

FSH Watch

RESEARCH
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CONNECTING THE COMMUNITY OF PATIENTS, FAMILIES, CLINICIANS AND INVESTIGATORS



TRANSLATIONAL RESEARCH

Journey toward developing a drug for FSHD

Perspectives and updates from recently funded FSH Society grantees

by **DARKO BOSNAKOVSKI, D.V.M., Ph.D.**
University Goce Delcev Stip, Macedonia

Although FSHD is considered one of the most common inherited neuromuscular diseases, there is no specific therapeutic practice for it. So what can we do about it? First, we have to understand the mechanisms of the disease and to identify the crucial links in the chain of molecular reactions that underline FSHD. Furthermore, we have to develop a system to screen various therapeutic approaches, and in the end to generate an animal model where clinical relevance of therapy can be determined. When I joined the FSHD group lead by Dr. Michael Kyba at University of Texas Southwestern six years ago to develop a specific therapy for FSHD all of the above was considered to be a big enigma. We started working with DUX4¹, the gene within D4Z4, due to the similarity of its homeodomains to those of the master myogenic genes Pax3 and Pax7². We postulated that both genes compete for the same target DNA sequences and with that the function of myogenic cells is impaired. At that time only a small portion of the scientific community, led by Alexandra Belayew

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Dr. Davide Gabellini with fellow researchers at the 2010 FSH Society FSHD research workshop



TRANSLATIONAL RESEARCH

A possible approach for treating FSHD with RNAi therapeutics

Perspectives and updates from FSH Society grantees

by **DANIEL PEREZ**
FSH Society

with contributions by **DAVIDE GABELLINI, Ph.D.**
Division of Regenerative Medicine, San Raffaele Scientific Institute, Milan, Italy, and

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Two exciting papers were recently published on possible approaches for treating FSHD using a disease gene silencing approach called RNA interference (RNAi). The details emerged within one month of each other in the journal *Molecular Therapy*. The two complementary studies were performed by different teams of

scientists: the Harper Lab at The Ohio State University and Nationwide Children's Hospital in Columbus, Ohio, with a collaborator in Modena, Italy; and the Gabellini and Chamberlain labs in Milan, Italy, and Seattle, Washington, respectively.

RNA interference is a natural cellular process that controls the levels at which certain genes are expressed. In this sense, it operates less like an on/off switch and more like a molecular volume control knob. Over the last several years, many scientists have been working to co-opt these natural gene-silencing strategies for therapeutic purposes. Indeed, the main triggers of RNAi in the cell, called inhibitory RNAs or microRNAs, can be rationally designed in the lab to knock down disease genes. In the two molecular therapy studies, the research teams rationally engineered FRG1-targeted inhibitory RNAs and then delivered them to muscles of dystrophic FRG1^(-high) mice using adeno-associated viral (AAV) vectors,

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Dear Friends,

This issue of the *FSH Watch* reports great advances and dedicated people making remarkable progress toward understanding and treating FSHD. We are proud of the work detailed in this issue, moving us closer to understanding and treating FSHD. The FSH Society has made excellent progress in bringing scientists into the field, allowing them to train, and in giving them opportunities to develop ideas in FSHD. We hope you agree.

In last year's research issue we highlighted Society seed-funded work by Drs. Lemmers et al. in their paper "A Unifying Genetic Model for Facioscapulohumeral Muscular Dystrophy," *Science*, August 19, 2010, that put forth a unified and long-sought explanation of the exact biological workings of a disease that affects an estimated one in 14,000 or 22,100 Americans and 490,000 individuals worldwide. Subsequently the Society announced that it helped to fund a second critical advance in determining the cause of FSHD in *PLoS Genetics*, "Facioscapulohumeral Dystrophy: Incomplete Suppression of a Retrotransposed Gene," on October 28, 2010, that shows that the disease is caused by the inefficient suppression of a DUX4 gene that is normally expressed only in early development.

The FSH Society's approach to solving FSHD is pragmatic. We are now studying the "FSHD causing targets" in our translational research phase to enable us to initiate therapeutic clinical trials. In this issue, we highlight efforts in each of the areas supported by the Society – mechanistic, translational, pre-clinical and clinical. The FSH Society continues to fund ground breaking and early-stage efforts in research, translational research, small molecule screening and clinical efforts. As you read about the projects we've recently funded and the updates from scientists all over the world, please remember that great things are happening, and they are only made possible by your financial donations and willingness to actively participate in research efforts.

Please consider participating in studies highlighted in this issue, including the natural history study and developing biomarkers for FSHD.

The FSH Society's research portfolio seeks a good balance between conservative and innovative approaches in developing our knowledge of how FSHD works. We are constantly readjusting between following the "published paradigm" to guide FSHD progress with that of setting out in new innovative directions to improve our understanding of the disease. Both attitudes are appropriate. In times like these, when the research community struggles with developing a high-confidence therapeutic target, a bias towards innovation is appropriate.

A top priority as outlined by the international community during the most recent Society research planning workshop is to independently verify and confirm the DUX4 hypothesis as published in *Science* in 2010. And if confirmed, move to understand normal DUX4 function, and finally, move towards understanding the naturally occurring variability of DUX4 to allow scientists to manipulate the disease in our favor. Currently, the published paradigm is that DUX4 is necessary and sufficient to cause FSHD. However, if it turns out that DUX4 is necessary but not sufficient or even not necessary to cause FSHD, then the choices of a target and pathway are markedly different. It is one thing to eliminate a protein that you are not supposed to have at all in muscle and another to modulate a protein that has a resolution near the noise floor of detection and may be expressed at different thresholds in individuals with FSHD and without FSHD.



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

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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.

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Two papers on possible approaches for treating FSHD using RNA interference (RNAi therapy) were recently published by the Harper Lab at The Ohio State University and Nationwide Children's Hospital in Columbus, Ohio, with a collaborator in Modena, Italy; and the Gabellini and Chamberlain labs in Milan, Italy, and Seattle, Washington, respectively in *Molecular Therapy*. The two complementary studies are the first successful proof of concept of a possible therapeutic approach for FSHD. Using a well known and published mouse model of FSHD, with an over expression of a possible candidate gene for FSHD the FRG1 gene, scientists used RNA interference to silence the unwanted by products of a gene associated with FSHD. Both groups acknowledged the strengths and weakness of FRG1 as the target gene for FSHD, the prospect of DUX4 being the target for FSHD, and the ease of changing the gene of choice to knock-down using this technique. The caveat being that, the up regulated FRG1 transcript hasn't been consistently found in humans with FSHD while the mouse FRG1 mouse model has a myopathic phenotype that is visibly reversed when it is knocked down. While the opposite is with DUX4 -- the DUX4 transcript is accessible in human FSHD tissues though it has been very difficult to create a viable DUX4 mouse, and additionally, a DUX4 mouse with a visible phenotype that can be tested using RNAi.

Patents are now being filed for various techniques of modifying gene and protein expression in FSHD. Large pharmaceutical and biotechnology companies are scrutinizing DUX4 as a target for FSHD. But the necessity remains to verify the DUX4 results presented in 2010 with a large number of FSHD samples. You can help here by donating blood, skin and muscle tissue to research centers needing human FSHD-biomaterials. The Society is trying to provide the reagents, findings and animal models used in the translational and pre-clinical research process. The Society has funded the creation of more than two-dozen transgenic animal models and we continue to push forward. We consistently ask our grantees to publish on these models (not always easy to do) and to distribute and collaborate on these models. We are hopeful that the large scale funding agencies will deem it acceptable for them to verify and vet out this DUX4 finding through translational and clinical trials efforts that are well

established for other dystrophies.

We remain deeply concerned that FSHD research efforts are not functioning at the proper levels of funding and running on a reduced economy as compared to peer-diseases and diseases of similar economic burden. We have made this evident in our Congressional testimony and report language. We are also working side-by-side and in contact with other FSHD-agencies worldwide (U.S. NIH, MDA USA, AFM France, Stichting FSHD Holland, FSHD Global Australia, Pacific Northwest Friends, Muscular Dystrophy Canada, Cooperative International Neuromuscular Research Group, Treat-NMD) to address resources and funding needed. We all need to amicably and collectively contribute to solve FSHD.

As we move forward into translational areas, it is absolutely necessary to verify the mechanism and target. We can then move forward with confidence with therapeutic trials. Not doing so might dilute efforts to achieve such especially in these tough economic times for research funding. The Society continues to educate and advocate for more funding from large funding agencies and the U.S. federal government. We will need your help in reaching out to members of Congress to let them know how FSHD affects you, that a well funded NIH is necessary, and that not only will cuts in spending slow things down for FSHD research progress but increases in revenue are needed in FSHD at this critical time.

Since our last issue of the *FSH Watch*, we have lost good friends and family members to the ravages of FSHD. I dedicate this issue to Mr. Edward M. Schechter, who passed away on July 2. Ed was a constant champion for everyday folks suffering and living with FSHD. Ed made the FSH Society's longstanding achievements in FSHD possible through his guidance and the funding of the Marjorie Bronfman post-doctoral fellowships. We cannot deny the terribly destructive and debilitating aspects of FSHD. Your support is vitally needed. Thank you again for your continued support of the FSH Society and our work. Please consider giving as generously as you can to enable us to continue the Society's educational and advocacy efforts and amazing progress made in research. FSH Watch

— Daniel Paul Perez
President & CEO

Beth Johnston joins the FSH Society Board of Directors

Beth joined the Board of Directors in 2011. A native of Colorado, she received a Master of Business Administration from University of Denver and a Bachelor of Science, Business Management & Finance from Colorado State University. She has worked in information technology, project management, high-technology consulting, telecommunications and real estate. Beth volunteers for several organizations in New York where she now lives; she currently serves as Vice President of the St. Augustine Home & School Board in Ossining New York and will become President for the 2011-2012 school year. Beth is co-chair of the annual FSH Society fundraiser, "A Festive Evening of Song." Beth's interest in the FSH Society stems directly from the fundraising efforts she has been overseeing during the last five years. Second only to supporting efforts to raise the critical funds necessary for FSHD research, she feels that raising awareness of both the disease itself and the function of the FSH Society are the vital areas where she can contribute. Beth serves as a director and on the development committee. Beth and her husband Jeff have two daughters, Nicole and Samantha.



Beth Johnston with her husband Jeff Johnston at the 2011 New York Festive Evening of Song

Rationale for natural history studies of FSHD

Ground work and preparations for clinical trials in FSHD

by JEAN K. MAH, M.D., M.Sc., FRCPC

On behalf of the Cooperative International Neuromuscular Research Group
 Alberta Children's Hospital, University of Calgary, Calgary, Alberta, Canada

Despite recent breakthroughs in understanding the molecular genetics of FSHD, the exact mechanism(s) responsible for the variability of the disease remain undefined, and there is currently no cure for FSHD. Emerging therapeutic trials will benefit from the availability of natural history data and reliable outcome measures for both children and adults with FSHD.

The study of natural history in medicine refers to a careful and systematic descriptive study of the uninterrupted progression of a disease that occurs in affected individuals over time, starting from the beginning (infancy or early childhood) and extending throughout the course of the illness (adolescence, adulthood) until the end (menopause, senescence). A thorough knowledge of the natural history of disease is important for developing effective strategies towards disease

prevention and treatment. It also provides essential information regarding potential biomarkers to disease expression and contributes to the development of a future clinical trials network.

In the case of FSHD, many individuals are generally healthy until adolescence or young adulthood when the onset of facial or shoulder weakness subsequently brings them to medical attention. Recognition of this preclinical phase of the disease is important as it provides a window of opportunity for potential disease modification. For example, genetically engineered strategies that are designed to halt the progression of FSHD can potentially keep these young individuals symptom-free for the rest of their lives. As well, if the rate or degree of change in the strength of individual or groups of muscles among untreated individuals with FSHD

over a defined period of time is known, the information can then be used as one of the outcome measures to test the effectiveness of new treatments for FSHD. Changes that can be easily (and consistently) detected in the majority of cases are especially valuable as they require less number of study participants (i.e. smaller sample size) and the clinical trials can be completed within a shorter period of time (thus reducing cost and effort). Furthermore, an awareness of the differences in the rate of progression of disease among individuals with similar genetic mutations may lead to the identification of environmental factors or other genetic modifiers of FSHD.

Your on-going support and participation will make it possible for us to understand the natural history of FSHD. Thank you for your consideration of our work. [FSH Watch](#)

► In Memory of Edward M. Schechter 1920 – 2011

Edward Schechter of Shavertown, Pennsylvania, died on July 2, 2011, at age 91 at his home with many members of his loving family at his side. He fought a long and courageous battle for more than forty years against FSHD.

