

# Facioscapulohumeral Muscular Dystrophy [FSHD] International Research Consortium 2008

Tuesday, November 11, 2008  
7:30 a.m. – 5:00 p.m.

Philadelphia Marriott® Downtown  
1201 Market Street, Philadelphia, Pennsylvania 19107 USA  
Salons A and B

**Co-Chairs:** Silvère van der Maarel, Ph.D.  
Kathryn Wagner, M.D., Ph.D.

**Organizers:** Daniel Paul Perez  
Silvère van der Maarel, Ph.D.  
Kathryn Wagner, M.D., Ph.D.



## Sponsored By:

FSH Society, Acceleron Pharma, Association Française Contre les Myopathies (AFM), NIH Sen. Paul Wellstone BBRI Muscular Dystrophy Cooperative Research Center, Muscular Dystrophy Association (MDA), Fields FSHD Center, and, Athena Diagnostics

**November 11, 2008**

## **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 60 people have registered for this workshop making this the “place to be” for anyone with a keen interest in FSHD. At the end of the day, we will hold a round table discussion to discuss the future needs of FSHD. We hope for a thoughtful and productive session 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. NIH Sen. Paul Wellstone BBRI FSHD Muscular Dystrophy Cooperative Research Center, the Association Française Contre les Myopathies (AFM), the Muscular Dystrophy Association (MDA), Acceleron Pharma, the Fields FSHD Center, 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.  
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](mailto:daniel.perez@fshsociety.org), website: <http://www.fshsociety.org>*

**7:30-7:55 a.m.**

**REGISTRATION & CONTINENTAL BREAKFAST**

**7:55-8:00 a.m.**

**CO-CHAIRS OPENING REMARKS & WELCOME**

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

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

***Silvère van der Maarel, Ph.D.***

*Leiden University Medical Center, Leiden, the Netherlands*

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

**KEYNOTE(S) & CHARGE TO THE MEETING ATTENDEES**

***Daniel Paul Perez***

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

***Jane Larkindale, Ph.D.***

*Muscular Dystrophy Association, Tucson, Arizona USA*

***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*

**8:30-9:45 a.m.**

**PLATFORM PRESENTATIONS I**

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

*Leiden University Medical Center, Leiden, The Netherlands*

**D4Z4 & DOWNSTREAM TRANSCRIPTS**

**8:30-8:45 a.m.**

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

*Department of Pediatrics and Lillehei Heart Institute, University of Minnesota, 312 Church Street SE, Minneapolis, Minnesota 55455 USA*

**DUX4 in Cells and Mice**

Darko Bosnakovski and Michael Kyba\*

Department of Pediatrics and Lillehei Heart Institute, University of Minnesota, 312 Church Street SE, Minneapolis, Minnesota 55455 USA

**8:45-9:00 a.m.**

***Stephen Tapscott, M.D., Ph.D.***

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

## **The Transcriptional and Post-transcriptional Landscape of the D4Z4 Region**

Amy Asawachaicharn, Lauren Snider, Ashlee Tyler, Linda Geng, Lisa M Petek, Dan Miller, Lisa Maves, Richard Lemmers, Sara Winokur, Rabi Tawil, Galina Filippova, Silvere van der Maarel, Stephen Tapscott  
Fred Hutchinson Cancer Research Center, Seattle, Washington 98109 USA

**9:00-9:15 a.m.**

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

*Center for Genetic Medicine Research, Children's National, Medical Center, Washington DC  
Department of Pediatrics, George Washington University, Washington DC USA*

## **Conditional Expression of PITX1 in Mouse Skeletal Muscles Causes Muscle Atrophy**

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

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

<sup>2</sup>. Department of Pediatrics, George Washington University, Washington DC USA

<sup>3</sup>. Center for Neuroscience Research, Children's National, Medical Center, Washington DC USA

**9:15-9:30 a.m.**

***Davide Gabellini, Ph.D.***

*Stem Cell Research Institute, DIBIT-HSR, Milano, Italy*

## **Transcription of the Region Proximal to D4Z4 could Regulate 4q35 Gene Expression in FSHD**

Daphne S. Cabianca, Paola Picozzi and Davide Gabellini  
Stem Cell Research Institute, DIBIT-HSR, Milano, Italy

**9:30-9:45 a.m.**

***Scott Q. Harper, 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 43205 USA*

## **DUX4 Over-Expression Causes Muscle Toxicity In Vivo**

Lindsay Wallace<sup>2</sup>, Sara Garwick<sup>3</sup>, 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

**9:45-10:00 a.m.**

**MORNING BREAK**

**10:00-11:00 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

**10:00-10:15 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, Amina Boussouar, Andrea Förster, Eric Gilson & Frédérique Magdinier

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

**10:15-10:30 a.m.**

**Yvonne D. Meijer-Krom, Ph.D.**

*Fred Hutchinson Cancer Research Center, Seattle, Washington USA  
Leiden University Medical Center, Leiden, 2333 ZA, The Netherlands*

### **Role of CTCF and Chromatin Structure in FSHD**

Natalia A. Rabaia<sup>1</sup>, Yvonne D. Krom<sup>1,2</sup>, James M. Moore<sup>1</sup>, Jessica de Greef<sup>2</sup>, Richard J. Lemmers<sup>2</sup>, Stephen J. Tapscott<sup>1</sup>, Silvere van der Maarel<sup>2</sup>, Galina N. Filippova<sup>1</sup>

<sup>1</sup>Fred Hutchinson Cancer Research Center, Seattle, Washington 98109, USA

<sup>2</sup>Leiden University Medical Center, Leiden, 2333 ZA, The Netherlands

**10:30-10:45 a.m.**

**Xueqing Xu, Ph.D.**

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

### **Facioscapulohumeral Muscular Dystrophy: Gene Discovery by DNase-chip**

Xueqing Xu<sup>1</sup>, Koji Tsumagari<sup>1</sup>, and Greg Crawford<sup>2</sup> and M. Ehrlich<sup>1</sup>

<sup>1</sup>Human Genetics, Tulane Medical School, New Orleans, Louisiana 70112 USA

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

**10:45-11:00 a.m.**

**Silvère M. van der Maarel, Ph.D.**

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

### **Comparative Methylation Analysis of the D4Z4 Repeat Array on Chromosome 4q35 and 10q26**

Jessica C de Greef<sup>1</sup>, Richard JLF Lemmers<sup>1</sup>, Rabi Tawil<sup>2</sup>, Rune R Frants<sup>1</sup>, Silvere M. van der Maarel<sup>1</sup>

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

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

**11:00-11:45 a.m.**

## **PLATFORM PRESENTATIONS III**

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

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

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

*Leiden University Medical Center, Leiden, The Netherlands*

## FSHD CENTERS UPDATES

**11:00-11:15 a.m.**

**Louis M. Kunkel, Ph.D.**

*Children's Hospital Boston and Harvard Medical School, Boston, Massachusetts USA*

*Howard Hughes Medical Institute, Children's Hospital Boston, Boston, Massachusetts USA*

### **Biomarkers for Therapy of FSHD**

Charles P. Emerson, Jr.<sup>1</sup>, Kathryn R. Wagner<sup>2</sup>, Mayana Zatz<sup>3</sup>, Robert J. Bloch<sup>4</sup>, Woodring E. Wright<sup>5</sup>, Jeffrey Boone Miller<sup>1</sup>, Daniel Paul Perez<sup>6</sup>, Louis M. Kunkel<sup>7,8</sup>

<sup>1</sup> Boston Biomedical Research Institute, Watertown, Massachusetts USA

<sup>2</sup> The Johns Hopkins University School of Medicine, Baltimore, Maryland USA

<sup>3</sup> Universidade de Sao Paulo, Sao Paulo, Brazil

<sup>4</sup> University of Maryland School of Medicine, Baltimore, Maryland USA

<sup>5</sup> The University of Texas Southwestern Medical Center, Dallas, Texas USA

<sup>6</sup> FSH Society, Inc., Watertown, Massachusetts USA

<sup>7</sup> Children's Hospital Boston and Harvard Medical School, Boston, Massachusetts USA

<sup>8</sup> Howard Hughes Medical Institute, Children's Hospital Boston, Boston, Massachusetts USA

**11:15-11:30 a.m.**

**Rabi Tawil, M.D.**

*University of Rochester Medical Center, Rochester, New York*

### **Fields Center for FSHD and Neuromuscular Research**

Rabi Tawil, M.D.

University of Rochester Medical Center, Rochester, New York

**11:30-11:45 a.m.**

**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*

### **Italian FSHD National Registry: a Tool for Genotype-Phenotype Correlation**

Costanza Lamperti<sup>a</sup>, Greta Fabbri<sup>b</sup>, Liliana Vercelli<sup>c</sup>, Roberto D'Amico<sup>d</sup>, Roberto Frusciante<sup>e</sup>, Emanuela Bonifazi<sup>b</sup>, Chiara Fiorillo<sup>f</sup>, Carlo Borsato<sup>g</sup>, Maura Servida<sup>a</sup>, Francesca Greco<sup>b</sup>, Leda Volpi<sup>i</sup>, Antonio Di Muzio<sup>j</sup>, Lucia Morandi<sup>k</sup>, Carlo Pietro Trevisan<sup>l</sup>, Gabriele Siciliano<sup>i</sup>, Luca Colantuoni<sup>m</sup>, Carmelo Rodolico<sup>h</sup>, Giuliana Galluzzi<sup>m</sup>, Lucio Santoro<sup>f</sup>, Giuliano Tomelleri<sup>n</sup>, Corrado Angelini<sup>g</sup>, Enzo Ricci<sup>e</sup>, Laura Palmucci<sup>e</sup>, Maurizio Moggio<sup>a</sup>, Rossella Tupler<sup>b,o</sup>

<sup>a</sup> UOS Malattie neuromuscolari, Department of Neurology IRCCS. Fondazione Ospedale Maggiore Policlinico Mangiagalli Regina Elena, Dino Ferrari Center - University of Milan, Italy

<sup>b</sup> Department of Biomedical Sciences, University of Modena and Reggio Emilia, Italy

<sup>c</sup> Center for Neuromuscular Diseases, Department of Neuroscience, University of Turin, Italy

<sup>d</sup> Unit of Statistics, Department of Oncology and Hematology, University of Modena and Reggio Emilia, Italy

<sup>e</sup> Department of Neurosciences, Università Cattolica Policlinico A. Gemelli, Rome, Italy

<sup>f</sup> Department of Neurological Sciences, University "Federico II", Naples, Italy

<sup>g</sup> Department of Neurosciences, University of Padua, Italy

<sup>h</sup> Department of Neurosciences, Psychiatry and Anaesthesiology, University of Messina, Messina, Italy

<sup>i</sup> Department of Neuroscience, Neurological Clinic, University of Pisa, Italy

<sup>j</sup> Neuromuscular Diseases Unit, Center for Excellence on Aging, G. D'Annunzio University Foundation, Neurological Clinic, Ospedale SS Annunziata, Chieti, Italy

<sup>k</sup> Division of Neuromuscular Diseases, National Neurological Institute "Carlo Besta", Milan, Italy

<sup>l</sup> Department of Neurological and Psychiatric Sciences, University of Padua, Padua, Italy

<sup>m</sup> Molecular Genetics Laboratory of UILDM, Lazio Section, IRCCS Santa Lucia Foundation, Rome, Italy

<sup>n</sup> Department of Neurological Sciences and Vision, University of Verona, Italy

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

**11:45 a.m.-12:00 p.m.**

## **POWERPOINT PRESENTATION OF POSTERS**

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

*Leiden University Medical Center, Leiden, The Netherlands*

*Presenting: In absentia*

### **About the Facioscapulohumeral, Type 2 (or the Facioscapulohumeral) Autosomal Dominant Muscular Dystrophy in the K. Kindred Once Again**

Reexamination in 1993 of the famous K. kindred described for the first time by Oransky in 1927 then

Davidenko, Kulkova in 1938 and Kazakov et al. 1975, 1976

Clinical and molecular genetic study.

