

# FSH Watch

RESEARCH  
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2010



CONNECTING THE COMMUNITY OF PATIENTS, FAMILIES, CLINICIANS AND INVESTIGATORS



## FSH Society hails major breakthrough in FSHD

First study to pinpoint genetic causes of most common form of muscular dystrophy

by FSH SOCIETY  
Watertown, Massachusetts

**D**aniel Perez, co-founder, President and CEO of the FSH Society and a 48-year-old patient with FSHD, the most common form of muscular dystrophy, hailed new findings,<sup>1</sup> published online on August 19, 2010, in the journal *Science* that revealed for the first time the biological mechanism causing various types of FSHD. "This is a long-sought explanation of the exact biological workings of a disease that affects an estimated one in 14,000 individuals, or roughly 22,000 Americans and 490,000 worldwide," he said, adding that this discovery "creates an enormous opportunity for research to develop ways to prevent or treat FSHD." The study, titled "A Unifying Genetic Model for Facioscapulohumeral Muscular Dystrophy," was conducted by researchers from Leiden University in The Netherlands, the Fred Hutchinson Cancer Research Center in Seattle, Washington, and the University of Rochester Medical Center in New York.

"We are calling on the National Institutes of Health to immediately find ways to confirm and exploit these important findings, which I believe will generate great hope where there has been none" he said. "It is truly a potential watershed moment and we are cautiously optimistic that we are entering a new phase of research."

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The Leiden University Medical Center FSHD Group. Back row (left to right) Richard J.L.F. Lemmers, Patrick van der Vliet, Rinse Klooster, Silvere van der Maarel and Rune Frants. Front row (left to right) Judith Balog, Kirsten Straasheijm, Yvonne Krom and Jessica de Greef



## International collaboration reveals a cause for FSHD

by SILVÈRE VAN DER MAAREL, PH.D.  
Leiden University Medical Center, Leiden, The Netherlands

**S**ince the discovery of the partial deletion of a repetitive DNA structure at the tip of chromosome 4q in patients with FSHD in the early nineties, the disease mechanism underlying FSHD has remained largely elusive. Contrary to most genetic diseases, the mutation in FSHD did not seem to directly affect the disease gene and over the years many different disease mechanisms were proposed, challenged and proven inconclusive.

Our increased understanding of the FSHD region on chromosome 4q only made things more complicated. First, it was shown that a similar DNA structure

exists on chromosome 10q but that deletions on this chromosome do not cause FSHD. In the past few years, it became apparent that there are many different variants of the tip of chromosome 4q, all differing by small changes in the DNA at the FSHD region on 4q. Close examination of disease chromosomes revealed that deletions in some, but not all of these chromosome 4 variants cause FSHD. Although at first sight this observation only further obscured the picture, it was at the basis of the recent breakthrough reported in *Science* magazine.

By the end of 2007, three groups decided to join forces: Dr. Rabi Tawil (University of Rochester Medical Center, Rochester), Dr. Stephen Tapscott (Fred Hutchinson Cancer Research Center, Seattle) and Dr. Silvere van der Maarel (Leiden University Medical Center, Leiden,

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Dear Friends,

As you read this annual research edition of the FSH Watch you will see the incredible progress that the FSH Society and FSHD researchers worldwide have made!

The recent breakthrough publication by Dr. Richard Lemmers and his colleagues (Lemmers et al. "A Unifying Genetic Model for Facioscapulohumeral Muscular Dystrophy," *Science*, published online August 19, 2010) finds its origins in many endeavors and aspects of research encouraged and supported by the FSH Society over the past two decades. The researchers involved in this effort are exceptionally talented and gifted clinicians and scientists. Additionally, there is a vital story to be told about how your donations to the FSH Society have grown the field of FSHD from almost nothing to where it is today. Over the years, your financial support has enabled the FSH Society to fund \$2.4 million in post-doctoral and research fellowships that would otherwise never have been initiated and that have collectively yielded insight into an extremely complex genetic mechanism that had previously been considered a simple Mendellian type of dominant inheritance.

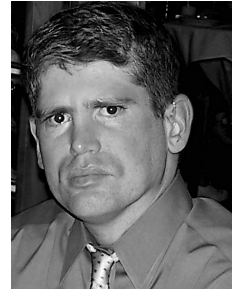
People are taking note of FSHD as the most prevalent muscular dystrophy and realizing the profound impact of this discovery on the way scientists view the human genome. We are inspired that Dr. Francis Collins, Director of the National Institutes of Health, took note of the discovery in Gina Kolata's article "Reanimated 'Junk' DNA Is Found to Cause Disease," on the front page of the August 20th *New York Times* by saying "If we were thinking of a collection of the genome's greatest hits, this (discovery) would go on the list."

We congratulate Drs. Richard J.L.F. Lemmers, Silvère van der Maarel, Rune R. Frants, George W.A.M. Padberg,

Rabi Tawil, Stephen Tapscott and their teams and colleagues on this major achievement in advancing understanding of FSHD. Drs. Lemmers and van der Maarel began their careers in FSHD with fellowships from the FSH Society. Several authors are past and present members of the FSH Society Scientific Advisory Board. We thank Richard T. Fields for providing the funding to continue this work the Society initiated. His philanthropic gift of \$7.1 million to create the Fields Center enabled researchers to scale-up research efforts required to achieve this finding. Be sure to read the article by Dr. van der Maarel, Co-Director of the Fields Center for FSHD, about the discovery. And also read perspectives and reaction to the discovery by several longstanding FSH Society-funded researchers.

We also include newly funded and ongoing FSH Society projects that continue to build the foundation for more advances, discoveries and inventions. The Society has helped to bring about success in research by providing support for excellence in science, innovative ideas, and for talented and creative scientists to pursue ideas even when they are not favored by reviewers in other funding agencies or scientific journals. DUX4 is an excellent example of just how challenging it has been for investigators to publish scientific papers and how the Society has persevered to keep alive investigations into the role of DUX4 in FSHD.

In this issue we highlight the 2009 International



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SUMMER/FALL 2010

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It is our editorial policy to report on developments regarding FacioScapuloHumeral Muscular Dystrophy (FSHD), but not to endorse any of the drugs or treatments discussed. We urge you to consult with your own physician about the procedures mentioned.

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Look for us on the internet at: [www.fshsociety.org](http://www.fshsociety.org)  
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Research Consortium workshops and the FSH Society's request to the U.S. Congress to increase funding on FSHD to \$25 million for fiscal year 2011. I continue to serve on the congressionally mandated federal advisory committee for muscular dystrophy research, the Muscular Dystrophy Coordinating Committee (MDCC), and have presented to that committee scientific opportunities and research needs for FSHD as identified by the research community.

FSHD funding from the National Institutes of Health continues to gain traction. For fiscal year 2009 actual FSHD funding increased to \$5 million out of an \$83 million portfolio for muscular dystrophy research (compared to \$3 million out of \$56 million the previous year). As this government fiscal year 2010 draws to a close on September 30, 2010, FSHD research funding has reached an estimated \$13 million!

What are the next steps, and what is a realistic assessment of what we expect to happen in the near term? First the results of the breakthrough study need to be verified; other researchers need to get the same results before we can move forward with pre-clinical and translational research. This process of re-confirming has begun. The FSH Society is holding our annual international research meeting in October 2010, where scientists can present results, exchange ideas and make a plan. Once the results are verified, compounds and other potential therapies, such as RNA interference and gene therapy, will be tested in vitro in cell models to treat the genetic package identified as causing FSHD; if one or more work, they will then be used to treat animal models with FSHD (animal models and cell models will need to be developed and shared by the research community). If none work, other compounds will be developed. After a compound or other therapy proves successful in animals, the investigator will apply to the FDA or similar agency to test it in humans. The FSH Society will help to identify patients who qualify to participate in a trial(s). At the conclusion of the trial, the efficacy will be determined.

It's obvious that the Society needs both funds restricted to research

and unrestricted funds (funds not earmarked for a specific purpose) so that we can continue.

What's our part? Here are four things we can do.

1. We can solicit contributions and memberships — from our mothers and fathers, sisters, brothers, aunts and uncles, cousins, close friends, business associates, etc. You can donate online at [www.fshsociety.org](http://www.fshsociety.org) (click Contribute).
2. We can make sure our primary care doctors, neurologists, physiatrists and therapists receive FSH Society literature, learn what our Society does, and are encouraged to join our Society.
3. We can make both restricted and unrestricted tax deductible contributions to our Society. We can look hard at what we contribute each year and consider making a substantial increase in this challenging year.
4. Finally, we can contact the FSH Society and the Office of the Patient Communication and Liaison of NIH Boston Biomedical Research Institute/Harvard Medical School Sen. Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD and volunteer to participate in current studies and future trials.

If we do our part, a year from now we will have a successful FSH Society achieving our research goals — finding the cause of our disease, and finding the means of reducing its effect upon our bodies and our lives. That hope is now within our grasp. The near and far reality of molecular therapeutics now lies before us. It is up to us to help get our Society there.

Thank you for your generous support — financial and otherwise. Thank you for helping us all to make progress happen on FSHD — past, present and future. FSH Watch

— Daniel Paul Perez  
*President & CEO*

## Michelle Mackay joins the FSH Society Board of Directors

Michelle joined the Board of Directors in 2010. A native of Australia, she received a degree in communication from Charles Sturt University, Bathurst, New South Wales, and in fine arts from Newcastle University, Central Coast, New South Wales. She has worked in the travel industry in Australia and in the UK. Michelle volunteers for several charitable organizations in Michigan where she now lives; she serves on the boards of the Food Bank of South Central Michigan (Kalamazoo), and the Gilmore Car Museum (near Battle Creek). She is eager to help advance the Society in the U.S. and abroad. Michelle and her husband, David, have two daughters. FSH Watch



**Michelle Mackay with William R. Lewis, Sr., M.D. at the 2010 FSH Society Patient and Researcher Network meeting Las Vegas, Nevada.**

## Introducing Raphaela Silverio

Raphaela Silverio joined the FSH Society in July 2010 as FSH Society Patient Liaison and Office Manager. Raphaela has strong administrative and bookkeeping skills in both non-profit and for-profit organizations. She is excited to be working for the FSH Society and looks forward to learning from the FSHD community. Raphaela was born in Rio de Janeiro, Brazil, grew up in New York City, and currently resides in suburban Boston, Massachusetts. Raphaela can be contacted through the Society's Executive & Development office:



**Raphaela Silverio**

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## About the FSH Society's role in developing the foundations for discovery in FSHD

The FSH Society is a 501(c)(3) non-profit patient-led research, education and advocacy organization, founded in 1991. The Society frequently testifies before Congress on the research needs in FSHD. It has funded \$2.4 million in \$30,000-45,000 a year fellowships to more than 60 researchers in 11 years, leading to several hundred publications in top tier peer-reviewed journals.

Researchers who published this *Science* paper have been recipients of FSH Society support and also currently serve and have served as members of the FSH Society Scientific Advisory Board. Both the first and last authors, Drs. Richard J.L.F. Lemmers and Silvere van der Maarel, began their careers in FSHD research as recipients of the FSH Society Marjorie Bronfman fellowship awards. Dr. van der Maarel was the first FSH Society Marjorie Bronfman fellow and Dr. Lemmers was the tenth FSH Society Marjorie Bronfman fellow. The FSH Society has provided the Leiden team and many of their partners with funds to support early phases of their work. Also noteworthy, Dr. Alexandra Belayew, who did pioneering work on DUX4, was awarded the FSH Society's initial inaugural grant as a FSH Society Delta Railroad research fellow.

The FSH Society is a small non-profit organization with a very dedicated and gifted group of scientific advisors, headed by Prof. David E. Housman of the Massachusetts Institute of Technology, and a board of directors composed mainly of FSHD patients. With the generous support of family foundations and thousands of affected FSHD families, the Society has helped to solve the mechanism causing FSHD. It has raised funds, carefully targeted many critical areas of

research, and built the foundation for discovery.

The FSH Society has initiated and funded many projects that helped to provide the corpus and foundation of knowledge needed to bring forth this insight regarding the genetic mechanism of FSHD. The FSH Society has also grown and sustained many aspects of research involved in this exciting area of research. Even when various theories were out of favor with major funding agencies or scientific journals, the FSH Society provided a way to allow continuity until such ideas could be proven or disproven. DUX4 was not well received until recent years. (See the articles by Belayew, Rosa and Hewitt in Perspectives on the recent breakthrough in understanding FSHD published in *Science*.) FSH Society funded researchers who have contributed to the overall work around the globe and who have published and worked on FSHD in recent years include: Drs. Alexandra Belayew, Darko Bosnakovski, Yi-Wen Chen, Frederique Coppee, Jessica de Greef, Melanie Ehrlich, Davide Gabellini, Meredith Hanel, Scott Harper, Jane Hewitt, Peter Jones, Yvonne Meijer-Krom, Michael Kyba, Cecelia Ostlund, Tonnie Rijkers, Alberto Rosa, Michael Rudnicki/Daniella Oliveria, Rossella Tupler, Kathryn Wagner, Sara Winokur, and Howard Worman. All of these findings and papers, along with the current breakthrough paper, enable us to finally seek a method for treating FSHD. This truly demonstrates the effectiveness of patient-driven and disease-focused organizations. **FSH Watch**

1. Lemmers *et al.* "A Unifying Genetic Model for Facioscapulohumeral Muscular Dystrophy," *Science*, published online August 19, 2010.

## An introduction to the NIH RePORT website and database

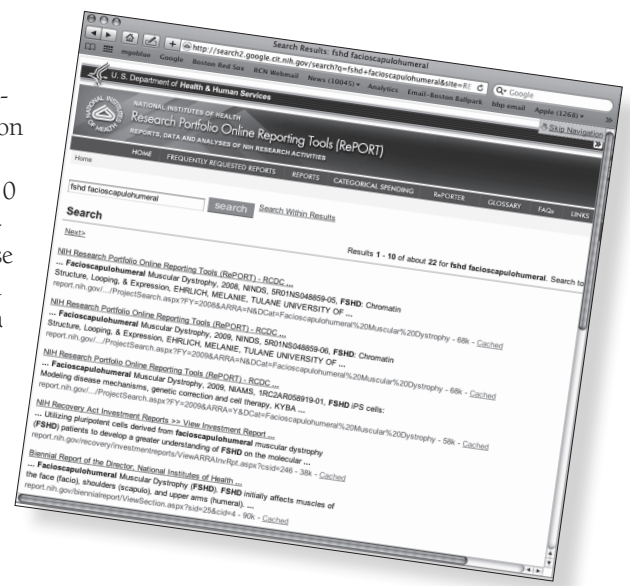
The National Institutes of Health (NIH) is the nation's medical research agency. Funded by U.S. tax dollars, the NIH supports researchers throughout the country and around the world as they work to improve people's health.

The NIH recognizes the importance of keeping the American people informed about how their tax dollars are spent to support medical research. To enhance public accessibility to reports, data and analyses of NIH research activities, the Research Portfolio Online Reporting Tools (RePORT) website gives the public a single access point to quickly and easily find data, including information on NIH expenditures and the results of NIH-supported research.

