



FSH Watch

A publication of the Facioscapulohumeral Muscular Dystrophy Society
 Connecting the community of patients, families, clinicians and investigators

FSH Society Helen Younger & David Younger fellowship grantee

Kyoko Yokomori, D.V.M., Ph.D.

FSH Society Helen Younger and David Younger fellow 1

FSHS-HDY-001 for *“The molecular characterization of the chromatin structure of the D4Z4 repeat associated with FSHD”*

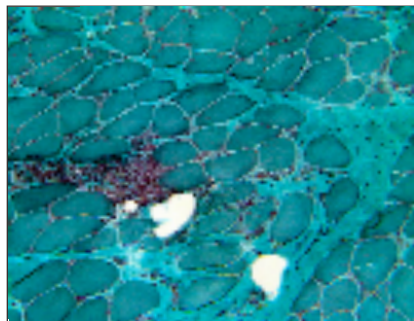
Total project \$90,000

by Kyoko Yokomori, D.V.M., Ph.D.

My name is Kyoko Yokomori. I am an Associate Professor in the Department of Biological Chemistry, School of Medicine at the University of California, Irvine. My colleagues include Drs. Leslie Lock, Sara Winokur, Virginia Kimonis and Doug Wallace, who are also interested in FSHD and other aspects of muscular dystrophy research. My specialty is in basic research, including molecular biology, biochemistry and cell biology. Specifically, my laboratory focuses on chromatin structure and how that affects genome functions, including DNA repair and gene expression. Dr. Weihua Zeng, a former graduate student and current postgraduate researcher in my laboratory, is a recent recipient

of the Helen and David Younger Fellowship from the FSH Society. We are interested in FSHD because we found evidence that chromatin structure plays an important role in FSHD

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FSHD muscle tissue from biopsy viewed under a microscope

FSH Society Jeff Jacobs Family Research fellowship grantee

Scott Harper, Ph.D.

FSH Society Jeff Jacobs Family research fellow 1 & FSH Society Landsman Charitable Trust fellow 2

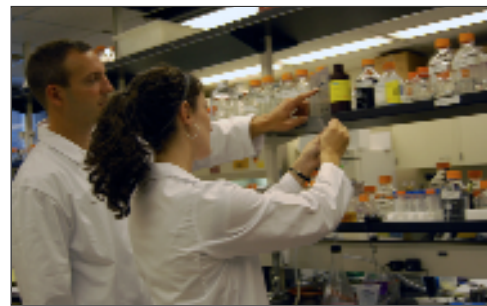
FSHS-JJFR-001 for *“Investigating DUX4 Structure and Function Using Rational Mutagenesis”*

Total project \$40,000

by Scott Harper, Ph.D.

I am an Assistant Professor of Pediatrics at The Ohio State University and Principal Investigator (PI) in the Center for Gene Therapy at the Research Institute at Nationwide Children’s Hospital in Columbus, Ohio. I spend most of my time doing research on FSHD and developing

novel molecular therapies to treat dominant muscular dystrophies, but I also teach graduate level classes in the OSU College of Medicine. As a recipient of FSH Society support from the Landsman and Jacobs Family fellowships, I am happy to discuss how the FSH Society has been instrumental in helping me develop my research program.



Scott Harper, Ph.D. and Lindsay Wallace, Ph.D. student, in the Molecular Cellular and Developmental Biology graduate program at OSU working on DUX4

The road to becoming a faculty member and PI at a major research institution is long. I spent five years earning my bachelor’s degree in biology while a member of the U.S. Naval

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I AM GRATEFUL TO THE FSH SOCIETY AND ITS DONORS

The FSH Society has been instrumental in helping me get my lab off the ground. I am grateful to the FSH Society and its donors, especially the Landsman and Jacobs Families for whom our fellowships are named, and I can assure you that we in the Harper Lab are humbled by your important contribution to our lab and work. If we are so lucky to have some success in the future, the FSH Society and its donors will deserve a great deal of credit. —Scott Harper

Volunteers with FSHD and unaffected family members needed for blood and muscle samples!

Please consider making the valuable gift of muscle tissue and blood samples to advance research efforts on FSHD. Muscle samples are in extreme short supply and tissue donors are needed. The FSH Society and Johns Hopkins School of Medicine, as part of the NIH BBRI Sen. Paul D. Wellstone MD CRC for FSHD, are recruiting volunteers with FSHD and their unaffected first degree relatives (for example: parent, brother, sister, or child of a person with FSHD) to provide muscle and

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What can YOU do?

by Daniel Paul Perez, President & CEO, FSH Society

It is great to be back with you one year after the last *FSH Watch Research Edition*.



Daniel Paul Perez,
 President & CEO,
 FSH Society

Exceptional progress is being made in all aspects of FSHD research worldwide, thanks to your support and our combined efforts and work!

We've asked the researchers and scientists of the fourteen FSH Society fellowship grants that have been completed,

initiated or continued since the last edition of the research newsletter to write in their own words: who they are, what they are doing, how this benefits you, why FSH Society programs, staff and funding are important to them, and how this has helped their careers and FSHD research. There's a wonderful spirit that pervades this group of remarkable scientists and it is evident that they are very near to the reality of breakthroughs in the molecular genetics of FSHD.

However, please know that help for FSHD researchers and patients must continue *at full speed ahead*. We are organizing programs, research and meetings to accomplish this. Please check www.fshsociety.org for updates on the 2009 international research workshops, the 2010 FSH Society International FSHD Patient-Researcher meetings and much more. I am available to consult with and help any corporation, individual, foundation, philanthropist, or organization with research programs and interests in FSHD. Please call to discuss how you'd like to help.

Much is happening at the U.S. federal level for research programs on FSHD. I continue to serve on the congressionally mandated federal advisory committee called the Muscular Dystrophy Coordinating Committee (MDCC). We are hard at work focusing and implementing the NIH Muscular Dystrophy Research and Education Plan submitted to Congress by the MDCC. This year we will strive to open access for

all of the dystrophies to the \$30 million of earmarks by the Department of Defense (DoD) available solely for Duchenne dystrophy in the Defense Health Program Research and Development Test and Evaluation appropriations. It simply is not good science to have only one type of muscular dystrophy research — not all types of muscular dystrophy — competing head-to-head in peer review. We will continue our efforts to open up the \$25 million spent on Duchenne-only programs through the Centers for Disease Control (CDC) epidemiology and surveillance projects to all of the dystrophies, and we will begin efforts to educate the Veterans Administration on FSHD to better serve veterans with FSHD.

We are utterly dismayed that the National Institutes of Health (NIH) have, to date, achieved an FSHD funding portfolio of \$3 million out of a total of \$56 million for muscular dystrophy research. Especially now, given that FSHD is published as the most prevalent muscular dystrophy of children and adults! (See article on page 22.)

We have gone on record with Congress and the NIH. Every now and again, I ask you to communicate with your Congressmen and Senators about FSHD. Each of us in the U.S. has two Senators and one Representative and they need to be made aware of what FSHD is and how it affects you, your health and health care, of the existence of our FSH Society, of the compelling need for the NIH to fund and support FSHD research at a much higher level and, finally, for the DoD and CDC to lift the restrictions barring FSHD. **Make no mistake — our voices will be heard!**

We must all understand that with the NIH now directly funding all types of muscular dystrophy research, an unanticipated difficulty has developed for the FSH Society and for our research. FSHD research funding at the federal level will not grow substantially unless FSHD researchers have sufficient data, findings, and numbers of eligible scientists to submit competitive high-quality grant applications to the NIH. The NIH

What can YOU do?

continues to say that it does not receive enough applications on FSHD. And why is this? Any number of reasons: first, we do not have the dollar resources to grow the field as quickly as we need to. As supporters of FSHD research, the Society and its members, must ensure that grantees aggressively pursue and apply for NIH grants to obtain federal funds rather than rely on soft money.

Secondly, for FSHD research to become a priority, more clinicians and researchers need to be trained and mentored, and of those, more need to commit to careers in FSHD research and pursue funding from NIH. It should concern anyone with FSHD that, despite the existence of a number of large FSHD research centers — that have been in existence for decades — we have not seen new clinicians and researchers appear from these institutions.

For an excellent illustration of how the FSH Society begins the process with seed money, and researchers ante up data and discovery to multiple large-scale funding sources, see the article on page nine: *Your dollars at work: An example of how we get it done.*

The NIH Boston Biomedical Research Institute/Harvard Medical School Sen. Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD, formed nine months ago, is off to a strong start. Co-directors Charles P. Emerson, Ph.D., BBRI, and Louis Kunkel, Ph.D., Harvard Medical School, are working diligently, and moving quickly to power-up the center — and they need our help to make research possible. This is a remarkable center as evidenced at its first

annual research retreat held in June. A key fact that FSHD patients must understand is that the lack of large numbers of well-defined, studied and characterized human tissue and cell lines are choking the growth of FSHD basic research and barring our way to clinical research.

The heart and core of the Wellstone MD CRC research is to break through the sound barrier of competitive exclusivity so that labs no longer go it alone. Instead, researchers can begin corroborating and reproducing results by working with the same standardized samples. These tissues and cells will help elevate the number and quality of applications to the NIH. The FSH Society serves as the Office of the Patient Communication and Liaison to the Wellstone MD CRC. We invite you to contact us and the center. Volunteers are urgently needed for muscle biopsy studies. Please contact us today!

It is obvious now why I told you all of this. The Society does not receive funding from any government source, including the new Wellstone Center for FSHD research. We are a grassroots non-profit committed to finding a cure for FSHD. Operating funds come solely from donations that are not earmarked for research. The Society needs both donations restricted to research and unrestricted funds so that we can continue.

If we all do our part, when we meet again a year from now, we will have a successful FSH Society leading us with power and assurance towards achieving our goals of finding the cause of our disease, and finding the means of

reducing its effect upon our bodies and our lives. That hope may now be within our grasp. It is up to us to help get our Society there.

And you and me, all of us — what's our part? Here are five things we can do:

1. We can solicit contributions and memberships — from our mothers and fathers, sisters, brothers, aunts and uncles, cousins, close friends, business associates, etc. You can donate online at www.fshsociety.org and click “Contribute.”
2. We can make sure our primary care doctors, neurologists, and physiatrists and therapists, receive our literature, are aware of what our Society does, and are given the opportunity to join our Society.
3. We can make both restricted and unrestricted tax free contributions to our Society.
4. We can look hard at what we contribute each year for our membership in this Society and then consider making a substantial increase in this challenging year.
5. We can contact the FSH Society and the Office of the Patient Communication and Liaison of NIH BBRI/Harvard Medical School Sen. Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD and volunteer to participate in current studies and future trials and raise funds for research.

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

The FSH Watch is published by the FSH Society and distributed by mail to its members and supporters. All material is copyrighted and may not be reproduced without written permission. To be placed on the mailing list or to submit an article, please write to: Nancy Van Zant, Executive Director, FSH Society, Inc., c/o BBRI R353, 64 Grove Street, Watertown, MA 02472 USA. Articles may be edited for space and clarity. Every effort has been made to ensure accuracy in the newsletter. If you wish to correct an error, please write to the above address. Look for us on the internet at: www.fshsociety.org

Editors: Nancy Van Zant and Daniel Paul Perez. Editorial Assistance: Carol Perez, Jenny Lazzaro, Charles C. Perez and Howard L. Chabner. Graphic design & editorial assistance: Susan L. Stewart, StewartBusinessStrategies.com

Jeff Jacobs Family Research, *continued from front page*

Reserves, and then started graduate school at the University of Michigan in Autumn of 1996. I earned my Ph.D. in Cellular and Molecular Biology in early 2002, and then went on to do a post-doctoral research fellowship at the University of Iowa to develop my skills as a scientist and also gain a greater understanding of how I would want to run my own independent laboratory. Once I felt I had enough experience, confidence, and evidence that I could be productive, I began interviewing for faculty positions in 2006. For the type of position I was seeking — that is, tenure-track faculty at an academic research institution — I had to develop a plan for what I was going to study and how I was going to accomplish the goals of my research program. I needed to convince my future colleagues and bosses that my proposed research program was potentially important and that I had the required experience and drive to pull it off. After about a year of traveling around the country to interview for various job openings, I accepted my current position and began working in June 2007.

As a new investigator, starting a new lab has been both exciting and a bit overwhelming. It has often been likened to developing a new business, and I agree with that analogy. On my first day, I was given the keys to an empty laboratory, and my first task was to fill it with the people, equipment, and supplies I needed to get my research moving. Obviously, this requires money. As an academic researcher, part of my job offer included “start-up” funds that I could use at my discretion to hire research staff/students and stock the lab. Within six years, I am expected to completely fund my entire lab (including salaries) with external research grants.

I wasted no time getting started and within a few months, I hired two research assistants and recruited a graduate student to my lab. We soon began working on several FSHD-related research projects. In the big picture, the goals of our research are to better understand the disease and to develop treatments. In the short term, our goals are to publish

manuscripts and get additional dollars from external funding sources such as the FSH Society, the Muscular Dystrophy Association, and the National Institutes of Health. In short, we need to generate quality data and ideas so that we can be competitive for research grants to keep our work moving forward. Accomplishing these short-term goals is essential to ultimately achieving the longer-term ones.

In my opinion, a lab’s productivity is directly related to the number of top-notch scientists working there. Of course the right supplies and equipment are also a necessity, but the people working in my lab are its number one asset. Not surprisingly, the salaries, tuition and benefits associated with maintaining my high-quality research staff are the most expensive items in my budget. Because of

the outstanding support from donors that make the FSH Society grants possible, I was able to hire an additional Ph.D.-level scientist this year. Moreover, we used FSH Society funds to generate preliminary data that I included in an application for an NIH Career Development Grant, which I was awarded in January of this year. The FSH Society has therefore been instrumental in helping me get my lab off the ground. I am grateful to the FSH Society and its donors, especially the Landsman and Jacobs Families for whom our fellowships are named, and I can assure you that we in the Harper Lab are humbled by your important contribution to our lab and work. If we are so lucky to have some success in the future, the FSH Society and its donors will deserve a great deal of credit.

