFM), the Muscular Dystrophy Association (MDAUSA), FSHD Global Research Foundation, Fields Center for FSHD and Neuromuscular Research, Acceleron Pharma, Genzyme, Genomic Vision, and Athena Diagnostics. It is truly a pleasure to bring the entire group together to accelerate solutions for facioscapulohumeral muscular dystrophy!

Thank you for coming. Thank you for sharing. Thank you for your extraordinary efforts and hard work on behalf of patients and their families.

Daniel Paul Perez  
FSH Society, Inc., Watertown, Massachusetts, USA

Kathryn Wagner, M.D., Ph.D.  
Kennedy Krieger Institute, Baltimore, Maryland USA  
The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Silvère van der Maarel, Ph.D.  
Leiden University Medical Center, Leiden, the Netherlands

*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, 11 Elmbrook Circle, Bedford, Massachusetts 01730 USA. Phone: (781) 275-7781, fax: (781) 275-7789, e-mail: daniel.perez@fshsociety.org, website: <http://www.fshsociety.org>*

## Day 1

**Monday, November 9, 2009**

Registration & Continental Breakfast	7:30-8:00	
<b>Welcome</b>	8:00-8:30	
Session 1	8:30-9:30	<b>SNPs &amp; Diagnosis</b> (3x20 minutes)
Session 2	9:30-10:30	<b>Chromatin</b> (3x20 minutes)
<b>Break &amp; Poster viewing</b>	10:30-11:00	
Session 3	11:00-12:20	<b>Biomarkers &amp; Genotype Phenotype</b> (4x20 minutes)
<b>Lunch &amp; Poster viewing</b>	12:20-13:50	
Session 4	13:50-14:50	<b>Model Systems</b> (3x20 minutes)
Session 5	14:50-15:50	<b>DUX4</b> (3x20 minutes)
<b>Break &amp; Poster viewing</b>	15:50-16:20	(moderators prepare for plenary session)
Session 6	16:20-17:40	<b>FRG1 &amp; CRYM</b> (4x20 minutes)
<b>Conclude</b>	17:40-18:00	
<b>Dinner</b> at Henrietta's Table	19:00-22:00	Private Dining Room, Charles Hotel, Harvard Square

## Day 2

Tuesday, November 10, 2009

**Continental Breakfast** 7:30-8:00

**Welcome &  
Charge to the Body** 8:00-8:05

**Co-Chairs:**

Kathryn Wagner, M.D., Ph.D.

Kennedy Krieger Institute, Baltimore, Maryland USA &

The Johns Hopkins University School of Medicine, Baltimore, Maryland USA &

Silvère van der Maarel, Ph.D.

Leiden University Medical Center, Leiden, the Netherlands

**Review** 8:05-8:20 **Review of significant insights November 9**

**Co-Chairs:**

Kathryn Wagner, M.D., Ph.D. & Silvère van der Maarel, Ph.D.

8:20-8:30 **Recap FSH Society 2006 Tactical & Strategic Plan**

**Facilitators:** Rune R. Frants, Ph.D., Leiden University Medical Center, the Netherlands &  
Michael R. Altherr, Ph.D., Los Alamos National Laboratory, New Mexico

**Outline available online at FSH Society, Inc.:**

<http://www.fshsociety.org/pages/resPSocPlan.html>

**Session 1** 8:30-9:15 **Scope of FSHD Research**

**Facilitators:** Kathryn Wagner, M.D., Ph.D. & Rune R. Frants, Ph.D.

**Discussion by entire Group**

**Suggested topics:**

Clinical Research: potential drugs, and important outcome measures, Critical experiments that need to be redone, corroborated, Epigenetics, Chromatin, Nuclear localization/signals, SNPs, Chromosome folding, Imprinting, and Genomic sequence

**Session 2** 9:15-10:15 **Assumptions on which solving FSHD rests**

**Facilitators:** Silvère van der Maarel, Ph.D. & Michael R. Altherr, Ph.D.

**Discussion by entire Group**

**Session 3** 10:15-11:15 **Requirements for solving FSHD**

**3 Breakout Sessions:**

1. **Clinical Research:** potential drugs, and important outcome measures

**Facilitators:** Kathryn Wagner, M.D., Ph.D. & Rabi Tawil, M.D. &  
John Porter, Ph.D., NIH NINDS

2. **Molecular genetics:** epigenetics, chromatin, genomics

**Facilitators:** Silvère van der Maarel, Ph.D. & Lou Kunkel, Ph.D. &  
Ljubisa Vitkovic, Ph.D., NIH Eunice Kennedy Shriver NICHD

3. **Cell biology:** cells, mouse, stem cells research

**Facilitators:** Davide Gabellini, Ph.D. & Leslie Lock, Ph.D. &  
Glen Nuckolls, Ph.D., NIH NIAMS

<b>Break &amp; Lunch</b>	11:15-11:45	
<b>Session 4</b>	11:45-13:30	<b>Tasks included and needing to be accomplished</b>
		<b>Facilitators:</b> Michael R. Altherr, Ph.D. & Rune R. Frants, Ph.D.
		<b>Discussion by entire Group</b>
<b>Session 5</b>	13:30-14:00	<b>Cost estimates, time and schedule</b>
		<b>Facilitators:</b> Silvère van der Maarel, Ph.D. & Kathryn Wagner, M.D., Ph.D.
		<b>Discussion by entire Group</b>
<b>Conclude</b>	14:00	

	<b>First Author</b>	<b>Presenting Author</b>	<b>Session</b>
8:30-8:50 a.m.	Tsumagari	Ehrlich	SNPs and Diagnosis
8:50-9:10 a.m.	Lemmers	Lemmers	SNPs and Diagnosis
9:10-9:30 a.m.	Sacconi	Sacconi	SNPs and Diagnosis
9:30-9:50 a.m.	Cabianca	Cabianca	Chromatin
9:50-10:10 a.m.	Ma	Ehrlich	Chromatin
10:10-10:30 a.m.	Zeng	Yokomori	Chromatin
10:30-11:00 a.m.			Posters & Break
11:00-11:20 a.m.	Fitzsimons	Fitzsimons	Biomarkers & Genotype Phenotype
11:20-11:40 a.m.	Rahimov	Wagner/J.B. Miller	Biomarkers & Genotype Phenotype
11:40-12:00 p.m.	de Greef	Tawil	Biomarkers & Genotype Phenotype
12:00-12:20 p.m.	Rahimov	Wagner	Biomarkers & Genotype Phenotype
12:20-1:50 p.m.			Posters & Lunch
1:50-2:10 p.m.	Krom	Butler-Browne	Model systems
2:10-2:30 p.m.	Kyba	Kyba	Model systems
2:30-2:50 p.m.	Block	D.G. Miller	Model systems
2:50-3:10 p.m.	Tassin	Tassin	DUX4
3:10-3:30 p.m.	Wallace	Wallace	DUX4
3:30-3:50 p.m.	Knopp	Zammit	DUX4
3:50-4:20 p.m.			Posters & Break
4:20-4:40 p.m.	Liu	Liu	FRG1 and CRYM
4:40-5:00 p.m.	Wuebbles	Long	FRG1 and CRYM
5:00-5:20 p.m.	Reed	Reed	FRG1 and CRYM
5:20-5:40 p.m.	Sun	Sun	FRG1 and CRYM

## Posters

[	Borgstein	Borgstein	Poster
	Corona	Rosa	Poster
	Dmitriev	Dmitriev	Poster
	Ehrlich	Ehrlich	Poster
	Hampson	Hampson	Poster
10:30-11:50 a.m.	Hanel	Jones	Poster
&	Knopp	Knopp	Poster
12:20-1:50 p.m.	Lachey	Lachey	Poster
&	Lunt	Lunt	Poster
3:50-4:20 p.m.	Nguyen	Walrafen	Poster
	Reed	Bloch	Poster
	Ricci	Ricci	Poster
	Sacconi	Sacconi	Poster
	Stadler	Stadler	Poster
]	Tupler	Tupler	Poster

**7:30-8:00 a.m.**

**REGISTRATION & CONTINENTAL BREAKFAST**

**8:00-8:10 a.m.**

**WELCOME**

*Daniel Paul Perez*

*President & CEO, FSH Society, Watertown, Massachusetts USA*

*Charles P. Emerson, Jr., Ph.D.*

*Boston Biomedical Research Institute, Watertown, Massachusetts USA &*

*U.S. NIH Eunice Kennedy Shriver NICHD Boston Biomedical Research Institute Senator Paul D.*

*Wellstone Muscular Dystrophy Cooperative Research Center*

**8:10-8:30 a.m.**

**CO-CHAIRS OPENING REMARKS &  
CHARGE TO THE MEETING ATTENDEES**

*Kathryn Wagner, M.D., Ph.D.*

*Kennedy Krieger Institute, Baltimore, Maryland USA &*

*The Johns Hopkins University School of Medicine, Baltimore, Maryland USA*

*Silvère van der Maarel, Ph.D.*

*Leiden University Medical Center, Leiden, the Netherlands*

**8:30 a.m.-9:30 a.m.**

**PLATFORM PRESENTATIONS I**

*Rabi Tawil, M.D., Moderator*

*Neuromuscular Disease Center, University of Rochester Medical Center &*

*Fields Center for FSHD and Neuromuscular Research, Rochester, New York USA*

**SNPs & DIAGNOSIS**

**8:30-8:50 a.m.**

*Melanie Ehrlich, Ph.D.*

*Human Genetics Program and the Department of Biochemistry, Tulane Medical School, New Orleans, Louisiana 70112 USA*

**An easy test for the presence of the 4qA161 FSHD-permissive haplotype and its application to studying the molecular genetics of FSHD**

Koji Tsumagari<sup>1</sup>, Desheng Chen<sup>1</sup>, Aaron D. Bossler<sup>2</sup>, and Melanie Ehrlich<sup>1</sup>

<sup>1</sup> *Human Genetics Program and the Department of Biochemistry, Tulane Medical School, New Orleans, Louisiana 70112 USA*

<sup>2</sup> *Molecular Pathology Laboratory, University of Iowa Hospitals and Clinic, Iowa City, Iowa 52242 USA*



**8:50-9:10 a.m.**

**Richard J.L.F. Lemmers, Ph.D.**

*Department of Human Genetics, Leiden University Medical Center, The Netherlands*

**The evolution of the subtelomeres of chromosomes 4q and 10q**

Richard JLF Lemmers, Patrick van der Vliet, Kristiaan J van der Gaag, Sofia Zuniga, Rune R Frants, Peter de Knijff, Silvère M. van der Maarel

Department of Human Genetics, Leiden University Medical Center, The Netherlands

**9:10-9:30 a.m.**

**Sabrina Sacconi, Ph.D.**

*Centre de référence des maladies Neuromusculaires, CHU, Nice, France*

**High frequency of hypomethylation in patients with FSHD like phenotype**

S. Sacconi<sup>1</sup>, J.C. de Greef<sup>2</sup>, R.J.L.F. Lemmers<sup>2</sup>, P. Camano<sup>3</sup>, S.M. van der Maarel<sup>2</sup> & C. Desnuelle<sup>1</sup>

<sup>1</sup> Centre de référence des maladies Neuromusculaires, CHU, Nice, France

<sup>2</sup> Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands

<sup>3</sup> Neuroscience Unit, Biodonostia Institute, San Sebastián, Spain

**9:30 a.m.-10:30 a.m.**

**PLATFORM PRESENTATIONS II**

**Jane E. Hewitt, Ph.D., Moderator**

*Institute of Genetics, School of Biology, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH United Kingdom*

**CHROMATIN**

**9:30-9:50 a.m.**

**Daphne Cabianca, M.S.**

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

**A ncRNA regulates chromatin conformation of the facioscapulohumeral muscular dystrophy region**

Daphne Cabianca<sup>1,2</sup>, Beatrice Bodega<sup>3</sup>, Victoria Neguembor<sup>1</sup>, Enrico Ginelli<sup>3</sup> and Davide Gabellini<sup>1,4</sup>

<sup>1</sup> Division of Regenerative Medicine, San Raffaele Scientific Institute, Milano, Italy

<sup>2</sup> UniSR-Open University International PhD Program in Cellular and Molecular Biology, Milano, Italy

<sup>3</sup> Department of Biology and Genetics for Medical Sciences, University of Milano, Italy

<sup>4</sup> Dulbecco Telethon Institute, Milano, Italy

**9:50-10:10 a.m.**

**Melanie Ehrlich, Ph.D.**

*Human Genetics Program and the Department of Biochemistry, Tulane Medical School, New Orleans, Louisiana 70112 USA*

**Chromatin modification at DNaseI hypersensitive sites in 4q35 in myoblasts**

Jing-Jing Ma<sup>1</sup>, Shao-Chi Chang<sup>1</sup>, Xueqing Xu<sup>1</sup>, Dmitri Loukinov<sup>2</sup>, Victor Lobanenkov<sup>2</sup>, and Melanie Ehrlich<sup>1</sup>

<sup>1</sup>Human Genetics Program and the Department of Biochemistry, Tulane Medical School, New Orleans, Louisiana 70112 USA

<sup>2</sup>Molecular Pathology Section, National Institute of Allergy and Infectious Diseases, Rockville, Maryland 20852 USA

**10:10-10:30 a.m.**

***Kyoko Yokomori, D.V.M., Ph.D.***

*Department of Biological Chemistry, School of Medicine, University of California, Irvine, California USA*

**Specific loss of histone H3 lysine 9 trimethylation and HP1gamma/cohesin binding at D4Z4 repeats is associated with facioscapulohumeral dystrophy (FSHD)**

