

# **Facioscapulohumeral Muscular Dystrophy International Research Consortium 2007**

**Tuesday, October 23, 2007**

**7:00 a.m. – 4:00 p.m.**

**San Diego Marriott Hotel & Marina  
333 West Harbor Drive, San Diego, California 92101 USA  
Marina Salons E and F**

**Co-Chairs: Silvère van der Maarel, Ph.D.  
Rabi Tawil, M.D.**

**Organizers: Daniel Paul Perez  
Rabi Tawil, M.D.  
Silvère van der Maarel, Ph.D.**

**Sponsored by:**

**FSH Society, Inc.**



**Association Française Contre les Myopathies (AFM)**



**Muscular Dystrophy Association (MDA USA)**



**Athena Diagnostics**



**October 23, 2007**

**PREFACE**

Dear Colleagues,

Welcome to the Facioscapulohumeral Muscular Dystrophy (FSHD) International Research Consortium 2007. Thanks to you we are seeing rapid developments on numerous fronts in FSHD. We are seeing increasing momentum in basic and clinical research initiatives as well as the development of promising new potential treatments for FSHD and other muscular dystrophies. Additionally, there is an increase in government, non-profit, and private funding and in international collaboration of volunteer health agencies and FSHD patients.

This rapid change in developments is well reflected in our program with no less than 16 platform presentations and 5 poster presentations or abstracts in absentia. The abstracts cover a wide range of new and exciting developments. We experience a revival of DUX4, we slowly uncover the function of FSHD Region Gene 1 (FRG1), and we have important clinical studies covered by our workshop. There will be substantial evidence presented that FSHD may be considered as a “chromatin disease” and we will be educated on new genetic developments in 4q-linked FSHD and non-4q-linked FSHD.

This year, we will again have a broad audience of clinicians, scientists, biotechnology companies, pharmaceutical companies, government research funding agencies, non-profit funding agencies, and patients themselves – altogether highlighting the translational character of the workshop. More than 50 people have registered making this workshop worldwide the “place to be” for anyone with a keen interest in FSHD. We have included at the end of our day a round table discussion with representatives of all stakeholders to discuss the future needs of FSHD. We are hoping for a lively and productive discussion in which all FSHD issues will be openly discussed to direct us into a new and better future for patients with FSHD.

This meeting is organized and sponsored by the FSH Society, Inc., the Association Française Contre les Myopathies (AFM), the Muscular Dystrophy Association USA (MDAUSA) 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.

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

Rabi Tawil, M.D.  
University of Rochester School of Medicine, Rochester, New York USA

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

*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](mailto:daniel.perez@fshsociety.org), website: <http://www.fshsociety.org>*

## **FSHD IRC Workshop 2007**

7:00 – 8:00 Registration  
7:30 – 8:30 Continental Breakfast  
8:00 – 8:15 **OPENING REMARKS**  
8:15 – 8:30 **KEYNOTE:** John Porter, NIH NINDS

8:30—10:10

Topic 1

### **D4Z4 DUX4 EXPRESSION**

Silvère van der Maarel, Ph.D. (moderator)

- 1) Darko Bosnakovski DUX4
- 2) Jane Hewitt DUX4
- 3) Michael Kyba DUX4
- 4) Yi-Wen Chen Pitx/DUX4
- 5) Alexandra Belayew DUX4/Pitx

10:10 – 10:30 Morning Break

10:30—11:50

Topic 2

### **D4Z4 CHROMATIN**

Sara T. Winokur, Ph.D. (moderator)

- 1) Frederique Magdinier CTCF/chromatin
- 2) Kyoko Yokomori chromatin
- 3) Melanie Ehrlich chromatin
- 4) Galina Filippova CTCF

11:50 – 12:00

Topic 3

### **POSTERS**

Silvère van der Maarel, Ph.D. (moderator)

- 1) Patrick Reed Mu crystallin
- 2) Valery Kazakov MRI
- 3) Kyle Siebenthall 4C
- 4) Hermien Kan MRI/MRS
- 5) Graham Kemp MRI/Creatine

12:00 – 12:40 Lunch

12:40—1:40

Topic 4

### **CLINICAL AND GENETIC STUDIES**

Rabi Tawil, M.D. (moderator)

- 1) Ted Abresch Pain
- 1) Richard Lemmers Genetics
- 3) Meena Upadhyaya Non-4q FSHD

1:40—3:00

Topic 5

### **FRG1 FUNCTION AND GENE EXPRESSION**

Jane E. Hewitt, Ph.D. (moderator)

- 1) Meredith Hanel FRG1
- 2) Joseph Marx FRG1
- 3) Rossella Tupler Myoblasts
- 4) Sara Winokur Expression

3:00 – 3:20 Afternoon Break

3:20 – 4:00

Topic 6

### **IDENTIFY TOP 5-10 PRIORITIES FOR 2008 FSHD RESEARCH DIRECTIONS AND COLLABORATIONS**

Silvère van der Maarel, Ph.D. (moderator)

Rabi Tawil, M.D. (moderator)

Rune R. Frants, Ph.D. (moderator)

William R. Lewis, Sr., M.D. (moderator)

John Porter, Ph.D. (moderator)

- 1) Group Discussion

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

**7:30-8:30 a.m.**  
**CONTINENTAL BREAKFAST (BUFFET)**

**8:00-8:15 a.m.**  
**OPENING REMARKS & CHARGE FOR THE MEETING**

***William R. Lewis, Sr., M.D.***

*Chairman of the Board, FSH Society, Inc. & Neurosurgeon, Monterey, California*

***Silvère van der Maarel, Ph.D.***

*Leiden University Medical Center, Leiden, The Netherlands*

***Rabi Tawil, M.D.***

*University of Rochester School of Medicine, Rochester, New York USA*

**8:15-8:30 a.m.**

**KEYNOTE**

***John D. Porter, Ph.D.***

*Executive Secretary, Muscular Dystrophy Coordinating Committee (MDCC)*

*Program Director, Neuromuscular Disease, Neurogenetics Cluster and the NINDS*

*Technology Development Program, National Institutes of Neurological Disorders and Stroke, Bethesda, Maryland*

**Translational Research in Muscular Dystrophy: NIH Activities from Target Identification to Trials**

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

**PLATFORM PRESENTATION(S) I**

*Silvère van der Maarel, Ph.D., Moderator*

*Leiden University Medical Center, Leiden, The Netherlands*

**D4Z4 DUX4 EXPRESSION**

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

***Darko Bosnakovski, D.V.M., Ph.D.***

*Department of Developmental Biology, UT Southwestern Medical Center, Dallas, TX 75390 USA*

**A small molecule screen identifies inhibitors of DUX4-mediated toxicity in myoblasts**

*Darko Bosnakovski<sup>1</sup>, Shuguang Wei<sup>2</sup>, Mingju Liu<sup>1</sup>, Michael Roth<sup>2</sup>, Rita Perlingeiro<sup>1</sup>, and Michael Kyba<sup>1</sup>*

<sup>1</sup>Department of Developmental Biology, UT Southwestern Medical Center, Dallas, TX 75390

<sup>2</sup>Department of Biochemistry, UT Southwestern Medical Center, Dallas, TX 75390

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

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

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

**Evolutionary conservation of D4Z4 and implications for understanding facioscapulohumeral muscular dystrophy**

Jannine Clapp<sup>1</sup>, Laura M Mitchell<sup>1</sup>, Marcel Wolfs<sup>1</sup>, Daniel J Bolland<sup>2</sup>, Anne E. Corcoran<sup>2</sup>, Paul J Scotting<sup>1</sup>, John A L Armour<sup>1</sup> and Jane E Hewitt<sup>1</sup>

<sup>1</sup>Institute of Genetics, School of Biology, The University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH, United Kingdom.

<sup>2</sup>Laboratory of Chromatin & Gene Expression, Babraham Institute, Cambridge, CB2 4AT, United Kingdom.

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

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

*Department of Developmental Biology, UT Southwestern Medical Center, Dallas, Texas 75390 USA*

**Genetic interactions between DUX4 and Pax3/Pax7 suggests a stem cell etiology for FSHD**

Darko Bosnakovski<sup>1</sup>, Zhaohui Xu<sup>1</sup>, Eun Ji Gang<sup>1</sup>, Cristi L. Galindo<sup>2</sup>, Mingju Liu<sup>1</sup>, Tugba Simsek<sup>1</sup>, Harold R. Garner<sup>2</sup>, Siamek Agha-Mohammadi<sup>3</sup>, Alexandra Tassin<sup>4</sup>, Frédérique Coppée<sup>4</sup>, Alexandra Belayew<sup>4</sup>, Rita Perlingeiro<sup>1</sup>, and Michael Kyba<sup>1</sup>

<sup>1</sup>Department of Developmental Biology, UT Southwestern Medical Center, Dallas, Texas 75390 USA

<sup>2</sup>Center for Biomedical Invention, UT Southwestern Medical Center, Dallas, Texas 75390 USA

<sup>3</sup>Division of Plastic Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania 15261 USA

<sup>4</sup>Lab. Biologie Moleculaire, Universite de Mons-Hainaut Pentagone, 7000 - Mons, Belgium

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

***Yi-Wen Chen, D.V.M., Ph.D.***

*Center for Genetic Medicine Research, Children's National, Medical Center, Washington DC USA*

**Characterization of a tet-repressible muscle-specific Pitx1 transgenic mouse**

Manjusha Dixit<sup>1</sup>, Jennifer Cabotage<sup>1</sup>, Rongye Shi<sup>1</sup>, Margret Sutherland<sup>2,3</sup>, Stephanie Muger<sup>2</sup>, Yi-Wen Chen<sup>1,3</sup>