Born in New York City in 1920, Ed was the son of Jacob and Meta Schechter. He grew up in New York and graduated from DeWitt Clinton High School and Dartmouth College. He attended Harvard Business School for two years, leaving shortly before graduation in 1942 to enlist in U.S. Army and fight in World War II. He was trained as an infantry officer with the 10th Mountain Division in Colorado and Washington, but he was deployed to the Pacific Theater where he served as an intelligence officer in battles throughout the islands of the South Pacific. He was among the first American soldiers to enter Hiroshima following the dropping of the atomic bomb. After seeing the devastation and suffering there, he turned in his weapons and led the rest of his life as a pacifist. Ed attained the rank of Captain and earned the Silver and Bronze Stars. Ed married Betty Jane Goodstein in 1944. Theirs was a long and loving marriage, and they celebrated their 67th wedding anniversary in January 2011.

Ed was truly a champion of FSHD research and many other just causes. He cared deeply about the FSH Society, patients and their families. He was a mentor, a great friend, a deep intellect, and an extraordinarily ethical and just man. We will miss his stunningly clear and booming voice, his direction, motivation and imperative to get things done in

FSHD. It was a true reflection of Ed's character, courage, kindness and strength that he made telephone calls in his last days to say goodbye to people and causes he cared deeply about. The FSH Society's successes in FSHD research to date are greatly owed to the support of Ed and his family. We will miss him constantly, and we regret we could not do more to alleviate FSHD during his lifetime.

The FSH Society is deeply indebted to Edward Schechter, brother of Mrs. Bronfman, of the Marjorie and Gerald Bronfman Foundation, and other members of their families for the advances that have been made possible in FSHD research worldwide. Edward Schechter deserves much gratitude for his stewardship of the Marjorie Bronfman grants to the FSH Society and their ensuing research breakthroughs.

This issue of the *FSH Watch* is dedicated to the memory of Ed Schechter, a rare gentleman and a true friend to the FSH Society, many of its members and his fellow FSHD patients.

At the wishes of Ed's family and friends, the FSH Society will establish an endowment fund to be called the "Ed Schechter Fund for FSHD," to recognize his many years of care and concern not only for the FSH Society and FSHD research, but also his keen interest in the individuals carrying out this research. When you make your membership gift at this time, please join with others in remembering Edward Schechter and make a gift to the FSH Society's Schechter Fund for FSHD to help support research.

International FSH consortium outlines research priorities for FSHD

Identifying priorities to solve FSHD

by **FSH SOCIETY**
 Watertown, Massachusetts

Scientists, patients, advocates, biotech and pharmaceutical companies, and clinicians from throughout the world gathered at Boston Biomedical Research Institute (BBRI) late October 2010 for the Research Planning Meeting for FSHD.

The meeting focused on collaborating to find new treatments and cures for FSHD. Meeting scientific clinical and research co-chairs were: Rabi Tawil, M.D., University of Rochester Medical Center & Fields Center for FSHD and Neuromuscular Research; and, Silvère van der Maarel, Ph.D., Leiden University Medical Center & Fields Center for FSHD and Neuromuscular Research. Daniel Paul Perez, President and co-founder of the FSH Society, co-organized the meeting. Professor David Housman, Chairman of the FSH Society Scientific Advisory Board (SAB) and many members of the Society's SAB were present and helped moderate the meeting. The meeting was co-hosted by Dr. Charles P. Emerson, Jr., distinguished scientist and Director of the BBRI, and Co-Director, along with FSH Society Board and SAB member Dr. Louis Kunkel of Harvard Medical School and Children's Hospital of the NIH Eunice Shriver Kennedy NICHD Sen. Paul D. Wellstone MD CRC for FSHD. Also joining the meetings was Ljubisa Vitkovic, Ph.D., program director for muscular dystrophy at the NICHD.

Sponsors for the event included Association Française Contre les Myopathies (AFM), The Fields Center for FSHD and Neuromuscular Research, FSH Society, FSHD Global Research Foundation, NIH Eunice Kennedy Shriver NICHD Boston Biomedical Research Institute Senator Paul D. Wellstone MDCRC, and the Muscular Dystrophy Association United States (MDAUSA).

The group recommended that, given the recent developments in the definition of the molecular mechanism of FSHD, the potential exists that within one or two years, evidence-based intervention strategies, therapeutics, and clinical trials could be planned and conducted. Hence, immediate priorities should be to independently-verify and confirm the DUX4 hypothesis as published in *Science* in 2010, and if this hypothesis is confirmed then understand normal DUX4 function, and finally, move towards understanding the naturally occurring variability of DUX4 to allow scientists to manipulate the disease therapeutically. The group stated that the FSHD research community needs to be prepared for this new era, by accelerating efforts in the following ten areas:

1. **Shareable Protocols.** There is a need for access to FSHD research protocols and experimental methods by FSHD researchers internationally. Readily available, clear and well defined research protocols are needed to allow verification, standardization and corroboration of research findings and publications.
2. **Common and shareable materials and data by the entire community.** There is a need for global and international biomaterials and data management. Schemas are needed to identify source and context of biomaterials and data, meaningful data identifiers, and easily accessible bio-repositories and data sources.



A pair of distinguished FSHD scientists provide a stimulating overview of a research area at one of eight topical working sessions at the 2010 FSH Society FSHD planning meeting

3. **Corroborate and verify DUX4 finding.** This line of work will be instrumental to pinpoint the real identity of FSHD1A (chromosome-4-D4Z4-contraction-linked cases) and FSHD1B (chromosome-4-non-D4Z4-contraction-linked cases & non-chromosome-4-linked cases). This information will form the basis for evidence-based intervention. There is a need to verify and reproduce the DUX4 finding using multiple sites and patient materials.
4. **FSHD alleles in the context of population genetics need to be defined.** There is a need to understand the normal function of the short DUX4 transcript in every human being and the abnormal function of toxic long-form of the DUX4 transcript.
5. **Biomarkers.** There is obvious need for monitoring intervention. There is the need to define biomarkers for clinical trials endpoints, to understand the FSHD at multiple omics levels {proteomics, genomics, transcriptomics, metabolomics} and to understand pathways and signaling of FSHD through "omics" analysis.
6. **FSHD clinical evaluation scales/systems need be defined under a single agreed standard.** There is an important need to have a comprehensive and single clinical evaluation standard to allow a list of clinical identifiers and parameters to be assembled into a thorough and robust dataset. This can be applied to subsequent systems biology and -omics areas.
7. **Working groups/Mouse model working group consortium.** There is a need for models and methods to interpret the current mechanistic paradigm of FSHD and fidelity of current data. An assessment of various modeling approaches is needed in order to achieve a consensus on the limits and capabilities of current modeling approaches.
8. **Model systems for mechanistic, intervention work and advancement to clinical trials.** We need to be able to understand the limits of both the data and the models. There is the need to address the complexity of FSHD in the context of mammalian

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The importance of having a well-funded U.S. National Institutes of Health

Report language to encourage NIH FSHD research efforts

by DANIEL PAUL PEREZ

FSH Society, Watertown, Massachusetts

In May 2011, a letter was sent on behalf of individuals and families with FSHD led by Representatives Sam Farr (CA), Edward Markey (MA) and John Tierney (MA) to the Chairman and Ranking Member Appropriations Subcommittee on Labor, HHS, Education asking the committee to add report language to the budget. We deeply appreciate the efforts of Congressmen Farr, Markey and Tierney on this critical issue. Their letter asked the committee to “recall, that during the subcommittee’s April 7, 2011 hearing there was testimony about FSHD. It is a form of muscular dystrophy, the most common form, in fact. With the passage of the Muscular Dystrophy Community Assistance, Research and Education (MD-CARE) Amendments of 2001 National Institutes of Health (NIH) funding for muscular dystrophy research quadrupled - from \$21 million in 2001 to \$86 million in 2010. Of this amount, FSHD gets around \$7 million for research. Duchenne Muscular Dystrophy (DMD) gets more than \$44 million even though FSHD is the more prevalent form of MD. In 2010, there were several studies that were published in recognized medical journals showing significant advances in research into understanding the origins of FSHD. To build on this new information FSHD advocates believe the mix of NIH funding that goes to the different kinds of MD needs to shift. Specifically, FSHD advocates believe FSHD research deserves more attention than it’s been getting especially now that there’s been breakthroughs. To emphasize the importance of these breakthroughs we suggest the committee add report language - like that below - that encourages NIH to expand and enhance its FSHD research.

The Committee is pleased to learn of research breakthroughs achieved last year in testing for causes of and research in FSHD. The Committee hopes such advances will be utilized as quickly as medically possible to develop possible treatments for FSHD. The Committee asks the Director of NIH to report back to the Committee within six months the actions NIH will take to address FSHD research.

Thank you for your attention to this important matter.” The FSH Society continues to advocate for an increase in NIH funding which in turn helps muscular dystrophy funding. As of this writing, the Congress is on recess and no Labor, HHS, Education



appropriations bill and report has been put forth. One scenario that is concerning, is that, the Labor, HHS, Education appropriations and report language will fall to the newly formed supercommittee and Congress will not accept the recommendation of the committee. The process would then default to a continuing resolution with last year’s budget and the “automatic trigger” in the debt ceiling reduction legislation will result in across the board cuts which would impact the NIH funding and its ability to fund research. Cuts in NIH funding will have a significant impact on the economy and biomedical research. The following links are to the Senate Appropriation Subcommittee webpage that contains written testimony from Dr. Francis Collins, the overall Director of the NIH. In each you will find that he references economic data pertaining to NIH research funding. This data is linked (via footnote) to the reports that detail the extent of economic benefit and job creation. FY2012 Budget Hearing: <http://appropriations.senate.gov/ht-labor.cfm?method=hearings.view&id=8a1dcace-6f68-4e35-ad94-4409966e2ffb> (starting on page 2). FY2011 Budget Hearing: <http://appropriations.senate.gov/ht-labor.cfm?method=hearings.view&id=ccfaba6-bbfe-46fa-8cf8-5925a7f89a1c> (starting on page 11).

The FSH Society encourages each of us affected by FSHD to communicate with your Senator or two Representatives your experiences with FSHD and the importance of funding research on FSHD via a well funded NIH. Every voice counts! 

MARJORIE BRONFMAN FSHD RESEARCH GRANT FOR 2011

The generosity and commitment of Marjorie Bronfman to FSHD research began in 1997 when she began to make research grants to the FSH Society. Mrs. Bronfman has renewed her commitment each year, including in 2011 with a new grant of \$50,000. Mrs. Bronfman, along with her late brother and one of her daughters, is affected with FSHD.

Through a process of review and recommendation by the Society's Scientific Advisory Board, grants are awarded for research fellowships (US\$30,000-US\$35,000/year) for research projects that show extraordinary promise to find the cause of FSHD. The 2011 contribution and the many grants that have preceded it have generated significant progress in FSHD research.

The FSH Society is deeply indebted to Mrs. Bronfman, to the Marjorie and Gerald Bronfman Foundation, and other members of her family for the advances that have been made possible worldwide over these years and for the opportunity to continue advances in 2010. Edward Schechter, brother of Mrs. Bronfman, deserves much gratitude for these research grants and for the research breakthrough. For more information about research fellowships, please contact Daniel Paul Perez, President & CEO of the FSH Society, at daniel.perez@fshsociety.org or call 781-275-7781 or 617-658-7811.

2011 NEW YORK FESTIVAL OF SONG FELLOWSHIP GRANTS

The FSH Society New York Music and Song Fellowship program was established in 2009 to help FSHD research efforts. The FSH Society is indebted to board members, Judith Seslowe of White Plains, New York, to Beth Johnston of Ossining, New York, and to their families and friends, for this groundbreaking effort on behalf of the FSHD community. As FSHD research moves ahead with new challenges, these awards have the potential to yield tremendous insights in FSHD treatment.

FSHD advocacy efforts on the U.S. President's Muscular Dystrophy Coordinating Committee

Ninth Annual Meeting highlights FSHD and translational research efforts

by DANIEL PAUL PEREZ
FSH Society, Watertown, Massachusetts

In the previous edition of the *FSH Watch* we highlighted the FSH Society's role and history in the MD-CARE Act and Daniel Paul Perez's role on the federal advisory committee mandated in the Act the Muscular Dystrophy Coordinating Committee (MDCC).

The FSH Society was instrumental in rewriting the MD CARE ACT 2001 to meet the needs of the entire community – people with all nine major types of muscular dystrophy - not just one type of muscular dystrophy. On December 18, 2001, Congress passed the Muscular Dystrophy Community Assistance Research and Education Act (MD CARE ACT), an unprecedented law mandating research, study and education on each type of muscular dystrophy. The law established the Muscular Dystrophy Coordinating Committee (MDCC) oversight committee to coordinate activities across the NIH, national research institutes and federal health programs relating to all forms of muscular dystrophy. In 2006, the MD-CARE Act expired. The entire muscular dystrophy community worked in support of re-authorization and Congress agreed it was important to continue the work in dystrophy. In 2008, the MD-CARE Act was re-authorized.

The FSH Society made suggestions and comments to strengthen the Act for all muscular dystrophies. Since passage of the MD-CARE Act, nearly an additional \$500 million has been funded by the NIH for muscular dystrophy research and education programs. The next time the MD CARE Act will need to be reauthorized is in 2013. Please contact the FSH Society if you are interested in helping with this effort.