If you go to the RePORT website at <http://projectreporter.nih.gov/reporter.cfm> and

type "fshd facioscapulohumeral" in the search box, and then select "Logic:" as "Or" you will see a comprehensive listing of NIH projects on FSHD and closely related to FSHD research. As of September 24, 2010 (the last month of the U.S. government fiscal year 2010), the database listed 38 projects on FSHD funded at a total of \$13,160,425. This is a significant increase over the fiscal year 2009 actual and fiscal 2010 estimate in RCDC!

In addition to the projects displayed as a hit list, you can also access tabs that show publications, patents and data visualization graphics for FSHD. Check it out! **FSH Watch**



# Educating on scientific opportunities in FSHD

Education and raising awareness of FSHD on the NIH MDCC

by DANIEL PAUL PEREZ

President and CEO, FSH Society, Watertown, Massachusetts, United States

The Muscular Dystrophy Community Assistance, Research, and Education Amendments of 2001 and 2008 (MD-CARE Act) authorized the establishment of the Muscular Dystrophy Coordinating Committee (MDCC) to coordinate activities across NIH and with other federal health programs and activities relevant to the various forms of muscular dystrophy. The MD-CARE Act directed the Committee to develop a plan for conducting and supporting research and education on muscular dystrophy through the U.S. national research institutes. FSHD is represented on the MDCC by Daniel Paul Perez, President & CEO of the FSH Society, who is one of five patient advocates on the 15-member federal advisory committee. For more information on the MDCC — organization and planning, information for patients and families, and information for researchers, please see the MDCC web site @ [http://www.ninds.nih.gov/find\\_people/groups/mdcc/index.htm](http://www.ninds.nih.gov/find_people/groups/mdcc/index.htm)

On November 30, 2009, the Eighth Annual Meeting of the Muscular Dystrophy Coordinating Committee was held in Bethesda, Maryland. The meeting began with introductions from Dr. Story Landis, MDCC Chair, NIH NINDS and Dr. John Porter, MDCC Executive Secretary, NIH NINDS. Introductions were followed by the NIH Annual Overview by Dr. Porter, and an update on the Paul D. Wellstone Muscular Dystrophy Cooperative Research Centers Network by Dr. Glen Nuckolls, NIAMS. Dr. Stephen Lynn, TREAT-NMD, United Kingdom, gave a talk titled: “Bringing Down the Barriers in Translational Medicine in Inherited Neuromuscular Diseases.” Reports from federal agencies were given by: Dr. Theresa San Agustin, National Institute of Disability and Rehabilitation Research/Department of Education (NIDRR);

Dr. Edwin Trevathan, Centers for Disease Control (CDC); Dr. Sara Copeland, Health Resources and Services Administration (HRSA); Dr. Marielena McGuire, Congressionally Directed Medical Research Programs/Department of Defense (DoD); Dr. Porter, Revisiting the MDCC Action Plan for the Muscular Dystrophies. Reports from patients and patient organizations were given, including a report on FSHD by Daniel Perez.

The FSHD report recapitulated the presentation topics, breakout discussions sessions and summary as presented by a majority of FSHD researchers who attended the FSH Society FSHD 2009 International Research Consortium & Research Planning Meetings held three weeks earlier on November 9-10, 2009. See the program book at [www.fshsociety.org](http://www.fshsociety.org), clicking on the “For Scientists” tab at top of page, then clicking on “FSH Society Annual International Research Consortium” on the left hand navigation, and then selecting “previous year’s program and abstract booklets” at bottom of page or visit <http://www.fshsociety.org/assets/pdf/FSHSocietyIRC2009ProgramAbstracts.pdf>.

Based on discussions that Mr. Perez had with key FSHD scientists at the research meeting in Watertown, Massachusetts, he informed the MDCC that a major breakthrough might be happening for FSHD and advised them to be prepared to move quickly in the pre-clinical, clinical and translational areas. He then reviewed the executive summary and recommendations (see box below) of the FSH Society 2009 Research Consortium and Research Planning meeting.

“During the past two decades, FSHD research has made

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To be prepared for this new FSHD-era, we need to update the priorities of the FSHD Roadmap as defined and updated in 2006 and 2008.

## 1. PATIENTS

There is a need for well-characterized registries with uniform data collection. Wellstone MD CRC, FSH Society, R.T. Fields Center and patient organizations are key to this process. These groups and registry and patient organizations are instrumental for:

- a. Work on natural history — identification of phenotype modifiers (genetic and environmental)
- b. Identification of the FSHD2 gene (contraction-independent FSHD)
- c. Biobanking (cell lines, etc.)

## 2. EPIGENETICS/GENETICS

This line of work will be instrumental to pinpoint the real identity of FSHD1 and FSHD2. This information will form the basis for evidence-based intervention.

- a. Modifying genes for FSHD1 (large inter-individual variation in symptoms)
- b. Identify the FSHD2 gene (common molecular pathway with FSHD1)
- c. Further work on the chromatin structure/function relationship

## 3. BIOMARKERS

There is obvious need for monitoring intervention

- a. Systems biology
  - i. transcriptomics, proteomics, metabolomics, etc.
- b. In situ (RNA, protein) to detect cellular heterogeneity
- c. Non-invasive monitoring (MRI, etc.)

## 4. MODEL SYSTEMS

Urgent need for more specific model systems for mechanistic, intervention work and advancement to clinical trials.

- a. Cellular
  - i. Biopsies
  - ii. Mosaics
  - iii. iPS
- b. Animal
  - i. Mouse — inducible/humanized mouse, etc.
  - ii. Other species”



## Working to increase large-scale and widespread funding on FSHD

It has been a long-term strategy of the FSH Society to increase awareness of FSHD, to generally increase the bandwidth of discovery and funding for FSHD, and in particular to increase U.S. federal funding on FSHD. When the FSH Society was founded, the U.S. NIH was spending \$4.3 million on all of the muscular dystrophies combined, and the MDA was spending three to four times that amount for research on all the diseases under its umbrella. It was our hope to open up funding to allow for greater allocations to FSHD research and clinical work.

Each American has two U.S. Senators and one U.S. Representative, and it is highly effective to let your elected officials and their health staff know that increases and decreases in federal funding for biomedical research through agencies such as the National Institutes of Health do affect those of us involved with FSHD. One might consider periodically checking in with your Congressional representative's offices and staff to thank them for the tremendous gains the NIH has made in research on FSHD research and funding and to let them know that you appreciate their support of vital programs that impact you and your family's health.

On April 16, 2010, Daniel Paul Perez submitted testimony to the U.S. House of Representatives Appropriations Subcommittee



on Labor, Health and Human Services, Education and Related Agencies about FY2011 appropriations for National Institutes of Health (NIH) research on FSHD. Mr. Perez's testimony covered five broad areas and facts about FSHD: 1. FSHD, the most prevalent form of muscular dystrophy; 2. NIH muscular dystrophy funding has quadrupled since the inception of the MD CARE Act 2001; 3. NIH funding of FSHD has remained level since the inception of the MD CARE Act 2001; 4. FSHD, the most prevalent form of muscular dystrophy is drastically underfunded at NIH; and 5. he then outlined areas of scientific

opportunity in FSHD that need NIH funding. Our request to the NIH Appropriations Subcommittee was that in "this year in FY2011, immediate help for those of us coping with and dying from FSHD. We ask NIH to fund research on FSHD at a level of \$25 million in FY2011."

For more information on advocacy and to read the entire U.S. House and Senate FY2011 testimonies please visit [www.fshsociety.org](http://www.fshsociety.org), click "FSH Society" at top of page, select "Advocacy" in left hand navigation and then click link to PDF file or visit [http://www.fshsociety.org/assets/pdf/FSHSociety\\_OWTestimony\\_USHouse\\_Approps\\_LHHSE\\_FY2011.pdf](http://www.fshsociety.org/assets/pdf/FSHSociety_OWTestimony_USHouse_Approps_LHHSE_FY2011.pdf).



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### A Unifying Genetic Model for Facioscapulohumeral Muscular Dystrophy

Richard J.L.F. Lemmers<sup>1</sup>, Patrick J. van der Vliet<sup>1</sup>, Rinse Klooster<sup>1</sup>, Sabrina Sacconi<sup>2</sup>, Pilar Camaño<sup>3,4</sup>, Johannes G. Dauwse<sup>1</sup>, Lauren Snider<sup>5</sup>, Kirsten R. Straasheijm<sup>1</sup>, Gert Jan van Ommen<sup>1</sup>, George W. Padberg<sup>6</sup>, Daniel G. Miller<sup>7</sup>, Stephen J. Tapscott<sup>5</sup>, Rabi Tawil<sup>8</sup>, Rune R. Frants<sup>1</sup>, Silvére M. van der Maarel<sup>1,\*</sup>

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**Abstract:** Facioscapulohumeral muscular dystrophy (FSHD) is a common form of muscular dystrophy in adults that is foremost characterized by progressive wasting of muscles in the upper body. FSHD is associated with contraction of D4Z4 macrosatellite repeats on chromosome 4q35 but this contraction is pathogenic only in certain "permissive" chromosomal backgrounds. Here we show that FSHD patients carry specific single nucleotide polymorphisms (SNPs) in the chromosomal region distal to the last D4Z4 repeat. This FSHD-predisposing configuration creates a canonical polyadenylation signal for transcripts derived from DUX4, a double homeobox gene of unknown function that straddles the last repeat unit and the adjacent sequence. Transfection studies revealed that DUX4 transcripts are efficiently polyadenylated and are more stable when expressed from permissive chromosomes. These findings suggest that FSHD arises through a toxic gain of function attributable to the stabilized distal DUX4 transcript.

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## EDUCATING ON SCIENTIFIC OPPORTUNITIES IN FSHD

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steady progress toward unraveling the molecular basis of this common muscle disease. The main line of research has focused on the extremely complex (epi)genetic enigma. This complexity has fascinated experts involved in related research. At the present moment the FSHD research field is covering a variety of multidisciplinary and complementary approaches.

Although the exact details of the molecular genetic basis of FSHD are still not in place, the general picture is coming into focus. Within one to two (1-2) years, evidence-based intervention

strategies will be on the drawing-board.

For those wishing to do more research, the detailed outline as presented in the FSHD Report to the 8th Annual Meeting of the Muscular Dystrophy Coordinating Committee Meeting, can be found online by going to [www.fshsociety.org](http://www.fshsociety.org), clicking on the "Research" tab at top of page, then clicking on "FSHD Research Plans" on the left hand navigation, and then selecting "U.S. NIH MDCC" from the drop down menu or visit [http://www.fshsociety.org/assets/pdf/MDCC2009\\_FSHSocietyDanPaulPerez.pdf](http://www.fshsociety.org/assets/pdf/MDCC2009_FSHSocietyDanPaulPerez.pdf). 

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## INTERNATIONAL COLLABORATION REVEALS A CAUSE FOR FSHD

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Netherlands), one of the first FSH Society fellows. A few months earlier, Drs. Tawil and Van der Maarel had already formed the Fields Center for FSHD and Neuromuscular Research with the support of Richard Fields. This collaboration between these three groups was based on the mutual understanding that true progress in FSHD can only be made by an open and multidisciplinary approach with the availability of ample relevant biological resources. The focus of this collaboration was therefore on collecting and stratifying biological resources, including detailed clinical evaluation, studying the genetic details of the FSHD locus and generating a detailed catalog of the different transcripts (RNA messages) emanating from this locus in patients and control individuals. Collectively, these three approaches were merged into a recently awarded multi-project grant by the NIH and led to the unifying genetic model presented in *Science*.

In the Leiden group, Drs. Van der Maarel and Lemmers, also a former FSH Society fellow, studied in detail the genetic variants of chromosome 4q with the objective of identifying common genetic variants that are shared by all patients with FSHD and which would separate disease-causing chromosome 4 ends from non-disease causing chromosome ends. To this end they studied DNA of patients collected by several centers, including that of Dr. Tawil, in whom the FSHD mutation was more complex than the average person with FSHD. In parallel, Dr. Tapscott focused on the different transcripts that are produced by the repeat structure in cultured muscle cells and in muscle biopsies collected by Dr. Tawil, looking for differences between individuals with FSHD and individuals who have no muscle disease. He not only confirmed earlier reported transcripts, but also identified new transcripts and measurable differences of some transcripts in muscle from individuals with FSHD.


When viewing the data collectively, a sequence variant was identified immediately adjacent to the repeat which was predicted to affect the stability of several transcripts emanating from the repeat. Previously, it was reported that the last unit of the repeat structure produces a unique transcript called DUX4 that makes use of a so-called polyadenylation signal to become stabilized. It was predicted that without this polyadenylation signal the transcript would be quickly degraded. The protein product of this DUX4 transcript was shown to be toxic to muscle cells. In the current study the authors show that while all disease chromosomes contain the stabilizing polyadenylation signal, on non disease-causing chromosomes this polyadenylation signal was mutated.

With these observations in mind, a model for FSHD was postulated that upon shortening of the repeat structure, the transcriptional activity would increase. But only if that chromosome contained the disease-causing variants, the resulting increased activity leads to increased levels of the DUX4 transcript while from non-permissive chromosomes, in the absence of polyadenylation, the transcripts would quickly get degraded.

Indeed, in muscle cell cultures of patients with FSHD, the DUX4 transcript is detected, while in cultures of control individuals, DUX4 is not detected. Moreover, when artificially introducing the FSHD locus from disease-causing chromosomes (with polyadenylation signal) into mouse muscle cells, a stable DUX4 transcript could be identified, while introducing the same locus from non-permissive chromosomes (without polyadenylation signal) did not yield detectable levels of DUX4 transcripts.

This finding provides a new and unifying model for FSHD. It explains the chromosome and chromosome-variant specificity of the disease, and unifies the genetic and epigenetic findings in all patients with FSHD known to this consortium. It also provides new directions for research that focuses on studies that determine whether the DUX4 transcript (or protein) causes FSHD, or whether other transcripts that make use of this polyadenylation signal are causally related to FSHD.

### **A Unifying Genetic Model for Facioscapulohumeral Muscular Dystrophy**

R.J.L.F. Lemmers, P.J. van der Vliet, R. Klooster, S. Sacconi, P. Camaño, J.G. Dauwerse, L. Snider, K.R. Straasheijm, G.J. van Ommen, G.W. Padberg, D.G. Miller, S.J. Tapscott, R. Tawil, R.R. Frants, S.M. van der Maarel. *Science* Aug 19, 2010. This study was supported by the Fields Center for FSHD and Neuromuscular Research; the Netherlands Organization for Scientific Research NWO 917.56.338; Breakthrough Project Grant by the Netherlands Genomics Initiative NWO 93.51.8001; the National Institutes of Health P01NS069539; the Muscular Dystrophy Association; the Shaw Family Foundation; a Marjorie Bronfman Fellowship grant from the FSH Society; the Pacific Northwest Friends of FSH Research; the Dutch FSH Society; Centro Investigación Biomédica en Red para Enfermedades Neurodegenerativas (CIBERNED); Basque Government (Fellowship grant, N° 2008111011); and Instituto Carlos III, ILUNDAIN Fundazioa. 

