

Facioscapulohumeral Muscular Dystrophy International Research Consortium 2006

Monday, October 9, 2006
10 a.m. – 6:00 p.m.

Hilton New Orleans Riverside
Two Poydras Street
New Orleans, Louisiana 70140 USA
Marlborough A & B Rooms – 2nd floor

Chair: Silvère van der Maarel, Ph.D.
Organizers: Daniel Paul Perez
William R. Lewis, Sr., M.D.
Silvère van der Maarel, Ph.D.

Sponsored by:

FSH Society, Inc.



Wyeth Pharmaceuticals



Association Française Contre les Myopathies (AFM)



Muscular Dystrophy Association (MDA USA)



October 9, 2006

PREFACE

Dear Colleagues,

Welcome to the Facioscapulohumeral Muscular Dystrophy (FSHD) International Research Consortium 2006. Thanks to you we are seeing rapid developments on numerous fronts in FSHD. We are seeing momentum in research laboratories, clinics, treatments and interventions, in government and private funding programs and in international collaboration of volunteer health agencies and FSHD patients.

The FSH Society is currently compiling a tactical and strategic research plan for FSHD. We continually ask ourselves -- how can we best accelerate the rate of discovery in FSHD research? Communication of what we know, what we do not know and what we need to know is key. We encourage the sharing of new data and new ideas to promote solutions, treatment and therapy for FSHD.

We ask you to keep updating all funding agencies with newly gained knowledge and insights. We need your highest level of collegiality and willingness to share. We need you to help us produce a series of uniform reagents (anti-bodies, cell lines and animal models) to provide consistent experimental materials. We need your willingness to propagate, share and bio-bank materials. The more insight you provide, the better we are able to deliver research and business plans, to target funding from philanthropists, foundations, businessmen, government funding agencies and volunteer health agencies.

FSHD research will see yet another banner year in 2006 and into 2007, as more researchers become involved and as more clinical researchers and drug companies gain experience in clinical trials on FSHD.

This meeting is sponsored by the FSH Society, Inc., Wyeth Pharmaceuticals, the Association Française Contre les Myopathies (AFM), and the Muscular Dystrophy Association USA (MDAUSA). It is truly a pleasure to bring the entire group together to accelerate solutions!

Thank you for coming.

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

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

William R. Lewis, Sr., M.D.
FSH Society, Inc., Monterey, California, USA

The Facioscapulohumeral Muscular Dystrophy Society (FSH Society) 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 are acknowledged for tax purposes. All inquiries should be addressed to the FSH Society, Inc., Carol Perez, 3 Westwood Road, Lexington, Massachusetts 02420 Phone: (781) 860-0501, fax: (781) 860-0599, e-mail: solvefshd@fshsociety.org, website: <http://www.fshsociety.org>

FSHD IRC Workshop 2006

9:55 – 10:00 Opening Remarks

10:00—11:20

Topic 1

Population & Genome Wide Studies, Quantitative & Qualitative Transcriptome Analysis

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

- 1) Meena Upadhyaya 10:00 – 10:20
- 2) Jessica de Greef 10:20 – 10:40
- 3) Amy Asawachaicharn 10:40 – 11:00
- 4) Joseph Marx 11:00 – 11:20

11:20–12:20

Topic 2

DUX4 & Therapy

Melanie Ehrlich, Ph.D. (moderator)

- 5) Yi-Wen Chen 11:20 – 11:40
 & Eugénie Annseau
- 6) Michael Kyba 11:40 – 12:00
- 7) Rossella Tupler 12:00 – 12:20

12:20 – 1:40 Lunch

1:40-3:00

Topic 3

Chromatin

Rossella Tupler, M.D., Ph.D. (moderator)

- 8) Melanie Ehrlich 1:40 – 2:00
- 9) Chunbo Shao 2:00 – 2:20
- 10) Koji Tsumagari 2:20 – 2:40
- 11) Frédérique Magdinier 2:40 – 3:00

3:00-3:20

Topic 4

Studies of A-type lamins and EDMD

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

- 12) Brian Kennedy 3:00-3:20

3:20 – 4:00 Break

4:00 – 4:20 Update FSHD planning meeting (William R. Lewis, Sr., M.D.)

4:20 – 5:30 Group Discussion (Silvère van der Maarel, Ph.D.)

WELCOME

9:55-10:00 a.m.

OPENING REMARKS & CHARGE FOR THE MEETING

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

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

10:00-11:20 a.m.

PLATFORM PRESENTATION(S) I

Silvère van der Maarel, Ph.D., Moderator

Leiden University Medical Center, Leiden, The Netherlands

Population & Genome Wide Studies, Quantitative & Qualitative Transcriptome Analysis

10:00-10:20 a.m.

Meena Upadhyaya, Ph.D.

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

A large patient study confirming that facioscapulohumeral muscular dystrophy (FSHD) disease expression is almost exclusively associated with an FSHD locus located on a 4qA-defined 4qter subtelomere.

Thomas, NST¹, Wiseman, K¹, Spurlock, Gill¹, MacDonald, M¹, Üstek, D² and Upadhyaya, Meena¹

¹Department of Medical Genetics, School of Medicine, Cardiff University, Heath Park, Cardiff, CF14 4XN, United Kingdom

²Division of Medical Genetics, Department of Pediatrics, Istanbul University, 34390, Capa, Istanbul, Turkey

Application of array comparative genomic hybridisation (CGH) to FSHD.

Kiran Mantripragada¹, Bisma Monem¹, Jan Dumanski², Meena Upadhyaya¹

¹Institute of Medical Genetics, Cardiff University, Heath Park, Cardiff, United Kingdom

²Department of Genetics, Howell and Elizabeth Heflin Center for Human Genetics, Comprehensive Cancer Center, Birmingham, Alabama, USA

10:20-10:40 a.m.

Jessica de Greef, Ph.D.

Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands

Comparative analysis of 4q-linked FSHD, non-4q-linked FSHD, and ICF syndrome.

JC de Greef¹, M Wohlgemuth², O Chang¹, RR Frants¹, C Weemaes³, GW Padberg², SM van der Maarel¹

¹Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands

²Department of Neurology, University Medical Center Nijmegen, Nijmegen, Netherlands

³Department of Pediatrics, University Medical Center Nijmegen, Nijmegen, Netherlands

10:40-11:00 a.m.