The MDCC is responsible for developing and implementing a plan for conducting and supporting research and education on muscular dystrophy, measuring progress, and periodically reviewing and revising the plan. The U.S. Action Plan for the Muscular Dystrophies was completed and submitted to Congress in 2005. The plan will be revisited, re-assessed and revised in late-2011. To read the current U.S. Action Plan go to www.fshsociety.org click on Research tab at top, then click on FSHD Research Plans/U.S. NIH Action Plan in left hand navigation or see <http://www.fshsociety.org/pages/resPNIHAction.html>

On April 20, 2011, the Ninth Annual Meeting of the Muscular Dystrophy Coordinating Committee was held in Rockville, Maryland. The meeting highlighted recent breakthroughs in understanding of the FSHD mechanism and efforts in translational research and preclinical research to translate findings into therapies. The meeting began with introductions from Dr. Story Landis, MDCC Chair, NIH NINDS and Dr. John Porter, MDCC Executive Secretary, NIH NINDS. Introductions were followed by the NIH Overall Activities and Funding Report ARRA Funding for Muscular Dystrophy by Dr. John Porter, NIH NINDS and an update on the Paul D. Wellstone Muscular Dystrophy Cooperative Research Centers Network by Dr. Glen Nuckolls, NIAMS.

Several informative presentations were given by the U.S. NIH and other federal agencies that included: "NIH Therapy Development Resources to Leverage for Muscular Dystrophy" by John McKew, of the newly formed NIH Center for Translational Therapeutics (NCTT); "Changes in the Rare Disease Landscape at the Food and Drug Administration (FDA) and NIH and Impact for Muscular Dystrophy" by Anne Pariser, FDA and Steve Groft, NIH Office of Rare Disease Research (ORDR); "New Federal Policies and Initiatives Impacting Muscular Dystrophy (Federal hESC policy update; FDA-NIH Joint Leadership Council; Other)" by Story Landis, NINDS; and "Challenges of Clinical Trials in Muscular Dystrophy [DMD]—Triaging Candidate Opportunities, Trial Recruitment, and Communication of Trial Rationale and Results" by Kurt Fischbeck, NINDS, Berch Griggs, University of Rochester, and Pat Furlong, Parent Project Muscular Dystrophy.

Most significantly, Dr. Stephen Tapscott, Fred Hutchinson Cancer Research Center, Seattle, Washington updated the committee on the recent breakthroughs in 2010 at his laboratory in understanding the FSHD mechanism in a talk titled: "Research Update: Building upon a Mechanistic Breakthrough—Lessons Learned for the Muscular Dystrophies." Other reports from non-NIH federal agencies included a talk given by: Dr. Mark Swanson,

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A POSSIBLE APPROACH FOR TREATING FSHD WITH RNAi THERAPEUTICS

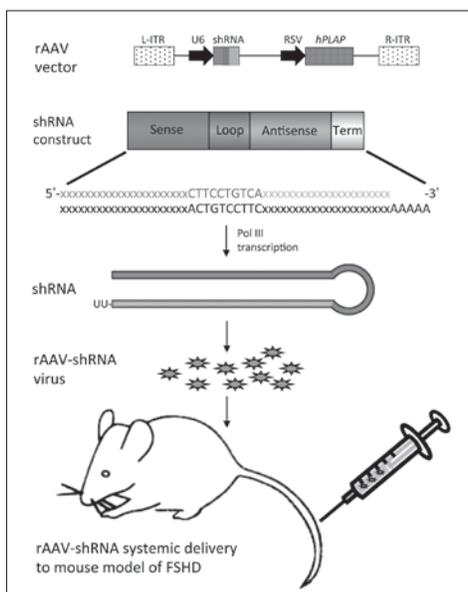
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which are benign in humans. FSHD Region Gene 1, otherwise known as FRG1, is a gene that is very near the deleted region of DNA associated with FSHD called the D4Z4 region and it has been widely studied as a possible candidate gene for FSHD.

The first of these studies, published by Wallace, et al., on July 5, 2011, reported that AAV-delivered artificial microRNAs reduced toxic FRG1 levels and improved histological and functional muscle abnormalities associated with FRG1 over expression in mice. Drs. Wallace and Harper write that since this disease allele-specific gene silencing using RNAi is feasible, this “work supports that RNAi-based gene therapy is a promising candidate strategy for treating dominant myopathies, regardless of the causal genetic mutation.” The Harper Lab is currently modifying this strategy to target another FSHD candidate gene, DUX4, as well as genes involved in other dominant muscular dystrophies, including some forms of Limb Girdle Muscular Dystrophy (LGMD).

In the second study, published on August 9, 2011, Bortolanza, et al., note that administering shRNA, “with a single, systemic delivery they reached all the skeletal muscles body-wide and obtained a specific and long term FRG1 silencing. This was associated to a significant rescue of the phenotype at histological, molecular and functional levels. Importantly, there was no sign of toxicity. More importantly, they treated adult animals that had already developed signs of muscular dystrophy to closely mimic possible future clinical settings. While in the paper we targeted the FRG1 gene, the same approach is easily applicable to DUX4 knockdown by simply exchanging the shRNA expression cassette. Hence our approach is applicable to any FSHD candidate gene.”

Both papers, authored by FSH Society funded researchers, are important for several reasons. First, they are the first successful proof of concept of a possible therapeutic approach for FSHD. Second, the approach is relevant for dominant myopathies in general. Mutations in at least 29 genes are responsible for a variety of genetically dominant muscle diseases. Considered as a group, these may affect as many as 1 individual in 2,400, making them the most common muscle disorders. Indeed, of the three most important muscle diseases (FSHD, Myotonic and Duchenne), FSHD and myotonic are genetically dominant disorders. Nevertheless, dominant myopathies have been largely neglected as targets of translational research because feasible molecular approaches for suppressing disease genes were unavailable until RNAi emerged a few years ago. Based on their results, these researchers predict that approaches similar to the one that they described could be applicable (with modifications depending on the specific disease) to a large number of patients affected by dominant myopathies. The financial and other support of the FSH Society was very important in obtaining the results described in these papers.



rAAV-shRNA systemic delivery to mouse model of FSHD

Drs. Gabellini, Harper, Wallace, Garwick-Coppens and Tupler are all past or current FSH Society fellows. Drs. Gabellini, Harper and Wallace began their careers in FSHD with grants from the FSH Society. While we remain cautiously optimistic about a treatment for FSHD, as the verification, corroboration and identification of the FSHD target (gene, RNA, protein) continues, this work is a major step forward in proof of concept of RNAi therapy in FSHD models. This work was made possible by a culmination over the years of a combination of FSH Society Marjorie and Gerald Bronfman fellowship grants, Jacobs Family and Friends research fellowship grants, FSH Society Grant Delta Railroad Construction Company fellowship grant and a FSH Society Landsman Charitable Trust fellowship grant.

► RNA Interference Improves Myopathic Phenotypes in Mice Over-expressing FSHD Region Gene 1 (FRG1)

Molecular Therapy, 2011 July 5

[Epub ahead of print]

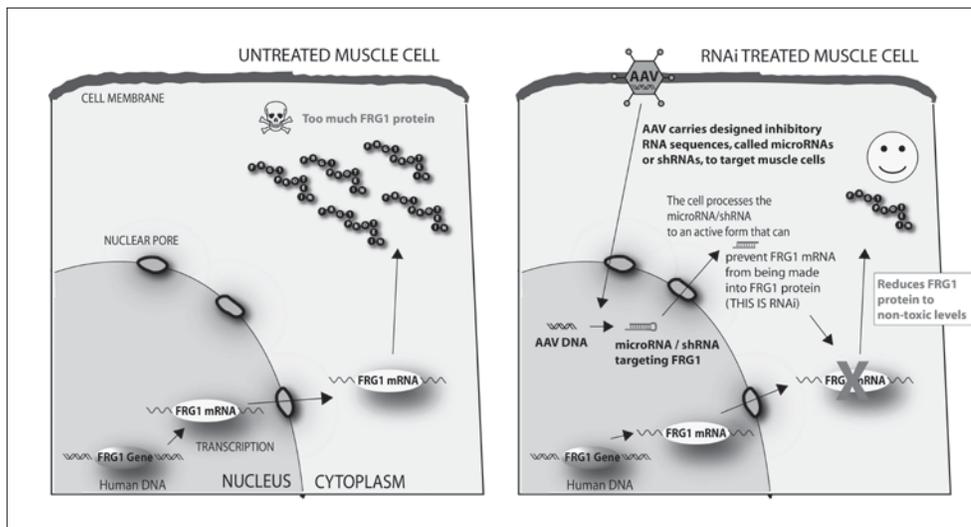
Wallace LM, Garwick-Coppens SE, Tupler R, Harper SQ

Molecular, Cellular, and Developmental Biology Graduate Program, The Ohio State University, Columbus, Ohio, USA. Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, USA.

Abstract: Muscular dystrophies, and other diseases of muscle, arise from recessive and dominant gene mutations. Gene replacement strategies may be beneficial for the former, while gene silencing approaches may provide treatment for the latter. In the last two decades, muscle-directed gene therapies were primarily focused on treating recessive disorders. This disparity at least partly arose because feasible mechanisms to silence dominant disease genes lagged behind gene replacement strategies. With the discovery of RNA interference (RNAi) and its subsequent development as a promising new gene silencing tool, the landscape has changed. In this study, our objective was to demonstrate proof-of-principle for RNAi therapy of a dominant myopathy in vivo. We tested the potential of adeno-associated viral (AAV)-delivered therapeutic microRNAs, targeting the human Facioscapulohumeral muscular dystrophy (FSHD) region gene 1 (FRG1), to correct myopathic features in mice expressing toxic levels of human FRG1 (FRG1^(high) mice). We found that FRG1 gene silencing improved muscle mass, strength, and histopathological abnormalities associated with muscular dystrophy in FRG1^(high) mice, thereby demonstrating therapeutic promise for treatment of dominantly inherited myopathies using RNAi. This approach potentially applies to as many as 29 different gene mutations responsible for myopathies inherited as dominant disorders.

[PubMed - as supplied by publisher]

PMID: 21730972



How RNAi works for FRG1 myopathy

<http://www.nature.com/mt/journal/vaop/ncurrent/full/mt2011118a.html>

The following are selected excerpts from Wallace, Harper et al paper's Discussion section: "We used the FRG1^(-high) mouse model in this study, which was initially developed to test the hypothesis that FRG1 over expression was a primary pathogenic insult underlying FSHD. Although the progressive myopathy produced in these mice strongly supported this hypothesis, there have been some conflicting data arguing against the involvement of FRG1 in FSHD, or at least minimizing its role as a primary pathogenic insult. Thus, it is fair to say that FRG1 is a controversial FSHD candidate gene. Nevertheless, for this study, we were unconcerned with this ongoing debate, because our primary goal was to demonstrate proof-of-principle for RNAi therapy of dominant myopathies in general, and the FRG1-high line was useful as an outstanding model of dominant muscle disease. We reasoned that its involvement in FSHD, or lack thereof, was irrelevant to the goal of this study. We therefore developed a gene therapy strategy to knockdown pathological levels of human FRG1 in FRG1-high mouse muscles. Here, we reported that AAV6-delivered artificial microRNAs reduced toxic FRG1 levels and improved histological and functional muscle abnormalities associated with FRG1 over expression in mice. Our work therefore supports the therapeutic potential of RNAi therapy for dominant myopathies in general. In addition, it could be applied to FSHD, if additional evidence supporting FRG1 involvement in the disease emerges; alternatively, our strategy could be modified to target other FSHD candidate genes, such as DUX4."

► AAV6-mediated Systemic shRNA Delivery Reverses Disease in a Mouse Model of Facioscapulohumeral Muscular Dystrophy

Molecular Therapy, 2011 August 9

Bortolanza S, Nisona A, Sanvito F, Maciotta S, Sitia G, Wei J, Torrente Y, Di Serio C, Chamberlain JR, Gabellini D

Dulbecco Telethon Institute and Division of Regenerative Medicine, San Raffaele Scientific Institute, Milano, Italy

Abstract: Treatment of dominantly inherited muscle disorders remains a difficult task considering the need to eliminate the pathogenic gene product in a body-wide fashion. We show here that it is possible to reverse dominant muscle disease in a mouse model of facioscapulohumeral muscular dystrophy (FSHD). FSHD is a common form of muscular dystrophy associated with a complex cascade of epigenetic events following reduction in copy number of D4Z4 macrosatellite repeats located on chromosome 4q35. Several 4q35 genes have been examined for their role in disease, including FRG1. Overexpression of FRG1 causes features related to FSHD. Here we show that systemic delivery of RNA interference expression cassettes in the FRG1 mouse, after the onset of disease,

led to a dose-dependent long-term FRG1 knockdown without signs of toxicity. Histological features including centrally nucleated fibers, fiber size reduction, fibrosis, adipocyte accumulation, and inflammation were all significantly improved. FRG1 mRNA knockdown resulted in a dramatic restoration of muscle function. Through RNA interference (RNAi) expression cassette redesign, our method is amenable to targeting any pathogenic gene offering a viable option for long-term, body-wide treatment of dominant muscle disease in humans.