Weihua Zeng<sup>1</sup>, Jessica C. de Greef<sup>2</sup>, Yen-Yun Chen<sup>1</sup>, Richard Chien<sup>1</sup>, Xiangduo Kong<sup>1</sup>, Heather C. Gregson<sup>1</sup>, Sara T. Winokur<sup>1</sup>, April Pyle<sup>3</sup>, Keith D. Robertson<sup>4</sup>, John A. Schmiesing<sup>1</sup>, Virginia E. Kimonis<sup>5</sup>, Judit Balog<sup>2</sup>, Rune R. Frants<sup>2</sup>, Alexander R. Ball, Jr.<sup>1</sup>, Leslie F. Lock<sup>1</sup>, Peter J. Donovan<sup>1</sup>, Silvere M. van der Maarel<sup>2</sup>, and Kyoko Yokomori<sup>1</sup>

<sup>1</sup>Department of Biological Chemistry, School of Medicine, University of California, Irvine, California 92697-1700 USA

<sup>2</sup>Leiden University Medical Center, Center for Human and Clinical Genetics, P.O. Box 9600, 2300 RC Leiden, The Netherlands

<sup>3</sup>Institute for Stem Cell Biology and Medicine, Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine at UCLA, 277A BSRB Box 951489, Los Angeles, California 90095-1489 USA

<sup>4</sup>Department of Biochemistry and Molecular Biology, University of Florida, Box 100245, Gainesville, Florida 32610 USA<sup>4</sup>;

<sup>5</sup>Division of Medical Genetics and Metabolism, Department of Pediatrics, University of California Irvine Medical Center, 101 The City Drive South, ZC4482, Orange California 92868 USA

**10:30 a.m.-11:00 a.m.**

**POSTER VIEWING & MORNING BREAK**

[FOR LISTING OF THE POSTERS PLEASE SEE LUNCH PROGRAM]

**11:00 a.m.-12:20 p.m.**

**PLATFORM PRESENTATIONS III**

***Michael Kyba, Ph.D., Moderator***

*Lillehei Heart Institute and Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota USA*

**BIOMARKERS & GENOTYPE/PHENOTYPE**

**11:00-11:20 p.m.**

***Robin B. Fitzsimons, M.B., B.S., B.Sc., (Med), Ph.D., FRACP***

*University of Sydney, Sydney, NSW, 2000 Australia*

**FSHD and signal patterns in retina and muscle: Can twentieth century embryology help 'decode' twenty-first century molecular enigmas?**

Robin B. Fitzsimons, M.B., B.S., B.Sc., (Med), Ph.D., FRACP  
University of Sydney, Sydney, NSW, 2000 Australia

**11:20-11:40 p.m.**

**Jeffrey Boone Miller, Ph.D.**

*Boston Biomedical Research Institute, Watertown, Massachusetts USA &  
NIH Eunice Kennedy Shriver NICHD BBRI Senator Paul D. Wellstone Muscular Dystrophy  
Cooperative Research Center for FSHD Research*

**FSHD biomarker studies: Using micro-qPCR arrays to analyze gene expression in FSHD and control muscle biopsies and myogenic cell cultures.**

Fedik Rahimov<sup>1,2</sup>, Jennifer Chen<sup>2,3</sup>, Vivek K. Vishnudas<sup>2,3</sup>, Kendal Hanger<sup>2,3</sup>, Oliver King<sup>2,3</sup>, Jeffrey B. Miller<sup>2,3</sup>, Kathryn Wagner<sup>2,4</sup>, Louis M. Kunkel<sup>1,2</sup>, and Charles P. Emerson<sup>2,3</sup>.

<sup>1</sup> Children's Hospital Boston, 300 Longwood Avenue, Boston, Massachusetts 02115 USA

<sup>2</sup> NIH Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD Research

<sup>3</sup> Boston Biomedical Research Institute, 64 Grove Street, Watertown, Massachusetts 02472 USA

<sup>4</sup> The Kennedy Krieger Institute & The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 USA

**11:40 a.m.-12:00 p.m.**

**Rabi Tawil, M.D.**

*Neuromuscular Disease Center, University of Rochester Medical Center, Rochester, New York USA*

**Genotype-phenotype studies in FSHD2**

JC de Greef<sup>1</sup>, RJLF Lemmers<sup>1</sup>, RR Frants<sup>1</sup>, SM van der Maarel<sup>1</sup>, R Tawil<sup>2</sup>

<sup>1</sup>Department of Human Genetics, Leiden University Medical Center, The Netherlands

<sup>2</sup>Neuromuscular Disease Center, University of Rochester Medical Center, USA

**12:00-12:20 p.m.**

**Kathryn Wagner, M.D., Ph.D.**

*The Kennedy Krieger Institute & The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 USA*

**Biomarkers of Myostatin Inhibition for Future Trials in FSHD**

Fedik Rahimov<sup>1,3</sup>, Oliver King<sup>3,4</sup>, Louis M. Kunkel<sup>1,2,3</sup>, Kathryn R. Wagner<sup>3,5,6</sup>

<sup>1</sup> Program in Genomics and <sup>2</sup>Howard Hughes Medical Institute, Children's Hospital Boston, Harvard Medical School, Boston, Massachusetts 02115 USA

<sup>3</sup> NIH Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD Research

<sup>4</sup> Boston Biomedical Research Institute, 64 Watertown, Massachusetts 02472 USA

<sup>5</sup> The Kennedy Krieger Institute and <sup>6</sup>The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 USA

**12:20 p.m.-1:50 p.m.**

**BUFFET LUNCH & POSTER VIEWING**

**Presenting: Niels Borgstein, M.D.**

*Acceleron Pharma, Cambridge, Massachusetts USA*

**Initial results from single subcutaneous administration of ACE-031, a form of the soluble activin type IIB receptor, in healthy postmenopausal volunteers**

Borgstein N.G.\*, Yang Y.\*, Condon C.H.\*, Wilson D.M.\*, Haltom E.\*, Larouche R. #, Lachey J.L.\*, Seehra J.\*, Sherman M.L.\*

\*Acceleron Pharma, Cambridge, Massachusetts, USA; #Anapharm, Montreal, Canada

**Presenting: Alberto Luis Rosa, M.D., Ph.D.**

*Laboratorio de Biología Celular y Molecular, Fundación Allende, Córdoba, Argentina*

**Subcellular trafficking of DUX4, a pro-apoptotic protein encoded at the facioscapulohumeral muscular dystrophy locus FSHD1A**

Edgardo Daniel Corona and Alberto Luis Rosa

Laboratorio de Biología Celular y Molecular, Fundación Allende, Hipólito Irigoyen 384 / 8vo Piso – 5000 - Córdoba, Argentina

**Presenting: Petr Dmitriev, Ph.D.**

*UMR 8126, Université Paris-Sud 11, CNRS, Institut de Cancérologie Gustave-Roussy, Villejuif, France*

**Krüppel-like factor KLF15 Interacts with the D4Z4 enhancer and up regulates the FSHD-related gene DUX4c**

Petr Dmitriev<sup>1</sup>, Andrei Petrov<sup>#,1</sup>, Eugenie Anseau<sup>#,3</sup>, Sébastien Charron<sup>#,3</sup>, Frédérique Coppée<sup>3</sup>, Alexandra Belayew<sup>3</sup>, Gilles Carnac<sup>2</sup>, Ahmed Turki<sup>2</sup>, Dalila Laoudj<sup>2</sup>, Marc Lipinski<sup>1</sup> and Yegor S. Vassetzky<sup>1</sup>

<sup>1</sup> UMR 8126, Université Paris-Sud 11, CNRS, Institut de Cancérologie Gustave-Roussy, F-94804 Villejuif, France

<sup>2</sup> INSERM EA 4202 ERI25, 371 Avenue du Doyen Gaston Giraud F-34295 Montpellier, France

<sup>3</sup> Service de Biologie Moléculaire, Université de Mons-Hainaut; 6, avenue du champ de Mars, 7000, Mons, Belgium

**Presenting: Melanie Ehrlich, Ph.D.**

*Human Genetics Program and the Department of Biochemistry, Tulane Medical School, New Orleans, Louisiana 70112 USA*

**A Window into secrets of myogenesis genes: whole-genome DNaseI hypersensitivity mapping**

Melanie Ehrlich<sup>1</sup>, Koji Tsumagari<sup>1</sup>, L. Song<sup>2</sup>, T. S. Fuery<sup>2</sup>, D. London<sup>2</sup>, A. P. Boyle<sup>2</sup>, G. E. Crawford<sup>2</sup>

<sup>1</sup> Human Genetics Program and the Department of Biochemistry, Tulane Medical School, New Orleans, Louisiana 70112 USA

<sup>2</sup> Institute for Genome Sciences & Policy, Duke University, Durham, North Carolina 27708 USA

**Presenting: Amanda Hampson, Ph.D.**

*Institute of Genetics, School of Biology, Queen's Medical Centre, University of Nottingham, Nottingham, NG7 2UH United Kingdom*

**Studying sequence variation at the human D4Z4 and mouse DUX loci**

Amanda Hampson, Laura M Mitchell, Gemma Dennis, Andreas Leidenroth, Joanne Pollinton and Jane E Hewitt.

Institute of Genetics, School of Biology, Queen's Medical Centre, University of Nottingham, Nottingham, NG7 2UH United Kingdom

**Presenting: Peter L. Jones, Ph.D.**

*Department of Cell and Developmental Biology; University of Illinois at Urbana-Champaign, Urbana, Illinois USA*

**Endogenous FRG1 protein expression in human and mouse skeletal muscle**

Meredit L. Hanel, Jessica Chia-Yun Sun, and Peter L. Jones

Department of Cell and Developmental Biology; University of Illinois at Urbana-Champaign; 601 S. Goodwin Ave, B107 Chemical and Life Sciences Laboratory; Urbana, Illinois 61801 USA

**Presenting: Jennifer Lachey, Ph.D.**

*Acceleron Pharma, Cambridge, Massachusetts USA*

**Long-term activin receptor type IIB inhibition improves strength and function of dystrophic muscle**

Lachey, J.L., Bogdanovich, S., Pistilli, E.E., Pullen, A.E., Khurana, T., Seehra, J.

**Presenting: Peter Lunt, Ph.D.**

*Clinical Genetics Dept, St Michaels Hospital, Bristol BS2 8EG United Kingdom*

**Experience in use of 4qA and 4qB in diagnostic testing for FSHD**

Peter Lunt<sup>1</sup>, Suzanne O'Shea<sup>2</sup>, Gemma Dennis<sup>2</sup>, Anne Gardner<sup>2</sup>, Maggie Williams<sup>2</sup>

<sup>1</sup> Clinical Genetics Dept, St Michaels Hospital, Bristol BS2 8EG United Kingdom

<sup>2</sup> Bristol Genetics Laboratories, Southmead Hospital, Bristol BS10 5NB United Kingdom

**Presenting: Pierre Walrafen, Ph.D.**

*Genomic Vision, Paris France.*

**Transferring the Molecular Combing test for FSHD in a routine diagnostics lab: lessons learned and new findings**

Pierre Walrafen<sup>1</sup>, Karine Nguyen,<sup>2,3</sup> Catherine Vovan<sup>2</sup>, Anne Vannier<sup>1</sup>, Emilie Renard<sup>1</sup>, Charlène Chaix<sup>1</sup>,  
Rafaëlle Bernard<sup>2,3</sup>, Aaron Bensimon<sup>1</sup>, Nicolas Lévy<sup>2,3</sup>

<sup>1</sup> Genomic Vision, Paris Santé Cochin, 29 rue du Faubourg Saint Jacques, 75014 Paris France.

<sup>2</sup> Département de Génétique Médicale, Hôpital d'enfants Timone, 265 rue St Pierre, Marseille France.

<sup>3</sup> Inserm UMR\_S910 "Génétique Médicale et Génomique Fonctionnelle", Université de la Méditerranée, Faculté de Médecine, 27, Bd Jean Moulin, Marseille France.

<sup>4</sup> These authors contributed equally to this work

**Presenting: Patrick Reed, Ph.D.**

*University of Maryland School of Medicine, Baltimore, Maryland USA*

*NIH Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD Research*

**Toward quantitative proteomic comparisons of skeletal muscles from FSHD patients and their unaffected, first-degree relatives**

Patrick W. Reed<sup>1,2</sup>, Kathryn Wagner<sup>2,3</sup>, and Robert J. Bloch<sup>1,2</sup>

<sup>1</sup> University of Maryland School of Medicine, Baltimore, Maryland USA

<sup>2</sup> NIH Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD Research

<sup>3</sup> The Kennedy Krieger Institute & The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 USA

**Presenting: Enzo Ricci, M.D.**

*Istituti di Neurologia e di Radiologia Università Cattolica -Policlinico Gemelli – Roma*

**Pelvic and lower limb involvement in FSHD: a muscle MRI study**

Ricci E., Frusciantè R., Tasca G., Iannaccone E., Monforte M., Renna R., Silvestri G., Mirabella M., Pelliccioni M., Padua L., Pescatori M., Di Lella G., La Schena F., Ottaviani P., Tonali P.A.