Valery Kazakov, Dmitry Rudenko

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

***Presenting: Nina Canki-Klain, M.D., Ph.D.***

*Croatian Institute for Brain Research, Zagreb University Medical School*

*Department of Neurology, Zagreb University Hospital Center, Croatia*

### **Possible Mechanisms Responsible for great Inter and Intra Familial Phenotypic Heterogeneity in FSHD**

Canki-Klain N.<sup>1,2</sup>, Zagar M.<sup>2</sup>, Lannoy N.<sup>3</sup>, Verellen-Dumoulin C.<sup>3</sup>

<sup>1</sup> Croatian Institute for Brain Research, Zagreb University Medical School

<sup>2</sup> Department of Neurology, Zagreb University Hospital Center, Croatia

<sup>3</sup> Unité de Génétique Médicale, UCL, Bruxelles, Belgique

***Presenting: Meredith L. Hanel, Ph.D.***

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

### **Investigating D4Z4 Mediated Gene Regulatory and Epigenetic Effects on FRG1 in a Telomeric Environment in the Developing Vertebrate *Xenopus Laevis***

Meredith L. Hanel and Peter L. Jones

Department of Cell and Developmental Biology, University of Illinois, Urbana-Champaign, Urbana, Illinois

***Presenting: Silvère M. van der Maarel, Ph.D.***

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

### **Comprehensive Expression Analysis of FSHD Candidate Genes Provides Evidence for Transcriptional Deregulation of FRG2 Only**

Rinse Klooster<sup>1</sup>, Kirsten Straasheijm<sup>1</sup>, Bharati Shah<sup>2</sup>, Janet Sowden<sup>2</sup>, Rune Frants<sup>1</sup>, Charles Thornton<sup>2</sup>, Rabi Tawi<sup>1,2</sup>, Silvère van der Maarel<sup>1</sup>

<sup>1</sup> Department of Human and Clinical Genetics, Medical Genetics Center, Leiden University Medical Center, Einthovenweg 20, 2333 ZC Leiden, The Netherlands

<sup>2</sup> Neuromuscular Disease Center, Department of Neurology, University of Rochester Medical Center, 601 Elmwood Ave, Rochester, New York 14642 USA

***Presenting: Julie Dumonceaux, Ph.D.***

*UMRS 787 Inserm-UPMC, Institut de Myologie, Paris, France*

### **FRG1 Over-expression Induces Severe Dystrophic Phenotype in the Adult Mouse**

Julie Dumonceaux, Solenne Marie and Luis Garcia

UMRS 787 Inserm-UPMC, Institut de Myologie, 105 Bd de l'Hôpital, 75013 Paris, France

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

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

**Mutagenesis Analysis of the DUX4 Nuclear Localization Signals NLS1 and NLS2**

Edgardo Daniel Corona and Alberto Luis Rosa

Laboratorio de Biología Celular y Molecular, Fundación Allende

Hipólito Irigoyen 384 / 8vo Piso – 5000 - Córdoba, Argentina.

*Presenting: In absentia*

**Pluripotent Stem Cells for FSHD Research**

Sara Winokur and Leslie F. Lock

Departments of Biological Chemistry and Developmental and Cell Biology

Sue and Bill Gross Stem Cell Research Center

University of California, Irvine, CA 92697

**12:00-1:15 p.m.**

**BUFFET LUNCH AND POSTER VIEWING**

**1:15-2:30 p.m.**

**PLATFORM PRESENTATIONS IV**

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

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

**CLINICAL & EXPRESSION STUDIES**

**1:15-1:30 p.m.**

**Nicolas Lévy, M.D., Ph.D.**

*Département de Génétique Médicale, Hôpital d'enfants Timone, Marseille, France*

*Inserm UMR\_S910 "Génétique Médicale et Génomique Fonctionnelle", Université de la Méditerranée,*

*Faculté de Médecine, Marseille, France*

**Direct and Simultaneous Visualization of D4Z4 Arrays on Distinct 4qA, 4qB and 10q Combed Alleles: Implication for FSHD Diagnosis and Physiopathology**

Pierre Walrafen<sup>1\*</sup>, Karine Nguyen,<sup>2,3\*</sup> Catherine Vovan<sup>2</sup>, Anne Vannier<sup>1</sup>, Nathalie Dufrane<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*

<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*

**1:30-1:45 p.m.**

**Patricia Arashiro, Ph.D.**

*University of São Paulo, São Paulo, Brazil*

**Trying to Understand the Clinical Variability in FSHD**

P. Arashiro<sup>1</sup>, I. Eisenberg<sup>2</sup>, A.T. Kho<sup>2</sup>, A. Cerqueira<sup>1</sup>, M. Canovas<sup>1</sup>, R. Pavanello<sup>1</sup>, L.M. Kunkel<sup>2</sup>, M. Zatz<sup>1</sup>

<sup>1</sup>. *University of São Paulo, São Paulo, Brazil*

<sup>2</sup>. *Harvard Medical School, Boston, Massachusetts, United States*



**1:45-2:00 p.m.**

**Melanie Ehrlich, Ph.D.**

*Human Genetics Program and Dept. of Biochemistry, Tulane Medical School, New Orleans, Louisiana*

**Guanine Quadruplexes, non-B DNA Structures, in D4Z4: Potential for Interactions**

Melanie Ehrlich, Desheng Chen, Koji Tsumagari, and Xueqing Xu

Human Genetics Program and Dept. of Biochemistry, Tulane Medical School, New Orleans, Louisiana 70112  
USA

**2:00-2:15 p.m.**

**Greta Fabbri, Ph.D.**

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

**Size and Number of D4Z4 Alleles Play a Role in FSHD Phenotype**

Greta Fabbri<sup>1</sup>, Chiara Fiorillo<sup>2</sup>, Elena Signaroli<sup>1</sup>, Francesca Greco<sup>1</sup>, Liliana Vercelli<sup>3</sup>, Laura Palmucci<sup>3</sup>, Giuliano Tomelleri<sup>4</sup>, Lucio Santoro<sup>2</sup>, Rossella Tupler<sup>1,5</sup>

<sup>1</sup>. Dipartimento di Scienze Biomediche, Università di Modena e Reggio Emilia, Modena, Italy

<sup>2</sup>. Università degli Studi di Napoli "Federico II", Napoli, Italy

<sup>3</sup>. Centro per le malattie neuromuscolari "P. Peirola", Università degli Studi di Torino, Torino

<sup>4</sup>. Ospedale Policlinico, Clinica Neurologica, Verona

<sup>5</sup>. Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, USA

**2:15-2:30 p.m.**

**Laura Hagerty, Ph.D.**

*Acceleron Pharma, Cambridge, Massachusetts, USA*

**Treatment with a Soluble Activin Receptor Type IIB Increases Muscle Mass and Strength in Wild-type Mice and Common Marmosets**

L. Hagerty<sup>1</sup>, S.M. Cadena<sup>1</sup>, L. M. Wachtman<sup>2</sup>, K. G. Mansfield<sup>2</sup>, E.E. Pistilli<sup>3</sup>, S. Bogdanovich<sup>3</sup>, E. Curran, T.S. Khurana<sup>3</sup>, R. S. Pearsall<sup>1</sup>, J.H. Lane<sup>1</sup>, J. L. Lachey<sup>1</sup>, J. Seehra<sup>1</sup>

<sup>1</sup>. Acceleron Pharma, 149 Sidney Street, Cambridge, Massachusetts, USA

<sup>2</sup>. New England Research Primate Research Center, Harvard School of Medicine Southborough, Massachusetts, USA

<sup>3</sup>. Department of Physiology and Pennsylvania Muscle Institute, University of Pennsylvania School of Medicine, Pennsylvania, USA

**2:30-3:30 p.m.**

**PLATFORM PRESENTATIONS V**

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

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

*Department of Pediatrics, George Washington University, Washington DC USA*

**FRG1 & CRYM**

**2:30-2:45 p.m.**

**Valentina Sancisi, Ph.D.**

*Department of Biomedical Sciences, University of Modena and Reggio Emilia, Italy*

**Structural and Functional Characterization of Muscle Fibres in the Novel Mouse Model of Facioscapulohumeral Muscular Dystrophy**

Valentina Sancisi<sup>1</sup>, Elena Germinario<sup>2,3</sup>, Valeria Ghiaroni<sup>1</sup>, Elisabetta Morini<sup>1</sup>, Daniela Danieli-Betto<sup>2,3</sup>, Rossella Tupler<sup>1,4</sup>

- <sup>1</sup>. Department of Biomedical Sciences, University of Modena and Reggio Emilia, Italy
- <sup>2</sup>. Department of Human Anatomy and Physiology, University of Padova, Padova, Italy
- <sup>3</sup>. Interuniversity Institute of Myology, Italy
- <sup>4</sup>. Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, Massachusetts USA

**2:45-3:00 p.m.**

***Ryan D. Wuebbles, Ph.D.***

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

**Muscular Dystrophy Candidate Gene FRG1 Functions in Angiogenesis and Muscle Development**

Ryan D. Wuebbles, Meredith L. Hanel and Peter L. Jones

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

**3:00-3:15 p.m.**

***William Mattox, Ph.D.***

*Department of Genetics, University of Texas, M.D. Anderson Cancer Center, Houston Texas USA*

**A Drosophila Model for the Effects of Increased FRG1 Expression**

Shihuang Su, Manli Shen, Allison Chin, Mo Madhavi and William Mattox

Department of Genetics, University of Texas, M.D. Anderson Cancer Center, Houston Texas USA

**3:15-3:30 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, Neil C. Porter MD, and Robert J. Bloch

University of Maryland School of Medicine, Baltimore, Maryland USA

**3:30-3:45 p.m.**

**AFTERNOON BREAK (REFRESHMENTS)**

**3:45-5:00 p.m.**

**GROUP DISCUSSION VI -- IDENTIFY TOP 5-10 PRIORITIES FOR 2009**

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

***Kathryn Wagner, M.D., Ph.D. (moderator)***

*Charlie Emerson, Jr., Ph.D.*

*Rune R. Frants, Ph.D.*

*John Porter, Ph.D.*

*Rabi Tawil, M.D.*

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

***Suggestions for focus areas are:***

*I. Animal and cellular models*

2. *Resources and repositories*
3. *Trials and patient registries*
4. *Chromatin and candidate genes*

**Identify Top 5-10 Priorities for 2009 FSHD Research Directions and Collaborations**

- 1.
- 2.
- 3.
- 4.
- 5.
- 6.
- 7.
- 8.
- 9.
- 10.