## Raising awareness of FSHD and visibility of the FSH Society

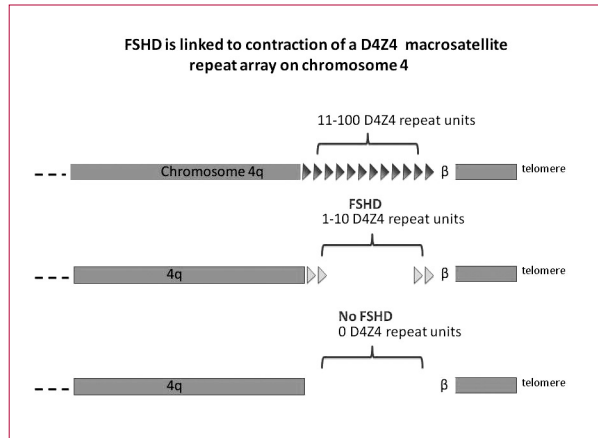
**F**SHD and the FSH Society recently made national and international headlines in a big way! Part of the mission of the FSH Society is to raise the visibility and awareness of FSHD, and we were very pleased to see the recent advance in understanding FSHD genetic research on the front page of the *New York Times*.

On August 19, 2010, the *New York Times* published an online story in its Science section titled “Reanimated ‘Junk’ DNA Is Found to Cause Disease,” by Gina Kolata, that gives insight into the significance of the breakthrough in understanding the genetic mechanism of FSHD as published online in the journal *Science* on August 19, 2010. The following day, the story appeared on the front page of the print edition.

Noteworthy are comments from Prof. David Housman, Chairman of the FSH Society’s Scientific Advisory Board (SAB), Dr. Francis Collins, Director of the National Institutes of Health, and Dr. Stephen Tapscott, one of the lead authors of the paper. Dr. Collins stated, “If we were thinking of a collection of the genome’s greatest hits, this (discovery) would go on the list.”

To read the article online please visit the FSH Society online at [www.fshsociety.org](http://www.fshsociety.org) or the *New York Times* at [http://www.nytimes.com/2010/08/20/science/20gene.html?\\_r=2&ref=science](http://www.nytimes.com/2010/08/20/science/20gene.html?_r=2&ref=science)

On August 19, 2010, the National Institutes of Health issued a press release on the paper that also mentions the role the FSH Society had in early work leading to this discovery as well as in supporting the current discovery. It explains that the “new study



**Normally the FSHD deletion causing region (D4Z4) contains from 10 to 100 repeating units of DNA, but in most people with FSHD, the array is smaller, with fewer than 10 repeats. The finding that individuals with zero repeats have no FSHD helped to further define the minimum region needed for FSHD. (Source: Dr. Stephen Tapscott’s 2010 FSH Society Patient and Researcher Network meeting presentation on August 1, 2010, Las Vegas, Nevada.)**

proposes a model that explains how the previous findings fit together. The study was led by Dr. van der Maarel (and Dr. Lemmers) in collaboration with Stephen Tapscott, M.D., Ph.D., at the Fred Hutchinson Cancer Research Center in Seattle, and Rabi Tawil, M.D., at the University of Rochester Medical Center in New York.”

“At one end of chromosome 4 is a chunk of repetitive DNA, called a tandem repeat array. Normally this region contains from 10 to 100 repeating units of DNA, but in most people with FSHD, the array is smaller, with fewer than 10 repeats. Within each repeating unit is a gene called DUX4.”

“The researchers found that in people with FSHD, the DUX4 gene generates a piece of RNA that is toxic to muscle cells. RNA is a sister molecule to DNA with many critical functions. The researchers also discovered

that variations on chromosome 4 are important because they affect the durability of DUX4 RNA. People with FSHD have chromosome variations that add a trailing segment to the RNA called a poly(A) tail. With the poly(A) tail attached, the RNA is more stable and more likely to cause damage.”

“The researchers came to these conclusions by creating artificial DNA constructs containing the short repeat array, in combination with different variations on chromosome 4. They inserted these constructs into muscle cells, and analyzed how the chromosome 4 variations affected the level of DUX4 RNA. They also studied FSHD families with unique chromosome rearrangements and showed that all families with FSHD shared chromosome 4 sequences encoding the poly(A) tail.”

“In another set of experiments, they found they could detect DUX4 RNA in muscle cells from individuals with FSHD but not in cells from unaffected individuals. Meanwhile, previous studies have shown that DUX4 can trigger muscle cell death.”


“This study provides evidence that DUX4 RNA is likely a key part of the disease process in FSH muscular dystrophy, and justifies further investigation of its role and how to silence its effects,” Dr. Tapscott said.

To read the article online please visit the FSH Society online at [www.fshsociety.org](http://www.fshsociety.org) or the National Institutes of Health at <http://www.nih.gov/news/health/aug2010/ninds-19.htm> or see the paper at *Science* by visiting: <http://www.sciencemag.org/scienceexpress/recent.dtl> and search on “Lemmers” 

## 2010 Delta Railroad Construction Research Fellowship Grants

**T**he FSH Society Delta Railroad Construction Company fellowship program continues to help FSHD research by awarding research grants that provide needed expansion of current work and innovative new work. The FSH Society is indebted to the Delta Railroad Construction Company of Ashtabula, Ohio, and to Larry and Ida Laurello and their



family for this groundbreaking effort on behalf of the FSHD community. Initiated in 1998, the Delta Railroad Research Fellowship Grants are yielding tremendous insights in novel areas of FSHD research and treatment. For more information about research fellowships, please contact Daniel Paul Perez at [daniel.perez@fshsociety.org](mailto:daniel.perez@fshsociety.org). 



## PERSPECTIVES AND REACTIONS

### An historical overview — D4Z4 to DUX4

by JANE E. HEWITT, PH.D.

University of Nottingham, Nottingham, United Kingdom

When asked to write about our work on DUX4, I decided to give something of an historical overview. My group was the first to identify D4Z4 in 1991, when human gene cloning was laborious as we had no genome sequence. To identify genes, we took advantage of the fact that different proteins that carry out similar functions are often encoded by similar DNA sequences (this is called conservation). A postdoctoral fellow in London at the time, I was trying to find a particular type of gene that contains homeobox sequences. These genes encode proteins that contain homeodomains, a sequence of amino acids that can bind to specific DNA sequences near the beginning of some genes and cause them to be turned on. I used short synthetic pieces of DNA corresponding to the homeobox sequence to “fish” these genes out of all the thousands present in the human genome. One of the genes that I found (the gene we now call DUX4) was unusual as it was present in many (up to 100) repeated copies. We mapped this gene to the very end of human chromosome 4. Then I met one of the Leiden group at a scientific meeting and we realized that our gene might help their search for the FSHD mutation — little did we appreciate at the time how important it would turn out to be! This chance meeting led directly to a collaboration and identification of the FSHD mutation in 1992. The repeated sequence containing the homeobox gene was called D4Z4 (this indicates a repeated DNA sequence on chromosome 4). Subsequently, Alexandra Belayew named the protein-coding part of this sequence DUX4; each D4Z4 DNA repeat unit contains one copy of the DUX4 gene.

Initially we thought that figuring out why this deletion caused FSHD would be simple; after all, we knew that the mutation was close to or within a potential gene. So our research questions were: Where and when in the human body is this gene active? What is the function of DUX4 protein and what other genes does it turn on or off? We are still trying to answer these questions! We also wanted to find the equivalent gene in the mouse, as this would be needed for an animal model of FSHD. This all was simple in theory but in practice turned out to be extremely difficult. There are several factors that contributed to this. There are many copies of DUX4 throughout the human genome, not just at chromosome 4. These non-4q copies have



Current Ph.D. students Andreas Leidenroth and Amanda Hampson



The first DUX group! London 1992.

similar DNA sequences to DUX4 but are dead genes that are no longer able to code for functional protein (these types of genes are called pseudogenes). However, the presence of these copies interfered with our experimental strategies. In addition, although almost all human genes have a mouse equivalent, we could not find any DUX4 genes in the mouse genome. Consequently, DUX4 was considered by some to be “junk” DNA.

However, in science “absence of evidence should not be considered evidence of absence.” By 2004, the completion of the genome sequences of human, mouse and several other organisms enabled us at last to identify DUX4 sequences in other species including mouse (the FSH Society funded part of this project) and elephants! Analyzing how the DUX4 DNA sequence has been conserved during evolution showed that DUX4 is most certainly not a “dead” gene or

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“ I was trying to find a particular type of gene that contains homeobox sequences. These genes encode proteins that contain homeodomains, a sequence of amino acids that can bind to specific DNA sequences near the beginning of some genes and cause them to be turned on. I used short synthetic pieces of DNA corresponding to the homeobox sequence to ‘fish’ these genes out of all the thousands present in the human genome. ”

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junk DNA (published 2007). DUX4 gene sequences show distinct properties or signatures that are not seen in genes that become dead. Around the same time as our data was published, the Belayew and Tapscott groups demonstrated that DUX4 is active and produces RNA. Andreas Leidenroth, a Ph.D. student in my lab, recently investigated the evolutionary history of four DUX4 related genes in mammals. Because DUX4 protein has been so difficult to study, some research groups have focused on some of these gene “cousins” instead. Andreas’ data shows that considering the family relationships between these cousins will be important for studies of DUX4 function in animal models.

The recent *Science* paper contains really exciting data from the Leiden group showing that DUX4 RNA is inappropriately produced in FSHD muscle cells. So it seems that most of the time, in most human cells, DUX4 is turned off (“silenced”) and does not produce a protein. However, our evolutionary data tells us that, even in people without FSHD, it must at some time produce DUX4 protein. My lab is still working to understand the functions of DUX4, 18 years on from that gene “fishing” experiment. [FSHWatch](#)

# PERSPECTIVES AND REACTIONS

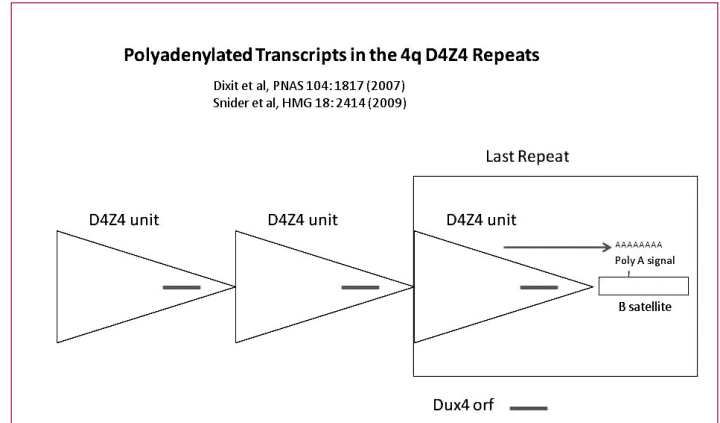
## The long walk towards a key role for DUX4 in FSHD

by **PROF. ALEXANDRA BELAYEW, PH.D.**  
*University of Mons, Mons, Belgium*

Just like the stress tests that are used to assess whether a bank could escape a financial crash, an international research consortium of eight laboratories has tested and validated the importance of DUX4 expression in the pathological mechanism of FSHD (Lemmers et al, 2010). On this occasion, I would like to remind you of the story of the long walk towards the DUX4 model. You have to go back 15 years, just after Professor Rune Frants's group identified the FSHD genetic defect as a D4Z4 repeat array contraction in 4q35. Then Jane Hewitt et al (1994) determined the sequence of D4Z4 elements in the DNA of a healthy person, and found a part that could possibly be the blueprint to build a protein. The blueprint had a special feature — it contained twice a homeobox, a sequence typical of genes acting in embryo development. The proteins made by such genes are named transcription factors; they can give instructions to several other genes and either switch them “on” or “off”, to build the different body parts such as muscles, nerves, skin, etc. It was very exciting to imagine that the protein of the FSHD gene might have such a function that might disturb many genes.

Besides the blueprint, a gene needs a start called a promoter. To demonstrate its activity, the researchers must detect the corresponding messenger RNA (mRNA) that carries a blueprint copy to the cell factory where the protein is assembled. Neither a promoter nor mRNAs could be defined in that study. It was very disappointing! My group was lucky to detect the missing promoter in J. Hewitt's gene by comparing its sequence with a large gene family we had just identified: all these genes had a blueprint containing a double homeobox and were thus named DUX (Ding et al, 1998). We also found the blueprint and its promoter in each of the two D4Z4 elements at the FSHD locus of a patient: since it was on chromosome 4, we named this gene DUX4 and proposed that its expression caused FSHD (Gabriëls et al, 1999). Our hypothesis looked weird because repeated elements were considered “junk DNA,” and for decades most researchers had thought they contained nothing useful, least of all a gene. That idea led several groups involved in FSHD study to focus on alternative 4q35 candidate genes.

If the D4Z4 element contained the DUX4 promoter and blueprint, it had nothing like a gene end. This is a short signal sequence that gets copied into the mRNA to which it triggers the addition of a shield (polyadenylation). It protects the mRNA from rapid destruction, thus leaving time for protein synthesis. We analyzed RNA extracted from myoblasts (cells grown in plastic dishes in the laboratory and derived from muscle biopsies of patients or healthy (control) donors). We identified an mRNA part containing the DUX4 blueprint in FSHD but not control myoblasts (Kowaljow et al, 2007). At this stage, it was not clear how this mRNA could be stable. In parallel we developed tools that allowed us to detect the DUX4 protein in FSHD but not control myoblasts (Dixit et al, 2007). So if the protein was made it meant a stable mRNA had to exist!