Istituti di Neurologia e di Radiologia Università Cattolica -Policlinico Gemelli – Roma

Radiologia –Istituto Dermopatico dell'Immacolata IRCCS - Roma

**Presenting: Sabrina Sacconi, Ph.D.**

*Centre de Référence des Maladies Neuromusculaires, Hôpital Archet 1, Nice, France*

**Electrostimulation training: an effective and safe treatment for FSHD patients**

Sabrina Sacconi<sup>1</sup>, Serge S. Colson<sup>2</sup>, Michaël Benchortane<sup>1</sup>, Véronique Tanant<sup>1</sup>, Claude Desnuelle<sup>1</sup>

<sup>1</sup> Centre de Référence des Maladies Neuromusculaires, Hôpital Archet 1, Nice, France

<sup>2</sup> Laboratoire de Motricité Humaine, Education, Santé, University of Nice-Sophia Antipolis

**Presenting: Guido Stadler, Ph.D.**

*Department of Cell Biology, UT Southwestern Medical Center at Dallas, Dallas, Texas USA*

*NIH Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD*

**Establishment of clonal myogenic cell lines from severely affected dystrophic muscles – tools for studying FSHD**

Guido Stadler<sup>1,4</sup>, Jennifer Chen<sup>2,4</sup>, Charles Emerson<sup>2,4</sup>, Kathryn Wagner<sup>3,4</sup>, Jerry Shay<sup>1</sup>, Woodring Wright<sup>1,4</sup>

<sup>1</sup> Department of Cell Biology, UT Southwestern Medical Center at Dallas, Dallas, Texas USA

<sup>2</sup> Boston Biomedical Research Institute, Watertown, Massachusetts USA

<sup>3</sup> Center for Inherited Muscle Disorders, The Kennedy Krieger Institute, Baltimore, Maryland USA

<sup>4</sup> NIH Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD

**Presenting: Rossella Tupler, M.D., Ph.D.**

*Dipartimento di Scienze Biomediche, Università di Modena e Reggio Emilia, Modena, Italia*

*Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, Massachusetts USA*

**Unexpected high percentage of subjects carrying D4Z4 reduced alleles and no clinical signs in FSHD families: which factors contribute to the disease mechanism?**

Emanuela Bonifazi<sup>1</sup>, Costanza Lamperti<sup>2</sup>, Chiara Fiorillo<sup>3</sup>, Liliana Vercelli<sup>4</sup>, Carlo Borsato<sup>5</sup>, Roberto Frusciantè<sup>6</sup>, Maura Servida<sup>2</sup>, Francesca Greco<sup>1</sup>, Ilaria Frambolli<sup>1</sup>, Luca Colantoni<sup>7</sup>, Giulia Ricci<sup>8</sup>, Leda Volpi<sup>8</sup>, Rita Di Leo<sup>9</sup>, Claudia Manzoli<sup>10</sup>, Paola Cudia<sup>11</sup>, Ebe Pastorello<sup>5</sup>, Leopoldo Ricciardi<sup>10</sup>, Monica Govi<sup>1</sup>, Isabella Scionti<sup>1</sup>, Michelangelo Cao<sup>5</sup>, Gabriele Siciliano<sup>8</sup>, Giuliana Galluzzi<sup>7</sup>, Morandi Lucia<sup>11</sup>, Di Muzio Antonio<sup>10</sup>, Trevisan Carlo Pietro<sup>5</sup>, Enzo Ricci<sup>6</sup>, Carmelo Rodolico<sup>9</sup>, Lucio Santoro<sup>3</sup>, Giuliano Tomelleri<sup>12</sup>, Corrado Angelini<sup>5</sup>, Laura Palmucci<sup>4</sup>, Maurizio Moggi<sup>2</sup>, Rossella Tupler<sup>1,13</sup>.

<sup>1</sup>Dipartimento di Scienze Biomediche, Università di Modena e Reggio Emilia, Via G. Campi 287, 41100 Modena. Tel. +39 059 2055414 - Fax +39 059 205 5426 – e-mail: rossella.tupler@unimore.it <sup>2</sup>UO Neurologia, Fondazione Ospedale Maggiore Policlinico, Mangiagalli Regina Elena, IRCCS Milano. <sup>3</sup>Dipartimento di Scienze Neurologiche, Università Federico II, Napoli. <sup>4</sup>Dipartimento di Neuroscienze, Università di Torino. <sup>5</sup>Dipartimento di Neuroscienze, Università di Padova. <sup>6</sup>Dipartimento di Neuroscienze, Università Cattolica Policlinico Agostino Gemelli, Roma. <sup>7</sup>Genetica molecolare, IRCCS Fondazione Santa Lucia, Roma. <sup>8</sup>Dipartimento di Neuroscienze, Università di Pisa. <sup>9</sup>Dipartimento di Neuroscienze, Psichiatria ed Anestesiologia, Università di Messina. <sup>10</sup>Dipartimento di Medicina e Scienze dell'invecchiamento, Osp. Civ. "SS Annunziata", Chieti. <sup>11</sup> Malattie Neuromuscolari, Neurologia, Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milano, <sup>12</sup> Dipartimento di Scienze Neurologiche e della Visione, Università di Verona, <sup>13</sup> Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester USA

**1:50 p.m.-2:50 p.m.**

**PLATFORM PRESENTATIONS IV**

**Charles P. Emerson, Jr., Ph.D., Moderator**

*Boston Biomedical Research Institute, Watertown, Massachusetts USA &*

*U.S. NIH Eunice Kennedy Shriver NICHD Boston Biomedical Research Institute Senator Paul D.*

*Wellstone Muscular Dystrophy Cooperative Research Center*

**MODEL SYSTEMS**

**1:50-2:10 p.m.**

**Gillian Butler-Browne, Ph.D.**

*Institute of Myology, UMRS 974 - UPMC University of Paris, Inserm, Paris, France*

**Isogenic immortalized myoblasts clones with or without mutation**

Yvonne Krom<sup>1</sup>, Kammel Machmaoui<sup>2</sup>, Bianca den Hamer<sup>1</sup>, Baziel van Engelen<sup>3</sup>, Vincent Mouly<sup>2</sup>, Rune Frants<sup>1</sup>, Rabi Tawil<sup>4</sup>, Gillian Butler-Browne<sup>2</sup>, Silvere van der Maarel<sup>1</sup>

<sup>1</sup> Department of Human Genetics, Leiden University Medical Center, Netherlands

<sup>2</sup> Institute of Myology, UMRS 974 - UPMC Univ. Paris 6 / U974 - Inserm / UMR7215 – CNRS, France

<sup>3</sup> Department of Neurology, Radboud University Nijmegen Medical Center, Netherlands

<sup>4</sup> Department of Neurology, University of Rochester Medical Center, Rochester, New York

**2:10-2:30 p.m.**

**Michael Kyba, Ph.D.**

*Lillehei Heart Institute and Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota USA*

**Investigating disease mechanisms and cell therapy with pluripotent cells bearing FSHD mutations**

Michael Kyba<sup>1</sup>, Darko Bosnakovski<sup>1</sup>, Abhijit Dandapat<sup>1</sup>, Radbod Darabi<sup>3</sup>, John Day<sup>2</sup>, Jessica C. de Greef<sup>4</sup>, Lynn M. Hartweck<sup>1</sup>, Antonio Filareto<sup>3</sup>, Richard J. Lemmers<sup>4</sup>, Ramiro Nandez<sup>1</sup>, Rita R. Perlingeiro<sup>3</sup>, Janet Sowden<sup>4</sup>, Matthew Struck<sup>1</sup>, Rabi Tawil<sup>5</sup>, Silvere M. van der Maarel<sup>4</sup>, Nathan Zaidman<sup>1</sup>, and Thomas P Zwaka<sup>6</sup>

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<sup>2</sup>Paul and Sheila Wellstone Center for Muscular Dystrophy and Department of Neurology, University of Minnesota, Minneapolis, Minnesota 55455 USA

<sup>3</sup>Lillehei Heart Institute and Department of Medicine, University of Minnesota, Minneapolis, Minnesota 55455 USA

<sup>4</sup>Department of Human Genetics, Leiden University Medical Center, Lund, The Netherlands

<sup>5</sup>Fields Center for FSHD and Neuromuscular Research and Department of Neurology, University of Rochester, Rochester, New York 14642 USA

<sup>6</sup>Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, Texas 77030 USA

**2:30-2:50 p.m.**

**Daniel G. Miller, M.D., Ph.D.**

*Department of Pediatrics, University of Washington, Seattle USA*

**Human and mouse developmental models of DUX4 transcriptional regulation**

Gregory J. Block<sup>1\*</sup>, Chrissie Cirovic<sup>1\*</sup>, Lisa M. Petek<sup>1\*</sup>, Angel Nelson<sup>2\*</sup>, Carol Ware<sup>2\*</sup>, Gala Fillipova<sup>3</sup>, Silvere van der Maarel<sup>4</sup>, Stephen J. Tapscott<sup>3</sup>, Daniel G. Miller<sup>1\*</sup>

<sup>1</sup> Department of Pediatrics, University of Washington, Seattle USA

<sup>2</sup> Department of Comparative Medicine, University of Washington, Seattle USA

<sup>3</sup> Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle USA

<sup>4</sup> Leiden University Medical Center, Leiden, The Netherlands.

\* Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle USA

**2:50 p.m.-3:50 p.m.**

**PLATFORM PRESENTATIONS V**

**Jane E. Hewitt, Ph.D., Moderator**

*Institute of Genetics, School of Biology, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH, United Kingdom*

**DUX4**

**2:50-3:10 p.m.**

**Alexandra Tassin, Ph.D.,**

*Laboratory of Molecular Biology, University of Mons, Mons, Belgium*

**Investigations on the molecular mechanism of FSHD by proteomic and metabonomic analyses of primary myotube cultures**

A. Tassin<sup>1</sup>, B. Leroy<sup>2</sup>, S. Sauvage<sup>1</sup>, J. Faille<sup>1</sup>, V. Erculisse<sup>2</sup>, S. Charron<sup>1</sup>, C. Vanderplanck<sup>1</sup>, M. Barro<sup>5</sup>, L. Vander Elst<sup>3</sup>, RN. Muller<sup>3</sup>, D. Laoudj-Chenivresse<sup>5</sup>, JM. Colet<sup>4</sup>, F. Coppée<sup>1</sup>, R. Wattiez<sup>2</sup>, and A. Belayew<sup>1</sup>.  
Laboratories of: <sup>1</sup> Molecular Biology, <sup>2</sup> Proteomics and Protein Biochemistry, <sup>3</sup> NMR and Molecular Imaging, and <sup>4</sup> Human Biology and Toxicology, University of Mons, Belgium  
<sup>5</sup> Unité INSERM ERI 13, University of Montpellier, France.

**3:10-3:30 p.m.**

**Lindsay Wallace, Ph.D.**

*Department of Pediatrics, The Ohio State University, Columbus, Ohio 43205 USA*

*Molecular, Cellular, and Developmental Biology Program, The Ohio State University, Columbus, Ohio 43205 USA*

*Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio USA*

**DUX4 promotes FSHD-associated pathology in vivo**

Lindsay Wallace<sup>2</sup>, Sara Garwick<sup>3</sup>, Wenyan Mei, Frederique Coppee<sup>5</sup>, Alexandra Belayew<sup>5</sup>, Jing Yang and Scott Q. Harper<sup>1,2,3</sup>

<sup>1</sup>Department of Pediatrics, The Ohio State University, Columbus, Ohio 43205 USA

<sup>2</sup>Molecular, Cellular, and Developmental Biology Program, The Ohio State University, Columbus, Ohio 43205 USA

<sup>3</sup>Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio 43205 USA

<sup>5</sup>Laboratoire de Biologie Moléculaire, Université de Mons-Hainaut, Académie Universitaire Wallonie-Bruxelles, Mons, Belgium

**3:30-3:50 p.m.**

**Peter S. Zammit, Ph.D.**

*King's College London, Randall Division of Cell and Molecular Biophysics, New Hunt's House, Guy's Campus, London, SE1 1UL, United Kingdom.*

**Assessing the effects of FRG1, DUX4 and DUX4c on muscle satellite cell function**

Paul Knopp and Peter S. Zammit

King's College London, Randall Division of Cell and Molecular Biophysics, New Hunt's House, Guy's Campus, London, SE1 1UL, United Kingdom.

**3:50 p.m.-4:20 p.m.**

**POSTER VIEWING & AFTERNOON BREAK**

[FOR LISTING OF THE POSTERS PLEASE SEE LUNCH PROGRAM]

**4:20-5:40 p.m.**

**PLATFORM PRESENTATIONS VI**

**Rune R. Frants, Ph.D., Moderator**

*Leiden University Medical Center, Leiden, The Netherlands*

**FRG1 & CRYM**

**4:20-4:40 p.m.**

**Qian Liu, Ph.D.**



*Department of Cell and Developmental Biology; University of Illinois at Urbana-Champaign, Urbana, Illinois USA*

**Facioscapulohumeral muscular dystrophy region gene-1 (FRG-1) is an actin bundling protein associated with muscle attachment sites**

Qian Liu, Takako Iida Jones, Vivian W. Tang, William M. Briehner, and Peter L. Jones

*Department of Cell and Developmental Biology; University of Illinois at Urbana-Champaign; 601 S. Goodwin Ave, B107 Chemical and Life Sciences Laboratory; Urbana, Illinois 61801 USA*

**4:40-5:00 p.m.**

***Steven W. Long, Ph.D.***

*Department of Cell and Developmental Biology; University of Illinois at Urbana-Champaign, Urbana, Illinois USA*

**The effects of elevated FSHD candidate gene expression on vertebrate development using *Xenopus laevis***

Ryan D. Wuebbles<sup>1,2</sup>, Steven W. Long<sup>1</sup>, Meredith L. Hanel, and Peter L. Jones

*Department of Cell and Developmental Biology; University of Illinois at Urbana-Champaign; 601 S. Goodwin Ave, B107 CLSL; Urbana Illinois 61801 USA*

**5:00-5:20 p.m.**

***Patrick Wayne Reed, Ph.D.***

*University of Maryland School of Medicine, Baltimore, Maryland USA*

**Mu-Crystallin and the Pathogenesis of FSHD**

Patrick W. Reed and Robert J. Bloch

*University of Maryland School of Medicine, Baltimore, Maryland USA*

**5:20-5:40 p.m.**

***Jessica Chia-Yun Sun, Ph.D.***

*Department of Cell and Developmental Biology; University of Illinois at Urbana-Champaign, Urbana, Illinois USA*

**Human FRG1 is a cytoplasmic actin bundling protein and a nuclear RNA associated protein**

Jessica Chia-Yun Sun, Michel Bellini, William M. Briehner, and Peter L. Jones

*Department of Cell and Developmental Biology; University of Illinois at Urbana-Champaign; 601 S. Goodwin Ave, B107 Chemical and Life Sciences Laboratory; Urbana, Illinois 61801 USA*

1.