Amy Asawachaicharn, Ph.D.

*Graduate Program in Molecular and Cellular Biology, University of Washington,
Seattle, Washington 98109 USA*

Expression profiling of MyoD-induced FSHD fibroblasts reveals limited expression overlap with FSHD muscle biopsies.

Amy Asawachaicharn^{1,2}, Yi Cao¹, and Stephen J. Tapscott¹

¹Graduate Program in Molecular and Cellular Biology, University of Washington, Seattle, Washington 98109 USA

²Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109 USA

11:00-11:20 a.m.

Joseph G. Marx, Ph.D.

Department of Biochemistry, University of Washington, Seattle Washington 98195 USA

Investigation of splicing defects in facioscapulohumeral muscular dystrophy (FSHD).

Joseph G. Marx, Steve D. Hauschka, and Brian K. Kennedy

Department of Biochemistry, University of Washington, Seattle WA 98195 USA

11:20-12:20 p.m.

PLATFORM PRESENTATION(S) II

Melanie Ehrlich,, Ph.D., Moderator

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

DUX4 & THERAPY

11:20-11:40 a.m.

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

*Center for Genetic Medicine Research, Children's National Medical Center, Washington,
DC, 20010 USA*

DUX4 transcriptionally regulates paired-like homeodomain transcription factor 1.

Manjusha Dixit¹, Aquanette Brown², Alexandra Belayew³, Yi-Wen Chen^{1,2}

¹Center for Genetic Medicine Research, Children's National Medical Center, Washington, DC, 20010 USA

²Department of Pediatrics, George Washington University, Washington, DC, USA

³Laboratory of Molecular Biology, University of Mons-Hainaut, Mons, Belgium

Also presenting for Eugénie Ansseau, Ph.d., Alexandra Tassin, Ph.D., Alexandra Belayew, Ph.D., et. al.

DUX4 gene expression in FSHD primary myoblasts.

Eugénie Ansseau¹, Alexandra Tassin¹, Sébastien Sauvage¹, Dalila Laoudj-Chenivresse², Marietta Barro², Denise Figlewicz³, Frédérique Coppée¹, and Alexandra Belayew¹

¹Laboratory of Molecular Biology, University of Mons-Hainaut, 7000-Mons, Belgium

²Centre de Recherche en Biochimie Macromoléculaire (C.R.B.M.), Montpellier, France

³Department of Neurology, University of Michigan, Ann Arbor, Michigan, USA

11:40-12:00 p.m.

Michael Kyba, Ph.D.

Center for Developmental Biology, University of Texas Southwestern Medical Center, 6001 Harry Hines Blvd, Dallas, Texas 75093 USA

Conditional gain of function analysis points to a key role for DUX4 in FSHD pathology.

Michael Kyba^{*1}, Rita C. R. Perlingeiro¹, Alexandra Belayew², Darko Bosnakovski¹, and Mingju Liu¹

¹Center for Developmental Biology, University of Texas Southwestern Medical Center, 6001 Harry Hines Blvd, Dallas, Texas 75093 USA

²Lab. Biologie Moleculaire, Université de Mons-Hainaut Pentagone, avenue du Champ de Mars 6B - 7000 – Mons Belgium

12:00-12:20 p.m.

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

Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, Massachusetts, 01605 USA & Dipartimento di Scienze Biomediche, Università' di Modena e Reggio Emilia, Modena, Italy

A strategy to develop potential therapeutic approaches for treating FSHD.

Marie McConkey¹, Bridget Wagner², Monica Salani¹, Valeria Ghiaroni³, Valentina Sancisi³, Paul Clemons², Rossella Tupler^{1,3}

¹Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, Massachusetts, 01605 USA

²Initiative for Chemical Genetics, Chemical Biology Program, Broad Institute of Harvard & MIT, Cambridge, Massachusetts, 02139 USA

³Dipartimento di Scienze Biomediche, Università' di Modena e Reggio Emilia, Modena, Italy

12:20 p.m.-1:40 p.m.

LUNCH

(Buffet Lunch Served)

1:40-3:00 p.m.

PLATFORM PRESENTATION(S) III

Rossella Tupler, M.D., Ph.D., Moderator

Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, Massachusetts, 01605 USA & Dipartimento di Scienze Biomediche, Universita' di Modena e Reggio Emilia, Modena, Italy

CHROMATIN

1:40-2:00 p.m.

Melanie Ehrlich, Ph.D.

Hayward Genetics Program and Department of Biochemistry, Tulane Medical School, Tulane University, New Orleans, Louisiana, 70112 USA

Differences in DNA Methylation within the D4Z4 Array, at Its First Repeat Unit, and Immediately Proximal to the Array in Normal Tissues and Cancers.

Melanie Ehrlich, Kesmic Jackson, Lixin Qi, and Michelle Lacey

Hayward Genetics Program and Department of Biochemistry, Tulane Medical School, and Department of Mathematics, Tulane University, New Orleans, Louisiana, 70112 USA

2:00-2:20 p.m.

Chunbo Shao, Ph.D.

Hayward Genetics Program and Department of Biochemistry, Tulane Medical School, New Orleans, Louisiana, 70112 USA

Need for a Comprehensive Analysis of 4q35 to Find the FSHD Master Gene.

Chunbo Shao¹, Koji Tsumagari¹, Vassilios Alexiadis², Mary Warren², and Melanie Ehrlich¹

¹Hayward Genetics Program and Department of Biochemistry, Tulane Medical School, New Orleans, Louisiana, 70112 USA

²Genpathway, Inc., San Diego, California 92121 USA

2:20-2:40 p.m.

Koji Tsumagari, Ph.D.

Human Genetics, Tulane Medical School, New Orleans, Louisiana, 70112 USA

The D4Z4 Array, Which Is Linked to Facioscapulohumeral Muscular Dystrophy, Has a Very Different Chromatin Structure than the Adjacent p13E11 Sequence.

Koji Tsumagari¹, Janet Sowden², Rabi Tawil², Stephen Hauschka³, and Melanie Ehrlich¹

¹Human Genetics, Tulane Medical School, New Orleans, Louisiana, 70112 USA

²Department of Neurology, University of Rochester School of Medicine, Rochester, New York, 14642 USA

³Department of Biochemistry, University of Washington, Seattle, Washington, 98195 USA

2:40-3:00 p.m.

