Facioscapulohumeral Muscular Dystrophy
FSHD

International Research Consortium Research Workshop Meeting 2004

Tuesday, October 26, 2004
10 a.m. - 6 p.m.

The Fairmont Royal York Hotel
100 Front Street W
Toronto, Canada M5I 1E3

Ontario Room

Chair: **Silvère van der Maarel, Ph.D.**
Organizers: **Daniel Paul Perez, Silvère van der Maarel, Ph.D.**

Sponsored by:

FSH Society, Inc.
Facioscapulohumeral Muscular Dystrophy Society, Inc.

Muscular Dystrophy Association (MDA USA)

Association Française Contre les Myopathies (AFM)

October 18, 2004

PREFACE

Dear Colleagues,

On behalf of the FSH Society, I welcome you to Toronto, Canada and to the Facioscapulohumeral Muscular Dystrophy (FSHD) International Research Consortium (IRC) Workshop 2004.

We are working hard toward a goal we know we can achieve. Today we are encouraging collaboration and mutual exploration from each and every participant. Our community is small in size and modest. Together with other colleagues who have contributed effort and time, we represent a substantially larger effort. I tell you this so you will understand the total dedication involved in every phase of this FSHD research program and community. We want our long range plan of solving the puzzle of FSHD to work and we know it can. And, given your solid participation and candid input – it will!

Once again, we stand before the deep mystery of facioscapulohumeral muscular dystrophy (FSHD) and amid the near and far reality of molecular genetics. Today we strive for advances, increased collaboration, renewed enthusiasm, and bold direction in understanding, treating and curing this devastating disease. FSHD is a problem enveloped by a multitude of extraordinary biology concepts each worthy of study independently. For the FSHD patients we must constantly ask ourselves if an interesting line of research and study will lead to a cure or a lessening the burden of disease. The answer to that question in itself will always help us refocus the research field for the benefit of FSHD sufferers. We must choose clear thinking and sound judgment over being mired in differences and the politic of research. If there are differences in the results and research directions, respect them as such and work towards understanding why and what they are. It is essential for us to now work quickly, diligently and co-operatively. FSHD needs a push to make progress happen quickly and this will only come by sharing new data, new theories and new ideas in the theater of the FSHD research consortium.

The workshop format will be platform presentations and interactive poster sessions complemented by active discussion and problem solving. The format does allow more time for plenary discussions following the platform presentations and ensures time and attention to the poster sessions. Platform lectures will be limited to 20 minutes. To encourage discussion, we request that presentations be approximately 10 minutes in length allowing for 10 minutes of discussion. To create more time for discussions, we ask that you do not include introductory slides of a redundant and familiar nature. We also ask that the moderators and presenters of each session stay on time to keep to our schedule. Posters will be presented and viewed in one group. Each presenter will have a few minutes to detail findings and explain the poster followed by a lively discussion by attendees.

Finally, as an attempt to initiate more scientific discussion to formulate better future research strategies for the FSHD field, we are asking in the final session of this meeting your attention to four issues that were raised during the last year and the workshop. Each of these issues is at the interface of basic molecular research and the clinic.

This meeting of the FSHD IRC could not have been organized without the support of our sponsors. I express my gratitude for the generous support of the FSH Society, the Muscular Dystrophy Association of the USA and the Association Française Contre les Myopathies (AFM France). We are honored to have several directors, program directors and senior staff from multiple institutes of the United States National Institutes of Health (NIH), the Canadian Institutes of Health Research (CIHR) and Muscular Dystrophy Canada. In particular, I am indebted to Daniel Paul Perez who took charge of most of the work organizing this meeting.

It is truly a pleasure to be meeting with the entire group and know that we will have a fast moving and interesting workshop!

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

WELCOME
9:55-10-00 a.m.

OPENING REMARKS
Daniel Paul Perez, President & CEO, FSH Society, Inc.

PLATFORM PRESENTATION I

MODEL SYSTEMS
10:00-11:00 a.m.
Silvère van der Maarel, Ph.D., Moderator
Leiden University Medical Center, Leiden, The Netherlands

10:00-10:20 a.m.
Sabrina Sacconi, Ph.D.
Fédération des Maladies Neuromusculaires and INSERM U638, Nice France

Myoblasts from Unaffected Muscle of Facioscapulohumeral Muscular Dystrophy Patients Demonstrate no Alteration in Proliferation, Differentiation and in vivo Regenerating Ability when Compared to Controls.
S Sacconi[1], JT Vilquin[2], JP Marolleau[3], J Larghero[3], C Desnuelle[1].
[1]Fédération des Maladies Neuromusculaires and INSERM U638, Nice France; [2]Inserm U582, Institut de Myologie, Groupe hospitalier Pitié-Salpêtrière, Paris, France; [3]Laboratoire de Thérapie cellulaire, Hôpital Saint Louis, Paris, France.

10:20-10:40 a.m.
Jane E. Hewitt, Ph.D.
Institute of Genetics, Queens Medical Centre, University of Nottingham, Nottingham, NG7 2UH, UK

Identification of Homologues of the D4Z4 Repeat.
J. Clapp[1], J. Fantes[2] and J.E. Hewitt[1]
[1]Institute of Genetics, Queens Medical Centre, University of Nottingham, Nottingham, NG7 2UH, UK
[2]MRC Human Genetics Unit, Western General Hospital, Edinburgh, EH4 2XU, UK

10:40-11:00 a.m.
Rossella Tupler, M.D., Ph.D.
University of Massachusetts Medical School, Worcester, Massachusetts, USA
Biologia Generale e Genetica Medica, Via Forlanini 14, Pavia, Italy

Analysis of 4q35 gene Over-expression in Transgenic Mice.
Davide Gabellini[1], Barbara Angeletti[2], Vladimiro Calvari[1], Maurizio Moggio[3], Michael R.Green[1], Rossella Tupler[1,2]
[1]Program in Gene Function and Expression, University of Massachusetts Medical School,Worcester, MA, USA
[2]Biologia Generale e Genetica Medica, Via Forlanini 14, Pavia, Italy
[3]Dipartimento di Scienze Neurologiche- Universita' degli Studi Milano, Via F. Sforza, 35, Milan, Italy

PLATFORM PRESENTATION II

MECHANISM(S)
11:00-12:00 p.m.
Sara Winokur, Ph.D., Moderator
University of California, Irvine, California USA

11:00-11:20 a.m.
Melanie Ehrlich, Ph.D.
Department of Biochemistry, Tulane Medical School, New Orleans LA 70112 USA

Analysis of the D4Z4 Repeat Array with a Highly Specific Hybridization Probe.
Melanie Ehrlich, Kesmic Jackson, Mary E. Ballestas, Guanchao Jiang, Koji Tsumagari, Sara Winokur[2], and Vettaikorumakankav Vedanarayanan[3]
Department of Biochemistry, Tulane Medical School, New Orleans LA 70112 USA; [2]Department of Biological Chemistry, University of California, Irvine CA 92697 USA; and [3]Department of Neurology, University of Mississippi, Jackson MS 39601 USA