## **An easy test for the presence of the 4qA161 FSHD-permissive haplotype and its application to studying the molecular genetics of FSHD**

Koji Tsumagari<sup>1</sup>, Desheng Chen<sup>1</sup>, Aaron D. Bossler<sup>2</sup>, and Melanie Ehrlich<sup>1</sup>

<sup>1</sup> *Human Genetics Program and the Department of Biochemistry, Tulane Medical School, New Orleans, Louisiana 70112 USA*

<sup>2</sup> *Molecular Pathology Laboratory, University of Iowa Hospitals and Clinic, Iowa City Iowa 52242 USA*

The identification of all the *cis*-acting elements at 4q35.2 that are necessary for pathogenesis in facioscapulohumeral muscular dystrophy (FSHD) is still uncertain. In all FSHD patients (~85) that were part of a recent study, Lemmers *et al.* (Am. J. Hum. Genet.:81, 884, 2007) found at least one copy of a haplotype, called 4qA161, that encompasses 4q35.2 sequences immediately proximal to D4Z4, distal to D4Z4, and at the first repeat unit of D4Z4. However, the 4qA161 haplotype is also present in ~60% of control individuals, and so can be referred to as FSHD-permissive. It should either contribute directly to pathogenesis or be linked to a polymorphism directly necessary for pathogenesis. Lemmers *et al.* mapped the frequency of the 4qA161 haplotype by the following procedure:

1. determination of the D4Z4-distal A or B type in Southern blotting analysis after pulsed field gel electrophoresis (PFGE) using A-type and B-type oligonucleotide probes;
2. and isolation of allelic 4q D4Z4 region bands by preparative PFGE on EcoRI digests blot-hybridized to a p13E-11 probe, amplification of the extracted DNA by PCR, and analysis of the product for a simple sequence-length polymorphism ~3 kb proximal to D4Z4 by capillary gel electrophoresis.

These procedures are time-consuming, and the harvesting of PFGE bands is challenging and too complicated for most clinical labs. Using sequence data from Lemmers *et al.*, we designed a restriction endonuclease-PCR method based upon single nucleotide polymorphisms (SNPs) to determine if a sample has one, two, or no 4qA161 alleles by a procedure that eliminates the signal from 10q alleles. We found no 4qA161 haplotype in ~30% of 20 controls and none of 20 PFGE-confirmed FSHD patients represented in Tulane's collection of DNA samples. From our Iowa collection of 15 FSHD samples, several had no 4qA161 haplotype but these samples had only been molecularly diagnosed by linear gel electrophoresis and Southern blotting with the p13E-11 as well as hybridization to D4Z4-distal A and B probes and the BlnI resistance test for the most proximal repeat unit. Therefore, it is likely that our PCR SNP test helps to clarify the identification of FSHD-associated alleles, especially when 4qA hybridization to an allele with a short D4Z4 array occurs in the absence of p13E-11 hybridization to this allele. The PCR products that we obtained from this assay are suitable for direct DNA sequencing. Initial sequencing results confirm the validity of our test and easily provide sequence information about the 4q D4Z4-proximal sub-region. Our method will aid clinicians in ruling out some false-positive FSHD patients, in prenatal screening, and in detecting rare patients with FSHD but without this haplotype. Our SNP test also has ramifications that can facilitate molecular genetic research on FSHD. (Funded in part by NIH grant R01 NS048859 to ME)

2.

## **The evolution of the subtelomeres of chromosomes 4q and 10q**

Richard J.L.F. Lemmers, Patrick van der Vliet, Kristiaan J. van der Gaag, Sofia Zuniga, Rune R.R. Frants, Peter de Knijff, Silvère M. van der Maarel

*Department of Human Genetics, Leiden University Medical Center, the Netherlands*

Subtelomeres are hot spots of inter- and intrachromosomal segmental duplications. Consequently, human subtelomeres are composed of blocks of homologous DNA sequences, called duplicons, which are dispersed over many different chromosome ends. FSHD is caused by the contraction of the macrosatellite repeat D4Z4 on the subtelomere of chromosome 4q35. We studied the human 4q and 10q subtelomeres, which contain the FSHD locus and which are identical over a region of on average >200 kb. Detailed sequence analysis of four polymorphic markers on 4q and 10q subtelomeres among samples from the African, European, and Asian HAPMAP panels revealed eighteen distinct 4q haplotypes and eight distinct 10q haplotypes. Haplotypes that were composed of D4Z4 repeat sequences that were otherwise specific for 4q and 10q were detected at frequencies >10% in all three populations, at first sight supporting a mechanism of and ongoing interchromosomal exchanges between chromosomes 4 and 10.

Further analysis allowed us to construct an evolutionary network of all haplotypes and to identify the 4q haplotype ancestral to all 4q and 10q haplotypes. Based on this network, we demonstrate that all haplotypes originate from only four discrete duplication events during recent human evolution. We also provide evidence that haplotypes with mixtures of 4q and 10q specific D4Z4 sequences (hybrid repeats) represent intermediate haplotypes in the transition from 4q to 10q haplotypes, rather than being the result of recent and recurrent translocations between both chromosomes. Haplotype distribution studies on a large number of globally dispersed human DNA samples (the HGDP-CEPH panel) supported our findings and show that all standard and non-standard haplotypes were already present in Africa before modern human migration out of Africa. Thus contrary to the earlier view of unconstrained exchanges between the of subtelomeric regions of chromosome 4 and 10, our studies suggest that only a few translocations have occurred between both chromosomes and that most probably intrachromosomal mutations explain the large number of standard and non-standard haplotypes.

**Funding:** This study was funded by the Fields Center, the Netherlands Organization for Scientific Research (NWO), the FSH Society and by the Prinses Beatrix Fonds. The authors declare no conflict of interest.

3.

### **High frequency of hypomethylation in patients with FSHD like phenotype**

S. Sacconi<sup>1</sup>, J.C. de Greef<sup>2</sup>, R.J.L.F. Lemmers<sup>2</sup>, P. Camano<sup>3</sup>, S.M. van der Maarel<sup>2</sup> & C Desnuelle<sup>1</sup>

<sup>1</sup> *Centre de référence des maladies Neuromusculaires, CHU, Nice, France*

<sup>2</sup> *Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands*

<sup>3</sup> *Neuroscience Unit, Biodonostia Institute, San Sebastián, Spain*

We studied 9 sporadic patients presenting with typical FSHD clinical phenotype and absence of D4Z4 deletion on chromosome 4q35 tested by classical Southern blot technique.

Clinical features included facial and shoulder girdle muscle weakness in association or not with pelvic, abdominal, and anterior forelegs muscle involvement. Muscle biopsies showed variable degree of muscular dystrophy, absence of abnormalities of cytoskeletal proteins routinely analyzed by immunohistochemistry and Western Blot.

We performed calpain 3 and valosin containing protein (*VCP*) mutation analysis by direct sequencing, extensive D4Z4 genotyping by PFGE analysis and DNA methylation studies of the D4Z4 repeat.

We found 2 patients presenting with already known calpain 3 mutations, 1 patient presenting with a *VCP* mutation, 1 patient carrying a deletion of the probe region used for FSHD diagnosis, 1 patient with a pathogenic complex rearrangement and 3 patients presenting with D4Z4 hypomethylation. For 1 out of 10 patients molecular diagnosis is still lacking.

We conclude that 1) even in absence of calpain3 deficiency detected by Western blot, this diagnosis has to be considered in patients with symmetric limb girdle muscular dystrophy and facial involvement, as well as the *VCP* diagnosis 2) PFGE analysis has to be performed routinely in FSHD diagnostic workout to exclude deletion of the probe region (alternatively these deletions can be detected by re-hybridizations with probe D4Z4) and more complex genetic rearrangements related to FSHD, and 3) the percentage of patients presenting with hypomethylation of D4Z4 not associated with D4Z4 deletion is very high and further studies are needed to better define this condition. We will provide a clinical and histological description of these 5 cases and a short discussion on features that they have in common.

Keywords: FSHD, Hypomethylation, Calpain 3, *VCP*

#### **Presenting author:**

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4.

### **A ncRNA regulates chromatin conformation of the facioscapulohumeral muscular dystrophy region.**

Daphne Cabianca<sup>1,2</sup>, Beatrice Bodega<sup>3</sup>, Victoria Neguembor<sup>1</sup>, Enrico Ginelli<sup>3</sup> and Davide Gabellini<sup>1,4</sup>

<sup>1</sup> Division of Regenerative Medicine, San Raffaele Scientific Institute, Milano, Italy

<sup>2</sup> UniSR-Open University International PhD Program in Cellular and Molecular Biology, Milano, Italy

<sup>3</sup> Department of Biology and Genetics for Medical Sciences, University of Milano, Italy

<sup>4</sup> Dulbecco Telethon Institute, Milano, Italy

FSHD, the third most common myopathy, is an autosomal dominant disorder whose genetic locus maps to chromosome 4 (4q35).

Unlike the majority of genetic diseases, FSHD is not due to a mutation within a protein-coding gene. Instead, FSHD is caused by reduction in the copy number of a 3.3 kb repeated sequence called D4Z4. Loss of D4Z4 causes an epigenetic alteration leading to de-repression of three 4q35 genes: FRG1, FRG2 and ANT1.

D4Z4 is highly polymorphic and in healthy subjects the number of repeats can range from 11 to 100 units, while FSHD patients carry less than 11 units. The number of D4Z4 repeats is a critical determinant of the age of onset and clinical severity of FSHD. In general, fewer repeats are associated with a more severe phenotype that presents in childhood. Paradoxically, individuals completely devoid of D4Z4 are normal suggesting that at least one copy of D4Z4 is required to cause FSHD, possibly through a gain-of-function mechanism.

We found that a chromatin-bound non-protein coding RNA (ncRNA) is transcribed proximally to D4Z4 selectively in the skeletal muscle of FSHD patients and in FSHD myotubes. Intriguingly, in several experimental systems production of the ncRNA correlates with de-repression of 4q35 genes. Notably, knockdown of the ncRNA prevents 4q35 gene de-repression.

We used chromosome conformation capture to study the spatial organization of the 4q35 chromatin. We found that 4q35 gene de-repression is associated to a reorganization of the 4q35 chromatin. Interestingly, the ncRNA is required for this chromatin reorganization to take place. Based on our preliminary results, we propose that the ncRNA activates the epigenetic cascade culminating with 4q35 gene de-repression in FSHD.

## Chromatin modification at DNaseI hypersensitive sites in 4q35 in myoblasts

Jing-Jing Ma<sup>1</sup>, Shao-Chi Chang<sup>1</sup>, Xueqing Xu<sup>1</sup>, Dmitri Loukinov<sup>2</sup>, Victor Lobanenko<sup>2</sup>, and Melanie Ehrlich<sup>1</sup>

<sup>1</sup>*Human Genetics Program and the Department of Biochemistry, Tulane Medical School, New Orleans, Louisiana 70112 USA*

<sup>2</sup>*Molecular Pathology Section, National Institute of Allergy and Infectious Diseases, Rockville, Maryland 20852 USA*

Evidence suggests that changes in chromatin structure at 4q35 play a major role in facioscapulohumeral muscular dystrophy (FSHD). Although there are many parameters defining chromatin structure, some are of special importance. Among these are dimethylation of histone H3 at lysine 4 (H3K4me2), which is enriched in enhancers and promoters, and binding of the transcription factor and insulator protein CTCF. In collaboration with Greg Crawford, our lab has begun studying the structure of chromatin throughout 4q35.2 using high-resolution methods. First, we mapped DNaseI-hypersensitive sites (DHS), markers of promoters, enhancers, and silencers that are active or poised for activity. From our analysis with tiling arrays on three FSHD and three control myoblast cell strains (Xu *et al.*, *Nucleic Acids Res.*, Oct 9, Epub ahead of print, 2009) and next-generation sequencing on another three control strains (unpublished results), we found several tissue-specific DHS in two large gene deserts in 4q35.2. One of these DHS was found preferentially in FSHD myoblasts and is located 272 kb proximal to the FSHD-linked D4Z4 tandem repeat. It was the closest unique-sequence DHS to D4Z4 other than the DHS at the promoter of *FRG1*.

In the present study, we used chromatin immunoprecipitation (ChIP) with specific antibodies, to test whether these DHS regions, all of which are far from genes, are enriched for H3K4me2 relative to randomly chosen non-gene and non-DHS sequences. Three myoblast cell strains were tested, namely, control fetal myoblasts, biopsy-derived control myoblasts, and myoblasts from a patient with clinical symptoms of FSHD but without a demonstrated short D4Z4 array. Two amplicons from each DHS were analyzed by real-time qPCR of the immunoprecipitate *vs.* the input chromatin. The FSHD-preferential DHS and three other gene-desert DHS were all enriched for H3K4me2, usually about 4-to-30 fold compared to the average signal from three negative-control amplicons. The enrichment for a transcription-associated chromatin mark at these DHS located more than 0.1 or 0.5 Mb from the nearest known gene indicates that even the gene deserts at 4q35.2 need to be considered for possible FSHD-related roles in *cis* to a short FSHD-causing D4Z4 array. It has been postulated that long distance looping at 4q35.2 may be involved in FSHD. Therefore, these DHS with marks of active chromatin are of interest to examine for looping with D4Z4. Preliminary results from ChIP with antibodies to CTCF, a protein implicated in long-distance looping, indicate that the FSHD-preferential DHS was enriched in CTCF in fetal myoblasts. This CTCF enrichment is of special interest because of the finding of Ottaviani *et al.* (*PLoS Genet.* 5:e1000394, 2009) that CTCF binds to D4Z4. To look for postulated pathogenic long-range interactions involving these and other sites in 4q35.2, we are testing for chromatin looping by two methods. (Supported by NIH Grant NS048859-S2)

6.