1.

## **DUX4 in Cells and Mice**

Darko Bosnakovski and Michael Kyba\*

*Department of Pediatrics and Lillehei Heart Institute  
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By targeting each FSHD candidate gene to a doxycycline-inducible locus in murine C2 myoblasts, we have shown that DUX4 is uniquely toxic, that this toxicity is caused by sensitizing cells to oxidative stress, and that both Pax3 and Pax7 act as dominant inhibitors of the DUX4 phenotype. At low, non-toxic, levels of expression, DUX4 interferes with the expression of myogenic regulators and inhibits differentiation, a feature shared by DUX4c. Using a set of deletion and frameshift mutants, we show that both repression of MyoD and inhibition of differentiation require translation of the homeodomains but not the c-terminus, while toxicity requires translation of both the homeodomains and the c-terminus. Based on these results, we introduced DUX4, in the context of the terminal D4Z4 repeat, into mouse ES cells downstream of a doxycycline-inducible promoter on the X chromosome, proximal to the ubiquitously-expressed HPRT locus. We had previously tried, unsuccessfully, to generate transgenic mice carrying DUX4 and D4Z4 in various constructs by pronuclear injection (random integration) without success. Using the X-linked inducible locus, we have now derived strains carrying D4Z4/DUX4, however surprisingly, the D4Z4/DUX4 X chromosome (XD) is male-specific lethal, even without dox. Female carriers are fertile but suffer a growth disadvantage and a severe skin phenotype. Although they do not display an obvious muscular dystrophy, these are the first mice to carry phenotypically active D4Z4 sequences. We hypothesize that D4Z4 sequences interact with our basal promoter, resulting in DUX4 expression, and consequent growth defects. We interpret previous failures to generate mice carrying active D4Z4 repeats to be due to strong selection against D4Z4 integrations into open chromatin where DUX4 may be expressed. Females may be partially protected from the dominant-lethal gene by X-inactivation, an unintended consequence of our X-linked approach. We discuss these results in the context of developing an animal model for FSHD.

\*presenting

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### **The Transcriptional and Post-transcriptional Landscape of the D4Z4 Region**

Amy Asawachaicharn, Lauren Snider, Ashlee Tyler, Linda Geng, Lisa M Petek, Dan Miller, Lisa Maves, Richard Lemmers, Sara Winokur, Rabi Tawil, Galina Filippova, Silvere van der Maarel, Stephen Tapscott

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Recent reports suggest that a DUX4 encoding transcript might contribute to FSHD pathophysiology. We have mapped this transcript and additional sense and anti-sense transcripts that are transcribed from the 4qA allele in control and FSHD muscle cells and biopsies. We have investigated the possibility that additional open reading frames might contribute to FSHD. In addition, we have sought to determine whether an RNA mediated mechanism might contribute. Our studies provide additional potential mechanisms for FSHD pathophysiology that will need to be further evaluated.

### **Conditional Expression of PITX1 in Mouse Skeletal Muscles Causes Muscle Atrophy**

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Using genome-wide expression profiling, we recently showed that paired-like homeodomain transcription factor 1 (PITX1) was specifically up-regulated in patients with facioscapulohumeral muscular dystrophy (FSHD) comparing to 11 other neuromuscular disorders. In addition, the double homeobox 4 (DUX4) gene located within each D4Z4 unit is a transcription regulator of Pitx1. 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 transactivator protein driven by muscle creatine kinase promoter (mCK-tTA). The TRE-Pitx1/mCK-tTA mice were kept on doxycycline (200µg/ml) until 3 weeks old. Over-expression of Pitx1 was induced by withdrawing doxycycline in the drinking water. The mice showed significant weight loss, muscle weakness, reduced muscle mass, reduction of vertical and horizontal movements comparing to their single transgenic siblings. Muscle-specific expression of Pitx1 was confirmed by quantitative RT-PCR and immunohistochemistry. Hematoxylin and Eosin staining showed large number of atrophic myofibers, some necrotic and central nucleated myofibers and mild inflammation infiltration. The phenotype and pathology of the Pitx1 model is reversible. The Pitx1 mice regained body and muscle weight with normal histology after Pitx1 expression was stopped by adding doxycycline in the drinking water. The results suggest that the gene may play an important role in muscle atrophy and in FSHD.

## **Transcription of the Region Proximal to D4Z4 could Regulate 4q35 Gene Expression in FSHD**

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant neuromuscular disorder that is not due to a classical mutation within a protein-coding gene. Instead, almost all FSHD patients carry deletions of an integral number of tandem 3.3 kilobase DNA repeats, termed D4Z4, located on chromosome 4q35.

Several observations suggest that FSHD may be due to an epigenetic alteration causing transcriptional deregulation of 4q35 genes proximal to D4Z4. Accordingly, we have found that D4Z4 contains a transcriptional silencer whose deletion leads to inappropriate de-repression in FSHD skeletal muscle of 4q35 genes located upstream of D4Z4. Interestingly, muscle-specific over-expression of the 4q35 gene FRG1 causes a disease remarkably similar to FSHD. Notably, we have found that in muscle of FRG1 transgenic mice and FSHD patients, specific pre-mRNAs undergo aberrant alternative splicing. Collectively, our results suggest that inappropriate over-expression of FRG1 and abnormal alternative splicing play an important role in FSHD.

D4Z4 copy number determines age of onset and clinical severity of FSHD. In general, fewer repeats are associated with a more severe, early-onset disease. However, individuals completely devoid of D4Z4 are healthy suggesting paradoxically that at least one copy of D4Z4 is required to cause FSHD possibly through a gain-of-function effect.

Transcriptional repression mediated by D4Z4 bears several parallels to Polycomb group (PcG) proteins-mediated silencing. First, D4Z4 contains a sequence, DBE for D4Z4 Binding Element, identical to a conserved sequence motif (PRE) required for PcG mediated silencing. Second, transcriptional repression at 4q35 requires association to DBE of YY1 and HMGB2 which are the human homologs of *Drosophila* Pleiohomeotic (PHO) and Dorsal switch protein 1 (DSP1), respectively. PHO is the only member of the PcG known to bind DNA in a sequence-specific manner, while DSP1 is required for binding of PcG to PREs. Recently, it has been shown that transcription of non-coding RNAs (ncRNAs) from PREs plays an important role in regulating PcG-mediated silencing. Intriguingly, it was recently reported that the first repeat of the array and the region immediately proximal to it display a more open chromatin structure than the bulk of the array.

Based on these evidences we decided to investigate if the first repeat of the array and the region immediately proximal to it were transcribed. Real-time quantitative RT-PCR using human muscle samples from normal individuals and patients affected by FSHD revealed that, while no signal was detectable in normal muscle, a specific transcript was present at a significant level in all FSHD muscle samples. Interestingly, we and others have previously shown the same expression pattern for FRG2 the closest protein-coding gene to D4Z4. Cytoplasmic, nuclear soluble and chromatin-associated RNA was prepared to test the sub-cellular localization of the identified transcript. Interestingly, while the majority of the RNA of the protein-coding gene Gapdh was present in the cytoplasmic fraction, the identified transcript was enriched in the chromatin fraction. Notably, a similar localization was displayed by the non-coding regulatory RNA Xist. On the basis of these results, we concluded that the first repeat of the array and the region immediately proximal to it generate a chromatin-associated RNA concomitantly with the de-repression of 4q35 genes. It is tempting to speculate that production of this transcript could play a role in regulating the epigenetic cascade culminating with 4q35 genes de-repression in FSHD. Functional characterization of the identified transcript will be presented.

## **DUX4 Over-Expression Causes Muscle Toxicity 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. However, the in vivo effects of DUX4 over-expression in muscle have not been reported. Here, we describe the first 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 observed a 40% transgenesis rate, and gene expression was restricted to somites with weaker staining in heart. In DUX4-injected fish, we observed evidence of muscular dystrophy, indicated by partial or complete loss of somite definition, abnormal fish body morphology, and impaired or completely absent mobility. These results are consistent with abnormalities reported in other zebrafish models of muscular dystrophy. We also observed cardiac hypertrophy in some fish, which is likely an artifact of the cardiac activity from the MHCK7 promoter. 33% of DUX4-injected fish died between 42 and 96 hours post-injection. We hypothesize that deaths arise from cardiomyopathy or DUX4 fish immobility, which prevents them from feeding. No abnormal phenotypes or deaths were observed in GFP-injected fish during the same time course. For mouse studies, we delivered adeno-associated viral vectors (AAV6) carrying DUX4 or control GFP vectors to neonate or adult muscle. DUX4 over-expression beginning at postnatal day 1 caused muscle atrophy up to 4 weeks post-injection (our latest time point). Specifically, DUX4-transduced muscles were significantly smaller than GFP-transduced or contralateral, untransduced controls. We also observed increased central nucleation, myofiber size variability, and inflammatory cell infiltration, which are all general features of muscular dystrophy. Similar histological indicators of muscle turnover were seen in DUX4-transduced young adult (6 week old) mouse muscle. DUX4 expression caused myofiber degeneration and massive mononuclear cell infiltration in muscle by 1 week post-injection. Untransduced or GFP transduced myofibers were normal. By three weeks post-injection, myofibers in DUX4-transduced mice, but not GFP or saline-injected animals, were almost exclusively centrally nucleated, an indication of muscle turnover. Using functional grip strength testing, we found that DUX4-injected muscles were significantly weaker than GFP or saline-treated controls. Together, these data support DUX4 muscle toxicity in vivo and justify further investigation of DUX4 in FSHD pathogenesis.

This work was supported by The FSH Society Landsman Charitable Trust, The Muscular Dystrophy Association, and Startup Funds from the Research Institute at Nationwide Children's Hospital.



## 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 position effect mechanisms.

Therefore, using several strategies, we reconstituted the basic genomic organization of the 4q35 locus involved in FHSD and analyzed the epigenetic effect mediated by D4Z4 after integration of the transgene into the human genome. We showed that the D4Z4 subtelomeric element is a bona fide insulator element protecting from position effect variegation and able to block enhancer-promoter communication.