Frédérique Magdinier, Ph.D.

*Laboratoire de Biologie Moléculaire de la Cellule, Ecole Normale Supérieure de Lyon,
CNRS UMR5161, INRA U1237, IFR128, Lyon, France*

D4Z4 behaves as an insulator element protecting gene expression from telomeric silencing and the influence of other 4q subtelomeric sequences.

Alexandre Ottaviani, Sylvie Rival-Gervier, Andrea Förster, Eric Gilson & Frédérique Magdinier
Laboratoire de Biologie Moléculaire de la Cellule, Ecole Normale Supérieure de Lyon, CNRS UMR5161,
INRA U1237, IFR128, Lyon, France

3:00-3:20 p.m.

PLATFORM PRESENTATION(S) IV

Silvère van der Maarel, Ph.D., Moderator

Leiden University Medical Center, Leiden, The Netherlands

Studies of A-type lamins and EDMD

3:00-3:20 p.m.

Brian K. Kennedy, Ph.D.

Department of Biochemistry, University of Washington, Seattle Washington 98195 USA

Studies of A-type lamins and Emery-Dreifuss Muscular Dystrophy (EDMD)

Brian K. Kennedy, Steve D. Hauschka, and Joseph G. Marx
Department of Biochemistry, University of Washington, Seattle WA 98195 USA

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

BREAK

4:00-4:20 p.m.

UPDATE FSHD PLANNING MEETING

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

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

4:20-5:30 p.m.

GROUP DISCUSSION

Silvère van der Maarel, Ph.D.

Leiden University Medical Center, Leiden, The Netherlands

ABSTRACTS SECTION

Abstract	First author	Assignment
1	Thomas	Platform
2	Mantripragada	Platform
3	De Greef	Platform
4	Asawachaicharn	Platform
5	Marx	Platform
6	Dixit	Platform
7	Ansseau	Poster
8	Kyba	Platform
9	McConkey	Platform
10	Ehrlich	Platform
11	Shao	Platform
12	Tsumagari	Platform
13	Ottaviani	Platform
14	Kazakov	Poster
15	Kennedy	Platform

1. A large patient study confirming that facioscapulohumeral muscular dystrophy (FSHD) disease expression is almost exclusively associated with an FSHD locus located on a 4qA-defined 4qter subtelomere.

Thomas, NST¹, Wiseman, K¹, Spurlock, Gill¹, MacDonald, M¹, Üstek, D² and Upadhyaya, Meena¹

¹Department of Medical Genetics, School of Medicine, Cardiff University, Heath Park, Cardiff, CF14 4XN, United Kingdom

²Division of Medical Genetics, Department of Pediatrics, Istanbul University, 34390, Capa, Istanbul, Turkey

Introduction: Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant muscle disorder that represents the third most common human muscular dystrophy. The FSHD disease locus is located at chromosome 4q35, where it is associated with large contractions of locus D4Z4, a highly polymorphic repeat array. In addition to the almost complete association between FSHD disease expression and the presence of large D4Z4 deletions, another biased molecular association with a specific 4qter subtelomeric sequence has recently been described in FSHD patients. Two distinct 4qter subtelomeres, defined as types 4qA and 4qB, have been identified, and these two allelic forms were found to be equally prevalent in the Caucasian population. However, in almost all chromosome 4-linked FSHD patients, disease expression is only found to occur when large, 4q35-located D4Z4 deletions are situated, in cis, on 4qA-defined 4qter subtelomeres.

Objective: To confirm and extend this FSHD disease association data by measuring the frequency of type 4qA- and 4qB-defined 4qter subtelomeres in a large cohort of 164 unrelated FSHD patients, all with known large D4Z4 deletions, derived from Turkey and the UK.

Results: An almost complete association was found between large, 4q35-located D4Z4 deletions located on 4qA-defined 4qter subtelomeres and FSHD disease expression in our extensive patient cohort. In addition, the failure of probes 4qA and 4qB to hybridise to two patient-derived DNA samples identified the presence of an additional rare type of 4qter subtelomeric sequence in humans.

Conclusions: We have greatly strengthened the FSHD disease association data, using the largest FSHD patient panel so far studied, to confirm that large D4Z4 deletions located in cis on 4qA-defined 4qter subtelomeres are essentially in complete association with FSHD disease expression. This confirmed data will have immediate practical value for the molecular diagnosis of FSHD in suspected patients.

2. Application of array comparative genomic hybridisation (CGH) to FSHD.

Kiran Mantripragada¹, Bisma Monem¹, Jan Dumanski², Meena Upadhyaya¹

¹Institute of Medical Genetics, Cardiff University, Heath Park, Cardiff, United Kingdom

²Department of Genetics, Howell and Elizabeth Heflin Center for Human Genetics, Comprehensive Cancer Center, Birmingham, Alabama, USA

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common muscular dystrophy. Frustratingly, while the FSHD locus was mapped to the 4q35 region more than a decade ago, the identity of the FSHD gene is still unknown. Novel approaches may therefore be required to 'tease out' the true FSHD gene/s from somewhere within the 4q35 candidate region, a genomic region containing many pseudogenes, duplications and repetitive sequences.

Microarray-based comparative genomic hybridisation (array-CGH) has been developed to detect variations in DNA copy number across the genome, and the methodology has been used to screen for the presence of micro-deletions and insertions in a number of disease-associated genes. A direct relationship between copy number and mRNA expression levels has been reported (Pollack et al., 2002). Many of these studies provide clear evidence that DNA copy number alterations can lead directly to global de-regulation of gene expression.

In our pilot study, we searched for DNA copy number changes in leucocyte DNA samples isolated from 9 FSHD patients. The 32K BAC array (Ishkanian, et al., 2004) was used, a microarray platform that makes use of overlapping genomic clones at a resolution of ~100 kb, to provide a comprehensive, contiguous coverage of the entire genome. Strict quality control criteria were used for the analysis of the data obtained from the microarray hybridizations. Specific copy number alterations were detected by more than a 1000 different clones across the array. In order to maximise the likelihood of identifying FSHD-specific alterations, the array-CGH data derived from analysing each of the 9 FSHD DNA samples were compared, and those clones that were similarly altered in at least 3 of the samples were then considered for further analysis. Using this threshold we identified 288 genomic clones, of which 258 were amplified and 30 were deleted, that were localised to 10 different chromosomes (1 >5, 7, 8, 16, 17 and 19). The gene content of the genomic regions contained in each of these clones was then determined by reference to the relevant NCBI and Ensembl databases.