PMID: 21829175

[PubMed - as supplied by publisher]

<http://www.nature.com/mt/journal/vaop/ncurrent/full/mt2011153a.html>

The following are selected excerpts from Bortolanza, Gabellini et al paper's Discussion section: "Although several intriguing FSHD candidates have been proposed, no single gene has been conclusively linked to FSHD development thus far. It was reported that the D4Z4 repeat contains an ORF encoding a double homeobox protein named DUX4. DUX4 has been detected in FSHD-derived primary myoblasts but not in controls, suggesting that D4Z4 may directly affect disease progression through the aberrant production of DUX4. Several functional studies described extreme general toxicity for DUX4. Cellular toxicity of DUX4 coupled with very low DUX4 expression in human cells poses a difficult challenge for modeling the human disease in mice. However, if a DUX4 mouse model is produced, our approach could be adapted for DUX4 knockdown in muscle through retargeting of the RNAi hairpin sequence to DUX4 mRNA.

"A growing understanding of its function, strongly suggests that FRG1 overexpression plays an important role in FSHD. Based on these data, FRG1 inhibition would be expected to lead to a therapeutic benefit in FSHD. Hence, we have used the only available FSHD mouse model to provide a proof of principle with respect to the use of RNAi therapeutic approaches for FSHD. In this study, we demonstrated long-term, dose-dependent reduction in FRG1 expression in all the muscles analyzed. Therapeutic benefits were observed in all of the mice treated with either low or high AAV6-

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Understanding the function of the DUX4 gene in FSHD

Perspectives and updates from recently funded FSH Society grantees

by **SCOTT Q. HARPER, Ph.D.**

The Research Institute at Nationwide Children's Hospital, Columbus, Ohio

The Harper Lab has gratefully received funding from the FSH Society in recent years for our work on understanding the function of the DUX4 gene in FSHD. I am honored to have the opportunity to update the FSH Society on our progress.

DUX4 is a transcription factor

Human beings have roughly 25,000 genes that give rise to proteins. These proteins have diverse functions. Some, like collagen, are raw materials used to structurally build cells and tissues. Others, like enzymes, may be used to help digest food and convert it to energy for powering our cells. The genes that encode these proteins are not always on or off in every cell, at every moment; control needs to be maintained over when, where, and how much these genes are expressed. To help accomplish this, cells use another group of proteins, called transcription factors, that switch other genes on or off in response to specific stimuli. The DUX4 gene, which has been recently implicated as a contributor to FSHD, produces a “transcription factor” protein.

Our FSH Society-funded projects on DUX4

We, and others in the FSHD research field, hypothesized that DUX4 is involved in FSHD. DUX4 appears to be normally dormant in muscle, but under specific conditions associated with FSHD, the gene can be turned on. Since it is a transcription factor, it can then turn on other genes that may not normally be expressed in muscle. If some of these genes were toxic, then DUX4 itself would be toxic.

Project 1 August 2009

Our initial goals for our FSH Society-funded work were to: 1.) test if DUX4 was toxic to mammalian (mouse) muscle and, 2.) determine if this toxicity was related to its function as a transcription factor. We indeed found that DUX4 was capable of damaging otherwise normal muscle, and that its ability to elicit this damage required transcription factor function. We then began trying to identify which toxic genes DUX4 could be activating to cause such damage. We found that DUX4 was stimulating genes involved in programmed cell death, which does not normally occur in healthy muscle cells. Our findings, together with many other published studies by other research groups, led us to conclude that DUX4 is



Drs. Takako Jones, Peter Jones and Lindsay Wallace (right) discuss research advances at the FSH Society 2010 FSHD International Research Consortium

toxic to mammalian muscle because it can activate genes that ultimately lead to programmed cell death.

We are happy to report that this work was recently published in the *Annals of Neurology*, and we have used this data to apply for additional funding from the National Institutes of Health and the Muscular Dystrophy Association. Moreover, this work represents a significant portion of the Ph.D. thesis of Ms. Lindsay Wallace, an Ohio State University graduate student in the lab. We were honored that Ms.

Wallace was chosen as the Outstanding Pre-Doctoral Trainee at the 60th meeting of the American Society of Human Genetics last year for this work. We are therefore grateful that the FSH Society and its donors have played an important part in the training of a promising young scientist, who will continue working in the field throughout her career.

Project 2 August 2010

We are continuing to work on understanding the pathways DUX4 controls. But we also turned an eye toward understanding how DUX4 could account for the pattern of muscle involvement often associated with FSHD. In other words, if DUX4 is involved in FSHD, how does it affect some muscles more than others? The most obvious idea is that DUX4 itself is turned on only in affected muscles. As we already mentioned, transcription factors are proteins that turn other genes on and off. They accomplish this by activating a switch, called a “promoter.” We therefore set out to test if DUX4’s promoter was active specifically in muscles affected by FSHD. This project is ongoing and we are beginning to see some interesting results, but we do not yet have conclusive data that we can confidently share with the greater FSHD community. We are excited to report our findings once we have a more complete story.

Ultimately, our goal is to better understand how DUX4 participates in the FSHD disease process, since this information is essential if we want to develop treatments for the disease. The FSH Society’s support has been critical for my pursuing this goal, and also in my lab’s growth and development in general over the last several years. As I mentioned, this support has included not only research funding but also the training of a new generation of scientists. For this, we are eternally grateful. 

Understanding the role of chromatin changes and positioning of the locus within the nuclear space in FSHD

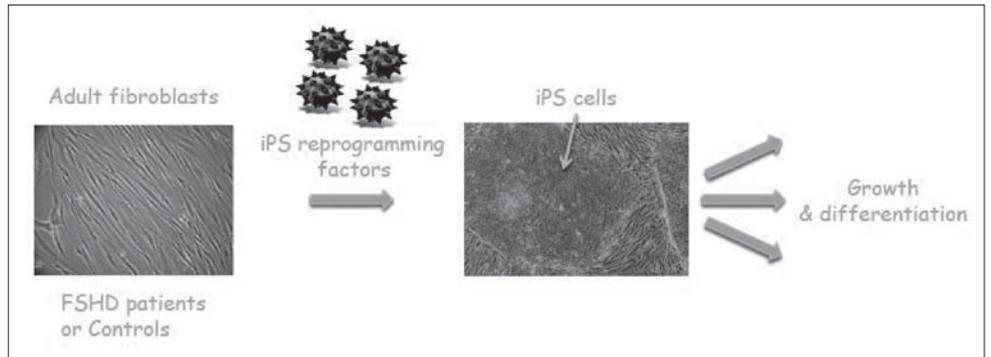
Perspectives and updates from FSH Society grantees

by **FREDERIQUE MAGDINIER, Ph.D.**
 Faculté de Médecine de la Timone, Marseille, France

The main subject of my team is the implication of chromatin and epigenetic changes in human diseases, from rare to common diseases. My research group is located in Marseilles, in the laboratory of Medical Genetics and Functional Genomics directed by Nicolas Lévy. My research project on FSHD began in 2003 in Lyon (France). In 2009, I obtained a one-year grant from the FSH Society (Delta Railroad Construction, Inc.), which was of tremendous importance in the creation of my research team in Marseilles and the development of new aspects of my research project.

FSHD is undoubtedly a very exciting albeit challenging disease to work on for any one interested in epigenetics. With regard to FSHD, we mainly focus on the role of chromatin changes and positioning of the locus within the nuclear space on the disease onset progression and penetrance. Since the discovery of the partial deletion of the D4Z4 repetitive DNA at the end of the long arm of chromosome 4 in patients with FSHD, the disease mechanism underlying FSHD has remained puzzling. Close examination of the 4q35 region greatly increased our understanding of FSHD onset with the recent breakthrough on the regulation of the DUX4 gene, contained within the D4Z4 repeats, proposed as a key player in FSHD.

In my initial work, we put a lot of effort in creating models mimicking the organization of the 4q35 locus either in patients or in healthy situations and tried to decipher the biological function of the D4Z4 repeat on chromatin regulation. As any model, ours has limitations and we now try to address new questions using cells from patients. This has been feasible during the last months thanks to a fruitful collaboration with the departments of Neurology and Genetics at La Timone Children's Hospital in Marseilles, a reference center in France for the diagnosis and exploration of FSHD patients. With the idea in mind that any exception to the rule might help in understanding the pathology, we tried to collect samples from atypical FSHD cases (twin sets, families with variable



Adult pluripotent stem cells (fibroblasts obtained from a skin biopsy) can be reprogrammed into induced pluripotent stem cells (iPS)

“We mainly focus on the role of chromatin changes and positioning of the locus within the nuclear space on the disease onset progression and penetrance. Since the discovery of the partial deletion of the D4Z4 repetitive DNA at the end of the long arm of chromosome 4 in patients with FSHD, the disease mechanism underlying FSHD has remained puzzling.”

penetrance, FSHD2 patients). Although not very productive in term of publications, the last nine months have been very active in term of samples collection and preparation of primary cell lines (myoblasts, fibroblasts isolated from biopsies and recently induced pluripotent cells) from FSHD patients and controls. Now, we want to learn more on these samples by comparing diseased and unaffected muscles at the level of chromatin fiber and compare when feasible muscle biopsies with primary myoblasts in culture. With regard to induced pluripotent cells, adult pluripotent cells (fibroblasts obtained from a skin biopsy) can be reprogrammed to a pluripotent state, i.e. with the capacity to be differentiated into new cell lineages.

These different types of cells are a very valuable resource, which will help us not only to obtain a better understanding of the disease but can also be used to develop and test therapeutic molecules for FSHD.

All cells in a given individual share the same genetic background. However, all genes do not necessarily lead to the production of a protein in every cell. To achieve this fine ON/OFF tuning, cells are regulated by epigenetic mechanisms, which can be defined as changes in gene expression caused by mechanisms other than changes in the underlying DNA sequence. Different modifications contribute to epigenetic regulations such as DNA methylation, The D4Z4 repeat contraction coincides with the loss of repressive chromatin marks in the 4q35 region and the transcriptional de-repression of the DUX4 gene in skeletal muscle. In some cases, the opening of the chromatin structure is independent from a contraction of the D4Z4 repeat array (FSHD2). Based on our promising preliminary data on patients' samples, we are continuing the analysis of the epigenetic changes occurring at the 4q35 locus in pathological samples. We try to make a link between changes in DNA methylation and DUX4 expression in FSHD1 and 2 patients. We are using iPS cells to investigate how epigenetics marks are deposited onto the D4Z4 repeat in different contexts. We are also pursuing the characterization of the long distance chromatin interactions involving the 4q35 gene within the nuclear space. FSHWatch

Creating a multicenter collaborative study on the clinical features, expression profiling, and quality of life of infantile onset FSHD

Perspectives and updates from recently funded FSH Society grantees

by JEAN K. MAH, M.D., M.Sc., FRCPC

Alberta Children's Hospital, University of Calgary, Calgary, Alberta, Canada

FSHD is an inherited neuromuscular disorder. Recent scientific advances have provided valuable insights into how genetic change (such as deletion of the D4Z4 repeat region on chromosome 4 for Type 1 FSHD) can adversely affect muscle development, leading to progressive wasting and weakness of the face, shoulder girdle, upper arms, abdominal, and lower limbs musculatures. Among individuals with genetically confirmed diagnosis of FSHD, there is a wide spectrum of expression of the disease, ranging from being unaffected to wheelchair-dependency because of significant weakness. Although FSHD typically manifests during the second or third decade of life with facial involvement, scapular winging, and limitation in shoulder and arm movements, atypical features with relative facial sparing and predominantly lower extremities weakness have been described. Environmental factors and other genetic modifiers likely play important roles in the variable expression of FSHD.

Less is known regarding the early childhood form of FSHD because it is uncommon. Infantile (onset less than 11 years of age) FSHD makes up about five percent of the total FSHD population, and affected individuals may have a more severe phenotype as well as extra-neuromuscular manifestations such as cognitive disability, seizures, visual impairment, and hearing deficit. Affected children may present with low muscle tone since infancy, with global developmental delay and poor growth due to feeding difficulties. Orthopedic complications such as hip displacement and spinal deformities may compound the muscle weakness leading to loss of independent ambulation at a young age. Even though early infantile

form of FSHD may be associated with smaller fragments size of D4Z4 repeats, anticipatory planning can be challenging, as it may arise from spontaneous mutation with no other affected family members. Because it is less common, the physical and psychosocial impact of infantile onset FSHD has not been formally assessed. As well, a prospective evaluation protocol for the natural history has been developed for adults but not for children with FSHD.

Therefore, the main objectives of this study are: 1.) to establish a standardized muscle testing protocol including both manual and quantitative muscle testing as well as function testing for use in children and adults with infantile onset FSHD; 2.) to describe the clinical phenotypes of infantile FSHD, separately for the early infantile group (onset before age five) and the late onset group (onset between five and ten years of age); 3.) to evaluate the impact of physical impairment, secondary health conditions, activity limitations and disability caused by FSHD on health-related quality of life and disability across different age groups; and 4.) to explore potential genetic modifiers of clinical phenotypes and disease progression in infantile FSHD by first comparing expression profiles in a most severe phenotype subgroup of patients to a mildest phenotype subgroup of patients and identifying genes and pathways that potentially modify the phenotypes using microarrays. The differentially regulated genes and pathways will then be validated using gene transcription and protein assays. The correlation between gene expression and disease severity will be determined.