**FSHD patients have chromosome variations called 4aA and pLAM that add a trailing segment to the RNA called a poly(A) tail. With the poly(A) tail attached, the RNA is more stable and more likely to cause damage. (Source: Dr. Stephen Tapscott's 2010 FSH Society Patient and Researcher Network meeting presentation on August 1, 2010, Las Vegas, Nevada.)**

After many attempts, we managed to characterize the full DUX4 mRNAs of FSHD myoblasts: they started in the last D4Z4 unit and unexpectedly ended in the region (called pLAM) outside of the repeated elements. Wasn't that extraordinary? In order to become a complete gene, DUX4 extended from the D4Z4 unit where it started, into the flanking pLAM region that provided its end! (Dixit et al, 2007). In addition, we found that a part of the pLAM region within the DUX4 gene was missing in the mRNA: this is an intron. It is found in many genes, is first copied into the mRNA and gets spliced out of it. We concluded that “at least part of a D4Z4 unit with one full copy of the DUX4 gene is required on the FSHD allele to cause the disease.” We further discussed that “differences between the (pathogenic) 4qA and (non pathogenic) 4qB alleles ... may affect both the splicing and polyadenylation signals”, and included data “suggesting no stable DUX4 transcript could derive from chromosome 10” (Dixit et al, 2007). Our model has now been validated by the study of Lemmers et al. (2010). Furthermore as expected from its homeoboxes, the DUX4 gene codes for a transcription factor that is toxic and deregulates many genes (Kowaljow et al, 2007; Dixit et al, 2007; Bosnakowski et al, 2008). [FSH Watch](#)

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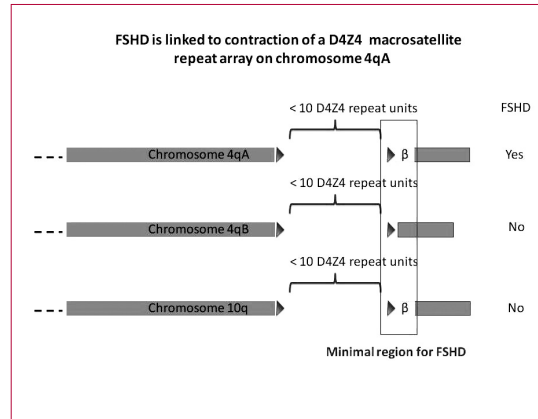
# PERSPECTIVES AND REACTIONS

## FSHD research attention focuses on the DUX4 gene

by **MICHAEL KYBA, PH.D.**

University of Minnesota, Minneapolis, Minnesota

My laboratory uses a combination of molecular genetics and developmental biology to study how stem cells are regulated. Our research on FSHD over the past six years has been focused principally on understanding the function of the DUX4 gene. This focus was initiated because of a simple observation: one part of the DUX4 protein is very similar to an equivalent part of the muscle stem cell regulator, Pax7. Mice with mutations in the Pax7 gene lose their muscle stem cells and experience profound muscle degeneration. This connection between a gene at the site of the FSHD mutation, and a gene required for muscle regeneration, seemed just too striking to be ignored. We hypothesized that if the DUX4 protein were present in muscle stem cells of individuals with FSHD, it might interfere with Pax7 and result in a malfunction that resembled the loss of Pax7, i.e. the loss of muscle regeneration. The analogy would be if you were to put together an electrical circuit, but inserted the wrong component at a certain place, the circuit could behave as if the necessary component were missing. The components in this case (DUX4 and Pax7) are proteins that bind to DNA and turn on or turn off the genes encoded by that bit of DNA to which they bind. The protein sequence shared by DUX4 and Pax7 is the DNA-binding part of each protein; in the electrical circuit analogy the part that determines the location of the component within the circuit. Over the past several years, we have been working to prove or disprove the hypothesis that DUX4 and Pax7 compete with each other for insertion into specific sites in the large and complex circuit that is the genome. Our work to date broadly supports this hypothesis for FSHD. We have developed cell-based systems in which competition between Pax7 and DUX4 can be observed, and we have identified several important muscle regeneration genes for which these factors compete. For example, an important muscle regulator named MyoD is turned on by Pax7 but turned off by DUX4. By antagonizing the system that allows muscle



**A minimum genetic region is required for FSHD. The Science paper shows one FSHD patient who has a very rare translocation of the minimum FSHD causing region from chromosome 4 that was moved to chromosome 10. Placing the minimum region into another chromosomal context DUX4 really brings DUX4 into the limelight, as it is very hard to explain by invoking any gene other than DUX4. (Source: Dr. Stephen Tapscott's 2010 FSH Society Patient and Researcher Network meeting presentation on August 1, 2010, Las Vegas, Nevada.)**

to regenerate when injured, DUX4 makes muscle tissue much more fragile and sensitive to stress.

When we began this work, there was no direct evidence that DUX4 was responsible for FSHD, and little interest in the significance of protein similarities with muscle regeneration genes. The field was mainly focused on genes nearby but not at the actual site of the FSHD mutation on chromosome 4. As there was no consensus on which gene was at the root of FSHD, funding agencies were unenthusiastic about research projects involving the deep study of any of these genes. My own laboratory was very fortunate to have received philanthropic support from the Pacific Northwest Friends of FSHD Research and the FSH Society to pursue research into DUX4 during this time.

Several important findings including work by Jane Hewitt showing that mice have a similar gene, implying that DUX4 is not junk DNA, and by Alexandra Belayew showing that the DUX4 gene is expressed and the protein is present in FSHD, as well as our own work on the pathological effects of DUX4, have pushed DUX4 into the fore. The recent publication by Silvere van der Maarel's research team in Leiden and collaborators in Seattle and Rochester demonstrating that FSHD can be linked to chromosome 10 if a copy of DUX4 jumps over from chromosome 4 really brings DUX4 into the limelight, as it is very hard to explain by invoking any gene other than DUX4. In concert with this accumulation of evidence in favor of DUX4, interest by the NIH in FSHD (measured by dollars spent) has also increased. This is a trend that will need to continue, as basic research naturally leads into the development of therapies targeting the DUX4 gene. Because of the lack of consensus on a specific mechanism, FSHD research funding has lagged far behind other muscular dystrophies. By all indications, with the recent work on DUX4, this tide appears to be changing, and we are finally entering into a hopeful time for research towards a therapy for FSHD. FSHWatch

## PERSPECTIVES AND REACTIONS: THE LONG WALK TOWARDS A KEY ROLE FOR DUX4 IN FSHD

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# PERSPECTIVES AND REACTIONS

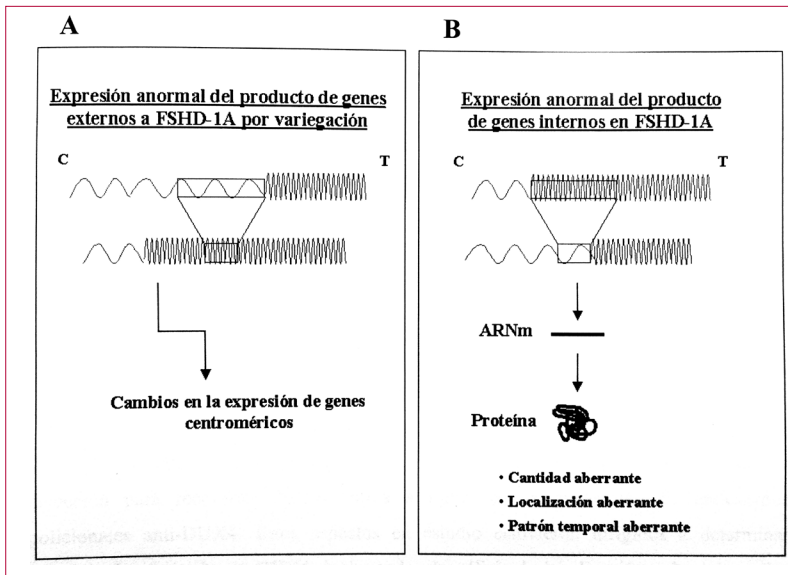
## FSHD and DUX4 gene expression: a genetic signature of FSHD families

by ALBERTO LUIS ROSA, M.D., PH.D.

Fundación Allend, Hipólito Irigoyen, Córdoba, Argentina

Clinical genetic research is not just a medical or scientific thing. It is part of the everyday life of patients carrying inherited diseases and their families. Scientific studies certainly contribute to the happiness and hope of patients all around the world. Patients, families, doctors and scientists interested in FSHD know about these feelings since (at least!) the year 1992<sup>1</sup>: in that year a fruitful collaboration between research groups from UK and The Netherlands recognized that FSHD is associated to shortening of a portion of chromosome 4 (i.e. D4Z4 alleles: contractions of the polymorphic tandem repeat D4Z4 at the tip of the large arm of chromosome 4). FSHD patients and their families received this exciting news with enthusiasm. A detailed molecular analysis of the D4Z4 region was later presented by the same research groups<sup>2</sup>. Those studies contributed to the beginning of an intense international research devoted to decipher the cryptic pathogenic mechanism underlying FSHD. The role of D4Z4 in FSHD was quickly revisited: in 1996 it was demonstrated that individuals carrying a complete deletion of the D4Z4 tandem do not have FSHD<sup>3</sup>, meaning that at least one residual D4Z4 unit is required to cause the disease.

The connection between D4Z4 and FSHD increased in complexity when scientists found the existence of interchromosomal exchanges between homologous regions of chromosomes 4 and 10<sup>4</sup>. Only contractions of chromosome 4-linked D4Z4 alleles appeared to cause FSHD. Scientists concluded that these observations “complicate the search for the FSHD gene but also imply the presence of a potentially novel molecular pathogenesis”<sup>5</sup>. Twelve additional years of intense and elegant formal and molecular genetic research, mostly



Original drawing presented at the University of Cordoba (Argentina) in 1998 by Drs. Kowaljow and Rosa challenging the more appealing hypothesis at that time (represented in panel A) which proposed that shortening of the tandem D4Z4 (rectangle) allows spreading of telomeric (T) heterochromatin (peaked waves) all over the centromeric (C) proximal region (smooth waves) altering the expression of genes external to D4Z4. On Panel B, Drs. Rosa and Kowaljow proposed an alternative hypothesis: shortening of the D4Z4 tandem leaves a relaxed chromatin environment at the residual D4Z4 units allowing binding of transcription factors and expression of DUX4. The drawing proposes that the hypothetical DUX4 gene (at that time considered a pseudogene) is epigenetically controlled and that abnormally expressed DUX4 protein (i.e. higher amounts, abnormal muscle tissue patterns and/or altered developmental expression) produces FSHD.



Current members of Dr. Rosa's laboratory in Argentina. From left to right: Dr. Alberto L. Rosa, postdoctoral student Dr. Daniela Jacquelin and Ph.D. student E. Daniel Corona

performed by Drs. Lemmers, van der Maarel, Frants and colleagues in Leiden, the Netherlands, revealed two puzzling characteristics of pathological FSHD chromosomes: 1) FSHD patients carry particular (“permissive”) DNA sequences at the end of the D4Z4 tandem repeat<sup>6,13</sup>, and 2) D4Z4 sequences in FSHD patients are less “peppered” by DNA methylation<sup>7</sup> (an epigenetic watermark of DNA). FSHD families around the world have been extremely valuable in all of these studies: DNA samples obtained from patients all over the world provide scientists with a large collection of chromosome variants and DNA diversity, allowing us to challenge hypotheses derived from initial examinations of a few clinical cases.

Along these years, many other excellent papers have been published concerning the cellular and molecular biology of FSHD. Two predominant ideas have influenced scientists to pursue their various research avenues:

1. contraction of the D4Z4 tandem lead to aberrant expression of a gene located outside of the D4Z4 tandem region gene<sup>8</sup>, and,
2. the D4Z4 tandem itself was just ‘junk’ DNA. Indeed, efforts from various laboratories around the world to recognize expression of a gene within

the D4Z4 tandem were unsuccessful. In 1998, however, two groups, Dr. Belayew's in Belgium and my own research group in Argentina, started a series of studies considering a risky (!) alternative hypothesis: “shortening of the D4Z4 tandem repeat modifies its chromosome configuration leading to abnormal expression of a D4Z4-encoded toxic protein that causes FSHD<sup>9,10</sup>. The hypothesis was independently formulated by Drs. Hewitt and Belayew in Europe and by my

laboratory in Argentina. A courageous doctoral (Ph.D.) student in my research group, Valeria Kowaljow, explored between 1998 and 2004 “the potential pathogenic role in FSHD of the putative toxic protein DUX4, encoded at the tandem repeat D4Z4 at 4q35”<sup>10</sup>.

Even when the idea was attractive among members of the FSHD scientific community, it was hard for our laboratories to link our interesting experimental data about the biology of DUX4 with the pathogenesis of FSHD. Likewise, it was also hard to convince reviewers from scientific journals about this potential relationship between DUX4 and FSHD. In 2007, however, the first paper showing that DUX4 is a toxic protein was published from a scientific collaboration between Belayew’s and Rosa’s laboratories<sup>11</sup>. Our findings increased the scientific interest in DUX4, triggering the current intense research on this protein. Various studies performed in other laboratories since 2008, confirmed and extended our results concerning the ubiquitous nuclear localization of DUX4 as well as its cellular toxic properties. Highlighting the potential value of DUX4, a dedicated scientific review refers to this protein as a “pearl in the junk”<sup>12</sup>. In a confluent line of research, recent elegant and rigorous genetic experiments<sup>14</sup> show that the distal D4Z4 unit of FSHD chromosomes is expressed as a DUX4 message (i.e. mRNA). Authors of this internationally collaborative study show that this DUX4 message is stable only in chromosomes carrying the above mentioned “permissive” DNA sequences<sup>7,13</sup>. It is suggested that this “stabilization” is mediated by DNA sequences (i.e. a “poly(A) signal”) present

only at these “permissive” chromosomes<sup>14</sup>. This beautiful piece of genetic research also has the signature of FSHD families: world-wide genetic diversity and complex D4Z4 alleles have been a major resource for this fundamental discovery. Moreover, this remarkable scientific contribution unifies two major findings on FSHD molecular pathology: contraction of the D4Z4 tandem is pathogenic only in “permissive” chromosomes<sup>14</sup>. Also, the reported expression of a D4Z4 unit containing the information for the protein DUX4 is in line with Hewitt-Belayew’s and Rosa’s hypothesis: FSHD is caused by altered expression of the toxic protein DUX4<sup>9,10,11</sup>. FSH Watch

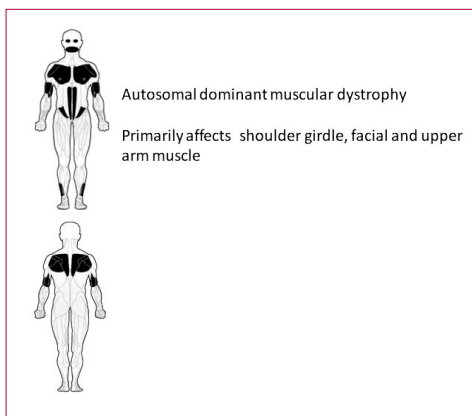
- <sup>1</sup> Wijmenga et al Nat Genet (1992) 2, 26-30;
- <sup>2</sup> Hewitt et al Hum Mol Genet (1994) 3, 1287-1295;
- <sup>3</sup> Tupler et al J Med Genet (1996) 33, 366-370;
- <sup>4</sup> van Deutekom et al Hum Mol Genet (1996) 5, 1997-2003;
- <sup>5</sup> Tawil et al Ann Neurol (1998) 43, 279-282;
- <sup>6</sup> Lemmers et al Nat Genet (2002) 32, 235-236;
- <sup>7</sup> van Overveld et al Nat Genet (2003) 35, 315-317;
- <sup>8</sup> Gabellini et al Cell (2002) 110, 339-48;
- <sup>9</sup> Gabriëls et al Gene (1999) 236, 25-32;
- <sup>10</sup> Kowaljow and Rosa (1998) University of Cordoba, Argentina;
- <sup>11</sup> Kowaljow et al Neuromuscular disord (2007) 17, 611-623;
- <sup>12</sup> Dmitriev et al Neuromuscular disord (2009) 19, 17-20;
- <sup>13</sup> Lemmers et al Am J Hum Genet (2007) 81, 884-894;
- <sup>14</sup> Lemmers et al Science (2010) (Epub ahead of print)

## FSH SOCIETY HAILS MAJOR BREAKTHROUGH IN FSHD

... from page 1

FSHD is a lifelong, progressive disease causing severe loss of all skeletal muscles (face, shoulders, trunk, upper arms, legs and feet). Crippling and potentially life shortening, it is transmitted to children both genetically from parents and spontaneously (by mutation). It affects entire family constellations. Until now, there has been little definitive evidence of the exact genetic package that triggers the disease.