11:20-11:40 a.m.
Kristen Bastress, Ph.D.
Marcy Speer, Ph.D.
Center for Human Genetics, Duke University Medical Center, Durham, North Carolina USA

D4Z4 fragment analysis and genome-wide SNP screening in Non-Chromosome 4-linked Facioscapulohumeral Muscular Dystrophy.
K.L. Bastress[1], J.M. Staijch[1], R. Lemmers[2], S.M. van der Maarel[2], M.C. Speer[1], J.R. Gilbert[1]
[1]Center for Human Genetics, Duke University Medical Center, Durham, NC
[2]Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands

11:40-12:00 p.m.
Yi-Wen Chen, Ph.D.
Center for Genetic Medicine Research, Children's National Medical Center, Washington, D.C. USA
Department of Pediatrics, George Washington University, Washington, D.C. USA

Over-expression of Pitx1 Gene Activates Muscle Atrophy Pathways.
Ling Li[1] and Yi-Wen Chen[1, 2]
[1]Center for Genetic Medicine Research, Children's National Medical Center, Washington, D.C. USA
[2]Department of Pediatrics, George Washington University, Washington, D.C. USA

POSTER PRESENTATION SESSION & LUNCH

12:00 p.m.-1:30 p.m.

(Buffet Lunch Served at 12:00 p.m.)

Silvere M. van der Maarel, Ph.D.
Leiden University Medical Center, Center for Human and Clinical Genetics, Department of Human Genetics, Leiden, The Netherlands

Contractions of D4Z4 on 4qB Subtelomeres Do Not Cause Facioscapulohumeral Muscular Dystrophy
Richard J. F. L. Lemmers,[1] Marielle Wohlgemuth,[2] Rune R. Frants,[1] George W. Padberg,[2] Eva Morava,[3,4] and Silvere M. van der Maarel[1]
[1]Leiden University Medical Center, Center for Human and Clinical Genetics, Department of Human Genetics, Leiden, The Netherlands; [2]Departments of Neurology and [3]Pediatrics, University Medical Center Nijmegen, Nijmegen, The Netherlands; [4]Department of Medical Genetics, University of Pecs, Pecs, Hungary

Cecilia Östlund, Ph.D.
Departments of Medicine and of Anatomy and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY, USA

Intracellular Trafficking and Dynamics of Double Homeodomain Proteins.
Cecilia Östlund[‡], Ruth M. Garcia-Carrasquillo[‡], Alexandra Belayew[§] and Howard J. Worman[‡]
‡ Departments of Medicine and of Anatomy and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY, USA; § Laboratory of Molecular Biology, University of Mons-Hainaut, Mons, Belgium

Alexandre Ottaviani, Ph.D.
Laboratory of Cell Molecular Biology, CNRS UMR 5161, Telomeric & epigenetic regulations group, Ecole Normale Supérieure de Lyon. 46, Allée d'Italie. 69007 Lyon,France

Role of the D4Z4 Sequence, Telomere Repeats and Subtelomeric Elements in the Etiology of the FacioScapuloHumeral Dystrophy (FSHD).
Alexandre Ottaviani, Eric Gilson, Frédérique Magdinier
Laboratory of Cell Molecular Biology, CNRS UMR 5161, Telomeric & epigenetic regulations group, Ecole Normale Supérieure de Lyon. 46, Allée d'Italie. 69007 Lyon,-France

Richard J.L.F. Lemmers, Ph.D.
Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands

Timing and Mechanism of Somatic D4Z4 Contractions; Causes and Consequences.
Richard J.L.F. Lemmers[1], Michiel J.R. van der Wielen[1], Egbert Bakker[1], George W. Padberg[2], Rune R. Frants[1], and Silvère M. van der Maarel[1]
[1]Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands; [2]Department of Neurology, University Medical Center Nijmegen, Nijmegen, The Netherlands

Denise A. Figlewicz, Ph.D.
Department of Neurology, University of Michigan, Ann Arbor, Michigan USA

Withdrawal From the Cell Cycle in FSHD Myoblasts.
D.A. Figlewicz, Y. Hong, I. Coltas
Department of Neurology, University of Michigan, Ann Arbor, Michigan USA

POSTER PRESENTATION SESSION & LUNCH (Continued)

Valery Kazakov, M.D., Ph.D.
Department of Neurology, Pavlov State Medical University, St. Petersburg, Russia

What is Scapuloperoneal or (Facio)scapuloperoneal Muscular Dystrophy with 4q35 Deletion: Is it an Independent Form or a Variant of a Facioscapulohumeral Muscular Dystrophy? What was Davidenkov's Opinion Concerning this Important Problem?

Valery Kazakov, Dmitry Rudenko
Department of Neurology, Pavlov State Medical University, St. Petersburg, Russia

PLATFORM PRESENTATION III

U.S. NIH INITIATIVES AND THE FSHD NATIONAL REGISTRY
1:30 p.m.-2:00 p.m.

1:30-1:45 p.m.
Glen H. Nuckolls, Ph.D., Program Director for Muscle Disorders and Therapies
National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Institutes of Health (NIH), DHHS, Bethesda, Maryland USA

National Institutes of Health (NIH) Initiatives in Muscle Disease Research.
Glen H. Nuckolls, Ph.D.
Extramural Programs, Muscle Biology Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Institutes of Health (NIH), DHHS, Bethesda, Maryland USA

1:45-2:00 p.m.
Rabi Tawil, M.D.
University of Rochester School of Medicine, Rochester, New York USA

The FSHD National Registry.
Rabi Tawil, Richard Moxley, Charles Thornton, Colleen Donlin-Smith, James Hilbert, Eileen Eastwood, Nancy Merriman, Karen Berk, Bill Martens, Tracy Forrester, Mike McDermott, and James Forrester
University of Rochester School of Medicine, Rochester, New York USA

PLATFORM PRESENTATION IV

PROTEINS
2:00-3:20 p.m.
Denise Figlewicz, Ph.D., Moderator
University of Michigan, Ann Arbor, Michigan USA

2:00-2:20 p.m.
Alexandra Belayew, Ph.D.
University of Mons-Hainaut, Mons, Belgium

Functional Study of the *DUX4* and *DUX4c* Genes.
Eugénie Ansseau, Frédérique Coppée, Eva D'Amico, Aline Marcowycz, Christel Mattéotti, Sébastien Sauvage, Jonathan Willocq, and Alexandra Belayew
Laboratory of Molecular Biology, University of Mons-Hainaut, 7000-Mons, Belgium

2:20-2:40 p.m.

Silvère M. van der Maarel, Ph.D.
Department of Human Genetics, Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands

The Interaction Between FRG1P and PABPN1 Implies a Common Molecular Pathway for the Muscular Dystrophies FSHD and OPMD.