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► THE FSH SOCIETY: Who We Are

The Facioscapulohumeral muscular dystrophy Society (the FSH Society) is a world leader in combating muscular dystrophy. Since 1997, it has provided nearly three million dollars in seed grants for pioneering FSHD research projects and education worldwide and created an international collaborative network of patients and researchers. The Society relies entirely on private grants, donations and grassroots philanthropy. Its purpose is to increase the awareness, understanding and conduct research and education on the most prevalent muscular dystrophy affecting men, women and children called FSHD.

The FSH Society offers basic research grants, research and post-doctoral fellowships to support research relevant to understanding the molecular genetics and cause of FSHD on an ongoing and ad-hoc basis. The FSH Society Scientific Advisory Board (SAB) provides strategy for FSHD research, recruits researchers, selects research proposals, evaluates research proposals, grants fellowships and monitors ongoing projects and research opportunities. The Society also organizes an annual symposium for researchers worldwide that yields major gains in

understanding FSHD. FSH Society grants have led to hundreds of publications acknowledging Society support in top-tier scientific journals.

In accordance with its primary purpose of serving the FSHD community in the United States and abroad, the FSH Society has brought together through education, patient-researcher network meetings, support group meetings, peer-support, and advocacy to more than 5,000 FSHD-affected families committed to working cooperatively. Through the FSH Society, its electronic bulletin board, social networking, chat room and quarterly newsletter, the FSH Watch, FSHD patients have found ways to be useful to medical and clinical researchers working on their disease. The support patients receive from one another through sharing their common experience is invaluable and immeasurable. The FSH Society acts as a clearinghouse for information on FSHD and on potential drugs and therapies designed to alleviate the effects of the disease. It fosters communication among FSHD patients, their families and caregivers, charitable organizations, government agencies, industry, scientific researchers, and academic institutions.



VOLUNTEERS NEEDED FOR MUSCLE BIOPSY STUDY

Please consider making the valuable gift of muscle tissue and blood samples to advance research efforts on FSHD.

Muscle samples are in extremely short supply and tissue donors are needed. The FSH Society and Johns Hopkins School of Medicine, as part of the NIH-funded Boston Biomedical Research Institute Sen. Paul Wellstone Muscular Dystrophy Cooperative Research Center for FSHD, are recruiting volunteers with FSHD and their first degree unaffected relatives (for example: parent, brother, sister, or child of a person with FSHD) to provide muscle and 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 of treatments and a cure for FSHD.

To date, more than 60 individuals in over 30 families with FSHD-affected volunteers and their unaffected relatives have participated in the Wellstone Center for FSHD research study. More groups are needed to help meet the goal of increasing the sample library to cover a wider range of FSHD. As of July 2011, the research study has increased in sample size from a target of 66 to 100 participants. Researchers at the Wellstone Center have asked the FSH Society to reach out especially to individuals with suspected FSHD related hearing loss, suspected FSHD related retinal issues, and FSHD-affected individuals of ethnic and racial minorities, but all are welcome and needed.

In order to determine eligibility, you will need to provide a copy of your genetic testing diagnostic result and medical records indicating FSHD diagnosis. If you have suspected FSHD related hearing loss, FSHD related retinal issues, are an FSHD patient or individual of minority race or ethnicity, please contact us at the contacts below. This additional information will not adversely affect your previous inquiry or consideration for inclusion into the study.

Go to www.fshsociety.org for more information, or contact Ms. Doris Walsh at the FSH Society, 617-658-7877 or doris.walsh@fshsociety.org or call Genila Bibat, M.D., at Johns Hopkins School of Medicine – Neurology, 707 North Broadway, Baltimore, MD 21205 at 443-923-2697.

Kennedy Krieger Institute, Center for Genetic Muscle Disorders

An interdisciplinary clinic dedicated to the diagnosis and treatment of FSHD

by **KATHRYN WAGNER, M.D., Ph.D.**
Kennedy Krieger Institute, Baltimore, Maryland

Editorial note: FSH Watch will occasionally highlight neuromuscular clinics that we believe might be of interest to patients and their families. This issue includes programs in Maryland and New York.

The Center for Genetic Muscle Disorders at the Kennedy Krieger Institute was founded by Kathryn Wagner, M.D., Ph.D., in 2009. The Center is a part of Kennedy Krieger's new outpatient center, thoughtfully designed in partnership with, and for, those with disabilities. Although the building and Center are new, Dr. Wagner is no newcomer, having cared for patients with FSHD for over a decade at Johns Hopkins prior to moving her practice to Kennedy Krieger. She also serves on the Scientific Advisory Board for the FSH Society and is a principal investigator in the Senator Paul Wellstone Muscular Dystrophy Cooperative Research Center project entitled "Biomarkers for Therapy of FSHD."

The Center for Genetic Muscle Disorders provides current interdisciplinary clinical care for patients by experts in muscle disease and also provides an environment for research programs that develop better and novel therapeutics to treat these disorders in the future. The collective expertise of the Center's staff provides these patients with an informed diagnosis and prognosis, as well as a unique perspective on how the disease will impact their day-to-day life. As leaders in their fields, medical professionals are able to work with patients to find practical solutions to the specific problems they are experiencing as well as work to slow the progression of the disease. Specialists in the field of neurology, rehabilitative medicine, social work and genetics counseling are available at every clinic visit. In addition, for those patients with complex medical needs, pulmonologists and endocrinologists participate in the clinic as needed. Enabled by the collaboration among Kennedy Krieger's various programs, patients who visit the Center also have easy access to a variety of clinical services to help manage their symptoms and improve their quality of life, such as audiology, speech therapy, aquatic therapy and assistive technology.

A unique strength of the Center for Genetic Muscle Disorders includes the option for participants to be involved in novel therapies and research. Kennedy Krieger is currently enrolling subjects with FSHD in a biomarker study (see sidebar, at left) as part of the NIH-funded, FSHD Wellstone centered at Boston Biomedical Research Institute. Previously, Dr. Wagner led a trial of the myostatin inhibitor, MYO-029, one of the first trials of a novel drug in FSHD. She has been a strong advocate for more clinical trials in FSHD and continues to work to bring industry attention to the disease.

Another strength of the Center is the ability for patients to remain in the program through the transition from childhood to adolescence and through adult life. Dr. Wagner is an adult neurologist who sees patients of all ages. Neurologist Diana Escolar, M.D., cares for pediatric neuromuscular patients and neurologist Doris Leung, M.D, cares for primarily adult patients with muscular dystrophy. Kennedy Krieger, a renowned institute for children with physical and developmental disabilities, also serves adults with a range of disorders affecting the brain and musculoskeletal system including adult onset muscular dystrophy, spinal cord injury, cerebral palsy and spina bifida. There is an active group of those with FSHD who participate in the clinic who convene outside of clinic to discuss common challenges and solutions as well as opportunities for the future.

For an appointment call Angie Lasseth at 443-923-9525. The clinic address is 801 North Broadway, Baltimore. 



Kathryn Wagner, M.D., Ph.D., Director, Kennedy Krieger Institute, Baltimore, Maryland with FSHD patients Lillien and Collin Walters

Unveiling D4Z4-connected pathways leading to over-expression of FSHD genes

Perspectives and updates from FSH Society grantees

by **DAPHNE CABIANCA, Ph.D.**
Università Vita Salute San Raffaele, Milan, Italy

I started studying FSHD about four years ago, at the beginning of my Ph.D. in Cellular and Molecular Biology at the Università Vita Salute San Raffaele in Milan, Italy, under the supervision of Dr. Davide Gabellini, who has a long-lasting experience in the FSHD field. Dr Gabellini's lab studies FSHD at different levels. Among the various projects available, I found myself extremely interested in the elucidation of the initial steps that characterize FSHD pathogenesis. In particular, I wanted to better understand the molecular mechanism linking the primary FSHD genetic defect (loss of D4Z4 repeats) to the pathology.



Daphne Cabianca, Ph.D.

For the first three years of my Ph.D. I was financed by the University, while for the last 18 months of my Ph.D. work I have been generously supported by the FSH Society, which awarded me a New York Symphony and Song Fellowship Grant. Recently, I defended my Ph.D. thesis focused on molecular aspects of FSHD with success.

At the beginning of my project, it was already known, thanks to studies performed by other groups, that FSHD patients display a reduced accumulation of several repressive marks at the disease-associated locus (4q35). Indeed, it has been proposed, and nowadays there is increasing evidence strongly supporting it, that an inefficient repression in FSHD patients leads to the inappropriate over-expression of 4q35 gene(s). Among these, DUX4 is currently the leading candidate for FSHD development. However, little is known about how the de-regulation of 4q35 genes occurs.

The research I conducted focused on the characterization of the molecular events driving the over expression of FSHD candidate genes in the disease. In particular, we found that D4Z4 is a target of the Polycomb Group (PcG) of proteins, factors with crucial cellular functions that act to shut off gene expression. Interestingly, FSHD patients show reduced enrichment of PcG proteins at D4Z4, suggesting that a weakening of PcG repressive function could be involved in increased expression of 4q35 genes. I found that deletion of D4Z4 repeats is associated to the production of a chromatin-associated, 4q35-localized long non coding RNA by the region immediately proximal to the repeat array. We named this RNA DBE-T. Remarkably, functional studies revealed that DBE-T is required for the over-expression of FSHD candidate genes. Indeed, by reducing the expression of DBE-T we could counteract the over-expression of 4q35 genes, including DUX4.

Compared to other genetic diseases, FSHD is extremely complicated as it involves novel cellular mechanisms that are challenging to study. Nevertheless, efforts by several groups are leading to important discoveries that are helping to shed light on FSHD pathogenesis. Our work is helping to unveil the pathway originating with D4Z4 deletion and leading to over-expression of FSHD candidate genes. By doing so, it contributes to the FSHD cause, adding new important pieces to the puzzle. Indeed, this study led to the identification of novel players whose action is potentially crucial for FSHD pathogenesis. Importantly, our results go beyond single candidate genes-generated defects. It is tempting to speculate that DBE-T might be a valid therapeutic target in order to achieve a general normalization of 4q35 genes expression in FSHD.

Work made possible by a FSH Society New York Festival of Music research fellowship grant. 

Isolating a biomarker for FSHD therapeutics

Updates from recently funded FSH Society grantees

by **JESSICA CHIA-YUN SUN, Ph.D., and PETER L. JONES, Ph.D.**
*Boston Biomedical Research Institute (BBRI)
 Watertown, Massachusetts*

One of the aims of our FSHD research program is to identify biomarkers for FSHD. Essentially these biomarkers are proteins and nucleic acids that are aberrantly expressed in FSHD muscle as compared with muscle in unaffected individuals. The rationale for this research is two-fold. First, biomarkers may in fact be the causal agents for pathology and identifying them may provide targets for therapeutic development. Secondly, FSHD biomarker discovery is important so that when treatments are developed for FSHD, we will have something measurable to indicate if the treatments are being effective. Finding consistent biomarkers for FSHD is vital.

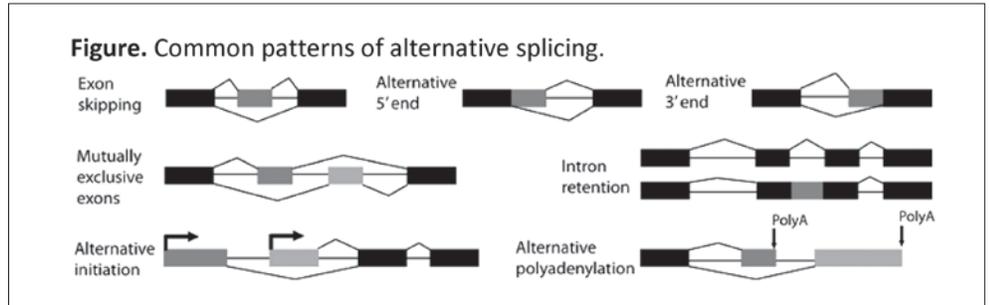
For this FSHD biomarker project we investigated protein expression in two cell lines derived from FSHD muscle and compared with two cell lines from unaffected, unrelated individuals. We found a previously unknown protein that was developmentally over-expressed in both FSHD lines compared with unaffected cells. This data formed the foundation of our proposal to identify and characterize this protein. However, as interesting as this may be, there were legitimate concerns about basing this work on such a small sampling of cells (two FSHD and two control). Thankfully the FSH Society Scientific Advisory Board approved partial funding for the project and requested that we investigate more FSHD and control cells to shore up our preliminary finding. Fortunately we have recently moved our laboratory to the Boston Biomedical Research Institute where the Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD is based. Since our move we have been able to utilize the unique collection of myogenic cells obtained from families of FSHD patients and their unaffected first-degree relatives to validate our initial biomarker hypothesis. Using these cells we have found that this unidentified protein is similarly developmentally over-expressed in all of the Wellstone FSHD cell lines we have analyzed compared with matched control unaffected cells. This data strongly suggests that we have indeed found a biomarker for FSHD cells and we are now eager to identify and further characterize it in the near future.