“These most recent findings begin to define a pathway to treatment for FSHD.” said Professor David Housman of Massachusetts Institute of Technology, Chairman of the FSH Society’s Scientific Advisory Board. “Understanding how a quiescent ancestral gene that is seemingly tucked away in a forgotten corner of the human genome can reemerge to cause muscle weakness and wasting in tens of thousands of people around the world is



**Diagram of typical muscle involvement in FSHD.**  
**(Source: Dr. Stephen Tapscott’s 2010 FSH Society Patient and Researcher Network meeting presentation on August 1, 2010, Las Vegas, Nevada.)**

a fascinating scientific story,” he said. “But more importantly, the knowledge that has

emerged from tracking this complex story of DNA slipping and sliding into a deadly configuration opens the door to new ways to prevent damage from being done and an eventual return to health for victims of this very common form of muscular dystrophy.”

Nancy Van Zant, Executive Director of the FSH Society, said: “Patients ask us every day if there is any hopeful research to share. We are always optimistic for them, but progress has seemed slow. Now, the reality that a specific genetic package of material leads to the toxicity causing the disease means that researchers can focus efforts to test medications and other therapies on this defined target. Speaking as one of the leaders of this organization, I look forward to participating in the coordination of financial resources and patient efforts to help investigators carry this finding forward into treatments.” FSH Watch

# PERSPECTIVES AND REACTIONS

## Appreciating the complex genomic structure of FSHD

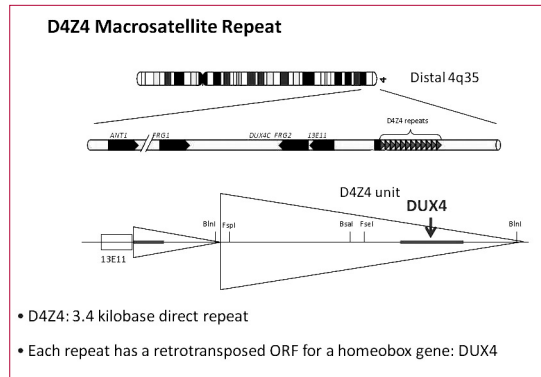
by **YI-WEN CHEN, D.V.M., PH.D.**

*Children's National Medical Center, Washington, D.C.*

The double homeobox protein 4 (DUX4) gene located in each D4Z4 repeat encodes a homeobox-containing protein. Homeobox-containing proteins are a group of proteins that bind to DNA using their homeoboxes and regulate the expression of other genes. Changes in DUX4 expression can lead to changes of a cascade of genes regulated by it. This and the fact that DUX4 resides inside the D4Z4 array made the DUX4 a plausible candidate gene of FSHD. However, due to the complex genomic structure of the chromosomal region where the DUX4 gene is located, the importance of DUX4 was not well appreciated until recently.

Dr. Chen's group is interested in understanding the molecular mechanisms of FSHD. The group uses a technology called "gene expression profiling" which can study gene activities of the whole genome using a small glass chip. The approach has been used to study the molecular events that occur in muscles of various muscle disorders. By comparing the gene expression profiles of FSHD to 11 other neuromuscular disorders, the group identified genes that were abnormally expressed in FSHD but not the other disorders. One of the genes was paired-like homeodomain transcription factor 1 (PITX1), of which the protein product was also a homeobox-containing protein. In 2007, based on a collaborative study with Dr. Alexandra Belayew, the collaborative team reported that the DUX4 protein was indeed aberrantly expressed in FSHD and was likely responsible for the disease-specific upregulation of PITX1 in muscles of patients with FSHD. In the same study, the team proposed that the DUX4 gene in the most distal D4Z4 element was likely to be the only one that could be transcribed into a polyadenylated RNA. Polyadenylation of RNA is a critical step to stabilize the RNA so a functional DUX4 protein which had been shown to be toxic to muscle cells can then be produced. The team hypothesized that a genetic variation that affects the polyadenylation might be able to influence the stability of the RNA, which then can affect whether an individual with a shortened D4Z4 array will have the disease or not.

This hypothesis was supported by the recent findings reported by Dr. Lemmers, van der Maarel and colleagues in the *Science* journal. In the study, the group showed that an individual needs to have both a contracted D4Z4 array and a genetic feature associated with stable DUX4 mRNA in the genome to have FSHD, which strongly suggested that aberrant expression of DUX4 is the cause of FSHD. The Chen group currently is conducting studies to understand the molecular basis of the DUX4 toxicity in muscles. An animal model overexpressing PITX1 was generated by the group (supported by the FSH Society) and showed muscular dystrophy phenotype. The mouse model is under evaluation for translational studies. The group is also testing a therapeutic strategy using a small molecule which can bind to the overexpressed gene such as DUX4 and suppress its expression in muscles. The same strategy can be applied to other genes in case additional genes are involved in the disease process. [FSH Watch](#)



**At the end of chromosome 4 in a region called 4q35, is a region comprised of equal length and similar repeating units called D4Z4 and within each repeating unit is a gene called DUX4. (Source: Dr. Stephen Tapscott's 2010 FSH Society Patient and Researcher Network meeting presentation on August 1, 2010, Las Vegas, Nevada.)**

Fellowship Grantees who have Completed or are Completing their Projects Since the Last Research Newsletter

*FSH Society Marjorie and Gerald Bronfman Fellowships*

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

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

*Towards the Discovery of Early Developmental Defects in FSHD*

**Total project \$105,000**

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

**Michael Kyba, Ph.D.**

**University of Minnesota, Lillehei Heart Institute, Minneapolis, Minnesota USA**

*Molecular Analyses of DUX4 and Interaction with Myogenic Regulators in FSHD*

**Total project \$21,488**

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

**INIMEC-CONICET, National Research Council of Argentina**

**Laboratorio de Biología Celular y Molecular, Córdoba, ARGENTINA**

*Analyses of functional domains in the pro-apoptotic protein DUX4*

**Total project \$59,040**

*FSH Society Connors and Jacobs Families and Friends Research Fellowship grant*

**Scott Q. Harper, Ph.D.**

**Center for Gene Therapy**

**The Research Institute at Nationwide Children's Hospital**

**The Ohio State University, Columbus, Ohio USA**

*Investigating DUX4 Structure and Function Using Rational Mutagenesis*

**Total project \$40,000**

*FSH Society Family Picnic Fellowship*

**Frédérique Magdinier, Ph.D.**

**Laboratory INSERM UMR 910**

**"Medical Genetics & Functional Genomics"  
 Faculté de médecine de la Timone, Marseille, FRANCE**

*Exploring the interplay between the nuclear envelope and the higher order chromatin organization of the D4Z4 array in control and FSHD cells*

**Total project \$30,000**

# Volunteers with FSHD and their unaffected family members are needed

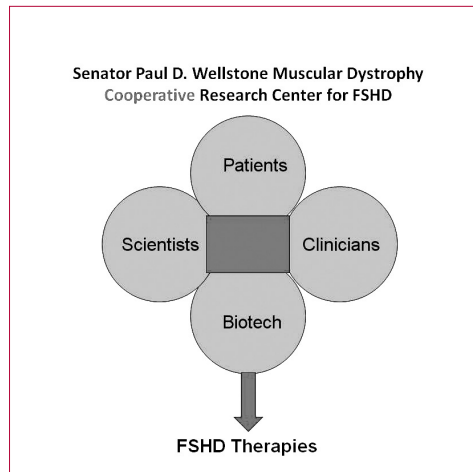
*With your help, we're one step closer to finding a cure*

The country's largest research project on FSHD is taking place now at the NIH Boston Biomedical Research Institute's Senator Paul Wellstone Center for FSHD Research. The teams at the center are making excellent progress on defining biomarkers that will be used to accelerate treatments and clinical trials on FSHD.

The FSH Society would like to answer the question often asked by many affected patients and their unaffected family members "How can I help the research and progress towards treatments?" One of the greatest contributions you can make at this time is to literally give of yourself. Researchers need to be able to compare large numbers of muscle, blood and cells from FSHD patients and their unaffected genetically related family members in order to determine the differences between FSHD-diseased muscle and unaffected muscle. Your help is urgently needed!

WHO is asking me to participate? As part of the NIH Boston Biomedical Research Institute's Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center ([wellstone.bbri.org](http://wellstone.bbri.org)), the FSH Society and Johns Hopkins School of Medicine are recruiting volunteers with FSHD and their first-degree unaffected relatives (for example: parent, sibling, or child of a person with FSHD) to provide muscle and blood samples.

WHAT will I be asked to do? Volunteers will be asked to provide muscle and blood samples at Johns Hopkins School of Medicine in Baltimore, Maryland. Volunteers who choose to participate in this study will make two visits to Johns Hopkins: one for screening, and another for muscle biopsy and research blood sample. However, for those who are travelling from a distance, both the screening and the biopsy can be performed during one extended stay by prior arrangement. The screening consists of a general history and physical examination, a neurological exam, and a collection of blood samples (about eight teaspoons of blood will be taken). At the conclusion of the screening the principal investigator may determine that the volunteer does not qualify for participation in this study.



**Diagram depicting cooperative paradigm for FSHD research at the NIH Boston Biomedical Research Institute's Senator Paul Wellstone Center for FSHD Research. (Source: Dr. Charles P. Emerson, Jr.'s 2010 FSH Society Patient and Researcher Network meeting presentation on August 1, 2010, Las Vegas, Nevada.)**

Muscle biopsies are taken from two locations (shoulder and upper arm) on the same arm and are performed in an operating room under local anesthetic similar to that used by a dentist. A small cut, about an inch long, is made and a small piece of the muscle, about the size of two pencil erasers, is removed. After the biopsy, the surgeon will stitch the area with dissolvable sutures and a pressure bandage will be applied. The volunteer will be educated on the proper care of the incisions.

The FSHD-Wellstone Muscular Dystrophy Cooperative Research Center has set a goal of recruiting 15 FSHD-affected volunteers and 15 relatives each year to participate in this study. All procedures, tests, drugs, and devices are part of this research and will be supplied free of charge.

WHY is this study important? Once blood and muscle samples have been collected, they will be sent to the tissue bank at Boston Biomedical Research Institute, headquarters for the Wellstone Research Center, and analyzed by FSHD researchers. The muscle tissue from the FSHD-affected participant remains paired with that of the unaffected relative so that familial and genetic differences can be observed. The collected samples will be used to learn more about how individuals with FSHD differ from individuals with

normal muscle. Collected samples will be available to the international community working on FSHD research to help accelerate the search for a treatment and a cure.

Although there is no direct benefit to the volunteer from participating in this study, participation will be a great asset to the multiple studies at the FSHD-Wellstone and the larger FSHD research community.

To date, twenty groups of FSHD-affected volunteers and their unaffected relatives have participated in this research study. While we have been successful in recruiting participants since the study's inception in December 2008, there is still a great need for FSHD-affected participants and their unaffected first degree relatives. At this time, the study especially needs individuals from minority races and ethnicities.

Although there are no direct tangible benefits to study participants, those who have participated have found it to be immensely rewarding, fascinating, empowering and fulfilling. For descriptions by several participants of their experiences, see Research Issue Summer 2009 FSH Watch with the articles by Don Burke and others, at [www.fshsociety.org](http://www.fshsociety.org), select top-menu "Community & Reference" then select "FSH Watch Newsletters" in the left hand navigation, or, go directly to [http://www.fshsociety.org/assets/pdf/FSHSociety\\_FSHWatch\\_Summer2009.pdf](http://www.fshsociety.org/assets/pdf/FSHSociety_FSHWatch_Summer2009.pdf).

HOW do I get involved? There are a few ways to let us know you are interested in participating in this muscle biopsy study:

For general information on the study, including participant travel reimbursement, please contact Raphaela Silverio at:

► Raphaela Silverio  
Patient Liaison  
FSH Society, Inc.  
64 Grove Street  
Watertown, MA 02472  
617-658-7877 Office  
617-658-7879 Fax  
[raphaela.silverio@fshsociety.org](mailto:raphaela.silverio@fshsociety.org)

If you would like more information the study, please contact the Research Study

... continued on page 19

## PERSPECTIVES AND REACTIONS

# The roles of FSHD candidate genes in muscle development

by CHIA-YUN JESSICA SUN, PH.D., TAKAKO JONES, PH.D., AND PETER L. JONES, PH.D.

Boston Biomedical Research Institute, Watertown, Massachusetts

All cells in the body require their own particular complement of proteins to function and develop properly. This includes both the expression of the proteins necessary for the cell's function and survival, as well as the repression or lack of unwanted and potentially deleterious proteins. However, it is not simply a matter of ON or OFF for each of the >40,000 genes encoded by the genome. The level of each expressed gene is critical and tightly regulated in every cell type. Unwanted changes in gene expression levels negatively affect cellular function and viability and can lead to disease. In respect to FSHD, it is generally well accepted that gene expression is altered such that a gene (or genes) are expressed in FSHD patient muscles at deleterious levels which eventually results in pathology. Our approach towards FSHD has been to first understand the normal expression and functions of FSHD candidate genes in vertebrate development, then determine which genes produce FSHD-like pathology in developing muscle when mis-expressed. With support from the FSH Society, a Landsman Charitable Trust Fellowship, and a Kelly Family Post-Doctoral Research Fellowship, our laboratory has made great progress investigating the function of several of the FSHD candidate genes, including FRG1, DUX4c, PITX1, and DUX4, and assaying the effect of their altered expression on muscle development<sup>1-3</sup>.

The system we use as a model for vertebrate muscle development is the early development of the African Clawed Frog (*Xenopus laevis*). Frogs have numerous advantages over other animal systems including cost, the relative ease of manipulating gene expression, and their external embryonic development (frogs develop from single cell embryos to adults in water), yet they are still vertebrate. Using this system we have determined both the requirements for FSHD candidate proteins, if any, in muscle development as well as assayed deleterious effects on muscle development due to over-expression of the FSHD candidate proteins. We have shown that the FRG1 protein, highly evolutionarily conserved, is localized to the muscle contractile machinery and is critical for the normal development of the musculature and vasculature; over-expression of FRG1 produces vasculopathy

and dystrophic muscles<sup>1,2,4</sup>. Conversely, the DUX4 protein, unique to humans, is extremely toxic to most cells and prevents normal development by inducing massive cell death<sup>3</sup>. The DUX4 protein as a whole does not appear to be necessary for development, and even very small levels of expression are catastrophic for normal vertebrates. Interestingly, expression of the highly similar DUX4c showed no noticeable effect on development. Taken together, our data suggests FSHD pathophysiology could be caused by increased expression of either FRG1, DUX4, or both.

Our work, based in developmental biology and biochemistry, has focused strictly on understanding the function and pathogenic potential of the FSHD candidate genes. The recent breakthrough work by Lemmers et al.<sup>5</sup>, based in human genetics, has answered several confounding issues in FSHD and has added needed perspective to work on the FSHD candidate genes. Mainly, this work suggests the primary underlying factor leading to FSHD is the stable mis-expression of the DUX4 mRNA. Consistent with what we see in our developmental system, this expression of DUX4 is toxic. While several questions remain to be addressed in respect to DUX4's normal developmental function and how (or if) DUX4 expression alone can account for the non-muscular pathologies exhibited by many FSHD patients, stable DUX4 mRNA expression appears necessary (and potentially sufficient) for FSHD progression. Other candidate genes such as FRG1 could be relegated to secondary roles, potentially contributing to the high degree of variable pathology and severity found among FSHD patients. Overall, the work by Lemmers et al.<sup>5</sup> is a great step forward for all of us in the FSHD field and patients alike. **FSH Watch**

### References

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- Liu, Q., et al. *J Cell Sci*, 2010. 123: p. 1116-1123.
- Lemmers, R.J., et al. *Science*, 2010.