A guide to acronyms

In the interest of readability and space, we would like to offer a list of acronyms for your reference. We will use these acronyms throughout the newsletter.

BBRI	Boston Biomedical Research Institute
CDC	Centers for Disease Control
CFC	Combined Federal Campaign
DM	Myotonic Muscular Dystrophy
DMD	Duchenne Muscular Dystrophy
EDMD	Emery-Dreifuss Muscular Dystrophy
FSHD	Facioscapulohumeral Muscular Dystrophy
LGMD	Limb-Girdle Muscular Dystrophy
MD	Muscular Dystrophy
MD-CARE	Muscular Dystrophy Community Assistance, Research and Education Act of 2008
MDA	Muscular Dystrophy Association
MDCC	Muscular Dystrophy Coordinating Committee
MDCRC	Muscular Dystrophy Cooperative Research Centers
NIAMS	DHHS NIH National Institute of Arthritis & Musculoskeletal & Skin Diseases
NICHD	DHHS NIH Eunice Kennedy Shriver National Institute of Child Health and Human Development
NIH	DHHS National Institutes of Health
NINDS	DHHS NIH National Institute of Neurological Disorders and Stroke
NIH BBRI Sen. Paul D. Wellstone MD CRC for FSHD	
	NIH Boston Biomedical Research Institute Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD

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Tides Foundation challenge is complete! New donors contribute to this success!

You may recall that in late 2008, the Tides Foundation of San Francisco awarded the FSH Society a \$30,000 matching grant. The conditions of the match were that we raise contributions from new individual donors or institutional funders between December 15, 2008, and December 15, 2009. Funds were to come from new sources and not from renewals or previous donors. The Tides Foundation

wanted to see the Society expand our "prospect pool."

We sent out this word to you, and you asked your family and friends to make gifts. Individuals with FSHD who had not previously been members of the Society also participated.

We thank you very much for making these gifts, for cultivating new sources and encouraging them to give in this time

period. All our energies and targeted approaches enabled the successful completion of this challenge well in advance of the deadline.

Tides Foundation funds together with new gifts from new donors added \$60,000 to the Society's income in the first half of 2009! We are grateful to the Tides Foundation for these funds and for their confidence in the Society's mission.

Volunteers needed for blood & muscle samples, *continued from front page*



Kathryn Wagner, M.D., Ph.D., of the NIH BBRI Sen. Paul D. Wellstone MD CRC for FSHD, who will lead the clinical programs of the center, with patient Don Burke during clinical trial on myostatin inhibition.

blood samples. The collected samples will be used to learn more about how individuals with FSHD differ from individuals with normal muscle, in the search for treatments and a cure for FSHD.

Volunteers will be asked to provide muscle and blood samples to a central laboratory dedicated to FSHD. This central lab will then provide the samples to researchers studying FSHD. Although there is no direct benefit to the volunteer from participating in this study, participation will be a great asset to the multiple studies at the NIH BBRI Sen. Paul D. Wellstone MD CRC for FSHD and the larger FSHD research community. Volunteers who choose to participate in this study will make two visits to Johns Hopkins School of Medicine: 1) for Screening, and 2) for Muscle Biopsy and Research Blood Sample. The screening consists of a general history and physical examination, a neurological exam, and a collection of blood samples (about 8 teaspoons of blood will be taken). At the conclusion of the screening the Principal Investigator may determine that the volunteer does not qualify for participation in this study.

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

pressure bandage will be applied. The volunteer will be educated on the proper care of the incisions. On the day of the muscle biopsy, blood will also be taken (about 2 teaspoons).

Volunteers can anticipate being in this study for about two months. This is the time it will take to receive the results of the pre-biopsy blood work, scheduling, and to perform the biopsy. Once blood and muscle samples have been collected, they will be permanently stored at the tissue bank at BBRI, headquarters for the NIH BBRI Sen. Paul D. Wellstone MD CRC for FSHD, and analyzed at various labs. Muscle cells isolated from the biopsies can be reproduced many times over, providing material for experiments by multiple research labs. The muscle tissue from the FSHD-affected participant remains paired with that of the unaffected relative so that familial and genetic

differences can be observed. The NIH BBRI Sen. Paul D. Wellstone MD CRC for FSHD has set a target of recruiting 15 FSHD-affected volunteers and 15 relatives each year to participate in this study. All procedures, tests, drugs, and devices are part of this research and will be supplied free of charge.

They include:

- History and physical
- Neurological exam
- Manual muscle strength testing
- CBC/Diff
- Coag Panel (PT, aPTTT)
- HIV
- Hepatitis B and C
- Muscle biopsy
- Operating room
- Lidocaine injection
- Research blood for DNA

FOR MORE INFORMATION ...

Volunteers will not be paid for their participation in this study. Volunteers can be reimbursed for their travel expenses (i.e., mileage, airfare, hotel) up to \$500, per person, per visit. Receipts for travel expenses must be submitted to Jenny Lazzaro at the FSH Society to receive reimbursement; her contact information is listed below. Reimbursement(s) can be expected 4-6 weeks after submission.

If you would like more information about this clinical research study, please contact Regina Brock-Simmons, at:

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 (410) 502-7220 Office • (410) 955-3294 Fax
 For Johns Hopkins School of Medicine details, IRB approval number and contact, please visit:

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

For information on the study, the NIH BBRI Sen. Paul D. Wellstone MD CRC for FSHD, the FSH Society and for participant travel reimbursement, please contact Jenny Lazzaro, at:

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Thank you for your consideration and generosity!

Helen Younger & David Younger fellowship, *continued from front page*

pathogenesis, and we are convinced that this will contribute to advances in the FSHD field.

FSHD is a debilitating disease with therapeutic options that are largely supportive. To devise new approaches to therapy, the underlying mechanism of the disease process must be better understood. The underlying cause of FSHD is more complex than the more common “defective gene” scenario that characterizes other conditions like cystic fibrosis or Huntington’s disease. Most FSHD disease cases are linked to a specific change in genomic DNA. Namely, FSHD is associated with a decrease in the number of D4Z4 DNA repeats in the subtelomeric region of chromosome 4q (4qter). The progressive muscle atrophy associated with FSHD is most likely caused by abnormal expression of certain genes. However, it is unclear how the D4Z4 repeat number change leads to this abnormal gene expression. Therefore, it is vital to understand the regulation and function of 4qter D4Z4 repeats in order to address the etiology and pathogenesis of FSHD.

Genomic DNA wraps around histone proteins and forms a “bead-on-a-string”-like structure termed “chromatin.” Modifications of histones and chromatin organization dictate significantly when, and in which cell types, particular genetic

information is expressed. For example, “heterochromatin,” a state of chromatin in which genes are not expressed (silenced), usually contains specific methylation of a certain amino acid of



*Kyoko Yokomori,
D.V.M., Ph.D.*

histone H3 which is then bound by proteins that mediate gene silencing. We have discovered a specific change in histone modification at the D4Z4 repeat sequences, which is detected in both 4q-linked (with D4Z4 repeat contraction) and phenotypic (no repeat contraction) FSHD patient cells. Importantly, this change is highly specific for FSHD; no

significant change of this histone modification was observed in Duchenne muscular dystrophy (DMD), limb-girdle muscular dystrophy (LGMD), oculopharyngeal muscular dystrophy (OPMD), inclusion body myopathy associated with Paget’s disease of bone and frontotemporal dementia (IBMPFD), or “immunodeficiency, centromeric instability and facial anomalies” (ICF) syndrome patient cells. Furthermore, this change is seen not only in affected muscle cells or fibroblasts derived from patient biopsy samples, but also in patient lymphoblasts. Thus, our study provides evidence that this chromatin change may induce abnormal gene expression and trigger FSHD and may also provide a very useful diagnostic test that is applicable to any suspected FSHD patient. This study

appeared in PLoS Genetics in July 2009 (Zeng et al., 2009).

With the generous support of the FSH Society, we were able to extend our initial preliminary finding, which led to two MDA research grants and a NIH R01 currently pending. The goal of my laboratory is to define the chromatin changes in the genomes of FSHD patients and then to identify how these alterations affect target genes. We are currently extending this initial finding by focusing on two questions. First, what are the factors that determine the heterochromatin organization in the 4qter D4Z4 region and how are their functions compromised in FSHD? Second, what genes are affected by the loss of heterochromatin in this region and how? By delineating the molecular pathway that leads to FSHD, we hope to identify possible therapeutic targets.

We could not have continued our research without the support from the FSH Society which encourages researchers to actively test new ideas and thus energizes the field. We believe that constant investigation of novel approaches and concepts is the best way to obtain clinically relevant information to serve patients. I believe that a full understanding of how this disease occurs is the best way to establish a firm basis for the development of future interventions and therapies.

Research materials are needed for embryonic stem cell research on FSHD

FSHD researchers are finding that FSHD occurs at very early stages of development and, therefore, they are in need of embryonic tissues to study FSHD. Couples who have had pre-implantation genetic diagnosis and in vitro fertilization (IVF PGD) are asked to consider making a valuable gift to research by donating remaining frozen IVF PGD embryos to FSHD tissue and stem cell repositories. Please contact Daniel Paul Perez at the Research Office of the Society or e-mail esc@fshsociety.org

Patients and families to gather in Las Vegas, Nevada July 30-August 1, 2010!

The 2010 International Patient/Researcher Network Meeting will be held at the Paris and Bally's Hotels in Las Vegas, July 30-August 1. These hotels have been selected for their accessibility, convenient location and good value. They are a part of the same complex and share meeting space.

The conference will begin with registration and lunch at noon on Friday, July 30, and conclude after lunch on Sunday, August 1.

Lectures and small group discussions are planned to bring the most current advances in FSHD research, including the work of the new NIH BBRI Sen. Paul D. Wellstone MD CRC for FSHD, to the FSHD community of patients, families and scientists. There will be both formal and informal forums to share experiences of living with FSHD, practical and clinical information for daily living such as pain management, breathing and respiration, nutrition, travel and leisure, exercise and physical therapy, patient advocacy, and many of the other topics you have requested.

We will also provide opportunities for



different age and care groups such as teenagers and young adults, parents, siblings and Infantile FSHD families to gather.

The program and the registration fee are in planning. We expect to offer one registration fee that covers primarily two breakfasts and three lunches; an optional festive dinner will be available for Friday night, likely at a venue outside the hotels. More details will be made available on the Society's website as planning proceeds and in the fall *Watch*.

For Bally's or Paris hotel reservations, you may call 877-603-4389. The hotels are holding a block of rooms at \$89 (Bally's) per night (single or double occupancy) and \$120 (Paris) per night (single or double occupancy), plus taxes. There is no charge for parking. The **group code** for our conference rate for Bally's is **SBIPRO** (last digit is zero) and for Paris, **SPIPRO** (last digit is zero). These facilities have many wheelchair-accessible guestrooms, including a total of 100 rooms with roll-in showers. For the best selection of accessible rooms and showers, please make your reservations early. The closing date for the Society's block of rooms is July 9, 2010.

Contact a member of our FSH Society Peer-to-Peer Team

We are here to help!

FSH Society volunteers are available to talk to you and to answer your questions. Our goal is to respond within 24 hours.

Whether you have a new diagnosis of FSHD or have been living with FSHD, we understand that you may experience a range of reactions including anger, sadness, fear of the future and feeling isolated with the disease. Or, you may be the parent of a child with FSHD or Infantile FSHD (IFSHD) and wish to speak with another parent. Whatever your feelings, they are normal and you are not alone.

Life with FSHD poses challenges for every member of the family. Whether you are affected with FSHD, the parent of an affected child, or a loved one of a person affected, it takes time to adapt to the day-to-day demands of the disease.

As we continue work towards treatments and a cure, our Peer-to-Peer Team is ready to talk! We are ready to listen and eager to share our own experiences with you. Although we are not healthcare professionals, we will try to direct you to the resources that you need to deal with issues around FSHD and to provide information to your doctor.

Please call the FSH Society at (617) 658-7878 to be connected with a member of our Peer-to-Peer Team. A new friend will call you shortly.

Be prepared to answer these questions when you call the Society:

Who has been diagnosed? When? By whom? Confirmed by genetic testing? Confirmed by a clinical diagnosis? You suspect you or someone close to you may have FSHD? Age? Gender? When did symptoms first appear?

Please also kindly provide us with a mailing address, e-mail and phone number.

Please call. We look forward to hearing from you.

2009 Delta Railroad Construction research fellowship grants



The FSH Society Delta Railroad Construction Company fellowship program continues to help FSHD research efforts by awarding research grants that provide

needed expansion of current work and innovative approaches in FSHD studies. The FSH Society is indebted to the Delta Railroad Construction Company of Ashtabula, Ohio, Larry and Ida Laurello, and their family for this groundbreaking effort on behalf of the FSHD community. Initiated in 1998, the Delta Railroad Research Fellowship Grants are yielding tremendous insights in new and novel areas of FSHD research and treatment.

For more information about research fellowships, please contact:

Daniel P. Perez, President & CEO.

FSH Society, Inc.

Research Programs Office

11 Elmbrook Circle

Bedford, MA 01730 USA

(781) 275-7781, (617) 658-7811 • daniel.perez@fshsociety.org

Your dollars at work: An example of how you get it done

On July 15, 2009 a significant paper on the FSHD mechanism emerged from the laboratories at the University of California at Irvine. It is a fine example of how the FSH Society initiates new emerging ideas in science and helps these ideas flourish. The FSH Society and its generous members enabled this work with a grant to Dr. Kyoko Yokomori. Through other FSH Society research, advocacy and education programs and meetings, we networked Dr. Yokomori with other FSH Society fellows to form coalitions of collaborators.

We also supported California Proposition 71 that led to the state-funded stem cell research program under the California Institute for Regenerative Medicine (CIRM) that now funds Dr. Yokomori \$600,000 to develop embryonic stem cell lines for FSHD. These are the results of your support and patience. It is interesting to note that the FSH Society initiated and funded this work alone, and along the way more and more people have become involved and added as authors to the recent paper and larger funding agencies are now funding the work. The authors read like a “Who’s Who” of the FSHD research world, and include leading experts in embryonic stem cell, induced pluripotent stem cell (iPS), and adult stem cell research.

