

FSH Watch

RESEARCH
ISSUE
2013



CONNECTING THE COMMUNITY OF PATIENTS, FAMILIES, CLINICIANS AND INVESTIGATORS

NEWS AND EVENTS

Michelle and David Mackay Host FSH Society Fundraiser

FSHing expedition reels in \$180,000

by JUNE KINOSHITA
FSH Society, Lexington, Massachusetts

Michelle Mackay and her husband, retired Kellogg's CEO David Mackay, have always been active members of their community in Battle Creek, Michigan, but over the past several years they have taken up a new cause—raising awareness and funding for FSHD research.

The Mackays, both Australian natives, have worked with the FSH Society for quite personal reasons: Mrs. Mackay
... continued on page 5



FSH Society Board member Michelle Mackay, and David Mackay

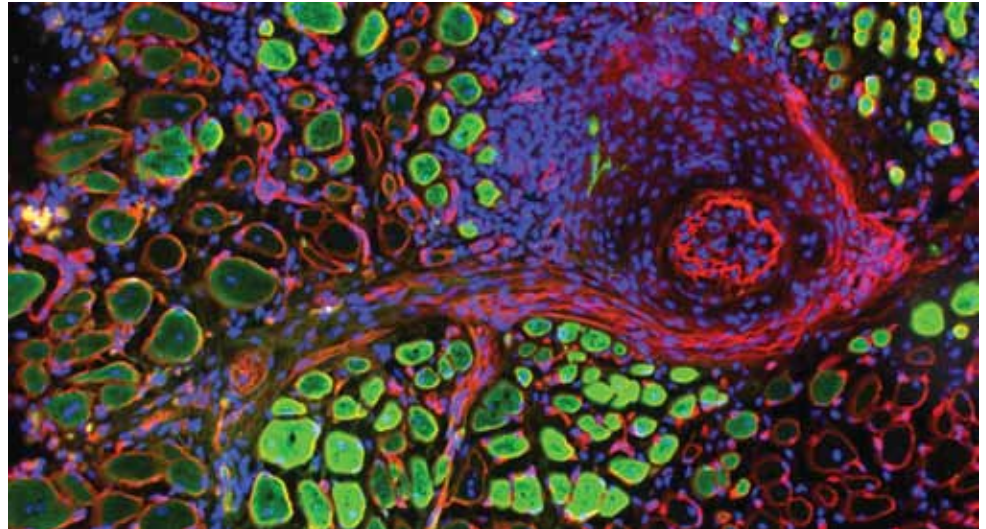


Image of a muscle stem cell transplantation assay. The picture shows repair of a damaged muscle (after injection of a toxin that destroys the tissue) in a mouse by muscle stem cell transplantation. Muscle stem cells are stained in green and muscle fibers are stained in red.

RESEARCH

FSH Society Annual Research Report

Staying nimble and strategic in a challenging landscape

by JUNE KINOSHITA and DANIEL PAUL PEREZ
FSH Society, Lexington, Massachusetts

This issue of FSH Watch contains our Annual Research Report, with updates and perspectives from recent grant award recipients. This is work that your gifts to the FSH Society have made possible.

Your support has fueled many significant advances. These range from fundamental investigations into the genetic regulation of *DUX4* and the cellular mechanisms of muscle cell death in FSHD to the development of mice, fish, and cells that model the disease more accurately, and better tools to

diagnose FSHD and measure the progression of symptoms—critically important for clinical trials.

We live in exciting times for FSHD research, but serious challenges loom ahead. Developing new drugs carries a hefty price tag. Getting the FSHD field ready for clinical trials will require unprecedented cooperation and collaboration among researchers—and with patients.

Yet, just when FSHD research requires greater investment, critically needed funding from the National Institutes of Health is drying up. Under sequestration, the NIH is being forced to slash its budget by \$1.7 billion in 2013 alone.

For a small field like FSHD research, the impact of such belt tightening is not

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Together we lead the way

Dear Friend,

Twenty-five years ago, when the late Steve Jacobsen and I first conceived the idea of the FSH Society, the worldwide constellation of patients, researchers, and clinicians working on FSHD could be counted on two hands. Today, the universe of researchers, clinicians, and companies actively engaged in FSHD reaches into the hundreds, and we now have access to nearly 10,000 patients and families affected by FSHD. Amazing!

Along with this incredible growth, we've transformed FSHD from a disease that received little support and hardly any interest to one of the most fascinating and hot areas of science and therapeutics. FSH Society members, families, and friends, members of the Board of Directors and the Scientific Advisory Board, Society staff, researchers and clinicians funded by the Society, and those involved with Society programs have contributed talent, energy, and funds to bring about remarkable advances in every aspect of FSHD.

We continue to work together for treatments and a cure. As a Society, we empower people by mobilizing patients and communities to take action. As a community, we improve on our knowledge about FSHD—together, we act as a driving force in the development of research directed toward treatments and a cure for FSHD. With improved knowledge, we increase the ability to connect and communicate, and to be the leading source of information and support for all FSHD patients and their families.

Many of you have contributed to the Society and



Daniel Paul Perez
 President & CEO

its events in the first half of 2013. Some of these events are reported within—they have themes of music and dancing, walking and rolling, as you will see or perhaps have experienced by attending one of these benefits. Other events are planned for later in the year. Please consider joining us!

When you make gifts to the FSH Society, 87 cents of every dollar go to FSHD research and education—funds that lead us closer to treatments and a cure. Funds that allow us to invest in research, education, and patient support. And your financial gifts provide us with the operational capacity to engage governmental and private sector organizations in expanding their funding of additional research and patient services.

This issue of FSH Watch reports on research results that have been made possible through your generous philanthropy. The investigators who were asked to write for this issue cite their appreciation for the resources you help provide. With your financial support, the Society convenes the largest yearly scientific conference focused solely on FSHD to speed up the exchange of knowledge and encourage collaboration. We ask scientists to engage in dialogue with patient advocates to determine the highest priorities for research.

The FSH Society is covering a wide range of research rather than focusing on just one gene, transcript, or protein. As you read this issue, you will see there are many compelling approaches and projects converging on the genetic mechanisms behind FSHD and how these develop into disease. Every project we fund is with an eye toward treatments and a cure.

The Society has taken a leadership role in FSHD Champions, a network formed in 2012 of all agencies worldwide that are funding research efforts in FSHD. The scale of translating discoveries in the laboratory to treatments for the disease dictates that we all work with

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FSH Watch

SUMMER/FALL 2013

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It is our editorial policy to report on developments regarding FSHD, but we do not endorse any of the drugs, procedures, treatments, therapies, or products discussed. We urge you to consult with your own physician about any medical interventions.

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one another. Together, we'll be able to treat the disease.

Speaking of which, volunteers are the most important part of the research enterprise. FSHD is a uniquely human disease, and we cannot make progress without patients and their unaffected family members who volunteer to be studied for research. Please do reach out to investigators seeking research subjects (see pages 4 and 9).

But while we wait for breakthroughs and effective therapies, life goes on, and many of us are busy living with FSHD. We realize that the time it takes to gain clarity on one of the most complex genetic human diseases is frustrating to many. We are funding "cure-oriented" research all the way up and down the line. It takes rigor in experimental design, proper biomaterials, access to patient sample collections, and adequately powered research. And we are getting there.

We're very appreciative of all of you—patients, friends, and families working together to support research and other work of the Society. Please help us by giving generously—of your volunteer time, energy, ideas, and funds—and helping to continue to make progress. All of us working on FSHD are giving our utmost for all of you. Please spread the word about our efforts.

With good wishes.



—Daniel Paul Perez
President & CEO



NEWS AND EVENTS

GET SOCIAL!

Join our online communities to get news, ask questions, and seek advice and support from fellow FSHD patients and family members. The FSH Society Yahoo! Groups forum has tens of thousands of searchable posts. Bookmark these pages and come back often. To find the FSH Society Facebook page and Yahoo! Groups, go to our homepage at www.fshsociety.org, click on the "Community & Reference" menu tab at the top of the page, and select "Online Community" in the left-side vertical navigation menu. You'll see links to take you directly to our Facebook page and Yahoo! Group. If privacy is a concern, you can use your account privacy settings to limit who can see your posts. You can also follow us on [Twitter @FSHSociety](https://twitter.com/FSHSociety).

RAZOO ONLINE FUNDRAISING

Razoo provides an easy way for you to create an online campaign. Your donors will enjoy the convenience of giving online and knowing that their gifts will go directly to the FSH Society. Razoo has built-in social media sharing, so you and your friends can help spread the word over Facebook, Twitter, and other social media. <http://www.razoo.com/story/Facioscapulohumeral-Society>

HAVE YOU MADE A GIFT TO THE SOCIETY IN 2013?

Thanks to the support from members like you, the FSH Society is a world leader in combating muscular dystrophy. It has provided nearly \$4.4 million in seed grants for pioneering research worldwide and developed an

international collaborative network of patients and researchers. **Your generous support is making a real difference!** Please return your gift in the enclosed envelope. Or contribute online at www.fshsociety.org. Thank you!

MATCHING GIFTS AND OTHER WORKPLACE GIVING

Many employers offer workers options for directing the company's funds to a charitable organization of their choice. When this opportunity is available to you, please consider how your workplace might make a gift to the FSH Society. This is a great way to double, triple, or even quadruple your gift!

UNITED WAY COMMUNITY CAMPAIGNS, FALL 2013

You may have an opportunity to support the FSH Society this fall when you make a United Way pledge for 2013. Check with your human resources department for more information.

CHARITY NAVIGATOR TOP PERFORMER

The FSH Society has been awarded its fifth consecutive Four-Star Award by one of the nation's leading charity watchdog organizations, Charity Navigator, and was named one of America's "Ten Charities Worth Watching."

The FSH Society's inclusion in this highly prestigious list was announced by Charity Navigator on its website. "These 10 charities all operate on less than \$2 million a year, but they all earn a four-star rating.... We encourage you to learn more about them."

Charity Navigator's Four-Star Award indicates that the FSH Society consistently executes its mission in a fiscally responsible way and outperforms most other charities in the United States. This "exceptional" designation from Charity Navigator demonstrates to the public that the Society is worthy of its trust. www.charitynavigator.org.



COMBINED FEDERAL CAMPAIGN (CFC), 2013 CAMPAIGN

The FSH Society has been approved by the Office of Personnel Management for the 2013 campaign. The CFC is the world's largest and most successful annual workplace charity campaign, with more than 300 CFC campaigns throughout the country and internationally to help raise millions of dollars each year. Pledges made by federal, civilian, postal, and military donors during the campaign season (September 1 to December 15) support eligible nonprofit organizations that provide health and human service benefits throughout the world. The FSH Society's identification number is 10239.

DOES THE SOCIETY HAVE YOUR CURRENT EMAIL ADDRESS?

If you want to be sure to receive breaking news and other up-to-the-minute information from the Society, please send us your email address at info@fshsociety.org.

Wellstone Muscular Dystrophy Cooperative Research Center for FSHD is recruiting families to discover factors that impact disease severity

DUX4 expression is necessary but not sufficient by itself for FSHD

by **DORIS G. LEUNG, MD, and KATHRYN WAGNER, MD, PhD**
Center for Genetic Muscle Disorders, Kennedy Krieger Institute, Baltimore, Maryland

The Center for Genetic Muscle Disorders at the Kennedy Krieger Institute is a member of the U.S. National Institutes of Health (NIH) Wellstone Muscular Dystrophy Cooperative Research Center, “Biomarkers for Therapy of FSHD.” This group includes basic science, clinical, and translational researchers at multiple academic institutions coordinated through the University of Massachusetts Medical School.

The Center is actively involved in several research studies focused on discovering the mechanisms of disease and developing improved methods of studying muscle in FSHD. One of their most successful efforts has been the muscle biopsy program, which was designed to systematically collect and characterize muscle tissue samples that could be shared among multiple FSHD researchers.

So far, the muscle biopsy program has succeeded in collecting muscle tissue from more than 80 individuals. These muscle samples are not only being used by researchers in the Wellstone, but they are also being transformed into cell lines that can be stored and shared with outside research groups and used in future studies of FSHD.

The Wellstone muscle biopsy program is now in the process of expanding to include a second site, the University of Massachusetts Medical School. The muscle biopsy program has also expanded within the Kennedy Krieger Institute to include a non-invasive imaging biomarker project (described in this issue of FSH Watch on page 25).

One of the unique features of the Wellstone study is the co-enrollment of unaffected family members along with individuals who have FSHD. This has proven to be a valuable asset to the Wellstone’s research efforts in that it allows researchers to more accurately judge whether genetic variations seen in a person’s

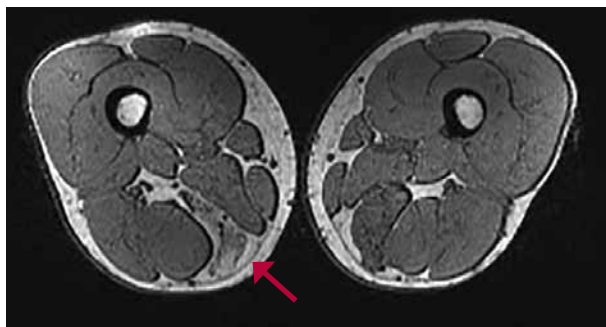


Figure: MRI of the thighs of a patient without any symptoms of FSHD and normal strength on muscle testing. The only abnormal muscle in this individual is the semi-membranosus (red arrow), which shows extensive atrophy and fat replacement, while all other muscles appear normal.

In order to identify and study these non-manifesting carriers of FSHD, researchers in the Wellstone are working on recruiting larger families of individuals with FSHD. They are particularly interested in recruiting individuals who have a first-degree relative (parent, child, or sibling) with FSHD but do not have any symptoms of muscle disease themselves

muscle tissue are related to FSHD or if they are normal variations that belong to that person’s family.

An unexpected outcome of the FSHD biopsy study was the discovery of several individuals who participated as the unaffected control family member but were found to have the gene mutation that causes FSHD. Many of these individuals, “non-manifesting carriers,” did not have weakness, although they did have the same mutation as their affected family members.


A new research project is now underway

to determine what factors cause some people with the mutation to have severe disease and others to have normal strength. It is possible that there are major genetic modifiers (including the SMCHD1 gene, which was recently found to be associated with FSHD2, also called FSHD1B, a less common type of FSHD) which do not cause disease by themselves but have a significant effect on the symptoms of the disease. These genetic modifiers not only would provide new information on the pathologic processes that govern

FSHD, but they could also provide new targets for therapies.

In order to identify and study these non-manifesting carriers of FSHD, researchers in the Wellstone are working on recruiting larger families of individuals with FSHD. They are particularly interested in recruiting individuals who have a first-degree relative (parent, child, or sibling) with FSHD but do not have any symptoms of muscle disease themselves. These individuals will be asked to undergo genetic testing for the mutation that causes FSHD in addition to neurological evaluation to confirm the absence of muscle weakness or other signs of FSHD. Those who are found to be non-manifesting carriers of FSHD may be asked to undergo further testing, such as muscle MRI, to assess for subtle signs of disease (see Figure).

This exploration of the milder end of the FSHD spectrum represents a new avenue of research in FSHD, and the investigators of the Wellstone are very hopeful that it will forge new collaborations within the FSHD research community and build on the tremendous progress that has been made in the field of FSHD research in recent years.

Individuals or families who would like to receive more information about this study should contact Genila Bibat, the study coordinator for the Center for Genetic Muscle Disorders, at 443-923-2697 or bibat@kennedykrieger.org. 

FSH SOCIETY ANNUAL RESEARCH REPORT


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just painful; it can be catastrophic. The loss of a single grant may force a lab to abandon the field—or close its doors. The arid funding landscape discourages young scientists and doctors from pursuing a career in research. Scientific careers cannot be turned on and off as quickly as a budgetary spigot. A few years of poor funding can thin the ranks of an entire generation of researchers.

At the FSH Society, we are committed to nurturing the most promising young scientists through our graduate and postdoctoral research fellowships. At the same time, we fund established researchers for their most innovative ideas, which face ever taller hurdles in receiving NIH support.

We also invest in critically important resources, for example, by covering more than \$55,000 in travel costs for volunteers who donated DNA and muscle biopsies for the NIH Wellstone Muscular Dystrophy Cooperative Research Center. We cannot stress enough the importance of this muscle tissue collection, comprising samples from FSHD patients and their unaffected family members. Several pivotal papers have already resulted from the analysis of these samples, and we expect more key studies in the future.

When you entrust your funds and time to the Society, we feel a great responsibility to invest your contributions wisely. We must react quickly to near-term challenges and opportunities. However, we must be sure also to invest in longer-term strategic resources and tools to help the entire field advance more quickly and effectively.

We hope this Annual Research Report fills you with appreciation and excitement for the impact we are having—together. 

MICHELLE AND DAVID MACKAY HOST FSH SOCIETY FUNDRAISER

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was diagnosed with FSHD in her twenties. For years, she was very secretive about her condition and did everything in her power to hide her symptoms, but her perspective shifted several years ago after she took a major fall that dislocated her hip.

“Something changed for me that day. I realized that hiding the disease was more painful than sharing my story,” said Michelle Mackay. “Once I began talking about life with FSHD, I found that people really wanted to listen—and more importantly, they wanted to help.” She has since become a vocal and active advocate in battling the disease and now serves on the Board of Directors of the FSH Society.

The Mackays hosted a festive event to raise awareness and funds for the FSH Society on Friday, July 5, at their lakeside home in Gull Lake, Michigan. The community was invited to attend the fundraiser, and 270 people showed up to enjoy a spit roast, dancing, an auction, and live music by the bluegrass band Small Town Son. Attendees paid \$75 per ticket and contributed additional donations and auction purchases, reaching over \$90,000, which the Mackays matched, for a grand total of more than \$180,000.

Michelle Mackay’s mother, a talented artist, created eye-catching fish-themed table decorations, which Michelle explained were a memory aid to help people remember the name of the disease: F-S-H. The invitation specified “FSH-ing attire,” which prompted friends to sport creative jewelry made from fishing lures and hats festooned with “facts about muscular dystrophy.”