### **Specific loss of histone H3 lysine 9 trimethylation and HP1 $\gamma$ /cohesin binding at D4Z4 repeats is associated with facioscapulohumeral dystrophy (FSHD)**

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Facioscapulohumeral dystrophy (FSHD) is an autosomal dominant muscular dystrophy in which no mutation of pathogenic gene(s) has been identified. Instead, the disease is, in most cases, genetically linked to a contraction in the number of 3.3 kb D4Z4 repeats on chromosome 4q. How contraction of the 4qter D4Z4 repeats causes muscular dystrophy is not understood. In addition, a smaller group of FSHD cases are not associated with D4Z4 repeat contraction (termed "phenotypic" FSHD), and their etiology remains undefined. We carried out chromatin immunoprecipitation analysis using D4Z4-specific PCR primers to examine the D4Z4 chromatin structure in normal and patient cells as well as in small interfering RNA (siRNA)-treated cells. We found that SUV39H1-mediated H3K9 trimethylation at D4Z4 seen in normal cells is lost in FSHD. Furthermore, the loss of this histone modification occurs not only at the contracted 4q D4Z4 allele, but also at the genetically intact D4Z4 alleles on both chromosomes 4q and 10q, providing the first evidence that the genetic change (contraction) of one 4qD4Z4 allele spreads its effect to other genomic regions. Importantly, this epigenetic change was also observed in the phenotypic FSHD cases with no D4Z4 contraction, but not in other types of muscular dystrophies tested. We found that HP1 $\gamma$  and cohesin are co-recruited to D4Z4 in an H3K9me3-dependent and cell type-specific manner, which is disrupted in FSHD. The results indicate that cohesin plays an active role in HP1 recruitment and is involved in cell type-specific D4Z4 chromatin regulation. Taken together, we identified the loss of both histone H3K9 trimethylation and HP1 $\gamma$ /cohesin binding at D4Z4 to be a faithful marker for the FSHD phenotype. Based on these results, we propose a new model in which the epigenetic change initiated at 4q D4Z4 spreads its effect to other genomic regions, which compromises muscle-specific gene regulation leading to FSHD pathogenesis.

7.

## **FSHD and signal patterns in retina and muscle: Can twentieth century embryology help ‘decode’ twenty-first century molecular enigmas?**

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Sir Andrew Huxley has said that if scientists in the mid-twentieth century had been less obsessed with the then new science of biochemistry and had also looked to the early twentieth century studies of muscle morphology, they would have discovered that ‘sliding filaments’ cause muscle contraction long before his own paper (and that of another group) on that subject appeared in ‘Nature’ in 1954.

The purpose of the present discussion is to correlate ‘traditional’ (1950s and earlier) studies of human embryology with both the clinical manifestations of FSHD (as it affects muscle and retina) and the most recent concepts of muscle development which focus on signaling pathways (wnt especially) and ‘patterns of transcription factors’.

The general association of FSHD with asymptomatic peripheral retinal vasculopathy which occasionally results in symptomatic ‘Coats syndrome’ is well established. ‘Coats Disease’ is part of a spectrum of sporadic or genetic vascular disorders which includes ‘Norrie Disease’ and Familial Exudative Vitreoretinopathy (FEVR). All are caused by similar developmental asymmetric abnormalities of peripheral retinal blood vessels. They can be caused by mutations affecting ligand (such as norrin) or receptors/co-receptors (such as frizzled-4 and LRP5) of wnt signaling pathways.

Given the complexity of wnt signaling pathways, and the pivotal role of both the canonical and planar polarity wnt pathways in myogenesis and regeneration, I previously postulated that aberration in other components of wnt signaling might cause muscle weakness by impairing regeneration in FSHD. There is a developing literature on the interactions between wnt pathways and transcription factors in proliferating myoblasts.

Not all facial muscles are normally affected in FSHD. Patterns of embryonic facial human development illustrated by R M Patten (1953) and others show that in humans the affected facial expression muscles derive virtually exclusively from the second branchial arch. Recent work of R G Kelly and others show that patterns of signaling, and of redundancies of myogenic transcription factors, in facial muscles differ radically from those in other muscles – AND that these patterns also differ in each of the four branchial arches which become face muscles. PAX3 is absent from embryonic face muscle.

There are further complexities. FEVR has been described in DiGeorge Syndrome (due to mutated TBx1 – a transcription factor which interacts with VEGF1 and is present in the first human two branchial arches). Coats Disease may occur in the Cornelia de Lange Syndrome (caused by mutation affecting cohesion - now implicated in FSHD).

Further knowledge about the selective spatial and temporal patterns of signaling and transcription factors in human muscles - especially those derived from the second branchial arch - selectively affected in FSHD may give clues to its pathogenesis and treatment. Might a regional absence of redundancy predispose to clinical expression?



8.

### **FSHD biomarker studies: Using micro-qPCR arrays to analyze gene expression in FSHD and control muscle biopsies and myogenic cell cultures.**

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As one of our approaches to identify and validate FSHD mRNA biomarkers, we are using a micro-qPCR array system (Fluidigm Biomark 48.48) to quantify and compare gene expression in FSHD and control muscle biopsies and cultured myogenic cells. In initial studies, we analyzed both biopsies and cell cultures for a panel of genes that consisted largely of FSHD candidates and housekeeping genes (for normalization). Cultures were additionally analyzed for a second set of genes that consisted largely of markers of terminal differentiation. The qPCR assays were highly reproducible for both biopsies and cultures, with standard errors of only ~0.1- 0.2 cycle over a wide range of expression levels. In addition, genes with generally stable expression in both biopsies and cultures were identified for use in normalization (*e.g.*, *HPRT1*, *PPIA*).

For the biopsies, we carried out quadruplicate qPCR analyses of a total of 25 biopsies, including biceps and deltoid samples from eight FSHD patients and four healthy siblings and an additional FSHD deltoid sample. Biopsies came from a total of six families. Initial results suggest that expression of particular genes may depend on family origin and extent of disease progression, as well as on D4Z4 status.

For cultured cells, we grew CD56-positive myogenic cells obtained from an FSHD patient, a healthy sibling, and an alpha-sarcoglycan (SGCA)-deficient LGMD2D patient. Cells were cultured in two different laboratories, two growth media, and two differentiation media. Cultures were harvested in the proliferation phase or after four days of differentiation. The qPCR assays showed that expression in proliferating myoblasts was sensitive to culture conditions. In this first experiment, expression of differentiation markers (*e.g.*, myosin heavy chains, MyoD, myogenin) was consistently lower in FSHD than in healthy or SGCA-deficient cultures.

In one case, we had data from biopsies and cultures derived from a paired FSHD patient and healthy sibling. We found that genes that were highly expressed in culture tended to be highly expressed in the corresponding biopsy. Also, a group of inflammation-related genes were more highly expressed in biopsies than in cultures, consistent with a non-myogenic cell origin in the biopsies. Though many genes showed very similar levels of expression in the cultures and the biopsies, other genes showed higher expression in the culture or in the biopsy. Though definitive conclusions await comparisons of larger numbers of FSHD and control biopsies and cultures, these initial experiments demonstrate that micro-qPCR arrays are a robust method for quantitative comparisons of gene expression in FSHD and control biopsies and cells.

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## Genotype-phenotype studies in FSHD2

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**Introduction:** In <5% of patients with facioscapulohumeral muscular dystrophy (FSHD) no D4Z4 repeat contraction on chromosome 4q35 can be identified. Yet, in common with FSHD1 patients with D4Z4 contraction, these so-called FSHD2 patients show loss of DNA methylation and heterochromatin markers at the D4Z4 repeat. This suggests that a defect in chromatin structure of the D4Z4 repeat underlies FSHD2.

**Objective:** To date there has been no comprehensive clinical study of FSHD2. The purpose of our study was to critically evaluate the clinical features in FSHD2 patients in order to establish whether these patients represent true phenocopies of FSHD1 and to establish (epi) genotype-phenotype correlations.

**Methods:** Using pulsed field gel electrophoresis we determined D4Z4 repeat size. We used methylation-sensitive Southern blot analysis to determine D4Z4 methylation on chromosomes 4 and 10. Using a standardized clinical evaluation form recently developed by the Fields Center, we clinically evaluated all patients.

**Results:** We identified 26 FSHD2 patients in 22 families carrying a normal-sized D4Z4 repeat ( $\geq 40$  kb) with prominent D4Z4 hypomethylation. All patients carry at least one D4Z4 repeat on the pathogenic haplotype 4qA161 and in most patients (23/26) the smallest D4Z4 repeat on the 4qA161 allele is <80 kb in size. In contrast, only 25% of 4qA161 alleles in the Dutch population is <80 kb. In contrast to FSHD1, most FSHD2 patients are sporadic (70%). The pattern of clinical involvement in FSHD2 is identical to FSHD1. In the present cohort there was a slight predominance of males (65%) but there were no gender differences in average age at onset or clinical severity scores.

**Conclusions:** We conclude that in order to develop FSHD2 a hypomethylated and medium-sized D4Z4 repeat on the pathogenic haplotype 4qA161 is necessary. Clinically, these patients are indistinguishable from patients with FSHD1. This new cohort of FSHD2 patients provides further evidence that FSHD2 represents a true phenocopy of FSHD1.

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## **Biomarkers of Myostatin Inhibition for Future Trials in FSHD**

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Inhibition or loss of the TGF-Beta family member, myostatin, leads to increased muscle mass and strength in animals and humans. In animal models of muscular dystrophy, myostatin inhibition stimulates regeneration and inhibits fibrosis. The first clinical trial of an inhibitor of myostatin in human disease was with a neutralizing antibody, MYO-029. Although this trial showed positive trends in FSHD, there were several limitations to the trial. The greatest limitation was that it remained unclear at the conclusion of the trial if the drug did not adequately inhibit myostatin or if it inhibited myostatin but did not have a significant effect on muscle strength. So that the FSHD community does not repeat this experience with novel therapeutics in development, it is critical to develop accurate biomarkers of myostatin inhibition. In our efforts to develop a specific biomarker of postnatal myostatin inhibition in skeletal muscle, we have compared the gene expression patterns of muscle from mice postnatally treated with ActRIIB.Fc (a broader inhibitor of TGF-Beta family members) with muscle from myostatin null mice. We assessed global gene expression profiling in quadriceps and soleus muscles obtained from mice treated with ActRIIB.Fc over one and two weeks, from *Mstn*<sup>-/-</sup> and control mice using the Affymetrix GeneChip® 1.0 ST arrays. There was a significant overlap in up and downregulated genes in both muscle types between mice treated for two weeks with ActRIIB.Fc and *Mstn*<sup>-/-</sup> mice ( $P < 0.0001$ ). Furthermore, we validated expression of 30 genes that showed the most significant up and downregulation on arrays with quantitative real-time PCR using TaqMan gene expression assays on Fluidigm's BioMark™ microfluidic dynamic arrays. There was a strong correlation between microarray results and quantitative real-time PCR with a correlation coefficient of over 0.9. Our results demonstrate the feasibility of developing a biomarker of myostatin inhibition in skeletal muscle.

**Isogenic immortalized myoblasts clones with or without mutation**

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Over the years, cell and animal models for FSHD have been generated by specific over-expression of individual candidate genes. However, given the controversy of transcriptional deregulation of these genes in FSHD muscle, the relevance of these models remains uncertain.

Moreover, there are currently no good human cellular models for FSHD. Primary myoblast cell cultures are available but they have limited replicative capacity and suffer from large inter-individual variation. Here we present preliminary results showing that the shortcomings of primary human myoblast cell cultures can be overcome by immortalization of myoblast cell culture derived from FSHD patients.

Taking advantage of the high frequency of somatic mosaicism in FSHD, we immortalized myoblast cell cultures derived from affected muscle of five mosaic FSHD patients. By clonal expansion, we were able to generate pairs of clonal myogenic cell lines with or without contraction. Thus, these cell lines are isogenic with exception of the D4Z4 contraction.

Preliminary studies demonstrate that these cell lines retain their myogenic properties, differentiate into multinucleated myotubes, and show no overt deregulation of the subtelomerically located FRG1 as a consequence of hTERT transgene expression.

We propose that their immortality and isogenicity will overcome issues of inter-individual variation and thereby make them ideal cell lines to further study the pathogenic mechanism of FSHD and to be used in high throughput small molecule screens.

## **Investigating disease mechanisms and cell therapy with pluripotent cells bearing FSHD mutations**

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There is currently no animal model bearing the actual FSHD mutation (D4Z4 contraction), and the lack of a suitable model system to study the effects of this mutation has severely hampered progress in understanding FSHD. In an effort to shed light on the disease mechanism and to speed a potential cell therapy, we have derived iPS cells from myoblast cultures taken from FSHD patients and controls. We present preliminary data on the differentiation of these cells through mesoderm and into muscle, and the expression of FSHD candidate genes along this developmental pathway. We compare these cells to mouse embryonic stem cells that we have engineered to carry FSHD mutation-bearing DNA constructs at a euchromatic site on the X chromosome and discuss the roadblocks to the application of iPS cells to cell therapy for FSHD.

## Human and mouse developmental models of *DUX4* transcriptional regulation

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The majority of individuals with Facioscapulohumeral Muscular Dystrophy (FSHD) have a shortened array of D4Z4 repeats linked to a specific inherited haplotype located on the long arm of chromosome 4. Shortened disease-causing D4Z4 arrays and D4Z4 arrays from individuals with FSHD2 have also been shown to be hypomethylated suggesting that aberrant transcriptional regulation of D4Z4 sequences may be an important component of FSHD pathology. Homeotic transcription factors are important regulators of stem cell fate and thus *DUX4* may normally have a spatiotemporally regulated expression pattern that is disrupted in individuals with FSHD. To determine the pattern of *DUX4* expression during development, we localized cells containing  $\beta$ -galactosidase activity during mouse embryogenesis using a transgenic mouse containing the human *DUX4* promoter and a *LacZ* reporter gene. In addition, we've generated and characterized induced pluripotent stem (IPS) cells from fibroblasts of FSHD-affected individuals. We demonstrate that these cells are pluripotent using a mouse teratoma assay, and show that they can be differentiated to *NCAM*(+) cell populations in culture providing a human cell culture model to study changes in developmentally-regulated transcription associated with D4Z4 array contraction and/or methylation.