<sup>1</sup>Center for Genetic Medicine Research, Children's National, Medical Center, Washington DC

<sup>2</sup>Center for Neuroscience Research, Children's National, Medical Center, Washington DC

<sup>3</sup>Department of Pediatrics, George Washington University, Washington DC

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

***Alexandra Belayew, Ph.D.***

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

**Further studies on the DUX4, DUX4c and PITX1 genes in FSHD**

Eugénie Anseau<sup>1\*</sup>, Alexandra Tassin<sup>1\*</sup>, Céline Vanderplanck<sup>1</sup>, Samuel Cloet<sup>1</sup>, Marietta Barro<sup>2</sup>, Dalila Laoudj-Chenivresse<sup>2</sup>, Manjusha Dixit<sup>3</sup>, Yi-Wen Chen<sup>3,4</sup>, Alexandra Belayew<sup>1</sup>, and Frédérique Coppée<sup>1</sup>

<sup>1</sup>Laboratory of Molecular Biology, University of Mons-Hainaut, Mons, Belgium

<sup>2</sup>INSERM ERI 25 "Muscle et Pathologies", University of Montpellier I, Montpellier, France

<sup>3</sup>Center for Genetic Medicine Research, Children's National Medical Center, Washington, DC

<sup>4</sup>Department of Pediatrics, George Washington University, Washington, DC

\* These authors contributed equally to this study

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

**MORNING BREAK**

**10:30-11:50 a.m.**

**PLATFORM PRESENTATION(S) II**

*Sara T. Winokur, Ph.D., Moderator*

*University of California Irvine, Irvine, California USA*

**D4Z4 CHROMATIN**

**10:30-10:50 a.m.**

***Frédérique Magdinier, Ph.D.***

*Laboratoire de Biologie Moléculaire de la Cellule, Ecole Normale Supérieure de Lyon, CNRS UMR5239, INRA U1237, IFR128, Lyon, France*

**CTCF as a new regulator of D4Z4 function**

Alexandre Ottaviani, Sylvie Rival-Gervier, Andrea Förster, Amina Boussouar, Eric Gilson &

Frédérique Magdinier

Laboratoire de Biologie Moléculaire de la Cellule, Ecole Normale Supérieure de Lyon, CNRS UMR5239, INRA U1237, IFR128, Lyon, France

**10:50-11:10 a.m.**

***Kyoko Yokomori, Ph.D.***

*Department of Biological Chemistry, School of Medicine, University of California, Irvine, California 92697-1700 USA*

**Specific loss of histone H3 lysine 9 trimethylation and HP1(gamma) /cohesin binding at D4Z4 repeats in facioscapulohumeral dystrophy (FSHD)**

Weihua Zeng<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>, Jessica C. de Greef<sup>2</sup>, April Pyle<sup>3</sup>, Keith D. Robertson<sup>4</sup>, John A. Schmiesing<sup>1</sup>, Virginia E. Kimonis<sup>5</sup>, Alexander R. Ball, Jr.<sup>1</sup>, Peter Donovan<sup>1</sup>, Silvère 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, 2801A Molecular Sciences Bldg. Box 951489, Los Angeles, CA 90095-1489 USA

<sup>4</sup>Department of Biochemistry and Molecular Biology and UF-Shands Cancer Center Program in Cancer Genetics, Epigenetics and Tumor Virology, University of Florida College of Medicine, Gainesville, Florida 32610 USA

<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

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

**Melanie Ehrlich, Ph.D.**

*Human Genetics and Biochemistry, Tulane Medical School, New Orleans, Louisiana USA*

**Special DNA and Chromatin Structures in and Adjacent to D4Z4 May Contribute to Its Biological Effects**

Melanie Ehrlich, Koji Tsumagari, Chunbo Shao, Lixin Qi, Kescmic Jackson, and Desheng Chen.  
Human Genetics and Biochemistry, Tulane Medical School, New Orleans, Louisiana USA

**11:30-11:50 a.m.**

**Galina N. Filippova, Ph.D.**

*Fred Hutchinson Cancer Research Center, Seattle, Washington 98109 USA*

**Role of CTCF and chromatin structure in FSHD**

Galina N. Filippova  
Fred Hutchinson Cancer Research Center, Seattle, Washington 98109 USA

**11:50 a.m.-12:00 p.m.**

**POSTERS AND ABSTRACTS IN ABSENTIA**

*Silvère van der Maarel, Ph.D., Moderator*

*Leiden University Medical Center, Leiden, The Netherlands*

**Abnormal expression of mu-crystallin in facioscapulohumeral muscular dystrophy**

Reed PW, Corse AM, Porter NC, Flanigan KM, Bloch RJ  
Department of Physiology, University of Maryland School of Medicine, 660 W. Redwood Street, Baltimore, MD 21201, USA

**Lower limb muscles MRI findings in patients with 4q35-linked facio-scapulo-limb, type 2 muscular dystrophy (FSLD2) [(or a facioscapulo-peroneal dystrophy (FSPD)]**

Valery M. Kazakov, M.D., Ph.D., D.Sc.<sup>1</sup>, Vladislav O.Kolynin<sup>2</sup>, M.D., Dmitry I. Rudenko, M.D., Ph.D.<sup>1,2</sup>, Alexander Pozdnyakov, M.D.<sup>3</sup>

<sup>1</sup>Department of Neurology, Pavlov State Medical University of St. Petersburg, Russia

<sup>2</sup>Second Neurological Department, City Hospital 2 of St. Petersburg, Russia

<sup>3</sup>Institute of Radiology, St. Petersburg, Russia

**Study of aberrant nuclear organization in facioscapulohumeral dystrophy (FSHD) using circular chromosome conformation capture (4C)**

Kyle T. Siebenthall & Barbara J. Trask

<sup>1</sup>Division of Human Biology, Fred Hutchinson Cancer Research Center, and

<sup>2</sup>Department of Genome Sciences, University of Washington, Seattle, Washington USA

**MR imaging in facioscapulohumeral muscular dystrophy at 3T – initial experience**

H.E. Kan, T.W. Scheenen, D.W. Klomp, M. Wohlgemuth, G.W. Padberg and A. Heerschap  
Department of Radiology, Radboud University Nijmegen Medical Centre, Nijmegen  
The Netherlands

**Oral creatine supplementation does not change muscle strength, body composition or muscle biochemistry in patients with FSH dystrophy**

Graham J Kemp<sup>1,2</sup>, Bryan RF Lecky<sup>3</sup>, William E Bimson<sup>2</sup>, Graeme L Close<sup>1</sup>, Neil Roberts<sup>2</sup>, Malcolm J Jackson<sup>1</sup>

<sup>1</sup>Division of Metabolic & Cellular Medicine, University of Liverpool, Liverpool, United Kingdom

<sup>2</sup>Magnetic Resonance & Image Analysis Research Centre, University of Liverpool, Liverpool, United Kingdom

<sup>3</sup>Walton Centre for Neurology & Neurosurgery, Liverpool United Kingdom

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

**LUNCH**

(Buffet Lunch Served)

**12:40-1:40 p.m.**

**PLATFORM PRESENTATION(S) III**

*Rabi Tawil, M.D., Moderator*

*University of Rochester School of Medicine, Rochester, New York USA*

**CLINICAL AND GENETIC STUDIES**

**12:40-1:40 p.m.**

***R. Ted Abresch, M.S.***

*Department of Physical Medicine and Rehabilitation, UC Davis, Davis California 95616 USA*

**Chronic Pain in Persons Facioscapulohumeral Dystrophy and other Neuromuscular Disorders**

R. Ted Abresch

Department of Physical Medicine and Rehabilitation, UC Davis, Davis California 95616 USA

**1:00-1:20 p.m.**

***Richard JLF Lemmers, Ph.D.***

*Department of Human Genetics, Leiden University Medical Center, Albinusdreef 2, 2333 ZA, Leiden, The Netherlands*

**Specific sequence variations within the 4q35 region are associated with FSHD**

Richard JLF Lemmers<sup>1</sup>, Patrick J van der Vliet<sup>1</sup>, Mariëlle Wohlgemuth<sup>2</sup>, Kristiaan J van der Gaag<sup>1</sup>, Peter de Knijff<sup>1</sup>, George W Padberg<sup>2</sup>, Rune R Frants<sup>1</sup>, and Silvere M van der Maarel<sup>1</sup>

<sup>1</sup>Department of Human Genetics, Leiden University Medical Center, Albinusdreef 2, 2333 ZA, Leiden, The Netherlands

<sup>2</sup>Department of Neurology, University Medical Center Nijmegen, P.O. box 9101, 6500 HB, Nijmegen, The Netherlands



**1:20-1:40 p.m.**

**Meena Upadhyaya, Ph.D.**

*Institute of Medical Genetics, Heath Park, Cardiff, United Kingdom*

**A clinically well characterised FSHD family not linked to 4q35**

Meena Upadhyaya, Gill Spurlock, Ian Frayling, Mark Rogers

Institute of Medical Genetics, Heath Park, Cardiff, United Kingdom

**1:40-3:00 p.m.**

**PLATFORM PRESENTATION(S) IV**

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

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

**FRG1 FUNCTION AND GENE EXPRESSION**

**1:40-2:00 p.m.**

**Merideth L. Hanel, Ph.D.**

*Chemical and Life Sciences Laboratory, Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 USA*

**Analysis of FRG1 in *Xenopus laevis* during development**

\*Hanel, M.L., \*Wuebbles, R. and Jones, P.L.