We further show that D4Z4 acts as a CTCF and A-type Lamins-dependent insulator. We also demonstrate that both anti-silencing activity of D4Z4 and CTCF binding are lost upon multimerization of the repeat above the threshold of 11 D4Z4. Our findings suggest a new mechanism by which D4Z4 contraction contributes to FSHD physio-pathology by acting as an insulator in patients but not in normal individuals and might thereby impact on the expression of the genes causing this peculiar muscular dystrophy.

## 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 loss of repressive chromatin marks including DNA methylation as well as changes in higher order chromatin organization at the 4qD4Z4 region may account for deregulation of gene expression observed in FSHD.

Because of the strong evidence for a change in chromatin architecture at D4Z4 in FSHD, we aimed to investigate a putative role for a chromatin insulator protein CTCF in this mechanism. CTCF is a multi zinc finger protein that, depending on the cellular and genomic context can act as transcriptional activator, silencer, or insulator. Recent evidence suggests that CTCF can also act to bring spatially separated chromatin domains in close proximity, both in cis and in trans. We have demonstrated a gain-of-function for CTCF in FSHD alleles. While D4Z4 is normally not occupied by CTCF, in disease alleles there is a discrete, yet significant binding of CTCF to at least three sequences within the D4Z4 repeat unit. Further mapping identified two clusters of CTCF binding sites within the D4Z4 repeat unit, one on each side of the transcription start site of DUX4, one of the candidate genes recently shown to be deregulated in FSHD. Interestingly, we demonstrated that CTCF binding to D4Z4 is largely methylation insensitive, which is consistent with the relatively high levels of CpG methylation at D4Z4 even in FSHD cells. Since CTCF has been shown to protect from the spreading of DNA methylation our findings suggest that aberrant CTCF binding at D4Z4 in FSHD may interfere with the setting of repressive epigenetic marks and thus prevent transcriptional silencing in the region. Indeed we observed a significant loss of DNA methylation at CTCF binding sites at D4Z4 in FSHD alleles in comparison to normal alleles. Moreover, down regulation of CTCF resulted in reduction of the DUX4 transcript levels in FSHD cells.

Notably, in a parallel study by Dr Frederique Magdinier and colleagues presented at this Meeting D4Z4 has been shown to act as a CTCF-dependent insulator in FSHD but not in control cells, supporting our hypothesis on the role for CTCF in protecting D4Z4 from transcriptional silencing, and thus providing a new mechanism for the FSHD pathogenesis.

## Facioscapulohumeral Muscular Dystrophy: Gene Discovery by DNase-chip

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There should be a disease-related sequence at 4q35.2 that is not present at 10q26.3 because facioscapulohumeral muscular dystrophy (FSHD) patients have one short D4Z4 array with <11 units of 3.3-kb repeats in D4Z4 at 4q35.2 while almost identical arrays at 10q26 are nonpathogenic despite high 4q/10q homology in and around D4Z4. No 4q35 sequence differentially expressed in FSHD vs. control muscle was seen in five published microarray expression analyses. However, expression microarrays are biased towards known genes and well-studied tissues. To discover undocumented 4q35.2 genes, including non-coding RNA genes (genes encoding RNAs but not proteins), and new transcription regulatory sequences, we looked for DNaseI hypersensitive (DH) sites, a gene hallmark. Searches for DH sites can lead to the positions of unknown genes as well as gene regulatory sequences distant from the gene itself. We analyzed the DNaseI-hypersensitive fraction from three FSHD and three control myoblast cell populations on high-resolution custom tiling arrays containing overlapping oligonucleotide probes (DNase-chip). The arrays contained the terminal 4 Mb of 4q (all of 4q35.2), the terminal 1 Mb of 10q, and some 4q35.1 sequences. The proximal 1.5 Mb of 4q35.2 had a normal density of DH sites, although some were in clusters far from known genes. This suggests the presence of undocumented genes in the proximal portion of 4q35.2. In the distal 2.5 Mb of 4q35.2, there was a much lower density of DH sites. One of these, at the 5' end of FRG1, was observed in all myoblast cell strains. Two others, that were located far from known genes, were seen preferentially in FSHD myoblasts.

By real-time quantitative RT-PCR (qRT-PCR), we tested for transcripts from sequences in and around the two FSHD-preferential DH sites. For this analysis, cDNA was synthesized from FSHD and control myoblast RNA with random priming or oligo(dT) priming. FSHD and control myoblast cell strains (2 – 4 cell strains each) had transcripts from unique sequences in the vicinity of both of the above DH sites despite being far from known genes. These amplicons had similar RNA levels in undifferentiated myoblasts from FSHD patients and controls. Transcription was not indiscriminate because some amplicons in these 4q35.2 subregions were consistently negative or only very weakly positive in qRT-PCR. Nonetheless, these results indicate that the two large gene deserts in 4q35.2 contain transcription units, which might encode functional ncRNAs that are active in myoblasts. Therefore, there might be an FSHD-related myogenesis-specific gene yet to be discovered in 4q35.2. (Supported in part by NIH Grant NS048859 and FSH Society Grant FSHS-MGBF-013)

## Comparative Methylation Analysis of the D4Z4 Repeat Array on Chromosome 4q35 and 10q26

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Significant hypomethylation of the proximal repeat unit of the D4Z4 repeat array is observed in patients with facioscapulohumeral muscular dystrophy (FSHD), both in patients with a contracted D4Z4 repeat array on one of their chromosome 4 alleles (4q-linked FSHD) and in a small group of patients without a D4Z4 contraction (phenotypic FSHD). At present, the precise role of D4Z4 hypomethylation in the FSHD disease mechanism remains unclear.

Using the methylation-sensitive restriction enzyme CpoI in a Southern blot analysis, we have performed a comprehensive methylation study of the D4Z4 repeat array, both at the proximal D4Z4 repeat unit and also at all D4Z4 repeat units of the repeat array. In addition, we have studied D4Z4 methylation on chromosome 10, which carries a highly homologous repeat array. For the development of FSHD, the D4Z4 contraction needs to occur on a specific genetic background. Only contractions associated with the 4qA161 haplotype seem to result in FSHD, while contractions in other haplotypes such as 4qA166, 4qB163 and 10qA166 are non-pathogenic.

Our results show that upon D4Z4 repeat array contraction below 40 kb, pronounced hypomethylation occurs at both the proximal D4Z4 repeat unit and also at all D4Z4 repeat units of the contracted allele. Importantly, this hypomethylation occurs irrespective of the genomic location of the D4Z4 repeat array. Thus, control individuals with a contracted D4Z4 repeat array on a non-pathogenic haplotype such as 4qA166, 4qB163 and 10qA166 also present with D4Z4 hypomethylation. Further, our data show that the methylation defect in phenotypic FSHD patients is not restricted to chromosome 4. Also on chromosome 10 alleles pronounced D4Z4 hypomethylation was observed, implying that a genetic defect responsible for D4Z4 methylation at chromosomes 4 and 10 may underlie phenotypic FSHD.

We speculate that loss of DNA methylation at D4Z4 is necessary, but not sufficient, to develop FSHD. Haplotype-specific SNPs or other epigenetic factors may have an important role in FSHD pathogenesis.

## Biomarkers for Therapy of FSHD

Charles P. Emerson, Jr.<sup>1</sup>, Kathryn R. Wagner<sup>2</sup>, Mayana Zatz<sup>3</sup>, Robert J. Bloch<sup>4</sup>, Woodring E. Wright<sup>5</sup>, Jeffrey Boone Miller<sup>1</sup>, Daniel Paul Perez<sup>6</sup>, Louis M. Kunkel<sup>7,8</sup>

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A new NIH-supported Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center has been established to further understanding of the underlying molecular and cellular pathology of facioscapulohumeral muscular dystrophy (FSHD) and to establish muscle tissue and cell repository biomarker databases as resources for FSHD research and evaluation of outcomes of FSHD clinical trials. The Center is led by Dr. Charles P. Emerson, at the Boston Biomedical Research Institute, and Dr. Louis Kunkel at Children's Hospital Boston and Harvard Medical School, and includes investigators at Johns Hopkins Hospital, Boston Biomedical Research Institute, University Sao Paulo, University of Maryland School of Medicine, and the University of Texas Southwestern Medical Center. The Center is partnered with the FSH Society for patient outreach activities and with two biotechnology companies, Acceleron Pharma and Genzyme/Myosix, for therapeutic development.

The Center currently sponsors four (4) multi-investigator research projects and three (3) cores:

**Project 1.** "Clinical Trials and Biomarkers of Myostatin Inhibition." This project, led by Drs. Kathryn Wagner and Louis Kunkel, partners with Acceleron Pharma to develop biomarkers for the myostatin inhibitor, ACE-031, in healthy human subjects and mice and to develop protocols for clinical trials using biomarkers to test the efficacy of myostatin inhibitors in FSHD patients.

**Project 2.** "Biomarker Discovery in Muscles from FSHD Patients." This project, led by Drs. Louis M. Kunkel, Mayana Zatz, and Robert J. Bloch, undertakes to identify mRNAs, miRNAs and protein biomarkers through gene expression array and proteomics analyses of biopsies of affected and unaffected muscles obtained from FSHD patients and first degree relatives. These studies undertake to define the molecular pathology underlying FSHD and identify disease biomarkers for FSHD clinical trials.

**Project 3.** "Myogenesis Studies for FSHD Biomarkers." This project, led by Drs. Charles Emerson and Woodring Wright, investigates the growth and differentiation characteristics of mortal and immortalized muscle progenitors derived from muscle biopsies of affected and unaffected muscles obtained from FSHD patients and first degree relatives. These studies undertake to identify cellular defects in myogenic cell lineages from affected FSHD muscles that affect their regenerative capacity and aging as well as to identify disease biomarkers expressed in FSHD muscle progenitor cells and newly differentiated fibers.

**Project 4.** "Mouse Model Studies for FSHD Biomarkers." This project, led by Drs. Jeffrey B. Miller and Robert J. Bloch, investigates muscle disease pathology and biomarker expression in existing and newly generated mouse models of FSHD to identify validated FSHD mouse models for studies of disease progression and pathology and for therapeutic development.

**Core A.** An “Administrative Core” provides the administrative and financial structure and fosters a communicative scientific environment within the Center. This Core is the information hub to communicate Center activities to the greater scientific community, governmental (NIH) agencies and foundations, other Wellstone Centers, and the lay and FSHD patient advocate communities. Core A, together with the FSH Society and Mr. Daniel Perez, also will organize scientific, patient and community outreach meetings.

**Core B.** An “Education and Training Core” oversees a pre- and postdoctoral fellowship program designed to train and mentor young scientists for careers in skeletal muscle and muscular dystrophy research, with a strong focus on training for FSHD research. This Core also will organize an annual Center retreat to enable trainees to present research findings and interact with Center scientists.

**Core C.** A “Cell Core” will establish a tissue and cell repository of muscle biopsies and immortalized and mortal muscle progenitor cells derived affected and unaffected muscles of FSHD patients and first degree relatives and establish a FSHD muscle biomarker database from investigations in Projects 1-4. This Core also will partner with Genzyme/Myosix to utilize and develop technologies for identification and isolation of muscle cell progenitors from FSHD muscles and will maintain a the cell and tissue repository of validated FSHD and control muscle for distribution to FSHD researchers for their basic and therapeutic studies.