Silvana van Koningsbruggen[1], Kirsten R. Straasheijm[1], Hans G. Dauwerse[1], Janet M. Cowan[2], Patricia G. Wheeler[2], Henk J. ter Laak[3,4], Baziel G.M. van Engelen[4], George W. Padberg[4], Rune R. Frants[1], Silvère M. van der Maarel[1]
[1]Department of Human Genetics, Center for Human and Clinical Genetics, Leiden University Medical Center, Netherlands; [2] Division of Genetics, Department of Pediatrics, Tufts-New England Medical Center, Boston, Massachusetts, USA; [3]Department of Pathology and [4]Department of Neurology, University Medical Center, Nijmegen, Netherlands

2:40-3:00 p.m.

Dalila Laoudj-Chenivesse, Ph.D.
Centre de Recherche en Biochimie Macromoléculaire, CNRS, 1919 route de Mende, 34293,Montpellier, France

Increased Levels of ANT1 Protein and Response to Oxidative Stress are Early Events in FSHD Muscle.

Dalila Laoudj-Chenivesse, Gilles Carnac, Catherine Bisbal, Gerald Hugon, Sandrine Bouillot, Claude Desnuelle, Yegor Vassetzky, and Anne Fernandez
Centre de Recherche en Biochimie Macromoléculaire, CNRS, 1919 route de Mende, 34293. Montpellier, France

3:00-3:20 p.m.

Sara Winokur, Ph.D.
Department of Biological Chemistry, University of California, Irvine, California USA

Characterization of CP39, a Candidate Gene for FSHD.

Sara Winokur, Ulla Bengtsson, Seung-Ah Chung, Jorge H. Martin
Department of Biological Chemistry, University of California, Irvine, California USA

BREAK
3:20-3:40 p.m.

(Tea, Refreshments, and Desserts Served at 3:20 p.m.)

DISCUSSION and CLOSURE
3:40-6:00 p.m.

From inventory to work plan: a pragmatic approach of four issues at the interface of basic molecular research and the clinic.

ISSUE ONE
The role of ANT1 is the FSHD phenotype - Can we relate a mitochondrial dysfunction to complaints of fatigue and pain? Which issues need to be addressed and how?
Moderator: Jane E. Hewitt, Ph.D.
Institute of Genetics, Queens Medical Centre, University of Nottingham, Nottingham, UK

ISSUE TWO
Muscle specificity of the disease - myoblasts from affected and non-affected tissue seem to have different phenotypic properties. Which issues need to be addressed and how?
Moderator: George W.A.M. Padberg, M.D.
Department of Neurology, UMC St Radboud, Nijmegen, The Netherlands

ISSUE THREE
Population genetics – mostly based on non-published data, there may be population differences in the occurrence and/or susceptibility to the disease. What is the relevance? Which issues need to be addressed and how?
Moderator: Silvère M. van der Maarel, Ph.D.
Leiden University Medical Center, Center for Human and Clinical Genetics, Department of Human Genetics, Leiden, The Netherlands

ISSUE FOUR
Unusual phenotypes and genotypes – over the last years, we have seen publications of unusual phenotypes and genotypes in FSHD. How can these contribute to our understanding of the pathogenesis of FSHD at large? Which issues need to be addressed and how?
Moderator: Peter W. Lunt
Department Clinical Genetics, Institute of Child Health, Bristol Children's Hospital, Bristol, England

CONCLUDING REMARKS
6:00 p.m.-

Silvère van der Maarel, Ph.D.

1. Myoblasts from Unaffected Muscle of Facioscapulohumeral Muscular Dystrophy Patients Demonstrate no Alteration in Proliferation, Differentiation and *in vivo* Regenerating Ability when Compared to Controls.

S Sacconi[1], JT Vilquin[2], JP Marolleau[3], J Larghero[3], C Desnuelle[1].

[1]Fédération des Maladies Neuromusculaires and INSERM U638, Nice, France
[2]Inserm U582, Institut de Myologie, Groupe hospitalier Pitié-Salpêtrière, Paris, France
[3]Laboratoire de Thérapie cellulaire, Hôpital Saint Louis, Paris, France

Facioscapulohumeral muscular dystrophy is an autosomal dominant disease characterized by a typical regional distribution, featuring a composed pattern of clinically affected and unaffected muscles. No treatment is currently available for this condition, which physiopathological mechanism is still unknown. Autologous myoblast transfer (MT) from unaffected to affected territories could be considered in view of stopping or slowing the disease progression.

The first mandatory step was to evaluate the feasibility of this approach lied in an extensive characterization of myoblasts prepared from unaffected muscles of FSHD patients. Harbouring the molecular defect, these cells could manifest the deleterious effect of D4Z4 deletion when submitted to a large-scale expansion in order to obtain a suitable amount of cells for MT.

In the present study, we explored proliferation ability, differentiation and animal implantation of myoblasts from clinically and hystologically unaffected muscle of five patients presenting classical FSHD phenotypes and 6 to 10 D4Z4 repeat units. The results were compared to that obtained with myoblasts from healthy muscle of 10 control donors.

Using a validated cell culture methodology, we were able to produce 10^9 FSHD and control cells within 16-23 days. More than 80% of these cells were myoblasts, as demonstrated by the labelling of CD56 and desmin myoblastes specific markers. FSHD myoblasts presented a doubling time equivalent to that of control cells; they kept high proliferation ability, sustained viability (greater than 95%), and a high proportion of CD56 and desmin-positive cells. They did not show telomere shortening. *In vitro* these cells were able to differentiate, expressing myotubes-specific antigens. Myoblasts and myotubes did not show any morphological aberration. This latter finding contrasted with a previous study [Winokur *et al*, 2003; Neuromusc Disord 13:322-333] on myoblastes carrying 3 to 6 D4Z4 repeat units and presenting with "vacuolar-necrotic" phenotype. We could not conclude about this discrepancy that could come from several parameters: different anatomical origin of the biopsy, different degree of dystrophy in these muscles, different genetic status of FSHD patients (i.e. D4Z4 repeat number), or different cell culture procedures.

Since no FSHD animal model is currently available, Rag immunodeficient mice were chosen to assess the *in vivo* regenerating ability of FSHD and control myoblasts. The use of other dystrophic animal models (e.g.: Mdx, calpainopathy, Dy/Dy) would have been irrelevant because these animals are immunocompetent and would have acutely rejected human myoblasts. A month after the injection, human dystrophin-positive fibers were found both in muscles that had received FSHD or control myoblasts. No significant differences were seen in the implantation rate.

In conclusion, our data suggest that myogenic cells expanded from unaffected FSHD muscles may be suitable tools in view of autologous cell transplantation clinical trials. Moreover, the use of pure FSHD myoblast culture models, which contrast with the very crude and variable nature of whole muscle biopsies, could offer several advantages in clarifying FSHD physiopathological mechanisms.