\*authors contributed equally to the work

B107 Chemical and Life Sciences Laboratory, Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 USA

**2:00-2:20 p.m.**

**Joseph G. Marx, Ph.D.**

*National Institutes of Health (NIH), National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), Bethesda, Maryland 20892 USA*

*University of Washington, Seattle, Washington 98195 USA*

**Characterization of FRG1's RNA associated activity and its implications in FSHD pathogenesis**

Joseph G. Marx<sup>1</sup>, Xylena Reed<sup>2</sup>, Stephen D. Hauschka<sup>2</sup>, and Brian Kennedy<sup>2</sup>

<sup>1</sup>National Institutes of Health (NIH), National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), Bethesda, Maryland 20892 USA

<sup>2</sup>University of Washington, Seattle, Washington 98195 USA

**2:20-2:40 p.m.**

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

*Dipartimento di Scienze Biomediche, Universita' di Modena e Reggio Emilia, Modena, Italia*

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

## **Selective muscle involvement in facioscapulohumeral muscular dystrophy: the role of 4q35 gene expression**

Sabrina Sacconi<sup>1</sup>, Greta Fabbri<sup>2</sup>, Barbara Angeletti<sup>2</sup>, Valeria Ghiaroni<sup>2</sup>, Jean-Thomas Vilquin<sup>3</sup>, Claude Desnuelle<sup>1</sup> and Rossella Tupler<sup>2,4</sup>

<sup>1</sup>Fédération des Maladies Neuromusculaires, CHU de Nice and Inserm U638 – Nice, France

<sup>2</sup>Dipartimento di Scienze Biomediche, Università di Modena e Reggio Emilia, Modena, Italia

<sup>3</sup>Inserm U582, Institut de Myologie, Groupe hospitalier Pitié-Salpêtrière – Paris, France

<sup>4</sup>Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, Massachusetts USA

**2:40-3:00 p.m.**

***Peter Masny, Ph.D. and Sara T. Winokur, Ph.D.***

*University of Colorado, Denver, Colorado USA*

*University of California, Irvine, California USA*

## **Allele specific detection of FSHD gene expression in single nuclei**

Peter Masny<sup>1</sup>, On Ying Chan<sup>2</sup>, Ulla Bengtsson<sup>3</sup>, Jessica de Greef<sup>2</sup>, Jane Hewitt<sup>4</sup>, Melanie Ehrlich<sup>5</sup>, Yi-Wen Chen<sup>6</sup>, Alexandra Belayew<sup>7</sup>, Rabi Tawil<sup>8</sup>, Silvere van der Maarel<sup>2</sup>, Leslie Lock<sup>3</sup>, Sara Winokur<sup>3</sup>

<sup>1</sup>University of Colorado, Denver, Colorado USA

<sup>2</sup>Leiden University, Leiden, The Netherlands

<sup>3</sup>University of California, Irvine, California USA

<sup>4</sup>University of Nottingham, Nottingham, United Kingdom

<sup>5</sup>Tulane University, New Orleans, Louisiana USA

<sup>6</sup>Children's National Medical Center, Washington DC USA

<sup>7</sup>University of Mons-Hainaut, Mons, Belgium

<sup>8</sup>University of Rochester, Rochester, New York

**3:00-3:20 p.m.**

**AFTERNOON BREAK (REFRESHMENTS)**

**3:20-4:00 p.m.**

## **GROUP DISCUSSION**

*Silvère van der Maarel, Ph.D. (moderator)*

*Rabi Tawil, M.D. (moderator)*

*Rune R. Frants, Ph.D. (moderator)*

*Leiden University Medical Center, Leiden, The Netherlands*

*William R. Lewis, Sr., M.D. (moderator)*

*John Porter, Ph.D. (moderator)*

## **Identify Top 5-10 Priorities for 2008 FSHD Research Directions and Collaborations**

- 1.
- 2.
- 3.
- 4.

- 5.
- 6.
- 7.
- 8.
- 9.
- 10.

## ABSTRACTS SECTION

| <b>Abstract</b> | <b>Presenter</b>     | <b>Assignment</b>      |
|-----------------|----------------------|------------------------|
| <b>1</b>        | <b>Bosnakovski</b>   | <b>Platform</b>        |
| <b>2</b>        | <b>Hewitt</b>        | <b>Platform</b>        |
| <b>3</b>        | <b>Kyba</b>          | <b>Platform</b>        |
| <b>4</b>        | <b>Chen</b>          | <b>Platform</b>        |
| <b>5</b>        | <b>Belayew</b>       | <b>Platform</b>        |
| <b>6</b>        | <b>Magdinier</b>     | <b>Platform</b>        |
| <b>7</b>        | <b>Yokomori</b>      | <b>Platform</b>        |
| <b>8</b>        | <b>Ehrlich</b>       | <b>Platform</b>        |
| <b>9</b>        | <b>Filippova</b>     | <b>Platform</b>        |
| <b>10</b>       | <b>Reed</b>          | <b>Poster</b>          |
| <b>11</b>       | <b>Kazakov</b>       | <b>Abstract/Poster</b> |
| <b>12</b>       | <b>Siebenthal</b>    | <b>Poster</b>          |
| <b>13</b>       | <b>Kan</b>           | <b>Abstract/Poster</b> |
| <b>14</b>       | <b>Kemp</b>          | <b>Abstract/Poster</b> |
| <b>15</b>       | <b>Abresch</b>       | <b>Platform</b>        |
| <b>16</b>       | <b>Lemmers</b>       | <b>Platform</b>        |
| <b>17</b>       | <b>Upadhyaya</b>     | <b>Platform</b>        |
| <b>18</b>       | <b>Hanel</b>         | <b>Platform</b>        |
| <b>19</b>       | <b>Marx/Kennedy</b>  | <b>Platform</b>        |
| <b>20</b>       | <b>Tupler</b>        | <b>Platform</b>        |
| <b>21</b>       | <b>Winokur/Masny</b> | <b>Platform</b>        |

## **1. A small molecule screen identifies inhibitors of DUX4-mediated toxicity in myoblasts**

Darko Bosnakovski<sup>1</sup>, Shuguang Wei<sup>2</sup>, Mingju Liu<sup>1</sup>, Michael Roth<sup>2</sup>, Rita Perlingeiro<sup>1</sup>, and Michael Kyba<sup>1</sup>

<sup>1</sup>Department of Developmental Biology, UT Southwestern Medical Center, Dallas, TX 75390

<sup>2</sup>Department of Biochemistry, UT Southwestern Medical Center, Dallas, TX 75390

DUX4 is an FSHD candidate gene located within the chromosome 4q D4Z4 repeats, and we have presented evidence that DUX4 is toxic to myoblasts and induces several FSHD-associated transcriptional changes.

As a first step towards developing a targeted therapy for FSHD, we have taken advantage of the conditional toxicity of DUX4-inducible myoblasts to develop a small molecule screening platform for identifying inhibitors of DUX4. We have conducted a high throughput screen of 44,000 small molecules, a subset of the UT Southwestern HTS 200,000 compound library, using an assay based on rapid toxicity of high level DUX4 expression, which leads to myoblast death within 24 hours. We identified approximately 500 compounds with significant rescue effect (60 to >100% cell survival). Several classes of compounds revert toxicity indirectly, including antioxidants. To identify direct inhibitors, we have conducted serial follow up assays, including secondary screens for protection against oxidative stress, reversion of toxicity in other DUX4-expressing cell types, and interference with the conditional gene expression system. We present a progress report of the current status of this screen, and highlight several interesting compounds.

## **2. Evolutionary conservation of D4Z4 and implications for understanding facioscapulohumeral muscular dystrophy**

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We have taken advantage of the extensive DNA sequence data from whole genome projects to re-examine the extent of D4Z4 evolutionary conservation. This evolutionary study has identified D4Z4 orthologues in primates (Apes, Old and New World Monkeys and Lemurs) and in Afrotheria (elephants and related species). The DUX4 ORF is conserved in these species and analysis of the primate sequences indicates evidence of selection at the codon level, which is indicative of a protein-coding function.

Phylogenetic analysis suggests that primate and Afrotherian D4Z4 arrays are orthologous and originated from a retrotransposed copy of an intron-containing DUX gene, DUXC. We have also identified murine (mouse and rat) homologues of D4Z4. Comparison of the organization of all these mammalian D4Z4 loci identifies two very striking properties. First, there is maintenance of the DUX4 ORF in all the species. In contrast, apart from the ape sequences, there is little nucleotide similarity outside of this ORF even between relatively closely related species such as humans and Old or New World monkeys. Second, all of the homologues are organized as multiple copies in a head to tail arrangement, where the repeat units within an array are almost identical within a species but differ between species. Examination of genome scaffold assemblies and physical mapping data indicate that these mammalian arrays typically contain at least ten repeat units. We will also present data on the expression analysis of the mouse Dux array by RT-PCR, RNA fluorescence and tissue in situ hybridization. Exogenous expression of mouse Dux protein results in a nuclear localization pattern that is consistent with its expected function as a transcription factor. The identification of a potential homologue of D4Z4 in the mouse that is expressed and encodes a similar homeodomain protein raises the possibility of engineering a mutation that might mimic the FSHD deletions and development of in vivo and in vitro models of the disease.