## 2010 New York Music and Song Fellowship Grants

The FSH Society New York Music and Song Fellowship program was established in 2004 to help FSHD research. The FSH Society is indebted to board member Judith Seslowe of White Plains, New York, to Beth Johnston of Ossining, New York, and to their families and friends for this groundbreaking effort on behalf of the FSHD community. As FSHD research moves ahead with new challenges, these awards have the potential to yield tremendous insights in FSHD treatment.

For more information about research fellowships, please contact:

Daniel P. Perez, President & CEO  
FSH Society  
Research Programs Office  
11 Elmbrook Circle  
Bedford, MA 01730 USA  
781-275-7781, 617-658-7811  
daniel.perez@fshsociety.org

FSH Society 2009 New York Music and Song Fellowship Grantees:

Daphne Cagianca, M.S.  
*A ncRNA Regulating the Epigenetic Switch at the Basis of FSHD*  
Total project \$45,000

Michael Kyba, Ph.D.  
*FSHD iPS cells: bioinformatics support*  
Total project \$45,000



## Research planning—helping to solve FSHD


Since the identification of the FSHD locus on chromosome 4 in the early 1990's, FSHD research has made steady progress in understanding the mechanism and function of the molecular, cellular and evolutionary biology of the disease. The recent paper in *Science* (see page 6 of this *Watch*) that puts forth a unifying model defining the genetic mechanism of FSHD gives us a much better understanding of the underlying biology of the disease - so much so that FSHD research has begun to enter the area between basic research and clinical trials known as translational research. Researchers and clinicians have begun to identify major biochemical pathways in muscle control and growth and high priority potential drug targets for the disease. FSHD cell and animal models have begun to be developed that help build and accelerate the rationale for preclinical testing of candidate drugs.

We continually ask ourselves: What do we know about FSHD? What do we not know? What do we need to know? What are the obstacles to complete understanding? What must we — the FSH Society, as well as the FSHD patient and professional community at large — do next to accelerate progress toward solving FSHD?

These questions are addressed by generating plans and strategies that come from the consensus of researchers, clinicians, patients, funding agencies and industry.

In 2006, in Cambridge, Massachusetts the FSH Society addressed these questions at a planning meeting of its Scientific Advisory Board (SAB) and other members of the FSHD research community. Among the outside advisors we invited to work with the SAB was Dr. Stephen Tapscott, at the University of Washington, who at that time was collaborating with FSH Society research fellow Dr. Sara Winokur of University of California at Irvine. Dr. Tapscott is a lead author on the recently published Lemmers *Science* paper. The Society plays an important role organizing science, patient and planning meetings and in bringing in outside perspectives to advances ideas and science. Those assembled in 2006 developed the FSH Society FSHD Tactical and Strategic Research Plan — a review of FSH Society grant projects, what they have accomplished, and an assessment of how to go forward.

This plan and related recommendations guided the direction of the FSH Society's research programs, and helped optimize FSHD research funding by federal agencies, non-profits and private funding sources. For more about the 2006 FSH Society FSHD Tactical and Strategic Research Plan, go to <http://www.fshsociety.org/pages/resPSocPlan.html>. In 2008 and 2009 the Society organized the FSH Society International Research Consortium Workshops and Research Planning days to help researchers share data, ideas, network and strategize about next steps in the research. The Society is currently organizing the 2010 International Research Consortium Workshops and Research Planning days to be held October 21-22, 2010 at BBRI in Watertown, Massachusetts.

The Muscular Dystrophy Community Assistance, Research and Education Amendments of 2001 and 2008 (MD - CARE Act) authorized the establishment of the Muscular Dystrophy Coordinating Committee (MDCC) to coordinate activities across the National Institutes of Health and with other federal health programs and activities relevant to the various forms of muscular dystrophy. Daniel Paul Perez, President and CEO of the FSH Society, has served as a member of the MDCC since its inception, one of five patient advocates on the committee. In 2005, the MDCC developed and submitted to Congress an Action Plan for the Muscular Dystrophies, which Congress approved. The Action Plan covers all nine major forms of muscular dystrophy. The MDCC is expected to revisit and update this plan in the next year or two. To read more about the MDCC and the Action Plan for the Muscular Dystrophies, see <http://www.fshsociety.org/pages/resP-NIHAction.html> 

### California Institute of Regenerative Medicine (CIRM) funding for muscular dystrophy

We recently asked the California Institute of Regenerative Medicine (CIRM), established several years ago by California Proposition 71, for information on its funding on FSHD and related diseases. Thus far, seven grants, totaling \$11,546,762, have been granted for research on muscular dystrophy. Of this total \$607,200 was granted for FSHD to Dr. Kyoko Yokomori at the University of California, Irvine, California. Dr. Yokomori had previously received an FSH Society fellowship to study chromatin and epigenetics in FSHD. This is one example of how the seed grants provided by the FSH Society enable researchers to leverage and scale up their research projects.

*Derivation and Characterization of Human ES Cells from FSHD Embryos*  
Kyoko Yokomori  
University of California — Irvine. \$607,200.

Readers wishing to receive complete Project Abstracts and Statement of Benefit to California, both provided by CIRM applicants, can request the report from the CIRM <http://www.cirm.ca.gov/> by searching grants by disease type muscular dystrophy.

## Ongoing FSH Society research fellowship grants

*FSH Society Stuart Lai Mouse Model Development fellows*

- ▶ Jeffery Boone Miller, Ph.D.  
Boston Biomedical Research Institute  
Watertown, Massachusetts USA  
NIH Sen. Paul Wellstone MD CRC for FSHD &
- ▶ Robert J. Bloch, Ph.D.  
Patrick Reed, Ph.D.  
University of Maryland School of Medicine  
Baltimore, Maryland USA  
NIH Sen. Paul Wellstone MD CRC for FSHD  
"The Boston Biomedical Research Institute Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD, Project 4 — Mouse Model Studies for FSHD Biomarkers"  
Total project \$160,000



## Learning and networking at the 2010 FSH Society Patient & Researcher Network meeting

by NEIL CAMARTA AND FAMILY  
Calgary, Alberta, Canada

It all started a few months ago when one of my daughters mentioned that the FSH Society was having its Patient and Researcher Network Meeting in Las Vegas. To be honest, I'm not sure if her motivation was purely to attend the meeting — or have some fun in Las Vegas! In any case, we agreed that it would be great to go. The idea caught fire in our family and by the time we got to Las Vegas we had eleven members in tow — including six of us with FSHD.

Our extended family has at least twelve members with FSHD over several generations. We are all keen to learn more about our disease. The Patient and Researcher Network Meeting looked like one-stop-shopping. We weren't disappointed. We all got what we came for — at several levels.

First of all, none of us had ever met

another person with FSHD outside of our own family. The meeting gave us the chance to share our experiences with a large number of other FSHD patients.

Secondly, we all wanted to become a lot smarter about the state of research into finding a cure. Of course, this is what the meeting was all about — and it met its objective. We now know what's working — and isn't working — on the research front. But the main thing is that we all came away very encouraged about the prospects of finding a cure — and soon!

Finally, my daughters were naturally keen to find out their odds of having children with FSHD. They got the opportunity to talk to the experts directly about this and many other subjects. That's the real power of getting patients and researchers together.

As far as improvements for the future

— we came up with a couple of ideas.

It would help to have a high level “State of the Research” presentation at the front end of the meeting. This presentation could include “FSHD 101,” a “Glossary” of technical terms and a “Road Map” of the research so that we can see how it all ties together.

There were many Canadians at the conference. The FSH Society should look at setting up a Canadian affiliate that is registered as a charitable organization in Canada. This would allow Canadians to make tax deductible donations to the Society.

The bottom-line is that the FSH Society Patient and Researcher Network Meeting was a great success for our family and we would like to thank the organizers and participants for making it happen. Well done!

And, yes, we did have a lot of fun in Las Vegas! [FSH Watch](#)



## RESEARCH

## Marjorie Bronfman FSHD Research Grant for 2010

The generosity and commitment of Marjorie Bronfman to FSHD research began in 1997 when she began to make research grants to the FSH Society. Mrs. Bronfman has renewed her commitment each year, including in 2010 with a new grant of \$50,000. Mrs. Bronfman, along with her brother and one of her daughters, is affected with FSHD.

Through a process of review and recommendation by the Society's Scientific Advisory Board, grants are awarded for research fellowships (US\$30,000-US\$35,000/year) for research projects that show extraordinary promise to find the cause of FSHD. The 2010 contribution and the many grants that have preceded it have generated significant progress in FSHD research.

Both the first and last authors of the paper published online in *Science*, August 19, and reported above in this issue of *Watch*, Drs. Richard Lemmers and Silvere van der Maarel, began their careers in FSHD research as recipients of the prestigious FSH Society Marjorie Bronfman fellowship awards. The FSH Society is deeply indebted to Mrs. Bronfman, to the Marjorie and Gerald Bronfman Foundation, and to other members of her family for the advances that have been made possible worldwide over these years and for the opportunity to continue advances in 2010. Edward Schechter, brother of Mrs. Bronfman, has been a careful steward of foundation resources, and he deserves much gratitude for these research grants and for the research breakthrough. For more information about research fellowships, please contact Daniel Paul Perez, President & CEO at the FSH Society or at [daniel.perez@fshsociety.org](mailto:daniel.perez@fshsociety.org). [FSH Watch](#)

### Ongoing & recently completed FSH Society Marjorie & Gerald Bronfman Foundation Fellowship Grantees

Richard Lemmers, Ph.D.  
*Refinement of the FSHD critical region on 4qA chromosomes*  
Total project \$105,000

Melanie Ehrlich, Ph.D.  
*Finding the 4q35 FSHD Gene*  
Total project \$70,000

Patrick Wayne Reed, Ph.D.  
*“Analysis of Changes in the Proteome in FSHD”*  
Total project \$30,000

Yvonne Meijer-Krom, Ph.D.  
*Towards the Discovery of Early Developmental Defects in FSHD*  
Total project \$105,000

Darko Bosnakovski, D.V.M., Ph.D.  
*Molecular Analyses of DUX4 and Interaction with Myogenic Regulators in FSHD*  
Total project \$21,488

Paola Picozzi, Ph.D.  
*Functional Characterization of D4Z4 in FSHD*  
Total project \$17,500

Alberto Luis Rosa, M.D., Ph.D.  
*Analyses of Functional Domains in the Pro-apoptotic Protein DUX4*  
Total project \$59,040

## Sharing knowledge and gaining understanding

by **SUSAN BLANKSCHAEN**

Mentor on the Lake, Ohio, United States

When my boyfriend, Ken, asked me to attend the FSH Society meeting in Las Vegas, I was very excited to do so. As a nurse, I hoped to learn more about this challenging and puzzling disease. As his girlfriend, I hoped to learn ways to be supportive and to better understand the difficulties Ken faces. For myself, I hoped to learn from others who had more experience as to the many facets of being a partner to someone with FSHD. The meeting exceeded my expectations on all accounts. I learned much about FSHD and the current research being done as well as the potential for future treatment options. Those in attendance had the opportunity to hear about many exciting breakthroughs in knowledge and technology and to ask questions of those on the forefront of this research.

There was also a focus on things that impact the lives of those with FSHD with talks on exercise and therapy, pulmonary function, and scapular fixation surgery. There were numerous breakout sessions on topics such as advocacy and disability rights, maintaining good nutrition, and traveling with disabilities. These sessions presented meaningful and practical information both for those affected and for those who care about them. Perhaps the most powerful part of the conference was the opportunity to informally network with one another during the breakout sessions and throughout the day. These conversations gave opportunities to ask questions, share knowledge, and gain understanding in a meaningful and collaborative way. For some participants, such as me, it was the first time we had met others who had firsthand experience with FSHD. The sharing that can occur between people with a mutual bond is very powerful.

The FSH Society is to be commended, not only for putting on this wonderful meeting, but also for their tireless work in so many areas surrounding FSHD. I would encourage anyone and everyone to attend the next meeting and to network with others in the organization. I would also encourage them to assist in raising money to support the work being performed. There were inspiring presentations on success stories in fund-raising efforts. It was amazing to see how small efforts over time and with much diligence, grew into significant fund raisers. The work that will be needed to discover and develop a cure or treatment for FSH will take significant funding. These efforts will be critical in helping to make this a reality. It has inspired us to look at what we can do in this area. Lastly, I am humbled by the efforts of those who are affected by the disease. Whether it is in their efforts to raise funds, to perform grassroots education and lobbying for change, or merely to overcome the numerous challenges they face daily, they inspire the rest of us to want to do more. **FSH Watch**

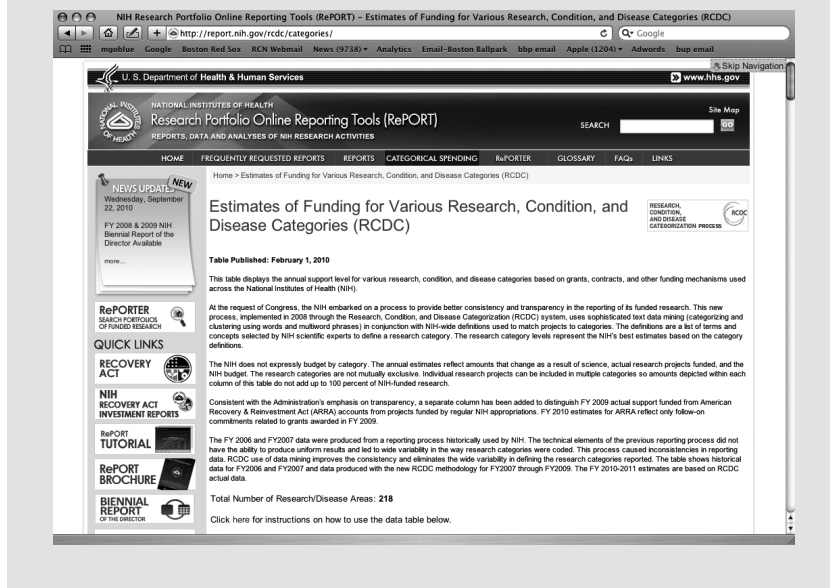
## An Introduction to the NIH RCDC Database

Research, Condition, and Disease Categorization (RCDC) reports are now included in the NIH's RePORT site. RCDC is a computerized process the NIH uses at the end of each fiscal year to sort and report the amount it funded in each of 215 historically reported categories of disease, condition, or research area.

According to the NIH, "RCDC provides consistent and transparent information to the public about NIH-funded research. For the first time, a complete list of all NIH-funded projects related to each category is available. By clicking on each of the categories, the public can access full project listings for that category and view, print, or download the detailed report."