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## Investigations on the molecular mechanism of FSHD by proteomic and metabonomic analyses of primary myotube cultures

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We identified a double homeobox gene (DUX4) within each D4Z4 unit at the FSHD locus that is activated in muscle cells following the FSHD deletion (Gabriels et al., 1999; Kowaljow et al., 2007; Dixit et al., 2007). Collaboration with M. Kyba and Y.W. Chen's groups showed that DUX4 was a transcription factor targeting a number of genes, some of which encode other transcription factors. Among others, DUX4 inhibited MYOD gene expression, leading to myoblast differentiation defects, inhibited genes involved in response to oxidative stress, and activated expression of PITX1 that is involved in muscle atrophy (Bosnakovski et al., 2008; Dixit et al., 2007).

Since we detected DUX4 expression in differentiating myoblasts, we chose to compare the proteome of FSHD and control myotubes by gel free differential mass spectrometry analysis using an isotope coded protein labelling (ICPL) on free amines (NH<sub>2</sub> termini and lysines).

In keeping with a differentiation defect in FSHD we observed increased amounts of vimentin, an intermediate filament protein only expressed in early myogenesis. A putative DUX4 binding site was found in the vimentin promoter. A 4-fold induction of luciferase activity was observed when we co-transfected C2C12 cells with a DUX4 expression vector and a luciferase reporter gene fused to the vimentin promoter. In addition, galectin-1 that is normally decreased during differentiation remained at higher levels in FSHD myotubes. Both the vimentin and galectin1 increases in FSHD myotubes were confirmed by specific immunodetection on Western blots.

In addition, the ICPL study indicated a general decrease of cytoskeletal and contractile proteins that might reflect an atrophy-associated proteolysis as well as differentiation defects. Our data also confirmed a mitochondrial dysfunction in FSHD: enzymes involved in fatty acid  $\beta$ -oxidation, Krebs's cycle and the electron transport chain were decreased. There was no change in glycolytic enzymes but an increase of lactate dehydrogenase in FSHD myotubes. This observation was validated by increased lactate accumulation in the culture medium that was detected by a metabonomics analysis (1H Nuclear Magnetic Resonance).

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## **DUX4 promotes FSHD-associated pathology in vivo**

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Insufficient understanding of the pathogenic mechanisms underlying FSHD has hindered development of effective therapies. RNAi therapy is emerging as an important strategy to treat dominant genetic disorders, like FSHD, but this approach requires specific gene targets. Toward this end, we are investigating the role of several candidate genes in FSHD development. DUX4 is an excellent FSHD candidate gene because of its location in the D4Z4 repeat, elevated expression in FSHD myoblast cultures, and pro-apoptotic function in vitro. Here, we describe in vivo evidence that DUX4 over-expression causes histological and functional deficits consistent with muscular dystrophy in two animal models, zebrafish and mice. For the former, we used the Tol2 transposon system and striated muscle-specific MHCK7 promoter to express DUX4 or control GFP genes in embryonic zebrafish muscle. Using GFP epifluorescence as an indicator, we found that gene expression was restricted to somites with weaker staining in heart. In DUX4-injected fish, muscle-specific DUX4 expression produced body malformations, impaired mobility, somite defects, and myofiber degeneration. These results are consistent with abnormalities reported in other zebrafish models of muscular dystrophy and indicate that DUX4 is toxic to developing eukaryotic muscle. To confirm DUX4 toxicity in a mammalian model more closely related to humans, we delivered adeno-associated viral vectors (AAV6) carrying DUX4 or control GFP vectors to neonate or adult mouse muscle. In neonatal mice, DUX4 caused significant muscle atrophy, increased central nucleation, myofiber size variability, fibrosis, fat replacement of muscle, and inflammatory cell infiltration, while control GFP or untransduced muscles were unaffected. These results confirm our findings in zebrafish, and indicate that DUX4 toxicity to developing muscle is conserved between eukaryotic species. In adult muscle, AAV6.DUX4 produced massive, dose-dependent myofiber degeneration, apoptosis, histological evidence of muscle turnover, mononuclear cell infiltration, and gross muscle weakness. Untransduced or GFP transduced myofibers were normal. To confirm the apoptotic nature of DUX4.V5 over-expression is a function of the protein and not a side effect of nonspecific over-expression, we made rational mutations in the DUX4 homeobox 1 DNA binding domain (HOX1 DBD). Specifically, we changed 5 highly conserved HOX1 DBD amino acids to alanines, determined protein expression by western blot and immunofluorescence staining, and confirmed that it was incapable of binding DNA using gel shift assays with a PITX1 promoter probe. Importantly, the DUX4.HOX1 mutant did not cause apoptosis in cultured cells, and when delivered to adult mouse muscle using AAV6 vectors, the HOX1 mutations prevented DUX4-mediated myotoxicity, as muscles were indistinguishable from wild type or control treated mice using several histological and functional tests. These data support that DUX4 toxicity is related to its ability to bind DNA and stimulate transcription, thereby suggesting that DUX4-stimulated downstream gene targets may contribute to FSHD. Moreover, together with



numerous recent studies, our data justifies further investigation into the potential role of DUX4 as an FSHD candidate gene.

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## Assessing the effects of *FRG1*, *DUX4* and *DUX4c* on muscle satellite cell function

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant condition, which in the majority of cases, is linked to contraction of the D4Z4 repeat array in the sub-telomeric region of chromosome 4q. It is unclear how this contraction results in FSHD though, but one theory is that it modifies the expression profiles of neighbouring genes, including *FRG1* and *DUX4c*. The D4Z4 region also contains an open reading frame that encodes for a double homeobox containing protein - DUX4.

While muscle fibres are clearly affected in FSHD, it is currently unclear whether satellite cell dysfunction also contributes to disease progression in this condition. In this study, we have examined the effects of several FSHD candidate genes (*FRG1*, *DUX4* and *DUX4c*) on satellite cell function, using our isolated murine myofibre model (Zammit et al., 2004). Retroviral-mediated constitutive expression of human *FRG1* had no obvious effects on satellite cell proliferation, differentiation or self-renewal. In contrast, both human DUX4 and DUX4c had marked effects on satellite cells. Constitutive expression of DUX4 was toxic to satellite cells, consistent with previous observations in C2C12 myoblasts (Bosnakovski et al., 2008). Human DUX4c inhibited myogenic progression and maintained satellite cells in a “quiescent-like” state. Preliminary results show that DUX4c-expressing satellite cells accumulated  $\beta$ -catenin protein in their nuclei. This may disrupt canonical Wnt signalling in satellite cells (Perez-Ruiz et al., 2008), thereby perturbing their myogenic differentiation and ability to effectively repair/regeneration muscle. Speculatively, altered expression of DUX4 and DUX4c in the satellite cells of FSHD patients would diminish the efficiency of muscle repair, contributing to muscle wasting and disease progression.

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- Perez-Ruiz, A., Ono, Y., Gnocchi, V. F., and Zammit, P. S. (2008).  $\beta$ -catenin promotes self-renewal of skeletal-muscle satellite cells. *J Cell Sci* 121, 1373-82.
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**Facioscapulohumeral muscular dystrophy region gene-1 (FRG-1) is an actin bundling protein associated with muscle attachment sites**

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In vertebrates, over-expression of facioscapulohumeral muscular dystrophy (FSHD) region gene 1 (FRG1) recapitulates the pathophysiology exhibited by FSHD patients, though FRG1's role in FSHD is still controversial and no precise function for FRG1 has been described in any organism. To gain insight on FRG1's function and potential role in FSHD, we analyzed the highly conserved *Caenorhabditis elegans* ortholog, *frg-1*. *C. elegans* body-wall muscles contain two distinct subcellular pools of FRG-1; nuclear FRG-1, concentrated in the nucleoli, and cytoplasmic FRG-1 associated with the Z-disk/costamere-like structures called dense bodies. Functionally, we demonstrate that FRG-1 is an F-actin bundling protein, consistent with its localization to dense bodies. This is particularly intriguing because it places FRG-1 along side the list of dense body components whose vertebrate orthologs are involved in myriad myopathies associated with disrupted costameres and Z-disks. Interestingly, over-expressed FRG-1 preferentially accumulated in the nucleus and, when over-expressed specifically from the *frg-1* promoter, disrupted the adult ventral muscle structure and organization. Together, these data further support a role for FRG1 over-expression in FSHD pathophysiology and identify a previously unsuspected role of FRG-1 being directly associated with the muscle structure and integrity.

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18.

**The effects of elevated FSHD candidate gene expression on vertebrate development using *Xenopus laevis***

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Although the genetic lesion leading to Facioscapulohumeral Dystrophy (FSHD) has been known for around fifteen years, the downstream epigenetic consequences and how they lead to patient pathology remain unclear. Up-regulation of a local 4q35 gene has emerged as the most likely model. Using the power of *Xenopus laevis* as a system for vertebrate development, our lab has previously studied the effects of loss and gain of one of the best candidates for FSHD, Frg1. We showed that FRG1 is critical to both muscle and vascular development. Further, we found that increased levels of FRG1 led to abnormal muscle and increased angiogenesis, the two most prominent symptoms of FSHD. Here, we have used the same model system to study the effects of other promising candidates including DUX4, PITX1, and DUX4C. We found that expression of DUX4 or PITX1 from embryonic mRNA injections led to massive cellular loss leading to abnormal development restricted to the expressing side of the embryos and these abnormalities were not muscle specific. Similarly, we examined the effects of DUX4C expression within our model and found no effects on muscle development. We conclude that only elevated Frg1 levels during *Xenopus* development, and not DUX4, DUX4C, or PITX1, leads to a phenotype resembling the pathology observed in FSHD patients.

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## **Mu-Crystallin and the Pathogenesis of FSHD**

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We have reported a large, specific increase in the levels of mu-crystallin (CRYM) in the soluble fractions prepared from deltoid muscles of patients with FSHD, but not from healthy controls or patients with inflammatory myopathies or other muscular dystrophies. Here we use electroporation to introduce mammalian expression plasmids encoding green fluorescent protein (GFP) or CRYM in the tibialis anterior skeletal muscles of mice to test the hypothesis that high levels of CRYM are toxic to skeletal muscle in vivo. We used the CMV promoter to drive high levels of gene expression.

In vivo gene transfection (IVGT) by electroporation led to high levels of CRYM or GFP, as assessed by immunofluorescence and immunoblotting. As electroporation alone causes muscle damage, we waited for 4 weeks or longer before examining transfected muscles. At 4 weeks after transfection, TA muscles expressing GFP showed little or no evidence of ongoing muscle pathology. Muscles expressing high levels of CRYM showed muscle pathology, as evidenced by inflammation, muscle fiber turnover (small caliber fibers expressing a development MHC), and increases in centrally nucleated fibers (CNFs). Many fibers expressing CRYM were adjacent to fibers that did not express the protein but that had central nuclei. Similar studies at longer times after transfection continued to show no evidence of ongoing pathology associated with GFP expression, but fibers expressing CRYM decreased in number gradually, until at 4 months no such fibers remained. CNFs persisted in the latter, however. These results suggest that muscle fibers that over-express CRYM die and are replaced with healthy fibers over a period of 4 months. We obtained similar results, but with a milder myopathy, when CRYM was expressed following IVGT under the control of the human skeletal actin promoter, which more closely matches expression levels with those we've observed in skeletal muscles of patients with FSHD. Our results suggest that sustained over-expression of CRYM at levels similar to those seen in muscles from patients with FSHD are sufficient to cause skeletal myopathy.

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**Human FRG1 is a cytoplasmic actin bundling protein and a nuclear RNA associated protein**

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FRG1 is one of the leading candidate genes for FSHD pathogenesis. The precise function of FRG1 is not known, however, several lines of evidence suggest FRG1 is a nuclear protein involved with aspects of RNA biogenesis. FRG1 is very highly conserved in vertebrates and invertebrates and putative domain analysis predicts two highly conserved nuclear localization signals (NLS), and a conserved fascin-like domain, yet sequence analysis of FRG1 fails to predict any RNA-binding motifs. To better understand the function of FRG1, we performed structure-function analysis of human FRG1. We found FRG1 associates with newly transcribed RNA using *Xenopus laevis* lampbrush chromosome spreads. Furthermore, in human cells we demonstrated FRG1 interacts with numerous RNA transcripts by RNA-IP. These data are consistent with a role for nuclear FRG1 in RNA biogenesis. The presence of a conserved fascin-like domain suggests FRG1 interacts with or bundles F-actin, a function normally reserved for cytoplasmic proteins. Interestingly, we have recently described a cytoplasmic pool of endogenous FRG1 in human, mouse, frog, and *C. elegans*. To determine if FRG1 with its fascin domain is a *bona fide* actin bundling protein, we performed *in vitro* actin bundling assays. Using human recombinant FRG1 we show that FRG1 robustly bundles F-actin *in vitro* and have characterized this activity. Overall, we conclude that human FRG1 is a RNA binding protein when in the nucleus and an actin bundling protein when in the cytoplasm, or when present at the muscle attachment sites.