We then compared our data to previous expression profiling data from FSHD patients (Winokur et al., 2003). Interestingly, the potential amplification of some genes (e.g., DNAJA3, DNAH17, DNAH3, COL5A3, POLR3F, TTLL5, RPL24, and ZNF protein family) were reflected in over-expression of their family member proteins. We have also identified several genes with skeletal-muscle specific functions, and which, therefore, may be considered as potential candidates for pathogenesis in FSHD. The ANKMY2 gene, for example, which contains ankyrin repeats and a MYND domain, was deleted in 5 out of the 9 FSHD patients. It is known that proteins containing the SET and MYND protein domains are specifically expressed in skeletal muscle. In addition, a

number of both the amplified genes (TITF1, HEBP2, RFWD2, TMEM43, TTL10, CDH5, RCC1, RCC2, FOXR1, VMD2, MOS, CACNA2D1, MIB1, UPK2, HMOX2, NOXO1, DNAH3, DOT1L, SBF1, SAPS2, SIX5, PLEKHA4, and PLEKHAJ1) and the deleted genes (OR2Z1, STOX2, KTN1, SNX13 and HEG1) are also of interest. Subsequent work will concentrate on minimising the number of candidate genes by profiling a much larger patient sample set (>50 FSHD samples), along with appropriate numbers of control samples, with the 32K microarray. Potential candidate genes that are then identified will be further characterised by direct mutation analysis to determine their potential role in FSHD pathogenesis.

3. Comparative analysis of 4q-linked FSHD, non-4q-linked FSHD, and ICF syndrome.

JC de Greef¹, M Wohlgemuth², O Chang¹, RR Frants¹, C Weemaes³, GW Padberg², SM van der Maarel¹

¹Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands

²Department of Neurology, University Medical Center Nijmegen, Nijmegen, Netherlands

³Department of Pediatrics, University Medical Center Nijmegen, Nijmegen, Netherlands

Facioscapulohumeral muscular dystrophy (FSHD) typically presents with weakness and wasting of the facial-, shoulder, and upper arm muscles and is caused by a partial deletion of the D4Z4 repeat in the subtelomere of chromosome 4q. Contraction of D4Z4 is associated with hypomethylation of this repeat suggestive for a chromatin restructuring at 4qter. Interestingly, hypomethylation of D4Z4 is also observed in non-4q-linked FSHD patients without contraction of D4Z4 and in patients suffering from the immunodeficiency, centromeric instability and facial abnormalities (ICF) syndrome, an unrelated disease not presenting with muscular dystrophy and caused by mutations in the DNMT3B gene, coding for a DNA methyltransferase.

In order to identify the gene defect in non-4q-linked FSHD and to unravel the pathogenic epigenetic pathway in FSHD, we have aimed to identify the differences and commonalities in FSHD, non-4q-linked FSHD and ICF syndrome. First, we have investigated whether non-4q-linked FSHD is caused by a defect in the same molecular pathway as ICF syndrome by examination of the methylation of non-D4Z4 repeat arrays in 4q-linked and non-4q-linked FSHD patients that were previously reported to be hypomethylated in ICF. Second, we have analyzed metaphase spreads of mitogen-stimulated lymphocytes of non-4q-linked FSHD patients for the presence of pericentromeric abnormalities of chromosomes 1, 9 and 16, which can be observed in ICF syndrome. Third, as ICF patients suffer from a defective B-cell differentiation, associated with hypogammaglobulinaemia, peripheral blood samples of non-4q-linked FSHD patients were tested for IgA, IgG and IgM levels. Fourth, we have performed mutational analysis of candidate genes involved in the biological process of DNA methylation in search for a second disease locus involved in the pathogenesis of non-4q-linked FSHD.

Our results do not show epigenetic or phenotypic commonalities between 4q-linked FSHD, non-4q-linked FSHD and ICF, other than the observed D4Z4 hypomethylation, suggesting that non-4q-linked FSHD is not caused by a defect in the same molecular pathway as ICF syndrome. We also did not find any mutations in the candidate genes tested for in several non-4q-linked FSHD patients.

4. Expression profiling of MyoD-induced FSHD fibroblasts reveals limited expression overlap with FSHD muscle biopsies.

Amy Asawachaicharn^{1,2}, Yi Cao¹, and Stephen J. Tapscott¹

¹Graduate Program in Molecular and Cellular Biology, University of Washington, Seattle, Washington 98109 USA

²Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109 USA

Transcriptional profiling of FSHD muscle biopsies previously revealed that the majority of genes misregulated in FSHD are involved in muscle differentiation and cell cycle control (Winokur et al., 2003). Specifically, many of the affected transcripts in FSHD represent genes that interact with, or are direct targets of the transcription factor, MyoD, suggesting a defect in normal myogenic differentiation program. Not only does the mechanism responsible for this gene dysregulation in FSHD remain unknown, but it is also unclear whether the aberrant transcripts detected result primarily from an intrinsic defect in FSHD muscle or from secondary dystrophic changes.

To determine whether global effects in myogenic genes result directly from a defect in MyoD-mediated gene activation, we have used a model system of MyoD-mediated conversion of human fibroblasts to skeletal muscle cells utilizing FSHD patients' fibroblasts that stably expressed inducible MyoD. We have found that these FSHD fibroblasts were able to differentiate into myotubes and expressed muscle specific genes upon induction of MyoD, suggesting that the transcriptional defects seen from muscle biopsies may not be due entirely to a defect in myogenic differentiation.

We then used microarray expression profiling to identify MyoD-mediated gene expression changes in these FSHD fibroblasts-derived muscle cells to determine whether we can recapitulate transcriptional changes shown in FSHD muscle biopsy study (Winokur et al., 2003). By comparing our result to the expression profile from FSHD muscle biopsies, we have shown that our tissue culture system shares a very limited gene expression overlap with the expression from FSHD muscle biopsies.