Corporate sponsors who lined up to support the Mackay event included the Battle Creek Community Foundation; Beam, Inc.; Courtesy Limousines; EPI Printers; Gilmore Keyboard Festival; Grand Rapids Art Museum; Heather Robilliard Ski Adventures and Silver Star Mountain Resort; Kalamazoo Institute of Arts; and Peruvian Connection.

“Hundreds of thousands of people around the world suffer from FSHD, but many—even those in the healthcare field—have never heard of FSHD,” said David Mackay. “We hope that our event, and many like it across the country, will help raise awareness about this disease. We are fierce in our resolve and will not stop until there is a cure.” 

Mark your calendar!

FSHD International Research Consortium meeting this October

Register now!

The 2013 FSH Society FSHD International Research Consortium workshop for research and clinical professionals will be held on Monday and Tuesday, October 21-22, 2013, at the Massachusetts Institute of Technology (MIT) in Cambridge. Tentatively, Monday will be a full-day workshop, and Tuesday will be a two-thirds day of research planning. The meetings will be held prior to the opening of the American Society of Human Genetics (ASHG) conference in Boston, Massachusetts.

David E. Housman, Chair of the FSH Society Scientific Advisory Board, has graciously offered to host the meeting at the David H. Koch Institute for Integrative Cancer Research at MIT. Joining Professor Housman in co-chairing the meeting are Stephen Tapscott, MD, PhD (Fred Hutchinson Cancer Research Center, Seattle, Washington) and Silvere van der Maarel, PhD (Leiden University Medical Center and Fields Center for FSHD, the Netherlands). Daniel Perez, President & CEO of the FSH Society, will serve as organizationa Chair.

Early registration deadline:

Friday, October 11, 2013

Late registration deadline:

Wednesday, October 16, 2013

Abstracts due:

Friday, September 20, 2013

Assignments for Poster/
Presentation made:

Friday, September 27, 2013



The David H. Koch Institute for Integrative Cancer Research

Please contact Daniel Paul Perez for further information at 781-301-6650 or daniel.perez@fshsociety.org. We expect this year’s meeting will be one of the best yet! Please pass this invitation on to colleagues and students who may have an active interest in FSHD. Space is limited to 100, so please reserve early.

2012 Research Publication Highlights

Progress and puzzles abound

by **DOUGLAS CRAIG, PhD, and DANIEL PAUL PEREZ**

Jersey City, New Jersey, and the FSH Society, Lexington, Massachusetts

We have selected significant FSHD research papers published in 2012. The papers are listed chronologically. Among these papers, two main themes emerge: 1) the refinement of the DUX4/D4Z4 hypothesis, and 2) the introduction of tools with which to test the hypothesis, support development of therapeutics, and support clinical studies. The FSH Society is acknowledged for support in five of the eight articles. Current and past FSH Society-funded fellows are listed as authors on all of these papers. Additionally, all of the discoveries were presented for discussion at the FSH Society's annual FSHD International Research Consortium (IRC) meetings prior to or concurrent with publication.

- **Geng LN, Yao Z, Snider L, Fong AP, Cech JN, Young JM, van der Maarel SM, Ruzzo WL, Gentleman RC, Tawil R, Tapscott SJ.** "DUX4 ACTIVATES GERMLINE GENES, RETROELEMENTS, AND IMMUNE MEDIATORS: IMPLICATIONS FOR FACIOSCAPULOHUMERAL DYSTROPHY." *Developmental Cell*. 17 January 2012 (Vol. 22, Issue 1, pp. 38-51).

Abstract: Facioscapulohumeral dystrophy (FSHD) is one of the most common inherited muscular dystrophies. The causative gene remains controversial and the mechanism of pathophysiology unknown. Here we identify genes associated with germline and early stem cell development as targets of the DUX4 transcription factor, a leading candidate gene for FSHD. The genes regulated by DUX4 are reliably detected in FSHD muscle but not in controls, providing direct support for the model that misexpression of DUX4 is a causal factor for FSHD. Additionally, we show that DUX4 binds and activates LTR elements from a class of MaLR endogenous primate retrotransposons and suppresses the innate immune response to viral infection, at least in part through the activation of DEF13, a human defensin that can inhibit muscle differentiation. These findings suggest specific mechanisms of FSHD pathology and identify candidate biomarkers for disease diagnosis and progression.

Significance: This is an interesting paper that helps in hypothesizing FSHD pathology and the role of immunity and immune response in FSHD.

- **Scionti I, Greco F, Ricci G, Govi M, Arashiro P, Vercelli L, Berardinelli A, Angelini C, Antonini G, Cao M, Di Muzio A, Moggio M, Morandi L, Ricci E, Rodolico C, Ruggiero L, Santoro L, Siciliano G, Tomelleri G, Trevisan CP, Galluzzi G, Wright W, Zatz M, Tupler R.** "LARGE-SCALE POPULATION ANALYSIS CHALLENGES THE CURRENT CRITERIA FOR THE MOLECULAR DIAGNOSIS OF FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY." *American Journal of Human Genetics*. 6 April 2012. 90(4): 628-635.

Abstract: Facioscapulohumeral muscular dystrophy (FSHD) is a common hereditary myopathy causally linked to reduced numbers

(≤ 8) of 3.3 kilobase D4Z4 tandem repeats at 4q35. However, because individuals carrying D4Z4-reduced alleles and no FSHD and patients with FSHD and no short allele have been observed, additional markers have been proposed to support an FSHD molecular diagnosis. In particular a reduction in the number of D4Z4 elements combined with the 4A(159/161/168)PAS haplotype (which provides the possibility of expressing DUX4) is currently used as the genetic signature uniquely associated with FSHD. Here, we analyzed these DNA elements in more than 800 Italian and Brazilian samples of normal individuals unrelated to any FSHD patients. We find that 3% of healthy subjects carry alleles with a reduced number (4-8) of D4Z4 repeats on chromosome 4q and that one-third of these alleles, 1.3%, occur in combination with the 4A161PAS haplotype. We also systematically characterized the 4q35 haplotype in 253 unrelated FSHD patients. We find that only 127 of them (50.1%) carry alleles with 1-8 D4Z4 repeats associated with 4A161PAS, whereas the remaining FSHD probands carry different haplotypes or alleles with a greater number of D4Z4 repeats. The present study shows that the current genetic signature of FSHD is a common polymorphism and that only half of FSHD probands carry this molecular signature. Our results suggest that the genetic basis of FSHD, which is remarkably heterogeneous, should be revisited, because this has important implications for genetic counseling and prenatal diagnosis of at-risk families.

Significance: This raises an important scientific challenge to the prevailing theory for the genetic basis of FSHD, arguing that a significant portion of the population with a non-FSHD phenotype has a 4q35 allelic genotype that is diagnostic for FSHD, and that even with some variability in the age of onset and penetrance, the discrepancy is too large to incorporate into that theory. It also raises important issues regarding the interpretation of the diagnostic test and genetic counseling. The impact of this paper is limited as the gene for FSHD2 was not tested. Moreover the 1.5 percent short 4qA alleles in the general population has been reported before (Overveld et al., *Human Mol. Genetics* 2000 Nov 22;9(19):2879-84). It is good that this figure has now been confirmed. The interpretation is that more factors than just the short allele lead to the phenotype. The bias of the genetic research is that it starts with patients and families already showing the phenotype. The fact that only a few large families have been reported and that most families are small suggests that either the presumed "other factors" make the disease disappear, or that "fitness" is very low. But even the lowest fitness estimate of 0.70 probably does not explain the number of small families. Finally, we do not counsel a genotype but a person in the context of a family with FSHD. We know that non-penetrance occurs in FSHD families and such cases should be counseled accordingly. However the percentage of non-penetrance is not precisely known and the 4A159/168PAS phenotypes need to be confirmed.

- **Cabianca DS, Casa V, Bodega B, Xynos A, Ginelli E, Tanaka Y, Gabellini D.**
“A LONG NCRNA LINKS COPY NUMBER VARIATION TO A POLYCOMB/TRITHORAX EPIGENETIC SWITCH IN FSHD MUSCULAR DYSTROPHY.”
Cell. 11 May 2012 (Vol. 149, Issue 4, pp. 819-831).

Abstract: Repetitive sequences account for more than 50% of the human genome. Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal-dominant disease associated with reduction in the copy number of the D4Z4 repeat mapping to 4q35. By an unknown mechanism, D4Z4 deletion causes an epigenetic switch leading to de-repression of 4q35 genes. Here we show that the Polycomb group of epigenetic repressors targets D4Z4 in healthy subjects and that D4Z4 deletion is associated with reduced Polycomb silencing in FSHD patients. We identify DBE-T, a chromatin-associated noncoding RNA produced selectively in FSHD patients that coordinates de-repression of 4q35 genes. DBE-T recruits the Trithorax group protein Ash1L to the FSHD locus, driving histone H3 lysine 36 dimethylation, chromatin remodeling, and 4q35 gene transcription. This study provides insights into the biological function of repetitive sequences in regulating gene expression and shows how mutations of such elements can influence the progression of a human genetic disease.

Significance: This paper goes quite a long way to re-invigorate the FRG1 mechanism for FSHD, and the researchers have data which helps to align this work with the DUX4 story. It looks all quite consistent now. This paper describes the first activatory lncRNA involved in a human genetic disease that in this case activates the epigenetic cascade that leads to gene de-repression at the chromosome 4q35 locus in FSHD.

- **Wallace LM, Liu J, Domire JS, Garwick-Coppens SE, Guckes SM, Mendell JR, Flanigan KM, Harper SQ.**
“RNA INTERFERENCE INHIBITS DUX4-INDUCED MUSCLE TOXICITY IN VIVO: IMPLICATIONS FOR A TARGETED FSHD THERAPY.”
Molecular Therapy. July 2012. 20(7): 1417-1423.

Abstract: No treatment exists for facioscapulohumeral muscular dystrophy (FSHD), one of the most common inherited muscle diseases. Although FSHD can be debilitating, little effort has been made to develop targeted therapies. This lack of focus on targeted FSHD therapy perpetuated because the genes and pathways involved in the disorder were not understood. Now, more than 2 decades after efforts to decipher the root cause of FSHD began, this barrier to translation is finally lowering. Specifically, several recent studies support an FSHD pathogenesis model involving overexpression of the myopathic DUX4 gene. DUX4 inhibition has therefore emerged as a promising therapeutic strategy for FSHD. In

this study, we tested a preclinical RNA interference (RNAi)-based DUX4 gene silencing approach as a prospective treatment for FSHD. We found that adeno-associated viral (AAV) vector-delivered therapeutic microRNAs corrected DUX4-associated myopathy in mouse muscle. These results provide proof-of-principle for RNAi therapy of FSHD through DUX4 inhibition.

Significance: This work is similar to the work done with overexpression of FRG1 in mice to produce a dystrophic phenotype, in which Drs. Wallace and Harper showed that concomitant expression of AAV-delivered siRNA to FRG1 would prevent development of the phenotype (“RNA interference improves myopathic phenotypes in mice over-expressing FSHD region gene 1 [FRG1].” *Mol Ther.* 2011...2048-54). They have now done the experiments with DUX4 and siRNA against DUX4. This paper represents a critical first examination of DUX4 and anti-DUX4 siRNA in a mouse model, and that is important.

- **Jones TI, Chen JC, Rahimov F, Homma S, Arashiro P, Beermann ML, King OD, Miller JB, Kunkel LM, Emerson CP Jr, Wagner KR, Jones PL.**
“FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY FAMILY STUDIES OF DUX4 EXPRESSION: EVIDENCE FOR DISEASE MODIFIERS AND A QUANTITATIVE MODEL OF PATHOGENESIS.”
Human Molecular Genetics. 15 October 2012. 21(20): 4419-4430.

Abstract: Facioscapulohumeral muscular dystrophy (FSHD), the most prevalent myopathy afflicting both children and adults, is predominantly associated with contractions in the 4q35-localized macrosatellite D4Z4 repeat array. Recent studies have proposed that FSHD pathology is caused by the misexpression of the DUX4 (double homeobox 4) gene resulting in production of a pathogenic protein, DUX4-FL, which has been detected in FSHD, but not in unaffected control myogenic cells and muscle tissue. Here, we report the analysis of DUX4 mRNA and protein expression in a much larger collection of myogenic cells and muscle biopsies derived from biceps and deltoid muscles of FSHD affected subjects and their unaffected first-degree relatives.

We confirmed that stable DUX4-fl mRNA and protein were expressed in myogenic cells and muscle tissues derived from FSHD affected subjects, including several genetically diagnosed adult FSHD subjects yet to show clinical manifestations of the disease in the assayed muscles. In addition, we report DUX4-fl mRNA and protein expression in muscle biopsies and myogenic cells from genetically unaffected relatives of the FSHD subjects, although at a significantly lower frequency. These results establish that DUX4-fl expression per se is not sufficient for FSHD muscle pathology and indicate that quantitative modifiers of DUX4-fl expression and/or function and family genetic background are determinants of FSHD muscle disease progression.

... continued on page 8

RESEARCH PUBLICATION HIGHLIGHTS

... from page 7

Significance: Potentially challenges the hypothesis that DUX4 alone is sufficient to cause FSHD, and suggests that there are additional critical molecular pathways (and potential therapeutic targets). See also our comments on the Scionti et al study about modifiers (see page 6).

- ▶ **Rahimov F, King OD, Leung DG, Bibat GM, Emerson CP Jr, Kunkel LM, Wagner KR.** “TRANSCRIPTIONAL PROFILING IN FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY TO IDENTIFY CANDIDATE BIOMARKERS.” *Proceedings of the National Academy of Sciences.* 2 October 2012. 109(40): 16234-16239.

Abstract: Facioscapulohumeral muscular dystrophy (FSHD) is a progressive neuromuscular disorder caused by contractions of repetitive elements within the macrosatellite D4Z4 on chromosome 4q35. The pathophysiology of FSHD is unknown and, as a result, there is currently no effective treatment available for this disease. To better understand the pathophysiology of FSHD and develop mRNA-based biomarkers of affected muscles, we compared global analysis of gene expression in two distinct muscles obtained from a large number of FSHD subjects and their unaffected first-degree relatives. Gene expression in two muscle types was analyzed using GeneChip Gene 1.0 ST arrays: biceps, which typically show an early and severe disease involvement; and deltoid, which is relatively uninvolved. For both muscle types, the expression differences were mild: using relaxed cutoffs for differential expression (fold change ≥ 1.2 ; nominal P value < 0.01), we identified 191 and 110 genes differentially expressed between affected and control samples of biceps and deltoid muscle tissues, respectively, with 29 genes in common. Controlling for a false-discovery rate of < 0.25 reduced the number of differentially expressed genes in biceps to 188 and in deltoid to 7. Expression levels of 15 genes altered in this study were used as a “molecular signature” in a validation study of an additional 26 subjects and predicted them as FSHD or control with 90% accuracy based on biceps and 80% accuracy based on deltoids.

Significance: As we understand it, if a potential treatment comes along for FSHD, it is likely that instead of reversing damage and restoring muscles, the treatment will slow down or halt the progression. Clinically, this raises the question of what to measure to evaluate efficacy. In the Duchenne Muscular Dystrophy study, investigators use the 6-minute walk test over several months, and are able to measure differences between controls and treated. However, with the variability and slow rate of progression in FSHD, such an approach is unlikely to produce a significant difference in a reasonable time frame. Biomarkers—particularly ones that can be measured non-invasively—will be required. This paper begins the process of using gene expression profiling in blood to identify potential clinical biomarkers to aid trials. This paper does not address the basic assumption that all members have the same genetic and metabolic profile and that the condition in the biceps and in the deltoid is the expression of the same dysregulation cascade. But if the genetic make-up of the

biceps and deltoid is different we might see DUX4 over-expression play out differently: i.e. DUX4 over-expression leads to the recognizable phenotype and the factor time reflects the resilience of the muscle to the DUX4 attack, possibly by different metabolic responses over time. If this is the case we should compare only the same muscle between studies.

- ▶ **Tassin A, Leroy B, Laoudj-Chenivesse D, Wauters A, Vanderplanck C, Le Bihan MC, Coppée F, Wattiez R, Belayew A.** “FSHD MYOTUBES WITH DIFFERENT PHENOTYPES EXHIBIT DISTINCT PROTEOMES.” *PLoS One.* ePub 18 December 2012. 7(12): e51865.

Abstract: Facioscapulohumeral muscular dystrophy (FSHD) is a progressive muscle disorder linked to a contraction of the D4Z4 repeat array in the 4q35 subtelomeric region. This deletion induces epigenetic modifications that affect the expression of several genes located in the vicinity. In each D4Z4 element, we identified the double homeobox 4 (DUX4) gene. DUX4 expresses a transcription factor that plays a major role in the development of FSHD through the initiation of a large gene dysregulation cascade that causes myogenic differentiation defects, atrophy and reduced response to oxidative stress. Because miRNAs variably affect mRNA expression, proteomic approaches are required to define the dysregulated pathways in FSHD. In this study, we optimized a differential isotope protein labeling (ICPL) method combined with shotgun proteomic analysis using a gel-free system (2DLC-MS/MS) to study FSHD myotubes. Primary CD56(+) FSHD myoblasts were found to fuse into myotubes presenting various proportions of an atrophic or a disorganized phenotype. To better understand the FSHD myogenic defect, our improved proteomic procedure was used to compare predominantly atrophic or disorganized myotubes to the same matching healthy control. FSHD atrophic myotubes presented decreased structural and contractile muscle components. This phenotype suggests the occurrence of atrophy-associated proteolysis that likely results from the DUX4-mediated gene dysregulation cascade. The skeletal muscle myosin isoforms were decreased while non-muscle myosin complexes were more abundant. In FSHD disorganized myotubes, myosin isoforms were not reduced, and increased proteins were mostly involved in microtubule network organization and myofibrillar remodeling. A common feature of both FSHD myotube phenotypes was the disturbance of several caveolar proteins, such as PTRF and MURC. Taken together, our data suggest changes in trafficking and in the membrane microdomains of FSHD myotubes. Finally, the adjustment of a nuclear fractionation compatible with mass spectrometry allowed us to highlight alterations of proteins involved in mRNA processing and stability.