PLoS Genetics. 2009 Jul;5(7):e1000559. Epub 2009 Jul 10.

Specific loss of histone H3 lysine 9 trimethylation and HP1 gamma/cohesin binding at D4Z4 repeats is associated with facioscapulohumeral dystrophy (FSHD).

Zeng W, de Greef JC, Chen YY, Chien R, Kong X, Gregson HC, Winokur ST, Pyle A, Robertson KD, Schmiesing JA, Kimonis VE, Balog J, Frants RR, Ball AR Jr, Lock LF, Donovan PJ, van der Maarel SM, Yokomori K.

Department of Biological Chemistry, School of Medicine, University of California, Irvine, California, USA

To view an abstract and link to free article go to PubMed and search for

PMID: 19593370 at <http://www.ncbi.nlm.nih.gov/sites/entrez>

Author Summary

“Most cases of facioscapulohumeral muscular dystrophy (FSHD) are associated with a decrease in the number of D4Z4 repeat sequences on chromosome 4q. How this leads to the disease remains unclear. Furthermore, D4Z4 shortening is not seen in a small number of FSHD cases, and the etiology is unknown. In the cell, the DNA, which encodes genetic information, is wrapped around abundant nuclear proteins called histones to form a “beads on a string”-like structure termed chromatin. It became apparent that these histones are modified to regulate both maintenance and expression of genetic information. In the current study, we characterized the chromatin structure of the D4Z4 region in normal and FSHD patient cells. We discovered that one particular histone modification (trimethylation of histone H3 at lysine 9) in the D4Z4 repeat region is specifically lost in FSHD. We identified the enzyme responsible for this modification and the specific factors whose binding to D4Z4 is dependent on this modification. Importantly, these chromatin changes were observed in both types of FSHD, but not in other muscular dystrophies. Thus, this chromatin abnormality at D4Z4 unifies the two types of FSHD, which not only serves as a novel diagnostic marker, but also provides new insight into the role of chromatin in FSHD pathogenesis.”

Funding

This work was supported in part by NIH RO1 HD49488, NIH PO1 HD47675, and CIRM RC1-00110 to PD [Peter Donovan]; the Netherlands Organization for Scientific Research NWO (016.056.338) to SvdM [Silvere van der Maarel]; NIH GM59150, MDA4026, the David and Helen Younger Research Fellowship from the FSH Society (FSHS-DHY-001); and a grant from the California Institute of Regenerative Medicine (RS1-00455-1) to KY [Kyoko

Yokomori]. WZ [Weihua Zeng] is a recipient of the FSH Society David and Helen Younger Research Fellowship (FSHS-DHY-002). RC [R Chien] is supported by NIH T32CA113265. None of the sponsors or funders listed above play any role in the design and conduct of the study, in the collection, analysis, and interpretation of the data, and in the preparation, review, or approval of the manuscript.”

To read full article at “PLoS Genetics.”

<http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1000559>

FSHD Future Fund growing

In early 2008, several members of the board of directors shared with FSH Society members their estate planning—that their wills or other trust documents include a bequest to the Society. By way of this good news, the Society launched the FSHD Future Fund, and declared these individuals charter members.

Since then, many other individuals and families have informed the Society of their inclusion in their estate plans.

We can all help the Society and its future work by becoming members of the FSHD Future Fund by including a bequest to the Society in our will or other estate planning documents.

If you have already included the FSH Society in your will, we hope you will let us know. If you will allow the Society to recognize your dedication in our *Annual Donor Report*, your example might inspire others. If you have questions about your planning and how it can support the work of the Society in the future, or if you would like a copy of the booklet, *Questions and Answers about Wills and Bequests*, let the Society office know by e-mail

nancy.vanzant@fshsociety.org.

Thank you!

Marjorie Bronfman FSHD research grant for 2009

The generosity and commitment of Mrs. Marjorie Bronfman to FSHD research began in 1997. Mrs. Bronfman has renewed her commitment through 2009 with a new grant of \$130,000. Through a process of review and recommendation by the Society's Scientific Advisory Board, grants are awarded for two-year research fellowships (US\$30,000-US\$35,000/year) for research projects that show extraordinary promise to find the cause of FSHD. The 2009 contribution, and the many grants that have preceded it, significantly aid progress in FSHD research. The FSH Society is deeply indebted to Mrs. Bronfman and the Marjorie and Gerald Bronfman Foundation for this new opportunity to advance FSHD research and for the advances that have been made possible worldwide over these years.

For more information about research fellowships, please contact:

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

FSH Society Marjorie & Gerald Bronfman Foundation fellowship grantees

Richard Lemmers, Ph.D.

FSHS-MGBF-010 for "*Refinement of the FSHD critical region on 4qA chromosomes*"
Total project \$105,000

Melanie Ehrlich, Ph.D.

FSHS-MGBF-013 for "*Finding the 4q35 FSHD Gene*"
Total project \$70,000

Patrick Wayne Reed, Ph.D.

FSHS-MGBF-014 for "*Analysis of Changes in the Proteome in FSHD*"
Total project \$30,000

Yvonne Meijer-Krom, Ph.D.

FSHS-MGBF-015 for "*Towards the Discovery of Early Developmental Defects in FSHD*"
Total project \$105,000

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

FSHS-MGBF-016 for "*Molecular Analyses of DUX4 and Interaction with Myogenic Regulators in FSHD*"
Total project \$21,488

Paola Picozzi, Ph.D.

FSHS-MGBF-017 for "*Functional characterization of D4Z4 in FSHD*"
Total project \$17,500

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

FSHS-MGBF-018 for "*Analyses of functional domains in the pro-apoptotic protein DUX4*"
Total project \$59,040

Richard Lemmers, Ph.D.

Marjorie Bronfman fellow grant number 10

Intrigued by the complexity of the genetic FSHD mutation — Characterization and specification of the pathogenic FSHD region

by Richard Lemmers, Ph.D.

As early as 1994, I started working on the genetics of FSHD in the group of professor Dr. Silvere van der Maarel and professor Dr. Rune Frants at the Leiden University Medical Center (LUMC) in Leiden, Netherlands. Currently I am working on the identification of the minimal DNA region that is responsible for the pathogenicity in FSHD.

When you think about genetics for an heritable disease such as FSHD the ultimate goal would be to find a way to cure the patients suffering from this disease. However, before researchers can even think about finding a cure they first have to identify the pathogenic gene. This identification might be very straightforward but for FSHD, it has turned out to be very complicated. When I started working on FSHD the chromosome region with the genetic mutation had just been identified; the D4Z4 region on chromosome 4. Since this discovery several genes have been identified that might be causally related to the disease, but for none of these genes a direct correlation has been established with FSHD. For many years I have been working on the D4Z4 repeat, which is a very unusual and complex DNA region. The D4Z4 repeat has a length in unaffected individuals varying between 11 and 100 D4Z4 units and in most patients with FSHD the repeat is contracted to a size between 1 and 10 units. Remarkably, the same repetitive DNA region is also present on chromosome 10, but a contraction of the D4Z4 repeat on chromosome 10 does not result in FSHD. This difference in pathogenicity between the same DNA region on chromosomes 4 and 10 has been the main focus of my research.

Ten years ago, we started studying the D4Z4 repeat on both chromosomes 4 and 10 by a technique called pulsed

field gel electrophoresis (PFGE). This could not be done by the standard electrophoresis methods as this does not allow the separation of the very large repeat fragments, such as the D4Z4 repeat. During my Ph.D. doctoral project, I searched for other levels of repeat complexity and we discovered two different variants of chromosome 4 (4qA and 4qB) which were almost equally present in the population. However, when we performed further analysis of this variation in FSHD patients we found that all disease chromosomes were of the 4qA variant. Further analysis in unaffected individuals showed that repeat contractions on the 4qB chromosome were not causing FSHD. Together with Patrick van der Vliet, a technician on this project, we extended this study and discovered more than 10 different variants of chromosome 4 based on DNA differences in and close to the D4Z4 sequence. Interestingly, one of the variants (4qA161) was found to be pathogenic, while repeat contractions on two other chromosome 4 variants seem non-pathogenic. Because the



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

D4Z4 repeats between pathogenic chromosomes 4 and non-pathogenic chromosome 10 also showed small sequence differences we hypothesized that these sequence variations are causing the difference in pathogenicity.

Follow-up studies were based on previous PFGE results, where we showed that more than 20% of the population carried exchanges between the repeats of chromosome 4 and 10. As a consequence some individuals carry 4-type D4Z4 repeats on chromosome 10 and others carry 10-type repeats on chromosome 4. Because of our publications on the genetic analysis of the D4Z4 repeat, neurologists from all over the world yearly sent us over 200 blood samples from FSHD patients and their family members for a detailed PFGE-based DNA analysis of the repeat. For some of these samples we showed that they carry a short (pathogenic) D4Z4 repeat on chromosome 4 that was mainly composed of 10-type sequences. Apparently, exchanges between chromosomes 4 and 10 also occur in FSHD repeats. As a consequence some FSHD patients were previously false negatively interpreted by the standard DNA analysis.

With the establishment of the Fields Center for FSHD and Neuromuscular Research

www.urmc.rochester.edu/fields-center/

we have now created in collaboration with Dr. Rabi Tawil (University of Rochester Medical Center) standardized clinical and genetic assessment protocols. These protocols are now fully implemented in the Fields Center and we hope these protocols will prevent future mistakes in DNA diagnosis. With the Fields

Center we are also planning for a best practice meeting for DNA diagnosis in FSHD early next year. The aim of the meeting is to educate diagnostic laboratories in the comprehensive DNA diagnosis of FSHD and to establish new and more accurate DNA diagnostic testing standards.

We have analyzed the DNA sequence in detail from a large number of unusual patients. Their DNA structure was compared with the DNA structure of other pathogenic and non-pathogenic repeats and from this comparison we were able to very accurately define the pathogenic region. If we could demonstrate that all unusual pathogenic repeats share the same pathogenic D4Z4 region then this would provide evidence that the FSHD gene is localized within the D4Z4 repeat. Currently, we are finishing these analyses and I would like to present our result at the next FSHD workshop.

The FSH Society Marjorie Bronfman Fellowship gave me the opportunity to analyze the genotype of these unusual FSHD patients. This research is very important and has already led to various publications on the mutational mechanism and genetic variations for FSHD and also on the improvement of the genetic diagnosis. Eventually, these findings might result in the discovery of the FSHD gene, which will hopefully provide a rational basis for FSHD therapy.

References:

- Lemmers RJ et al. (2007) Specific sequence variations within the 4q35 region are associated with FSHD. *Am J Hum Genet.* 81:884-894
- Lemmers RJ et al. (2004) Contractions of D4Z4 on 4qB Subtelomeres Do Not Cause FSHD. *Am J Hum Genet.* 75:1124-1130
- Lemmers RJ et al. (2004) Mechanism and timing of mitotic rearrangements in the subtelomeric D4Z4 repeat involved in facioscapulohumeral muscular dystrophy. *Am J Hum Genet.* 75:44-53

For the Ehrlich report, please see pages 27-28

Melanie Ehrlich, Ph.D.

FSHS-MGBF-013 for “Finding the 4q35 FSHD Gene”

Patrick Wayne Reed, Ph.D.

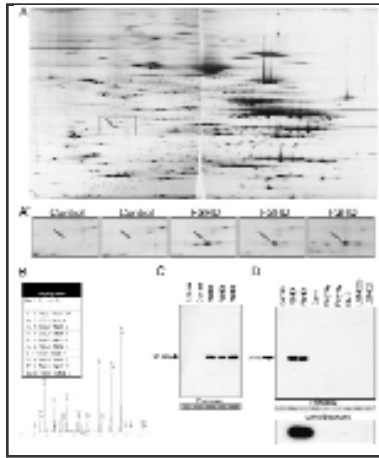
Marjorie Bronfman fellow grant number 14

by Patrick Wayne Reed, Ph.D.

I am currently a research associate in the Department of Physiology of the University of Maryland, School of Medicine, in Baltimore, Maryland. Dr. Robert J. Bloch and I have used seed funds provided by the FSH Society to study changes that occur in the structure of FSHD muscle and in its protein composition, known as the “proteome.” We have so far published two papers based on our work on FSHD. (In addition, we published a third paper and are working on a fourth, in which we developed the methods needed for our studies of FSHD).

The first paper described the structural changes of the muscle cell membrane or “sarcolemma” of patients with FSHD, which

we compared to changes seen in other muscular dystrophies, studied more extensively by Dr. Bloch's lab. For these studies, we needed to develop methods to examine the organization of muscle fibers in human biopsies, which are typically not fixed and so remain able to contract. It took a considerable effort to learn how best to work with them. Once we had developed these

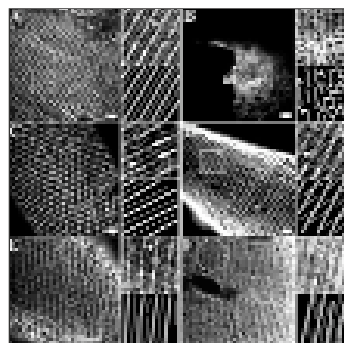


In the “2D gels FSHD” image, the arrow is pointing to the spot corresponding to mu-crystallin and the western blot gel images are detecting the protein band corresponding to mu-crystallin in FSHD but not in several other types of muscle diseases.

were greatly increased in FSHD muscle. Our findings indicated that the relationship between the sarcolemma and the contractile proteins to which it is attached is altered in muscle from patients with FSHD. These alterations can account, at least in part, for the increased weakness and fragility of the muscle. We published these results in “The Annals of Neurology” [Ann Neurol. 2006 Feb;59(2):289-97].