5. Investigation of splicing defects in facioscapulohumeral muscular dystrophy (FSHD).

Joseph G. Marx, Steve D. Hauschka, and Brian K. Kennedy

Department of Biochemistry, University of Washington, Seattle Washington 98195 USA

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common form of muscular dystrophy. FSHD presents with muscle weakening and fatigue in face, back/shoulders, and leg muscles. About 95% of the cases involve deletion of a sub-telomeric region (D4Z4 repeats). How reduction of these 3.2kb D4Z4 repeats leads to FSHD is unknown. D4Z4 repeats contain a potential repressor element, which binds the YY1 - HMGB2- nucleolin complex, effectively silencing downstream genes; thus loss of repeats likely leads to abnormal gene expression. However, expression studies of three nearby genes (FRG1, FRG2, and ANT1) have led to contradictory data (Gabellini et al. 2002; Winokur et al. 2003; Jiang et al. 2003). Recent transgenic studies suggest that FRG1 over-expression results in the mis-splicing of skeletal troponin and myotubularin related protein-1 and a muscular dystrophy phenotype (Gabellini et al. 2006). We are investigating FRG1 expression during myoblast growth and differentiation in vitro. We are also studying potential interactions between FRG1 and the D4Z4 repressor complex; and we are developing an in vitro splicing assay to analyze FRG1's role in MTMR1 splicing. This work is supported by the Pacific Northwest Friends of FSH and NIH NS-46788.

6. DUX4 transcriptionally regulates paired-like homeodomain transcription factor 1.

Manjusha Dixit¹, Aquanette Brown², Alexandra Belayew³, Yi-Wen Chen^{1,2}

¹Center for Genetic Medicine Research, Children's National Medical Center, Washington, DC, 20010 USA

²Department of Pediatrics, George Washington University, Washington, DC, USA

³Laboratory of Molecular Biology, University of Mons-Hainaut, Mons, Belgium

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disease linked to a partial deletion of the 3.3 kb D4Z4 repeat arrays at chromosome 4q35. The only known gene in each of the D4Z4 repeat unit is encoding a double homeodomain protein, DUX4, that was shown expressed in the myoblasts of patients with FSHD but not in controls. In this study, we determined whether paired-like homeodomain transcription factor 1 (PITX1) which was shown specifically up-regulated in FSHD was a direct transcriptional target of DUX4. To test whether DUX4 directly interacted with the Pitx1 promoter, we first amplified a 318-bp fragment with a putative DUX4 binding site in the Pitx1 promoter region, and inserted the fragment into the pGL3-basic luciferase reporter vector (Promega). We co-transfected C2C12 mouse myoblast cells with the pGL3-Pitx1 construct and the pCIneo-DUX4 and pCIneo-DUX4c expression vector, respectively, then measured the luciferase activities at 24 hours post-transfection. Co-transfection with either pCIneo-DUX1 (expressing a non-pathological homologue) or the pCIneo vector without insert was performed as controls. We observed a 7.4-fold increase (n=4, p=1.6x10⁻²¹) of the luciferase activity when pGL3-Pitx1 was co-transfected with pCIneo-DUX4 compared to samples co-transfected with the insertless vector. Co-transfection with pCIneo-DUX4c led to 4.3-fold (p=0.002) up-regulation of the luciferase activity, but the changes were not significant for pCIneo-DUX1 co-transfection. To validate the interaction between the DUX4 and Pitx1 promoter, the putative DUX4 binding site was mutated by site directed mutagenesis. Results showed that the luciferase activity was significantly decreased (p=1.7x10⁻¹⁷) when C2C12 cells were co-transfected with pCIneo-DUX4 and the mutant pGL3-Pitx1. Co-transfection with pCIneo-DUX4c led to a mild decrease in luciferase activity (p=0.047), and co-transfection with pCIneo-DUX1 showed no effect.

In conclusion, our results showed that DUX4 could specifically activate the Pitx1 promoter and that this was abolished by mutations of a putative DUX4 binding site. Although DUX4c could slightly activate the Pitx1 promoter, the binding site mutation did not affect the activation while the DUX1 protein that has a different target sequence did not activate the promoter. This is the first study identifying a direct transcription target of DUX4. Moreover, the fact that the PITX1 gene is specifically up-regulated in FSHD and associated to muscle atrophy and left/right asymmetry provides a direct link between the genetic defect in 4q35 and the pathophysiology of the disease.

7. DUX4 gene expression in FSHD primary myoblasts.

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Several years ago we have identified the DUX4 double homeobox gene within each unit of the D4Z4 repeat array that is contracted in FSHD. Demonstration of DUX4 expression in patient muscle cells proved technically very challenging because of its low level, its toxicity, and its homology to hundreds of DUX genes unlinked to FSHD. We could demonstrate expression of the homologous non-toxic DUX4c protein encoded by an isolated D4Z4 element 42 kb centromeric of the repeat array: it was induced by differentiation in control and FSHD myoblasts and was expressed at higher levels in biopsies of patients with low D4Z4 copy numbers.

For DUX4 mRNA studies we used retro-transcription (RT) and amplification by PCR (see below). We cloned all the products we obtained in the pCR4 plasmid and sequenced individual clones to confirm they were identical to DUX4. We first set up optimal conditions on total RNA's extracted from mouse C1C12 cells transfected with a pGEM plasmid containing either a single D4Z4 element, or a 13.5-kb EcoRI genomic fragment from the contracted 4q35 allele of a patient with only two D4Z4 units left. The extracted total RNA was digested with desoxyribonuclease I to eliminate putative DNA contamination. The same experimental conditions were then applied to human primary myoblasts. By 5' RACE we found one major transcription initiation site mapping 48 bp downstream from a TACAA box, as well in transfected cells as in FSHD and control myoblasts. We performed RT with a DUX4-specific primer followed by amplification with DUX4-specific primers flanking its open reading frame. We could detect by agarose gel electrophoresis the expected 1.4-kb RT-PCR product as well in transfected cells as in primary myoblasts: the signal was much stronger in differentiated versus proliferating FSHD myoblasts but hardly detectable in control myoblasts. No product different from DUX4 was found upon sequencing, and no product was obtained upon omission of the RT step.