Significance: This is an interesting paper and should be read in conjunction with Tassin et al: *J. Cell. Mol. Med* 17, 79-89, 2013, because in the latter the authors demonstrate that in FSHD-myotubes DUX4 is expressed in five times more myonuclei than in myoblasts (probably by a diffusion mechanism), and suggest that DUX4 expression pulses enforce a transcriptional amplification cascade. The former study demonstrates two forms

of FSHD myotubes: atrophic or disorganized, possibly depending on the level of DUX4 expression. Also DUX4 protein expression was demonstrated in FSHD muscles, particularly in non-affected muscles, suggesting that DUX4 expression is an early event in FSHD pathogenesis.

► **Lemmers RJ, Tawil R, Petek LM, Balog J, Block GJ, Santen GW, Amell AM, van der Vliet PJ, Almomani R, Straasheijm KR, Krom YD, Klooster R, Sun Y, den Dunnen JT, Helmer Q, Donlin-Smith CM, Padberg GW, van Engelen BG, de Greef JC, Aartsma-Rus AM, Frants RR, de Visser M, Desnuelle C, Sacconi S, Filippova GN, Bakker B, Bamshad MJ, Tapscott SJ, Miller DG, van der Maarel SM.**

“DIGENIC INHERITANCE OF AN SMCHD1 MUTATION AND AN FSHD-PERMISSIVE D4Z4 ALLELE CAUSES FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY TYPE 2.”

Nature Genetics. December 2012. 44(12): 1370-1374.

Abstract: Facioscapulohumeral dystrophy (FSHD) is characterized by chromatin relaxation of the D4Z4 macrosatellite array on chromosome 4 and expression of the D4Z4-encoded DUX4 gene in skeletal muscle. The more common form, autosomal dominant FSHD1, is caused by contraction of the D4Z4 array, whereas the genetic determinants and inheritance of D4Z4 array contraction-independent FSHD2 are unclear. Here, we show that mutations

in SMCHD1 (encoding structural maintenance of chromosomes flexible hinge domain containing 1) on chromosome 18 reduce SMCHD1 protein levels and segregate with genome-wide D4Z4 CpG hypomethylation in human kindreds. FSHD2 occurs in individuals who inherited both the SMCHD1 mutation and a normal-sized D4Z4 array on a chromosome 4 haplotype permissive for DUX4 expression. Reducing SMCHD1 levels in skeletal muscle results in D4Z4 contraction-independent DUX4 expression. Our study identifies SMCHD1 as an epigenetic modifier of the D4Z4 metastable epiallele and as a causal genetic determinant of FSHD2 and possibly other human diseases subject to epigenetic regulation.

Significance: Provides a solution to the puzzle of why around 5 percent of FSHD patients do not have a contraction (deletion) of D4Z4 on 4q35. This newly described genetic mechanism ties into the hypothesis that FSHD is linked to a change in chromatin structure allowing the expression of DUX4. The discovery of this genetic mutation provides a new diagnostic test and enables genetic counseling of FSHD2 patients. **FSH Watch**

Doug Craig, PhD, was Director of Mechanistic Studies at Lundbeck Research US and is currently a freelance medical writer and consultant to pharmaceutical and biotech companies.



CLINICAL RESEARCH

Cooperative International Neuromuscular Research Group recruiting patients with infantile-onset FSHD

Seeking patients of all ages who were diagnosed with FSHD in childhood

by **ZOË SUND**

Clinical Research Manager, Cooperative International Neuromuscular Research Group (CINRG), Children's National Medical Center, Washington, DC

The Cooperative International Neuromuscular Research Group (CINRG) site at the Children's National Medical Center in Washington, D.C., is recruiting patients with infantile-onset FSHD. To date, we have identified 30 eligible participants from participating CINRG sites globally; we look forward to further enrollment and data collection over the coming months. During a third year no-cost extension, Mah and Yi-Wen Chen are designing new research projects based on this growing cohort of individuals with a relatively rare but more severe form of FSHD and are moving forward with data analysis as well as collecting further samples for expression-profiling studies to be done at Dr. Chen's lab. *(For the grant information, see page 28.)*

Study name: A multicenter collaborative study of the clinical features, expression

profiling, and quality of life of infantile-onset FSHD.

Study overview: This study is being run by the CINRG. More information about CINRG and its research studies can be found at <http://www.cinrgresearch.org/>. This study is an observational study that aims to advance our knowledge of infantile-onset FSHD. The study will include 50 participants of all ages who have presented with symptoms of FSHD between birth and 10 years of age. Study participation will involve a single day of assessments at one of the participating CINRG centers (to include physical exam, cognitive testing, eye exam, hearing test, strength testing, and speech evaluation). The procedures may be split over additional days for scheduling purposes.

Inclusion criteria: Clinical diagnosis of FSHD including the presence of all of the following features based on review of medical records and/or direct examination: 1) onset of symptoms involving the facial or shoulder girdle muscles, and 2) genetic confirmation (according to the study-defined criteria for FSHD).

How to become involved: For more information about the study and a list of participating CINRG sites, please view *ClinicalTrials.gov* (<http://clinicaltrials.gov/ct2/show/NCT01437345?term=fshd&rank=3>) or the CINRG website.

You may also contact Zoë Sund, Project Manager, at 202-476-4110 or zsund@childrensnational.org to determine if you are eligible and to be referred to a participating CINRG site. **FSH Watch**

Priorities for FSHD Research: 2013 and Beyond

From the 2012 FSH Society FSHD International Research Consortium Workshop

by **DANIEL PAUL PEREZ**
 FSH Society, Lexington, Massachusetts

Approximately 80 scientists, clinicians, patients, and representatives of funding organizations, and biotech and pharmaceutical companies from around the world gathered on November 6, 2012, in San Francisco at the FSHD International Research Consortium and Research Planning meeting to share the latest progress in FSHD research. The meeting was a satellite of the Annual Meeting of the American Society of Human Genetics.

With Louis Kunkel (FSH Society Scientific Advisory Board and Board of Directors member) serving as moderator, we reviewed the previous year's research priorities and at the end of the day reprioritized them in light of recent developments. It was a very successful workshop with a positive, constructive, and collaborative atmosphere where new and unpublished findings were presented and with excellent interaction between the researchers and funding agencies.

The group concluded that given the recent developments in the genetics of FSHD Type 1 (or 1A) and Type 2 (or 1B), there is a need to ramp up the preclinical enterprise and establish the infrastructure needed to conduct clinical trials. Our immediate priorities should be to confirm the DUX4-fl hypothesis, understand normal DUX4 function, investigate potential toxicity from therapies to block DUX4, and comprehend the naturally occurring variability in FSHD symptoms and progression.

There was a consensus on the need to prepare for this new era in FSHD science by accelerating efforts in the following five areas:

1. Genetics and epigenetics. There is general acceptance that transcriptional deregulation of D4Z4 is central to FSHD1 and FSHD2. The gene *SMCHD1* accounts for approximately 80 percent of FSHD2 cases. There is a need to better understand the factors that modulate D4Z4/DUX4 activity and disease penetrance.



2. FSHD molecular networks. D4Z4 chromatin relaxation on FSHD-permissive chromosome 4 haplotypes leads to activation of downstream molecular networks. In addition to considering DUX4 as the “target,” downstream and upstream processes—molecular events that trigger or are triggered by DUX4—are equally important. Detailed studies of these processes are crucial for insight into the molecular mechanisms of FSHD pathogenesis and may contribute to explaining the large intra- and interfamilial clinical variability. Importantly, such work may lead to intervention (and possibly also prevention) targets. Additional FSHD genes and modifiers are still likely to exist. Apart from chromatin modifiers, these include, but are not limited to, *CAPN3* and the *FAT1* gene that were recently suggested to be involved in FSHD.

3. Clinical trial readiness. Recent advances in the molecular genetics of FSHD have opened up avenues for intervention along different avenues. Intervention trials are

envisaged within the next several years. The FSHD field needs to be prepared for this crucial step. There is an increasing need to improve the translational process. This includes, but is not limited to, the need for consensus on data capture and storage, overcoming national and international barriers, definition of natural history, identification of (meaningful) and sensitive outcome measures, biomarkers, and meaningful functional measures. There is a need to work more closely with the FDA to help define meaningful outcome measures for trials.

4. Model systems. There exists a variety of cellular and animal models, based on different pathogenic (candidate gene) hypotheses, but many basic questions must still be answered for further translational studies: When and where is DUX4 expressed in skeletal muscle and what regulates DUX4 activity? It was recognized that there still exists a gap in our knowledge linking the basic genetic and molecular findings with the observed muscle pathol-



Achieving rigor and statistical power in FSHD research studies

The NIH Wellstone FSHD cell repository and its research value

by **OLIVER KING, PhD**

NIH Wellstone MDCRC FSHD Program, University of Massachusetts Medical School, Worcester, Massachusetts

ogy. The FSHD Wellstone Center and the Fields Center continue to generate human cellular resources. These resources deserve attention and need to be replenished. Recent progress in embryonic stem cell technology, including induced pluripotent stem cell lines, allows for intergroup distribution and dedicated molecular (epi)genetic studies.

5. Sharing. Timely sharing of information and resources remains a critical contributor to the progress in the field. The Wellstone Center and the Fields Center continue to share their resources with the scientific community. The Fields Center website also continues to share other information (e.g., protocols, guide to FSHD muscle pathology, etc.).

Meeting organizers and attendees. Silvère van der Maarel (Leiden University Medical Center, the Netherlands, and co-PI of the Fields Center for FSHD and Neuromuscular Research) was the European research co-Chair. Rabi Tawil (University of Rochester Medical Center, New York, and PI of the Fields Center for FSHD and Neuromuscular Research) was the clinical co-Chair. Daniel Perez, President & CEO of the FSH Society, served as the organizational Chair. Other research directors attending the meeting were Sanjay Bidichandani and Paul Muhlrud of Muscular Dystrophy Association United States (MDAUSA), Bill Moss and Alan Watts of FSHD Global Research of Australia, Julie Dumonceaux of Association Française Contre les Myopathies (AFM), and Dan Miller of Friends of FSH Research, Seattle, Washington. Sponsors included AFM, FSH Society, FSHD Global Research Foundation, NIH Wellstone FSHD Center, MDA, and Genzyme.

The 2012 program and abstracts are available on our website (www.fshsociety.org). Click on the “For Scientists” tab and then on “FSH Society International Research Consortium” in the left-hand sidebar.

We thank our members and donors for their financial support of the FSH Society FSHD International Research Consortium Workshop.

Beginning in 2008, the U.S. National Institutes of Health (NIH) has funded a Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center with a focus on FSHD research. One of the goals of this Center has been the establishment of a tissue and cell repository. This consists of muscle biopsies from individuals with FSHD and their unaffected first-degree relatives (parents, children, and siblings) and cell lines derived from these biopsies.

The Center has to date collected muscle biopsies from more than 30 patients and their family members—totaling over 80 individuals. Primary cell lines have been derived and characterized for over a dozen of the families, with more in progress. Researchers in the Center are thankful to all the volunteers who contributed muscle samples, and to the FSH Society for its role in outreach, recruiting, and funding patients’ travel costs.

These samples have enabled a variety of studies into FSHD both by investigators within the Center (a collaborative group including members at the University of Massachusetts Medical School, Children’s Hospital in Boston, Boston University School of Medicine, Johns Hopkins School of Medicine, University of Maryland School of Medicine, and University of Texas Southwestern Medical Center) and by other groups to whom cell lines have been distributed.

One of the main uses of the samples so far has been to look for genes whose expression levels are altered in FSHD compared to controls. Such genes can provide insight into the processes that are dysregulated in FSHD and serve as molecular markers for monitoring potential therapeutics. Because there is natural variability in gene expression levels among individuals (both those with FSHD and those without), having a large collection of samples helps when assessing whether any observed differences between the FSHD and control groups may just be due to this natural variability. In statistical parlance, it increases the power to detect differences that are associated with FSHD.

Having more samples allows the detection of smaller, between-group differences and differences in genes that have higher within-group variability. Having more samples also allows for the use of more stringent criteria when assessing the significance of differences. This is particularly useful in genomewide studies, because when 20,000 genes are being examined, differences genuinely associated with FSHD could otherwise be swamped by false positives, which make it difficult to prioritize which leads to pursue. Having FSHD and control samples from the same families also helps reduce variability due to differences in genetic background that could obscure differences that are associated with FSHD.

In addition to genomewide studies, samples from the Wellstone repository have been used in targeted studies on expression of DUX4 and other candidate FSHD-related genes, and on the relationship between telomere length and expression of 4q35 genes. Cell lines from the repository are available to FSHD researchers, and it is hoped that they will continue to be a useful tool for both discovery and validation. More information is available at <http://www.umassmed.edu/wellstone/>.

The FSH Society is a key partner, fully integrated into the research and patient recruitment activities of our Center. Daniel Paul Perez serves as the Center’s Director of the Office of Patient Communication and Liaison (OPCL). The FSH Society has been instrumental in helping patients and their families connect with the study coordinator and in defraying expenses for patient travel and lodging by reimbursing cohort study members. Without these vital funds (approximately \$55,000 to date) and the support of the FSH Society and its members for research programs such as this, the burden on patients to participate in research would be far greater and we would not be making the tremendous strides in research we are seeing today.

Perspectives from the field: FRG1 is an important player in FSHD

It's important to keep diverse hypotheses alive

by **DAVIDE GABELLINI, PhD**

Division of Regenerative Medicine, San Raffaele Scientific Institute, Milan, Italy

I have been studying FSHD for the past 14 years. I started studying the disease in the summer of 1999 as a postdoctoral fellow at the University of Massachusetts Medical School in Worcester, thanks to a fellowship from the FSH Society. A few months after my arrival at UMASS, I had the privilege to meet periodically with and get to know Daniel Perez and the late Carol Perez. I was impressed by their strength and resilience. They have been a source of inspiration for all these years.

The Society has also supported my work after I moved back to Italy at the end of 2006 to establish my own group at the Division of Regenerative Medicine, San Raffaele Scientific Institute in Milan. The FSH Society is currently providing a pre-doctoral fellowship to a PhD student in my lab.

In writing this article, I went back to what little was known about FSHD in 1999. During the following years, our knowledge of FSHD has advanced dramatically, and we are now finally in the position to think about possible therapeutic approaches.

We now know that the disease onset is due to malfunctioning of the D4Z4 molecular switch. D4Z4 is a piece of DNA that is repeated many times toward the end of chromosome 4q35. Studies from many groups have shown that the normal function of D4Z4 is to keep the activity of nearby FSHD candidate genes repressed. In healthy subjects, D4Z4 acts as a switch in the “off” position or as a brake. In FSHD patients, the switch is moved to the “on” position (or the brake is lifted), and the activity of the FSHD candidate genes is activated. The idea is that the aberrant activity of one or more genes is responsible for FSHD onset and progression.

My group is currently working toward finding a cure for FSHD by investigating several avenues. We are studying the mechanism of action of D4Z4 with the hope of finding ways to bring it back to the “off” position in FSHD patients. We are characterizing what the genes aberrantly expressed in FSHD are doing and how their aberrant activity leads to muscular dystrophy. This way, we hope to be able to block the downstream effects of the D4Z4 malfunction in FSHD.



GE & MD Gene Expression Muscular Dystrophy

Davide Gabellini [lower left] and his group in Milan, Italy. Kneeling front, L to R: Davide Gabellini, Maria Victoria Neguembor, Claudia Huichalaf, Giulia Ferri, and Elisabetta Maria Cattafi. Standing back, L to R: Stefano Micheloni, Roberta Caccia, Valeria Runfola, Valentina Casà, and Mathivanan Jothi.

Finally, we are testing possible therapeutic approaches in mice we have generated that show features of FSHD.

Several investigators have tried to mimic FSHD in a laboratory mouse by playing with the expression of FSHD candidate genes in transgenic mice. So far, transgenic mice for ANT1, DUX4, FAT1, FRG1, and FRG2 have been described. Intriguingly, the only FSHD candidate gene that is capable of causing muscular dystrophy in the mouse is a gene called *FSHD region gene 1 (FRG1)*. Moreover, the type of muscular dystrophy developed in FRG1 mice is similar to FSHD in several aspects. For example, FRG1 mice develop the same stereotypical distribution of muscle

weakness as do FSHD patients, with the same muscles that are affected by the disease and those that are spared. This and other features of FRG1 mice convinced us that the gene plays an important role in FSHD, and it is important to go deeper in understanding its function.

We have recently found that FRG1 is aberrantly expressed specifically in the muscles of FSHD patients as compared to healthy controls or patients affected by other muscular dystrophies. By characterizing the defects responsible for disease onset in FRG1 mice, we have found that FRG1 regulates the biology of muscle stem cells. As a result, the aberrant expression of FRG1 causes prob-

lems in the development of muscles and in the ability to repair muscles after damage. These results suggest that a reduced ability to build and repair muscles, over time, could contribute to the development of FSHD.

To pinpoint the pathways affected in the disease, we have recently identified the genes that are expressed differently between healthy and FRG1 mice. Surprisingly, we have found that FRG1 mice display aberrant expression of the same group of genes that are altered in FSHD patients. This result suggests that the same pathways are altered in FRG1 mice and FSHD patients. Thus, FRG1 mice are a good tool for understanding what goes awry in FSHD patients and testing possible therapeutic avenues. Indeed, through our work we think we have identified important players for the pathology that can be targeted for therapeutic purposes.

Nevertheless, FSHD is a complex disease, and the malfunction of D4Z4 has several consequences in FSHD. Together with FRG1, there are for sure other important players contributing to the disease. For example, recent work from other groups strongly suggests that DUX4 and FAT1 are two other FSHD candidate genes with an important role in the disease. Understanding the relative contribution of these and possibly other FSHD candidate genes to the disease is one of the FSHD scientific community's current challenges.