The Center will be seeking collaborations with researchers in the international community to foster disease and therapeutic research on FSHD as well as engaging new industrial partners committed to develop therapeutics and undertake clinical trials for treatment of FSHD.

## **Fields Center for FSHD and Neuromuscular Research**

Rabi Tawil, M.D.

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The Fields Center for FSHD and Neuromuscular Research was established by the Fields Family Foundation in October of 2007. The Foundation, interested in long term funding of FSHD research, has committed to seven years of funding as well as the establishment of an endowment fund to be tapped at the end of that period. The principle aim of the Fields Center is to energize research in FSHD by: 1) Creating a seamless collaborative partnership between the University of Rochester Medical Center and Leiden University's Center for Human Genetics, a center without walls, 2) Increasing the availability of patient derived samples for research in FSHD, and 3) Developing clinical care standards as well as clinical outcome measures, critical in clinical trial readiness. From within this infrastructure framework, the Fields Center investigators will address specific scientific questions. As the Rochester-Leiden partnership is consolidated, the Fields Center will seek to expand its collaboration with other investigators. To date, the Fields Center has established a number of collaborations with Dr. Stephen Tapscott (Hutchinson Cancer Research Center, Seattle) and Drs. George Padberg and Baziel van Engelen (St. Radboud University, Nijmegen) as major collaborators on the molecular mechanisms and clinical research fronts respectively.

### Italian FSHD National Registry: a Tool for Genotype-Phenotype Correlation

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Facioscapulohumeral muscular dystrophy (FSHD) is the third most common myopathy. The disease has been causally related to deletion of tandemly arrayed 3.3 kb repeat units (D4Z4) on chromosome 4q35. The number of D4Z4 repeats varies from 11 to 150 in the general population, whereas less than 11 repeats are present in sporadic and familial FSHD patients. The number of D4Z4 repeats is thus considered diagnostic for the disease. 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 a high percentage of non-penetrant gene carriers. Compound heterozygous patients for the FSHD-sized alleles have also been described as well as FSHD subjects carrying D4Z4 alleles of 38-45 kb in size, considered a borderline D4Z4 allele present also in the normal population. In addition, analysis of subtelomeric polymorphisms suggests that in order to cause FSHD, D4Z4 deletion must be associated with a specific haplotype resulting from contemporary presence of 4qA, G variant of the D4Z4 SNP and 161 bp-sized SSLP. Collectively, these observations indicate that several factors might influence the clinical outcome in FSHD patients.

Through a multicenter research group composed by two diagnostic laboratories and 11 Clinical Partners representing the Italian centers studying neuromuscular disorders, we constituted the Italian FSHD National Registry which collects 1037 index cases, over 2000 individuals carrying D4Z4



deletion, 113 subjects carrying alleles between 38-45 kb in size, 26 compound heterozygotes carrying two D4Z4 deleted alleles, 88 FSHD-like patients.

Our multicenter research group also developed a standardized protocol that allows the quantification of muscle weakness in FSHD patients through the functional evaluation of six muscle groups specifically affected in FSHD. The protocol will be used to measure the degree of disability in FSHD patients and to generate a score defining the severity of the disease.

The ability to define numerically the clinical severity of the disease will permit to investigate the genotype-phenotype correlation in large cohorts of FSHD patients and families enrolled in the Registry and representing the Italian population of subjects affected by FSHD.

This study will bring a more precise estimate of FSHD prevalence and improve prognostic tools. It will also provide a well-characterized population suitable for studying novel therapeutic strategies for FSHD.

### **About the Facioscapulohumeral, Type 2 (or the Facioscapulohumeral) Autosomal Dominant Muscular Dystrophy in the K. Kindred Once Again**

Reexamination in 1993 of the famous K. kindred described for the first time by Oransky in 1927 then Davidenkov, Kulkova in 1938 and Kazakov et al. 1975, 1976  
Clinical and molecular genetic study.

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Additional study of the K. kindred which were previously investigated by Oransky in 1927, then Davidenkov and Kulkova in 1938 and then Kazakov et al. in 1969-1976 was carried out. On compare with data published by Oransky and Davidenkov - Kulkova we can examined in 1993 of 20 members from IV, V and VI generations of the K. kindred. Two patients of IV generation and six ones of V generation were re-examined by us (V.K.) after 24 years, in 1993. One patient of V generation and eleven members of VI generation had never been examined earlier. In 14 pre-symptomatic (Pr) patients from V and VI generations and one Pr from V generation the P13E-11 EcoRI/BlnI DNA analysis was carried out (using conventional linear gel electrophoresis) (Dr. K. Arahata). In 3 Pr (V-10, V-17 and VI-10) the DNA fragment sizes were 24/21, 33/30 and 33/30 kb (double digestion), respectively; in other 12 Pr the DNA fragment sizes were >50 kb. Besides, we knew the peculiarities of the clinical picture and duration of the disease between 1969-1979 in some patients from IV generation who we examined earlier and who were described by Oransky, and Davidenkov - Kulkova. Almost in all the patients of K. kindred from different generations in the early stage of the disease the slight affection of some facial muscles, a weakness and atrophy of the shoulder girdle and peroneal group muscles in the different degree were established. Disease in the K. kindred has been inherited as an autosomal dominant type for six generations. The penetrance of the gene is almost complete. However, the expressivity of the gene varies greatly from abortive to severe forms of the disease. As well necessary to remark that all reexamined and examined for the first time patients from V and VI generations and three patients from IV generation were pre-symptomatic. Thus it happened so that in K. kindred we can see the phenomena reverse of the progressing hereditary. In patient from III and IV generations who had severe developed disease the (F)SP or the FSP phenotype predominated in the clinical picture during a many long time. Later after 12-16 years the posterior thigh muscles (but not quadriceps), gluteus maximus (but not gluteus medius) and abdominal muscles were involved. The clinical and genetic data allowed at the present time, as well as earlier in 1974, to consider that the muscular dystrophy in K. kindred is the special type which we prefer to call “facioscapulohumeral muscular dystrophy, type 2 (FSLD2), a descending with a “jump”, with initial facioscapulohumeral or (facio)scapuloperoneal phenotypes” and (F)SP and FSP phenotype constituted merely a stage in the development of FSLD2.

Oransky V. Uber einen hereditaren Typus progressiver Muskeldystrophie. Dt. Z. NervHeilk. 1927; 99: 147-155. Davidenkov S.N. and Kulkova E.F. On nosological place of scapulo-peroneal amyotrophy. Zh. Nevropat. Psikhiat. 1938; 7: 11-20. Kazakov V.M., Bogorodinsky D.K., Skorometz A.A. Myogenic scapuloperoneal syndrome – Muscular dystrophy in the K. Kindred. Reexamination of the K. Family described for the first time by Oransky in 1927. Eur. Neurol. 1975; 13: 350-359. Kazakov V.M., Bogorodinsky D.K., Skorometz A.A. The myogenic scapulo-peroneal syndrome. Muscular dystrophy in the K. kindred: Clinical study and genetics. Clinical Genetics 1976; 10: 41-50.

## Possible Mechanisms Responsible for great Inter and Intra Familial Phenotypic Heterogeneity in FSHD

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### BACKGROUND

FSHD is caused by deletions within a tandem array of D4Z4 repeats on chromosome 4q35. A correlation between repeat contraction and clinical severity is well known. However, a remarkable phenotypic variability of clinical expression between and even within the same family is observed. This can be explained by several known and still hypothetical mechanisms. As illustration we present two unrelated Croatian families.

### CASE REPORTS

The first family consisted in three differently affected members: the proband had infantile onset of muscular weakness, but he was still ambulant at age of 17 years. He was intelligent, very tall (190cm) and thin (51 kg) adolescent with extremely hypotrophic facial, shoulder girdle, upper arm and upper leg muscles, scoliosis, pectus excavatum, pronounced lumbar hyperlordosis, and end-stage Coats disease in the blind right eye since the age of 2 years. His 11-year-old brother had facial weakness since early infancy, showed mild scapular winging and slight lumbar lordosis. He was active in sport and clinically in good condition. Minimally affected 40-year-old mother has facial asymmetry with right side muscular hypotrophy and slight frontal muscle weakness. In both sons, EMG pattern was myopathic, CK was twice normal. There was no hearing loss detected by audiogram. Fluorescein angiography of retina was normal in younger brother. The proband's angiogram showed only left eye with very prolonged interval arm-retina (25 sec) and was not adequate for assessment. DNA analysis of both sons and parents were done by Southern blotting using EcoRI and EcoRI/BlnI digested DNA and hybridization with p13E-11. The results have discovered that both sons and their mother have one short EcoRI/BlnI fragment of 12,0 kb.

The second family was presented by a 49-year-old proband from a four generation family in which at least thirteen individuals in three generations were affected. He had moderate sensorineuronal hearing loss, a slowly progressive, late onset weakness of facial, shoulder and pelvic girdle muscles and he was ambulant. His all three children: 25-year-old daughter, 21-year-old son and 10-year-old daughter had very mild symptoms without functional impairment. Proband's 53-year-old brother had classical but slowly progressive form of FSHD. His 25-year-old daughter showed only mild facial weakness and his 21-year-old son (not examined) would have some moderate muscular weakness. In proband's both parents families hearing loss was noticed. Southern blot analysis using probe p13E-11 showed in probands and his 53-year-old brother two shortened fragments of 35 kb and 32 kb. Proband's 25-year-old daughter as well as his 21-year-old son had a fragment of 32 kb and one normal fragment. The 10-year-old daughter was not tested even she had mild disease's symptoms. Very mildly affected daughter of older brother had one normal allele and the other with short fragment of 35 kb.

### DISCUSSION

To explain the observed inter and intra familial heterogeneity in presented families different genetic and epigenetic mechanisms can be raised:

*A correlation between repeat contracture and clinical severity* is well known.

Possible *maternal mosaicism* in first family, which was not excluded by pulse field electrophoresis. In addition maternal family history seems negative for FSHD.

To explain severe infantile form in older brother and relatively mild form at age of 11 in younger (follow up was not available) could be explained by *hypothetical modifier genes and epigenetic disease mechanism* (S.M. van der Maarel, 13th WMS, 2008)

Intrafamilial variability in second family can be explained by *compound heterozygous patients* for short deleted alleles and their progeny with only one short deleted allele. Although such families seem to be very rare, accurate molecular study are needed to confirm this hypothesis. It is interesting to mention that *the size and number of D4Z4 alleles play a role in FSHD phenotype* (G.Faabri et al., 13th WMS, 2008) who hypothesizes that the more severe clinical expression of the disease observed in compound heterozygotes might be related to transcriptional depression of genes at 4q35 occurring at both deleted D4Z4 alleles, supporting the current model for FSHD.