### **3. Genetic interactions between DUX4 and Pax3/Pax7 suggests a stem cell etiology for FSHD**

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Facioscapulohumeral muscular dystrophy is thought to be caused by deregulation of genes in the vicinity of the D4Z4 repeats on chromosome 4. The candidate gene responsible for the FSHD phenotype and its mechanism are unknown. We use a novel genetic tool, inducible cassette exchange, to isogenetically modify myoblasts with conditional variable FSHD candidate gene expression. This screen, which encompassed all proposed FSHD candidate genes, identified only one gene with deleterious effects: DUX4, encoding a protein with two homeodomains, similar to those of the myogenic master regulators, Pax3 and Pax7. DUX4 expression recapitulates key features of the FSHD molecular phenotype, including MyoD and glutathione redox gene repression, and sensitivity to oxidative stress. Because the DUX4 homeodomains are most similar in sequence to those of Pax3 and Pax7, the muscle stem cell master regulators, we investigated potential interactions between DUX4 and Pax3/7 in myoblasts. We demonstrate competition between DUX4 and Pax3/Pax7: when either Pax3 or Pax7 is expressed at high levels, DUX4 is no longer toxic. We show that key toxicity-associated and myogenic target genes are antipodally regulated by DUX4 and Pax3/7. As Pax7 is required for maintenance of the myogenic stem cell pool, we propose that DUX4 interferes with muscle regeneration at two levels. Specifically, we propose that DUX4 expression (1) limits self-renewal of the myogenic stem cell pool leading to premature loss of regenerative ability, and (2) impairs regeneration by (a) sensitizing myoblasts to oxidative stress and (b) reducing their differentiation capacity through interference with MRF expression. This model posits that FSHD is the first example of a human muscular dystrophy with a stem cell etiology.

#### 4. Characterization of a tet-repressible muscle-specific Pitx1 transgenic mouse

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Paired-like homeodomain transcription factor 1 (PITX1) is a homeobox transcription factor, which plays a critical role in hindlimb specification during embryonic development, but its function in postnatal muscles is not known. We recently showed that the PITX1 gene was specifically up-regulated in FSHD compared to 11 neuromuscular disorders. In addition, the gene can be regulated transcriptionally by DUX4. In this study, we hypothesized that up-regulation of Pitx1 in muscles activated molecular pathways involved in muscle atrophy. Tet-repressible muscle-specific Pitx1 transgenic mice were generated by crossing Pitx1 transgenic mice (TRE-Pitx1) with transgenic mice expressing tetracycline activator driven by mouse creatine kinase promoter (mCK-tTA). The TRE-Pitx1/mCK-tTA mice were kept on doxycycline (200µg/ml) until 3 weeks old. Five weeks after withdrawing doxycycline, the mice showed significant weight loss, muscle weakness, muscle atrophy, reduction of vertical and horizontal movements comparing to single transgenic sibling (tTA). Over-expression of Pitx1 was confirmed by immunohistochemistry in myonuclei of skeletal muscles, while it was not observed in other tissues except few positive nuclei in the heart muscle. Hematoxylin and Eosin staining showed angular atrophic myofibers, necrotic myofibers and mild inflammation infiltration. The results suggest that the gene may play important role in muscle atrophy and in FSHD.



## 5. Further studies on the DUX4, DUX4c and PITX1 genes in FSHD

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Several years ago our group identified the DUX4 gene within each unit of the D4Z4 repeat array. We have demonstrated its expression at the mRNA and protein levels in FSHD myoblasts but not in controls. We have also shown that the homologous DUX4c gene mapped 42 kb of the D4Z4 array was expressed in control and FSHD myoblasts. The DUX4c protein was found at higher levels in biopsies of patients with FSHD and low D4Z4 copy number.

We performed additional studies on the DUX4 mRNA 3' ends in control and FSHD myoblasts. The products of 3'RACE experiments were cloned and sequenced, and we could only find DUX4 mRNAs in FSHD myoblasts. We found two introns 3' from the DUX4 stop codon: the first one was alternatively spliced and mapped in the D4Z4 unit, and the second one was always spliced and mapped in the pLAM region where a poly-A addition signal was used. We could amplify by RT-PCR a 1.7-kb product covering the full DUX4 ORF with a forward primer in the start codon region and a reverse primer 3' of the pLAM intron in 4 FSHD myoblast lines, with increased signal upon differentiation. Sequence analyses confirmed the DUX4 identity. These results suggested that only the DUX4 gene from the last D4Z4 unit can express a stable mRNA, by use of pLAM sequences.

We previously identified Pitx1 as a direct transcription target of the DUX4 protein in vitro. The PITX1 gene (on 5q31) is specifically up-regulated in FSHD and associated to muscle atrophy and left/right asymmetry, providing a direct link between the genetic defect in 4q35 and the pathophysiology of the disease. In order to evaluate whether DUX4 could also activate the endogenous Pitx1 gene we transfected C2C12 cells with pCIneo-DUX4 and performed a co-immunofluorescence staining of DUX4 and Pitx1. Both proteins were detected in the same nuclei 24 h post-transfection, while only background signals were observed in cells transfected with the insertless pCIneo. We similarly evaluated DUX4/PITX1 expression in FSHD primary myotubes and could detect DUX4 positive nuclei, some of which were also stained for PITX1. By Western blot, we detected high levels of 52-kDa DUX4 in muscle biopsies from 3 patients with FSHD but not 2 controls. We could only detect the PITX1 protein in a severely affected FSHD muscle biopsy.

In a functional study we transfected TE671 rhabdomyosarcoma cells with pCIneo expression vectors for either DUX4, DUX4c, DUX1 or without insert. By

immunofluorescence we observed a strong up-regulation of PCNA (proliferating cell nuclear antigen) 24h post-transfection in DUX4c expressing cells only. When we added a differentiation medium (2% horse serum), DUX4c expressing cells continued to proliferate instead of aligning to fuse into myotubes like the other transfected cells. Together with previous data showing that DUX4c specifically induced the Myf5 transcription factor, these experiments suggest a role in the maintenance of the satellite cell pool. We propose that as well an excess (in patients with low D4Z4 copy number) as a reduced amount (in families where the D4Z4 deletion removes the DUX4c gene) of DUX4c expression could affect muscle regeneration and contribute to the FSHD pathology.

## 6. CTCF as a new regulator of D4Z4 function

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Several hypotheses have been proposed in order to decipher the FSH muscular dystrophy and a link between position effect and the 4q telomeric region rearrangement is a popular hypothesis to explain the molecular mechanism of this pathology. Our goal was to test the function of D4Z4 on the regulation of telomere silencing.

Using the telomere fragmentation strategy, we reconstituted the basic genomic organization of the 4q35 locus involved in FSHD and analyzed the epigenetic effect mediated by D4Z4 in a telomeric context after integration of the transgene into the human genome. We showed that the D4Z4 subtelomeric element is a bona fide insulator element protecting from TPE and able to block enhancer-promoter communication. In order to understand the meaning of this subtelomeric rearrangement observed in patients with FSHD, we engineered additional constructs containing up to 12 D4Z4. Interestingly, when the number of repeated elements increases, the D4Z4 array facilitates telomeric position effect. Thus, multiple homologous copies of an insulator can have a repressive effect upon gene expression in mammalian systems. We showed that CTCF participates in D4Z4 insulation mechanisms

## **7. Specific loss of histone H3 lysine 9 trimethylation and HP1(gamma) /cohesin binding at D4Z4 repeats in facioscapulohumeral dystrophy (FSHD)**

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Disruption of normal epigenetic signatures, represented by an altered histone code and/or DNA methylation irregularities, are associated with various developmental disorders and cancer malignancy. Therefore, characterization of the epigenetic chromatin changes is critical for understanding the underlying mechanisms of a multitude of human diseases. Facioscapulohumeral dystrophy (FSHD) is an autosomal dominant muscular dystrophy with most cases resulting from a contraction in the number of 3.3 kb D4Z4 repeats on chromosome 4q. The molecular mechanism by which contraction of D4Z4 repeats causes FSHD remains undetermined. We found specific loss of heterochromatin marks (H3K9 methylation and HP1(gamma) /cohesin binding) at D4Z4 in FSHD cells, not only in those with D4Z4 contraction but also in the minor population with no D4Z4 contraction. The results indicate that FSHD is an “epigenetic abnormality” disease, in which the loss of H3K9 heterochromatin at D4Z4 plays a critical role in pathogenesis. Interestingly, similar changes were observed in Emery-Dreifuss and limb-girdle muscular dystrophies, suggesting that a defect(s) in a common pathway contributes to the muscular dystrophy phenotype.

## **8. Special DNA and Chromatin Structures in and Adjacent to D4Z4 May Contribute to Its Biological Effects**

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Examining D4Z4 methylation in cancers vs. control tissues, we found three unexpected patterns of CpG methylation that were associated with chromosome position or an unusual DNA sequence. The identified sequence or position features were as follows: potentially atypical secondary structures within each repeat unit, an apparently different chromatin structure at the proximal end of the D4Z4 array compared with the bulk of the array, and an unusual DNA sequence outside and proximal to the D4Z4 array. In a companion analysis, we found that chromatin immediately proximal to D4Z4 arrays (including the p13E-11 region) showed an unexpectedly large difference in DNase I sensitivity relative to D4Z4 chromatin in both FSHD and control myoblasts and lymphoblastoid cells. The p13E-11 chromatin region was not only more sensitive to DNaseI than D4Z4 chromatin, but also more sensitive than unexpressed euchromatin standards in all cells examined despite the lack of a gene-like sequence in this region. We infer a boundary-type element in the beginning of the D4Z4 array and at the immediately proximal sequence. This might result from a distortion of chromatin structure around the junction of the high (G+C) D4Z4 and the low (G+C) sequence proximal to it. We propose that an unusual chromatin structure at the proximal end of the array and atypical DNA secondary structures within each 3.3-kb repeat unit contribute to topological constraints that confer pathogenicity on short 4q D4Z4 arrays and make long ones phenotypically neutral. In addition, there might be long-distance cis interactions at 4q35.2 between similar atypical secondary structures in D4Z4, in sequences distal to the array, and in sequences proximal to it and these interactions may be relevant to FSHD.