We have come a long way in the past 14 years. All these years of hard work have allowed us to understand many things about this fascinating disease. While we have still a lot to do, we know where we are and what needs to be done to finally bring a therapy to FSHD patients.

For those wanting more technical information, we recently published a third important paper on FRG1.¹ Together with two other recent publications from my group in 2013,^{2,3} our main findings are as follows:

- FRG1 is specifically overexpressed in muscle biopsies of FSHD patients as

compared to healthy controls and patients affected by other muscular dystrophies.

- FRG1 regulates the biology of muscle stem cells.
- FRG1 overexpression inhibits muscle differentiation and development.
- FRG1 does the above through at least two mechanisms: control of epigenetic gene expression and control of RNA splicing.
- The transcriptome of FRG1 mice is remarkably similar to that of muscles of FSHD patients (based on all the publicly available microarray data on FSHD muscles), indicating that FRG1 mice are similar to FSHD also at the molecular level.
- We have identified key FRG1 targets selectively altered in FSHD that contribute to the pathology and can be targeted for therapeutic purposes.

Altogether, these results build a very strong case in favor of an important role of FRG1 in FSHD.

With this in mind, we are most thankful that the FSH Society retains a broad range of

funding in its research portfolio. We all recognize that investing in just one gene or target at this time runs the serious risk of not finding an effective therapy for the disease. **FSH Watch**

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LIVING WITH FSHD

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FSHD Patient Brochure (in English)

http://www.fshsociety.org/assets/pdf/PatientBrochure_May2009.pdf

FSHD Patient Brochure (in Spanish)

<http://www.fshsociety.org/assets/html/PatientBrochureSpanish.html>

Physical Therapy and FSHD

<http://www.fshsociety.org/pages/patHlExer.html>

FSHD: A Guide for Schools

http://www.fshsociety.org/assets/pdf/FSHSociety_FSHDPubs_AGuideForSchools.pdf





BASIC SCIENCE AWARDS

Understanding disease mechanism is the key to solving FSHD

Introduction by JUNE KINOSHITA. Researcher perspectives compiled and edited by DANIEL PAUL PEREZ
FSH Society, Lexington, Massachusetts

▶ ANNUAL
RESEARCH
REPORT

The human body is a miracle of complexity, its proper functioning the result of an almost incomprehensible web of interacting chemical events across a lifespan. Disease results when mistakes enter into this complex web: a wrong molecule, an imposter, a garbled message, a broken connection. To treat a disease, it helps to understand what went wrong so that researchers can engineer a fix.

Because the system is so complex, it's easy for researchers to get misled and follow a suspect down the wrong alley. That's why it's critically important to make sure studies are rigorous, statistically robust, and repeatable. It's essential to approach even the most convincing hypotheses with skepticism and an eye to alternative interpretations of the data.

Pharmaceutical companies look to basic scientists to provide well-tested hypotheses in which to invest. As leading ideas move into the drug development pipeline, it's important to make sure there are fresh ideas to fill the pipeline. The FSH Society provided seed grants that helped give rise to the concept that the DUX4 protein is toxic to muscle—a hypothesis that now enjoys strong support from many large funders. The Society continues to support research on DUX4, especially studies that delve deeper into the story while also planting seeds for the future by funding promising new ideas.

▶ **D4Z4 DNA SEQUENCE ENCLOSES THE KEY FOR THE PROPER REGULATION OF FSHD GENES**

Perspective by Valentina Casà, MS

Grant title: Role of Polycomb Group Proteins in Facioscapulohumeral Dystrophy

Investigators: Valentina Casà, MS, and Davide Gabellini, PhD, Division of Regenerative Medicine, Fondazione Centro San Raffaele, Milan, Italy

From August 2012: \$45,000 over 18 months



Valentina Casà, MS

Although there is a genetic defect at the basis of FSHD, several aspects of the disease suggest that genetics is not sufficient to explain why individuals develop FSHD. What else is going on in this pathology? Which other elements compose the whole picture of the mechanisms underlying FSHD? The need for a deeper understanding is surely shared by anyone who is interested in this disease.

When I joined the FSHD group led by Davide Gabellini at San Raffaele Scientific Institute in Milan, Italy, to start my PhD, it was already known that, besides the genetic lesion (a loss of DNA

repeats called D4Z4 at the 4q35 region on chromosome 4), the epigenetic status of the FSHD locus was relevant for the development of the disease.

Epigenetics refers to heritable changes in gene function that occur without changes in DNA sequence. For example, epigenetics is what makes monozygotic (identical) twins different, or what makes cells and tissues of our body have different shapes and functions, though they all share a common DNA code. Several epigenetic alterations have been known for a long time in FSHD, including reductions in modifications that normally promote gene repression occurring either on DNA (methylation) or on histones (the DNA packaging proteins).

Thanks to the work I'm conducting in Davide Gabellini's lab, I identified a novel epigenetic alteration in the FSHD locus. In particular, I found that a histone mark known as H3K27me3 is significantly reduced in progenitor muscle cells derived from FSHD patients. Importantly, this histone modification is a hallmark of a group of epigenetic factors called Polycomb, which mediate gene repression and are crucial for several fundamental processes.

Polycomb proteins, conserved from flies to humans, are normally very selective when binding to DNA, and particular combinations of DNA motifs are normally present on their DNA targets, thus contributing to their specific recruitment. It was very surprising to me to discover that D4Z4 repeats contain DNA motifs very similar to the ones of well-characterized Polycomb targets. Indeed, D4Z4 repeats are sufficient to recruit Polycomb proteins. This is extremely important because it is suggesting that each D4Z4 repeat encodes for the specific recruitment of proteins able to establish and maintain gene repression. In other words, D4Z4 repeats contain the key for the correct regulation of gene expression within their sequence.

Given the intrinsic ability of D4Z4 repeats to recruit Polycomb, I hypothesized that the genetic loss of D4Z4 repeats could be linked to the epigenetic loss of Polycomb binding in the disease locus. Indeed, I found that muscle cells from patients, carrying a reduced number of D4Z4 units, display a reduced binding of Polycomb to the locus.

Moreover, by inhibiting Polycomb activity in healthy muscle cells to mimic the disease situation, I observed a consequent increase of gene expression of 4q35 genes, thus suggesting that a weakening of Polycomb repressive function could be involved in the overexpression of genes responsible for the disease.

One of the most ambitious challenges in FSHD is linking the genetic defect of the FSHD locus to its epigenetic alterations, as an interplay of genetic and epigenetic components is most likely at the basis of FSHD. Still, we need to learn a lot from this disease to unveil additional elements that contribute to the pathogenesis of

FSHD. Nevertheless, the good work from the FSHD scientific community, much effort, and the precious financial support from charities such as the FSH Society are contributing to a more and more precise picture of the complex mechanisms taking place in this disease and are driving toward the development of therapeutic approaches for FSHD.

► AUTOPHAGY DEFECTS IN FSHD

Perspective by Sachchida Pandey

Grant title: Autophagy Defects in FSHD

Investigator: Sachchida

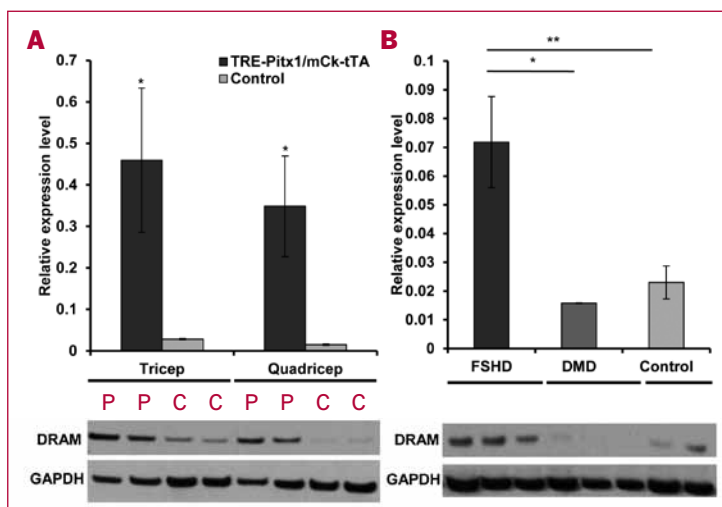
Pandey, PhD, Children's Research Institute, Washington, D.C.

From August 2012: \$99,599 over two years

Autophagy is a critical process for the degradation of cytoplasmic components including damaged and aging organelles to maintain the cell homeostasis. Upon induction of autophagy, cytoplasmic cargos are engulfed by a double-membraned structure called phagophore to form an autophagosome. The autophagosome then fuses with the lysosome, forming an autophagolysosome. The cytoplasmic cargos are degraded by lysosomal hydrolases in the autophagolysosome.

The molecular mechanistic aspects of autophagy are strikingly conserved in higher eukaryotes. There are several unrelated autophagy "ATG" genes known that are each required for autophagy. Loss of any one of the ATG genes blocks autophagy. While overexpressing autophagy genes leads to cell death, a base level of autophagy activity is critical for cell survival.

Studies have identified an upregulation of autophagy following traumatic brain injury, in numerous neurological disorders including spinal bulbar muscular atrophy (SBMA), Huntington's, Parkinson's, Alzheimer's, and prion diseases. In muscular disorders, activation of autophagy was reported to be involved in inclusion body myositis, limb girdle muscular dystrophy, and Danon's disease. On the other hand, reduction of the basal autophagy activities, which



Immunoblotting of DRAM protein. (Figure A) After overexpression of PITX1 for five weeks, muscles showed high expression of DRAM in PITX1 transgenic mice (mCk-tTA/TRE-PITX1) in comparison to controls (mCk-tTA). Immunoblot showing DRAM protein in PITX1 transgenic mice in column marked with "P" and controls in column marked with "C." (Figure B) DRAM protein was significantly upregulated in FSHD muscles compared to DMD and healthy controls. (*p < 0.05).

is necessary for maintaining cellular homeostasis, was reported to contribute to the myopathy of collagen VI myopathy. It has been also shown that autophagy inhibition induces abnormality in glycogen delivery to lysosomes in Pompe's disease, causing deposition of glycogen and autophagic buildup in muscle fibers.

Our group previously reported that PITX1 (paired-like homeodomain transcription factor 1) is a transcriptional target of DUX4. To further study the role of PITX1 in FSHD, our lab has generated transgenic mice—work supported by the FSH Society. In a study, the p53-dependent cell death pathway was identified in DUX4 expression-induced myopathy, although the mechanistic

link between DUX4 and p53 was not clear. PITX1 has been shown to bind directly to the regulatory elements of the p53 promoter and activates p53 transcription with subsequent apoptosis in human mammary carcinoma cells. Recently, we have showed that PITX1 is the missing link in the DUX4-activated p53 pathway. Further, expression profiling data of PITX1 transgenic mice showed that autophagy genes, including DRAM, were misregulated in the muscle overexpressing PITX1. We also confirmed the higher expression of DRAM in muscle from PITX1 transgenic mice by protein analysis (Figure A). Our study suggested that a molecular pathway involving DUX4, PITX1, p53, and p53-dependent autophagy is involved in FSHD.

Our preliminary data showed disease-specific upregulation of a master autophagy regulator, namely, damage-regulated autophagy modulator (DRAM), in FSHD muscle biopsies, but not in Duchenne's muscular dystrophy (DMD) and controls (Figure B), suggesting autophagy activation in FSHD.

We further characterized the autophagy state in differentiated immortalized FSHD myoblasts. We observed higher expression of DRAM and sequestosome 1 (p62), as well as a lower LC3B-II/LC3B-I ratio (a marker for autophagy induction), which indicates suppression of autophagy in the immortalized FSHD myoblasts. The suppression of autophagy is also supported by accumula-

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tion of ubiquitinated protein in the immortalized FSHD myoblasts. While the activation of DRAM should activate the downstream autophagy pathways, we observed defects in autophagosome formation. Interestingly, the upregulation

of lysosomal-associated membrane protein 1 (LAMP1) and 2 at the mRNA level in muscle biopsies of patients with FSHD suggests that the lysosomal system is activated and ready for the later steps of forming autophagolysosomes. However, the autophagy process is somehow disrupted in immortalized FSHD myoblasts.

While abnormally activated autophagy causes diseases, insufficient autophagy activities have also been shown to cause muscular disorders. Hence, we hypothesize that defects in autophagy are part of the pathological pathways of FSHD. In addition, autophagy defects are directly induced by aberrant expression of DUX4. The goals of this current project are to understand the mechanism of FSHD and identify molecular pathways for treatment development.

► THE 4Q35 GENE FAT1: A NEW PLAYER IN FSHD

Perspective by Angela Zimmermann, PhD, and Françoise Helmbacher, PhD

Grant title: Specific Silencing of FAT1: Role in Pathogenesis of FSHD
Investigator: Angela K. Zimmermann, PhD, Centre National de la Recherche Scientifique, IBDML—Development Biology Institute of Marseille, Campus de Luminy, France
From August 2012: \$140,000 over two years

The group led by Françoise Helmbacher, PhD, at IBDM, Aix-Marseille University, France, studies the mechanisms involved in building muscles and wiring nerve-muscle connections during embryonic development. The team uses genetically modified mouse models to understand how alterations of the underlying molecular programs perturb neuromuscular development and lead to devastating neuromuscular pathologies in humans.

The team recently discovered that a gene called FAT1 was a key determinant of muscle development. Genetic disruption of FAT1 in mice leads to abnormalities in the shape of muscles of the face and shoulder, and to regionalized muscle wasting at adult stages. Furthermore, disruption of the FAT1 gene causes vascular abnormalities in the retina and malformations of the inner ear. Strikingly, these defects mimic with a surprising accuracy the clinical picture of FSHD. Although complete abrogation of FAT1 gene function in mice also causes additional symptoms that do not occur in FSHD, preventing the gene from functioning only in muscles appears sufficient to reproduce some of the shape abnormalities.

Together with the group of Nicolas Levy, the Helmbacher team has thus explored the potential links between FSHD and this gene in humans. They found several ways by which partial alterations of the human FAT1 gene were occurring in FSHD.

First, the amount of FAT1 protein was reduced in fetal FSHD muscles compared to controls, while preserved in other organs

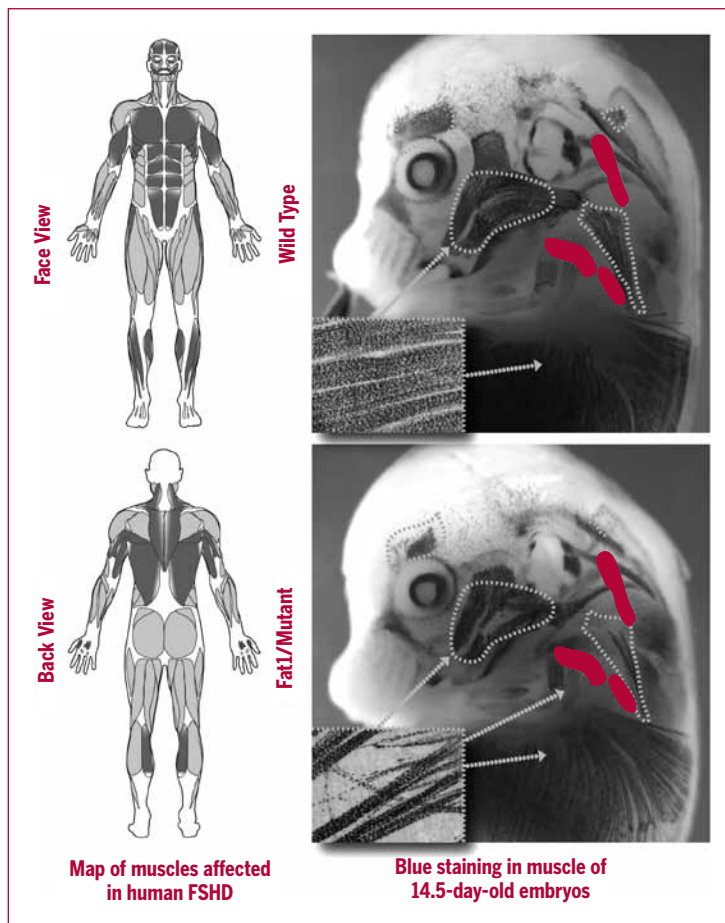


Figure: Loss of function of the *Fat1* gene alters the shape of selective subsets of muscles in the face and shoulder, also affected in FSHD. (Left) Map of human muscles subject to degeneration in FSHD patients. Affected muscles are represented in dark grey. Top: face view. Bottom: back view. (Right) Skeletal muscles are visualized in mid-gestation mouse embryos (14.5 days of development) owing to the dark staining. (Top) Wild-type embryo. (Bottom) Embryo deficient for the functions of the *Fat1* gene. Light dotted lines indicate muscles reduced in the mutants. Red sections indicate supernumerary muscles found in mutants. These differences, including muscles around the eye, in subcutaneous muscles of the face, in the region between the proximal forelimb and the scapular area, reproduce precisely the map of muscles affected in FSHD. Observation of these abnormal muscles at higher magnification shows the appearance of misoriented muscle fibers in mutant muscles.

and in adults. Second, genetic alterations in the FAT1 gene were present more frequently among FSHD patients than in control individuals. This genetic link was true not only for patients with the classical FSHD1 diagnosis, but also for a significant proportion of FSHD patients without abnormality on chromosome 4. Finally, DUX4, the toxic protein considered central to FSHD pathogenesis, was capable of shutting down expression of the FAT1 gene in muscle cells in vitro.

Thus, the alterations of FAT1 gene functions occurring in FSHD can result from either the classical DUX4 overproduction or the less frequent alternative mutations our teams identified. Furthermore, these alterations appear to affect FAT1 functions only in selective organs, such as muscles or retinal vasculature, while

preserving them in other organs.

Behind the apparent complexity of this disease, these findings identify the FAT1 gene as a common denominator to the various genetic mechanisms leading to FSHD. FAT1, therefore, represents an exciting novel therapeutic target, in addition to the ones previously identified by other groups.