Alternative splicing as an important layer of gene misregulation in FSHD and infantile FSHD

Perspectives and updates from FSH Society grantees

by **YI XING, M.D., Ph.D**
University of Iowa, Iowa City, Iowa

Proteins are encoded by mRNAs which, on average, are approximately 2,500 bases long. However, RNA initially transcribed from genomic DNA (pre-mRNA) can be over 100 kilobases in length. To attain a final form, pre-mRNAs must be processed: intervening sequences (introns) must be removed and exons (which contain the protein code) must be defined and concatenated. This searching and joining process is called RNA splicing and is performed by a huge cellular machine—the spliceosome—that contains over 100 proteins. Depending on the cellular environment—determined by the tissue type, developmental stage, or extra-cellular cues—different exons will be recognized in a single pre-mRNA. This crucial regulatory process, termed alternative splicing, can produce multiple, distinct mRNA products from a single gene, allowing one gene to generate a myriad of proteins (see Figure). Splicing must be tightly regulated in order to produce the correct (functional) gene product. Genetic mutations that disrupt splicing cause a broad range of human diseases, including cancers, neurodegenerative diseases, and certain types of muscular dystrophy. One candidate gene of particular interest in the pathophysiology of FSHD and infantile FSHD (IFSHD) is FRG1 (FSHD region gene 1). FRG1 is located in close proximity to the D4Z4 region and encodes a component of the spliceosome. Important work by Dr. Davide Gabellini and colleagues showed that transgenic mice selectively overexpressing FRG1 in skeletal muscle



Various types of alternative splicing being considered in FSHD

develop phenotypes that resemble FSHD in human patients. This raises the possibility of a global alternative splicing defect in FSHD/IFSHD.

Dr. Xing's laboratory at the University of Iowa Carver College of Medicine studies the regulation of alternative splicing in healthy and diseased cells. In collaboration with Dr. Katherine Mathews at the University of Iowa Wellstone Muscular Dystrophy Cooperative Research Center, his group is differentiating primary skin fibroblast or myoblast cell cultures collected from FSHD/IFSHD patients and healthy individuals into muscle cells. On RNAs extracted from these differentiated muscle cells, the scientists are using a variety of cutting-edge high-throughput genomic technologies to examine the activities of proteins that regulate splicing, as well as the alternative splicing patterns of all human genes in normal and diseased cells. For example, by deep sequencing of mRNA molecules using ultra-fast next-generation DNA sequencers,

it is now possible to generate hundreds of millions of RNA sequences from any patient sample to globally analyze gene expression and alternative splicing at the nucleotide resolution. The scientists hope that these studies will reveal splicing as an important layer of gene misregulation in FSHD/IFSHD, and find novel genes and molecular pathways amenable to therapeutic development. Importantly, by comparing FSHD and IFSHD cells, scientists might gain significant novel insight into the molecular mechanism underlying the early onset and severe symptoms of IFSHD. *Editorial note: Many of the primary skin fibroblast or myoblast cell cultures were collected from FSHD/IFSHD patients and healthy individuals who attended the 2008 biennial FSH Society Patient Researcher meeting in Coralville, Iowa.*

Work is made possible by a FSH Society Aubrie Lee Family Research Grant for Infantile FSHD & Fire Island & Fabiola Bertinotii research fellowship grant [FSH Watch](#)

▶ Investigating gene-expression differences in FSHD stem cells as they develop

by **MICHAEL KYBA, Ph.D**
Lillehei Heart Institute, University of Minnesota, Minneapolis, Minnesota

We are studying primary cells from muscles of FSHD-affected individuals, as well as induced pluripotent stem (IPS) cells derived from these muscle cells. IPS technology is a relatively recent development in the stem cell field that allows cells from adults to be “reprogrammed” in vitro so that they resemble embryonic stem (ES) cells. IPS cells are essentially ES cells but since they are not derived from embryos their use is not controversial. Importantly, they have the potential to differentiate

into any cell type, and we have used FSHD IPS cells to generate cells of different types, and at different stages of differentiation. Support from the FSH Society is allowing us to investigate gene-expression differences, that may give insight into the cause of FSHD, and thereby helping us to understand in which cells and at what stage in their development changes occur that may cause this disease. With the FSH Society's support we now have an individual doing our project's bio-informatics analysis and we hope to have results that could be discussed in the near future. *Work made possible by a FSH Society New York Festival of Music research fellowship grant*

Continuing to make progress in understanding and treating FSHD

Since 1998, the FSH Society has transformed FSHD research by providing grants for vital start-up funding for investigators in FSHD and research projects on FSHD. The FSH Society has two rounds of grant applications each year, with deadlines in February and August. Grant applications are thoroughly analyzed and vetted by the SAB. An initial letter of intent is submitted, which is reviewed by Professor David Housman, Chair of the SAB. If a letter of intent is accepted, the applicant submits a full application. The main section where researchers describe the proposed work and workflow is around 12 pages long. Upon receipt of all full grant applications for a particular round, Professor Housman assigns teams of two or more members of the SAB to critique each proposal. Any potential conflicts of interests are noted, and SAB members who may have a conflict are not assigned to review, and do not vote on, the particular proposal. The two reviewers review the application in depth and provide a detailed written description and recommendation to the other members. Initial critiques are due within three weeks of the assignment and a full meeting of the SAB is held around two weeks thereafter. Grant applications are reviewed and voted upon by the entire SAB, with discussion led by the two primary reviewers. SAB recommendations for approved applications are then sent to the Society's Board of Directors for a vote. When the SAB disapproves an application, it provides the applicant with a detailed description of the reasons for disapproval, and the applicant may resubmit the application for consideration in a later round. SAB members and the chair serve without pay.

Upon acceptance by the Society's board, the grantee receives a letter of acceptance and a grants policies and procedures document. The grantee is then asked for written confirmation indicating their intention of accepting or declining the fellowship knowing that the grant is administered in accordance with the FSH Society's policies document. It is understood that the funds awarded have not been provided for any other purpose than research on FSHD. The grantee is asked to reply within two weeks where upon a check is issued in advance for the first six months with equal installments to follow at subsequent six month intervals based on review of requested progress reports.

The milestones and insights gained are significant. The fellowship program allows innovative and entrepreneurial research to develop, prove successful, and ultimately to attract funding from large funding sources such as the US National Institutes of Health (NIH) and large private sources. We are very pleased to list the grantees funded in the February 2011 and August 2010 cycles. You can also read more about FSH Society funded research projects by going to www.fshsociety.org clicking on the "Research" menu tab at top of page and then selecting "Research Fellows & Grantees" in the left hand vertical navigation menu.

FSH Society grants recently awarded

February 2011 and August 2010 rounds

by FSH SOCIETY
Watertown, Massachusetts

The FSH Society has two rounds of grant applications each year, with deadlines in February and August. Grant applications are thoroughly analyzed and vetted by the SAB. For a description of the review and approval process, see box left.

The Scientific Advisory Board (SAB) met in November to review grant applications received for the August 2010 round of FSH Society grants funding. Below is a list of the funded projects, including project descriptions as submitted by grant applicants.

► February 2011 Round

1. Antisense strategies against DUX4 as therapeutic approaches for FSHD

Eugénie Anseau, Ph.D./Alexandra Belayew, Ph.D.

Universite de Mons, Mons, Belgium

\$70,500 over 2 years, \$25,000 year 1, \$45,500 year 2

Summary (Provided by Applicant): FSHD is a muscle degeneration disease genetically linked to contractions of the D4Z4 repeat array on the 4q35 subtelomeric region. Our group has identified the double homeobox 4 (DUX4) gene within each unit of the D4Z4 repeat array and shown that the encoded protein was expressed in primary myoblasts and biopsies of patients with FSHD but not in non-affected individuals. We found that the only stable DUX4 messenger RNAs derive from the last unit and extend to the flanking pLAM sequence that provides a polyA addition signal. This signal is required to develop FSHD as independently confirmed by an eight-laboratory consortium which studied genetic polymorphisms in hundreds of patients and thousands of healthy individuals. In aggregate our collaborative studies with four different groups have shown that the DUX4 protein is a transcription factor that targets a large set of genes, some of which encode other transcription factors that in turn target additional genes. Globally, DUX4 activation at the FSHD locus initiates a transcription cascade leading to muscle atrophy, inflammation, decreased differentiation potential and oxidative stress, the key features of the disease. By differential protein, RNA and gene studies we keep identifying additional FSHD biomarkers and define whether they are direct or indirect DUX4 targets.

Strikingly, we found that DUX4 expression in human myoblast induces an atrophic myotube phenotype and atrophy markers. The rationale of our on-going project is that inhibition of DUX4 expression should prevent the global gene deregulation process and allow muscle regeneration in patients. We have first developed small inhibitory RNAs (siRNAs) and conditions to suppress DUX4 protein expression either in primary myoblast cultures transfected with a DUX4 expression vector, or in primary FSHD myoblasts. Addition of DUX4 siRNA to FSHD myoblasts allowed recovery of a normal myotube phenotype with a decrease of atrophy markers. We have started a collaboration with Prof. Steve Wilton (ANRI, Australia) because of his expertise in the exon

skipping therapeutic approach with antisense oligonucleotides (AOs) in Duchenne muscular dystrophy. Prof. Wilton provided us with 7 AOs directed against various parts of the DUX4 mRNA characterized in our group: the aim was to either block translation or induce mRNA degradation to prevent DUX4 protein expression. We were able to identify conditions for selective DUX4 inhibition by 3 AOs as done for the siRNAs above in human primary myoblast cultures. Moreover DUX4 mRNA inhibition also affects the expression of several FSHD markers such as μ -crystallin, -catenin and TP53. These results constitute a proof of concept in myoblast cultures that DUX4 inhibition might reverse the FSHD phenotype. In the present project we want to validate these results by other techniques (RNA and protein expression profiling) and to test the effect of these AOs and siRNA in mouse models *in vivo*.

A FSH Society New York Festive Evening of Music research fellowship grant

2. Humanized mouse model for the study of Facioscapulohumeral Dystrophy

Marietta Barro, Ph.D./Charles P. Emerson Jr., Ph.D.
Boston Biomedical Research Institute, Watertown, Massachusetts
\$40,000 over 1 year

Summary: FSHD is genetically caused by the contraction of D4Z4 DNA repeats located on chromosome 4 in 4q35. Although the genetic defect was identified 20 years ago, the exact molecular mechanism causing the disease is unknown, and there is currently no mouse disease model. To provide such a valuable tool, we will develop a humanized mouse model for FSHD, obtained by the engraftment of FSHD patient-derived myoblasts into mouse muscle. Engrafted human cells are able to form muscle fibers in the host mouse muscle, thus allowing pioneering studies in an *in vivo* context. Because of the dominant nature of FSHD, we hypothesize that the engrafted fibers will display a disease phenotype and recapitulate pathological molecular mechanisms associated with FSHD that will allow us to study the development of the disease. Our preliminary studies have already established the feasibility of this project. Through the cell repository of the Boston Biomedical Research Institute (BBRI) Wellstone Center, we have the unique opportunity to access early passage myoblast cells from cohorts of FSHD probands and their appropriate controls, i.e., a first degree relative. We will graft these standardized cultured cells into mouse muscle to obtain the FSHD humanized mouse model, thereby generating a well-controlled *in vivo* model for the study of FSHD. The very pressing issue in the field today is the verification of the current DUX4 model. The humanized mice produced will be used to investigate the hypothesis that DUX4 gene expression is a major cause of FSHD pathogenesis. In the obtained model, DUX4 expression will be evaluated during *in vivo* regeneration, and the consequence of its expression on fiber turnover and satellite cell renewal

will be assessed. This work will contribute to the understanding of the role of DUX4 *in vivo*, thus providing a better understanding of FSHD pathogenesis.

The proposed project will be completed following 2 specific aims:
Specific Aim 1: Optimization of the FSHD humanized mouse model. We will improve results obtained in preliminary experiments by designing more efficient transplantation strategies. In order to fully interpret the disease model, we will seek to increase the amount of muscle formed from implanted human cells, by devising more efficient transplantation strategies. The cell repository of the BBRI Wellstone Center provides access to freshly isolated FSHD and their appropriate control muscle cells sorted for CD56 expression, which are expected to have particularly high engraftment potential. However, the timing between the toxin injection and the cell injection, as well as depletion of endogenous satellite cells by irradiation of the mouse legs, may affect the ability of implanted cells to regenerate the murine muscle and will be optimized during this aim. Upon establishment of an effective mouse model, we will look for disease characteristics, as described in Specific Aim 2.