The second paper identified a single protein, called “mu-crystallin,” in extracts of deltoid muscles from FSHD patients, that was not detectable in healthy controls or in extracts of muscles showing other forms of muscular dystrophy or myopathy. For these experiments, we had to improve the available methods to examine the protein composition (“proteome”) of muscle. The approach we chose is called “large-format, two-dimensional gel electrophoresis,” which separates proteins according to their sizes and electrical charges. Once we had introduced our improvements, we were able to compare more than 3000 different proteins in healthy and FSHD muscle.

methods, we used them to examine FSHD muscles. Our experiments demonstrated a reorganization of the cytoskeleton underlying the sarcolemma and the proteins of the sarcolemmal membrane that are distinct from the reorganization seen in other models of muscular dystrophies. We also examined the distances between the sarcolemma and the nearest contractile structures and found that these



Reorganization of the costameres in the muscle membrane cytoskeleton in FSHD muscles from Annals of Neurology paper. In this picture, panels D and F show reorganization of the costameres in FSHD, panel C shows organization in Duchenne's MD, panels A, C, and E show normal organization in healthy muscle.

In our initial experiments, we found an abnormally high expression of a protein called mu-crystallin in muscle from patients with FSHD, but not in samples from healthy patients or patients with other types of muscle diseases. Mu-crystallin is also linked to defects in hearing and vision, which are commonly seen in FSHD. This and other characteristics of the protein suggest that it may play a role in the disease process in FSHD. We published the results of this study in “Experimental Neurology” [Exp Neurol. 2007 Jun;205(2):583-6. Epub 2007 Mar 21].

Since then, we have studied mu-crystallin further. We showed that mu-crystallin is indeed increased in amount in the cytoplasm of the muscle fibers in FSHD muscle, but not in other cell types in the muscle. Although we are currently testing additional tissue samples, generously provided by YOU to the NIH BBRI Sen. Paul D. Wellstone MD CRC for FSHD, high expression of mu-crystallin in muscle from patients with FSHD appears to be a consistent finding in the limited number of tissue samples we have most recently examined. We have also been expressing high levels of this protein in muscles of mice to see if it can produce muscle pathology on its own, using a technique called in vivo electroporation. Our preliminary results suggest that high levels of mu-crystallin can indeed cause myopathy. With the help of the FSH Society, we have just begun the process of preparing “transgenic” mice — mice that have been manipulated genetically, in our case to make large amounts of mu-crystallin in skeletal muscles. We hope to study these animals in the fall and winter of 2009.

Our work is important for FSHD research because the true changes in the muscle that lead to FSHD are unknown. The two-dimensional electrophoresis research will lead to new biomarkers for FSHD muscular dystrophy and possibly will identify proteins involved specifically in the disease process, which can become drug targets to treat the disease. Indeed, mu-crystallin may be such a biomarker, and, if our preliminary results are supported by future work, may be a potential drug target for treatment of FSHD. This of course will need testing in an appropriate animal model, possibly provided by our transgenic mice. With these mice, we can use our observations of changes in the sarcolemma as a test of how accurate an animal model of FSHD might be. Importantly, all of this initial work was made possible initially by funds from the FSH Society. Our ongoing studies are supported by funds provided by the FSH Society through the NIH BBRI Sen. Paul D. Wellstone MD CRC for FSHD to Dr. Bloch, and by a fellowship to Dr. Reed from the Muscular Dystrophy Association. We are especially grateful to the FSH Society and Mr. Daniel Perez for

support to initiate our studies, for continuing support through the Wellstone Center, and for their unfailing efforts to make FSHD research a priority nationally and internationally. Finally, thank YOU for donating the money and muscle tissue to make this work possible.

Yvonne Krom, Ph.D.

Marjorie Bronfman fellow grant number 15

by Yvonne Krom, Ph.D.

In 2000, I started my career as a Ph.D. student at the department of Human Genetics in Leiden, the Netherlands, on a project



Yvonne Krom, Ph.D.

focusing on atherosclerosis. During these five years as a Ph.D. student, I became aware of and fascinated by the research performed in the lab of professor Dr. Silvere van der Maarel and professor Dr. Rune Frants to elucidate the pathogenic cause of FSHD. In 2006 I joined their FSHD group as a postdoctoral fellow and a fellowship of the FSH Society allowed me to explore and establish a novel research line within the FSHD research program.

Though symptoms in FSHD patients often do not appear before the age of 20, several observations raise the intriguing possibility that a defect in the myogenic program early in embryogenesis contributes to the pathogenesis of FSHD later in life. Thus far, research towards the identification of such a developmental defect is strongly hampered by the absence of an appropriate model. We have set up several different cellular and mouse models to get insight in the role of early myogenesis in FSHD.

With my FSH Society Marjorie Bronfman fellowship, I was able to join the lab of professor Dr. Stephen Tapscott in Seattle, Washington for three months, which was great for several reasons. First, it allowed me to implement techniques operational in Seattle to compare myogenesis between FSHD and control individuals on a transcriptome level. To do so, we applied the myogenic transcription factor MyoD to primary fibroblasts of both control and FSHD patients. Second, it broadened my knowledge of the complex gene expression pathways that unfold during myogenesis. Last but not least, during my visit in Seattle I met many scientists, leading to additional insights and new and exciting experiments. At the moment, I still have an ongoing collaboration with the labs of professor Dr. Stephen Tapscott and Dr. Gala Filippova. In fact, this collaboration is now further consolidated in the Fields Center for FSHD and Neuromuscular Research at the University of Rochester Medical Center headed by Professor Rabi Tawil. Together with our additional collaborators, professor Gillian Butler-Browne (Institute of Myology, Paris) and professors Baziél van Engelen and Dr. George Padberg (University Medical Center Nijmegen), and together with Bianca den Hamer, a technician on this project, we have now also generated immortalized myogenic cell lines from muscle biopsies of FSHD patients. I expect that these cell lines

will enable me to better study the myogenesis of FSHD.

During these years it has become clear to me that the underlying mechanism leading to FSHD is complicated. FSHD research operates at the edge of our current biological knowledge and technical possibilities. From a scientific view, it feels like we are facing important breakthroughs. Knowledge of this disease is increasing rapidly. There is consensus about the primary defect in FSHD, D4Z4 contraction-mediated changes in chromatin structure of the pathogenic 4qA161 allele. At the moment, Dr. Richard Lemmers and Patrick van Vliet are uncovering the allelic sequence variations on chromosomes 4 and 10, which seem essential for pathogenicity. Recent publications from other groups shed new light on a role for DUX4, a pro-apoptotic protein, in pathogenesis of FSHD. Recently, in vivo expression of DUX4 transcripts has been found, in which a truncated form of DUX4 inhibits myogenesis. Considering that DUX4 belongs to the family of homeobox proteins that often play essential roles in development, I am trying to merge these findings into my studies with model systems for FSHD, including the previously mentioned cellular models and our mouse models containing the FSHD locus. Although these mice do not show an obvious muscle phenotype, the integrated FSHD locus shares many commonalities with the human locus with respect to its chromatin structure.

From a patient point of view, I can imagine that it is frustrating; the FSHD locus (contraction of D4Z4 on chromosome 4) being discovered almost 20 years ago, but still the causative gene/protein has not been found and no cure is available. Most likely there is still a long road ahead of us before a treatment will become available, but research is progressing fast now. Therefore, we are very grateful that individuals, patients with FSHD, and also their healthy relatives are willing to participate in research studies, providing us with the necessary blood, skin and/or muscle biopsies.

The FSH Society Marjorie Bronfman Fellowship gave me the opportunity to set up cellular models in order to improve our insight of the myogenic program early in embryogenesis. These studies have led to attractive new models in which we hope to further uncover the disease mechanism of FSHD. In due time, I hope to update you on my research activities in the *FSH Watch*.

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

Marjorie Bronfman fellow grant number 16

[Editor's note Dr. Bosnakovski did not submit an article at this time]

Paola Picozzi, Ph.D.

Marjorie Bronfman fellow grant number 17

[Editor's note Dr. Picozzi left academia to go into industry after 6 months]

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

Marjorie Bronfman fellow grant number 18

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

I was born and educated in Argentina. I obtained a degree in Biochemistry (1983) and a Ph.D. in Molecular Biology (1987). In 1988, when I was 28 years old, I started my own research laboratory to study basic genetic phenomena in lower eukaryotic cells. Twelve Ph.D. students trained in my laboratory in those years obtained top scientific positions in Argentina, the United States and Europe. I continued my studies at the School of Medicine and, in 1994, I obtained a Medical Degree and, in 2002, a second Ph.D. (this time in Medical Genetics), all at the University of Cordoba. This University has a large tradition in medicine and science, being the oldest university in Latin America. During 1997-2002 I was awarded as International Researcher of the Howard Hughes Medical Institute from the U.S.

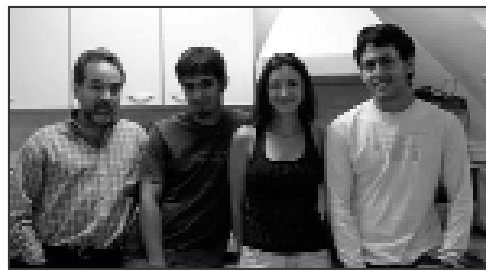
After obtaining my M.D., I wished to develop in Argentina a link between the fields of basic science and clinical medicine. To pursue these objectives, in 1997, I spent a year in France working on ICF, a human syndrome connected with DNA methylation, which is a form of epigenetic control in mammalian cells. My previous research interest in lower eukaryotic cells was the epigenetic bases of gene expression. Also, in 1997, I visited the laboratory of Dr. Judith Melki at Strasbourg (France). Dr. Melki had directed the group that discovered the gene *SMN1* (for the disease called *spinal muscular atrophy*). At the scientific library in Strasbourg, I found a copy of the recent thesis work by Dr. Judith van Deutekom (Leiden, Netherlands), concerning the genetic characterization of the locus FSHD at 4q35. That day I read the entire thesis and become fascinated with the scientific challenge offered by a potential epigenetic control underlying the pathogenesis of FSHD. Just a few months later, my laboratory in Argentina started offering molecular testing for FSHD. Together with Dr. Mayana Zatz (from Brasil), we are still the only laboratories performing molecular testing for FSHD in Latin America.

Based on my previous expertise with inherited dominant traits in lower eukaryotic cells (as it is observed in FSHD), I suspected that the disease should not be explained by a “repressive” mechanism but based on a “toxic gain of function” phenomenon, perhaps explained by abnormal epigenetic control of a gene in a relaxed chromatin environment. In January 1998, I proposed to a young Ph.D. student from my laboratory, Valeria Kowaljaw, to explore “the potential pathogenic

role in FSHD of a putative toxic protein potentially encoded by the tandem repeat D4Z4 at 4q35.” It was a very risky project. A few months later, a paper was published by the group of Dr. Alexandra Belayew (Belgium) proposing a similar hypothesis. I met Dr. Belayew for the first time at the International Workshop of the FSH Society in Los Angeles, in 2003. I was not in the scientific program but I approached the meeting and asked Dr. Sara Winokur –Chair of the FSH Workshop in 2003– to let me present something I considered exciting research news in FSHD: my PhD student Valeria Kowaljaw had found in Argentina that DUX4 is a toxic protein when expressed in cultured cells. We showed that DUX4 is located in the cell nucleus—a finding also presented by Dr. Belayew’s group—and kills the cells by a phenomenon called apoptosis. The audience at the meeting was excited by this news.

We joined efforts with Dr. Belayew to continue studying the toxic effect of DUX4. The FSH Society and the AFM (Association Francaise contre les Myopathies) were key players of the last years, supporting research in our laboratories. This support included my visit in 2005 to Dr. Belayew’s laboratory in Belgium. Our combined efforts lead to the confirmation of the apoptotic nature of DUX4-mediated cell death. Our results were published in a collaborative paper in the journal “Neuromuscular Disorders” (NMD 2007 vol 17, pages 611 to 623).

Taken together, our studies show that DUX4 is a major candidate for the pathogenesis of FSHD. Current work performed in my laboratory, as well as in many other laboratories now interested in DUX4, would increase our knowledge about this mysterious and interesting protein as well as its potential role in FSHD.



Albert Luis Rosa lab personnel
from left to right: A.L. Rosa, E.D. Corona, J. Barbosa,
N. Nunez

FSH Society Kelly Family Fellowship grantee

Ryan D. Wuebbles, Ph.D.

FSH Society Kelly Family fellow 1

FSHS-KF-001 for “Testing the effects of elevated DUX4, DUX4c, and PITX1 in the *Xenopus laevis* vertebrate model system”

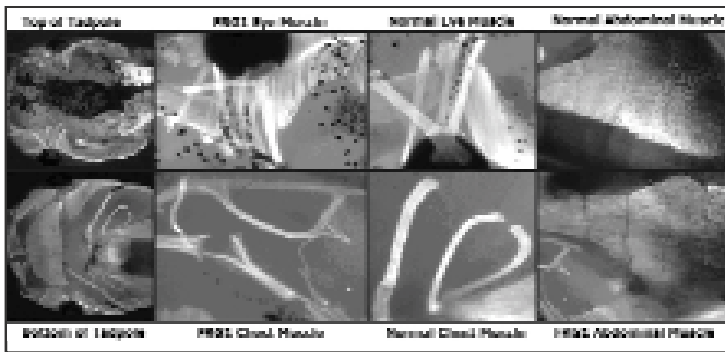
Total project \$15,000

by Ryan Wuebbles, Ph.D.

I was diagnosed with FSHD in 1999, at the age of 20, while I was attending the University of Illinois with a major of Electrical Engineering. Due to this life changing

event, my interest switched to the pursuit of finding a cure for FSHD. To accomplish this, I became a graduate student in Dr. Peter Jones' lab at the University of Illinois. Like most people, Dr. Jones had never heard of FSHD before I came and spoke with him about joining his lab. As he was an expert in epigenetics, and FSHD is an epigenetic disease, Dr. Jones allowed me to begin researching FSHD using the frog model system. My work gradually began shifting away from epigenetics towards studies of FSHD candidate protein function.

With the help of Dr. Meredith Hanel, we began to examine the best candidate for FSHD, a gene called FRG1, which is well conserved evolutionarily in the frog. By altering levels of FRG1 in our frog model and examining the effects on the developing tissues, we determined that proper levels of FRG1 are critical to the normal development of muscle and vasculature. Further, our studies showed that increased levels of FRG1 led to dystrophic muscle and abnormal vasculature, similar to the symptoms presented by FSHD patients. This research was published in two separate articles in the journals of "Developmental Dynamics" and "Disease Models and Mechanisms." This research strongly suggests FRG1 plays a role in the development of FSHD,



Pictures of a half-transgenic tadpole's muscle with too much FRG1 on one half of the animal. Increased FRG1 leads to dystrophic muscle.

providing an invaluable target for drug therapy. Due to this research, I recently received my Ph.D. from the University of Illinois in Cell and Developmental Biology.