Supported in part by NIH grant R01 NS048859 and an FSH Society Grant.

## 9. Role of CTCF and chromatin structure in FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant neuromuscular disorder linked to partial deletion of the D4Z4 repeat array within the subtelomeric region of chromosome 4q35. Although the causal relationship between deletions of D4Z4 and FSHD is well established, how this deletion triggers the disease remains unclear. Recent studies have suggested that the chromatin structure of the 4qD4Z4 region, including DNA methylation and chromatin loop organization, is affected in FSHD alleles. Several other findings, including upregulation of 10q genes in FSHD and hypomethylation of both 4q alleles in phenotypic FSHD, also point to the role of complex epigenetic interactions, including trans-allelic and inter-chromosomal interactions, in the pathogenesis of FSHD.

The known chromatin insulator protein, CTCF, has been recently implicated in mediating long-range intra- and inter-chromosomal interactions. We have identified two clusters of CTCF binding sites within the D4Z4 repeat unit and demonstrated that CTCF binds to the 4qD4Z4 region *in vivo*. Moreover, our initial studies indicated that CTCF binds to 4qD4Z4 *in vivo* in FSHD cells, but not in control cells, suggesting a role for CTCF in chromatin structuring of the 4qD4Z4 region in FSHD. Since CTCF binding is regulated by methylation, our findings suggest that epigenetic changes at the 4qD4Z4 repeats in FSHD alleles, including loss of DNA methylation, make these repeats accessible for CTCF binding, providing chromatin insulation in the region and altering long-range chromatin interactions that could account for both cis- and trans-effects in deregulation of gene expression observed in FSHD.

Collaborators: Silvere van der Maarel (LUMC, the Netherlands), Stavros Lomvardas (UCSF, USA), Stephen Tapscott (FHCRC, USA), and Barb Trask (FHCRC, USA)

## **10. Abnormal expression of mu-crystallin in facioscapulohumeral muscular dystrophy**

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To identify proteins expressed abnormally in facioscapulohumeral muscular dystrophy (FSHD), we extracted soluble proteins from deltoid muscle biopsies from unaffected control and FSHD patients and analyzed them using two-dimensional electrophoresis, mass spectrometry and immunoblotting. Muscles from patients with FSHD showed large increases over controls in a single soluble, 34 kDa protein (pI=5.08) identified by mass spectrometry and immunoblotting as mu-crystallin (CRYM. Soluble fractions of biopsies of several other myopathies and muscular dystrophies showed no appreciable increases in mu-crystallin. Mu-crystallin has thyroid hormone and NADPH binding activity and so may influence differentiation and oxidative stress responses, reported to be altered in FSHD. It is also linked to retinal and inner ear defects, common in FSHD, suggesting that its up-regulation may play a specific and important role in pathogenesis of FSHD.

Poster

## **11. Lower limb muscles MRI findings in patients with 4q35-linked facio-scapulo- limb, type 2 muscular dystrophy (FSLD2) [(or a facioscapulo-peroneal dystrophy (FSPD)]**

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The aim of this study was to describe and correlate the clinical and MRI data in different FSLD2 families' members at different stages of the disorder.

### **Patients and Methods**

We studied 16 FSLD2 patients (9 men and 7 women, 17-61 years old) from 10 families with 4q35 p13E11 EcoRI/BlnI fragment size deletion ranging between 13 and 28 kb. 5 patients were pre-symptomatic (Pr) and 11 were symptomatic with clinical facioscapulo-peroneal (FSP) phenotype (4 patients) and final facio-scapulo-peroneal-femoro (posterior thigh muscles)–gluteal (gluteus maximus) (FSPFG) phenotype (7 patients). MRI of 20 muscles bilaterally in observed patients was performed on a 1.5-tesla Siemens Magnetom Vision system using axial T1 weighted (T1W) images.

### **Results**

MRI study of Pr patients did not show any definite pathological changes of the lower limb muscles. T1W images in lower legs from patients with clinical FSP phenotype showed severe involvement of anterior compartment muscles with relative sparing of gastrocnemius and soleus in all patients, except one and with complete sparing of peroneus longus and deep posterior compartment muscles in all patients. In thighs we could see various degrees of involvement of posterior thigh muscles with sparing of sartorius, gracilis and quadriceps in all patients, except rectus femoris, which was severely involved in three patients. T1W images in lower legs from patients with clinical final FSPFG showed total involvement of the anterior compartment and gastrocnemius (medial head) muscles in all patients, and relatively milder affection of soleus in two patients with relative sparing of the peroneus longus and complete sparing of deep posterior compartment muscles in all patients. In thighs we could see severe involvement of hamstrings and adductors with sparing of quadriceps in all patients excluding rectus femoris in one patient and vastus medialis and intermedius in four patients which were partly involved more clearly on the right side. The sartorius and gracilis muscles were asymmetrical involved only in 3 patients from 7 ones.

The radiological muscle pattern does not fully correlate with clinical pattern of muscle weakness. In patients with FSP phenotype the posterior thigh muscles clinically had a normal strength, although the different degree involvement of semimembranosus, biceps femoris (long head) and semitendinosus muscles in all patients and partly affection of adductor longus and magnus in 3 patients was revealed on MRI study. As well, in all observed patients the quadriceps muscle clinically showed a normal strength, although



the total/severe involvement of rectus femoris (in 7 patients) and partly affection of vastus medialis and intermedius (in 4 patients) was revealed on MRI study. In 10 patients the different degree involvement of medial gastrocnemius and a lesser degree of soleus muscles were revealed on MRI. However, clinically these muscles had a normal strength. Asymmetry affection in muscles on the right and left side was found in 7% on MRI and 4% on manual strength muscle testing ( $p < 0,001$ ). MRI findings there were not correlated with DNA fragment size ( $r = - 0.29$ ;  $p > 0.05$ ), patient's age ( $r = - 0.42$ ;  $p > 0.05$ ), disease severity ( $r = 0.43$ ;  $p > 0.05$ ), disease duration ( $r = -0.26$ ;  $p > 0.05$ ) and manual muscle strength testing of the quadriceps, hamstrings (only in patients with FSP phenotype) and gastrocnemius (approximately  $r = -0.39$ ;  $p > 0.05$ ). From the other hand, there was a close correlation MRI finding with phenotype ( $r = 0.60$ ;  $p < 0.02$ ), daily life work disability ( $r = 0.69$ ;  $P < 0.004$ ), muscle strength of tibialis anterior ( $r = -0.59$ ) and extensor digitorum longus ( $r = -0.64$ ) as well as hamstring (only in patients with FSPFG phenotype) (approximately  $r = - 0,62$ ;  $p < 0.001$ ). Thus, our clinical and MRI data show that the 4q35-linked FSLD2, (the same as a facioscapulooperoneal muscular dystrophy) is a clinical entity with specific clinical and MRI patterns of muscle involvement.

## **12. Study of aberrant nuclear organization in facioscapulohumeral dystrophy (FSHD) using circular chromosome conformation capture (4C)**

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The genetic lesion that causes FSHD has been known for many years, but the molecular mechanism underlying this disease remains a mystery. It is becoming apparent that epigenetic phenomena are involved in FSHD pathogenesis, with differences seen in the DNA methylation in the first repeat unit and the activity of a matrix attachment region (the FRR-MAR) proximal of the D4Z4 array in FSHD cells compared to normal cells. While two groups have shown that 4q35 consistently resides at the nuclear periphery and is physically tethered to the nuclear lamina in both FSHD and normal cells, we hypothesize that the FSHD locus comes into contact with other genomic loci and impacts their regulation. In this model, the deleted locus and normal locus would have different interaction partners at the nuclear periphery. In order to test this hypothesis, we will use the FRR-MAR and first proximal D4Z4 unit as ‘bait’ in Circular Chromosome Conformation Capture (4C) assays, which capture sequences in close physical proximity in three-dimensional nuclear space. We will then characterize the captured loci using high-throughput Solexa sequencing to create a complete picture of the potential interaction space of the FSHD locus in normal and FSHD myoblasts. We will present details of our experimental plan for discussion with other workshop attendees. This is a one-year pilot study funded by the Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center, and it represents the first large-scale study of nuclear interactions of a disease locus in normal and diseased cells.

Poster

### **13. MR imaging in facioscapulohumeral muscular dystrophy at 3T – initial experience**

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#### **Background**

In patients suffering from muscular dystrophies, individual muscle involvement can be difficult to assess due to the presence of unaffected synergistic muscle groups. Magnetic resonance imaging (MRI) allows excellent non-invasive visualization of individual muscles, without the disadvantage of ionizing radiation. Apart from the assessment of individual muscle involvement, MRI can also be used in the diagnosis of specific muscular dystrophies, as many of these have characteristic muscle involvement. In many muscular dystrophies, fatty infiltration and atrophy of muscles can be detected with MRI and especially in patients suffering from inflammatory myopathies, edema is also present in skeletal muscles (2). MRI is not commonly applied in patients with facioscapular muscular dystrophy (FSHD), only 3 recent studies report MRI at a field strength of 1.5T(1, 3, 4). Dedicated imaging of muscular edema and imaging at a field strength of 3T, allowing increased resolution, has, to our knowledge, never been applied. The aim of the present study was to apply different MRI methods to detect lower leg muscle involvement in patients with FSHD at a field strength of 3T.