Specific Aim 2: Characterization of the FSHD humanized mouse model to evaluate the role of DUX4 during *in vivo* muscle regeneration. The model obtained in Specific Aim 1 will be characterized by establishing differences between the fibers generated from FSHD cells and fibers from their appropriate control cells in injected muscles. Recent breakthroughs in the field suggest that DUX4, a gene identified inside D4Z4 repeats, expresses a toxic protein in the muscles of patients with FSHD, thus causing the disease. DUX4 may have a normal role during development and the FSHD pathology might involve incomplete developmental silencing of DUX4. However, the precise molecular and cellular mechanisms involving DUX4 remain to be uncovered. The BBRI Wellstone Center, currently investigating DUX4 expression in muscle samples from its cohort collection, has been able to detect DUX4 transcripts in FSHD samples, and these cohorts will be selected for the generation of the humanized FSHD mouse model. Initially, the expression of DUX4 at the mRNA and/or protein levels will be assessed in FSHD- and control transplanted muscles. This will be followed with experiments designed to compare the biological characteristics of the resulting muscle fibers. Finally, we will develop a dynamic approach to investigate the current DUX4 model in following the evolution of the engrafted fiber over time using *in vivo* bioluminescence live imaging. Murine models surpass *in vitro* limitations due to their ability to reproduce complex *in vivo* environment thereby providing a deeper understanding of disease mechanisms. Our model for creating humanized FSHD fibers in murine muscle will recapitulate the mechanisms of pathological fiber formation *in vivo*, allowing us to fully characterize the disease progression and test potential therapeutic agents.

A FSH Society New York Festive Evening of Music research fellowship grant

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3. Testing a therapeutic approach for FSHD: evaluation of the efficacy of AOs blocking DUX4 in a mouse model of isolated myofibres

Alexandra Tassin, Ph.D./Alexandra Belayew, Ph.D.

Universite de Mons, Mons, Belgium

\$15,000 over 1 year

Summary: FSHD is considered the most frequent hereditary muscle disorder in adults, affecting one individual in 20,000. It is associated with contractions of the D4Z4 repeat array in the 4q35 subtelomeric region. In non-affected individuals, this array comprises 11-100 tandem copies of the 3.3-kb D4Z4 element while in patients, only 1-10 D4Z4 copies are left (Wijmenga et al., 1992). Our group has identified the double homeobox 4 (DUX4) gene within each unit of the D4Z4 repeat array (Gabriels et al., 1999) and several studies have now demonstrated the causative role of DUX4 in FSHD. We have demonstrated that the stable full-length DUX4 messenger RNA (mRNA) is produced from the last D4Z4 unit in FSHD, using a polyadenylation signal in the flanking pLAM region, located telomeric to the distal repeat (Dixit et al., 2007) as recently confirmed by a study of genetic polymorphisms in hundreds of patients and thousands of non-affected individuals (Lemmers et al., 2010). This polyadenylation site is necessary to develop FSHD on a contracted allele therefore called “permissive chromosome” (Lemmers et al., 2010). The mRNA from this distal D4Z4 unit contains the entire DUX4 open reading frame (ORF) and 1 or 2 alternatively spliced introns in the 3'UTR (DUX4-fl). In addition, a short DUX4 mRNA terminates at the previously described polyadenylation site in the pLAM region but uses a cryptic splice donor site within the DUX4 ORF (DUX4-s). DUX4-fl was only detected in FSHD muscle cells and biopsies, whereas DUX4-s is detected both in control and some FSHD samples (Snider et al., 2010). A long DUX4 mRNA was detected in induced pluripotent stem cells (iPS cells) and human testis where the gene contains 4 additional exons and a more distal polyadenylation signal. Expression of this DUX4 mRNA was suppressed during differentiation of control iPS cells to embryoid bodies whereas expression of full length DUX4 mRNA persisted in differentiated FSHD iPS cells (Snider et al., 2010). These data, together with the conservation of the DUX4 ORF through evolution (Clapp et al., 2007) suggests a possible role of DUX4 in human development.

Dr. Tassin intends to undertake a post-doc for 3 months in 2011 at King's College London, to initiate a collaborative research project between our lab and that of Dr. P. Zammit. In agreement with Dr. Zammit, our collaborative project will consist of testing antisense oligonucleotides (AOs) directed against the 3'UTR of the DUX4 gene that we have developed in our laboratory, in collaboration with Prof. S. Wilton (ANRI, University of Western Australia). These AOs have undergone preliminary screening in cell culture, but require more extensive testing. Dr. Zammit has developed mouse myofibre models that provide an ideal system to further test our AOs. The satellite cells associated with the isolated myofibres will be infected with retroviral vectors encoding DUX4, and the effects on myogenic progression and apoptosis of AO administration analysed. We want specially to focus on the pLAM region responsible for the stabilisation of the DUX4 mRNA leading to FSHD. This

system will allow better understanding of the action AOs, for evaluating their potential suitability as a human therapy. We believe that this collaboration will give us new insights into a potential therapy for FSHD.

A FSH Society California Walk 'n' Roll research fellowship grant

4. Investigating mouse models of FSHD

Paraskevi Sakellariou, Ph.D./Robert J. Bloch, Ph.D.

University of Maryland School of Medicine, Baltimore, Maryland

\$40,000 over 1 year

Summary: There is a great need for a valid mouse model for FSHD. Such an animal model would provide a valuable tool for exploring the effects of newly cloned genes and novel proteins on the pathophysiology of this disease. It would also greatly facilitate research towards the development and testing of new therapeutic approaches to FSHD. We propose to examine two possible mouse models of FSHD, the FRG1 over-expressor, from Drs. Davide Gabellini and Rossella Tupler, and mu-crystallin over-expressor, developed by Drs. Patrick Reed and Robert Bloch. I will breed these mice and test them for their physiological and morphological characteristics, and their susceptibility to injury and ability to recover from injury. I will also initiate xenografting studies to create mice with humanized normal and FSHD ankle dorsiflexor muscles, combining methods that are routine in the Bloch laboratory with unique reagents provided by collaborators in the Wellstone Muscular Dystrophy Cooperative Research Center (MDCRC), “Biomarkers for Therapy of FSHD.” These experiments should reveal the usefulness of available transgenic models for the study of FSHD, and promote the development of humanized mouse muscles for the study of the pathophysiology of FSHD in situ.

A FSH Society New York Festive Evening of Music research fellowship grant

5. Epigenetic abnormality in FSHD

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

University of California, Irvine, California

\$8,875 for 3-month extension

Summary: Our preliminary findings indicate that D4Z4 repeat regions indeed interact with other genome regions, and that these interactions are indeed disrupted in FSHD. With a three-month extension of my fellowship, I plan to perform a high-throughput identification of potential target genes that interact with D4Z4 using the recently developed “Chromatin Interaction Analysis using a Paired-End Tag” (ChIA-PET) technique. This strategy enables the genome-wide detection of chromatin interactions mediated by specific factors that are normally assembled at D4Z4. Identification of additional FSHD pathogenic genes other than FRG1 and DUX4 is important to explore future therapeutic targets to improve or prevent the clinical symptoms of FSHD.

Previously, with the support from the FSH Society in 2010, we found that a set of factors that normally assemble at D4Z4 repeats do not bind to these repeats in FSHD cells. Interestingly, these factors are known to function in gene silencing and long-distance genomic interactions, which appear to be particularly important for coordinated developmental gene regulation in human cells. Two candidate genes, FRG1 in a neighboring region and DUX4 encoded within D4Z4, have been identified whose artificial over expression did cause muscular dystrophy in vivo or a myoblast differentiation defect in vitro, respectively. The loss of chromatin structure associated with gene silencing at D4Z4 may explain the abnormal expression of these genes in the disorder. However, FSHD patient muscle cells do not always over express these genes. Thus, there are likely to be additional unidentified genes and signaling pathways involved in the pathogenesis of FSHD. Our hypothesis is that D4Z4 normally spreads a silencing effect to target genes through genomic interactions mediated by D4Z4-bound factors. This function is lost in FSHD, resulting in the abnormal over expression of a set of target genes that leads to clinical manifestations of the disorder. I am taking two strategies to test this model; (1) screen for any genes that might have lost factors similar to those that are lost from D4Z4 in FSHD by high-throughput genome-wide chromatin immunoprecipitation (ChIP)-sequencing, and (2) directly search for genomic regions that interact with D4Z4 using biochemical chromatin conformation capture (3C)-related methods. Any candidate genes identified by these assays will be tested for their effect on cell viability, proliferation/differentiation, and muscle-related downstream gene expression. I will try to re-create the expression change detected in FSHD cells in normal human myoblasts (by over expression or repression) and compare it to the phenotypes of FSHD myoblasts to determine whether the candidate gene contributes to the FSHD cellular phenotype. My research aims to decipher the epigenetic abnormality mechanism in FSHD, which should provide novel insight into the disease mechanism and thus potentially present new therapeutic strategies.

A FSH Society Sanford Batkin & Helen Younger and David Younger research fellowship grant

6. Analysis of DUX4-fl expression

Peter L. Jones, Ph.D.

Boston Biomedical Research Institute, Watertown, Massachusetts
\$7,500 for 1 year

Summary: We request support from the FSH Society for our pilot project investigating DUX4 expression in unaffected and FSHD subjects. The DUX4-fl expression model for FSHD has not been independently validated, likely due to the lack of quality clinical resources in the field. At this point in FSHD research, validating and expanding upon the DUX4-cytotoxicity model for pathogenesis is vital to the entire field and we are best positioned to do the necessary experiments with the unique set of highly controlled reagents being generated by the NIH Wellstone Muscular Dystrophy CRC for FSHD at BBRI. Each Wellstone cohort consists of an FSHD affected

subject and an unaffected first-degree relative. Each subject donated two biopsies, one from the biceps and one from the deltoid. A portion of each biopsy was used to derive myogenic cell cultures. Quite surprisingly, in our initial preliminary results using 4 cohorts we found some inconsistencies with the published DUX4 expression results that have warranted further investigation. Therefore we have begun a much larger effort to analyze DUX4-fl mRNA and protein expression in a larger set of Wellstone cohorts using RT-PCR and immunostaining (ICC). However, this project is not funded at all in my lab or in the original Wellstone budget and my lab receives no financial support from the Wellstone Center. The Wellstone has supported us by providing us with cells, which we culture, and RNA which the Louis Kunkel lab purified from biopsies (we do not actually work with the biopsies) and we have been fortunate to receive these Wellstone samples. At this point, to ensure that our results are statistically meaningful, we need to analyze many more cells and biopsy RNAs and it has become cost prohibitive. Therefore I am requesting financial support for consumables and services (DNA sequencing) to conduct these experiments.

A FSH Society Cape Cod Walk 'n' Roll fellowship research fellowship grant

► August 2010 Round

1. Small Molecule Screen to Identify Inhibitors of DUX4-mediated Toxicity, Therapeutic Approach for FSHD

Darko Bosnakovski, D.V.M., Ph.D.
University "Goce Delcev" Stip
Faculty of Medical Sciences
Stip, R. Macedonia
\$90,000 over 2 years

Summary (Provided by Applicant): The goal of this proposal is to discover a chemical compound that efficiently inactivates the DUX4 protein and to work towards a drug for a therapeutic approach to FSHD. The aims of the proposed study targets the most crucial topic and urgent needs of FSHD patients: specific and direct pharmacological therapy. First, the project help to narrow focus from 82 potential compounds to inactivate DUX4, implicated as necessary to cause FSHD, as a result of high-throughput screening to the most promising direct DUX4 inhibitors. Second, scientists will evaluate effectiveness of DUX4 inhibition and study the properties of the selected compounds.

A FSH Society New York Festive Evening of Music research fellowship grant

2. Defining the Tissue and Cell Specificity of the Human DUX4 promoter in Mice

Scott Harper, Ph.D.
The Research Institute at Nationwide Children's Hospital,
Columbus, Ohio
\$50,000 over 1 year

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Summary: Since animal models, particularly mice, are crucial tools for studying disease pathogenesis and developing potential therapeutics, the absence of an FSHD mouse model is a fundamental problem in the FSHD field. A major goal of the Harper lab is to generate an FSHD mouse model expressing a single FSHD-permissive human D4Z4 repeat, and to use this model to understand the role of the D4Z4-resident gene, DUX4, in FSHD pathogenesis, and develop RNAi therapeutics targeting DUX4. This project will carefully define the developmental and cell specific expression patterns of DUX4p-GFP mice, and develop an AAV vector to determine whether a viral-mediated vascular delivery approach can produce the same expression patterns. These studies are important first steps toward developing an AAV-mediated D4Z4 mouse model.

A FSH Society Jacobs Family and Friends, California Walk 'n' Roll, and Kelly Family and Friends research fellowship grant

3. Identification of a Novel FSHD Biomarker [an unknown 50 kDa polypeptide highly expressed in FSHD samples]

Jessica Sun, Ph.D./Peter Jones, Ph.D.