2. Identification of Homologues of the D4Z4 Repeat.

J. Clapp[1], J. Fantes[2] and J.E. Hewitt[1]

[1]Institute of Genetics, Queens Medical Centre, University of Nottingham, Nottingham, NG7 2UH, UK
[2]MRC Human Genetics Unit, Western General Hospital, Edinburgh, EH4 2XU, UK

D4Z4 contains an open reading frame (ORF) potentially encoding a homeodomain protein. DNA hybridization techniques have so far only identified homologues of D4Z4 in higher primates, consistent with a non-coding function of D4Z4. Here we show that bioinformatics analysis of draft genome sequences has enabled us to identify potential homologues of D4Z4 in rhesus macaque, mouse and rat. The sequence and organisation of mouse D4Z4 (mD4Z4) has been confirmed by a combination of PCR and physical mapping. The mD4Z4 unit is 4.9kb, compared to 3.3kb in human. mD4Z4 contains an ORF of 2025bp, potentially encoding two homeodomains with 55% amino acid similarity to those encoded by human D4Z4. There is no significant sequence conservation outside the coding region. The mD4Z4 repeat units are arranged in large tandem arrays, with FISH analysis suggesting a single chromosome locus. The rat homologue of D4Z4 potentially encodes a homeodomain protein with 66% amino acid similarity to the mouse protein. Macaque D4Z4 is longer than human due to an insertion, however the potential ORF is intact. The conservation of the ORF between these species and the identification of several mouse ESTs suggest a coding function for D4Z4. Furthermore, our findings provide potential for the development of a mouse model of FSHD.

3. Analysis of 4q35 gene Over-expression in Transgenic Mice.

Davide Gabellini[1], Barbara Angeletti[2], Vladimiro Calvari[1], Maurizio Moggio[3], Michael R.Green[1], Rossella Tupler[1,2]

[1]Program in Gene Function and Expression, University of Massachusetts Medical School,Worcester, MA, USA
[2]Biologia Generale e Genetica Medica, Via Forlanini 14, Pavia, Italy
[3]Dipartimento di Scienze Neurologiche- Universita' degli Studi Milano, Via F. Sforza, 35, Milan, Italy

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common hereditary myopathies. The disease has been causally related to deletion of tandemly arrayed 3.3 kb repeat units (D4Z4) on chromosome 4q35 possibly affecting chromatin organization and nearby gene expression. Consistently, we have observed that three genes mapping at 4q35, FRG2, FRG1 and ANT1, are over-expressed in the FSHD affected muscle.

Investigating the possibility that deletion of D4Z4 repeats initiates transcriptional misregulation, we showed that an element within D4Z4 specifically binds a multi-protein complex consisting of YY1, a known transcriptional repressor, HMGB2, an architectural protein, and nucleolin. We demonstrated that this multi-protein complex binds D4Z4 *in vitro* and *in vivo* and mediates transcriptional repression of 4q35 genes (Gabellini et al, 2002 (Cell, 110, 339-349, 2002)).

Based upon these results we proposed that deletion of D4Z4 leads to the inappropriate transcriptional de-repression of 4q35 genes resulting in disease. In normal individuals, the presence of a threshold number of D4Z4 repeats leads to repression of 4q35 genes by virtue of a DNA-bound multi-protein complex that actively suppresses gene expression. In FSHD patients, deletion of an integral number of D4Z4 repeats reduces the number of bound repressor complexes and consequently decreases (or abolishes) transcriptional repression of 4q35 genes. The model suggests that deletion of repeated elements in the subtelomeric region of 4q may act in *cis* on neighboring genes by de-repressing their transcription and thus starting a cascade of events, which ultimately lead to FSHD.

To test the proposed pathogenic model *in vivo*, we generated transgenic mice selectively over-expressing ANT1, FRG1, and FRG2 in skeletal muscle; these are the genes that were found over-expressed in muscle tissues affected by FSHD. To this purpose, ANT1, FRG1, and FRG2 have been cloned into an expression vector carrying the human skeletal actin (HSA) promoter that is expressed in all skeletal muscle fibers of the adult mouse, with minor fiber to fiber variation. The transgene expression profiles were analyzed at either the mRNA level or the protein level in skeletal muscle, heart, brain, kidney and liver. The HSA-FRG1, HSA-FRG2, and HSA-ANT1 transgenes were expressed uniquely in skeletal muscle tissues. Interestingly mice over-expressing the HSA-FRG1 transgene develop signs of muscular dystrophy.

We expect functional analysis of genes in the FSHD chromosomal region to provide relevant information to understand the molecular basis of FSHD. Furthermore, animal models reproducing some features of FSHD will be a valuable and endless source of biological material to study the biological effects of 4q35 gene over-expression in muscle. Eventually they can be used to gauge the efficacy of therapeutic treatments.

4. Analysis of the *D4Z4* Repeat Array with a Highly Specific Hybridization Probe.

Melanie Ehrlich, Kesmic Jackson, Mary E. Ballestas, Guanchao Jiang, Koji Tsumagari, Sarah Winokur, and Vettaikorumakankav Vedanarayanan*

Department of Biochemistry, Tulane Medical School, New Orleans LA 70112; Department of Biological Chemistry, University of California, Irvine CA 92697; and Department of Neurology*, University of Mississippi, Jackson MS 39601

The presence of many *D4Z4*-like sequences at locations other than 4q35 and 10q26 has interfered with methylation analysis of *D4Z4* by Southern blotting. Therefore, we tested subfragments of the 3.3-kb repeat to try to find a hybridization probe that is specific for the *D4Z4* sequences on chromosomes 4 and 10 (Chr4 and Chr10). We identified a sub-fragment that extends from 385 to 1391 bp after the *Kpn*I site as a specific probe for Southern blot hybridization. When this probe was used in Southern blots with human-rodent somatic cell hybrid (SCH) DNAs at 14□C below the T_m, there was hybridization only to human Chr4- or Chr10-containing DNAs. With *Kpn*I digests of these hybrid DNAs and human genomic DNA, only the expected 3.3-kb band was obtained. We also used this probe for digests made with *Apo*I, which, like *Kpn*I, is insensitive to CpG methylation, but unlike *Kpn*I is specific for the 4q *D4Z4* repeats. With these digests, the 3.3-kb band was the only band obtained from four different Chr4-containing hybrids and only high-molecular-weight signal was seen in a Chr10-containing hybrid, as predicted. When analogous *EcoR*I and *Hind*III double digests were analyzed with this probe, only high-molecular-weight signal was obtained from Chr4- or Chr10-containing hybrids and human genomic DNA, which is consistent with cleavage only outside the 4q and 10q *D4Z4* arrays. In contrast to the specificity of this *D4Z4* sub-fragment in Southern blotting, no PCR primer-pairs from six different subregions of *D4Z4* were specific for Chr4 and Chr10 upon amplification of a panel of SCH DNAs.