#### **Methods**

Seven patients with diagnosed FSHD (age  $46 \pm 17$  years) and 5 healthy age and sex matched volunteers ( $48 \pm 15$  years) were measured in a 3T magnet (Magnetom trio, Siemens Medical Solutions). A home-built, dedicated lower leg coil was used for imaging. Of all subjects images were recorded with 4 mm slices (17 slices) and a field of view (FOV) of  $135 \times 180$  mm. Three sets of images were recorded: T1 weighted (repetition time (TR) = 552 ms, echotime (TE) = 16 ms), fat suppressed T2 weighted (TR = 3430, TE = 76 ms) and TIRM (TR = 4000, TE = 41 ms, inversion time = 180 (n=4 patients) or 220 ms and FOV  $150 \times 150$  mm (n=3 patients). After the data acquisition, MRC scores for both legs and the RICCI score was obtained.

#### **Results**

Of the 7 patients, 5 showed marginal to severe muscle involvement on T1 weighted images, denoted by an increased signal intensity in one or more muscles (fig 1B-D)). The two patients that did not show muscle involvement had normal MRC scores of the measured leg. Most affected muscles were the medial gastrocnemius muscle (4/5) tibialis anterior muscle (4/5) and EDL (4/5). Hyperintensity due to edema was visible in two patients on T2 weighted or TIRM. Interestingly, multi slice T1 weighted imaging indicated that some muscles are non-uniformly affected, both in the sagittal as in the longitudinal direction.

## Conclusion and discussion

Our results show that T1 weighted muscle MRI is a valuable technique in the characterization of individual muscle involvement in patients with FSHD. Possibly, the technique could be useful in identifying pathophysiological mechanisms, like the non-uniform distribution of fatty replacement of muscles. Muscular edema was visible in 2 from the 5 affected patients, indicating that T2 weighted or TIMR imaging provides a useful addition to the imaging protocol. In the future, correlations with MRI findings and MRC scores will be performed, hoping to arrive at biomarkers for clinical studies.

## References

[1] Iosa M et al, Clin Biomech (Bristol, Avon), 2007.[2] Mercuri E et al, J Magn Reson Imaging 25: 433-440, 2007.[3] Olsen DB et al, J Neurol 253: 1437-1441, 2006.[4] Sookhoo S et al, Clin Radiol 62: 160-165, 2007.

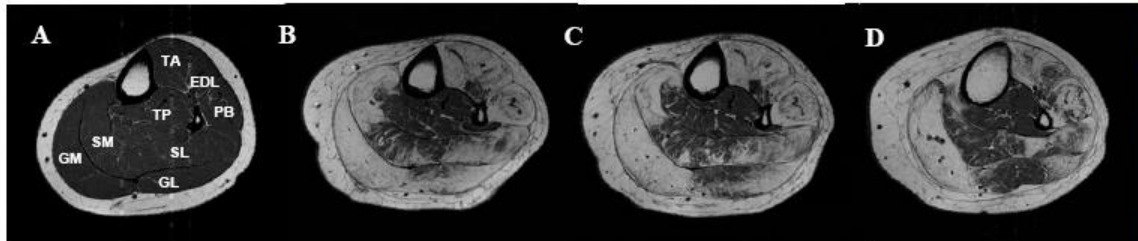


Figure 1. T1 weighted images of the proximal part of the calf in a healthy volunteer (A) and of a moderately affected FSHD patient (B – D, 3 slices from the distal to the proximal end of the upper calf). The acquisition time of this scan is less than 2 minutes and it allows excellent visualization of the different muscles. Note the non-uniform distribution of the affected muscles in the patient, both in the sagittal as well as in the longitudinal direction (mainly the medial gastrocnemius). Abbreviations: TA = tibialis anterior; EDL = extensor digitorum longus; PB = peroneus brevis; TP = tibialis posterior; SM, SL = soleus medialis and lateralis; GM, GL = gastrocnemius medialis and lateralis.

## **14. Oral creatine supplementation does not change muscle strength, body composition or muscle biochemistry in patients with FSH dystrophy**

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### **Aim**

To examine the effects of oral creatine supplementation on markers of ROS (reactive oxygen species) damage and defence mechanisms, body composition and muscle strength in facioscapulohumeral dystrophy (FSHD).

### **Background**

Creatine supplementation has been reported to increase strength in some muscular dystrophies, but this has not been specifically examined in FSHD. There has been interest in ROS mechanisms in this and other dystrophies, and creatine-mediated enhancement of mitochondrial function is a possible beneficial mechanism.

### **Methods**

4 female and 7 male genetically confirmed FSHD patients aged 34-64 y received 5g/d creatine orally for 3 months. They were assessed before and after by spirometry; manual muscle testing and quantitative isometric strength testing (grip, neck, elbow, shoulder, hip, knee, ankle); time to travel 30 feet; SF-12; body composition assessment by body mass, bioimpedance and quantitative MRI; plasma creatinine and liver function tests; and in biceps muscle biopsy, markers of ROS damage (malondialdehyde) and protective mechanisms (reduced and total glutathione, catalase, superoxide dismutase and glutathione peroxidase) and total creatine content. One subject withdrew.

### **Results**

Creatine supplementation had no significant effect except to increase plasma creatinine by 7% ( $P < 0.05$ ). In particular there was no significant change in muscle strength or muscle cross-sectional area, in spirometry, bioimpedance measurements and quality of life or in ROS measures. There was no significant change in muscle creatine content ( $175 \pm 50$  vs  $150 \pm 50$  mmol/kg protein,  $P = 0.7$  by paired t-test). Of technical interest is the strong correlation ( $r = 0.96$ ) between aggregate MRI measures of muscle cross-sectional area and average isometric strength, which both correlate ( $r = 0.7$ ) less well with fat-free mass by bioimpedance

### **Conclusion**

Creatine supplementation at 5 g daily for 12 weeks had no effect on muscle mass or strength in FSHD, probably because of failure of muscle creatine uptake. It had no effect on measures of ROS damage and defence. Similar lack of clinical effect of creatine

supplementation in myotonic dystrophy is also likely due to defective muscle creatine uptake (Tarnopolsky et al. (2004) Muscle Nerve 29: 51-58). Further study of muscle creatine uptake in muscle disease is desirable.

Funded by an FSH Society Roberts Foundation Nutrition Research Grant

## **15. Chronic Pain in Persons Facioscapulohumeral Dystrophy and other Neuromuscular Disorders**

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### **Introduction**

Recent preliminary research suggests that pain may be a significant problem for many persons with FSHD. For example, Bushby et al. recently reported on four individuals with FSHD who identified pain as their most disabling symptom and complained of between three to seven separate pain complaints. In addition, our group found that 83% of a sample of 811 individuals with various neuromuscular diseases (NMDs), including 64 persons with FSHD, reported at least some ongoing pain problems. Moreover, the frequency and severity of pain in their combined sample of patients with FSHD, MMD, and a sample of patients with limb-girdle syndrome was significantly greater than levels of pain reported by the general US population. In a more recent our group recently surveyed 193 individuals with a variety of NMDs, including 18 patients with FSHD and 26 patients with MMD, and found that 73% of the sample as a whole (89% of patients with FSHD and 69% of those with MMD) reported pain problems, with 27% of the overall sample reporting severe pain (19% of patients with FSHD and 50% of patients with MMD). We found that pain was reported to interfere moderately with a number of activities of daily living across all of the NMD diagnostic groups (range of interference ratings, 2.6 to 4.63 on 0 – 10 interference ratings scales) and to occur all over the body (least common, abdomen/pelvis at 16%; most common, back at 49%). However, we were unable to examine pain interference, pain sites, and pain treatments as a function of diagnostic group due to the low sample sizes of the individuals NMD diagnostic groups in our previous study.

Although the preliminary findings from our group and others indicate that chronic pain can be a serious problem for many persons with FSHD, much remains unknown about the nature and scope of pain in these patient populations. Importantly, most of the research on pain that has been performed with patients with FSHD has reported findings from a mixed population of patients with limited sample sizes for particular diagnoses. This limits both the reliability and generalizability of the available findings. Descriptive analyses regarding pain with larger samples of patients with specific diagnoses would provide for greater reliability of the findings, and would allow us to confirm (or question) previously published data concerning pain in patients with these conditions. Moreover, because FSHD is a progressive disease, it is possible that the onset of pain, and the severity of pain once it develops, is related to a patient's age. This study sought to address the need for more information about the nature and scope of pain in persons with FSHD and myotonic muscular dystrophy

### **Methods**

Retrospective, cross sectional survey performed using a community-based survey. Participants were recruited from the NIH-funded National Registry of Myotonic Dystrophy and Facioscapulohumeral Muscular Dystrophy Patients and Family Members

(n=296); the University of Washington NMD Clinic list (n=87); the Quality of Life Pediatric Survey Study (n=8); and four participants who independently contacted study personnel. A total of 296 potential subjects with MMD or FSHD contacted us. Of these, 235 (93%) completed and returned a mail survey questionnaire on the nature and scope of their pain. The survey included questions asking about demographic information, NMD-related information, pain intensity, pain interference, pain location, and pain treatments. All participants provided basic demographic information about their gender, age, race/ethnicity, educational level, marital and employment status. They also provided information about their NMD diagnosis, including approximate date of diagnosis, type of physician who made the diagnosis, whether or not they had received a DNA confirmation of diagnosis, and their use of assistive devices for ambulation.