This project received support from the FSH Society through a postdoctoral fellowship for Angela Zimmermann (Helmbacher lab), who will study mouse models, genetically altering Fat1 gene functions only in selective organs such as muscles, to mimic selective subsets of FSHD symptoms and to evaluate disease progression. The project also aims to generate a mouse model that reproduces the genetic alterations in FAT1 that the researchers identified in FSHD patients. Selective ablation of Fat1 in mice thus represents an excellent model to identify new drug treatments and to assay their efficacy in symptom prevention and/or alleviation and, ultimately, to improve quality of life in FSHD patients.

Publication: Caruso N, Herberth B, Bartoli M, Puppo F, Dumonceaux J, Zimmermann A, Denadai S, Lebossé M, Roche S, Geng L, Magdinier F, Attarian S, Bernard R, Maina F, Levy N, Helmbacher F. Deregulation of the protocadherin gene FAT1 alters muscle shapes: implications for the pathogenesis of Facioscapulohumeral dystrophy. *PLOS Genetics* 2013, 10.1371/journal.pgen.1003550

► **PURSUIING NOVEL CONCEPTS IN FSHD PATHOPHYSIOLOGY:
THE CADHERIN PROTEIN FAT1**
Perspective by Julie Dumonceaux, PhD

Grant title: FAT1 Roles in Muscular Physiology and FSHD Onset
Investigators: Virginie Mariot, PhD, Julie Dumonceaux, PhD, and Gillian Butler-Browne, PhD, *Thérapie maladies du muscle strié/ Institut de myologie, Paris, France*
From February 2012: \$68,000 over one year

In 2012, I was lucky enough to obtain a grant from the FSH Society for the research that my group is currently carrying out on FSHD. With the funds received, I was able to hire a postdoctoral fellow for one year to perform experiments in our laboratory; the goal is to try to decipher the mechanisms leading to FSHD. Even though during recent years the research on the onset and progression of FSHD has seen extraordinary advances, it is still unclear exactly how the gene deletion present since birth leads to this adult-onset disease. In addition, the exact mechanisms underlying the clinical onset of the muscle pathology still need to be deciphered.

In particular, I would like to highlight our recent findings concerning downregulation of a new gene called FAT atypical cadherin 1 (FAT1) in FSHD fetal biopsies. Very recently, in a collaborative effort involving three French laboratories, we were able to demonstrate that FAT1 may be involved in FSHD onset. One could have thought that FAT1 is just another misregulated protein that is not directly and importantly involved in FSHD onset, but since the downregulation of FAT1 in a mouse recapitulates most of the FSHD hallmarks (atrophy of a specific set of muscles, asym-

metry, non-muscular symptoms such as retinal vasculopathy), this provides striking new data suggesting that FAT1 may play an important role in FSHD pathology.

Surprisingly, nothing was yet known about this new gene concerning how it may be involved in normal muscle development, physiology, or regeneration, and consequently in FSHD onset. Therefore, the project I proposed to the FSH Society in 2012 was to better understand these two last points. Understanding the natural role of FAT1 in muscle cells is crucial in order for us to determine how its misregulation plays a role in FSHD.

These experiments are very important, since the implication of FAT1 in FSHD onset may reveal some new pathophysiological mechanisms, improve FSHD diagnosis, and/or highlight some new therapeutic strategies.

The grant that we received from the FSH Society was extremely important because in France, it is difficult to obtain funding from governmental agencies for research on rare diseases like FSHD. Without the FSH Society, it would not have been possible to develop this project, so I am very grateful to the Society, Daniel Perez, and all the people who have made donations to the FSH Society for giving us this chance to work on FSHD, which is a very challenging but very exciting field of research.

► **STUDYING EARLY MOLECULAR DEFECTS ARISING DURING
MYOGENESIS OR EARLY DIFFERENTIATION IN FSHD**
Perspective by Frédérique Magdinier, PhD

Grant title: Tri-dimensional Organization of the FSHD Locus During Proliferation and Differentiation of Muscle Cells in FSHD Patients and Controls
Investigators: Marie Gaillard, MS, and Frédérique Magdinier, PhD, *INSERM UMR_S 910, Epigenetics, chromatin & diseases team, Faculté de Médecine de Marseille, FRANCE*
From February 2012: \$30,000 over one year

FSHD is not only associated with a gene abnormality leading to a change of function of a key protein, but is also linked to a complex rearrangement involving the repetitive D4Z4 sequence in a gene-poor region at the tip of the long arm of chromosome 4.

Our team is interested in understanding how and why this rearrangement occurs and how this chromosomal abnormality contributes to the disease, why so many cases arise de novo in non-affected families, and why penetrance is variable from one individual to another.

Gene localization in the nucleus is not arbitrary, but governed by a dynamic process. Unlike most of the other human telomeres, the 4q35 region is localized at the periphery of the cell nucleus, a highly specialized compartment enriched in heterochromatic factors and implicated in numerous biological phenomena. The molecular basis of this association is still poorly understood, but likely involves a network of interactions that will influence the regulation of the “FSHD gene(s).”

By developing an innovative method of fluorescence in-situ

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▶ ANNUAL RESEARCH REPORT

hybridization in three dimensions (3D-FISH), we have shown in the past that the D4Z4 repeat plays a key role in organizing genomic regions, especially telomeres, specialized structures at the end of chromosomes that protect against genomic

loss and fusion of chromosome, especially during aging.

The FISH method is usually employed to detect a specific region of the DNA molecule. We perform the 3D-FISH on cells plated on glass slides in conditions that preserve the conformation of the cell nucleus in order to have a view in 3D of how the DNA molecule, and especially the 4q35 FSHD locus, behaves in cells from patients and control cells (Figure 1C).

Our hypothesis is that the tri-dimensional organization of the 4q35 locus is altered in patients and is responsible for deregulation of the disease's gene expression. Thanks to the pre-doctoral fellowship awarded by the FSH Society to Marie Cecile Gaillard, a PhD student in my lab, we are now comparing the tri-dimensional organization of the FSHD locus in patients and control cells.

We have recently shown that expression of several genes located in the 4q35 region is modulated as early as the fetal stage. We are now trying to investigate how the tri-dimensional organization of the D4Z4 array might affect gene expression by comparing the topology of the 4q35 region in induced pluripotent cells from patients and controls (Figure 1A) during proliferation of muscle precursors and differentiation into several lineages (teratomas, embryoid bodies, differentiated cells; Figure 1B).

These approaches should bring further insights into the underlying mechanisms of FSHD and the link between the number of D4Z4 units and the muscular dystrophy.

▶ UNDERSTANDING THE LIFE AND TRAVELS OF DUX4 PROTEIN IN MUSCLE FROM ITS INCEPTION TO DEATH Perspective by Richard J. L. F. Lemmers, PhD

Grant title: Identification of the Epigenetic Mechanisms That Regulate DUX4 Activity in Skeletal Muscle

Investigators: Richard J. L. F. Lemmers, PhD, and Silvère van der Maarel, PhD, Leiden University Medical Center (LUMC), Department of Human Genetics, the Netherlands

From August 2011: \$80,000 over two years

I am a senior researcher in the group of Silvère van der Maarel in the Department of Human Genetics, Leiden University Medical Center in the Netherlands. My colleagues include Patrick van der Vliet, Judit Balog, and Yvonne Meijer-Krom. I am responsible for the detailed genotyping of patients with an unusual or unexplained genotype. This work has been essential for the identification of the genes that cause FSHD1 (FSHD1A) and FSHD2 (FSHD1B). Currently, I am mainly focusing on the consequences of FSHD gene mutations and other genes that might be involved in FSHD.

FSHD is caused by inappropriate expression of DUX4 that is thought to be toxic and damages the muscle in patients. The mechanisms that regulate DUX4 expression in the affected muscle are largely unknown, and currently we do not know how a protein that is expressed in minute amounts causes a chronic and progressive muscle wasting.

Other researchers have studied the toxic effect of the DUX4 protein by placing the bare DUX4 gene in an overexpression system. However, the expression of DUX4 seems to be regulated by

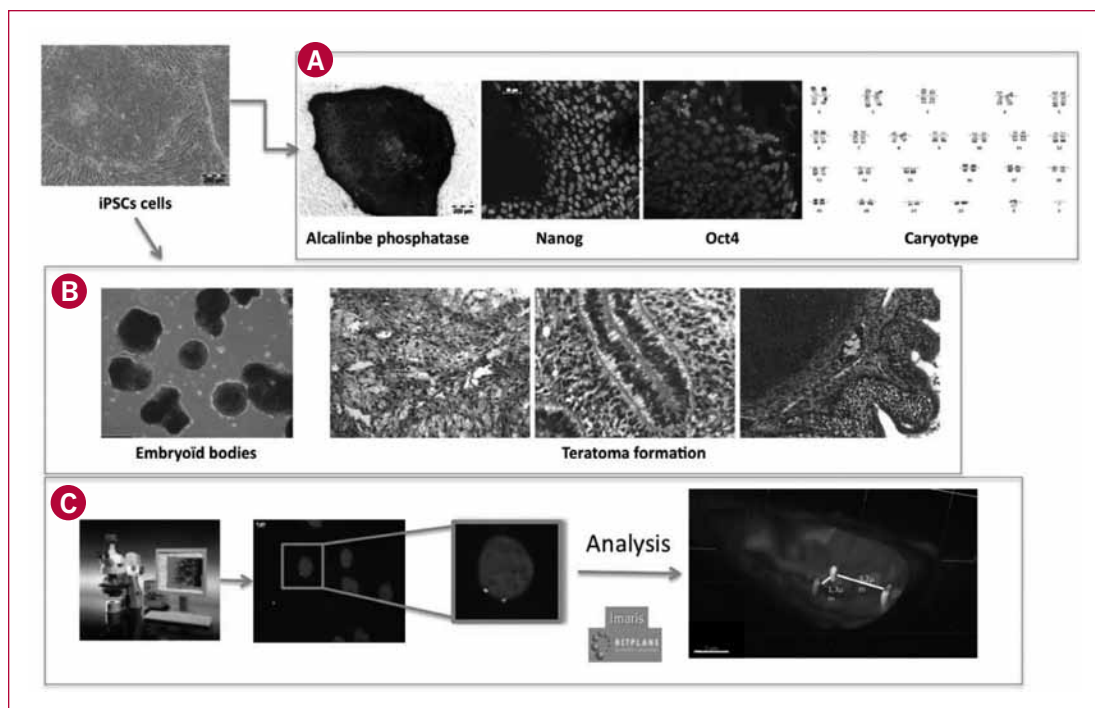


Figure: In order to investigate the regulation of the 4q35 region, we developed several clones of induced pluripotent cells. These cells express stem cell-specific markers such as phosphatase alkaline, Nanog, or Oct4 (Panel A) and have the capacity to differentiate into the three primordial layers present in early embryogenesis. This capacity to differentiate is verified in vitro by formation of embryoid bodies or in vivo by formation of teratomas (Panel B). Panel C. After plating, cells are stained with DAPI and hybridized with probes specific for different parts of the 4q35 region. After acquisition by confocal microscopy, images are processed with the Imaris software. The volume, position, and relative distances are determined for each FISH signal, as shown on the right panel.



Richard Lemmers conducting FSHD experiments in Leiden

genetic factors on both sides of the gene—factors that are typically not considered in overexpression systems.

We found that when the DUX4 gene is retained within its natural genomic organization, i.e., in the context of the FSHD repeat, the protein shows a low expression level comparable to levels seen in muscle cells obtained from FSHD patients. Therefore, we aimed to develop so-called reporter constructs in which we replaced the DUX4 gene within the FSHD repeat with a fluorescent protein that can be easily visualized by microscopy to follow the expression in living muscle cells. This system would allow us to study

the timing of protein expression and the initiation cascade that precedes DUX4 activation.

While the initial data were very promising and formed the basis of our proposal, we have encountered unexpected hurdles with the many different reporter constructs we have generated thus far. For example, we created a series of DUX4 reporter constructs in which we replaced the DUX4 protein with a fluorescent protein while maintaining the original regulatory region behind the FSHD gene. These constructs were tested in transient cell systems and gave the expected fluorescence reporter signals. However, the reporter signal was lost after we created stable lines from the same constructs.

In searching for an explanation, we determined precisely the sequence of the reporter RNA, an intermediate molecule copied from the DNA, which forms a reading template to produce the fluorescence protein. This showed that the reporter RNA sequence was not correctly processed, making it impossible to produce the fluorescent protein. This was completely unexpected, as this had not been reported before, and our preliminary studies showed that these constructs were faithfully producing the fluorescent protein. Currently, we are working on alternative constructs in an attempt to prevent these problems.

We don't believe that these constructs are entirely useless. Clearly, the processing of DUX4 RNA is complex and context dependent, as was demonstrated previously by the different DUX4 variants that can be produced in muscle and germline. We believe it is important to learn more about the factors that regulate DUX4 RNA processing and have faith that the stable cell lines we have already generated, while not useful as reporter constructs for their original application, may prove useful for the delineation of factors involved in DUX4 RNA processing.


► **DUX4-FL EXPRESSION IS NECESSARY BUT NOT SUFFICIENT BY ITSELF FOR FSHD**

Adapted from *FSH Watch*, Fall 2012

Grant title: Analysis of DUX4-fl Expression

Investigator: Peter L. Jones, PhD, Boston Biomedical Research Institute, Watertown, Massachusetts

From February 2011: \$7,500 over one year

Recent studies have proposed that FSHD is caused by the production of an abnormal protein, DUX4-fl. A high-profile paper published in *Human Molecular Genetics* in July 2012 reported that while DUX4-fl is indeed significantly overproduced in muscle from FSHD patients, the protein is also found at lower levels in relatives who are genetically unaffected by the disease. This discovery supports the hypothesis that DUX4-fl is necessary to cause FSHD, but other factors regulating the amount of DUX4-fl are involved in determining disease progression. What does this mean for future treatments? "DUX4 is still a great therapeutic target," said lead author Peter Jones, "but there are also going to be additional targets. This is great news." 



FUNDING FOR RESEARCH TOOLS

Animals and cells that model FSHD

Introduction by JUNE KINOSHITA. Researcher perspectives compiled and edited by **DANIEL PAUL PEREZ**
FSH Society, Lexington, Massachusetts

To study a disease thoroughly, and to test ideas for treatment, we need “model systems”—cells, laboratory animals, and other biological entities that scientists can investigate and manipulate to figure out precisely what is going on. In a disease such as FSHD, which boasts a magnificently complex genetic mechanism, creating these tools can be very challenging.

Also, it is not enough simply to churn out an “FSHD cell line” or mouse. Model systems are not a carbon copy of a human disease. They have quirks based on how they were made, and the expression of the disease isn’t completely identical across different species (e.g., humans vs. animals), so each model needs to be thoroughly characterized before researchers can interpret the data generated by the model.

Developing these tools is cutting-edge science. What makes them “tools” is that they are necessary to get the job done and that, ideally, they can be shared among labs. Glory goes to the scientists who develop tools that become the standard workhorses (or workmice)!

▶ **GENERATING NEW FRONTIERS FOR FSHD: CREATING HUMAN INDUCED PLURIPOTENT STEM CELL (HIPSC) LINES FROM FSHD**
Perspective by **Gabsang Lee, PhD**

Grant title: Derivation of Human Induced Pluripotent Stem Cells From FSH Patient Fibroblasts

Investigator: Gabsang Lee, PhD, Johns Hopkins University, Baltimore, Maryland

From August 2012: \$49,705 over one year

FSHD is caused by chromatin relaxation of the polymorphic D4Z4 macrosatellite repeat array containing a conserved open reading frame for the DUX4 retrogene. The stabilization and translation of DUX4 are necessary to develop FSHD. DUX4 is a transcription factor of the double homeobox family and is normally expressed in the human germline and germ cell development. It seems that the expression of the DUX4 retrogene is developmentally regulated in germline development and possibly in aspects of early embryonic muscle development. Understanding how the DUX4 gene expres-

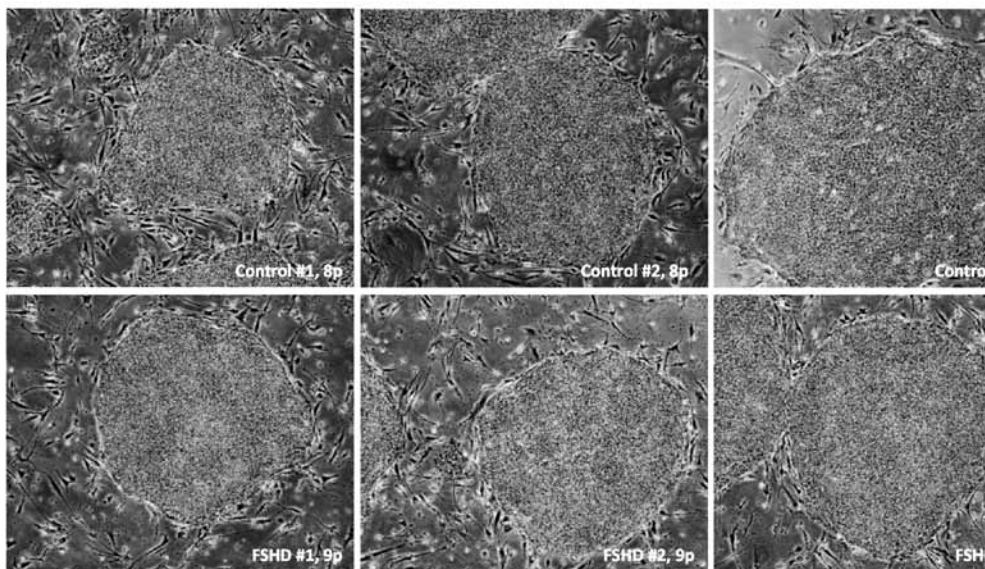
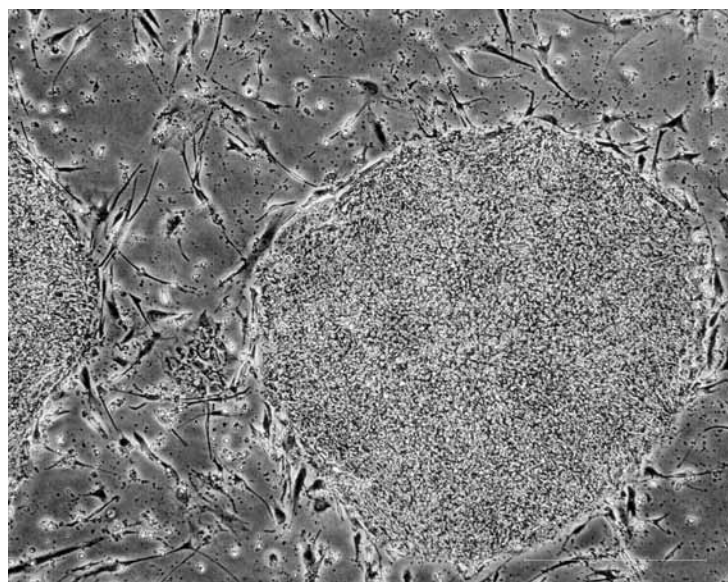


Figure 1. The morphology of control and FSHD hiPSC clones



FSH Society-supported research helps produce FSHD-human induced pluripotent stem cell (hiPSC) clones

sion is controlled during human embryonic muscle cell development should give us better insight on molecular and cellular pathogenesis of FSHD.