We used this 1-kb hybridization probe to examine CpG methylation at *Eag*I sites by comparing *Eag*I, *Bln*I-*Eag*I, *Apo*I-*Eag*I, *Bln*I and *Apo*I digests of heart, lung, and cerebellum DNA from two autopsy samples. While most of the *D4Z4* copies were methylated at *Eag*I sites, an appreciable number were unmethylated at all examined sites. Furthermore, there was evidence of some tissue-specificity and individual variation in methylation levels that might reflect array sizes. The latter conclusion was consistent with results from blot hybridization of the same set of digests from four skeletal muscle samples, two FSHD and two non-FSHD biopsies. For all the muscle samples, again there was a high degree of methylation at *Eag*I sites in *D4Z4*, but a considerable portion of *D4Z4* copies had unmethylated *Eag*I sites. We also analyzed two FSHD and two non-FSHD lymphoblastoid cell lines for which we know the copy number of all the *D4Z4* arrays. In these cell lines, we consistently saw a much higher degree of methylation, regardless of FSHD or control origin, than in the somatic tissue DNAs. Surprisingly, Southern blot analysis of 15 Wilms tumors and various normal tissues digested with either of two CpG-methylation sensitive restriction endonucleases, *Eag*I or *Sma*I, revealed that 20% of the tumors were hypomethylated and 27% were hypermethylated in their *D4Z4* arrays. That almost half of the tumors had dramatic changes in *D4Z4* methylation, like satellite DNAs, suggests that there is some aspect of the structure of *D4Z4* arrays *in vivo* which resembles that of satellite DNA. We are using the 1-kb *D4Z4* hybridization probe to analyze the chromatin structure within the *D4Z4* repeat arrays by DNaseI chromatin sensitivity assays to determine if the arrays themselves are condensed like satellite DNA heterochromatin. Supported in part by NIH Grants R21 NS 43974 and R01 NS048859.

5. D4Z4 fragment analysis and genome-wide SNP screening in Non-Chromosome 4-linked Facioscapulohumeral Muscular Dystrophy.

K.L. Bastress[1], J.M. Staijch[1], R. Lemmers[2], S.M. van der Maarel[2], M.C. Speer[1], J.R. Gilbert[1]

[1]Center for Human Genetics, Duke University Medical Center, Durham, NC, USA
[2]Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands

Facioscapulohumeral muscular dystrophy (FSHD) is a disease of skeletal muscle, with symptoms including both facial and shoulder girdle weakness. In the majority of FSHD cases, the molecular basis is a contraction of the D4Z4 repeat on the end of the long arm of chromosome 4. However, there is no linkage to 4q35 in approximately 5% of FSHD families. This is referred to as FSHD1B or non-chromosome 4-linked FSHD in this small group of families. Efforts to identify linkage to other areas of the genome have been of limited success. Fragment analysis of the D4Z4 repeat has revealed variability of fragment size within some of the FSHD1B families, where some of the affected individuals show D4Z4 contraction (> 30 kb). However, several affected individuals within these families do not have D4Z4 fragment sizes in the FSHD range by EcoRI/BlnI analysis. This apparent discrepancy in fragment size can be found within branches of families and even among sibships. Interestingly, these affected individuals lack contraction of D4Z4, but have clinical features of FSHD including facial weakness, proximal muscle weakness, scapular winging, neck flexor weakness, elevated CK levels and myopathic EMG. In one non-chromosome 4-linked FSHD family (DUK 1392) of 18 affected members tested for D4Z4 fragment size, all 18 had large D4Z4 fragments (>50 kb). We preformed a 10K SNP chip genomic screen on a subset of individuals of 2 FSHD families (DUK 689 and DUK 1361), stratifying for individuals where there was confirmed absence of an FSHD-size D4Z4 allele (> 45 kb) or the 4q haplotype associated with a contracted allele in these families. For the combined family set, peak 2pt lod scores were obtained on chromosomes 7 (lod = 2.1), 10 (lod = 2.3), and 17 (lod = 2.5). Regions of interest by a microsatellite screen of the stratified population also identified a region of interest on chromosome 10 (lod =2.67). Since only a subset of each family was used in the SNP screen analysis, we plan to expand these pedigrees and to include additional individuals in the screen. We are currently characterizing the LD patterns between the SNPs used in the genomic screen and performing multipoint analysis to further verify and narrow these regions of interest.

6. Over-expression of Pitx1 Gene Activates Muscle Atrophy Pathways.

Ling Li[1] and Yi-Wen Chen[1, 2]

[1]. Center for Genetic Medicine Research, Children's National Medical Center, Washington, DC
[2]. Department of Pediatrics, George Washington University, Washington, D.C.

Muscle atrophy and weakness are the most common presenting manifestations of facioscapulohumeral muscular dystrophy (FSHD). We previously showed that paired-like homeodomain transcription factor 1 (Pitx1) was specifically up-regulated in FSHD patients and hypothesized that up-regulation of Pitx1 contributed to the pathological changes in FSHD. To identify the molecular targets of Pitx1, we over-expressed Pitx1 in mouse skeletal muscles by electroporation, followed by expression profiling using Affymetrix Mouse Genome 430A 2.0 microarrays. In addition, we investigated the effect of Pitx1 on myoblasts proliferation and differentiation using C2C12 cells transfected with Pitx1 gene. The control samples were transfected with insertless vector in both in vivo and in vitro studies.

At day 3 after gene delivery by electroporation, immunofluorescence staining localized the over-expressed *Pitx1* protein in the myonuclei. By comparing the expression profiles of Pitx1-trasfected samples to controls, we identified 22 and 15 known genes significantly up- and down-regulated in Pitx1-transfected muscles, respectively ($p<0.01$, $n=5$). Five of the 22 genes were members of the ubiquitin-proteasome pathways, including atrogin-1/MAFbx which has been reported strongly induced in skeletal muscle atrophy. The up-regulation of atrogin-1 was subsequently confirmed by real-time quantitative RT-PCR (4.6 fold, $p<0.05$, $n=5$). The change was not found in muscles over-expressing LacZ gene ($n=5$). In addition, 4 enzymes responded to oxidative stress, including glutathione peroxidase 3, were significantly increased. We further identified NADH staining-positive myofibers in the Pitx1-transfected muscles at day 7 after the gene delivery. To determine the effect of over-expressing PITX1 on cell proliferation, cell doubling time as well as total cell count at 0, 24, 48, and 72 hours were determined using C2C12 cells. The doubling time of PITX1-transfected cells was significantly higher compared to cells transfected with insertless vector ($n=4$, $p<0.01$). The total cell count also showed that cell proliferation was significantly inhibited in the PITX1 transfected cells at 24, 48, and 72 hours ($n=4$, $p<0.05$). The Pitx1-transfected cells started differentiated earlier compared to the controls, which is similar to what had been observed in myoblasts from FSHD patients. We concluded that up-regulation of Pitx1 activated molecular pathways that were involved in muscle atrophy in myofibers and affected cell proliferation and differentiation in myoblasts, which are likely involved in the pathological changes observed in FSHD.