Average pain intensity over the past week was assessed using an 11-point numerical rating scale (0="no pain" to 10="pain as bad as could be") taken from the Grading of Chronic Pain scale (GCP). Pain interference with daily activities was assessed using a 12-item interference scale adapted from the Brief Pain Inventory Pain Interference scale (BPI).<sup>19</sup> Participants were asked to indicate whether or not they experience bothersome pain in one or more of 17 specific body sites (head, neck, shoulders, upper back, lower back, arms, elbows, wrists, hands, buttocks, hips, chest, abdomen/pelvis, legs, knees, ankles and feet). Participants were asked to indicate if they were currently using or had ever used any of 25 specific pain treatments (physical therapy, nerve blocks, biofeedback/relaxation training, acupuncture, magnets, massage, hypnosis, counseling/psychotherapy, mexiletine, neurontin, tricyclic antidepressants, narcotics/opioids, acetaminophen, aspirin/ibuprofen, valium, tegretol, baclofen, TENS units, anticonvulsants, chiropractic adjustments, heat, ice, marijuana, strengthening exercises or range of motion exercises).

## **Results/Discussion**

More individuals with FSHD (82%) than with MMD (60%) reported pain. The most frequency pain sites for both diagnostic groups were lower back (66% MMD, 74% FSHD) and legs (60% MMD, 72% FSHD). Moreover, the average pain severity reported in patients with FSHD in our sample (4.40 out of 10 in the current sample) and percent of patients with FSHD who report severe pain (23% in the current sample) also replicate previous findings. These pain problems are chronic with a mean duration of pain being 11-13 years in our samples. This finding, when considered in light of both the high frequency of pain in general, and the existence of subgroups of patients (about 25% in both samples) who report severe pain, underscores the need to identify and provide effective pain treatments for patients with these neuromuscular diseases.

Both FSHD and MMD patients endorsed generally similar levels of interference of pain with functioning, although there was a slight trend for patients with MMD (range of interference ratings, 2.14 to 4.17/10) to report higher levels of interference with some activities than patients with FSHD (range, 1.14 to 3.65/10). Pain was reported to have a moderate degree (3.73 and 3.53/10) of interference with enjoyment of life. Moreover, the strength of the associations found between pain severity and interference with the life activities tended to be strong (correlation coefficients greater than .50 for six of the 12 activities; the correlation coefficient was never less than .30). Modern biopsychosocial



pain rehabilitation treatments focus not only on the pain itself, but also on the extent to which pain interferes with function. The significant pain interference reported by the patients in this study, when considered in light of the multi-domain focus of contemporary pain treatments, raises the possibility that patients with neuromuscular disease and chronic pain might benefit from pain rehabilitation approaches. Overall, the sites of pain reported by these patients reflect the body areas that are commonly affected by these MDs (e.g., low back and legs as most common, chest, buttocks, and head as relatively less common). The most frequent pain site for both diagnostic groups was the low back. This reflects the fact that low back pain is a common site of pain in the able-bodied adult population. In both FSHD and MMD the degree of back pain may be exacerbated by the fact that the trunk and neck flexors are among the weakest muscle groups in both of these disorders. Moreover, in both diseases there is a significant imbalance between the extensors and flexors of the neck and the trunk. As the individuals become weaker, the biomechanical stresses are increased and pain may become more pronounced. This is supported by the fact that subjects with FSHD reported a significantly older age at which pain began in their hands and ankles compared to the subjects with MMD.

No single treatment for pain has been shown to be widely effective for subjects with FSHD and MMD. No treatment was currently used by more than 46% of all of the patients reporting pain, or by more than 42% of the patients reporting severe pain. The most common treatments were ibuprophen or aspirin (used by 46% of patients with pain), acetaminophen (used by 34%), and strengthening exercises (used by 29%). Of those treatments that had been tried, the most effective (rated as providing at least 5/10 relief) were ibuprophen/aspirin, opioids, massage, chiropractic manipulation, nerve blocks, heat and marijuana. However, it should also be noted that many of these treatments also have significant drawbacks. For example, opioids, which were rated as the most effective (6.49/10) in this sample, had been tried by 25% of the sample, but were only currently being used by 8% of the sample. This data suggests that the pain relief gained from the opioids did not outweigh their side effects (grogginess and constipation) when taken at the doses required to provide substantial relief. Similarly, marijuana, although reported to be highly effective (6.00/10), was still used by only a little over half of the patients who had tried it (4% of the sample using, 7% had tried). The significant side effects (such as decreased motivation) and significant problems with access may decrease the desirability of this treatment. The other treatments that were rated as being relatively highly effective tend to be short lasting. This may explain the fact that many of the patients who had ever tried massage, chiropractic manipulation, and nerve blocks no longer receive these treatments. The only treatment that was relatively highly effective and was still being used by a substantial number of patients (26%) was heat. Perhaps this is because heat is an extremely accessible treatment (most people own a hot water bottle or heating pad) that has few, if any, negative side effects. Overall, the findings suggest that there remain too few options for pain relief for patients with MMD and FSHD and chronic pain. There is a substantial need for the development of effective and long-lasting pain treatments for persons with NMD and FSHD that can be made easily available and that have few negative side effects.

Pain is likely related, at least in part, to fatigue. Our results are consistent with a recent study of NMD patients that included 139 subjects with FSHD and 322 subjects with MMD. Severe fatigue was reported by 61-74% of these patients and the severity of the fatigue was correlated with an increase in the number of problems with physical functioning, mental health, and bodily pain. Although the causal relationship is not clear, it is likely that physical disability leads to both pain and fatigue conjointly, but chronic pain would certainly worsen fatigue symptoms.

### **Conclusion**

The findings from this study indicate that pain is a common problem in both FSHD and MMD, with the majority of adults with these conditions reporting pain. The most frequent pain sites for both diagnostic groups were lower back and legs. Significant differences between diagnostic groups in frequency of pain at specific sites were found in shoulders, hips and feet, with participants with FSHD reporting pain more often in their shoulders and hips, and participants with MMD reporting pain more often in their feet and hands. These findings highlight the need to identify and provide effective pain treatments for patients with FSHD and MMD. Future work needs to address chronic pain in a variety of other neuromuscular diseases.

## 16. Specific sequence variations within the 4q35 region are associated with FSHD

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FSHD is caused by contraction of the macrosatellite repeat D4Z4 on chromosome 4q35. The D4Z4 repeat is very polymorphic in length, and D4Z4 rearrangements occur almost exclusively via intrachromosomal gene conversions. Several disease mechanisms have been proposed, but none of these models can comprehensively explain FSHD, because repeat contraction alone is not sufficient to cause disease. Almost identical D4Z4-repeat arrays have been identified on chromosome 10q26 and on two equally common chromosome 4 variants, 4qA and 4qB. Yet only repeat contractions of D4Z4 on chromosome 4qA cause FSHD; contractions on the other chromosomes are nonpathogenic. We hypothesized that allele-specific sequence differences among 4qA, 4qB, and 10q alleles underlie the 4qA specificity of FSHD. Sequence variations between these alleles have been described before, but the extent and significance of these variations proximal to, within, and distal to D4Z4 have not been studied in detail. We examined additional sequence variations in the FSHD locus, including a relatively stable simple sequence-length polymorphism (SSLP) proximal to D4Z4, a single-nucleotide polymorphism (SNP) within D4Z4, and the A/B variation distal to D4Z4. On the basis of these polymorphisms, we demonstrate that the subtelomeric domain of chromosome 4q can be subdivided into nine distinct haplotypes, of which three carry the distal 4qA variation. Interestingly, we show that repeat contractions in two of the nine haplotypes, one of which is a 4qA haplotype, are not associated with FSHD. We also show that each of these haplotypes has its unique sequence signature, and we propose that specific SNPs in the disease haplotype are essential for the development of FSHD. This genotype study was performed on individuals of European descent. To obtain information on the flow and evolution of the different 4q haplotypes we extended this study to a worldwide panel. Analysis of the 4q SSLP showed that individuals from African descent displayed all haplotypes that have been identified in the Caucasian population, albeit with a different distribution. The presence of all haplotypes in the African population suggests that these haplotypes arose before the modern humans migrated out of Africa. Furthermore, 4qA-type haplotypes seem to be more abundant among the Africans, while Amerindians almost exclusively seem to carry D4Z4 alleles that belong to the 4qB haplogroup. The different distribution of 4q haplotypes among the ethnic groups may have important implications for the worldwide distribution of FSHD.