To view NIH spending and dollars allocated to FSHD (see facioscapulohumeral muscular dystrophy) please visit the NIH RCDC database at: <http://report.nih.gov/rcdc/categories/>

For fiscal year 2009 actual dollars spent on FSHD the NIH reports \$5 million!



The screenshot shows the NIH RePORT website interface. The main heading is "Estimates of Funding for Various Research, Condition, and Disease Categories (RCDC)". Below this, it states "Table Published: February 1, 2010". The table displays the annual support level for various research, condition, and disease categories based on grants, contracts, and other funding mechanisms used across the National Institutes of Health (NIH). The interface includes a search bar, navigation links (HOME, FREQUENTLY REQUESTED REPORTS, REPORTS, CATEGORICAL SPENDING, RePORTER, GLOSSARY, FAQs, LINKS), and a sidebar with quick links to RePORTER, RECOVERY ACT, NIH RECOVERY ACT INVESTMENT REPORTS, RePORT TUTORIAL, and BIENNIAL REPORT OF THE DIRECTOR.

## VOLUNTEERS WITH FSHD AND FAMILY MEMBERS NEEDED

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Coordinator, Dr. Genila M. Bibat, at:

- ▶ Genila M. Bibat, M.D.  
Research Study Coordinator  
Center for Genetic Muscle Disorders  
Kennedy Krieger Institute &  
Research Associate  
Johns Hopkins School of Medicine  
707 North Broadway  
Baltimore, MD 21205 USA  
443-923-2778 Office • 443-923-2779 Fax

To help expedite evaluation and enrollment in the study, it is helpful if you have, or have good access to, your medical records and genetic testing results. For Johns Hopkins School of Medicine details, IRB approval number and contact, please visit:

<http://www.fshsociety.org/assets/pdf/JohnsHopkinsFSHDWellstoneKathrynWagner.pdf>

Thank you for your consideration and generosity!

For more information about this and other research studies, please visit [www.fshsociety.org](http://www.fshsociety.org). **FSH Watch**

## FSH Society grants recently awarded

**F**SH Society research and fellowship grant awards provide vital start-up funding for investigators in FSHD and research projects on FSHD. The research milestones and insights gained are demonstrably significant. The fellowship program allows innovative and entrepreneurial research to develop, prove successful, and ultimately to attract funding from large funding sources such as the US National Institutes of Health (NIH) and large private sources. The FSH Society meets an important need in funding and developing new ideas and supporting new investigators in FSHD research by giving them the funds needed to develop data and to carry their ideas to the next stage of development. Without the FSH Society research and fellowships program, many key initiatives in FSHD research might never have seen the light of day. Well over 300 peer-reviewed papers have been published by FSH Society-funded researchers.

Since 1998, the FSH Society has transformed FSHD research. The field is on the verge of significant breakthroughs. It is essential to fund new ideas and support new investigators and new lines of investigation when tackling a disease as complex as FSHD.

The scientific engine of the FSH Society is its Scientific Advisory Board. Scientific progress requires having the most qualified panel of experts who not only evaluate the merit of new proposals they receive, but are also actively engaged in thinking proactively and comprehensively about the scientific problem. Peer-reviewed science is key to research success, and the peer review must be just that — review by scientists and doctors who have comprehensive, up to date command of the science and can evaluate the proposed investigations in light of the science.

Two times per year, in February and in August, the FSH Society accepts grant applications. We are very pleased to list the grants and grantees funded in the August 2009 and February 2010 grant cycles.

► **Yi Xing, Ph.D.**

**The University of Iowa, Iowa City, Iowa USA**  
**FSH Society Aubrie Lee Family Research Grant for Infantile FSHD & Fire Island Fellowship for “Genome-wide analysis of FRG1-mediated splicing defects in FSHD and iFSHD”**  
**Total project \$39,998**  
**Special Class: Infantile FSHD funds**

**Goal: (Provided by Applicant):** The goal of this project is to systematically examine FRG1-mediated splicing defects in FSHD and infantile FSHD (iFSHD). FRG1 is a component of the spliceosome and several lines of evidence suggest its involvement in RNA processing. It is proposed that due to the deletion of a transcriptional silencer within D4Z4, FRG1 is overexpressed in the skeletal muscle of FSHD patients. Transgenic mice selectively overexpressing FRG1 in skeletal muscle develop phenotypes that resemble FSHD in human patients. Moreover, in skeletal muscle of FRG1 transgenic mice and FSHD patients, aberrant splicing of two muscle-expressed genes (Tnnt3, Mtmr1) was observed. However, the global impact of FRG1 overexpression on splicing, as well as its role in FSHD pathogenesis, remains poorly understood, and other models of pathogenesis are also attractive. We plan to test the hypothesis that overexpression of FRG1 in FSHD patients will result in global disruption of pre-mRNA splicing. Further, if this hypothesis is correct, we would predict that the

severe clinical phenotype of iFSHD is correlated with more profound and widespread transcriptome dysregulation at the level of splicing than is seen in milder, adult onset FSHD.

To test these hypotheses, we will utilize a high-density Affymetrix exon array (HJAY array), with 4.6 million probes for 315,137 exons and 260,488 exon junctions in the human genome to identify aberrant splicing events in FSHD and iFSHD. Our collaborator Katherine Mathews has collected skin fibroblast cells from FSHD patients, iFSHD patients and healthy controls and these are available through the Iowa Wellstone Center Core B, run by Steven Moore. After MyoD-induced myodifferentiation of fibroblasts, we will extract RNA and use exon arrays to identify splicing differences between healthy controls, FSHD patients and iFSHD patients. We intend to examine at least four individuals from each group. Following exon array analysis, candidate diseasespecific splicing events will be validated by RT-PCR/qPCR. We will also test the hypothesis that diseasespecific aberrant splicing events preferentially impact genes important for muscle or other organs known to be affected in FSHD/iFSHD. Together, results from this project will lead to improved understanding of FSHD/iFSHD pathophysiology, and reveal novel disease markers and therapeutic targets.

► **Alberto Rosa, M.D., Ph.D.**

**Cellular and Molecular Biology, Fundacion Allende, Córdoba, ARGENTINA**  
**FSH Society Tango Small Research Grant for “Genome DUX4-mediated control of PITX1 gene expression”**  
**Total project \$3,649.85**  
**Special Class: Small Grant**

**Goal: (Provided by Applicant):** Identify specific amino acids from H1/H2 homeodomains controlling the expression of the Pitx1 gene. Hypothesis: Specific amino acids from the DUX4 H1/H2 homeodomains bind the promoter region of the gene Pitx1 to control its expression. Rationale: DUX4 wild type binds the sequence CCGATGCTGTCT-TCTAATTAGT-TTGGACCC, located at the promoter region of the gene Pitx1. Homeobox motifs bind a core motif TAAT present in the promoter region of their specific target genes. In this project we will identify amino acids from H1 and/or H2 motifs participating in the control of Pitx1 gene expression mediated by DUX4.

► **Daphne Cabianca, M.S. / Davide Gabellini, Ph.D.**

**Division of Regenerative Medicine, Centro San Raffaele del Monte, Milano, ITALY**  
**FSH Society Grant FSHS-NYFS-003 New York Festival of Music and Song Fellowship Grant for “A ncRNA regulating the epigenetic switch at the basis of FSHD”**  
**Total project \$45,000**

**Goal: (Provided by Applicant):** Facioscapulohumeral muscular dystrophy (FSHD), the third most common myopathy, is an autosomal dominant neuromuscular disorder characterized by progressive weakness and atrophy affecting specific muscle groups. FSHD is not due to a mutation within a protein-coding gene, but is caused by contraction of the 3.3 kb macrosatellite repeat D4Z4 in the subtelomeric region of chromosome 4q35. The mechanism through which contraction

of D4Z4 repeats causes muscular dystrophy is currently not clear, but there is a general agreement that reduction of D4Z4 activates an epigenetic cascade leading to 4q35 chromatin re-organization and altered gene expression.

My preliminary results suggest that a non-protein coding RNA (ncRNA) transcribed proximally to D4Z4 regulates 4q35 gene expression in FSHD. Furthermore, I found that the trithorax protein Ash1 is recruited to the region selectively in FSHD patients and is involved in 4q35 gene de-repression. It is tempting to speculate that production of the ncRNA activates an epigenetic switch culminating with 4q35 gene de-repression in FSHD. An attractive hypothesis would be that transcription of the region proximal to D4Z4 plays a role in de-condensation of the 4q35 genomic region, setting the stage for activation of 4q35 genes and, most importantly, preventing re-repression of the region. Here, I propose to rigorously investigate the role played by the ncRNA in regulating the epigenetic state of D4Z4 and in 4q35 gene de-repression.

**Specific aim:** To elucidate the mechanism underlying control of gene expression at 4q35. Understanding the mechanism through which the ncRNA is inducing 4q35 gene de-repression in FSHD will generate novel insights into the biological role of ncRNAs in chromatin structure regulation in higher eukaryotes. Moreover, it will help to elucidate the molecular pathways that become altered in FSHD, provide useful molecular markers of FSHD and favor the identification of potential therapeutic targets.

► **Julie Dumonceaux, Ph.D./Gillian Butler Browne, Ph.D.**

Association Institut de Myologie, Paris, FRANCE

FSH Society Grant Delta Railroad Construction Company Fellowship Grant for

“Molecular mechanisms involved in FSHD”

Total project \$37,800

**Goal: (Provided by Applicant):** The global deregulation of muscle genes in facioscapulohumeral dystrophy (FSHD) is still poorly understood : despite the identification of a contraction in the D4Z4 repeats in the chromosome 4 shared by the patients, the molecular mechanisms responsible for the disease have not yet been resolved. Our aim is to increase our understanding of these mechanisms.

We will focus our studies on the abnormal expression of miRNA and on Dux4 expression in FSHD myoblasts and myotubes in order to determine the transcriptional alterations mediated by the D4Z4 contraction observed in the FSHD patients.

The effects of Dux4 over-expression will be analyzed in normal and immunodeficient mice after transduction of the whole muscle by a Dux4 coding AAV vector. The Dux4 gene has been cloned under the control of a tetracycline dependent promoter (collaboration with Alexandra Belayew). This system allows us to control the expression level of Dux4 mRNA and to stop the Dux4 over-expression at any time. Our preliminary results shows that after a massive over-expression of Dux4, the majority of the muscle fibers have a centrally located nuclei, suggesting a toxicity of the transgene. We will now confirm this result on more mice and determine if this Dux4 over-expression induces a miss-regulation of other genes (FRG1, p21, Pitx1,

etc) or of miRNAs. We will also modulate the expression of Dux4 to a lower and less toxic level.

Using immortalized FSHD myoblasts we have observed that some miRNAs are miss-regulated in these FSHD compared to DMD or control clones. We would like to confirm these results using a unique and rare material: immortalized clones of myoblasts generated from mosaic human muscle biopsies which will allow us to eliminate the extremely high inter individual variations classically observed among FSHD patients. Many clones have been isolated and have been sent to our collaborators Sylvere Van der Maarel who provided the muscle biopsies and Stephen Tapscott. We will also receive from Nicolas Levy a skin biopsy from 2 highly interesting cases: identical twins of 32 years old, of whom one is totally asymptomatic whereas the other one is in a wheelchair. Both of them carry the same deletion: a southern blot of the peripheral lymphocytes has revealed that they both carry 2 D4Z4 units. We would like to understand how 2 identical twins have a totally different phenotype. We will immortalized the skin fibroblasts, transduced them using a lentivirus encoding MyoD under the control of an inducible promoter and analyse the expression of Dux4, FRG1, p21 etc in these cells. Moreover, all the immortalized clones we will generate (from the mosaic patients, as well as from the twins) will be injected into regenerating tibialis anterior muscles of immunodeficient mice to analyse their fusion potential and the mRNA and miRNA mis-regulation in an in vivo context.

This work will contribute to a better understanding of the molecular mechanisms leading to FSHD. Some therapeutical targets as well as some bio-markers of the disease may be identified which would be essential for a cure.

► **Scott Q. Harper, Ph.D.**

Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital

The Ohio State University, Columbus, Ohio, USA

FSH Society Connors and Jacobs Families and Friends Research Fellowship & 2009 Cape Cod: Walk 'n' Roll for FSHD Research Fellowship grants for

“Investigating DUX4 structure, function, and expression using rational mutagenesis and the human DUX4 promoter”

Total project \$40,000

**Goal: (Provided by Applicant):** The pathogenic mechanisms underlying facioscapulohumeral muscular dystrophy (FSHD) are unclear. We hypothesize that DUX4 over-expression in muscle contributes to FSHD development. In our pilot study which was partially funded by FSH Society in 2008 (FSHD-LCT-002; \$10,000), we showed the first in vivo evidence that DUX4 caused apoptosis and phenotypes associated with muscular dystrophy in zebrafish and mice. In a follow-up study, also funded by the FSH Society in 2009 (FSHS-JJFR-001; \$40,000), we began to define the biochemical function of the DUX4 protein as it related to apoptosis and muscle toxicity, using rational mutagenesis. We have so far demonstrated that DUX4-mediated cell death and dystrophy were dependent upon its ability to bind DNA, and presumably transactivate downstream pro-apoptotic cascades. These results suggested that DUX4 toxicity is

... continued on page 22

## FSH SOCIETY GRANTS RECENTLY AWARDED

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related to increased activity of natural DUX4 function and not simply to non-specific effects caused by over-loading cells with excessive protein. Despite these promising results, we will not complete all the goals of this proposal in the 1-year time frame supported by our current FSH Society fellowship. We are therefore submitting this application to renew funding of the original Specific Aim funded in our 2009 fellowship, as well as to support a second Specific Aim that represents a modification of the *in vivo* expression strategy in our previous fellowship.

**Specific Aim 1:** To define DUX4 domains necessary for stimulating apoptosis and muscle toxicity *in vitro*. In our preliminary work, we over-expressed DUX4 in mouse muscle using adeno-associated viral vectors (AAV). In parallel experiments, we also generated DUX4-expressing zebrafish embryos. Our data support that DUX4 induced apoptosis *in vitro* and caused dystrophic phenotypes in two different animal models (mice and zebrafish) *in vivo*. To gain a better understanding of DUX4 structure and function, we rationally mutagenized 8 predicted DUX4 functional domains or residues to investigate the functional effects of these changes on DUX4-induced apoptosis. In our most definitive data to date, we showed that DNA binding by the DUX4 homeodomain 1 (HOX1) is required to induce cell death *in vitro* and dystrophy-associated phenotypes *in vivo*. In this Aim, we will continue to investigate DUX4 structure-function relationships pertaining to DUX4 pro-apoptotic activity *in vitro* using DUX4 mutants we have already generated or new constructs that we will generate. These studies will be an important step toward understanding DUX4 structure and function relationships as they pertain to stimulation of apoptosis and muscle toxicity.