7. Contractions of D4Z4 on 4qB Subtelomeres Do Not Cause Facioscapulohumeral Muscular Dystrophy.

Richard J. F. L. Lemmers,[1] Marielle Wohlgemuth,[2] Rune R. Frants,[1] George W. Padberg,[2] Eva Morava,[3,4] and Silvere M. van der Maarel[1]

[1]Leiden University Medical Center, Center for Human and Clinical Genetics, Department of Human Genetics, Leiden, The Netherlands
[2]Departments of Neurology and [3]Pediatrics, University Medical Center Nijmegen, Nijmegen, The Netherlands
[4]Department of Medical Genetics, University of Pecs, Pecs, Hungary

Facioscapulohumeral muscular dystrophy (FSHD) is associated with contractions of the D4Z4 repeat in the subtelomere of chromosome 4q. Two allelic variants of chromosome 4q (4qA and 4qB) exist in the region distal to D4Z4. Although both variants are almost equally frequent in the population, FSHD is associated exclusively with the 4qA allele. We identified three families with FSHD in which each proband carries two FSHD-sized alleles and is heterozygous for the 4qA/4qB polymorphism. Segregation analysis demonstrated that FSHD-sized 4qB alleles are not associated with disease, since these were present in unaffected family members. Thus, in addition to a contraction of D4Z4, additional cis-acting elements on 4qA may be required for the development of FSHD. Alternatively, 4qB subtelomeres may contain elements that prevent FSHD pathogenesis.

8. Intracellular Trafficking and Dynamics of Double Homeodomain Proteins.

Cecilia Östlund[‡], Ruth M. Garcia-Carrasquillo[‡], Alexandra Belayew[§] and Howard J. Worman[‡]

‡ Departments of Medicine and of Anatomy and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY, USA
§ Laboratory of Molecular Biology, University of Mons-Hainaut, Mons, Belgium

Double homeodomain (DUX) proteins are encoded by a family of 3.3-kilobase repeated elements dispersed in the human genome. One of these elements named D4Z4 is found in a tandem repeat array on chromosome 4 that is partially deleted in facioscapulohumeral muscular dystrophy. We have evaluated the trafficking and mobility of two DUX proteins, DUX1 and DUX4. We transfected C2C12 myoblasts with cDNA encoding these proteins fused to the green fluorescent protein and studied their intracellular localization and diffusional mobilities using fluorescence recovery after photo-bleaching and fluorescence loss in photobleaching. We also studied truncated forms of the proteins, containing one or both homeodomains or a region outside the homeodomains. We show that both full-length proteins are actively transported into the nucleus, and that the homeodomains contain the signals required for this localization. DUX1 is more mobile than DUX4 within the nucleus (t1/2=4.8 s for DUX1 and 13.4 s for DUX4), suggesting differences in the way the two proteins interact with nuclear components.

9. Role of the D4Z4 Sequence, Telomere Repeats and Subtelomeric Elements in the Etiology of the FacioScapuloHumeral Dystrophy (FSHD).

Alexandre Ottaviani, Eric Gilson, Frédérique Magdinier

Laboratory of Cell Molecular Biology, CNRS UMR 5161.
Telomeric & epigenetic regulations group.
Ecole Normale Supérieure de Lyon. 46, Allée d'Italie. 69007 Lyon, France
Tel: 33 4 72 72 86 63. Email: Frederique.Magdinier@ens-lyon.fr

Human telomeric position effects have been found recently in mammalian cells. Heterochromatin regions and telomeres in various organisms are associated with strong silencing of genes located in their vicinity. The telomere position effect (TPE) is closely related to position effect variegation (PEV) and involves the remodelling of chromatin architecture. A link between position effect and the 4q telomeric region rearrangement is a popular hypothesis to explain the molecular mechanism involved in the FSHD disease. Indeed, key to understanding the pathogenesis leading to FSHD is elucidation of how deletion of a tandemly repeated fragment in the subtelomeric region of 4q35 to a critical threshold is responsible for this myopathy.

Therefore, we constructed different models in order to investigate the effects of TPE on FSHD by putting D4Z4 near a GFP reporter at a subtelomeric position. In this simple experimental model, the effect of a single D4Z4 on transcription was analyzed. Preliminary results show that D4Z4 exhibit a strong enhancer activity on gene expression. However, previous report described the presence of a repressor within this element suggesting that D4Z4 is a mosaic of regulatory sequences probably involved in long-range chromatin interaction. Further experiments are needed to explain this phenomenon.

Using half-YACs containing the 4q region, we are constructing a new system which will allow us to study, in a subtelomeric context, the effect of the different number of repeats observed in FSHD patients. Both cis- and trans-sensing effects of the 4q subtelomeric sequences on expression of the reporter gene will be examined.

Experimental models and preliminary result will be presented here.

10. Timing and Mechanism of Somatic D4Z4 Contractions; Causes and Consequences.

Richard J.L.F. Lemmers[1], Michiel J.R. van der Wielen[1], Egbert Bakker[1], George W. Padberg[2], Rune R. Frants[1], and Silvère M. van der Maarel[1]

[1]Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands; [2]Department of Neurology, University Medical Center Nijmegen, Nijmegen, The Netherlands

Autosomal dominant facioscapulohumeral muscular dystrophy (FSHD1A) is associated with contractions of the polymorphic D4Z4 repeat array on chromosome 4qter with a high frequency of new mutations of mitotic origin. Although somatic mosaicism in FSHD is mainly reported in asymptomatic parents, mitotic mutations leading to somatic mosaicism occur equally frequently in patients and parents. Explaining this apparent discrepancy, we here demonstrate that somatic mosaicism in FSHD patients goes largely undetected using the standard diagnostic technique, indicating that linear electrophoresis is unsuitable to identify mosaic patients. As a consequence, the phenotype of mosaic patient's offspring will be underestimated, whereas the recurrence risk in the symptomatic mosaic individuals will be overestimated.

Detailed D4Z4 analysis of mosaic individuals with FSHD revealed a mosaic mixture of a contracted FSHD-sized allele and the unchanged ancestral allele in three quarter of individuals, which is suggestive of a mitotic gene conversion without crossover. However, in the remainder, the D4Z4 rearrangement resulted in two different-sized D4Z4 repeats, indicative of a gene conversion with crossover. In all cases, DNA markers proximal and distal to D4Z4 showed no allelic exchanges, suggesting that, despite the observation of increased frequencies of translocated D4Z4 repeats on chromosome 10 in mosaic individuals, all rearrangements were intrachromosomal. We propose that D4Z4 rearrangements occur via a synthesis-dependent strand annealing model that relatively frequently allows for crossovers. Furthermore, the distribution of different cell populations in mosaic patients with FSHD suggests that mosaicism here results from D4Z4 rearrangements occurring during the first few zygotic cell divisions after fertilization Interestingly, there seems to be a preference for the mitotic rearrangement of the maternal D4Z4 repeat.

11. Withdrawal From the Cell Cycle in FSHD Myoblasts.

D.A. Figlewicz, Y. Hong, I. Coltas.