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21. [P]

**Initial results from single subcutaneous administration of ACE-031, a form of the soluble activin type IIB receptor, in healthy postmenopausal volunteers**

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ACE-031 is a novel, fully human, fusion protein derived from the extracellular domain of the human activin receptor type IIB (ActRIIB) linked to the Fc portion of human IgG1. ACE-031 binds to and prevents myostatin and other members of the TGF- $\beta$  superfamily from signaling through ActRIIB. In numerous animal studies, administration of an ActRIIB-Fc fusion protein results in increased muscle mass and strength and increased bone mineral density, in both normal animals and models of disease. In this first-in-human, randomized, double-blind Phase 1 study, the safety and tolerability of single, escalating doses of ACE-031 in healthy postmenopausal women were evaluated. Secondary outcomes included assessments of the pharmacokinetic and pharmacodynamic properties of ACE-031. Forty-eight subjects received ACE-031 or placebo subcutaneously at dose levels ranging from 0.02 to 3 mg/kg. ACE-031 was safe and well tolerated with a linear PK profile and a t<sub>1/2</sub> ranging from 10-15 days.

Increases in lean body mass (LBM) as measured by DXA were observed as early as 15 days following ACE-031 administration and were maintained at 2 months post treatment (2.6 % mean maximum increase from baseline). Changes in serum biomarkers of pharmacologic activity were observed including increases in adiponectin and decreases in leptin consistent with increased fat metabolism. Increases in bone formation biomarker (bone specific alkaline phosphatase) and decreases in the bone resorption biomarker (s-CTX) were also observed.

Treatment with a single-dose of ACE-031 was well-tolerated and resulted in significant, rapid, and sustained increases in lean body mass. These data support the development of ACE-031 in neuromuscular diseases such as DMD, FSHD and ALS to increase muscle strength and improve physical function.

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22. [P]

## **Subcellular trafficking of DUX4, a pro-apoptotic protein encoded at the facioscapulohumeral muscular dystrophy locus FSHD1A**

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DUX4 is a pro-apoptotic protein, leading to cell death in cell transfection assays<sup>1,2</sup>. A DUX4-related protein is endogenously expressed in human muscle derived cells<sup>1,2</sup>, being the DUX4 gene specifically transcribed in FSHD myoblasts<sup>3</sup>. These studies, as well as recent results obtained by others<sup>5</sup>, support the hypothesis that DUX4 is a main potential pathogenic protein in FSHD<sup>6</sup>. We have proposed that abnormal temporal or spatial expression of DUX4 during muscle development may be toxic for muscle cells, causing FSHD<sup>3</sup>. DUX4 is a nuclear protein<sup>1-4</sup> and its subcellular localization may be required for its function and/or pro-apoptotic effect. In this report we analyze the subcellular trafficking of DUX4 using specific mutants for the two previously described<sup>4</sup> DUX4 nuclear localization signals, NLS1 (RRRR23) and NLS2 (RRKR98) as well as a third potential NLS (i.e. NLS3; RRARHPG151) recognized by visual inspection of the DUX4 amino acid sequence. Single, as well as combined double and a triple deletion NLSs- mutants, mostly localize to the cell nuclei indicating that subcellular trafficking of DUX4 is not mediated by these NLSs<sup>4</sup>. The effect of mutations in NLS1, NLS2 and NLS3 on DUX4-mediated apoptosis was studied using a GFP-based co-transfection assay developed in our laboratory<sup>1-3</sup>. The toxic effect of DUX4 was partially abolished in the analyzed mutants. These studies address the questions about the molecular domains involved in the normal trafficking of DUX4 as well as the domains required for DUX4-mediated cell death.

<sup>1</sup>Kowaljow W (2003) PhD Thesis, Argentina; <sup>2</sup>Rosa AL (2003) FSHD IRCM, Los Angeles, California;

<sup>3</sup>Kowaljow et al (2007) NMD 17, 611-623; <sup>4</sup>Ostlund et al (2005) Biochemistry 44, 2378-2384;

<sup>5</sup>Bosnakovski et al (2008) EMBO J 27, 2766-2779; <sup>6</sup>Dmitriev et al (2009) NMD 19, 17-20.



23. [P]

### **Krüppel-like factor KLF15 Interacts with the D4Z4 enhancer and up regulates the FSHD-related gene DUX4c**

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Facioscapulohumeral muscular dystrophy (FSHD), a dominant hereditary disease with a prevalence of 1 in 20,000 individuals, is caused by a partial deletion in the sub-telomeric D4Z4 repeat array on chromosome 4q. Here we show that a strong transcriptional enhancer within the D4Z4 repeat unit interacts with the Krüppel-like factor KLF15 and that the expression level of KLF15 regulates the activity of the D4Z4 enhancer which, in turns, induces expression of DUX4c in a KLF15-dependent manner. During muscle differentiation, increased expression of KLF15 activates the D4Z4 enhancer leading to activation of the promoter of the DUX4c gene. Furthermore, we show that KLF15 is expressed at higher levels in muscle biopsies from FSHD patients compared to controls. Higher expression of KLF15 in muscles of FSHD patients could induce untimely over-expression of DUX4c with deleterious effects on muscle cell differentiation. We suggest that the KLF15-controlled D4Z4 enhancer may play a role in normal and pathological development of muscular cells.

Key Words: Facioscapulohumeral dystrophy (FSHD)/Transcriptional regulation / Krüppel factor 15/ D4Z4 /DUX4c

## A Window into secrets of myogenesis genes: whole-genome DNaseI hypersensitivity mapping

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Subregions of 4q35.2 chromatin involved in facioscapulohumeral muscular dystrophy (FSHD), including D4Z4, a tandem disease-linked array, probably have special features in skeletal muscle that help to make this a predominantly muscle disease. Despite many expression microarray and RT-PCR studies, we lack sufficient understanding of FSHD-related gene dysregulation at 4q35.2 and in the rest of the genome. To approach these questions from a different vantage point, we began mapping DNaseI-hypersensitive sites (DHS) throughout the genomes of myoblast cell strains from unaffected individuals before and after differentiation to myotubes to compare these cell types to each other and to various non-muscle cell populations. DHS are predominantly known as marks of transcription control elements that are nucleosome free or have a loose nucleosome structure. Our analysis of DHS used next-generation DNA sequencing (DNase-seq). All active genes and genes poised for activity should have a DHS at their promoter. Other active transcription control elements also can have overlapping DHS.

We analyzed myoblast cell strains from three controls and myotubes populations from the same three batches of cells. We are comparing their genome-wide DHS maps to those from lymphoblastoid cells, hepatoma cells, myeloid cells, osteoblasts, and fibroblasts. No muscle cell-specific DHS were seen at the 4q35.2 *FRG1*, *FRG2*, or D4Z4. Just as we found by tiling array analysis, DNase-seq revealed muscle-specific DHS in several gene desert regions of 4q35.2 and at genes in the proximal portion of 4q35.2. We have begun data mining across the rest of the genome. Among our findings are the following:

- 1.) muscle-specific genes with unexpected far-upstream DHS, which are likely to be enhancers;
- 2.) muscle-associated genes with myotube-specific DHS;
- 3.) muscle-associated genes with myoblast-specific DHS;
- 4.) disease-associated genes with muscle-specific internal DHS that probably overlap enhancers, silencers, or alternative promoters;
- 5.) known muscle-specific genes with adjacent uncharacterized genes that have a muscle-specific DHS at their promoter.

These results demonstrate the power of high-resolution mapping of DHS to discover new transcription control sequences and unanticipated muscle-associations of poorly characterized genes. They lay the foundation for functional analyses and for an ongoing DNase-seq study of FSHD myoblasts and myotubes in comparison to controls. (Supported by NIH Grants NS048859 and HG003169, the FSH Global Research Foundation, and the National Human Genome Research Institute).

25. [P]

### **Studying sequence variation at the human D4Z4 and mouse DUX loci**

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by contraction of the D4Z4 array located on 4q35. Although FSHD is generally believed to be a chromatin-associated disease, the exact molecular mechanism is still unclear. Although some models propose that the deletions alter expression of genes outside the array; recent studies point towards a direct role for a potential homeobox gene, *DUX4*, that has a copy in each D4Z4 repeat unit. We were intrigued by recent genetic studies that demonstrated the requirement of the D4Z4 deletions to occur on particular 4q35 haplotypes (notably the 4qA161 haplotype) in order for the deletions to cause FSHD. This indicates the importance of one or more specific variants *in cis* for development of FSHD.

Our group is interested in the evolutionary history of DUX genes; we hope that this will inform us about the functions of these unusual genes. We recently identified a conserved DUX homeobox array in the mouse that may be functionally equivalent to D4Z4. Ectopic expression of high levels of either the human or mouse DUX open reading frame causes cell toxicity. We have previously demonstrated transcription from the mouse array. Like the human D4Z4 array, transcription in the mouse appears limited to short fragments and we detect both sense and antisense transcripts. It is currently unclear whether all or only some of the ~40 DUX copies of the mouse DUX repeat are transcribed and are functional.

We will present data from our study of sequence variations within the human and mouse DUX loci. We have detected a number of variants, some of which are within the coding region and could result in functional different DUX proteins. Mapping the relationships between these variants and expressed sequences will be important for understanding the normal functions of human and mouse DUX genes. Furthermore, such variation within the human array may be important mechanistically in FSHD.

26. [P]

### **Endogenous FRG1 protein expression in human and mouse skeletal muscle**

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FRG1 is a candidate gene proposed to be up-regulated in FSHD. While up-regulation of FRG1 has not been consistently shown in patient samples, over-expression of FRG1 in mouse, *Xenopus*, and *C. elegans* have all resulted in muscle phenotypes, demonstrating that mis-regulation of FRG1 is deleterious to muscle. To better understand the function of FRG1 we have developed novel highly specific antibodies against FRG1 and performed immunostaining for FRG1 in human and mouse tissues and cultured cells. Interestingly we detect both nuclear and cytoplasmic staining for FRG1. FRG1 is expressed in skeletal muscle fibers. Other tissues with prominent FRG1 staining include smooth muscle, sweat glands and epidermis. This is consistent with previous reports that FRG1 is expressed in a wide range of tissue types and points to a common basic cellular function. In support of this our group has shown that FRG1 functions in F-actin bundling. In longitudinal sections of human and mouse skeletal muscle FRG1 resides in the sarcomeres giving a striated pattern. This localization is conserved down to *C. elegans* where our laboratory has found FRG-1 localized to the body wall muscle dense bodies, structures analogous to the vertebrate Z-disk/costameres. While a potential role for FRG1 in the nucleolus in RNA biogenesis has been proposed, our results suggest an additional role for FRG1 in muscle cytoplasm and at the sarcomeres. This work places FRG1 at the heart of the conserved machinery responsible for making muscle attachments and maintaining muscle integrity; structures so often disrupted or malfunctioning in many human myopathies.

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**Both DUX4c and a truncated DUX4 variant perturb beta-catenin localisation in myogenic cells**

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant condition, which in the majority of cases, is linked to contraction of the D4Z4 repeat array in the sub-telomeric region of chromosome 4q. It is unclear how this contraction results in FSHD. Each D4Z4 repeat array contains an open reading frame that encodes a double homeobox containing protein - DUX4, which may be aberrantly expressed in FSHD. In addition, the contraction may modify the expression dynamics of neighbouring genes including DUX4c. Both DUX4 and DUX4c have been shown to inhibit myogenic progression in cell culture (Bosnakovski et al., 2008a; Bosnakovski et al., 2008b).

In this study, we have examined the effects of DUX4, DUX4c and several truncated DUX4 transcripts (a generous gift from S. Tapscott - Snider et al., 2009) on satellite cell function. Retroviral-mediated constitutive expression of human DUX4c in mouse satellite cells resulted in a nuclear accumulation of Beta-catenin protein and inhibited myogenic progression. A proposed truncated DUX4 variant lacking the carboxy terminus was also able to induce a similar redistribution of Beta-catenin in satellite cells, while full-length DUX4 was not. Therefore, the presence of DUX4c and/or truncated species of DUX4 may disrupt canonical Wnt signalling in satellite cells (Perez-Ruiz et al., 2008), so perturbing their myogenic differentiation and ability to effectively repair dystrophic muscle.

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28. [P]

### **Long-term activin receptor type IIB inhibition improves strength and function of dystrophic muscle**

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The absence of the membrane stabilizing protein dystrophin characteristic of Duchenne muscular dystrophy increases vulnerability of muscle to contraction-induced damage. One strategy to prevent mechanical stress-induced damage in dystrophic muscle is to promote muscle fiber hypertrophy. Inhibition of activin receptor type IIB (ActRIIB) signaling induces skeletal muscle hypertrophy and results in increased muscle mass and strength in wild-type mice. Previous work also describes a functional benefit of increasing myofiber size in dystrophic muscle. To determine the efficacy of chronic ActRIIB inhibition in improving dystrophic muscle, 7-week old mdx mice were treated with vehicle or RAP-031, a fusion protein comprised of a form of the ActRIIB extracellular domain fused to a murine Fc, for 42 weeks. Body composition, grip strength and extensor digitorum longus (EDL) contraction force were assessed. Over the course of the study, RAP-031 significantly ( $p < 0.001$ ) increased lean tissue mass compared to the vehicle cohort. Grip strength, normalized for lean tissue mass, was 28.3% higher ( $p < 0.01$ ) in RAP-031 treated mice compared to the control group. EDL absolute twitch and tetanus contraction forces were increased 30% ( $p < 0.01$ ) and 42.5% ( $p < 0.01$ ) respectively in the RAP-031 group compared to controls. The forces generated during an eccentric contraction (ECC) protocol were significantly greater ( $p < 0.01$ ) in the RAP-031 treated group in 5 trials compared to controls and there was no significant change in the force deficits resulting from the lengthening contractions. While the rate of force loss is similar, the finding that the absolute forces were greater throughout the ECC protocol could support a role for RAP-031 in increasing the degree of damage a muscle can withstand before contraction force is diminished to a critically low level. Overall, these data support a therapeutic benefit provided by chronic ActRIIB inhibition to dystrophic muscle.