I am currently working with Dr. Jones as a post-doctoral fellow, examining the effects of several other potential FSHD candidate genes called DUX4, DUX4C, and PITX1 in our frog model. This work has been generously funded through the FSH Society by the Kelly Family Post-Doctoral research fellowship grant. Hopefully, this research will shed light on a mechanism in which these genes contribute to the pathology of FSHD patients. As with FRG1, a better understanding of the role these candidates play in the disease will lead to better designed experiments for drug screens. The FSH Society funding has been critical to these studies. Further, it has allowed me to gradually transition from a graduate student at Illinois and find the right lab for me to continue research into the mechanisms of FSHD as a post-doc. I hope to find a lab where I can use the experience and knowledge I've gained from working with Dr. Jones and Dr. Hanel to begin drug screens to help FSHD patients, as well as myself.

Our lab is now almost exclusively focused on FSHD and we are pursuing it from several different angles. Currently Dr. Jones'

lab has three graduate students: Steven Long, Jessica Sun, and Qian Liu, who are each working on different aspects of FSHD. Two other post-docs are also involved in FSHD research, Dr. Meredith Hanel and Dr. Takako Iida Jones. Both are excellent researchers and their help and advice was invaluable to attaining my Ph.D. As I begin to transition into another laboratory, I hope this lab continues to grow as I believe the research being conducted will be critical to the discovery of a treatment for FSHD.

FSH Society Helen Younger and David Younger fellowship grantees

For the Yokomori report, please see the front page

Kyoko Yokomori, D.V.M., Ph.D.

FSHS-HDY-001 for "The molecular characterization of the chromatin structure of the D4Z4 repeat associated with FSHD"

Total project \$90,000

Weihua Zeng, Ph.D.

FSH Society Helen Younger and David Younger fellow 2

FSHS-DHY-002 for "Epigenetic abnormality in FSHD"

Total project \$35,500

by Weihua Zeng, Ph.D.

My name is Weihua Zeng. I am a former graduate student and now postdoctoral fellow in Dr. Kyoko Yokomori's lab at the University of California, Irvine. The major research focus of our lab is the organization and maintenance of proper chromatin structure ("chromatin" is the cell's DNA combined with certain proteins), which is very important for cellular functions such as gene expression, DNA repair, and cell replication.

My work has focused on learning more about DNA repeat sequences, which make up a large part of our chromatin. I have specifically worked on D4Z4 repeats, which are directly related to FSHD. In the vast majority of FSHD cases, there are far fewer of these repeats on chromosome 4 compared to an unaffected person. As I mentioned above, chromatin is composed of DNA (its sequence contains information for protein products), proteins called "histones," and other protein factors bound to the DNA. All these protein factors help organize the DNA and facilitate its function. Furthermore, these proteins, particularly the histones, can be chemically modified by enzymes in the cell to control whether certain genes are expressed or not. We can detect these chemically-modified histone (and non-histone) proteins by using special antibodies which bind to them.

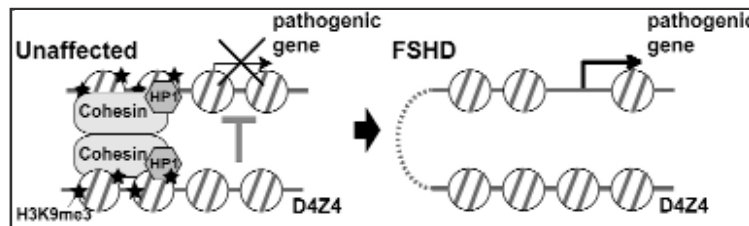


A picture of our lab members. Our PI is Dr. Kyoko Yokomori (front row, second from left). Assistant project scientist Dr. Alexander Ball (back row, third from left), Specialist Dr. Xiangduo Kong (back row, first left), graduate students Yen-Yun (Michelle) Chen (front row, far right) and Richard Chien (back row, far right) also contribute to this work, and they are all the co-authors of the paper. Weihua Zeng (front row, far left) is the first author of the paper.

Therefore, the major research method I use to study D4Z4 repeats is the chromatin immunoprecipitation (ChIP) assay. In this experiment, I use these specific antibodies to bind to their target proteins on chromatin and simultaneously the chromatin fragments interacting with the target proteins are also selected. By this method, I can study what kind of proteins are bound to D4Z4 repeats, and what changes happen to this binding pattern in FSHD patients. This is important for the diagnosis of FSHD. We were very excited to see that the changes in histone modification seen in patients with FSHD applied not only to the large number of patients with D4Z4 deletions, but also to the smaller group of patients who have FSHD but no D4Z4 deletion. We found that the histone H3 lysine 9 trimethylation modification is present in unaffected people's D4Z4 repeats, but is not present in FSHD. This change happens in FSHD specifically and does not occur in other muscular dystrophies. This result was published in our recent paper "Specific Loss of Histone H3 Lysine 9 Trimethylation and HP1/Cohesin Binding at D4Z4 Repeats Is Associated with Facioscapulohumeral Dystrophy (FSHD)" in "PLoS Genetics."

Histone H3 lysine 9 trimethylation is a modification on DNA-bound histone H3, and this modification is usually a marker for heterochromatin. Heterochromatin is a condensed state of chromatin and the genes in this

region are usually inactive. Loss of histone H3 lysine 9 trimethylation on D4Z4 not only serves as a potentially clinically useful diagnostic marker for FSHD, but also offers insight into FSHD pathogenesis. We think that this histone H3 modification recruits other proteins to D4Z4 repeats and these proteins help to bring different pieces of chromatin together, somewhat like a ring holding two pieces of string together. Once in contact, the modified histones on one length of chromatin can affect how the genes on the other piece of chromatin are expressed. In normal cells, the interaction results in the modified histone H3 at the heterochromatic D4Z4 region telling genes on the other piece of chromatin to be silent (i.e., not expressed). In FSHD, the loss of the H3 modification means that this silencing signal is gone, and the target genes may be expressed inappropriately. Some labs are looking at the expression of genes immediately adjacent to the D4Z4 repeats, but the results are not clear. With our model, it is necessary to look at all of the chromatin for target genes. To do this, we plan to do histone H3 lysine 9 trimethylation ChIP assays on both unaffected and FSHD muscle cells and hybridize the samples to whole genome arrays. With this approach, we can look for the effects of the loss of the H3 modification all over the genome. This is a challenging project and thus the funding from the FSH Society is very important. This



A possible model for the spreading of the change at D4Z4 to other genomic regions in FSHD. Two proteins, HP1 and cohesin, may contribute to the physical interactions of the heterochromatic D4Z4 region with other genomic regions leading to the spreading of the silencing effect to putative target genes in unaffected individuals. In FSHD, the loss of H3K9me3, HP1, and cohesin from D4Z4 results in loss of chromatin interaction and derepression of these genes, leading to muscular dystrophy. This figure is modified from the original version in our PLOS Genetics paper.

gracious support is critical for many reasons. First, the specific medium to grow muscle cells and the antibodies for the ChIP assays are all expensive (in fact, the fellowship has benefited my "PLoS Genetics" paper by helping me to purchase these reagents, which is stated in the Acknowledgement portion of the paper). Second, the array hybridization and data analysis from the array are also expensive. Third, further experiments are necessary to verify the interaction between the identified candidate genes and D4Z4 repeats, and the pathogenic roles of these candidate target genes must be verified in mouse models. The funding from the FSH Society can help a researcher like me to further address the elusive mechanism of FSHD pathogenesis, especially in the current tough economic situation. We hope that our findings can pave the way to new understanding of the disease and lead to novel approaches to diagnosis and treatment of FSHD.

FSH Society Landsman Charitable Trust fellowship grantees

For the Harper report, please see the front page

Scott Q. Harper, Ph.D.

FSHS-LCT-002 for “*In Vivo Investigation of DUX4 as a Candidate FSHD Gene*”

Total project \$10,000

Meredith Hanel, Ph.D.

FSH Society Landsman Charitable Trust fellow 1

FSHS-LCT-001 for “*An in vivo Xenopus System for Studying D4Z4 Mediated Chromatin and Gene Expression*”

Total project \$30,000

by Meredith Hanel, Ph.D.

I studied human genetics for my Ph.D. at the University of Alberta, Canada. After completing my Ph.D., I wanted to branch out and learn some developmental biology and did a post doctoral fellowship in a laboratory at University College Dublin, Ireland, where I used

tadpoles (species: *Xenopus laevis*) to study eye development. I was impressed by the power of using *Xenopus* to model and test the roles of particular genes in the formation of tissues and organs. I first learned about FSHD when I was looking for a second post doctoral fellowship, hoping to combine my interest in human genetics with using the developing tadpole to study human disease. It turned out the laboratory of Dr. Peter L. Jones at the University of Illinois was a perfect fit.

When I learned more about FSHD, I realized that it was a very complex disease. The location of the genetic disruption is known, but how this causes muscular dystrophy has not been worked out. I was attracted to the challenge of trying to solve this disease and also felt excited that research into such a complex disease mechanism would really further biology in general.

Since I joined the lab, I have been working on two different

aspects of FSHD. FSHD is proposed to result from a mis-regulation of a gene or multiple genes located close to the mutation near the end of chromosome 4. The first aspect of my work, a joint effort together with Dr. Ryan Wuebbles, who has now completed his Ph.D. in our laboratory, focuses on the candidate gene, FRG1. While the mis-regulation of FRG1 is proposed to result in FSHD, human samples have not consistently shown up or down regulation of FRG1. To evaluate the likelihood of FRG1 mis-regulation as a disease mechanism, we asked whether manipulating levels of FRG1 was capable of causing FSHD-like effects in the tadpole. If FSHD is indeed caused by having too much FRG1, experimentally increasing the amount of FRG1 in tadpoles would be expected to result in visible changes to the muscle. If FRG1 has an important role in muscle development, knocking down levels should also be detrimental to the muscle. Our results clearly showed that both too much and too little FRG1 lead to severe complications in muscle development. Since muscle development and the muscle growth and regeneration that occur throughout our lifetime are very similar processes, we think that mis-regulation of FRG1 in adults disrupts the ability of muscle to repair and grow. Another exciting finding that came from our studies was that the pattern of blood vessel formation was also dependent on levels of FRG1. Since many FSHD patients have increased abnormal vascular growth in the retina, too much FRG1 can explain both the muscular dystrophy and the vascular abnormalities. We think that besides the vasculature in the eyes, FSHD patients could potentially have altered vasculature in the muscle. This could be

important since muscle repair involves communication between the muscle cells and the vascular cells. In our laboratory investigations into the function of FRG1 are ongoing. Once we can pinpoint how FRG1 exerts its effects on muscle, it will be possible to think about specific drugs and therapies which could be beneficial.

The other logical therapeutic target would be at the level of regulation of FRG1. Therefore the second aspect of my research is to understand how FRG1, or other genes in the region, could become mis-regulated in FSHD. The FSHD mutation is a loss of DNA containing a repeated sequence called D4Z4, located near the end of chromosome 4. There are a number of ways that we imagine that this loss of DNA

could lead to gene mis-regulation. First, the D4Z4 repeats can bind proteins that enhance or repress the levels at which genes are turned on. Second, repeated DNA sequences can become wound up tightly and inhibit genes from being turned on, and this tight conformation can spread a good distance along the chromosome. Loss of a certain number of these repeats may change the DNA conformation so it becomes less tightly wound and more open, allowing genes to become activated. My research

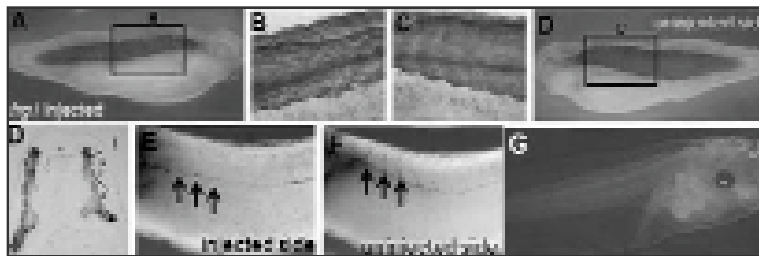


Figure 1. (A,B) Tadpole with FRG1 overexpressed on right side only shows disorganized muscle, stained in brown. (C,D) Uninjected left side of same tadpole has normal well organized muscle. (D) Cross section through tadpole body shows that the injected side (i) has many detached muscle cells. (E) Tadpole with FRG1 levels knocked down has inhibited vascular growth, stained in blue (arrows), compared with (F) the control side of the tadpole that shows sprouting of veins (arrows). (G) Live transgenic tadpole with the human FRG1 regulatory region directing expression of green fluorescent protein. Figures above were published in: Wuebbles, R.D., Hanel, M.L., Jones, P.L. *Dis Model Mech.* (2009) 2:267-74. Hanel, M.L., Wuebbles, R.D., Jones, P.L. *Dev Dyn.* (2009) 238:1502-12.

centers around answering the question: How do DNA sequences in the vicinity of FRG1 affect the level at which it is turned on? I am using transgenic tadpoles to test how the DNA sequences surrounding FRG1 affect its ability to be turned on. By engineering transgenes with the regulatory region of FRG1 attached to green fluorescent protein, I am able to get a visual read out on the levels of FRG1. I am experimenting with the addition of different numbers of D4Z4 repeats. There are also some DNA sequence elements which are always linked with the disease chromosome and I am testing how these elements impact on FRG1. The FSHD region is at the end of chromosome 4. The ends of chromosomes, called telomeres, have a specific DNA sequence that tends to inactivate genes that are close-by. Our system takes this into consideration by the addition of telomeres to our transgenes. We have already learned something interesting about the regulation of FRG1 — that FRG1 seems to be immune to the repressive effects of the telomeric sequence, remaining quite active. A better understanding of how FRG1 is regulated will help us to understand what goes wrong in the disease.