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

Facioscapulohumeral dystrophy (FSHD) is an autosomal dominant disorder resulting from deletion of 3.3 kb ("D4Z4")repeats on chromosome 4q35. In recent years, our attention has focused on studies of FSHD myoblasts, which display an aberrant phenotype in both the undifferentiated and differentiated states. FSHD myoblasts upregulate the cyclin dependent kinase inhibitor, p21, divide less robustly, and leave the cell cycle and fuse more readily than controls. While p21 is upregulated downstream of MyoD and initiation of the myogenic differentiation program in control myoblasts, we have determined, using real-time PCR, that the p21:MyoD ratio is nearly 3 fold elevated in FSHD myoblasts. This suggests that a pathway other than normal differentiation may be responsible for withdrawal from the cell cycle in FSHD myoblasts. Recent identification of D4Z4-binding proteins has suggested a new direction for study. The D4Z4-binding proteins include YY1, a member of the Class I polycomb protein complex. Acetylation of members of the Class I polycomb complex leads to activation of the Class II group of polycomb group proteins, which localize to the surface of condensed chromatin domains. Perhaps the functionally most important member of this group is the polycomb protein Bmi1. Not only is Bmi1 association with chromatin and heterochromatin inversely related to progression of the cell cycle, it has been shown to promote proliferative capacity in several cell types, including human fibroblasts. Nothing is known about the potential role of Bmi1 in control of myoblast proliferation. We quantitated expression levels of Bmi1 in undifferentiated control and FSHD myoblasts; Bmi1 expression is significantly decreased in FSHD myoblasts. Bmi1 in other cell types suppresses the expression of tumor suppressor protein p16. We are currently investigating whether p16 mRNA levels are altered in undifferentiated FSHD myoblasts.

12. What is Scapuloperoneal or (Facio)scapuloperoneal Muscular Dystrophy with 4q35 Deletion: Is it an Independent Form or a Variant of a Facioscapulohumeral Muscular Dystrophy? What was Davidenkov's Opinion Concerning this Important Problem?

Valery Kazakov, Dmitry Rudenko

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

In the literature the opinion was advanced that Davidenkov isolated neural scapuloperoneal amyotrophy as a special variant of the peroneal muscular atrophy of Charcot-Maria-Tooth. An analysis of Davidenkov's works shows that his idea of the nosology of scapuloperoneal amyotrophy changed with time. Since 1935 Davidenkov has argued "scapuloperoneal amyotrophy was probably an independent disease with the features of both Charcot-Marie-Tooth amyotrophy and Landouzy-Dejerine myopathy but significantly closer to the latter". In 1938 Davidenkov wrote: "It is possible to suppose that actually scapulo-peroneal amyotrophy and Landouzy-Dejerine amyotrophy are connected with the action of the same basic gene but different phenotypes may be due to a modified action of the changing familial gene pool…". In 1962 when critical analysis of some autosomal dominant families with scapuloperoneal amyotrophy described earlier by him was carried out, Davidenkov wrote: "scapuloperoneal amyotrophy cannot be considered a variant of neural amyotrophy of Charcot-Maria-Tooth. It is a well outlined, peculiar form which belongs to a myopathy and approaches mostly to the myopathy of Landouzy and Dejerine, although it has some features which differ substantially both from the classical myopathy Landouzy-Dejerine and from the other variants of myopathy". Our studies re-analyzed the material concerning some probands and their relatives whom Davidenkov has examined as patients with scapuloperoneal amyotrophy by detailed analysis of the pattern of muscle involvement at different stages of the disease, using needle EMG, motor and sensory nerve conduction velocities, muscle biopsy, CT of muscles and DNA analysis. This confirmed Davidenkov's view of the nosological independence of scapuloperoneal amyotrophy the same as a (facio)scapuloperoneal type of muscular dystrophy. At present we prefer to call this disease "facioscapulolimb muscular dystrophy, type 2 (FSLD2), a descending with a "jump", with initial facioscapuloperoneal or (facio)scapuloperoneal phenotypes, autosomal dominant with DNA fragment sizes between 13-35 kb (or equal 37 kb) (double digestion) assigned with chromosome 4q35". Apparently, the 4q35 deletion is associated not only with classical FSHD but also with a FSLD2 and the detected DFS cannot be the criterion for differentiation FSLD2 from classical FSHD.

Valery Kazakov, M. D., Ph. D., Dr. med. Sc.
Professor of Clinical Neurology
Department of Neurology
Pavlov State Medical University
L. Tolstoy Str. 6/8
197089 filial 1 St. Petersburg, Russia
Tel. +7 812 510 9381, fax: +7 812 234 0125, e-mail: valerykazakov@mail.ru

13. Functional Study of the *DUX4* and *DUX4c* Genes.

Eugénie Ansseau, Frédérique Coppée, Eva D'Amico, Aline Marcowycz, Christel Mattéotti, Sébastien Sauvage, Jonathan Willocq, and Alexandra Belayew

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

The FSHD candidate gene (*DUX4*) we propose maps within each of the D4Z4 units repeated in tandem at 4q35. We have shown that a double homeodomain protein with the apparent molecular weight (MW: 52 kDa) and charge (pI: 8.6) of DUX4 was expressed in FSHD but not control primary myoblasts. The *DUX4* functional studies are done in parallel on the homologous *DUX4c* gene located in a single truncated D4Z4 element 42 kb centromeric of the D4Z4 array. A rabbit antiserum directed against a DUX4c-specific peptide detected a protein with the apparent DUX4c size (47 kDa) on a Western blot prepared with nuclear extracts of FSHD but not control myoblasts.

The DUX4 and DUX4c proteins are 424- and 374-residue long, respectively, and present only 2 mismatches over their 342 amino-terminal residues. Their putative toxicity was evaluated in TE671 cells (human rhabdomyosarcoma) transfected with *pCINeoDUX* expression vectors. DUX4c induced cell proliferation and the Myf5 *trans*-factor that inhibits myoblast terminal differentiation. In contrast, DUX4 was toxic as evidenced by leakage of cytoplasmic lactate dehydrogenase leading to 50% cell death in 48h. DUX4 expression also activated the apoptosis effector caspases 3 and 7. In addition, DUX4 led to premature fusion of the surviving cells.

We used the yeast two hybrid system to identify DUX protein partners. DUX4 could not be used as a bait because it presented a very strong transcriptional activity. In contrast, DUX4c could be used to screen a cDNA library derived from human skeletal muscle, yielding 187 positive clones. Among the 150 ones analysed, two classes of putative DUX partners were identified: (i) cytoskeleton proteins: 105 clones encoded desmin, and 3 encoded alpha actinin 3, (ii) transcription factors with a "zinc finger" domain either of the LIM (13 clones encoded FHL3 and 2 clones a LIM-like domain) or MYND type (2 different clones). A clone encoding importin/karyopherin 13 was also found: this protein mediates nuclear import of homeoproteins. A fraction of this cDNA library had previously been screened with DUX1 (a non-pathological homologue) and had yielded 35/42 positive clones encoding desmin. Reconstruction experiments in yeast had shown that the desmin interaction was mediated by the double homeodomain of either DUX1 or DUX4/4c. Confirmation of this interaction *in vitro* was obtained by GST pull down experiments and co-immunoprecipitations from extracts of cells transfected with a DUX expression vector. A DUX/desmin co-localisation was detected by immunofluorescence in these cells on narrow regions at the nuclear periphery.