## 17. A clinically well characterised FSHD family not linked to 4q35

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Facioscapulohumeral muscular dystrophy (FSHD) is the commonest form of inherited muscular dystrophy, affecting 1 in 10,000 individuals. The condition is characterised by progressive weakness and atrophy of the musculature of the face, upper arms and shoulder girdle. One in five cases are due to newly arisen mutations. The FSHD gene locus (FSHD1A) maps to the long arm of chromosome 4 (4q35). In the majority of FSHD cases, the molecular basis of the disease is identified as a large deletion within the D4Z4 repeat arrays, although the precise genetic basis for the disease is still unknown. In >95% of FSHD cases, a contraction of the D4Z4 repeat below a threshold of 11 units when located on a 4qA allele is associated with disease. Several FSHD families unlinked to 4q35 have been reported and a provisional linkage to chromosome 15 was reported, however, on subsequent molecular analysis this finding proved to be untenable because the presence of proximal extended deletions encompassing probe p13E11 in these patients prevented the identification of contracted allele. We now report a three-generation FSHD family with the bona-fide clinical features of FSHD but in which the FSHD locus is not linked to 4q35. Almost all the affected individuals in this family had facial muscle weakness, difficulty in raising arms, exhibited asymmetry of the face, shoulder girdle, scapular or limb muscle and scapular winging. An extensive analysis of skeletal muscle-specific proteins in the proband failed to detect any abnormality. FSHD diagnostic molecular analysis with probe p13E11 identified a BlnI-sensitive (10q derived) 17 kb fragment. The BlnI resistant fragment in each affected individual was >48 kb. The presence of a genomic deletion involving the probe p13E11 region was excluded with Hind III/probe 4qA analysis. The family was further analysed with probes 4qA and 4qB and there was no direct association of probe 4qA with the disease expression. This family would appear to represent an evidence of second FSHD-associated locus unlinked to 4q35. A genome-wide linkage analysis may identify the location of a potential second FSHD locus in this family and may help to further define the molecular pathogenesis of FSHD.

## 18. Analysis of FRG1 in *Xenopus laevis* during development

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Facioscapulohumeral region gene 1 (FRG1) is a leading candidate gene for FSHD. Localized to the FSHD region 100 kb centromeric to the 4q35D4Z4 repeats, it is proposed that the contraction of the D4Z4 repeat array leads to altered expression levels of FRG1 protein. While FRG1 is very highly conserved evolutionarily between humans and other organisms, the normal function of FRG1 protein during vertebrate muscle development is still unknown. In order to better understand the normal function of FRG1 we have cloned and analyzed the expression of the FRG1 homolog in the African clawed frog *Xenopus laevis*, a model organism well suited for studying muscle development. Although *Xenopus* is a vertebrate, all of *Xenopus* embryonic development is external and amenable to analysis of embryonic events. In addition, analysis of muscle development is aided by the fact that myogenesis in *Xenopus* is separated with primary myogenesis occurring in the tadpole and later secondary myogenesis occurring during metamorphosis.

We have analyzed the temporal and spatial expression pattern by RT-PCR and whole mount in situ hybridization. *Frg1* mRNA is expressed in egg and during early development throughout primary myogenesis, and is downregulated at late tadpole stages after primary muscle differentiation is largely complete. In order to address function we performed over-expression and morpholino knock down of *Frg1* in the developing tadpole. Our analysis has provided insight into the role of FRG1 in a developing vertebrate organism and suggests that disruption of normal FRG1 expression may in fact lead to the muscle deterioration seen in FSHD, possibly a result of an inability of muscle precursors to repair and regenerate normal muscle.

## 19. Characterization of FRG1's RNA associated activity and its implications in FSHD pathogenesis

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Facioscapulohumeral muscular dystrophy (FSHD) is a muscular dystrophy characterized by the progressive muscle weakness in the face, back, and arms. FSHD usually appears around puberty and exhibits a stepwise decline throughout the patient's lifespan. Patients typically have a normal lifespan; however, their quality of life is significantly impacted. The cause of FSHD is due to the loss of repeated DNA elements (termed D4Z4 repeats) on the subtelomeric region of 4q. D4Z4 repeats are 3.2kb in length and are believed function as a heterochromatin nucleation site; unaffected individuals have between 11-100+ D4Z4 repeats but unaffected individuals have less than 10. While D4Z4 repeat loss is clearly associated with the disease, how their loss results in FSHD is unclear. The leading hypothesis suggests an epigenetic mechanism. D4Z4 repeats recruit heterochromatin factors, which likely silence nearby (and probably distal) genes. In FSHD, reduced D4Z4 repeats presumably leads to reduced heterochromatin formation and thus up-regulation of silenced genes. However, which gene(s) are affected is unknown. One leading candidate is a nearby uncharacterized protein termed FSHD region gene 1 (FRG1). Studies suggest FRG1 maybe increased in FSHD patients and mice over-expressing FRG1 develop muscular dystrophy; however, the function of FRG1 is unknown.

In our preliminary studies, we found FRG1 interacts with heterologous ribonucleoprotein U (hnRNP-U). hnRNP-U is involved in a wide range of activities ranging from transcription to mRNA stability and splicing. As both FSHD patients and FRG1 transgenic animals have alternative splicing defects, we focused on RNA associated functions of hnRNP-U. Utilizing an RNA immunoprecipitation procedure, we found, similar to hnRNP-U, FRG1 could bind specific RNA transcripts. We are currently testing whether this binding is direct and if this interaction affects RNA stability and protein levels. We hypothesize that FRG1 results in muscular dystrophy by altering key RNA processing events in muscle.

## 20. Selective muscle involvement in facioscapulohumeral muscular dystrophy: the role of 4q35 gene expression

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We previously described the over-expression of three genes, ANT1, FRG1 e FRG2, located at 4q35 in FSHD affected muscles. However, three other laboratories failed to detect any over-expression of these candidate genes in FSHD muscle. Since different experimental designs were applied, one could imagine various reasons for conflicting data on expression of those genes, such as, sample variability or a multiplicity of FRG1-like genes. We undertook an experimental procedure aimed to clarify conflicting data and to establish an adequate procedure to analyze the expression of 4q35 genes.

From what we know about FSHD, it is clear that the selection of muscle specimens to be used for studying muscle gene expression is crucial. In FSHD, not all muscles are affected, asymmetry of muscle weakness is often observed, and there are gender differences in age of onset and clinical progression. Furthermore, the penetrance of the disease is age-dependent and also related to the number of repeated elements: more severe cases appear to be associated with a lower number of D4Z4 repeats. It is also known that not all body skeletal muscles are equal, due to differences in architecture and fiber type composition. Finally, expression profile variability is a known feature of muscle tissue, and a different site of the biopsy might generate discrepancies in 4q35 gene expression.

In order to understand the basis of different expression results obtained, we standardized the samples for our study on the basis of the following criteria:

- a) Clinical expression of the disease. Two sets of muscle specimens were obtained from patients presenting null involvement of leg muscles or from patients with affected limb girdle muscles.
- b) Length of the tandemly repeated D4Z4 sequence. As previously described, the level of expression of FSHD inversely correlates with number of repeated elements. Thus the repeat length was considered in our analysis.
- c) Sex. In FSHD males are more affected than females. As first step we decided to analyze muscle samples obtained from male FSHD patients.
- d) Site of biopsy. To investigate whether specific muscle might respond differently to the effect of D4Z4 deletion, we selected specimens originating from the vastus lateralis muscle and subscapularis muscle. Histological examination of muscle samples was performed.
- e) Healthy Controls. We carefully selected sex- and age-matching control specimens obtained from healthy donor.

Myoblasts were isolated and cultured from selected muscle specimens. Cells obtained from vastus lateralis and subscapularis muscle biopsies from FSHD patients and matched controls were selected and CD56 positive cells seeded in proliferation medium. Expression of FRG2, FRG1 and ANT1 genes was analyzed by real-time PCR. The number of D4Z4 repeat was assessed on peripheral blood cells at the time of patients diagnosis, then on cell culture at the highest passage we underwent biological and molecular studies (P8). No difference within these two values was evident. These determinations were done in order to exclude the possibility of somatic mosaicism. Control cell cultures were also tested to exclude the presence of D4Z4 deletion in healthy control subjects.

Results of this study indicate that myoblasts obtained from clinically and histologically affected territories are more susceptible to the alteration control of gene expression associated with D4Z4 contraction when compared to myoblasts issued from unaffected territories.



## 21. Allele specific detection of FSHD gene expression in single nuclei

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by epigenetic alterations in 4qter chromatin resulting from contraction of the D4Z4 repeat array and associated DNA polymorphisms. The precise mechanism by which deletions of D4Z4 influence gene expression in FSHD is as yet unresolved. Regulatory models include a cis effect on proximal gene transcription (position effect), DNA looping, and nuclear localization / trans effects. In order to directly test whether deletions of D4Z4 affect gene expression in cis, we investigated nascent RNA transcription in single nuclei so that we could measure expression from each allele independently. FSHD and control myotubes (differentiated myoblasts) were subjected to sequential RNA-DNA FISH. Sixteen genes in the FSHD region (DUX4, FRG2, TUBB4Q, FRG1, FAT, KLK3/FXI, CYP4V2, TLR3, SORBS2, PDLIM3 (ALP), LRP2BP, SNX25, SLC25A4 (ANT1), HELT, CASP3 and IRF2) and were examined independently by RNA-FISH, and subsequently with DNA-FISH using a unique chromosome 4q35 probe so that nascent RNA emanating specifically from the 4q alleles was detected. A D4Z4 probe was labeled with a third fluorochrome in order to identify the contracted and normal alleles. We designed a novel software program, Census, in order to capture and quantitate allele-specific gene expression in single nuclei. Our data is not consistent with an altered cis effect on RNA transcription in FSHD myotubes, even for those genes in which such an effect has been proposed. As there are many indications that FSHD involves a developmental component, we have initiated gene expression analysis in embryonic stem cells (hESCs). Expression of several genes implicated in FSHD, including DUX4, FRG1 and PITX1, were detected in hESCs by immunofluorescence. Results of these expression studies will be presented, and include the nuclear localization of the FSHD region in the earliest stages of human development.