Researching FSHD in the laboratory of Dr. Jones has been a great experience. The great support and enthusiasm from Dr. Jones provide a positive and stimulating work environment. I have spent a good deal of my time working in collaboration with Dr. Ryan Wuebbles, a recent Ph.D. graduate who is living with FSHD. Ryan is not just a co-worker but also a friend, and working with him has given me a unique perspective. His limitless optimism and determination to figure out this disease have been truly inspirational. I am happy to say that our work has resulted in two publications to date and we are expecting several more publications from our laboratory this year.

I am very grateful for the financial support I received from the FSH Society. The research in our laboratory is at the level of trying to understand the basic science behind the disease, and it is great that the FSH Society values basic science research along with more clinical research. Once we have a better understanding of why the disease progresses as it does, and what all the components are, we will have clear direction for clinical interventions.

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Your gift can go even further if your company makes matching donations. Check it out!

FSH Society Family Picnic fellowship grantee

Frédérique Magdinier, Ph.D.

FSH Society Family Picnic Fellow 1

FSHS-NYFP-001 for “Exploring the interplay between the nuclear envelope and the higher order chromatin organization of the D4Z4 array in control and FSHD cells”

Total project \$30,000

by Frédérique Magdinier, Ph.D.

Since my Ph.D., I have always been interested in the regulation of gene expression by epigenetic mechanisms and, more particularly, the consequences of epigenetic dysregulation in the onset of human pathologies. During my post doc at the National Institutes of Health in Bethesda Maryland, in the laboratory of Dr. Gary Felsenfeld, I contacted Dr. Eric Gilson at the Ecole Normale Supérieure in Lyon, who offered me a position in his lab to participate in a new collaborative project launched by the Association Française contre les Myopathies (AFM) focused on the molecular mechanisms of FSHD. In the meantime, I applied to INSERM (Institut National Scientifique pour l'Etude et la Recherche Médicale), obtained a permanent position as an associate researcher and started the project on FSHD in January 2003.

The proper development and health of an organism are the result of a complex interplay between regulatory pathways. The phenotype (appearance) of a cell is not only the result of the expression of the genes it contains but also depends on a precise equilibrium between activation mechanisms and repressive effect that allows the fine tuning of gene expression patterns. Epigenetics refers to changes in phenotype or gene expression pattern caused by mechanisms other than changes in the underlying DNA sequence. Alterations of the epigenetic regulation and subsequent defects in either transcriptional activation or silencing can have severe consequences and lead to a wide range of pathologies.

Because of my background and the specificity of Dr. Eric Gilson's team in the field of telomeres, this project was extremely interesting for me as it combined epigenetics, telomere biology and, more importantly to my eyes, human pathology. The FSHD locus, which is localized at a subtelomeric position, i.e., close to the telomere (the tip of human chromosomes), has a particular structure and the disease is due to the rearrangement of repeated elements rather than to a “classical” mutation of a gene encoding a protein. Our goal was to investigate the role of telomere proximity in the physio-pathological mechanisms of FSHD and to investigate the hypothesis of a “position effect” mechanism or, in simple words, whether increasing the distance between the telomere and the 4q35-specific

sequences by adding a certain number of D4Z4 up to a threshold of 11 repeats would alter the expression of a nearby gene and the function of D4Z4 by epigenetic and telomere-dependent effects.

To address this hypothesis, we created a large collection of constructs (engineered DNA molecules containing a set of genomic tools easy to manipulate) which, upon insertion into the human genome, would mimic what is observed in patients suffering with FSHD or healthy controls in terms of epigenetic regulation and chromatin architecture. Using this approach, we recently showed that *D4Z4* exhibits anti-silencing activities and displaces a telomere toward the nuclear periphery. These properties of *D4Z4* depend upon the vertebrate insulator CTCF protein and A-type Lamins, two key components of the nucleus. Moreover, CTCF-binding and perinuclear positioning are lost upon multimerization of the repeats, suggesting a new mechanism for the FSHD pathogenesis based on a switch of activity from an anti-silencing to a neutral activity depending on the dosage of CTCF (Ottaviani *et al.* "PloS Genetics," 2008; Ottaviani *et al.*, "EMBO J.," In press).

Unlike most of the other human telomeres, the 4q35 region is localized at the periphery of the nucleus, a region implicated in numerous biological phenomenon, but the molecular basis of this association with the nuclear periphery, a highly specialized compartment enriched in heterochromatic factors, is still poorly understood but likely involves a network of interactions that will influence the "FSHD gene(s)."

Thanks to the funding granted by the FSH Society, we plan to continue the deciphering of the mechanism of FSHD by investigating:

The role of A-type Lamins and components of the lamina or the nuclear pore in the peripheral positioning of the 4q35 locus and the consequence of this positioning in gene regulation and their role in the coalescence of higher-order chromatin structure and nuclear positioning.

The role of CTCF and A-type Lamins in the control of epigenetic marks (DNA methylation, histone modifications, variants) that decorate *D4Z4* in patients or normal cells and the regulation of these marks during muscle differentiation.

We believe that this project should open new paths in the understanding of the regulation of the *D4Z4* array and 4q35 region and its role in the development of FSHD and lead to the identification of proteins that would regulate the biological function of short and long *D4Z4* array.

I recently moved to the laboratory led by Pr. Nicolas Lévy, M.D., Ph.D., who is also directing the department of Medical Genetics, Timone Hospital in Marseille, France. This medical department is the center of reference for the diagnosis of FSHD in France. In the last 10 years, this team explored a cohort of more than 400 patients and collected clinical and molecular data for all of them. Thus, cells from patients harboring *D4Z4* contractions ranging from 1 to 11 repeated units but also from patients with typical FSHD features but without the shortening of the *D4Z4* array will be available through our collaboration. This grant is of great importance for me since it will allow me to continue my work on FSHD as an independent investigator and will, I hope, lay new ground for the understanding of the FSHD pathology and open new avenues for a targeted therapeutic strategy of the FSHD pathology.

FSH Society Stuart Lai Mouse Model Development fellowship grantee

Jeffrey Boone Miller, Ph.D., Robert J. Bloch, Ph.D.

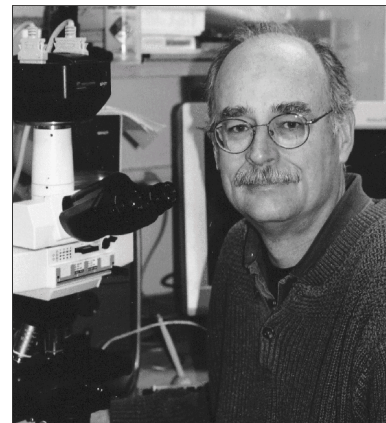
FSH Society Stuart Lai Mouse Model Development fellows
FSHS-SLMM-001 for "The Boston Biomedical Research Institute Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD, Project 4 — Mouse Model Studies for FSHD Biomarkers."

Total project \$160,000

Developing animal models of FSHD

by Patrick Reed, Ph.D., Robert Bloch, Ph.D., Jeffrey Boone Miller, Ph.D.

Studies of FSHD in the laboratory have been difficult because there is no naturally occurring or experimental animal with a muscular dystrophy that is genetically similar to FSHD in humans. Many other forms of muscular dystrophy have either



Jeffrey Boone Miller, Ph.D.

been identified in natural populations (for example in mice, dogs and cats) or created by genetic manipulation (typically in mice). These animals have been extremely useful in experiments to understand the nature of the pathogenic disease mechanisms in the muscular dystrophies and are now being used in studies of potential treatments. Having a mouse model of FSHD would greatly accelerate the

progress in our understanding of this disease and the development of therapies to treat it.

While moving towards the development of an animal model of FSHD, investigators have so far been obliged either to study cells derived from the muscles of patients with FSHD and grown in the laboratory, or to study muscle biopsies. Studies of muscle cells cultured in the laboratory have provided some unique insights into FSHD, but it will be some time before we know if they directly address the defects in the muscles of young adults when they are first diagnosed with FSHD. Muscle biopsies are much more difficult to obtain and are available in only limited amounts. Through our new *NIH BBRI Sen. Paul D. Wellstone MD CRC for FSHD*, together with Drs. Emerson, Kunkel, Wagner, Wright, and Zatz, we will be continuing to pursue both of these established approaches. With additional support from the FSH Society, we will also develop models of FSHD in mice.

Our focus in developing an animal model is based on our finding that a single protein, mu-crystallin, is present at abnormally high amounts in extracts of deltoid muscles from FSHD patients, compared to healthy muscle and muscles from

Stuart Lai Mouse Model Development, *continued from page 19*

patients with several other muscle diseases. Experiments in which we forced muscle fibers in mice and rats to make high levels of mu-crystallin showed that the protein led to fiber loss and muscle pathology. This supports the idea that mu-crystallin may play a role in the pathogenesis of FSHD, but our experiments to date do not control for the amounts of mu-crystallin that we express in rodent muscles. We are therefore creating mice in which we can control the levels of expression in mu-crystallin to model the levels that we see in FSHD muscle. These “transgenic” mice should be available in the autumn of 2009, and we hope to begin our studies of them shortly thereafter, with the expectation that they will serve as a good animal model of FSHD.

The FSH Society, through the Wellstone Center, is also supporting the acquisition and study of other strains of mice that have been proposed as models of FSHD, including several strains of mice that express low to high levels of FRG1 (FSHD-region gene 1, linked to the deletions of D4Z4 repeats at the end of chromosome 4 that are diagnostic in most cases of FSHD), FRG2, ANT1, PitX1 and, when they are available, DUX4. Examination

of these mice, and the new mouse strains that we are making, should allow us to determine which, if any, can serve as a good model for FSHD, for use in studies of pathogenesis and tests of potential therapies.

The contributions of the FSH Society have been central to all our efforts. Without the initial funding it provided to enable us to initiate our studies, we would never have been able to progress to this point. Specifically, it provided Delta Railroad and Marjorie Bronfman Awards to Dr. Bloch that supported our initial studies of adult FSHD muscle and then supported the development of methods that made possible our studies of the proteins that change in FSHD. It then provided a Marjorie Bronfman Fellowship Grant to Dr. Reed that allowed him to identify mu-crystallin as the most prominent protein that changes in FSHD and to show that high levels of the protein are pathogenic to muscle. Its recent support of the creation and acquisition of transgenic mice is crucial to our future progress, and to the success of the Wellstone Center devoted to the identification of biomarkers of FSHD.

Eighteenth FSH Society Marjorie Bronfman fellowship

The FSH Society is pleased to announce the commencement of a new exciting research project and the eighteenth FSH Society Marjorie Bronfman Research Fellowship award. The FSH Society and its members are deeply indebted to the generosity of Mrs. Marjorie Bronfman for allowing FSHD research and solutions to flourish. This unique program, offered solely by the FSH Society and through the efforts of its peer review and Scientific Advisory Board, has rapidly accelerated the understanding of FSHD.

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

FSHS-MGBF-018 “*Analyses of functional domains in the pro-apoptotic protein DUX4*”
Total project \$59,040

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

DUX4 is a protein encoded within the 3.3-kb polymorphic tandem-repeat called D4Z4, at the locus FSHD1A (facioescapulohumeral muscular dystrophy 1A) on the human chromosomal region 4q35. We have shown that DUX4 is pro-apoptotic and is potentially related to the pathogenesis of FSHD. In this project we will use mutagenesis analyses to study the requirement of two hypothetical nuclear location signals (NLS1/NLS2) and two homeobox motifs (H1/H2) in the phenomenon of apoptosis mediated by DUX4. The studies will contribute to the understanding of the biology of DUX4 and its potential pathogenic role in FSHD.

Specific aims

Aim 1. Characterize NLS1 and NLS2, two putative nuclear signal localization sequences of DUX4. Hypothesis: Nuclear trafficking of DUX4 requires the sequences NLS1 and/or NLS2. Rationale: DUX4 is a nuclear protein. Analysis of its amino acid sequence shows two putative nuclear localization signals (NLS1 and NLS2). In Specific Aim 1, we will use site-directed mutagenesis to study the functionality of NLS1 and NLS2.

Aim 2. Determine whether the NLS1/NLS2 and the H1/H2 homeoboxes have a role in DUX4-mediated apoptosis. Hypothesis: The normal function(s) of DUX4 requires NLS1 and/or NLS2 as well as H1 and/or H2 sequences. The DUX4 pro-apoptotic pathway is dependent on the function of the NLS1/NLS2 and/or H1/H2. Rationale: DUX4 is a pro-apoptotic protein. Functional domains recognized in DUX4 are the NLS1/NLS2 and H1/H2. In Specific Aim 2, we will use site-directed mutagenesis to study the role of these sequences in DUX4-mediated apoptosis.

FSH Society grant summary — current grantees

► Marjorie & Gerald Bronfman Foundation Fellowship Grants

Richard Lemmers, Ph.D.

FSHS-MGBF-010 for “Refinement of the FSHD critical region on 4qA chromosomes”

Total project \$105,000

Melanie Ehrlich, Ph.D.

FSHS-MGBF-013 for “Finding the 4q35 FSHD Gene”

Total project \$70,000

Patrick Wayne Reed, Ph.D.

FSHS-MGBF-014 for “Analysis of Changes in the Proteome in FSHD”

Total project \$30,000

Yvonne Meijer-Krom, Ph.D.

FSHS-MGBF-015 for “Towards the Discovery of Early Developmental Defects in FSHD”

Total project \$105,000

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

FSHS-MGBF-016 for “Molecular Analyses of DUX4 and Interaction with Myogenic Regulators in FSHD”

Total project \$21,488

Paola Picozzi, Ph.D.

FSHS-MGBF-017 for “Functional characterization of D4Z4 in FSHD”

Total project \$17,500

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

FSHS-MGBF-018 for “Analyses of functional domains in the pro-apoptotic protein DUX4”

Total project \$59,040

► Stuart Lai Mouse Model Development Fellowship Grant

Jeffrey Boone Miller, Ph.D.

Robert J. Bloch, Ph.D.

FSHS-SLMM-001 for “The Boston Biomedical Research Institute Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD, Project 4 — Mouse Model Studies for FSHD Biomarkers”

Total project \$160,000

► Kelly Family Fellowship Grant

Ryan D. Wuebbles, Ph.D.