The induction of pluripotency in somatic cell types via overexpression of reprogramming factors has been one of the great break-

throughs in stem cell biology. Human induced pluripotent stem cells (hiPSCs) can provide a unique window into early human development in the context of FSHD. In addition, patient-specific skeletal muscle cells after genetic intervention should be a new cellular material for transplantation-based therapeutic approaches.

For these reasons, my lab started to reprogram myoblasts of FSHD patients as well as age- and sex-matched healthy donors. We used a non-integrating and “footprint-free” gene-delivery system, which does not have any remnant transgene (MYC, SOX2, OCT4, and KLF4) expression after reprogramming (hiPSC stage). As shown in Figure 1, our first FSHD hiPSC clones and control clones have characteristic morphology. Currently, we have 18 different hiPSC clones from FSHD myoblasts (acquired from a 19-year-old male), and now we have a total of 111 frozen vials. The control hiPSCs were reprogrammed from myoblasts from a healthy donor (an 18-year-old male) and a total of 53 frozen vials.

Now we are attempting to reprogram other FSHD patient myoblasts to generate additional hiPSC clones, which should be done by this fall. Further, the FSHD hiPSCs will be put through a battery of assays addressing pluripotency, including extensive immunofluorescence/FACS with pluripotent stem cell markers, confirmation of transgene absence, karyotypical normality, cell line authentication, and their differentiation potentials. Once the characterization is finished, we will deposit the hiPSC clones to the Stem Cell Core at Johns Hopkins for distribution to other laboratories.

► **A TRANSGENIC MOUSE MODEL OF DUX4-MEDIATED FSHD**
Comments by Peter Jones, PhD, from his grant proposal

Grant title: A Transgenic Model of DUX4-Mediated FSHD
Investigator: Peter Jones, PhD, University of Massachusetts Medical School, Worcester, Massachusetts (previously at Boston Biomedical Institute)
From February 2012: \$105,000 over two years

The most critical need in the FSHD field is a reliable and faithful mouse model of FSHD. This has been inhibited in the past by lack of a consistent and consensus understanding of the gene misregulation in the human condition that leads to FSHD pathology.

Now that there is widespread agreement about the involvement of DUX4-fl in FSHD pathology, there are different barriers: the severe cytotoxicity of DUX4 and its lack of conservation in mammals. As such, the field has so far failed to generate a genetic mouse model based on DUX4 expression that recapitulates the DUX4-fl expression profile and FSHD-like pathophysiology.

This project proposes to generate a regulatable and tunable strain of D4Z4/DUX4 transgenic mice by using the Cre/lox system and targeted transgenesis into the ROSA26 locus. Importantly, this model incorporates the downstream cis-regulatory elements and DUX4 splicing and polyadenylation of the FSHD-associated 4q35 locus. This is different from any of the mouse models discussed at meetings (few are published) that fail to show any phenotype.

The targeting construct has already been generated and shown to function properly in human and mouse myogenic cell culture and myotubes. With this construct, we believe we can manipulate DUX4-expression in mice 1) to a range of cells in a population (1:50 down to 1:5,000) in the developmental profile of DUX4 expression and/or 2) in any select tissue or spatiotemporal pattern desired.

These mice will prove invaluable for therapeutic screening and understanding DUX4 function. As such, once they are generated and initially characterized, we will make these mice available to the FSH community at large in a timely manner for those with therapeutic approaches.

► **DEVELOPING AN IN-VIVO MODEL FOR FSHD RESEARCH**
Perspective by Hiroaki Mitsuhashi, PhD

Grant title: Expression of Human DUX4 in Zebrafish Development
Investigators: Hiroaki Mitsuhashi, PhD, and Louis Kunkel, PhD, Children's Hospital Boston, Massachusetts
From February 2012: \$60,000 over one year

The Kunkel lab is grateful for funding received from the FSH Society for our work on a zebrafish model of FSHD. Our goal is to use the power of zebrafish genetics and our knowledge of its development to characterize how human DUX4-fl perturbs development and leads to the human disorder. We believe that these studies should lead to a better understanding of FSHD pathology and rational approaches to therapy.

We focused on the impact of DUX4-fl expression on vertebrate development for the following reasons:

1. Unlike other muscular dystrophies, FSHD patients develop muscle weakness on the face, shoulder, and upper arm muscles that often shows asymmetry. One possible explanation for this unique selectivity of muscle involvement is that the pathological events may arise in the muscle progenitors that give rise to those specific muscles during development.

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FUNDING FOR RESEARCH TOOLS

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2. Although most typical FSHD patients show some clinical symptoms in the second decade, some patients show more severe symptoms in early childhood known as infantile FSHD.

3. DUX4, a leading candidate gene for FSHD, produces a protein containing homeobox domains. Usually, these homeobox domain-containing proteins play an important role in early development.

To examine the effect of DUX4 expression during vertebrate development, we introduced human DUX4 mRNA into zebrafish fertilized eggs. We used two types of DUX4 mRNA: DUX4-fl (full-length transcript), which is predominantly expressed in FSHD patients' muscle, and DUX4-s (short transcript variant), which is expressed in normal individuals. When we introduced a high dose of DUX4 mRNAs (10 picograms per egg), DUX4-fl-injected embryos died within 24 hours, while DUX4-s-injected ones were almost normal. This result showed that the form of DUX4 expressed in FSHD patients' muscle is severely toxic to developing embryos.

Next, we decreased the amount of injected DUX4 mRNA to be more consistent with the low levels of DUX4 observed in human FSHD muscle. It has been reported that approximately one cell per 1,000 in FSHD muscle expresses DUX4 protein. We introduced very small amounts of DUX4-fl mRNA, which would be expected to match the levels observed in the patients during development of fertilized zebrafish eggs. The small quantities of introduced DUX4-fl caused asymmetric abnormalities of facial, fin, and/or trunk muscle, as well as ear and eye abnormalities in zebrafish embryos. Since hearing loss and retinal abnormality are known as FSHD symptoms, we considered that these phenotypes mirror FSHD. We also found that some muscle progenitor cells are mislocalized in the developing embryos following injection of the small quantities of DUX4-fl. Our results suggest that the disturbance of normal development by DUX4-fl plays an important role in FSHD pathogenesis.

Another exciting finding from our study was that DUX4-s has a potential to inhibit the toxic function of DUX4-fl and ameliorate the phenotype. When we co-introduced DUX4-fl with

20-fold more DUX4-s mRNA into zebrafish fertilized eggs, most of the abnormal phenotypes seen in the zebrafish embryos with single DUX4-fl introduction were rescued. This indicates that it might be possible to improve FSHD phenotypes by modifying DUX4 isoforms from toxic DUX4-fl to protective DUX4-s.

Using this zebrafish model, we are investigating the precise mechanism of how DUX4-fl perturbs normal development and how it leads to the phenotype that mirrors what is seen in FSHD. We also expect that zebrafish will be an important animal model to develop therapeutic approaches to FSHD. This work was made possible by the FSH Society research fellowship grant.

▶ HUMANIZED MOUSE MODELS OPEN THE DOOR FOR PRECLINICAL RESEARCH TO DEVELOP FSHD THERAPEUTICS

Perspective by James Windelborn, PhD, and Charles Emerson, PhD

Grant title: A Humanized Mouse Model for Investigations of FSHD Pathology and Therapeutic Development

Investigators: James Windelborn, PhD, and Charles Emerson, PhD, University of Massachusetts Medical School (UMMS), Worcester
From February 2012: \$60,000 over one year

Grant title: Humanized Mouse Model for the Study of Facioscapulohumeral Dystrophy

Investigators: Marietta Barro, PhD, and Charles P. Emerson Jr., PhD, Boston Biomedical Research Institute, Watertown, Massachusetts

From February 2011: \$40,000 over one year

A grant from the FSH Society has enabled significant progress toward the development of a humanized mouse model of FSHD. Mouse models are an essential tool for preclinical research on human genetic diseases and development of therapeutics. However, because the D4Z4 4q chromosome region and DNA deletions associated with FSHD disease are absent from the mouse genome, traditional genetic engineering approaches to generate FSHD-related DNA mutations are not feasible. This has been a major impediment to FSHD research.

To solve this critically important problem, James Windelborn, a fellow working in the laboratory of Charles Emerson at the Wellstone Center at the University of Massachusetts Medical School (UMMS) in Worcester, has developed a novel approach. Windelborn, together with other members of the Emerson lab, has developed methods to engraft muscle stem cells obtained from FSHD patients into regenerating mouse muscles, where they grow and differentiate to form innervated FSHD muscle fibers.

Significantly, his animal imaging studies show that these engrafted FSHD muscles remain stable in the mouse for over four months, and these grafts express FSHD biomarkers associated with D4Z4 genetic deletions and FSHD muscle disease. These findings validate the FSHD stem cell engraftment model and open the door for preclinical studies to investigate and under-



Zebrafish embryos just after hatching at 2 days post-fertilization. They look almost normal, but some of them show body curvature or asymmetric loss of eye color.

stand FSHD muscle pathology and develop therapeutics. This is a major step forward toward FSHD clinical trials.

Therapeutic development studies are now underway using the FSHD muscle stem cell engraftment model as well as a related humanized mouse muscle engraftment model developed by Kathryn Wagner of the Kennedy Krieger Institute in Baltimore, Maryland, who is also a member of the UMMS Wellstone Center research team.

Windelborn's research has been made possible by donors to the FSH Society and by committed FSHD patients and their family members who have contributed muscle biopsies to build and populate the muscle stem cell repository of the UMMS Wellstone Center. This is another example of the vital role that FSHD patients and family members can play by donating tissue to help scientists unlock the mysteries of FSHD.

► TOWARD THE DEVELOPMENT OF A NOVEL XENOGRAFT MODEL OF FSHD

Perspective by Paraskevi Elvina Sakellariou, PhD

Grant title: Investigating Mouse Models of FSHD
Investigators: Paraskevi Sakellariou, PhD, and Robert J. Bloch, PhD, University of Maryland School of Medicine, Baltimore
From February 2011: \$40,000 over one year

Developing and testing therapeutics for FSHD would be significantly advanced if a valid mouse model of the disease were available. Ideally, a murine version of FSHD would reproduce all the features of FSHD muscle and retain the morphological, physiological, and genomic differences found in fresh biopsies. As the pathophysiological mechanisms that lead to FSHD are still unclear, it would be best if such a model were constructed from FSHD tissue itself, rather than by manipulating particular genes or gene products that, although involved, may not be sufficient for pathogenesis. I have been developing such a model, with support from a postdoctoral fellowship from the FSH Society. I have used human muscle precursor cells provided by a collaborator, Woodring Wright, University of Texas Southwestern Medical School, in transplant experiments with immunocompromised mice to create humanized muscles. The cells are injected into the anterior portion of the mouse hindlimb, which I treated earlier to remove the muscle normally present there.

I have combined a novel set of procedures to promote engraftment of the cells and found that the muscle tissue they form is highly differentiated and only slightly contaminated with mouse cells. Moreover, the fibers in the human muscle tissue in the graft are innervated and physiologically active—that is, they contract upon electrical stimulation.

This level of engraftment has never been achieved before. I am now testing modifications of my procedures to promote the formation of even larger grafts and generating grafts of human muscles from cells prepared from individuals with FSHD. The mice carrying these grafts should be very useful in studying the

molecular basis for FSHD and testing pharmaceutical approaches to its treatment.

Editor's note: As with the research of James Windelborn described in this issue, the research described here would not be possible without tissue donations from individuals with FSHD.

► DEFINING THE TISSUE AND CELL SPECIFICITY OF THE HUMAN DUX4 PROMOTER IN MICE

Perspective by Scott Q. Harper, PhD

Grant title: Defining the Tissue and Cell Specificity of the Human DUX4 Promoter in Mice
Investigator: Scott Harper, PhD, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio
From August 2010: \$50,000 over one year


With prior funding from the FSH Society in 2008, we tested the effects of DUX4 gene expression in otherwise normal mouse muscle. We found that the DUX4 gene was extremely damaging. This work supported the hypothesis that DUX4 was involved in FSHD.

These data led us to ask, Can the unique involvement of specific muscle groups in FSHD be caused by preferential expression of DUX4 only in those muscles? In 2010, we were awarded a grant by the FSH Society to address this question. The goal of this project was to determine if the promoter of DUX4 (a promoter is a piece of DNA that serves as a sort of on/off switch for genes) was preferentially “on” in affected muscles and “off” in unaffected muscles.

Our strategy involved creating a line of “reporter gene” mice. Essentially, we inserted the DUX4 promoter in front of the jellyfish green fluorescent protein (GFP) gene and created a new strain of mice whose cells glowed green only where the DUX4 promoter was “on.” As a result, we could then determine which cells and muscle groups were susceptible to DUX4 expression by tracking which ones were green glowing.

Making new lines of mice is costly and time consuming, taking years to produce and analyze. The FSH Society provided us with the initial seed funds to produce these mice, and we used the preliminary data from this study to apply for a larger three-year grant from the Muscular Dystrophy Association (MDA), which was funded in 2011.

This MDA grant extends the FSH Society study so that we are now in the process of using a similar strategy to produce mice that express DUX4 in FSHD-affected muscles. We hope that these animals will be useful for better understanding FSHD disease and, importantly, for developing treatments. We expect to publish some of these findings in the next half-year.

We are forever grateful to the FSH Society for its incredible financial support of our research program. Moreover, we are indebted to Daniel Paul Perez for the moral and intellectual support he has provided to the Harper lab over the last five years. 



PAVING THE WAY FOR CLINICAL TRIALS

Paving the way for clinical trials

Introduction by **JUNE KINOSHITA**. Researcher perspectives compiled and edited by **DANIEL PAUL PEREZ**
FSH Society, Lexington, Massachusetts

The current price tag to develop a new drug is \$100 million. Serious money. Serious risk for pharmaceutical companies. Companies turn to academic researchers and patient advocacy organizations such as the FSH Society to help “de-risk” their investment. They want to know how well supported the disease hypotheses are, whether there are validated therapeutic targets, what compounds look promising, and what “clinical trial endpoints” have been established. Are there changes in patients’ health and function that can be measured that would show if a drug has had an effect—ideally in less than 12 months? For FSHD, that is a tall challenge, because symptoms are different from patient to patient, and the muscle weakness progresses in unpredictable fits and starts. The FSH Society is investing in both development of therapeutic strategies and biomarker/clinical endpoint development in preparation for clinical trials in the near future.

▶ CLINICAL TRIAL READINESS FOR FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY

Perspective by Jeffrey Statland, MD

Grant title: Evaluation of an FSHD-Specific Patient Reported Outcome Measure and a Disease Specific Functional Rating Scale

Investigator: Jeffrey Statland, MD, University of Rochester, New York
From August 2012: \$59,185 over two years

Grant title: Pilot Study of Electrical Impedance Myography in Facioscapulohumeral Muscular Dystrophy

Investigator: Jeffrey Statland, MD, University of Rochester, New York
From February 2013: \$48,909 for one year

As our understanding of the molecular pathophysiology of FSHD advances, researchers are identifying potential therapeutic targets. Consequently, it has become imperative that appropriate clinical trial tools be in place for FSHD. The need to develop such outcome measures was recognized at the FSH Society-sponsored International Research Consortium’s annual meetings as well as at the 171st European Neuromuscular Center workshop on standards of care and management of FSHD.

The aims of our projects are to test the reliability, validity, and preliminary responsiveness to change of three new FSHD outcome measures: 1) a disease-specific, patient-reported health inventory (FSHDHI); 2) a disease-specific functional rating scale (FSHD-FO); and 3) a novel quantitative technique for measuring changes in muscle structure electrical impedance myography (EIM).

Our general research approach follows a three-tiered strategy. We use open-ended patient interviews to identify areas of high

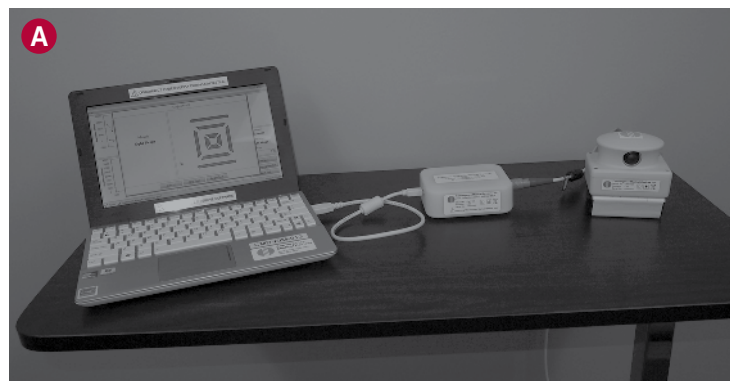


Figure 1. Electrical impedance myography. (A) A portable device with a simple hand-held wand is used to measure impedance over selected muscles. **(B)** Each measurement takes seconds.



impact on FSHD. We review the existing FSHD literature. Then we develop novel clinical trial instruments by using a combination of existing standardized measurements and novel FSHD-specific questionnaires.