29. [P]

### **Experience in use of 4qA and 4qB in diagnostic testing for FSHD**

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The Bristol Genetics Laboratory is one of the three (3) United Kingdom laboratories offering molecular diagnostic testing in FSHD, now receiving over 350 sample requests per year. Primarily the testing is based on *EcoRI* /*BlnI* +/- *ApoI* digestion using probe p13E-11. Supplementary testing with 4qA and 4qB probes (*HindIII*) has been introduced, but only on specific request in response to our explanatory comments on the initial report, and has accordingly been added to approximately 5% of cases. This is proving to be helpful in at least four different situations:

- 1) Patients with clinical FSHD who have 2 shortened D4Z4 fragments
- 2) Patients with clinical FSHD, and a suspected proximal deletion involving p13E11, as they have no shortened fragment detected with p13E-11
- 3) Patients with a neuromuscular condition but an intermediate range D4Z4 fragment where the clinician wishes to exclude FSHD
- 4) Patients where a previous NM diagnosis has been changed by finding a shortened D4Z4 fragment, and absolute confirmation is essential for considering pre-implantation or prenatal genetic diagnosis.

We present here case illustrations of these, in the context of our summary service data, and discuss whether introduction of fuller 4q35 haplotyping, or potentially a methylation based assay such as chromatin immunoprecipitation analysis, should be a target for research into diagnostic improvement.

## **Transferring the Molecular Combing test for FSHD in a routine diagnostics lab: lessons learned and new findings**

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Molecular pathophysiology of FSHD involves the heterozygous contraction of a number of D4Z4 sequences, 3.3-kb tandemly repeated units on chromosome 4. Molecular diagnostics of FSHD is an everlasting challenge due to several factors, namely: the variability of the repeat array size within a wide range (from 3.3 kb to more than 300 kb), the presence of a homologous repeat array on chromosome 10, and the existence of two haplotypes of chromosome 4, of which only one is potentially pathogenic. Indeed, FSHD is associated with repeat arrays with less than 10 repeats on 4qA chromosomes. Contractions on 4qB or 10q chromosomes are silent. Beside these general difficulties, specific cases may yet add to the complexity: somatic mosaicism, translocations between 4q and 10q chromosomes and deletions of nearby sequences are quite common events. The current diagnostics procedure, based on Southern blotting, is time-consuming, expensive, and may still lead to diagnostics errors. Therefore, there is an obvious need for a test that will allow simple and reliable diagnostics of FSHD.

We have presented last year the preliminary results of the test we are developing for the diagnostics of FSHD using the Molecular Combing technology. With this technology, DNA molecules are linearly stretched and immobilized on a glass substrate, where they can be hybridized with fluorescent probes. This allows high resolution (~1 kb) observation of loci up to several hundreds of kilobases observed molecule by molecule. Thus, mapping of even the most complex regions is straightforward using this technology.

Following the initial development, we have focused on improving the design of the test and adapting it to routine use in diagnostics laboratories. Indeed, the analysis of Molecular Combing results requires intensive fluorescence microscopy acquisition and image processing and analysis. The new design allows for an improved reliability, while sparing on acquisition and analysis time. Besides, dramatic improvement in hands-on time has been achieved thanks to thorough automation of the most time- and effort-consuming steps. Image acquisition is now fully automated thanks to the adaptation of a fluorescence scanner, in collaboration with its manufacturer. We have also developed a fully automated image processing and analysis software, which we are now in the process of validating. This will allow for simple interpretation by the clinician, with very little workload for the technical staff.

Along with these developments, we have transferred the whole test in our routine diagnostics laboratory, where we have been able to test its suitability in this environment. We will present practical considerations involved, as well as the first results obtained in this environment. We have found several unexpected features in the D4Z4 region, which we will present as well. Last, we will discuss ongoing developments and perspectives for routine use of this test in replacement of Southern blotting, which should prove to be a tremendous progress in simplicity, reliability, turnover time and cost.



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**Toward quantitative proteomic comparisons of skeletal muscles from FSHD patients and their unaffected, first-degree relatives**

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We are studying the changes in the proteome that occur in FSHD and have been using large-format, two-dimensional gel electrophoresis in our initial studies. We have introduced several modifications to published methods that improve protein solubility and recovery, minimize proteolysis, reduce the loss of resolution due to contaminants and cross-linking, and enhance analysis of faint spots. Key modifications are: (i) the use of 7M urea + 2 M thiourea, instead of 9M urea, in preparing samples and gels for isoelectric focusing; (ii) the use of a strong reducing agent followed by 4-vinylpyridine, to block sulfhydryl groups and prevent non-specific protein cross-linking; (iii) digital enhancement of spots that are faintly labeled with stain; (iv) large gels that enable all proteins from the first dimension to be resolved in the second dimension without cutting the gel, and proteins with molecular masses less than 30 kDa to be resolved. These changes make 2-D gel electrophoretic analysis of the proteome more comprehensive, reproducible and sensitive, with minimal artifacts. We have begun to use our improved methods to examine fresh, snap frozen, age- and sex-matched biopsies of deltoid and biceps muscles from patients with FSHD and from their first degree affected and unaffected relatives, as well as myoblast cell lines prepared from control and affected samples. Our analyses reveal ~3,000 protein spots in biopsy samples. Very few spots change significantly between FSHD and unaffected controls, suggesting that the levels of expression of the vast majority of proteins prominent in skeletal muscle are not altered by FSHD.

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### **Pelvic and lower limb involvement in FSHD: a muscle MRI study**

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**Introduction:** FSHD is an autosomal dominant disease in which lower limb involvement always follows that of facial and shoulder muscles and represents an unequivocal index of disease progression and severity. However, limited data are available on the extent and severity of pelvic and lower limb muscle involvement.

**Objectives:** 1) To identify the pattern of muscle involvement most frequently observed in FSHD. 2) To identify the distribution and severity of muscle involvement across different ages to get clues about the natural history of the disease. 3) To verify the correlation between MRI-derived indexes and clinical scores. 4) To characterize at the histopathological level the signal abnormalities in T2-W sequences, which can be found in the majority of muscles during the course of the disease.

**Methods:** We studied 204 patients, mean age  $44 \pm 16$ , with a genetically confirmed diagnosis of FSHD (pathogenic EcoRI/BlnI-resistant fragment ranging between 10 and 40 kb). Muscle examination was performed on a 1.5-Tesla MR scanner, with T1-W SE images (TR/TE=500/35 msec) and T2-W STIR images (T1=1 50 msec). Axial slices were obtained from psoas to distal foot muscles and 40 muscles were evaluated on each side. Open and needle muscle biopsies were performed from muscles with normal T1-W signal and both normal and hyperintense T2-W STIR sequences.

**Results:** 1) Abdominal muscles, semimembranosus, biceps femoris (long head), adductor magnus, tibialis anterior, and soleus were more frequently and more severely affected, while popliteus, tibialis posterior, ileopsoas, flexor digitorum longus were only rarely involved on T1-W scans. A number of T1-W unaffected muscles randomly present T2-W hyperintensities, which are usually supposed to account for muscle inflammation/oedema. 2) A correlation was found between both age and size of the pathogenic EcoRI/BlnI-resistant fragment and severity of muscle involvement assessed by clinical severity scale and MRI-derived indexes. 3) Moreover, the overall severity of muscle involvement significantly correlates with the level of clinical impairment. MRI sensitivity is higher than physical examination in detecting the involvement of individual muscles. 4) Immunological characterization shows that inflammation seems to be a constant finding in T2-STIR positive muscles.

**Conclusions:** Muscle MRI examination is a powerful and reliable tool in the evaluation of FSHD patients and may be helpful in clinical trials for the disease.

## **Electrostimulation training: an effective and safe treatment for FSHD patients**

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**Objective:** to demonstrate safety, feasibility and efficacy of electrostimulation training in facioscapulohumeral muscular dystrophy (FSHD) patients.

**Design:** non-controlled and before-after trial

**Participants:** 9 volunteer FSHD patients (3 women and 6 men; mean age 55.7±10.4 years) clinically characterized by shoulder girdle and quadriceps femoris muscles weakness. **Interventions:** all patients underwent five sessions per week of electrostimulation during five months consecutively. Each session last 23 minutes. The muscles stimulated were deltoids, trapezius and quadriceps femoris.

**Main outcome measures:** tolerance was assessed by pain and fatigue visual analog Scales (VAS), average length of electrostimulation sessions (ALES), average intensity of electrostimulation sessions (AIES) and plasma creatine kinase (CK) value. Feasibility was assessed by patients global participation rate to study (GPR) and individual participation rate to sessions (IPR). Efficacy was assessed by manual muscle testing (MMT), maximum volunteer isometric force (MVIF), Barre test, six minutes walking test (6MWT) and, by a subjective autoevaluation.

**Results:** Regarding tolerance: monthly averages of session pain and fatigue VAS were not significant ranging respectively from 0.8 to 1.7 cm and, from 1.1 to 1.7 cm, the ALES was 23 minutes for every month, and average CK rate tended to decrease (from 290±46 to 245±25 UI/L). In term of feasibility: GPR was 100% at the end of the study and MPR ranged from 77% to 89%. For efficacy, a significative improvement (p<0.05) was observed for: MMT of shoulder flexion, shoulder extension and knee extension, MVIF of shoulder flexion and abduction, and 6MWT (from 305.7±40.1 s to 333.8±44.9 s). Barré test improved from 57.11±14.36 to 77±21.3 but not significantly.

**Conclusions:** These data suggest that electrostimulation is a safe and effective method to improve strength and functionality of FSHD patient muscles, in view of reducing the progression of the disease. A multicentric controlled study is required to confirm these conclusions.

**Key words:** FSHD, force, electrostimulation, functionality, rehabilitation.

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### **Establishment of clonal myogenic cell lines from severely affected dystrophic muscles – tools for studying FSHD**

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A hallmark of dystrophic muscles is their replacement by connective tissue, and muscle biopsies from FSHD patients often contain only a minority of bona fide muscle cells. Since the primary cause that leads to muscular dystrophy is a defect within myogenic cells it is important to study this particular cell type (and not cells of the connective tissue) to understand the myopathy.

We show that it is possible to establish clonal myogenic cell lines from FSHD muscle that mainly consists of non-myogenic cells by overexpression of cdk4 and hTERT, and a subsequent cloning step. These cell lines are valuable tools to reproducibly study the effect of the FSHD mutation within myoblasts without the confounding influence of variable amounts of contaminating connective tissue cells.

## Unexpected high percentage of subjects carrying D4Z4 reduced alleles and no clinical signs in FSHD families: which factors contribute to the disease mechanism?

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Facioscapulohumeral muscular dystrophy (FSHD) is characterized by wide variability in clinical spectrum. Penetrance is considered complete by the age of 50. The FSHD molecular defect consists in reduced number of a 3.3 kb sequence (D4Z4) tandemly arrayed at 4q35 locus. Fewer than 9 repeats are present in sporadic and familial FSHD patients. However, since molecular analysis has become a widely used tool for FSHD diagnosis, several observations have emerged to complicate the evaluation of FSHD patients and genotype-phenotype correlation.

The great variability of FSHD clinical outcome appears to be more pronounced than expected with the presence of non-penetrant gene carriers, compound heterozygous patients for the FSHD-sized alleles, FSHD subjects carrying D4Z4 alleles of 9-11 units, which are also present in the normal population. As a result, a correlation between the number of the D4Z4 repeats with the severity of the disease is difficult to establish.

To collect clinical information suitable for statistical analysis in patients affected by FSHD a clinical evaluation form has been designed. The FSHD clinical form allows to obtain the functional evaluation of six muscle groups affected in FSHD through a questionnaire and to assign a disability score ranging from 0 (no signs) to 15 (wheelchair bound) (available at [www.fshd.it](http://www.fshd.it)).

In 2008 we established the Italian Registry for FSHD, which gathers 1037 molecularly diagnosed index cases. The clinical network, composed by 11 clinical centres, has collected the majority of the FSHD Italian family.

A preliminary study was conducted on 492 subjects carrying D4Z4 deleted alleles. As expected the FSHD score, a measure of disability, increases with age. Notably, in the group including subjects younger than 40 years the number of males exceeds the number of females suggesting a milder outcome of the disease in the latter group. Additionally, our analysis revealed that in the group of subject older than 70 years, 15% show no signs of FSHD.

As expected, correlation between D4Z4 units number and clinical expression of the disease quantified by FSHD score showed that the percentage of subjects with no signs of FSHD inversely correlates with the D4Z4 repeat number. However, genotype-phenotype correlation conducted on 96 FSHD families revealed that only in 26 families all the subjects carrying the D4Z4 molecular defect display clinical signs of FSHD. Interestingly in 9 families, with 3 or more subjects carrying FSHD-sized alleles carriers, only the proband displays clinical signs of FSHD.

Analysis of haplotype variants using D4Z4 repeat length, 4qA/4qB bi-allelic polymorphism, SSLP centromeric to D4Z4, G/C SNP variants revealed novel haplotypes associated with the disease. Interestingly, we detected 4 novel SSLP alleles in our population. One novel haplotype associated with the disease is represented by 33qA D4Z4 allele associated with the C D4Z4 SNP variant. A second novel haplotype (4qA163) co-segregates with a 25 Kb D4Z4 allele and is associated with FSHD phenotype. In both families the disease is fully penetrant.

The presence of a high number of subjects carrying the D4Z4 deletion with no signs of the disease emphasizes the complexity of the mechanisms underlying FSHD pathophysiology. Identification and definition of the weight of additional factors in the development of FSHD will lay the basis for a more precise prognosis and future therapeutic intervention. Additionally our analysis of 4q haplotypes in the Italian population indicates a wider genetic variability than that previously observed. This information is critical for genetic counseling and suggests the necessity of in-depth study to ascertain the prognostic role of 4q haplotypes.