FSHS-KF-001 for “Testing the effects of elevated DUX4, DUX4c, and PITX1 in the *Xenopus laevis* vertebrate model system”

Total project \$15,000

► Jeff Jacobs Family Research Fellowship Grant

Scott Q. Harper, Ph.D.

FSHS-JJFR-001 for “Investigating DUX4 Structure and Function Using Rational Mutagenesis”

Total project \$40,000

► Helen Younger and David Younger Fellowship Grant

Kyoko Yokomori, D.V.M., Ph.D.

FSHS-HDY-001 for “The molecular characterization of the chromatin structure of the D4Z4 repeat associated with FSHD”

Total project \$90,000

Weihua Zeng, Ph.D.

FSHS-DHY-002 for “Epigenetic abnormality in FSHD”

Total project \$35,500

► Landsman Charitable Trust Fellowship Grant

Meredith Hanel, Ph.D.

FSHS-LCT-001 for “An in vivo *Xenopus* System for Studying D4Z4 Mediated Chromatin and Gene Expression”

Total project \$30,000

Scott Q. Harper, Ph.D.

FSHS-LCT-002 for “In Vivo Investigation of DUX4 as a Candidate FSHD Gene”

Total project \$10,000

► Family Picnic Fellowship Grant

Frédérique Magdinier, Ph.D.

FSHS-NYFP-001 for “Exploring the interplay between the nuclear envelope and the higher order chromatin organization of the D4Z4 array in control and FSHD cells”

Total project \$30,000

FSH Society testifies to Congress on FSHD research funding

On May 25, 2009 the FSH Society submitted congressional testimony to the U.S. Senate Appropriations Subcommittee on Labor, Health and Human Services, Education and Related Agencies on the subject of FY2010 Appropriations for NIH research on FSHD. Similar testimony was submitted to the U.S. House Subcommittee on Labor, Health and Human Services, Education and Related Agencies on May 1, 2009.

Daniel Paul Perez, President & CEO of the FSH Society, submitted written

testimony on the need for funding for FSHD and how the disease affects those who live with it. He noted that FSHD is now recognized as the most prevalent form of muscular dystrophy and continues to be drastically under-funded at NIH. He reminded the committee that NIH muscular dystrophy funding has nearly tripled since the inception of the MD CARE Act in 2001 — from \$21M to \$56M — and that NIH FSHD funding has remained level during the same time period — \$3M for FSHD out of \$56M for

muscular dystrophy.

We requested immediate help for people coping with and dying from FSHD. We asked NIH to fund research on FSHD at a level of \$10 million in FY2010 — a level where it rightfully should be. For the full testimony, please visit the FSH Society website www.fshsociety.org, click on “The FSH Society” tab at top and select “Advocacy” from the left hand navigation.

New publication ranks FSHD most prevalent dystrophy!

On January 30, 2009, a European government epidemiology report ranked FSHD as the most prevalent muscular dystrophy.

A very interesting, informative web site on disease prevalence can be found at <http://www.orpha.net/consor/cgi-bin/index.php> that is backed by INSERM (Insitut National de la Santé Et de la Recherche Medicale). It has a wealth of information.

Notable is the “Orphanet Reports Series.” Orphanet reports are a series of texts covering topics relevant to all rare diseases.

Please see the *“Prevalence or reported number of published cases listed in alphabetical order of disease”* in the November 2008 - Issue 10. An updated report will be published regularly and will replace the previous version.



This update contains new epidemiological data and modifications to existing data based on new information. This new information ranks FSHD as the most prevalent muscular dystrophy, followed by DMD/BMD and then DM. FSHD has historically been

thought of as the second or third most prevalent muscular dystrophy — this new data ranks it as the most prevalent.

Estimated prevalence (cases/100,000)

- ▶ 7 FSHD
- ▶ 5 Duchenne (DMD) and Becker Muscular dystrophy (BMD)
- ▶ 4.5 Steinert myotonic dystrophy (DM)

This is very much in line with the experience of the FSH Society over the past two decades of working in muscular dystrophy.

FSHD now greater target for funding

This new data is significant as it clarifies the high economic cost of having FSHD and the burden of the disease on society. For a pharmaceutical or biotechnology company it signals that FSHD is a substantial market for drug therapy and has the largest group of consumers within the dystrophies. The historical thinking was that the order of one, two and three by prevalence was DMD, DM and FSHD; now the order is FSHD, DMD, and DM. For the NIH, DoD, CDC and other federal agencies funding muscular dystrophy, it means that they need to take a very hard look at why funding levels for FSHD are so low and, in some agencies, non-existent.

Excerpts from U.S. Senate testimony of May 25, 2009

The Most Prevalent Form of Muscular Dystrophy is now Markedly Under-funded at NIH

It is a fact that FSHD is now published in the scientific literature as the most prevalent muscular dystrophy in the world. The incidence of the disease is conservatively estimated to be 1 in 14,285. The prevalence of the disease, those living with the disease, ranges to two or three times as many as that number based on our increasing experience with the disease and more available and accurate genetic diagnostic tests.

The French government research agency INSERM (Insitut National de la Santé et de la Recherche Medicale) is comparable to the NIH, and it recently published prevalence data for hundreds of diseases in Europe. Notable is the “Orphanet Series” reports covering topics relevant to all rare diseases. The “Prevalence or reported number of published cases listed in alphabetical order of disease” November 2008 - Issue 10 report can be found at

http://www.orpha.net/orphacom/cahiers/docs/GB/Prevalence_of_rare_diseases_by_alphabetical_list.pdf

This update contains new epidemiological data and modifications to existing data for which new information has been made available. This new information ranks facioscapulohumeral muscular dystrophy (FSHD) as the most prevalent muscular dystrophy followed by Duchenne (DMD) and Becker Muscular dystrophy (BMD) and then in turn myotonic dystrophy (DM). FSHD is historically presented as the third most prevalent muscular dystrophy in the Muscular Dystrophy Community

Assistance, Research and Education Amendments of 2001 and 2008 (the MD-CARE Act). This new data ranks FSHD as the most prevalent.

Estimated (cases/100,000)

- ▶ 7 FSHD
- ▶ 5 Duchenne (DMD) and Becker Muscular dystrophy (BMD)
- ▶ 4.5 Steinert myotonic dystrophy (DM)

NIH Muscular Dystrophy Funding Has Nearly Tripled Since the Inception of the MD CARE Act (\$21M to \$56M)

Between fiscal year 2006 and 2007, NIH overall funding for muscular dystrophy increased from \$39,913,000 to

\$47,179,000, an 18 percent increase.

Between fiscal year 2007 and 2008, NIH overall funding for muscular dystrophy decreased as shown in the “Estimates of Funding for Various Research, Condition, and Disease Categories (RCDC)” report on the new Research Portfolio Online Reporting Tool (RePORT) from \$58 million to \$56 million, a 3 percent decrease. These figures are from the new “2007/2008 NIH Revised Method” columns. The same RCDC RePORT system report shows \$47 million as the 2007 figure under the “2007 NIH Historical Method” column, a 23 percent increase and restatement when converting to the new system.

Figures from the RCDC RePORT and the NIH Appropriations History for

continued on page 24

National Institutes of Health (NIH) Appropriations History		
Sources: NIH/OD Budget Office & NIH OCPL (Dollars in millions) & NIH RCDC RePORT (see page 27)		
Fiscal Year	FSHD Research Dollars	FSHD % of MD
2002	\$1.3	5%
2003	\$1.5	4%
2004	\$2.2	6%
2005	\$2.0	5%
2006	\$1.7	4%
2007	\$3.0	5%
2008	\$3.0	5%

Combined Federal Campaign

Federal employees and military personnel can donate to the FSH Society through the Combined Federal Campaign (CFC). Please consider making a contribution to the FSH Society through the CFC. The CFC is operated by the United States Government Office of Personnel Management. The FSH Society CFC code is #10239. For more information about the CFC you may visit the OPM website at: <http://www.opm.gov/cfc/index.htm>

Congressional testimony, *continued*

Muscular Dystrophy report historically provided by NIH/OD Budget Office & NIH OCPL show that from the inception of the MD CARE Act 2001, funding has nearly tripled from \$21 million to \$56 million for muscular dystrophy.

NIH FSHD Funding Has Remained Level Since the Inception of the MD CARE Act (\$3M/\$56M)

Between fiscal year 2006 and 2007, NIH funding for FSHD increased from \$1,732,655 to \$4,108,555. In fiscal 2007, FSHD was 8.7% [previous figures from old NIH reporting system — non-RCDC coding schema] of the total muscular dystrophy funding (\$4.109M/\$47.179M).

Between fiscal year 2007 and 2008, NIH funding for FSHD decreased from \$4,108,555 to \$3 million under the “2007 and 2008 NIH Revised Method.” The “2007 NIH Historical Method” was restated to \$3 million. In fiscal 2008 under “NIH Revised Method,”

FSHD was 5.3% of the total muscular dystrophy funding (\$3M/\$56M). The previous years 2006/2007 figures are revised and restated under “2007 NIH Historical Method” as (\$3M/\$58M) which is 5.1% of the total muscular dystrophy funding. FSHD funding has merely kept its ratio in the NIH funding portfolio and has not grown in the last seven years.

We highly commend the Director of the NIH on the ease of use and the accuracy of the Research Portfolio Online Reporting Tool (RePORT) report “Estimates of Funding for Various Research, Condition, and Disease Categories (RCDC)” with respect to reporting projects on FSHD.

The MD CARE Act 2008 mandates the

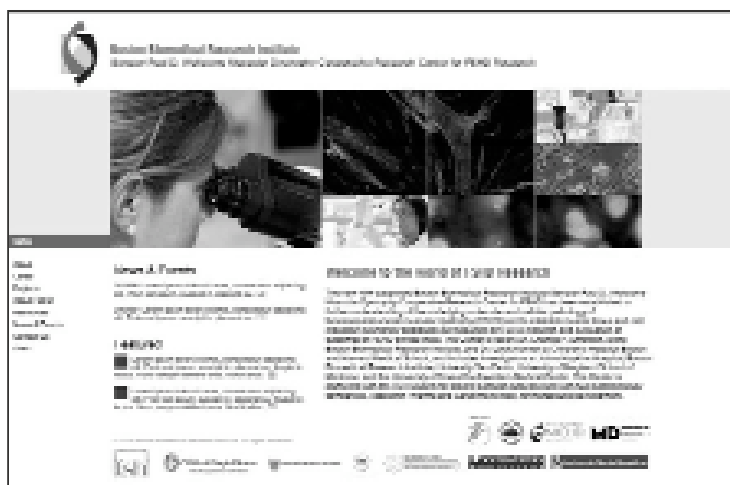
NIH Director to intensify efforts and research in the muscular dystrophies, including FSHD, across the entire NIH. It should be very concerning that in the last seven years muscular dystrophy has tripled to \$56 million and that FSHD has remained at five (5) percent of the NIH muscular dystrophy portfolio or \$3 million. Only three of the institutes at the NIH are funding FSHD. OD, NHLBI, NIGMS, NIBIB, NIDCD, NHGRI, NEI,

most prevalent dystrophy myotonic dystrophy (DM), received \$9 million from NIH. In 2008, the most prevalent dystrophy, facioscapulohumeral muscular dystrophy (FSHD), received \$3 million from NIH. It is now time to flip the stack and to make sure that FSHD, with its equal burden of disease and highest prevalence, gets more funding, stimulus and that NIH program staff initiates request for applications specifically in

FSHD. It is crystal clear, if not completely black and white, that the open mechanism program announcement and investigator driven model are not achieving the goal mandated by the MD CARE Acts 2001/2008 and by the NIH Action Plan for the Muscular Dystrophies as submitted to the Congress by the NIH. Efforts of excellent program staff and leadership at NIH, excellent reviewers and study sections, excellent and outstanding researchers working on FSHD and submitting applications to the NIH, and extraordinary efforts of the volunteer health agencies working in this area

have not yet enabled FSHD funding to increase at the NIH. It is time for NIH requests, contracts and calls for researcher proposals on FSHD to bootstrap existing FSHD research worldwide.

I am here once again to remind you that FSHD is taking its toll on your citizens. FSHD illustrates the disparity in funding across the muscular dystrophies and recalcitrance in growth over twenty years despite consistent pressure from appropriations language and Appropriations Committee questions, and an authorization and a reauthorization from Congress mandating research on FSHD.



A view of the home page of the new NIH BBRI Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD. Coming soon!

NIA, NCI and NCRR are all aware of the high impact each could have on FSHD. FSHD is certainly still far behind when we look at the breadth of research coverage NIH-wide.

Now, FSHD is published as the most prevalent muscular dystrophy, and given the extraordinary interest of the scientific and clinical communities in its unique disease mechanism, it defies gravity that it still remains the most prevalent and yet one of the most underfunded dystrophies at the NIH and in the federal research agency system (CDC, DoD and FDA). In 2008, the third most prevalent dystrophy, Duchenne (DMD) and Becker Muscular dystrophy (BMD) type, received \$22 million from NIH. In 2008, the second

The case for supporting research through the FSH Society

The strength of the FSH Society resides in its renowned Scientific Advisory Board and patient-driven Board of Directors. In the span of eleven years, (the Society awarded its first grant in 1998), the FSH Society has transformed the face of FSHD research. Today, we are on the verge of a series of significant breakthroughs. It is essential to fund new ideas and give support to new investigators, new data generation, and new lines of investigation when tackling a disease as complex as FSHD.

It is also essential to have and house a historical, unified and global view of FSHD research by a uniquely qualified panel of experts. These experts are not only judging the merit of new proposals they receive, but are also actively engaged in thinking globally and strategically

about the scientific problem over a period of several decades. Peer-reviewed science is key to research success, and peer review must be just that — reviewed by scientists and doctors who have a comprehensive command of the proposals and science being judged.

We know that research grants reviewed by the FSH Society Scientific Advisory Board will be absolutely top rate and necessary science. The NIH has a peer review system that prides itself on funding the “best science.” The Society is often surprised at the number of missed opportunities (even by the NIH) to fund first rate and progressive FSHD projects.