POSTER

## **Investigating D4Z4 Mediated Gene Regulatory and Epigenetic Effects on FRG1 in a Telomeric Environment in the Developing Vertebrate *Xenopus Laevis***

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The genetic lesion associated with FSHD is a contraction of the subtelomeric D4Z4 repeats localized to 4q35.2. The repetitive nature of these repeats coupled with their subtelomeric location suggest functional roles for D4Z4 repeats acting in regulation of genes in cis through epigenetic mechanisms. Supporting a regulatory role, FSHD disease pathology is caused by a mis-regulation of a 4q35-localized gene(s) and the leading candidate is FRG1 (FSHD gene 1). Over-expression of FRG1 in mouse results in an FSHD phenotype and misexpression of FRG1 in *Xenopus* results in muscular and vascular defects, fitting with FSHD pathology. In order to understand how FRG1 and other local genes could be misregulated in FSHD, we are modelling components of the FSHD 4q region by engineering transgenes to test the role of D4Z4 repeats on FRG1 regulation, comparing telomeric and non-telomeric insertions. Experiments will test potential D4Z4 mediated enhancer activity, repressor activity and insulator activity. We have previously shown that transgenes containing telomeric repeats are effectively integrated into the *Xenopus* genome producing de novo telomeres, a process well tolerated in *Xenopus* likely due to its pseudotetraploid nature. Transgenic tadpoles with the human FRG1 promoter drive reporter expression in a similar pattern to endogenous *Xenopus* FRG1 expression. Epigenetic regulation of *Xenopus* genes and its genome is conserved with humans, making potential findings applicable to humans. *Xenopus* provides a unique opportunity to observe developmental stage and tissue specific differences in epigenetic and gene regulation. Here we present preliminary data and outline the future goals of this study.

POSTER

## **Comprehensive Expression Analysis of FSHD Candidate Genes Provides Evidence for Transcriptional Deregulation of FRG2 Only**

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In facioscapulohumeral muscular dystrophy (FSHD) the majority of patients (>97%) carry a macrosatellite repeat contraction in the subtelomere of chromosome 4q. Since this discovery, several disease mechanisms have been proposed to explain how repeat contraction causes muscular dystrophy. All proposed mechanisms predict a chromatin change from closed to a more open chromatin structure followed by loss of control over expression of genes in or close to the repeat. Although initially a distance- and residual repeat size-dependent upregulation of the candidate genes FRG2, FRG1 and ANT1 was observed, later expression studies have both disputed as well as supported the transcriptional deregulation of 4qter genes. Moreover, chromatin studies have failed to provide evidence for a cis-spreading mechanism operating at 4qter in FSHD. In part, this inconsistency may be explained by differences in techniques used, and the use of RNA samples obtained from different muscle groups.

The aim of the present study is to comprehensively and uniformly study the expression of all FSHD candidate genes. To this end, we analyzed gene expression of DUX4, FRG1, FRG2, ANT1, ALP (PDLIM3), PITX1 and LRP2BP at the RNA and protein level in primary myoblasts, myotubes, and quadriceps muscle. All RNA and protein sources were identically processed and examined to minimize variability in expression due to technical differences. Expression was compared between samples obtained from FSHD patients, normal controls and disease control patients (Myotonic Dystrophy type 1 or DM1). We could not observe consistent changes in FSHD compared to normal controls and Myotonic Dystrophy type 1 (DM1) disease control patients, except for FRG2 RNA expression levels. Although we could not find evidence for deregulation of most FSHD candidate genes in the FSHD samples we examined, deregulation of these same genes might still play a role during specific developmental stages or regeneration of muscle fibers in FSHD.

POSTER

## Mutagenesis Analysis of the DUX4 Nuclear Localization Signals NLS1 and NLS2

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Our laboratory discovered that DUX4 is a toxic protein leading to apoptosis in cell transfection assays<sup>1,2</sup>. Additional studies demonstrated that this toxic effect is specific for DUX4 and it is not observed in cell transfections with the homologous protein DUX1<sup>3</sup>. Our laboratory also found that a DUX4-related protein is endogenously expressed in human muscle derive cells<sup>1,2</sup>, being the *DUX4* gene specifically transcribed in FSHD myoblasts<sup>3</sup>. It is hypothesized<sup>3</sup> that abnormal temporal or spatial expression of DUX4 during muscle development may be toxic for muscle cells, causing FSHD. DUX4 is a nuclear protein<sup>1,4</sup> and its subcellular localization may be required for this toxic effect. To analyze if nuclear localization of DUX4 is required for DUX4-mediated cell death, the potential pathogenic phenotype we have identified for this protein<sup>1-3</sup>, we constructed specific deletion mutants for two putative DUX4 nuclear localization signals: NLS1 (RRRR<sup>23</sup>) and NLS2 (RRKR<sup>98</sup>). Also, amino acid residues R<sup>96</sup> and K<sup>97</sup> from NLS2 were independently replaced with threonines. The subcellular distribution of DUX4 wild type and deletion mutants NLS1<sup>-</sup> and NLS2<sup>-</sup>, as well as the double mutant NLS1<sup>-</sup>/NLS2<sup>-</sup>, is being characterized in transiently transfected cells. The consequence of these mutations on DUX4-mediated apoptosis is studied using a GFP-based co-transfection assay previously developed in our laboratory<sup>1-3</sup>. Results presented here address the question if nuclear entrance of DUX4 is required for DUX4-mediated cell death.

<sup>1</sup>Kowaljow W (2003) PhD Thesis, University of Cordoba, Argentina; <sup>2</sup>Rosa AL (2003) FSHD International Research Consortium Meeting, Los Angeles, CA; <sup>3</sup>Kowaljow *et al* (2007) Neuromuscul Disord 17, 611-623; <sup>4</sup>Ostlund *et al* (2005) Biochemistry 44, 2378-2384.

POSTER

## **FRG1 Over-expression Induces Severe Dystrophic Phenotype in the Adult Mouse**

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It has been shown by Rossella Tupler and coll that the over expression of human FRG1 in transgenic mice induces a severe muscular dystrophy with features characteristic of the human disease (Gabellini et al, Nature 2006).

Here, we have tested the consequences of an over-expression of the mouse-FRG1 in normal adult mice (C57B6). Indeed, if mouse and human FRG1 proteins are close, their amino acids sequences are not identical, resulting in differences in the prediction of phosphorylated or N-glycosylated amino acids. We asked if the use the human orthologous in the transgenic mice could provoke a bias.

Thus, we have intramuscularly injected AAV vectors carrying either a mouse- or human-FRG1 cDNA under the control of the CMV promoter. As soon as one month after injection, we observe muscle atrophy and severe dystrophy in both treated muscles. Nevertheless, sarcolemmal integrity was preserved since dystrophin and dystroglycan labellings were not affected. Three months after injection, there was no more massive inflammatory response, although the presence of centralized myonuclei in many fibers reflects a massive necrosis regeneration phenomenon. Aberrant alternative splicing is under investigation.

In conclusion, these results confirm the toxic effect of an excess of mouse- or human-FRG1 in the skeletal muscle. It also demonstrates that it is possible to induce the disease after birth. We are now developing strategies to inhibit FRG1 expression.

POSTER



## **Pluripotent Stem Cells for FSHD Research**

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We have established an in vitro developmental model of FSHD using pluripotent stem cells 1) to compare the development of FSHD and normal cells from the pluripotent embryonic cell through differentiated myotubes and 2) to test whether appropriate manipulations of expression of gene(s) suspected to be responsible for FSHD pathology can induce FSHD-related abnormalities in normal cells and rescue FSHD cells. The parameters to be tested include alterations in cell morphology, chromatin structure, muscle-specific and FSHD-specific gene expression. The model system employs induced pluripotent stem (iPS) cells isolated from FSHD patient and control dermal fibroblasts. iPS cells were successfully generated through ectopic expression of the transcription factors OCT4, SOX2, KLF4 and NANOG. iPS cells are similar to embryonic stem (ES) cells in that they self-renew and can differentiate into cells of all three germ layers. Unlike adult stem cells that have restricted developmental capacity, iPS cells allow us to study FSHD cells during their entire developmental history. Isolation of iPS cells is a critical step in increasing our understanding of muscle and non-muscle contributions to FSHD pathology. These iPS cells will be used to determine the relationship between FSHD pathology and altered gene expression during development and the role of candidate genes in the FSHD pathology. The results of this study will fill important gaps in knowledge about FSHD and will be provided to those in the FSHD community for collaborative research.

POSTER

## **Direct and Simultaneous Visualization of D4Z4 Arrays on Distinct 4qA, 4qB and 10q Combed Alleles: Implication for FSHD Diagnosis and Physiopathology**

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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. D4Z4 are mainly located at subtelomeric regions on 4qter and 10qter and range from 1 to 150 units. FSHD is associated with a range of 1-10 units at 4q35 whereas, in normal controls, they usually range between 11 and 150 units. Several factors dramatically complicate FSHD molecular diagnosis, the most important being the cis-segregation of D4Z4 deletion with a specific distal genomic sequence on 4q35 (4qA allele), while D4Z4 shortening is silent both on alleles 4qB and 10q. In addition, D4Z4 arrays are spreaded at diverse positions in the genome possibly generating cross-hybridizations, deletions including the genomic p13E-11 sequence and part of D4Z4 have been reported as well as translocations between 4q and 10q D4Z4 repeats. Moreover, somatic mosaicism is frequently present in patients and asymptomatic carriers. All together, molecular diagnosis of FSHD, not only is time- and cost-consuming, but is also extremely challenging; it depends on sizing the repeats number and length at 4q35 (qA), usually by using the proximally adjacent p13E-11 probe and specific 4qA and 4qB probes after pulsed-field or linear gel electrophoresis followed by Southern blot.

To bypass these difficulties and provide FSHD patients with a faster, accurate and simple diagnosis, we have designed a molecular combing-based assay. This approach allows the characterisation of large genomic regions at the kilobase level of resolution. By performing a single fluorescent in situ hybridization using a specific set of probes corresponding to 4q, D4Z4, qA, qB, and 10q on combed DNA from patients and controls, we could directly visualise the probes positions on numerous DNA molecules from a same patient, directly identify 4qA-D4Z4 alleles, distinguish them from 4qB/D4Z4 and 10q/D4Z4 alleles and measure the D4Z4 number and length. In our opinion, this approach by providing a direct visualisation of pathogenic and silent alleles associated to D4Z4, not only constitutes a pertinent and reliable diagnosis tool but will also allow to explore and detect unexpected rearrangements such as 4q/10q translocations. This application of molecular combing as well as future developments of the technology are thus being actively considered for diagnosis of FSHD and the molecular exploration of the associated 4qA/4qB telomeric region in complex cases.