The FSHDHI was developed from large-scale survey input from 328 FSHD patients, including 48,000 direct patient responses. Individual questions for the patient-reported questionnaire were screened and selected based on their reported high prevalence and importance to the FSHD population.

The FSHD-FO, on the other hand, took each of these high-impact areas and combined existing standardized measures of motor function into a comprehensive, FSHD-specific battery of tests. Our current study will test how reliable these measures are, how well they relate to other existing muscular dystrophy outcomes not specific to FSHD, and how much they change over time.

In addition, the FSH Society has allowed us to expand our focus to include a novel technique to measure muscle structure. Electrical impedance myography (EIM) is a fast, non-invasive way to obtain quantitative information about muscle structure which may correlate with motor strength and function in FSHD.

The device (Figure 1), manufactured by Skulpt, Inc., of Boston,

Massachusetts, uses a low-intensity electrical current to obtain information about underlying muscle structure by taking advantage of the relationship of muscle structure to the impedance of current flow through the muscle. EIM is well suited toward investigation of muscles important to FSHD but not easily testable by traditional strength measures, including facial, abdominal, and paraspinous muscles.

The funding from the FSH Society makes projects like this possible. The hardest part of any project is obtaining money to get the projects off the ground: The FSH Society grant has enabled us to start recruiting participants to test these outcomes and allowed us to apply for additional funding from organizations such as the National Institutes of Health to complete this project.

It is of vital importance for the FSHD research community that development of outcome measures parallels advancements in molecular pathophysiology and drug development. At the completion of this project, we expect to have three valuable new FSHD-related outcome measures for future clinical trials.

For more details about the pilot study, please visit our website www.fsh-society.org or <http://www.prweb.com/releases/2013/8/prweb10998990.htm>. If you or someone you know has FSHD and is interested in potentially participating in the study, please contact us directly at 781-301-6651 or doris.walsh@fshsociety.org.

► **EFFORTS TO CREATE A BETTER AND MORE ACCURATE DIAGNOSTIC TECHNOLOGY FOR FSHD**
Perspective by Kyoko Yokomori, DVM, PhD, and Shohei Koide, PhD

Grant title: Development of a Novel ChIP-Based Diagnostic Assay for FSHD

Investigators: Kyoko Yokomori, DVM, PhD, University of California, Irvine; and Shohei Koide, PhD, The University of Chicago, Illinois

From February 2013: \$40,000 for one year

The goal of the project is to develop an accurate and robust diagnosis for FSHD. Although FSHD is reported to have a one-in-20,000 incidence, there is great concern that the actual number of affected individuals is significantly higher due to undiagnosed cases (with a likely incidence of one in 7,000).

Most cases of FSHD involve mono-allelic deletion of macrosatellite D4Z4 repeat sequences at the subtelomeric region of chromosome 4q (4qter D4Z4) (FSHD1), while the remaining <5 percent of cases demonstrate no D4Z4 repeat contraction (FSHD2).

Proper diagnosis depends initially on recognizing clinical signs and symptoms and differentiating FSHD cases from other muscu-

lar dystrophies. Molecular studies have been used to reinforce the clinical impression. The primary approach is detection of 4qD4Z4 repeat contraction by pulsed-field gel electrophoresis (PFGE). However, this method cannot identify phenotypic FSHD2, and certain band patterns can prove difficult to interpret. Therefore, we have an urgent need for a better diagnostic technology.

The Yokomori group previously found a specific change in histone modification (histone H3 lysine 9 trimethylation [H3K9me3]) at the D4Z4 repeat sequences that is detected in both FSHD1 and FSHD2 patient cells. Importantly, this change is highly specific for FSHD and is seen also in patient cells from blood samples. Thus, in this project, we plan to test the possibility of using detection of the loss of H3K9me3 in patient chromatin as a diagnostic method for FSHD.

We plan to use peripheral blood mononucleocytes (PBMCs) from patient blood samples. Detection of H3K9me3 loss will be assessed by a biochemical method (chromatin immunoprecipitation [ChIP]). We will assess the specificity of our protocol by testing blood samples from healthy individuals, patients of different ages and disease severities, and individuals with unrelated muscular dystrophies or unrelated diseases.

Related previous grant: Epigenetic Abnormality in FSHD
Investigators: Weihua Zeng, PhD, and Kyoko Yokomori, PhD, University of California, Irvine

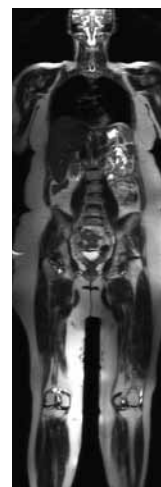
From February 2011: \$8,875 for three-month extension

► **MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY IN FSHD**
By Doris G. Leung, MD

Grant title: Magnetic Resonance Imaging and Spectroscopy Biomarkers in FSHD
Investigators: Doris G. Leung, MD, and Kathryn R. Wagner, MD, PhD, Hugo W. Moser Research Institute at Kennedy Krieger, Baltimore, Maryland
From August 2011: \$100,550 over two years

The Center for Genetic Muscle Disorders at the Kennedy Krieger Institute was awarded the Irene Lai Research Grant from the FSH Society in 2011, and this grant was renewed in 2012. This research study was designed with the purpose of using novel magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) tech-

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Whole-body magnetic resonance imaging of an individual with FSHD



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niques to study skeletal muscle disease in FSHD.

MRI is routinely used in clinical practice to produce anatomical images of different body tissues by using high-powered magnetic fields. MRS utilizes the

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same magnet to measure the concentration of different chemicals in tissues. This project was developed as an ancillary study of the work that is being done by the National Institutes of Health (NIH) FSHD Wellstone Muscular Dystrophy Cooperative Research Center, whose primary goal is to discover disease biomarkers from muscle tissue.

The objective of the MRI/MRS study is to determine whether non-invasive imaging can be used to accurately assess the severity of muscle disease in FSHD. It is anticipated that these techniques could be valuable outcome measures in determining the natural history of disease in observational studies and measuring response to therapy in clinical trials.

Since this study was initiated, 24 FSHD participants have been successfully enrolled. Each of these volunteers has undergone high-resolution anatomic and spectroscopic imaging (using MRS), and the data generated from this study include information about the concentration of fat, water, and other chemical compounds contained in muscle.

The Center for Genetic Muscle Disorders has also recently developed a new collaboration with the Department of Radiology at Johns Hopkins Hospital, which has allowed the incorporation of whole-body MRI into the study protocol. The addition of whole-body MRI will not only allow for the examination of a far greater number of individual muscles and muscle groups, but it may also help detect muscle involvement that occurs prior to the development of symptomatic weakness.

All new patients enrolled in the study are being scanned using whole-body MRI, and the study protocol has been expanded to allow participants who were enrolled prior to the inclusion of whole-body MRI to return for a second study that does include whole-body imaging. These repeat studies will also provide valuable longitudinal data about how the disease in muscle changes over time.

Efforts for the summer and fall of 2013 are focusing on the recruitment of normal controls. The inclusion of healthy controls is crucial to the success of the MRI/MRS project because studying normal individuals without disease will allow researchers to determine what types of imaging and spectroscopy findings are normal or abnormal in the FSHD population.

The control group will consist of healthy volunteers with no history of neuromuscular disease and who are willing to undergo genetic testing to confirm that they do not have FSHD. Based on the existing group of participants, we are particularly looking for healthy volunteers in the following groups: females ages 66-70, 56-62, 26-32, or 20-25; and males ages 67-73, 58-61, 49-55, 42-48, or 32-38. Doris Leung can be contacted at 443-923-9512 or leungd@kennedykrieger.org, and additional information on the trial can be found on www.clinicaltrials.gov (NCT01671865).

The Center for Genetic Muscle Disorders would like to express

our deepest gratitude to the research volunteers for their participation, and to the FSH Society for its continued support of our research endeavors.

▶ DISCOVERING DRUGS THAT PROTECT CELLS FROM DUX4 Perspective by Michael Kyba, PhD

Grant title: Additional Support for Medicinal Chemistry Developing Anti-DUX4 Therapeutics for FSHD

Investigator: Michael Kyba, PhD, Lillehei Heart Institute, University of Minnesota, Minneapolis

From August 2011: \$25,000 over two years

Progress in the development of a therapy for FSHD was hampered for many years by a disconnect between the genetic mutation that is associated with FSHD and the specific gene whose activity is responsible for muscle pathology. The reason for this disconnect is that the mutation that causes FSHD, unlike the mutations that cause most genetic diseases, does not actually cause the loss of a critical protein. Rather, it changes the number of copies of a DNA sequence that is repeated in many places in the genome.

In the past several years, it has become clear that the FSHD mutation is actually doing the opposite of what most mutations do. Rather than eliminating a critical protein, it is causing the acquisition of a pathogenic protein. The discovery that this protein, named DUX4, is expressed in FSHD patients, combined with the observation that this protein can have very deleterious effects on muscle cells in the petri dish, even when present in only miniscule quantities, has given us a target to aim for in the race to develop a treatment for FSHD.

My laboratory has been engaged in the search for ways to disable the DUX4 protein. One approach we have taken is to screen a large "chemical library," a collection of 200,000 small drug-like chemical compounds. Within this more or less random collection of molecules, we have identified approximately 1,000 compounds that had the effect of allowing cells to survive toxic levels of DUX4 protein.

With grant support from the NIH, combined with supplemental support from three foundations (the FSH Society, the Friends of FSH Research, and the FSHD Global Research Foundation), we have begun sieving through these compounds with the hope of finding one or more that might be suitable for development into a drug that could protect the muscle of FSHD-affected individuals from the damaging effects of DUX4. These compounds can act in a number of ways. For example, they might modify the state of the cell, making it resistant to DUX4, or they might bind to and block the function of DUX4 itself. Determining precisely what these compounds are doing is the major current focus of this research.

We hope to narrow down the size of this large set of compounds to two or three specific groups that are active against the DUX4 protein and suitable for next-phase drug development studies. Philanthropic support, including that of the FSH Society, has been of great benefit to this research.

► **THERAPEUTIC INROADS: DEPLOYING THE ANTISENSE OLIGOMER (AO) THERAPEUTIC STRATEGY AGAINST FSHD DISEASE-CAUSING TARGETS**

Perspective by Eugénie Anseau, PhD, and Alexandra Tassin, PhD

Grant title: Antisense Strategies Against DUX4 as Therapeutic Approaches for FSHD

Investigators: Eugénie Anseau, PhD, and Alexandra Belayew, PhD, Université de Mons, Belgium

From February 2011: \$70,500 over two years

Grant title: Testing a Therapeutic Approach for FSHD: Evaluation of the Efficacy of AOs Blocking DUX4 in a Mouse Model of Isolated Myofibres

Investigators: Alexandra Tassin, PhD, and Alexandra Belayew, PhD, Université de Mons, Belgium

From February 2011: \$15,000 over one year

Although the precise mechanism that causes FSHD is not fully understood yet, it clearly involves increased activity of the DUX4 gene that we discovered in our laboratory over 15 years ago. The DUX4 gene carries the recipe to make a protein that acts as a sort of crazy orchestra conductor. This DUX4 protein activates many genes that are normally not active in muscle cells and silences others that are needed to help protect muscle or repair damage. The resulting gene deregulation in FSHD muscle cells can explain the main features of the disease such as muscle wasting. It thus follows that a reduction of DUX4 amounts may offer a treatment for FSHD.

Toward this goal, we collaborated with Steve Wilton, now at Murdoch University, Western Australia, who is a world expert in the therapeutic use of a synthetic DNA form named antisense oligomer (AO), which can interfere with the making of a protein. This AO therapeutic strategy does not target the gene itself but interferes with the protein synthesis process.

Because the gene cannot leave the cell nucleus, it is copied on a messenger RNA (mRNA), which carries the protein recipe outside the nucleus to the place in the cell where the protein is to be made. We designed AOs targeting the DUX4 mRNA that can suppress DUX4 protein expression in human muscle cells grown in the laboratory and, most interestingly, also interfere with the wasting process induced by DUX4. These cells were kindly provided by Dalila Laoudj-Chneivess, University of Montpellier, France.

The current FSH Society support to Eugénie Anseau, PhD, is helping us to test whether additional AOs targeting other features of the DUX4 mRNA or AO cocktails might be able to suppress the DUX4 protein as efficiently when used in lower amounts.

As a next step in the preclinical development of a putative therapy for FSHD, the best AOs have to be tested in an animal model. However, to date no mouse model fully recapitulates the FSHD disease features, and we have decided to test our AOs in several models, none of which is ideal but which each present different useful properties.

With an FSH Society fellowship, Eugénie Anseau was able to spend three months in the laboratory of Scott Harper, Ohio State University, Columbus, to perform a first experiment to test three of those AOs in a first mouse model. Harper has modified a virus to produce DUX4 and injected it into a mouse leg muscle, causing an FSHD-like toxicity. Importantly, co-injection with our AOs reduced DUX4 messenger RNA in the mouse muscle, thereby supporting further development of our AO strategy in this animal model.

Alexandra Tassin, PhD, is creating other mouse models. Aline Derenne, a student in Alexandre Legrand's laboratory, University of Mons, Belgium, is funded to train on models and has recently established two additional models with funding from the Association Française contre les Myopathies (AFM). In these animal models, the DUX4 gene is injected as naked DNA (no virus) in a mouse leg muscle and followed by an electric shock, or injected under pressure in a leg muscle vein; both methods allow DNA distribution in the whole muscle.

Additional FSH Society funding has allowed Alexandra Tassin to start collaborating with Peter Zammit, King's College, London, United Kingdom, with the aim of establishing yet another mouse model in an ongoing project. The AOs will be tested in these models to evaluate their activity and lack of toxicity in a live animal. Overall, funding from the FSH Society has thus provided a major help in the development and evaluation of these AOs, which we hope will lead to therapeutic applications.

► **PROTECTIVE TAILS AND HOW TO REMOVE THEM**

Perspective by Antoine de Morrée, PhD

Grant title: Toward Therapeutics for FSHD: Understanding mRNA Processing

Investigators: Thomas A. Rando, MD, PhD, and Antoine de Morrée, PhD, Department of Neurology and Neurological Sciences, Stanford University School of Medicine, California

From August 2010: \$100,000 over two years, with matching funds from the Stanford Office of Medical Development and Gary Steinberg, Stanford Institute for Neuro-Innovation & Translational Medicine (SINTN)

FSHD is a muscle disease for which no treatment exists. It is not clear exactly what causes the disease, but recent research findings suggest that a toxic protein called DUX4 is involved. Not much is known about the DUX4 protein, or how and why the muscle makes it, given that it is toxic. A better understanding of how cells make DUX4 will help in the design of treatments that would prevent the muscle from making it. Therefore, we wanted to know more about DUX4 production.

Proteins are encoded in DNA in the form of genes. Each gene codes for a particular protein. When a cell decides to make that particular protein, it turns to the corresponding gene and uses the information in the gene to write a message to the protein production machinery. This message comes in the form of a molecule aptly

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named messenger RNA, or mRNA, and functions as a straightforward recipe to build the protein. In the case of FSHD, the DUX4 gene gives rise to DUX4 mRNA messages that enable the cell to make the toxic DUX4 proteins.

Strikingly, the muscles of healthy individuals do not make DUX4 protein because they do not have any mRNA for it. They do have the DUX4 gene and write the messages. However, these messages are unstable and are destroyed before they ever reach their target—the protein production machinery. The DUX4 gene lacks information that is important to stabilize the DUX4 messages and prevent them from destruction. Thus, healthy muscles are protected from making too much of the toxic protein.

FSHD patients, on the contrary, have a mutation in the DUX4 gene that allows the cells to protect the DUX4 mRNA messages by stabilizing them. Accordingly, the muscles of FSHD patients do not destroy the mRNA messages and instead accumulate them. These messages reach the protein production machinery and, as a result, the muscles make too much of the DUX4 protein at the wrong time. If we could prevent the stabilization and accumulation of the mRNA, that would stop the production of DUX4 and allow the muscles to “detoxify.” This might be a way to turn an FSHD cell into a healthy cell.

Cells stabilize mRNA messages by adding a protective tail to the end of the message. We investigated how cells choose whether or not to add a protective tail to a message. We noticed that, for most genes, the cells can choose where to attach the protective tail. Specific information written in the messages tells the cell where it can add a tail and where it cannot.

By comparing mRNA messages from many different species, we discovered information that is conserved by evolution. We expect that this conserved information is important and is what lets the cell know where it can add the protective tail. If we find a way to censor this information so that the cell can no longer read it, the cell will not know where to add the tails. In that case, the DUX4 messages would no longer be stabilized and would be destroyed before giving rise to toxic proteins.

We used synthetic reporter genes to investigate which parts of information change the cell’s ability to add a protective tail. We now hope to use this knowledge to coax the cells into thinking that the DUX4 messages do not need a protective tail and thereby change an FSHD cell to a healthy cell. These discoveries can therefore lead to the development of future treatments that will reduce the toxicity in the muscles of patients with FSHD.

Research support from agencies such as the FSH Society, though small, is very important. It stimulates unique research into specific disease mechanisms and helps beginning scientists build their careers and research focus.

- ▶ **Grant title: A Multicenter Collaborative Study on the Clinical Features, Expression Profiling, and Quality of Life of Pediatric Facioscapulohumeral Muscular Dystrophy**
Investigator: Jean Mah, MD, Alberta Children’s Hospital, Calgary
From August 2010: \$96,669 over two years.
Project is being co-funded by the Muscular Dystrophy Canada FSHD Fund.