For DUX4 protein detection, we have raised a monoclonal antibody against its carboxyl-terminal domain (297 residues) outside of the homeodomains that specifically detects the DUX4 (52 kDa) and homologous DUX4c (47 kDa) proteins on Western blots performed with extracts of cells transfected with pCI-neo-DUX expression vectors. The Western blot sensitivity was recently increased about 20-fold by use of a new peroxydase substrate (Pierce) and allowed detection of DUX4 in 6 additional FSHD myoblast lines. In conclusion these data underscore our hypothesis that the DUX4 gene is expressed in patients with FSHD.

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8. Conditional gain of function analysis points to a key role for DUX4 in FSHD pathology.

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We have undertaken a large-scale comparative phenotypic screen of candidate genes from 4q35. We find that uniquely among the genes tested, DUX4 is exquisitely toxic to myoblasts. With conventional gene expression methods, toxicity makes it impossible to do careful studies on homogeneous cell populations. We have adapted an inducible, conditional gain of function system, originally developed for our work with embryonic stem (ES) cells, to lentiviral vectors. C2C12 myoblasts were modified with a single copy of a doxycycline-inducible locus carrying cre flanked by incompatible loxP sites, which enables cassette exchange to insert any gene of interest downstream of the inducible promoter. All derivative cell lines are clonally related, and contain integrated transgenes at the identical genomic site, thus they are directly comparable. In this system, high DUX4 expression is followed sequentially by accumulation of the protein in euchromatic regions of the nucleus, gene expression changes, cell morphological changes, and apoptosis by 24 hours. At lower levels of DUX4, these changes are drawn out and differentiation is induced. We present microarray transcriptional profiling data from uninduced, 4-, and 12-hour induced samples, which demonstrate striking similarities to the Winokur et al. data from FSHD patient biopsies and myoblast cultures. To generate conditional gain of function mice, we have made an equivalent ES cell line. In vitro differentiation of these cells demonstrates that high levels of DUX4 are toxic to many but not all cell types. Thus, DUX4 represents a highly attractive candidate for the proximate cause of FSHD.

9. A strategy to develop potential therapeutic approaches for treating FSHD.

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Muscular dystrophies are among the most difficult diseases to treat, even though the underlying molecular defects are mostly known. So far, therapeutic approaches have been mainly focused on Duchenne Muscular Dystrophy, a fatal disorder, whereas FSHD has been, in this respect, neglected. Only a few therapeutic trials have been performed in FSHD patients so far. The anabolic effects on muscle of albuterol, a beta-adrenergic agonist, have been tested in FSHD patients in three randomized trials (Kissel et al, 1998; Kissel et al, 2001; van der Kooi et al, 2004). In all studies, albuterol appears to have limited positive effects on muscle strength and volume and its routine prescription is precluded. At present no effective therapy is available for FSHD.

Based on our previous observations (Gabellini et al, 2002), we generated transgenic mice over-expressing ANT1, FRG1, or FRG2, three 4q35 genes centromeric to D4Z4, and observed that mice over-expressing FRG1 develop muscular dystrophy with features of the human disorder (Gabellini et al, 2006). Our discovery pointed to a crucial role played by FRG1 in FSHD pathogenesis. We therefore reasoned that by suppressing/reducing the biological activity of FRG1 in muscles affected by FSHD, we might prevent or slow disease onset and/or progression.

To accomplish this goal, we considered that pharmacological strategies have intrinsic advantages: they are easy to deliver systemically and can circumvent many of the immunological and/or toxicity problems that are connected to vector and cell-based therapies. Thus we decided to undertake high-throughput screens (HTS) to identify compounds that will suppress/reduce the biological activity of FRG1 in muscles affected by FSHD and, as a consequence, will prevent or slow the disease progression. To this purpose we have designed three high-throughput screens based on different aspects of FRG1 biology. Specifically we are searching for small molecules that: a) suppress/reduce FRG1 promoter activity (dual luciferase assay), b) rescue differentiation ability in FRG1 over-expressing muscle cells, or c) bind recombinant FRG1.

Three sources of small molecules are utilized: diversity-oriented synthesis (DOS), bioactives (characterized in the literature), and other commercially available compounds. The DOS small molecules have the built in advantage of diverse complex structures grouped by similar chemistries that can be utilized for structure analysis. Moreover, as part of the post-screening modifications, DOS compounds can be optimized through appropriate chemical modifications.

These screens will allow us to explore different and alternative ways to counteract the pathogenic actions of FRG1 over-expression as well as different aspects of FRG1 biological activity. Selected compounds will be tested in FRG1 over-expressing mice, a testable model system to develop potential therapeutic approaches for treating FSHD. We envision that through the analysis of the results of the proposed HTSs we will acquire novel elements to understand FRG1 biological function in muscle tissue.

10. Differences in DNA Methylation within the D4Z4 Array, at Its First Repeat Unit, and Immediately Proximal to the Array in Normal Tissues and Cancers.

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DNA methylation is often associated with chromatin structure such that more methylation of cytosine indicates more chromatin condensation and transcriptionally repressive modifications of histones. Changes in chromatin structure in and near D4Z4 at 4q35 are probably critical to FSHD. D4Z4 methylation has already been shown by van Overveld et al. to be significantly decreased in FSHD patients. Methylation of D4Z4 at 4q35 and 10q26 requires DNA methyltransferase 3B. Other tandem repeats methylated by this enzyme are frequently hypo- or hypermethylated in cancer. Therefore, we studied methylation at CpG-containing sites (EagI, SmaI, MluI, BsaAI, HpyCH4IV, BstUI, and HpaII) in D4Z4 arrays from diverse normal tissues and cancers. For BsaAI, HpyCH4IV, BstUI, and HpaII sites, we compared methylation within the bulk of the D4Z4 array (2, 6, 37, and 71 sites per 3.3-kb repeat unit, respectively) and in the most proximal D4Z4 repeat unit or at the sequence outside and immediately proximal to the array (0, 1, 1, and 3 sites, respectively). Methylation analysis was by blot hybridization with a D4Z4 sub-fragment or a proximal sequence (p13E-11) as a probe under conditions specific for detection of 4q35 and 10q26 sequences. The examined CpG sites in D4Z4 were largely, but incompletely, methylated within the bulk of the array in normal somatic tissues and often displayed major increases or decreases in methylation in ovarian carcinomas and Wilms tumors. Importantly, cancers that showed hypermethylation in the bulk of the D4Z4 array were usually resistant to this hypermethylation or underwent hypomethylation, the opposite change, at analogous sites in the first repeat of the array and immediately proximal to the array. The difference in methylation status depending on the position in the array cannot be attributed to differences in the D4Z4 sequence because of the unusually high conservation of sequence throughout the array, even at its proximal end. Therefore, the observed position-dependent differences in methylation of D4Z4, seen strikingly in cancers, are likely to reflect an effect of chromatin conformation on the DNA methylation apparatus. These results suggest a different chromatin structure at the proximal end of D4Z4 array vs. the rest of the array. The inferred abrupt change in chromatin structure at the D4Z4/non-D4Z4 proximal junction may be important in the interaction of a short FSHD-causing D4Z4 array and its postulated immediate target gene (FSHD master gene) at 4q35. (Supported in part by NIH grant R01 NS048859)