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### Trying to Understand the Clinical Variability in FSHD

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FSHD is characterized by a great clinical inter and intrafamilial variability. Usually males are more severely affected than females. Approximately 10-20% of patients eventually become wheelchair-bound while 20-30% with a shortened D4Z4 array, remain asymptomatic or minimally affected. Interestingly, these cases seem to be concentrated in some particular families, suggesting that modifier genes could be segregating in these genealogies modulating the course of the disease in these individuals. In order to try to explain this clinical variability between FSHD patients, we compared the gene expression profiles of muscle tissue from three members (affected, asymptomatic carrier and normal control) from five unrelated FSHD families through expression microarrays. Our preliminary results showed that some genes on chromosome 4q are quantitatively expressed according to the residual number of D4Z4 in affected patients. We also observed that specific chemokines are up-regulated in asymptomatic carriers as compared to their related affected and normal controls, suggesting that they could play a role in a “protecting” mechanism from the pathogenic effects of the disease. The study of gene expression profiles from asymptomatic carriers is a novel approach that is revealing new and interesting results. Understanding such mechanisms is a great challenge, but will certainly lead to the development of new tools for prognosis and also for future treatment

## Guanine Quadruplexes, non-B DNA Structures, in D4Z4: Potential for Interactions

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Potential G-quadruplexes (PQS) are non-B structures containing four equal-length runs of G residues interacting by Hoogsteen base-pairing. They are thought to have the highest thermodynamic stability per base-pair of any secondary structure found in nucleic acids. They can take the form of hairpin structures formed from four clustered G runs, bimolecular-type structures from two clustered G runs, or tetramolecular structures (especially at telomeres) from four G runs on different DNA strands. D4Z4 arrays at 4q35.2, which are tightly linked to facioscapulohumeral muscular dystrophy (FSHD), contain a high density of PQS, consistent with their very high G+C content (73%). The 3.3-kb repeat unit of D4Z4 contains seven oligonucleotides that exactly match the PQS hairpin consensus sequence:

$G_{3-5}N_{1-7}G_{3-5}N_{1-7}G_{3-5}N_{1-7}G_{3-5}$ , where the length of the four G runs is equal.

Circular dichroism (CD) analysis of these seven D4Z4 sequences tested as single-stranded oligonucleotides revealed the characteristic spectra for G-quadruplexes. In addition, CD analysis provided evidence for at least six more PQS-like sequences from D4Z4 DNA that can form G-quadruplexes. These include two that should be interacting as bimolecular-type quadruplexes. Non-denaturing polyacrylamide gel electrophoresis of several of these sequences demonstrated that they can interact with each other, probably forming the bimolecular-type quadruplexes. Further evidence for quadruplex formation from PQS came from attempts to clone two D4Z4 subregion fragments containing PQS by the use of overlap extension and PCR. From one of the subregions, all four analyzed recombinant DNA clones had 7-12 base-substitution mutations localized to G residues in the PQS. From the other subregion, all four DNA clones had deletions almost precisely overlapping the PQS. The deletions and base substitutions were not observed upon overlap extension and PCR when the template had been experimentally mutated in the PQS. These findings and our comparison of five different thermostable DNA polymerases have technical implications for PCR and sequencing of D4Z4.

Lastly, our chromatin immunoprecipitation experiments on fetal myoblasts using D4Z4 primer-pairs and a nucleolin antibody provide evidence that nucleolin, a DNA-binding protein with a remarkably high affinity for G-quadruplexes, binds to multiple sites in D4Z4. This confirms a previous finding by Gabellini et al. (Cell 110:339, 2002) of nucleolin binding to a sequence immediately upstream of the *DUX4* open reading frame within D4Z4. Nucleolin is a chromatin remodeling protein and histone chaperone. Because D4Z4 chromatin topology probably plays a crucial role in FSHD pathogenesis, we propose that this quadruplex-binding protein and G-quadruplexes are involved in chromatin interactions of clinical importance to FSHD. (Supported in part by NIH grant R01 NS048859)

## Size and Number of D4Z4 Alleles Play a Role in FSHD Phenotype

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Autosomal dominant facioscapulohumeral muscular dystrophy (FSHD) is associated with a contraction of the repetitive element D4Z4 array on chromosome 4. A correlation between repeat contraction and clinical severity was found. However, a remarkable phenotypic variability of clinical expression even within the same family is observed. We investigated 9 FSHD families, in which at least one compound heterozygous patient, a rare condition for this disease, was present.

Our study includes 11 compound heterozygous patients for short deleted alleles. Clinical examination of FSHD families, and molecular studies of D4Z4 region were performed. A dedicated clinical form was designed to investigate FSHD patient's clinical history and to obtain the functional quantification of muscle weakness through a questionnaire that allows to assign a disability score specifically modified for FSHD patients, ranging from 0 to 15. The final score is the result of the evaluation of 6 distinct muscle groups (facial shoulder arm leg hip abdominal muscles). The clinical disability score (called FSHD score) based on functional evaluation of each muscular groups affected, was used to define the severity of disease expression.

Our study shows that heterozygosity for FSHD-sized alleles contributed to a more severe phenotype (FSHD score  $7.4 \pm 3.7$ ) with respect to relatives (FSHD score  $1.8 \pm 2.4$ ), carrying a single deletion on chromosome 4. We observed that 33 cases (70%) carrying the deletion on a single chromosome 4 were asymptomatic or showed a very mild phenotype. Besides, in compound heterozygotes FSHD was fully penetrant. This was independent of age and telomeric polymorphisms 4qA/4qB. Significantly, more females than males resulted protected from developing the disease.

Based on these observations, it is possible to hypothesize that the more severe clinical expression of the disease observed in compound heterozygotes might be related to transcriptional derepression of genes at 4q35 occurring at both deleted D4Z4 alleles, supporting the current model for FSHD pathogenesis.

## **Treatment with a Soluble Activin Receptor Type IIB Increases Muscle Mass and Strength in Wild-type Mice and Common Marmosets**

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Facioscapulohumeral muscular dystrophy (FSHD) is a genetic disorder which causes progressive weakening of skeletal muscle. Patients suffering from FSHD may experience speech impediments, an inability to raise their arms and difficulty walking. A potential strategy to alleviate these debilitating symptoms is to therapeutically increase muscle size and strength. An emerging target for this type of therapeutic intervention is the Activin receptor type IIB (ActRIIB). ActRIIB is the high affinity receptor for GDF-8 (a negative regulator of skeletal muscle growth) and other related GDF family members. To assess the effects of ActRIIB inhibition, C57BL/10 mice (n=10, male) were treated with a soluble ActRIIB-Fc fusion protein (ACE-031). Animals were dosed biweekly via intraperitoneal injection with 0.3, 1.0, 3.0, or 10 mg/kg ACE-031. Treatment for 2 and 4 weeks resulted in dose dependent increases in body weight and concomitant increases in lean tissue mass (assessed by NMR). At 4 weeks, the ACE-031 treated mice demonstrated significantly increased skeletal muscle mass (gastrocnemius, rectus femoris, pectoralis). To determine if these pharmacological effects would extend to higher species, ACE-031 was administered to marmosets (n=6/sex) via subcutaneous injection. Weekly administration of ACE-031 at 3 mg/kg resulted in significantly increased lean tissue mass (DEXA and Echo MRI) at 2 and 4 weeks. At 4 weeks, the treated group had gained 4.3 times more lean tissue (vehicle:  $+3.45 \pm 7.7$  g, ACE-031:  $+14.83 \pm 8.5$  g) as compared with control animals. To assess the functional relevance of the increases in muscle mass, ex vivo force measurements were performed on extensor digitorum longus (EDL) muscle from treated and untreated cohorts. Both absolute force (mN) and specific force (mN/muscle CSA) of twitch and tetanic contractions in EDL muscles from treated marmosets were significantly greater, confirming that ACE-031 treatment indeed produces a functional gain of strength. Demonstration of biologic activity in nonhuman primates strongly suggests utility for ACE-031 in the treatment of human muscle disorders, thereby offering hope for otherwise untreatable conditions such as FSHD.

## **Structural and Functional Characterization of Muscle Fibres in the Novel Mouse Model of Facioscapulohumeral Muscular Dystrophy**

Valentina Sancisi<sup>1</sup>, Elena Germinario<sup>2,3</sup>, Valeria Ghiaroni<sup>1</sup>, Elisabetta Morini<sup>1</sup>, Daniela Danieli-Betto<sup>2,3</sup>, Rossella Tupler<sup>1,4</sup>

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<sup>3.</sup> *Interuniversity Institute of Myology, Italy*

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

Facioscapulohumeral muscular dystrophy (FSHD) is a hereditary neuromuscular disorder characterized by progressive weakness and atrophy of the facial, shoulder, abdominal and pelvic girdle muscles. Evidence indicates the over-expression of genes mapped at distal long arm of chromosome 4 (4q35) as responsible for FSHD.

Transgenic mice over-expressing FRG1 (FSHD Region Gene 1) at low, medium and high level were utilized to confirm this hypothesis. Indeed FRG1 transgenics develop a progressive muscular dystrophy whose degree of severity correlates with the level of expression of the transgene. FRG1 transgenics showed reduced tolerance to exercise, a phenomenon that might correlate to muscle weakness. We also observed that the alternative splicing pattern of skeletal muscle troponin T (Tnnt3) pre-mRNA was altered in muscle of FRG1 mice and FSHD patients. Tnnt3 regulates muscle contractility and we considered the possibility that alterations of the splicing pattern of Tnnt3 pre-mRNA might correlate with muscle weakness.

To investigate the basis of this phenomenon, contractile properties of soleus, extensor digitorum longus and biceps muscles were studied in vitro. Calcium-sensitivity and specific tension of single fibres from soleus, vastus lateralis (VL) and biceps muscles were also analyzed. Functional properties were correlated to the expression of myosin heavy chains (MyHC), troponin C (TnC) and troponin T (TnT) isoforms.

All muscles showed a progressive loss of twitch and tetanic tensions, confirmed by the lower specific tension recorded in single fibres. The pCa-tension relationship of VL and biceps fibres was significantly shifted to the right, particularly in mice expressing FRG1 at the highest level. Muscle contractile proteins display a high variability of isoforms, due to multiple genes and/or alternative splicing. To investigate the molecular basis of physiological alterations observed in FRG1 mice we are analyzing isoform expression of myofibrillar proteins in transgenic and WT animals. SDS-PAGE and WB analyses of dystrophic muscles show a general shift toward a slow phenotype of both MyHC and TnC isoforms and an altered expression of fast TnT isoform.

This analysis will allow correlations of molecular alterations with physiological properties of dystrophic muscle.