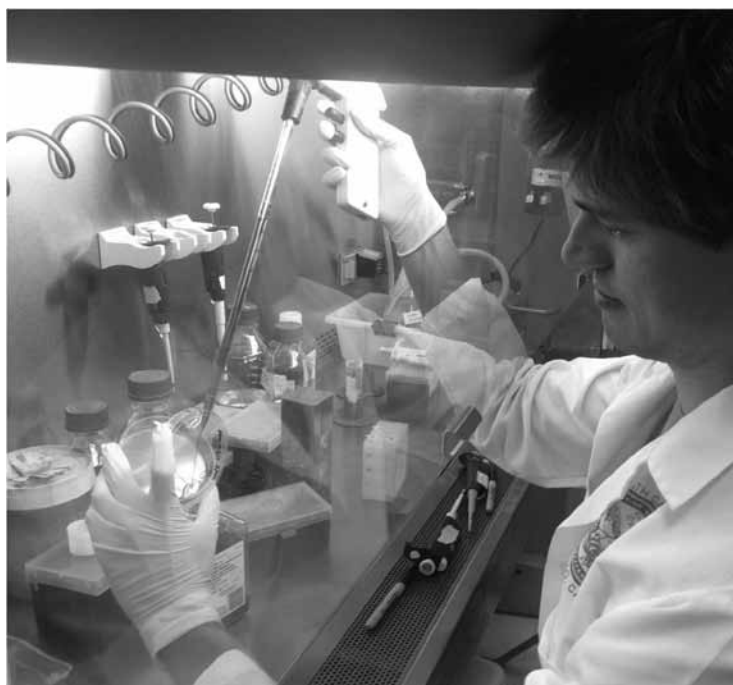
For more information on the study and recruitment, please see the article “Cooperative International Neuromuscular Research Group recruiting patients with infantile-onset FSHD” by Zoë Sund on page 9.

- ▶ **FSH SOCIETY SANDY BATKIN SMALL RESEARCH GRANT TO GENILA BIBAT AT THE KENNEDY KRIEGER INSTITUTE REVITALIZES MID-ATLANTIC SUPPORT GROUP**
Perspective by June Kinoshita

- ▶ **Grant title: FSH Society Mid-Atlantic Patient Outreach, Education and Support Group**
Investigator: Genila Bibat, MD, Kennedy Krieger Institute, Baltimore, Maryland
From February 2013: \$20,000 over two years

The FSH Society has awarded a grant of \$20,000 over two years to Genila Bibat to establish a patient support group program serving FSHD patients in the mid-Atlantic region. We included this award under “Paving the way for clinical trials” because networking and educating patients about their role and their voice in clinical research are an essential part of preparing for clinical trials.

Patients with FSHD in Maryland, Northern Virginia, and Southern Pennsylvania have been gathering for over a decade in private homes to discuss common problems and solutions. These gather-



Antoine de Morrée at work under the hood

ings were initially organized by the late Karen Johnson (a longtime member of the FSH Society's Board of Directors) and more recently by Lilleen Walters. On occasion, speakers have presented informally at these gatherings on topics of interest to the FSHD community.

These events have been very well attended, but there have been some limitations, the greatest of which have been the lack of a dedicated organizer to ensure regular meetings and no fully accessible meeting place in a location central for patients in the mid-Atlantic region.

Under this grant, Bibat and Walters have established a well-organized support group for families, friends, and patients with FSHD which is held regularly at the Kennedy Krieger Outpatient Center in Baltimore, Maryland. The center is fully accessible, with contiguous parking, and has a cheerful atmosphere for meeting. Its location is well known to many in the FSHD community who are patients at KKI and is within a reasonable drive from Northern Virginia, Southern Pennsylvania, Washington, DC, Delaware, and Maryland.

Each meeting features invited speakers who share information about FSHD research as well as practical discussion about living with FSHD, including surgical treatments, genetic counseling, family planning, adaptive sports, pursuing higher education, disability rights, and employment issues. At many of these meetings, patients are also offered opportunities to contribute data to ongoing studies. The meetings are held quarterly and livestreamed over the Internet.

Advertisement-free livestreaming is underwritten by a gift from Friends of FSH Research.

SCREENING FOR SMALL MOLECULE INHIBITORS OF DUX4 Summary provided by Darko Bosnakovski

- **Grant title:** Small Molecule Screen to Identify Inhibitors of DUX4-Mediated Toxicity, Therapeutic Approach for FSHD
Investigator: Darko Bosnakovski, DVM, PhD, University "Goce Delcev" Stip, Faculty of Medical Sciences, Macedonia
From August 2010: \$90,000 over two years

The goal of our project is to discover a chemical compound that efficiently inactivates the DUX4 protein and to work toward a drug for a therapeutic approach to FSHD. The aims of the proposed study target the most crucial topic and urgent need of FSHD patients: specific and direct pharmacological therapy. The work of the project will first narrow down a small subset of compounds from 82 potential compounds to inactivate DUX4, implicated as necessary to cause FSHD, as a result of high-throughput screening to the most promising direct *DUX4* inhibitors. Second, scientists will evaluate the effectiveness of *DUX4* inhibition and study the properties of the selected compounds. [FSH Watch](#)

FSHD Champions

Connecting and communicating on FSHD

FSHD Champions is an informal, international group consisting of 14 FSHD advocacy and research funding organizations working together to promote collaboration and transparency in FSHD research. Kees van der Graaf of Stichting FSHD and FSHD Europe, and Daniel Perez of the FSH Society co-organized the inaugural meeting, which was held in November 2012 alongside the FSHD International Research Workshop in San Francisco, California. The meeting was a resounding success! A charter for the FSHD Champions group was developed with the goal of forming a voluntary, informal federation open to all organizations funding FSHD research. The mission is to:

- promote communication and collaboration between FSHD researchers and FSHD research organizations/charities;
- have the opportunity to work together to fund research projects that are beyond the scope of the individual organizations;

- work together to advocate for greater FSHD research funding from organizations such as the U.S. National Institutes of Health, governmental agencies and NGOs in the European Union, and muscular dystrophy associations and charities worldwide;
- work collaboratively to promote international awareness of FSHD and the need for FSHD research funding;
- share expertise and best practices for the overall promotion and success of the individual FSHD research initiatives;
- work toward the availability of affordable and effective treatments for FSHD, and advocate for reimbursement of such treatments.

Since our initial meeting we have had monthly webinar/teleconferences and a variety of working groups. We're making excellent progress on a number of fronts. Members of FSHD Champions include: Association

Française Contre les Myopathies (AFM), Carrino Foundation, Friends of FSH, FSHD Canada, FSHD Europe, FSHD Global Research Ltd., FSHD Stichting, FSH Society, Muscular Dystrophy Association (MDA), MD Campaign (UK), U.S. National Institutes of Health (NIH), Prinses Beatrix Fund, Shaw Fischer families, and Vereniging Spierziekten Nederland (Netherlands Neuromuscular Diseases Association—VSN).

The next Champions in-person meeting will be held on October 23, 2013, in Cambridge, Massachusetts, the day after the FSHD International Research Consortium meeting. Members from each organization's executive staff and Board of Directors will attend to discuss how to achieve faster progress by working more closely together. Kees van der Graaf and Daniel Paul Perez will be the co-conveners of the meeting. [FSH Watch](#)

Science Terms

4q35. A cytogenetic term that defines the “address” of the chromosomal deletion associated with FSHD Type 1. The deletion is on chromosome 4, on the “q” (long) arm at region 35.

4q35 genes. A group of genes located in the 4q35 region, some whose activity is regulated by the D4Z4 repeat array.

Allele. An alternative genetic sequence at a given chromosome location, governing the same physical trait. Typically, you inherit one allele from your mother and one allele from your father.

Antibody. A protein produced by the immune system which binds specifically to unique features of other proteins and macromolecules. Exploited by the immune system to destroy foreign materials and by scientists to identify specific molecules.

Antisense oligomer. A single-stranded chain of nucleotides that bind to a specific sequence of RNA, thereby preventing its genetic information from being expressed.

Autophagy. The degradation of unnecessary or dysfunctional cellular components via lysosomes (compartments inside the cell that digest cellular waste).

Binding. The interaction of one molecule to another, which causes a change in the shape and function of the target molecule.

Biomarker. An objective measurement that indicates a patient’s health status, such as a test of muscle strength, a blood test, imaging, etc. Diagnostic biomarkers show if a person has a disease.

Therapeutic biomarkers show if a therapy is having the desired effect.

Chromatin. The combination of DNA and proteins (primarily histones) that make up the contents of the nucleus of a cell. The main functions of chromatin are to package DNA into a smaller volume, strengthen the DNA to allow cell division, prevent DNA damage, and control gene expression and DNA replication.

Chromosome. A recognizable organization of chromatin containing genes, regulatory elements, and other nucleotide sequences exploited in the study of cytogenetics. In humans, each cell nucleus contains 23 distinct pairs of chromosomes.

Clinical trial. A test of drugs, diagnostics, and other health interventions in human volunteers, conducted to generate safety and efficacy data.

Cytoplasm. The jelly-like part of the cell lying between the cell membrane and the nuclear membrane.

D4Z4. A repeated DNA segment of approximately 3,300 nucleotide bases. The number of repeats can vary significantly in different individuals and can be used to predict FSHD status. Loss of these repeats at chromosome locus 4q35 is associated with FSHD Type 1. Each D4Z4 contains a DUX4 gene.

Deletion. Loss of part of a DNA sequence, as in “deletion of D4Z4 repeats at 4q35” associated with FSHD.

Differentiation. The process by which a less specialized cell (e.g., in an embryo) becomes a more specialized cell type, such as skin,



heart, muscle, or neuron. In adults, stem cells divide and differentiate into daughter cells during wound healing and normal cell turnover.

DNA. The molecule that defines our genetic inheritance. It contains all the instructions for constructing and operating a living organism. DNA is made of four simple units, or “nucleotide bases,” whose specific sequence “spells out” genetic information.

DUX4 (double homeobox 4). A copy of the DUX4 gene is located within each D4Z4 repeat array on chromosome 4. Inappropriate expression of DUX4 in muscle cells is thought to be a contributor to FSHD.

Epigenetics. Refers to chromatin modifications that change gene function without changing the underlying DNA sequence. Examples of these modifications are DNA methylation and histone modification, both of which serve to regulate how a gene becomes expressed as protein. Some of these changes have been shown to be heritable.

Expression profiling. The first step in converting genetic information to an observable trait involves the transfer of information from gene to protein through an intermediate molecule called RNA. Measuring all of the RNA produced in one definable moment to create a global picture of cellular function produces a profile of total expression activity.

FISH (fluorescent in-situ hybridization). A laboratory technique, commonly employed in the field of cytogenetics, that uses fluorescent probes to detect and localize the presence of specific DNA sequences on chromosomes.

Gene. The basic unit of heredity defined by specific segments of DNA. Genes specify the instructions needed to produce an observable trait.

Gene expression. The initial step in converting the information contained in a gene, as DNA, to an observable character through an intermediate molecule RNA.

Gene locus. The physical location of a gene on a chromosome.

Genotype. The actual DNA sequence of a cell, organism, or individual, usually with reference to a specific characteristic under consideration.

Germline. The collection of biological cells that give rise to gametes (eggs or sperm).

Histone. The chief protein component of chromatin, acting as a spool around which DNA winds. It plays a role in gene expression.

Homeobox. A DNA sequence found within genes that are involved in the regulation of patterns of anatomical development in organisms.

Immortalized cells. Cells that continue to divide indefinitely, unlike normal cells which, with normal aging, cease to divide.

Immunocompromised. A state in which the immune system is defective and unable to mount an

immune response against disease pathogens or other foreign cells.

Innervate. The formation of a functional connection between nerve and muscle or other target tissue.

Longitudinal study. A research study involving repeated observations of the same variables in the same individuals over long periods of time.

Lysosome. A cellular organelle that contains enzymes that break down waste materials and cellular debris.

Magnetic resonance imaging (MRI). A medical imaging technique using the property of nuclear magnetic resonance to image the nuclei of atoms inside the body. MRI can create more detailed images of the human body than are possible with X-rays.

Magnetic resonance spectroscopy (MRS). A research technique using nuclear magnetic resonance to provide information about chemical properties of atoms and molecules.

Methylation. In biological systems, the addition of a methyl group to DNA, which can regulate the expression of genes. Some methylation changes are heritable.

Molecular pathway. A chain of chemical reactions occurring within a cell.

Mutation. A change in the nucleotide sequence in the genome of an organism with perceived deleterious effects.

Myocyte. A long, tubular cell found in muscle. Myocytes develop from myoblasts to form skeletal muscle fibers (myotubes) in a process known as myogenesis.

Nucleus. A large cellular organelle enclosed by a membrane that contains genetic material (DNA).

Organelle. A specialized subunit within a cell that has a specific function.

Pathophysiology. Physiological processes or mechanisms whereby disease develops and progresses.

Phenotype. The composite of an organism's observable characteristics or traits, such as its physical form, growth, biochemical and physiological properties, and behavior.

Pluripotent cells. A stem cell that has the potential to differentiate into diverse types of cells. Induced pluripotent stem cells (iPS cells or iPSCs) are a type of pluripotent stem cell artificially derived from an adult somatic cell by inducing a "forced" expression of certain genes.

Polycomb. A family of proteins that can alter chromatin such that genes are epigenetically silenced (unable to be expressed).

Protein. A biological molecule consisting of linear chains of amino acids. Proteins are the principal products of genetic information, and they do the bulk of work required for life. They perform a vast array of functions within living organisms, including providing cells with structure, mediating biochemical reactions, transmitting biological signals, and transporting molecules from one location to another.

Protein synthesis. The cellular production of proteins using information encoded in genes. The genetic code on DNA is transcribed into messenger RNA and then trans-

lated into a chain of amino acids to form protein.

Reprogramming. Conversion of a cell from one cell type to another. This involves conversion first to a pluripotent state, then re-differentiation to the new cell type.

RNA (ribonucleic acid). A family of large biological molecules that perform vital roles in the coding, decoding, regulation, and expression of genes. Like DNA, RNA is assembled as a linear chain of nucleotide "bases" but is usually single stranded.

Somatic cell. In multicellular organisms, any biological cell forming the body, other than a stem cell or germ line (reproductive) cell.

Stem cell. An undifferentiated biological cell that can differentiate into a variety of specialized somatic cells (pluripotent) and divide to produce more stem cells.

Target. A protein or nucleic acid (DNA or RNA) whose activity can be modified by an external stimulus, such as a drug or another biological molecule (e.g., hormone, antibody, neurotransmitter, etc.).

Telomere. A region of repetitive nucleotide sequences at the tip of the arm of a chromosome which protects the end of the chromosome.

Transcription. The copying of DNA into messenger RNA in gene expression.

Transgenic. An organism that has had exogenous genetic material (or transgene) introduced into its genome so that it will exhibit a new property. FSH Watch

Introducing the next generation of doctors to FSHD

Getting poked and prodded can be empowering

by **ASIFA LALJI**

Vancouver, British Columbia, Canada

Those of us with FSHD can often feel very powerless and frustrated by the lack of knowledge and understanding about our complicated disease. With all the ground-breaking advances in research over the last few years, FSHD is still a mystery to the broader medical community. While we all hope more researchers are inspired to continue the great work of a few dedicated people, I believe we can all do something to help this along.

I have participated in clinical neurology sessions as well as the patient-physician at-home interviews for medical students at the University of British Columbia. In these sessions, I am able to represent patients with



Asifa Lalji

FSHD to these emerging medical professionals and give them the opportunity to come face to face with this complicated disease.

While it can be unsettling to offer yourself up to be poked and prodded by 30 young strangers, especially when you are not sure you are ready to tell the world you have FSHD, I have found

the experience to be very empowering and would recommend it to anyone who has the opportunity.

The insights you can provide from a physical and emotional perspective are invaluable. How better to find out about what we are going through than to ask one of us? I know

this to be true when I see the same look of surprise and intrigue in each person I talk to about FSHD. The idea of not being able to perform simple tasks like washing your hair or holding a drink for too long seems very odd to hear from a person who just seems like everyone else.

This opportunity came to me through my neurologist, who is also a professor at the University of British Columbia Faculty of Medicine. My guess is that your neurologist might have a similar connection. Just volunteer! It's a great way to raise awareness of FSHD and create a personal connection. That personal connection is one of the reasons researchers choose to continue working to find a treatment for FSHD. FSH Watch



NEWS AND EVENTS

SHIFT Communications to help get our message out

National PR firm joins the FSH Society team

The FSH Society's newest partner, SHIFT Communications, came onboard at the beginning of the summer to provide pro bono assistance to the Society in spreading awareness about FSHD and the organization's educational and research efforts to find a treatment.

The award-winning Boston-based firm, with offices in New York City and San Francisco, has offered the Society access to a wide variety of experienced media professionals who bring extensive knowledge of health-care along with a mastery of public relations, digital media, and creative needs.

With over 100 full-time staff members across the country who have worked with everything from consumer products to pharmaceutical companies, SHIFT will be an important partner as we shape a call to action to draw attention to FSHD and garner more support. You can learn more about SHIFT at www.shiftcomm.com. FSH Watch



The SHIFT Communications team

2013 FSH Society Events

Register now! For details, visit www.fshsociety.org/pages/conEvents.html

Hustle4Muscle Golf Tournament

Friday, October 4

7:15 a.m.-9:00 p.m.

Abilene Country Club
Abilene, Texas

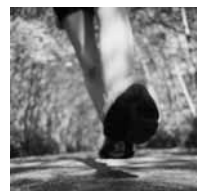


Fourth Annual Celebrity Charity Walk 'n' Roll for FSH Muscular Dystrophy

Sunday, October 6

10:00 a.m.-2:00 p.m.

Preceded on October 5 by a Fireside Chat with Gregory Block, PhD
Heritage Park
Irvine, California



Fifth Annual Cape Cod Walk 'n' Roll for FSH Muscular Dystrophy

Saturday, October 19

Noon-4:00 p.m.

Harwich Community Center
Harwich, Massachusetts