**Specific Aim 2:** To define DUX4 domains necessary for stimulating apoptosis and muscle toxicity *in vivo* using the human DUX4 promoter. DUX4 has been detected in human FSHD patient muscle biopsies, but its normal expression pattern in humans is unknown. Our preliminary data support that DUX4 induces apoptosis and phenotypes associated with muscular dystrophy in two different animal models, suggesting it contributes to FSHD development. For our preliminary studies, we expressed DUX4 using vectors containing the ubiquitously active CMV promoter or the engineered, muscle-specific MHCK7 promoter. Both of these promoters have well-characterized expression patterns, but it is unknown whether their cell-type and developmental specificity overlaps with that of natural DUX4. In our previous fellowship, we proposed to express DUX4 or mutant DUX4 constructs in zebrafish using the engineered muscle-specific MHCK7 promoter. Here, we have modified this expression strategy to more faithfully model DUX4 expression. We hypothesize that *in vivo* expression of DUX4 from its own promoter will produce phenotypes associated with muscular dystrophy *in vivo*. In this Aim, we will first investigate the developmental and cell-type expression patterns of the human DUX4 promoter by generating eGFPreporter zebrafish. We will then investigate DUX4 structure-function relationships pertaining to DUX4-induced muscular dystrophy *in vivo* by expressing wild type and mutant human DUX4 constructs from the DUX4 promoter in zebrafish. This work will help us understand temporal and cell-type specificity of DUX4 expression, and ultimately better define a potential role for DUX4 in FSHD pathogenesis.

► **Michael Kyba, Ph.D.**

**Minnesota Medical Foundation, University of Minnesota, Minneapolis, Minnesota, USA**

**FSH Society Grant New York Festival of Music and Song Fellowship for "FSHD iPS cells: bioinformatics support"**

**Total project \$45,000**

**Goal: (Provided by Applicant):** Facioscapulohumeral muscular dystrophy (FSHO) is a genetically dominant progressive myopathy affecting approximately 25,000 individuals in the United States. It is the third most common muscular dystrophy by incidence with a prevalence near or surpassing Duchenne's. The DNA lesion associated with this disease is a contraction within a series of 3.3 kb repeats - (D4Z4 repeats) near the telomere of 4q. It is not understood how this contraction results in disease, however it appears to modify the chromatin configuration of 4q35E2 and this has been proposed to lead to derepression of nearby genes. There is currently no animal model bearing the actual FSHD mutation (D4Z4 contraction), and the lack of a suitable model system to study the effects of this mutation has severely hampered progress in understanding FSHD. In an effort to shed light on the disease mechanism and to speed a potential cell therapy, we have recently derived iPS cells from myoblast cultures taken from FSHD patients and controls. The overall goal of this research program is to take advantage of the unique tool represented by pluripotent FSHD-affected cells to accelerate our path towards a molecular understanding of this disease. To address this goal, we will combine *in vitro* differentiation of iPS cells with assays for chromatin status and gene expression at 4q35.2. We will use a combination of high throughput, in some cases whole genome assays, which will generate a large quantity of bioinformatics data. Funding is requested to support a bioinformatics specialist to generate chromatin maps based on this data, which will allow us to pinpoint where in the genome, and at what stage in development, and in what developmental lineages, chromatin changes take place in FSHD. This data will provide a critical and currently missing link between the genetic damage which ultimately causes FSHD (the D4Z4 repeat array contraction) and the eventual myopathic phenotype.

► **Weihua Zeng, Ph.D. / Kyoko Yokomori, Ph.D.**

**University of California, Irvine, California, USA**

**FSH Society Helen Younger and David Younger Fellowship Research Grant for "Epigenetic abnormality in FSHD"**

**Total project \$35,500**

**Goal: (Provided by Applicant):** Based on our new data and our promising ongoing analyses, specific aims for the next grant period are: 1) to continue the analysis of the genome-wide epigenetic changes linked to FSHD pathogenesis, 2) to characterize the genome-wide long-distance chromatin interaction changes in FSHD, and 3) to characterize the regulation and function of the candidate genes identified in Aims 1 and 2. I believe that my research is making steady progress towards deciphering the epigenetic abnormality mechanism in FSHD, which should provide novel insight into the disease mechanism and thus potentially present new therapeutic strategies. Aim 1. Analysis of the genome-wide epigenetic changes linked to FSHD pathogenesis. 1) ChIP-sequencing In order to get a genome-wide pattern for these epi-

genetic markers, I am currently analyzing cohesin, HP1 $\gamma$ , H3K9me3 and H3K27me3 ChIP sequencing in both normal and FSHD myoblasts to identify genomic regions where these factors are specifically enriched. Some of the patient and control myoblasts spontaneously lost MyoD expression, which negatively impacted my assays. I set up a routine expression and differentiation screening to ensure the quality of myoblasts used for the experiments. Thus, myoblasts used in these studies have been tested. I have received the sequencing data and have begun to analyze them. With an extension of funding, I should be able to complete this analysis, including the manual confirmation.

**Aim 2:** Genome-wide characterization of the long-distance chromatin interaction changes in FSHD by Hi-C and ChIA-PET assays. This is a continuation of the previous Aim 3. Based on our epigenetic studies and preliminary 3C data by us and others, alteration of higher-order chromatin organization in FSHD is likely to be an important molecular change underlying FSHD pathogenesis. Chromatin interactions have typically been examined by cytological and biochemical methods: colocalization in the nucleus by 3D-fluorescent in situ hybridization (3D-FISH), and chromatin conformation capture (3C), respectively. 3C entails chemical crosslinking of interacting chromatin domains followed by restriction enzyme digestion and intramolecular ligation of the crosslinked DNA fragments. Crosslinking is then reversed and the ligated chromatin domains are analyzed by PCR using specific DNA primers. The frequency of interaction can be measured by the amount of PCR product. 3C can be done in combination with ChIP, which allows the enrichment of chromatin interactions that involve a particular protein (ChIP-loop). Variations of 3C that allow screening of unknown interactions were developed (e.g., 4C and 5C). However, these techniques did not have the capability to analyze interactions genome-wide. With the recent availability of deep sequencing technologies, it is now possible to directly sequence the 3C products in an unbiased manner. Two recent papers describe the techniques termed “Hi-C” and “Chromatin Interaction Analysis by Paired-End Tag sequencing (ChIA-PET)” that offer new approaches to study 3D chromatin organization in the nucleus. Hi-C (without immuno-enrichment for interactions involving a specific factor) requires enormous amounts of sequencing in order to analyze the data at a resolution higher than 1Mb (27), which is too costly and impractical. I will take two strategies: 1) Use a 4C method to PCR amplify those interactions that involve D4Z4 using primers designed based on my sequence analysis of D4Z4 homologs to distinguish 4q and 10q D4Z4 as opposed to D4Z4 homologs, and 2) perform a ChIA-PET-based high-throughput sequencing protocol to analyze genome-wide chromatin interactions involving HP1 $\gamma$ , cohesin, or condensin II. The data from FSHD myoblasts will be compared to control myoblasts, which may link epigenetic changes at D4Z4 and gene expression alterations critical for FSHD pathogenesis.

**Aim 3.** Characterization of the regulation and function of the candidate genes identified in Aims 1 and 3. This is the next step that I plan to take once a candidate gene(s) is identified in Aims 1 and 2. The functional significance of the identified candidate gene(s) in Aims 1 and 2 whose chromatin, expression, and chromatin interaction are specifically altered in FSHD will be analyzed further. I will prioritize and focus initially on factors that may likely be upstream of the dystro-

phic pathways, such as transcription factors, or some structural genes whose critical function in skeletal muscle function may be obvious. I will simulate the expression change seen in FSHD cells in normal human myoblasts (by over-expression with expression plasmid transfection or depletion by small interfering RNA (siRNA) transfection). The effect on the expression of potential downstream genes and cell viability, proliferation, and differentiation will be examined and compared to the phenotypes of FSHD myoblasts to determine whether the candidate gene contributes to the FSHD cellular phenotype. A similar analysis was recently done with Dux4 overexpression in mouse C2C12 cells. If promising, our future plan (beyond the scope of the current project) will be to use a transgenic mouse strategy to recapitulate the expression change of the mouse homolog of the candidate gene in skeletal muscles to test whether it may lead to muscular dystrophy. If the dystrophic phenotype can be recreated in these mice, they can serve as a powerful and versatile disease model with which treatment strategies can be screened more freely than in patients. However, it will be important to analyze human myoblasts in parallel since signaling pathways are sometimes different between mice and humans. [FSH Watch](#)

## FSHD International Research Consortium Meeting is scheduled

*October 21 & 22, 2010, Watertown, Massachusetts, USA*

The FSH Society FSHD International Research Consortium workshop will be held on Thursday & Friday, October 21-22, 2010, at the Boston Biomedical Research Institute (BBRI), in Watertown, Massachusetts. Thursday will be a full day workshop and Friday will be a half day of research planning. The Society organizes, sponsors and funds this meeting annually and there are no registration fees to attend the meeting. Other 2010 co-sponsors to date are NIH NICHD BBRI Sen. Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD, FSHD Global Research Foundation, and Fields Center for FSHD.

These are exciting times in FSHD research! The recent breakthrough by Lemmers et al., “A Unifying Genetic Model for Facioscapulohumeral Muscular Dystrophy,” in *Science* (published online August 19, 2010) gives rise to the potential to ameliorate the onset of FSHD. This will be an important time for the research and clinical community to convene to discuss these findings and to consider how to move forward.

Silvère van der Maarel, Ph.D. (Leiden University Medical Center and Fields Center for FSHD, Leiden, the Netherlands) and Rabi Tawil, M.D. (The University of Rochester and Fields Center for FSHD, Rochester, New York) will be the scientific and clinical co-chairs, respectively, and Daniel Perez (President & CEO of the FSH Society) will serve as scientific/organizational chair. Dr. Charles Emerson, Jr., Director of BBRI and co-Director of the NIH NICHD BBRI Sen. Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD, has graciously offered to host the meeting at BBRI in Watertown, MA.

We expect around 90 to 100 FSHD researchers and clinicians. This year's meeting is sure to be one of the best ever!



## 11th biennial International Patient and Researcher Network Meeting a success!


The 2010 FSH Society International Patient Researcher Network meeting held on July 30 to August 1, 2010, in Las Vegas, Nevada was a resounding success! 200 individuals joined together to learn about advances in treatment and research, to renew old friendships and to make new friends. The general feedback is that attendees really enjoyed the meeting, both scientifically and personally. And that it was great to meet with fellow patients, families, researchers, and clinicians, and for FSHD patients and families to be able to express to the professionals that patients really value the work they are doing.

The meeting was extraordinarily successful in increasing awareness of clinical work and research advances for FSHD. We thank the academic faculty from research and clinical centers and from industry for their excellent presentations and for their generosity with their time. Talks were given on:

- *Developing Novel Protein Therapeutics for the Treatment of Muscle Wasting Diseases*, by H.Q. Han, M.D., Ph.D.
- *FSHD, Clinical Trials and Myostatin* by Kathryn Wagner, M.D., Ph.D.
- *Advances in FSHD Research and Genetic Testing*, by Rabi Tawil, M.D.
- *Breathing and Respiratory Health for People with FSHD*, by Joshua O. Benditt, M.D.
- *Scapular Fixation Surgery for People with FSHD*, by Leigh Ann Curl, M.D.
- *FSHD and Stem Cells: Approaching Understanding and Therapy through iPS Adult Stem Cells*, by Michael Kyba, Ph.D.
- *FSHD Research at the NIH NICHD BBRI Wellstone MD CRC: Systems Biology and Biomarker Development*, by Charles P. Emerson, Jr., Ph.D.
- *New Tools to Aid Molecular Diagnosis and New Approaches to Understanding Gene Malfunction in FSHD Muscle*, by Melanie Ehrlich, Ph.D.
- *FSHD Mechanistic Models Involving DUX4*, by Scott Q. Harper, Ph.D.
- *Developments in FSHD Transcriptional Research*, by Stephen J. Tapscott, M.D., Ph.D.
- *Physical Activity and Exercise: A Physician's and a Patient's Perspectives*, by Craig M. McDonald, M.D., Ph.D. and Nils Hakansson, Ph.D., patient

The nearly twenty topical breakout sessions and discussion groups were highly valued by the attendees. The session "Successful Fundraising Initiatives for FSHD Research: You Can Do It, Too!", a series of presentations by volunteers successful in their communities and among family and friends, was especially informative and engaging. Special thanks go to Rich Holmes, Chris and Ellen Stenmon, Judy Seslowe, Beth Johnston and Rod Fulmer.

We thank the members of the Society, FSHD patients, their families, the educational faculty and the following sponsors for their help and support to make this conference and educational programming possible: Acceleron Pharma, Association Française Contre les Myopathies (AFM), Athena Diagnostics, Dorr Foundation, FSHD Global Foundation, Genzyme, NIH NICHD Boston Biomedical Research Institute Sen. Paul D. Wellstone MD Cooperative Research Center for FSHD Research, Permobil, Inc., Respironics and RideAway.

For more information and final program please visit our website at [www.fshsociety.org](http://www.fshsociety.org). We look forward to the 12th biennial International Patient and Researcher Network in 2012! 



**H.Q. Han, M.D., Ph.D., Executive Scientific Director, Metabolic Disorders, Amgen, Thousand Oaks, California delivers keynote address at 2010 Patient Researcher Network meeting**



**Presentations given by volunteers successful in their communities and among family and friends. Left to right: Rich Holmes, Chris and Ellen Stenmon, Beth Johnston, Judy Seslowe, and Rod Fulmer.**

### DO YOU FOLLOW THE SOCIETY'S FACEBOOK AND YAHOO! GROUPS PAGES?



Since its launch in early 2008, the Society's Facebook page has attracted hundreds of viewers and fans. Go to [www.facebook.com](http://www.facebook.com) and sign up. It is free and easy. Search on FSH Society and join the discussion. Bookmark it and come back often. You can also access the Facebook page and FSH Society Yahoo! Groups forum by going to [www.fshsociety.org](http://www.fshsociety.org) clicking on the "Community & Reference" menu tab at top of page and then selecting "Online Community" in the left hand vertical navigation menu.

### HAVE YOU MADE A GIFT TO THE SOCIETY IN 2010?

The FSH Society is a world leader in combating muscular dystrophy. It has provided over \$2.4 million in seed grants for pioneering research worldwide and has developed an international collaborative network of patients and researchers. If you are not already a member, won't you join in this effort? Please return your membership gift, or another gift, in the enclosed envelope. Or contribute online at [www.fshsociety.org](http://www.fshsociety.org). Go to Contribute, and select the gift category you wish to make. Thank you.

### VOLUNTEERS ARE STILL NEEDED FOR MUSCLE BIOPSY STUDY

Go to [www.fshsociety.org](http://www.fshsociety.org) for more information, or contact Raphaela Silverio at the FSH Society, 617- 658-7877 or [raphaela.silverio@fshsociety.org](mailto:raphaela.silverio@fshsociety.org) or Genila Bibat, M.D., at Johns Hopkins, 443-923-2778.

### DOES THE SOCIETY HAVE YOUR CURRENT E-MAIL ADDRESS?

If we do not have your current e-mail address and if you want to be sure of receiving up-to-the-minute information from the Society as news breaks, please send your e-mail address to us at [info@fshsociety.org](mailto:info@fshsociety.org). Thank you.