11. Need for a Comprehensive Analysis of 4q35 to Find the FSHD Master Gene.

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Facioscapulohumeral muscular dystrophy (FSHD) is associated with shortening of the D4Z4 array on chromosome 4q35.2, which is a gene-poor region according to the annotation in GenBank. Indirect evidence suggests that there should be an FSHD master gene, which is located at 4q35 and responds in cis to an abnormally short D4Z4 array, thereby leading to FSHD. The identity of this gene or genes is not clear. ANT1, FRG1, and FRG2 at 4q35 have been reported to be overexpressed at the RNA level in FSHD skeletal muscle by one lab but not by several others. From studies of commercial and custom-made expression microarrays, no additional candidates for the FSHD master gene have been proposed. In the most complete oligonucleotide expression microarray (Affymetrix U133) used for analysis of FSHD and control muscle samples, the 3-MB 4q35.2 region was represented only by a small number of known or hypothetical genes, including DUX4 and DUX4C. We have recently shown that there is little or no expression of DUX4 and DUX4C in FSHD and control myoblasts. Using expression microarrays, which are based upon the currently annotated human genome, for discovery of the FSHD master gene has the caveat that some genes will be missed because they are undocumented, unconventional, or have important, unrepresented splice variants. For example, although there are few known 4q35.2 genes, there are many more predicted genes based upon AceView models or Genscan. To look for the FSHD master gene in an unbiased fashion, we will assay the transcriptional activity of about one hundred well-spaced 0.2-kb subregions of the 1-MB region proximal to the 4q35.2 D4Z4 array. We will exclude the two segmental duplication regions, which are ~100 to 200 kb and ~300 to 450 kb proximal to the D4Z4 array, as well as the subtelomeric repeat region which is a ~100 kb region immediately proximal to the D4Z4 array. We have begun designing 4q35.2-specific PCR primers that can amplify ~0.2-kb sequences for use in real-time PCR assays in chromatin immunoprecipitation (ChIP) experiments with an antibody to the large subunit of RNA polymerase II. This ChIP assay, RNAPol-ChIP, measures the engagement of RNA polymerase II at a given subregion of chromatin as a direct measurement of transcription. We designed seven pairs of PCR primers located ~270 to 300 kb proximal to the D4Z4 array in 4q35.2. All primer-pairs were shown to be specific for chromosome 4 by conventional PCR using a panel of somatic cell hybrid DNAs. Moreover, upon real-time PCR, the products obtained had a single-peak melting curve, also indicating their specificity. These and other 4q35.2-specific primer-pairs will be used in RNAPol-ChIP analysis of FSHD and control myoblast samples, firstly, to look for new genes in 4q35.2 and, secondly, to test for FSHD-specific differences in transcription in this D4Z4-containing subband of chromosome 4. (Supported by FSH Society grant).

12. The D4Z4 Array, Which Is Linked to Facioscapulohumeral Muscular Dystrophy, Has a Very Different Chromatin Structure than the Adjacent p13E11 Sequence.

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To test whether D4Z4 arrays, which are associated with facioscapulohumeral muscular dystrophy, have the proposed heterochromatin-like structure, we used *in vivo* assays for chromatin DNaseI sensitivity. The chromatin DNaseI sensitivity assays included a comparison of the chromatin structure of D4Z4 arrays to that of p13E11, an adjacent, non-repeated sequence, which is only 0.1 kb proximal to D4Z4. In myoblasts, fibroblasts, and lymphoblastoid cells lines, we found conditions for DNaseI treatment of nuclei or lysolecithin-permeabilized cells that allow the relative accessibility (degree of condensation) of the test DNAs (D4Z4 and p13E11) in chromatin to be determined relative to that of standard DNA sequences. The DNA standards were constitutively expressed genes, HMBS, and B2M; genes unexpressed in the examined cell populations, CST5 and GHRHR; and satellite 2, the constitutive heterochromatin standard. After *in vivo* treatment with six different concentrations of DNaseI (usually 0 – 100 units/ml for 5 - 15 min at 37°C), the DNA was isolated, digested with StyI, and blot-hybridized. Partial digestion of these sequences by DNaseI *in vivo* was quantitated by the phosphorimager-determined decrease in intensity of the corresponding 1-to-3 kb StyI fragment in a single rehybridized Southern blot for each sample. We found that in fetal myoblasts, FSHD and control lymphoblastoid cell lines, and control fibroblasts, D4Z4 on both 4q35 and 10q26 had a DNaseI sensitivity intermediate to that of constitutive heterochromatin and unexpressed genes. We conclude that D4Z4 arrays are more condensed than unexpressed euchromatin but less than constitutive heterochromatin. The p13E11 sequence immediately adjacent to D4Z4 was much more sensitive to DNaseI and even more sensitive than were unexpressed genes. Analysis of FSHD and control myoblasts and comparison of short and long D4Z4 arrays is currently underway. Therefore, the p13E11 sequence seems to have a much looser chromatin structure than the adjacent D4Z4 array and is unexpectedly looser in its conformation than are unexpressed gene standards. This suggests that there is a strain in the chromatin structure at the border between D4Z4, with its 73% G+C content, and the adjacent p13E11 sequence, with its 39% G+C content. This apparent discontinuity in chromatin structure at the proximal border of the D4Z4 array may be important in the hypothesized looping between a short D4Z4 array at 4q35 and the currently unknown primary FSHD gene located *in cis*. (Supported in part by NIH grant R01 NS048859)

13. D4Z4 behaves as an insulator element protecting gene expression from telomeric silencing and the influence of other 4q subtelomeric sequences.