We further studied the *DUX4* proximal promoter fused to a luciferase reporter gene in transient transfection experiments and evaluated several mutations in the YY1 and MyoD *cis*-elements. Binding of these proteins was analysed in electrophoretic mobility shift assays, and their impact on *DUX4-LUC* activity shown by co-transfection with the respective expression vectors. These experiments confirmed that YY1 had no impact on the *DUX4* promoter activity in muscle cells, in contrast to MyoD that was a very potent inhibitor in TE671 cells. Since the *DUX4* promoter was previously shown to be activated by Sp1, we expect DUX4 expression to occur in FSHD satellite cells or myoblasts, not in differentiated muscle fibers.

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14. The Interaction Between FRG1P and PABPN1 Implies a Common Molecular Pathway for the Muscular Dystrophies FSHD and OPMD.

Silvana van Koningsbruggen[1], Kirsten R. Straasheijm[1], Hans G. Dauwerse[1], Janet M. Cowan[2], Patricia G. Wheeler[2], Henk J. ter Laak[3,4], Baziel G.M. van Engelen[4], George W. Padberg[4], Rune R. Frants[1], Silvère M. van der Maarel[1*]

[1]Department of Human Genetics, Center for Human and Clinical Genetics, Leiden University Medical Center, Netherlands,
[2] Division of Genetics, Department of Pediatrics, Tufts-New England Medical Center, Boston, MA, USA
[3]Department of Pathology and [4]Department of Neurology, University Medical Center Nijmegen, Netherlands.

FRG1 is considered a candidate gene for facioscapulohumeral muscular dystrophy (FSHD) based on its location at chromosome 4qter and its possible up-regulation in FSHD muscle. The FRG1 protein (FRG1P) is localized in nucleoli, Cajal bodies and speckles, but its exact function is unknown.

Since the nature and level of FRG1 deregulation are still controversial, we studied the expression and the localization of the FRG1 protein (FRG1P) in cryosections of control, FSHD, Duchenne muscular dystrophy (DMD) and myositis muscles. Our results showed a slight up-regulation of FRG1P in FSHD and a more prominent up-regulation in DMD and myositis muscle sections, which may suggest that FRG1P up-regulation is general for muscular dystrophies rather than a primary event in FSHD. Since the FRG1 RNA levels do not seem to correlate with protein levels, we emphasize the importance of studying protein levels and/or tissue morphology in addition to transcriptional deregulation.

Further functional analysis of the FRG1 protein demonstrates that transient expression of VSV-FRG1 redistributes endogenous PABPN1, the protein mutated in oculopharyngeal muscular dystrophy (OPMD), to nucleolar aggregate-like structures. These nucleolar aggregates are composed of PABPN1, ubiquitin, proteasomes and poly(A) RNA and are resistant to proteinase K treatment, similar to the nuclear aggregates found in OPMD myonuclei. Co-immunoprecipitation experiments confirmed an interaction between both proteins, which is suggestive for a common pathogenic pathway in both disease entities.

15. Increased Levels of ANT1 Protein and Response to Oxidative Stress are Early Events in FSHD Muscle.

Dalila Laoudj-Chenivesse*, Gilles Carnac, Catherine Bisbal, Gerald Hugon, Sandrine Bouillot, Claude Desnuelle, Yegor Vassetzky, Anne Fernandez
*Corresponding Author

Centre de Recherche en Biochimie Macromoléculaire, CNRS, 1919 route de Mende, 34293. Montpellier, France

One major mechanism proposed to account for FSHD pathogenesis is that decreased D4Z4 repeat numbers results in inappropriate up-regulation of adjacent putative 4q35 genes in FSHD muscle. However such model has been recently disputed in two reports on the basis of an apparent lack of up-regulation of any 4q35 genes. Amongst the genes coded at 4q35 is the heart and muscle-specific isoform of the adenine nucleotide translocator (ANT1). In view of the ongoing controversy concerning a possible deregulation and increased expression of 4q35 genes in relation to FSHD pathogenesis, we have analyzed the levels of ANT1 in muscle biopsies from FSHD patients. We show that ANT1 protein levels are markedly increased in comparison to control healthy muscles, especially in muscles that are clinically unaffected. Comparative protein expression analysis between healthy, DMD and FSHD muscle shows that proteins involved in mitochondrial function and protection from oxidative stress, are also reproducibly and specifically modified in all FSHD muscles, including in FSHD muscles that are typically spared. In addition, we observed high levels of activated caspase 3 only in samples from clinically affected FSHD muscles. Together, these data support that deregulation of 4q35 genes and in particular ANT1 is an initial event in FSHD pathogenesis. We propose that high levels of ANT1 may be sufficient to induce changes in oxidative capacity and subsequent increased oxidative stress. Such increased oxidative stress could lead to a fiber type-related differential oxidative injury, and specifically targets mitochondrial function. Our results support that deregulation of a mitochondrial pathway, namely ANT1 will eventually lead to muscle cell death.

16. Characterization of CP39, a Candidate Gene for FSHD.

Sara Winokur, Ulla Bengtsson, Seung-Ah Chung, Jorge H. Martin

Department of Biological Chemistry, University of California, Irvine, CA

The gene prediction program GenomeScan identified a hypothetical gene that we have termed CP39 (chromosomal protein, 39 kD) proximal to the D4Z4 repeat. We have characterized the genomic structure and expression pattern of this candidate gene. PCR on a single chromosome mapping panel demonstrates that CP39 is chromosome 4 specific and represents the closest known single-copy gene to the D4Z4 repeat. A polyclonal antibody to CP39 was generated using synthetic peptides derived from regions predicted to be hydrophilic. CP39 is expressed in multiple cell types including myoblasts, Hela cells and a rhabdomyosarcoma cell line, as determined by both Western blotting and immuno-cytochemistry using this anti-CP39 antibody. The specificity of anti-CP39 was verified by peptide competition experiments, in which each CP39 peptide alone, and non-specific peptides corresponding to BAF were not able to block CP39 antibody-antigen association, but the use of both CP39 peptides effectively competed this interaction.

Immunofluorescence in myoblasts reveals a nuclear pattern of CP39 expression, with a slight preferential distribution to the periphery in interphase cells. As the cells enter prometaphase and metaphase, CP39 is diffusely distributed to condensed chromatin. During late anaphase, CP39 displays a strong punctuate localization to chromosomes, with preference for specific sites including telomeres. This association with chromosomal sites remains throughout telophase and early cytokinesis. Co-IF experiments with TRF2 confirms co-localization of CP39 with some telomeric sites. CP39 appears to be cell-cycle regulated, with the strongest expression during M and G2 and the weakest expression in G0 phases. Although emerin, BAF and CP39 are associated with chromosomes at similar phases of the cell cycle, co-IF experiments do not reveal precise overlap in their nuclear localization. CP39 expression is reduced upon differentiation of myotubes. As FSHD exhibits proliferation / differentiation defects, and CP39 is the closest chromosome 4 specific candidate gene to D4Z4, we are actively investigating the potential role of CP39 in the disease. In preliminary analysis of a single FSHD myoblast culture, the pattern of CP39 association varied from normal myoblasts, with increased association with telomeric sites and a stronger association with prophase and metaphase chromosomes.