Respect for the scientific review process and discipline in the scientific method and critical thinking are paramount. The Society enforces and

abides by this principle. Individuals working with broad-based organizations supporting neuromuscular disease research may want to contact the Society to make sure their efforts are effectively targeted at finding a cure for FSHD. Individuals thinking of making a donation directly to a researcher should keep in mind that by donating those funds through the FSH Society, he/she ensures that the gift is used to advance FSHD research. Time-tried experts, who judge the science while considering FSHD research as a whole, vet all research proposals. This prevents duplicating efforts, working on an issue that has already been resolved, or spending valuable resources pursuing a hypothesis that lacks sufficient promise.

Introducing NIH RCDC & RePORT databases

The Research Portfolio Online Reporting Tool

The NIH is the nation’s medical research agency. The NIH has created online access to reports, data, and analyses of NIH research activities, called the Research Portfolio Online Reporting Tool (RePORT). The Report website gives access to NIH expenditures and the results of NIH-supported research. Visit their site at:

<http://projectreporter.nih.gov/reporter.cfm>

The Research, Condition, and Disease Categorization process

The NIH also created the new Research, Condition, and Disease Categorization (RCDC) reports to report the amount it funded in each of 215 historically reported categories of disease, condition, or research areas.

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

To view the annual dollars being spent on various types of muscular dystrophy including FSHD, please see

<http://report.nih.gov/rcdc/> or

<http://report.nih.gov/rcdc/categories/>

Save the Date

For gatherings of FSH Society members and friends!

- ▶ **New York City, New York**
Trunk Show: Shopping for a Cure for FSH Muscular Dystrophy
Thursday, September 17
- ▶ **Fire Island, New York**
Long Island Gathering of Families
Late Summer 2009
- ▶ **Cape Cod, Massachusetts**
Walk ‘n’ Roll for FSH Muscular Dystrophy
Saturday, October 10

For more information, contact the FSH Society office, info@fshsociety.org or (617) 658-7878.

Society-initiated research leads to first paper on FSHD & miRNA

An FSH Society-initiated fellowship to Dr. Patricia Arashiro working with Drs. Mayana Zatz and Louis Kunkel, led to the publication of a paper in top-rated "Proceedings of the National Academy of Sciences" journal – the first on miRNA and FSHD.

"Transcriptional regulation differs in affected FSHD patients compared to asymptomatic related carriers."

Proc Natl Acad Sci U S A. 2009 Apr 14;106(15):6220-5. Epub 2009 Apr 1.

Arashiro P, Eisenberg I, Kho AT, Cerqueira AM, Canovas M, Silva HC, Pavanello RC, Verjovski-Almeida S, Kunkel LM, Zatz M.

Human Genome Research Center, Department of Genetics and Evolutive Biology, Institute of Biosciences, University of São Paulo, 05508-090, São Paulo, Brazil.

Excerpts from PubMed

Our study is unique in comparing the gene expression profiles from related affected, asymptomatic carrier, and control individuals. Our results suggest that the expression of genes on chromosome 4q is altered in affected and asymptomatic individuals. Remarkably, the changes seen in asymptomatic samples are largely in products of genes encoding several chemokines, whereas the changes seen in affected samples are largely in genes governing the synthesis of GPI-linked proteins and histone

acetylation.... Interestingly, our results also suggest that the miRNAs might mediate the regulatory network in FSHD. Together, our results support the previous evidence that FSHD may be caused by transcriptional dysregulation of multiple genes, in cis and in trans, and suggest some factors potentially important for FSHD pathogenesis. The study of the gene expression profiles from asymptomatic carriers and related affected patients is a unique approach to try to enhance our understanding of the missing

link between the contraction in D4Z4 repeats and muscle disease, while minimizing the effects of differences resulting from genetic background.

For more information, see the FSH Society website

www.fshsociety.org/pages/comNewsEvents_ArashiroZatzKunkelPNAS.html

Or PubMed at

www.ncbi.nlm.nih.gov/sites/entrez search for PMID: 19339494

Become a member of the FSH Society today!

Join with thousands of other patients with FSHD and their families who recognize the importance of building constituency, education, advocacy, outreach and research for FSHD!

Your membership donation is vital to continuing the remarkable achievements of the FSH Society. Join or renew today for a year full of great benefits! The FSH Society, founded in 1991, is made up of members who contribute at least \$50 a year to the Society and its programs.

All gifts are tax-deductible

With a gift of \$50 or more, you:

- ▶ Receive news on research, the Watch newsletter, and other communication about FSHD
- ▶ Join together with others in the FSHD community to find treatments for patients, to improve their quality of life, and to help provide hope for a cure through the promise of research.

- ▶ Will be acknowledged in the Annual Donor report, unless we hear otherwise.

To become a Member online, go to:

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Please call (617) 658-7878

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BBRI R353
64 Grove Street
Watertown, MA 02472 USA

Society now on Facebook

The FSH Society has a Facebook page. To become a Facebook "fan" of the Society, visit www.facebook.com, search on FSH Society, and click the "become a fan" link in the upper right-hand corner.



Many Ways to Contribute to Society's work

At the FSH Society, we accomplish our work of helping to solve FSHD through your membership, gifts, bequests and many other forms of giving. Some of our work is accomplished with the help of dedicated volunteers who carry out fundraising projects to promote the FSH Society and its endeavor to find treatments and a cure for FSHD. You can become part of this vital work for the benefit of everyone who lives with FSHD by becoming a member, making another gift, or as a volunteer.

Bronfman Foundation fellowship grantee

Melanie Ehrlich, Ph.D.

Marjorie Bronfman fellow grant number 13

So, why is FSHD a chromosome-4 disease? by Melanie Ehrlich, Ph.D.

I am a biochemist who studies DNA and chromosomes. I stumbled (happily) into research on FSHD while investigating another disease that involves repeated DNA sequences. My first FSH Society grant about seven years ago was critical for my entry into the field of FSHD genetics. Daniel Perez's advocacy for NIH funding for FSHD was essential for NIH providing funds for FSHD grants, including grants which I obtained. FSHD research brought me a new appreciation of the importance of basic research to real patients for development of clinical tools and treatments.

The following article and figures were prepared by myself and one of my postdoctoral fellows, Dr. Xueqing Xu. He and Dr. Koji Tsumagari (who also made major contributions to this project), and Drs. Desheng Chen and Jingjing Ma work on FSHD in my lab at Tulane Medical School in New Orleans.

The Science

FSHD is a very complicated disease at the DNA level but we know one thing with no doubt about the mutation that causes it. When a string of repeated 3300 base-pair units of DNA, the so-called D4Z4 repeat array, is too short on chromosome 4 (chr4), this almost always causes the disease, while a mostly identical short D4Z4 array on chromosome 10 (chr10) never has any effect on health (Figure 1). Surprisingly, this is true even though chr4 and chr10 have very similar DNA in and around D4Z4. So, why is FSHD caused by a short D4Z4 array on chr4 and not also from a short D4Z4 array on chr10?

Is there a gene that is present on chr4 and not on chr10 somewhere near a short disease-causing D4Z4 array that gets abnormally turned on?

We are not sure. Maybe the gene is an unusual one that has not yet been identified (part of our FSH Society-funded study) or maybe the affected stage in muscle development has not been

studied.

Are there a small number of important disease-related differences in DNA sequences within D4Z4 arrays on chr4 compared with chr10?

We know that D4Z4 is used to make very small amounts of RNA from different parts of the repeat sequence including a part that looks mostly like a standard gene¹⁻³. A very small number of differences between the sequences of D4Z4 on chr4 and chr10 have been found^{4,5}. However, there are exchanges that occur between chr4 and chr10 so that their D4Z4 sequences get jumbled^{6,7}. Nonetheless, only the D4Z4 arrays on chr4 cause disease when they contract too much in size. If sequence differences between chr4 D4Z4 and chr10 D4Z4 matter for FSHD, it might be that the first repeat unit is the most important^{5,8}.

Are there a small number of differences in the DNA sequences next to the D4Z4 arrays on chr4 and chr10 that

are relevant to the disease?

Yes, there is variation among individuals in the DNA sequence at the far end of chr4, past D4Z4, and very small variations on the other side of D4Z4⁵.

Surprisingly, some of the variants are seen all the time in patients but also in about half of unaffected individuals. Therefore, these variants do not cause FSHD, but a chr4 DNA sequence variant seems to be permissive for the disease for some unknown reason.

Is there some special FSHD-related feature of a large part of the end of chr4 where the D4Z4 is found (Figure 1) that makes short D4Z4 arrays on chr4, but not on chr10, able to cause disease?

By fluorescence microscopy and DNA replication analysis in FSHD or control muscle cells (myoblasts)⁹, we showed that the end of chr4 (300,000 or 800,000 base-pairs away from D4Z4) is not very tightly condensed (is not constitutive heterochromatin). Therefore overall high

continued on page 28

Walk 'n' Roll for FSHD

To benefit the FSH Society — Please help "Unlock the Code"

Cape Cod, Massachusetts • Late Reg: 12:00 p.m. • Walk Begins: 1:00 p.m.

Come join us on scenic Cape Cod for a two mile Walk 'n' Roll down the Cape Cod Rail Trail, and raise funds for the FSH Society!

► Course Info

The Walk 'n' Roll starts at Stony Brook Elementary School on Underpass Road in Brewster, Massachusetts, and ends at the entrance to Nickerson State Park. Participants who wish to go only part of the distance can be picked up at the intersection of the Cape Cod Rail Trail and Thad Ellis Road; about a third of the route. Those who wish may walk or roll from the beginning to the end and back again, or arrange to have someone pick them up at the park to return them to their car at the school. Parking and accessible bathroom facilities are available at Stony Brook Elementary.

► Sign-Up & Sponsor Info:

Complete the registration form and mail or fax back to the FSH Society, along with your registration fee, at: FSH Society, Inc., 64 Grove Street, Watertown, MA 02472, ATTN: Walk 'n' Roll; Fax: (617) 658-7879

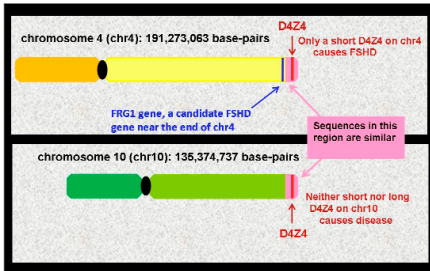
► Registration form can be found at:

www.fshsociety.org/assets/pdf/FSHSociety_WalknRoll_Registration%20Form_28July09.pdf

All registered walk 'n' rollers raising \$50 or more will receive an event T-shirt. Bring your sponsor form (located on the registration form) and collected pledges with you the day of the event. For directions, please put Nickerson State Park in your favorite map search engine.

Ehrlich, continued from page 27

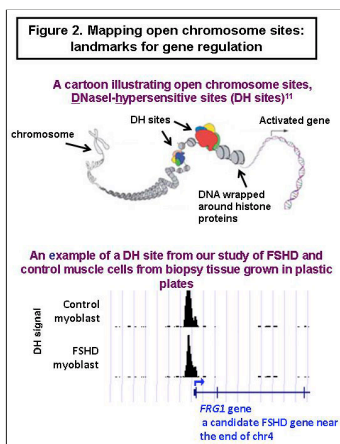
Figure 1. The position of FSHD-causing D4Z4 arrays. The D4Z4-containing ends of chromosome 4 and chromosome 10 are not to scale here but they are in figure 3.



condensation is not a property of this end of chr4 that distinguishes it from chr10 nor does it distinguish FSHD and control cells.

By biochemical analysis, we demonstrated that D4Z4 itself is only moderately condensed in FSHD and control blood or skin cells and that the region immediately next to it has only a low level of condensation¹⁰.

In collaboration with Greg Crawford of Duke University, we are using a powerful new technique (DNase-chip¹¹, Figure 2) to examine chromosome structure in the vicinity of D4Z4 in FSHD and control myoblasts. We are looking for small chromosomal sites (DNaseI-hypersensitive sites or DH sites) that are



open, unlike most DNA which is wrapped around a kind of protein called histones. These DH sites can be signals allowing gene expression. We think they may also help control the local shape of chromosomes.

There may be a combination of one or more factors from this list that makes

FSHD only a chr4 disease.

Our hypothesis is that there is a change in the overall shape at one end of the D4Z4 array when the array is too short. This could explain why a D4Z4 array on chr4 can be short enough to cause FSHD, but another that is only slightly larger and in the same position has no effect. Our DNase-chip study has shown us that there are

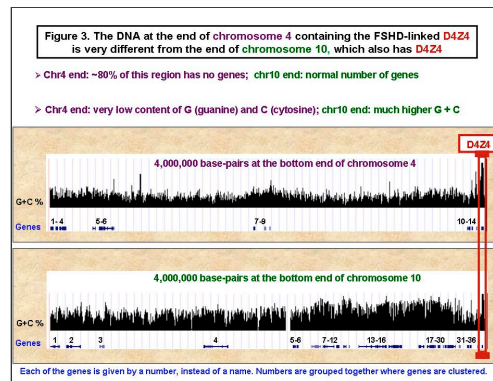
unusually few open sites (DH sites) at the D4Z4-containing end of chr4, many fewer than at the end of chr10. Some of these DH sites that are far from genes are specific for muscle cells. In these regions of chr4 and chr10, differences between the distributions of DH sites and the nature of the DNA (Figure 3) may provide clues as to how short D4Z4 arrays on chr4, but not on

chr10, cause disease. They may also explain earlier findings about the special location of the end of chr4 within the nucleus of FSHD & control myoblasts¹².

To help connect the dots between short D4Z4 arrays, chr4, and genes on other chromosomes, we are studying

expression of genes in FSHD and control muscle cells (myoblasts and myotubes) and other aspects of chromosome structure in this 4-million base-pair region of chr4 (Figure 4). In addition, with Dr. Crawford, we have begun mapping these

DH sites on all the chromosomes with an even more powerful and related technique, DNase-seq. This allows us to look at the genes on any chromosome involved in muscle development and genes involved secondarily in the muscular dystrophy symptoms of FSHD.



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