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The human subtelomeric regions are mosaics of genomic elements shared by different chromosomes. These regions are highly dynamic and may participate in the regulation of the telomere homeostasis but also, be associated with human pathologies. One of the best characterized human subtelomeric elements is the D4Z4 repeat involved in the Facio-Scapulo-Humeral dystrophy (FSHD). Several hypotheses have been proposed in order to decipher this muscular dystrophy and a link between position effect and the 4q telomeric region rearrangement is a popular hypothesis to explain the molecular mechanism of this pathology. Our goal was to test the function of D4Z4 on the regulation of telomere silencing.

Using the telomere fragmentation strategy, we reconstituted the basic genomic organization of the 4q35 locus involved in FSHD and analyzed the epigenetic effect mediated by D4Z4 in a telomeric context after integration of the transgene into the human genome. We showed that the D4Z4 subtelomeric element is a bona fide insulator element protecting from TPE and able to block enhancer-promoter communication.

A very few number of insulator elements have been described in the human genome so far and the mechanisms underlying their boundary activity and their putative implication in human pathology remain puzzling. D4Z4 is present at a subtelomeric position on chromosome 4 but also on several other loci where it is always localized at the boundary between heterochromatin and non-condensed chromatin suggesting that this element has a broad insulator function in partitioning permissive chromatin domains in the human genome. To address this hypothesis, we also examined telomeric position effect in the presence of different portions of the 4q35 subtelomeric locus and observe a strong silencing for some of the 4q35 subtelomeric elements. This silencing can be counteracted when D4Z4 is placed between this element and the reporter gene suggesting that the 4q chromosome end is a mosaic of regulatory element involved in epigenetic regulation. Thus, the D4Z4 array likely modulates the activity of the other subtelomeric sequences of the 4q35 locus.

In conclusion, we identified a new function for the D4Z4 subtelomeric element associated with the FSHD as an insulator element and mechanisms mediating this activity are under investigation.

14. Clinical, CT and MRI peculiarities in 4q35 linked facioscapuloperoneal muscular dystrophy. Report of two Russian families.

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Abstract. We give the description of two autosomal dominant with 4q35 linked facioscapuloperoneal muscular dystrophy (FSPD) families in which the patients were re-examined by V.K. after 24 - 28 and 35-36 years and the typical changes of the pattern of muscle affections on the different stages of the disease were established and confirmed by muscle CT or MRI.

In observed patients the disease began with initial involvement of the isolated facial muscles or their parts and shoulder girdle muscles and some time later of the peroneal group (anterior tibial) muscles. The developed scapulo-peroneal phenotype with slight or severe affection of the isolated facial muscles or their parts existed in the clinical picture a very long period. The muscles of the thigh (posterior group, namely), pelvic girdle (gluteus maximus, namely) and biceps brachii (slight weakened) are involved in two probands much later and comparatively less degree than the muscles of the scapulo-peroneal region. In connection with this, the name "facioscapulolimb muscular dystrophy, type 2 (FSLD2), a descending with a "jump" with initial FSP phenotype instead the name facio-scapulo-peroneal muscular dystrophy would be more correct. The scapulo-peroneal phenotype with slight or severe affection of isolated facial muscles or their parts (and it is more correctly to call it the FSP phenotype) constitute merely a stage in the development of FSLD2.

The muscle CT and MRI of FSLD2 patients showed more often and severe involvement of anterior compartment of lower leg muscles (tibialis anterior, extensor digitorum longus and extensor hallucis longus), posterior thigh muscles (semimembranosus, long head of biceps femoris and semitendinosus), rectus femoris and some time later of adductors of thighs and gastrocnemius (medial heads) and less degree soleus with sparing of peroneus longus and deep posterior compartment of lower leg, quadriceps, gracilis and sartorius muscles.

The radiological muscle pattern does not fully correlate with clinical pattern of muscle weakness. In patients with FSP phenotype the posterior thigh muscles and quadriceps clinically had a normal strength although the total/severe involvement of some hamstrings and rectus femoris was revealed on MRI and CT study. Asymmetry of the degree of affection in the same muscles on the right and left sides was evident. We suppose that classical AD scapulo-peroneal muscular dystrophy with minimal/slight affection of facial muscles (FSPD) (or FSLD2-descending with a "jump" type, author's note) is an independent form of muscular dystrophy which differs from classical FSHD (or FSLD1- gradually descending type, author's note) although it is connected with the same 4q35 chromosomal deletion.

15. Studies of A-type lamins and Emery-Dreifuss Muscular Dystrophy (EDMD).

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Mutations in the LMNA gene have been linked to a number of dystrophic and progeroid syndromes, including forms of Emery-Dreifuss Muscular Dystrophy and Limb-Girdle Muscular Dystrophy. LMNA encodes A-type nuclear lamins, including lamin A and lamin C. Interestingly, A-type lamins are expressed at high levels in differentiating tissues, leading to speculation that they act to control the balance between cell proliferation and differentiation. Cell biological approaches have suggested possible links between A-type lamin function and normal chromatin regulation of chromosome 4Q35 associated with FSHD.

We have developed cell culture models to address the role of A-type lamins in muscle differentiation. *Lmna*^{-/-} immortalized myoblasts were generated and their in vitro differentiation potential was determined. After induction of differentiation, *Lmna*^{-/-} muscle cells, relative to litter-matched controls, undergo delayed cell cycle withdrawal and delayed appearance of a terminal differentiation protein, myosin heavy chain (MyHC). These phenotypes can be recapitulated by siRNA-enforced reduction of lamin A/C or emerin expression in *Lmna*^{+/+} myoblasts. Several changes in proteins important for muscle differentiation are apparent in *Lmna*^{-/-} myoblasts including reductions in levels of desmin, MyoD and the retinoblastoma protein (pRB). Stable reintroduction of MyoD or desmin restores myogenic potential to *Lmna*^{-/-} myoblasts.

Our findings indicate that A-type lamins are required for proper patterns of gene expression in myoblasts derived from adult skeletal muscle. Further, they suggest that decreased muscle regeneration coupled with increased degeneration underlie muscular dystrophies linked to LMNA mutation. Currently we are initiating studies in *Lmna*^{-/-} mice using transgenic and gene therapy approaches to determine whether reintroduction of desmin or MyoD delays the onset or severity of muscular dystrophy.

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