**Muscular Dystrophy Candidate Gene FRG1 Functions in Angiogenesis and Muscle Development**

Ryan D. Wuebbles, Meredith L. Hanel and Peter L. Jones

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

The leading candidate gene responsible for facioscapulohumeral muscular dystrophy (FSHD) is *FRG1* (*FSHD region gene 1*). However, the correlation of altered FRG1 expression levels with disease pathology has remained controversial and the precise function of FRG1 is unknown. Here we carried out a detailed analysis of the normal expression patterns and effects of FRG1 mis-expression during *Xenopus* embryonic development. We show that *frg1* is expressed in and essential for the development of the vasculature and musculature. Depletion of FRG1 led to inhibited myotome growth and decreased angiogenesis. Elevated FRG1 led to abnormal epaxial and hypaxial muscle formation, dilated vasculature, and increased angiogenesis. Beyond dystrophic muscle, FSHD patients also often exhibit retinal vasculopathy and an increase in vasculature mRNA transcripts. Thus, *frg1* over-expression in *Xenopus* links aberrant skeletal muscle development with increased abnormal vasculature, two clinical aspects of FSHD, supportive of a causal role underlying the pathology exhibited in FSHD patients.



**A Drosophila Model for the Effects of Increased FRG1 Expression**

Shihuang Su, Manli Shen, Allison Chin, Mo Madhavi and William Mattox

*Department of Genetics, University of Texas, M.D. Anderson Cancer Center, Houston Texas*

We have developed a Drosophila model to study the effects of increased expression of FRG1. Transgenic strains were generated in which the Drosophila orthologue of this gene (CG6480) is expressed under the control of the GAL4-UAS system. Increased levels of FRG1 targeted specifically to the indirect flight muscle resulted in muscle loss, wing position abnormalities and a flightless phenotype. In contrast expression of FRG1 in other tissues (eyes, wing epithelia and sensory organs) had no obvious phenotypic effects suggesting that muscle is unusually sensitive to FRG1 levels. Flies with ubiquitous FRG1 displayed reduced viability in the larval-pupal stages and a similar lethal phase was observed when FRG1 was expressed in larval muscle. This also supports the above idea. We will report preliminary results from genetic screens in which second site mutations suppressing FRG1 lethality were identified. Among the suppressors are mutations affecting the fly orthologue of PABPN1 the primary factor in OPMD.

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

Patrick W. Reed, Neil C. Porter MD, and Robert J. Bloch

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

We have been studying the proteome of muscle from patients with Facioscapulohumeral muscular dystrophy (FSHD) to learn how the deletions at 4q35-ter alter protein expression to cause disease. Using Large Gel 2-D electrophoresis, we found that mu-crystallin is present in high amounts in FSHD muscle but present at much lower levels in healthy and disease controls. Immunoblots confirmed its high levels in FSHD muscles compared to controls and other muscular dystrophies. The fact that mu-crystallin is highly over-expressed is consistent with FSHD's autosomal dominant character, which is normally associated with a "gain of function" mutation. As T3 plays a central role in muscle differentiation, the over-expression of mu-crystallin could contribute to pathogenesis in part by altering T3 activity. Remarkably, the inner ear and the retina are both affected in FSHD patients, and mu-crystallin has been associated with retinal defects and deafness in monkeys and humans, respectively. Thus, the evidence suggests that mu-crystallin is a good candidate for a pathogenic protein in FSHD. Mu-crystallin is encoded from 16p13.11-p12.3, and so any muscle pathology caused by its over-expression would act downstream of the primary genetic defect at region 4q35. It is important to replicate the expression levels of mu-crystallin in animal muscles to levels similar to those observed in FSHD muscles to determine its importance in FSHD. We have been using *in vivo* electroporation to introduce mammalian expression plasmids encoding mu-crystallin under the control of the CMV promoter to learn whether high levels of expression of mu-crystallin can account for some or all of the muscle pathology seen in patients with FSHD. Preliminary results, to be reported, indicate that muscles electroporated to overexpress mu-crystallin show several signs of pathology, including an increase in the number of central nuclei and in the variation of fiber diameter.

Supported by the FSH Society and the Muscular Dystrophy Association.

**7:30 – 7:55            REGISTRATION & CONTINENTAL BREAKFAST**

**7:55 – 8:00            OPENING REMARKS & WELCOME**

*Kathryn Wagner*

*Silvère van der Maarel*

**8:00-8:30            KEYNOTES & CHARGE TO THE MEETING**

*Daniel Perez, FSH Society*

*Jane Larkindale, MDA*

*John Porter, U.S. DHHS NIH NINDS*

Abstracts listed by first author followed by presenting author (designated by asterisk)

**8:30—09:45            Topic 1   D4Z4 and DOWNSTREAM TRANSCRIPTS**

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

- |  |                           |
|--|---------------------------|
| <b>1) Darko Bosnakovski</b>  | <b>Michael Kyba *</b>     |
| DUX4 in cells and mice   |                           |
| <b>2) Amy Asawachaicharn</b>   | <b>Stephen Tapscott *</b> |
| The Transcriptional and Post-transcriptional Landscape of the D4Z4 Region                |                           |
| <b>3) Yi-Wen Chen</b>  | <b>Yi-Wen Chen *</b>      |
| Conditional expression of Pitx1 in mouse skeletal muscles causes muscle atrophy          |                           |
| <b>4) Daphne Cabianca</b>  | <b>Davide Gabellini *</b> |
| Transcription of the region proximal to D4Z4 could regulate 4q35 gene expression in FSHD |                           |
| <b>5) Lindsay Wallace</b>  | <b>Scott Q. Harper *</b>  |
| DUX4 Over-Expression Causes Muscle Toxicity In Vivo                                      |                           |

**09:45 – 10:00            MORNING BREAK**

**10:00—11:00            Topic 2   CHROMATIN**

Jane Hewitt, Ph.D. (moderator)

- |  |                                 |
|--|---------------------------------|
| <b>1) Alexandre Ottaviani</b>  | <b>Frederique Magdinier *</b>   |
| CTCF as a New Regulator of D4Z4 Function   |                                 |
| <b>2) Natalia A. Rabaia</b>  | <b>Yvonne Meijer-Krom *</b>     |
| Role of CTCF and Chromatin Structure in FSHD   |                                 |
| <b>3) Xueqing Xu</b>   | <b>Xueqing Xu *</b>             |
| Facioscapulohumeral muscular dystrophy: gene discovery by DNase-chip                   |                                 |
| <b>4) Jessica C de Greef</b>   | <b>Silvère van der Maarel *</b> |
| Comparative methylation analysis of the D4Z4 repeat array on chromosome 4q35 and 10q26 |                                 |

**11:00 – 11:45            Topic 3   FSHD CENTERS UPDATES**

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

- |   |                          |
|---|--------------------------|
| <b>1) NIH Sen. Wellstone FSHD MD CRC</b>                                  | <b>Louis Kunkel *</b>    |
| Biomarkers for Therapy of FSHD  |                          |
| <b>2) Fields FSHD Center</b>  | <b>Rabi Tawil *</b>      |
| Fields Center for FSHD and Neuromuscular Research                         |                          |
| <b>3) Italian Patient Network</b>   | <b>Rossella Tupler *</b> |
| Italian FSHD National Registry: a Tool for Genotype-Phenotype Correlation |                          |

**11:45 – 12:00            POWERPOINT PRESENTATION OF POSTERS**

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

- |  |                           |
|--|---------------------------|
| <b>1) Valery Kazakov</b>   | <b>n/a *</b>              |
| About the facioscapulohumeral, type 2 (or the facioscapulohumeral) autosomal dominant muscular dystrophy in the K. kindred once again                    |                           |
| <b>2) Nina Canki-Clain</b>   | <b>Nina Canki-Clain *</b> |
| Possible Mechanisms Responsible for Great Inter and Intra Familial Phenotypic Heterogeneity in FSHD  |                           |
| <b>3) Meredith Hanel</b>   | <b>Meredith Hanel *</b>   |
| Investigating D4Z4 mediated gene regulatory and epigenetic effects on FRG1 in a telomeric environment in the developing vertebrate <i>Xenopus laevis</i> |                           |

- 4) **Rinse Klooster** **Silvère van der Maarel \***  
Comprehensive expression analysis of FSHD candidate genes provides evidence for transcriptional deregulation of FRG2 only
- 5) **Edgardo Daniel Corona** **Alberto Rosa \***  
Mutagenesis analysis of the DUX4 nuclear localization signals NLS1 and NLS2
- 6) **Julie Dumonceaux** **Julie Dumonceaux \***  
FRG1 over-expression induces severe dystrophic phenotype in the adult mouse
- 7) **Leslie Lock** **n/a \***  
Pluripotent Stem Cells for FSHD Research

**12:00 – 1:15      BUFFET LUNCH and POSTER VIEWING**

**1:15—2:30      Topic 4   CLINICAL AND EXPRESSION STUDIES**

Kathryn Wagner, M.D., Ph.D. (moderator)

- 1) **Pierre Walrafen** **Nicolas Lévy \***  
Direct and simultaneous visualization of D4Z4 arrays on distinct 4qA, 4qB and 10q combed alleles : implication for FSHD diagnosis and physiopathology
- 2) **Patricia Arashiro** **Patricia Arashiro \***  
Trying to Understand the Clinical Variability in FSHD
- 3) **Melanie Ehrlich** **Melanie Ehrlich \***  
Guanine Quadruplexes, non-B DNA Structures, in D4Z4: Potential for Interactions
- 4) **Greta Fabbri** **Greta Fabbri \***  
Size and Number of D4Z4 alleles play a role in FSHD phenotype
- 5) **Laura Hagerty** **Laura Hagerty \***  
Treatment with a Soluble Activin Receptor Type IIB Increases Muscle Mass and Strength in Wild-type Mice and Common Marmosets

**2:30—3:30      Topic 5   FRG1 and CRYM**

Yi Wen Chen, D.V.M., Ph.D. (moderator)

- 1) **Valentina Sancisi** **Rossella Tupler \***  
Structural and functional characterization of muscle fibres in the novel mouse model of Facioscapulohumeral Muscular Dystrophy
- 2) **Ryan Wuebbles** **Ryan Wuebbles \***  
Muscular dystrophy candidate gene FRG1 functions in angiogenesis and muscle development
- 3) **Shihuang Su** **William Mattox \***  
A Drosophila model for the effects of increased FRG1 expression
- 4) **Patrick Reed** **Patrick Reed \***  
Mu-Crystallin and the Pathogenesis of FSHD

**3:30 – 3:45      AFTERNOON BREAK**

**3:45 – 5:00      Topic 6   IDENTIFY TOP 5-10 PRIORITIES FOR 2009**

Silvère van der Maarel, Ph.D. (moderator)  
Kathryn Wagner, M.D., Ph.D. (moderator)  
Rabi Tawil, M.D.  
Rune R. Frants, Ph.D.  
John Porter, Ph.D.  
Yi Wen Chen, D.V.M., Ph.D.  
Charlie Emerson, Ph.D.

**IDENTIFY TOP 5-10 PRIORITIES FOR 2009 FSHD RESEARCH DIRECTIONS AND COLLABORATIONS**

**Group Discussion:**

1. **Animal and cellular models**
2. **Resources and repositories**
3. **Trials and patient registries**
4. **Chromatin and candidate genes**