Boston Biomedical Research Institute, Watertown, Massachusetts
Partial funding for preliminary data \$10,000 over 1 year

Summary: Screening FSHD patient-derived myoblasts, control myoblast, and muscle samples for expression changes at the proteomic level produced an unknown 50 kDa polypeptide highly expressed in FSHD samples compared to controls. Interestingly, this polypeptide is equally expressed in both normal and FSHD-patient derived myoblasts and early myotubes, however, unlike in control cells where its expression decreases, this unknown polypeptide remains highly expressed in differentiated muscle suggesting it is developmentally regulated and this regulation is disrupted in FSHD. Identifying this protein will provide insight into FSHD pathophysiology, will be a useful FSHD biomarker, and may be one of the first proteins consistently and specifically up regulated in viable FSHD muscle. Therefore, generating specific and standardized antibodies to this protein will provide a useful resource for clinicians and basic FSHD researchers.

A FSH Society Cape Cod Walk 'n' Roll research fellowship grant

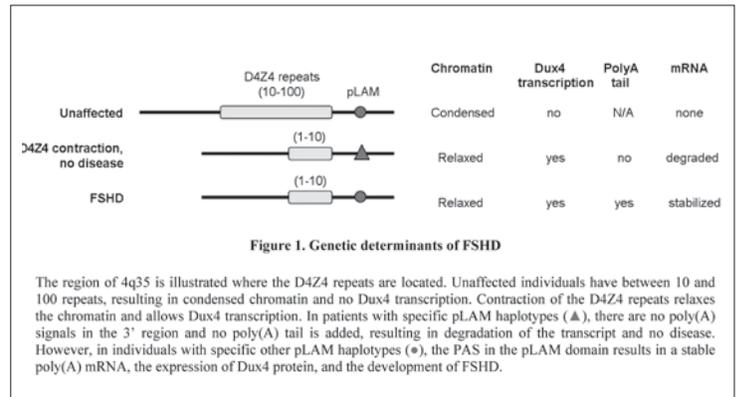
4. Toward Therapeutics for FSHD: Understanding mRNA Processing

Thomas A. Rando, M.D., Ph.D./Antoine de Morree, Ph.D.

Department of Neurology and Neurological Sciences
Stanford University School of Medicine, Palo Alto, California
\$200,000 over 2 years

This research project is being matched dollar for dollar up to \$100,000 by the Stanford Office of Medical Development and Dr. Gary Steinberg, Stanford Institute for Neuro-Innovation and Translational Medicine (SINTN).

Summary: Recently, the group of Dr. van der Maarel reported in the journal *Science* their findings of the high resolution haplo-



Dr. Thomas Rando describes the genetic determinants of FSHD leading to expression of DUX4

type mapping of patients and unaffected individuals with D4Z4 contractions. Their findings provide evidence that the disease develops in individuals who have BOTH a D4Z4 repeat contraction AND a specific sequence in the pLAM domain at the 3' end of the D4Z4 array (Figure 1). The D4Z4 repeat contraction results in “relaxed chromatin”, and allows the transcription of the DUX4 gene in the final D4Z4 repeat. However, it is the sequence in the pLAM domain that creates a site that is recognized by the cellular machinery allowing cleavage of the mRNA and the addition of a poly(A) tail. Without a poly(A) tail in the 3' untranslated region (3' UTR), transcripts are rapidly degraded and never translated into proteins. With these tails, transcripts are stabilized and appropriately localized in the cell, allowing for protein translation. In individuals who have D4Z4 contractions but a single base change in the distal sequence, the cell does not recognize it as a “polyadenylation signal” (PAS) site, no poly(A) tail is added to the 3'UTR of the transcript, the DUX4 transcript is unstable, no DUX4 protein is made, and the individuals are protected from getting the disease. Within this cascade are several opportunities, at least theoretically, to treat or even prevent FSHD in susceptible individuals. Any intervention that prevents the addition of the poly(A) tail to the DUX4 transcript is a potential therapeutic approach for FSHD.

The research project aims for a direct line to a novel therapeutic approach. The toxicity leading to FSHD depends of effective mRNA processing in which the DUX4 transcript is cleaved and modified by the addition of a poly(A) tail. If one of these processes could be blocked, then the mRNA would be destabilized and the FSHD genotype would yield a normal phenotype. Clearly, it is untenable to interfere with mRNA processing in general because of the toxicity to the cell. Therefore, understanding the mechanisms by which a cell can bypass a specific PAS site would suggest a mechanism for selectively blocking the PAS site in the pLAM domain in the DUX4 gene without generally affecting cellular mRNA processing. This would be an effective treatment for patients with FSHD.

A FSH Society William R. Lewis, Sr., M.D. and Family research fellowship grant

5. A multicenter collaborative study on the clinical features, expression profiling, and quality of life of pediatric FSHD

Jean Mah, M.D.

Alberta Children's Hospital, Calgary, Alberta, Canada

US\$96,669 over 2 years, Year 1 US\$51,434 & Year 2 US\$45,235

Project is being co-funded by the FSHD Fund Muscular Dystrophy

Canada FSHD Fund at CDN\$65,000.

Summary: FSHD is one of the most common types of muscular dystrophy. Despite recent advances in the understanding of the molecular genetics of FSHD, the exact mechanism responsible for the disease remains unknown, and presently there is no cure. As well, the prevalence, clinical variability, cross cultural presentation, and the psycho-

social impact of FSHD on affected individuals constitute a significant public health concern. Emerging therapeutic trials will benefit from the availability of natural history data and reliable outcome measures for both children and adults with FSHD. The main objectives of this study are: 1) to establish a standardized muscle testing protocol for use in children and youth with FSHD; 2) to describe the clinical phenotypes of pediatric onset FSHD; 3) to evaluate the impact of FSHD on health-related quality of life and disability across different age groups; and 4) to explore potential genetic modifiers of clinical phenotypes and disease progression in FSHD.

A FSH Society New York Festive Evening of Music research fellowship grant [FSH Watch](#)

Ongoing or completed FSH Society research fellowship grants since last research newsletter

Genome-wide analysis of FRG1-mediated splicing defects in FSHD and iFSHD

Yi Xing, Ph.D.

The University of Iowa, Iowa City, Iowa

\$39,998 over one year

A FSH Society Aubrie Lee Family Research Grant for Infantile FSHD & Fire Island & Fabiola Bertinotii research fellowship grant

A ncRNA regulating the epigenetic switch at the basis of FSHD

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

Division of Regenerative Medicine, Centro San Raffaele del Monte, Milano, Italy

\$45,000 over eighteen months

A FSH Society New York Festival of Music research fellowship grant

Molecular mechanisms involved in FSHD

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

Association Institut de Myologie, Paris, France

\$37,800 over one year

A FSH Society Grant Delta Railroad Construction Company research fellowship grant

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

Frédérique Magdinier, Ph.D.

Laboratory INSERM UMR 910 Medical Genetics & Functional Genomics,

Faculté de médecine de la Timone, Marseille, France

\$30,000 over one year

A FSH Society Family Picnic research fellowship grant

FSHD iPS cells: bioinformatics support

Michael Kyba, Ph.D.

Minnesota Medical Foundation, University of Minnesota, Minneapolis, MN

\$45,000 over one year

A FSH Society New York Festival of Music research fellowship grant

The Boston Biomedical Research Institute Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD, Project 4 – Mouse Model Studies for FSHD Biomarkers

Jeffery Boone Miller, Ph.D.

Boston Biomedical Research Institute, Watertown, Massachusetts

NIH Sen. Paul Wellstone MD CRC for FSHD

& Robert J. Bloch, Ph.D./Patrick Reed, Ph.D.

University of Maryland School of Medicine, Baltimore, Maryland

NIH Sen. Paul Wellstone MD CRC for FSHD

\$160,000 over four years

A FSH Society Stuart Lai Mouse Model Development research fellowship grant

Analyses of functional domains in the pro-apoptotic protein DUX4

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

INIMEC-CONICET, National Research Council of Argentina

Laboratorio de Biología Celular y Molecular, Cordoba, Argentina

\$59,040 over two years

A FSH Society Marjorie and Gerald Bronfman research fellowship grant

Investigating DUX4 Structure and Function Using Rational Mutagenesis

Scott Q. Harper, Ph.D.

The Research Institute at Nationwide Children's Hospital, Columbus, Ohio

\$40,000 over one year

A FSH Society Jacobs Family and Friends research fellowship grant

Tissue Donation for Research

Become a registered FSHD tissue donor

by **H. RONALD ZIELKE, Ph.D**

*The Eunice Kennedy Shriver NICHD Brain and Tissue Bank for Developmental Disorders
University of Maryland, Baltimore, Maryland, USA*

FSHD does not yet have a cure. One area open to research is a careful analysis of the multiple muscles that are affected in FSHD. To obtain a sufficient number and size of samples, these specimens are collected immediately after someone with FSHD dies. Tissue donation is recognized as a magnanimous act by all major religions. However, the decision to donate is a very personal one. No one answer is appropriate for all. But for those individuals and families that make this decision to donate tissue, a procedure is available by which you can leave a legacy for FSHD research.

The National Institutes of Child Health and Human Development (NICHD) funded the NICHD Brain and Tissue Bank at the University of Maryland to work with families who have made the decision to donate tissue following the death of the affected individual. The decision should be made with the active participation of the individual with FSHD. Equally important, the legal next of kin has to actively participate and agree to the decision. This is critical because once the Bank is informed of the death of the donor, the next of kin has to give final consent for tissue donation. In fact, it is most beneficial if both the donor and the next of kin sign the consent form.

To initiate the process of donation a donor may pre-register with the Bank via the internet at www.btbankfamily.org or by calling the Bank at 800-847-1539 to request a registration packet. This is also the time to ask questions. After the registration is complete, it is important to keep the Bank informed when the health of the potential donor changes. Since the quality of the tissue, and therefore its potential for important FSHD research, deteriorates with time after death, it is important to contact the

Bank within hours after death has occurred. Ideally the tissue has to be recovered within 24 hours after death. If it hasn't been done already, the family has to provide the Bank with contact information for the funeral home selected by the family as well as the names of local hospitals in which the donor received care. The latter is important because most hospitals will not assist in the tissue donation if the donor was not seen at that hospital. If the donor rather than the next of kin signed the consent form, then the next of kin has to sign a new form. The consent form can be processed via e-mail or fax. It is vital that the consent form be completed and returned as soon as possible because the Bank cannot contact a pathologist until the completed form is returned. The tissue recovery from a FSHD donor is more complex than for most tissue donations. The Bank has to recruit a pathologist willing and capable of recovering the various muscles. There is no cost to the family for the tissue donation. Tissue donation is completely compatible with an open viewing.

The Brain and Tissue Bank for Developmental Disorders at the University of Maryland in Baltimore is a tissue resource established to further research aimed at improved understanding, care and treatment of developmental disorders. The Brain and Tissue Bank serves as an intermediary between people who wish to have tissue donated for research upon the time of their death and the researchers who need this tissue for their vital work (www.btbank.org). Tissues listed on file at the BTB to be collected are deltoid, gastrocnemius, wrist flexors flexor carpi radialis, flexor digitorum superficialis), vastus lateralis, vastus medialis, hamstrings, upper trapezius, biceps, triceps, tibialis anterior, rhomboids, lower trapezius, retina, cochlea, and brain motor cortex). **FSH Watch**

► Understanding how D4Z4 chromatin is altered in FSHD

by **WEIHUA ZENG, Ph.D.**

University of California, Irvine, California

FSHD is one of the most prevalent muscular dystrophies. The majority of FSHD cases are linked to a decreased copy number of D4Z4 macro-satellite repeats at the subtelomeric region of chromosome 4q (FSHD1). A much smaller number of FSHD cases have no repeat contraction (FSHD2). Understanding the molecular pathway of a disease is important for the development of mechanism-based treatments. Despite the recent breakthrough discovery of the critical link of FSHD to the de-repression of the DUX4 gene, the molecular mechanism of FSHD is still not completely understood. With the generous support of the David and Helen Younger Research Fellowship from the FSH Society, we previously found that the D4Z4 repeat cluster normally contains a "heterochromatin"



Weihua Zeng, Ph.D.

structure specific for repressing gene expression, and that this structure is lost in FSHD patient cells. We reported that histone H3 lysine 9 tri-methylation (H3K9me3) is specifically lost from D4Z4 in FSHD patient cells (but not in other tested muscular dystrophy cells). This epigenetic change was observed even in patient peripheral blood-derived lymphoblasts, making it potentially useful as a diagnostic marker. Importantly, this histone methylation loss is observed in both FSHD1 and FSHD2, and is more FSHD-specific than (and distinct from) the previously identified DNA hypomethylation.

We also identified several factors in this pathway, including the histone methyl transferase responsible for H3K9me3 at D4Z4 (i.e. SUV39H1) as well as the downstream targets HP1-gamma and cohesin, which work together to form transcriptionally repressive heterochromatin. While no apparent mutation or abnormality was observed in SUV39H1 in FSHD, HP1-gamma and cohesin binding to D4Z4, which is dependent on H3K9me3, is lost in FSHD. Interestingly, the binding of these two effectors is cell type-specific, suggesting their role in cell

Development of a Mouse Xenograft Model for FSHD

Updates from recently funded FSH Society grantees

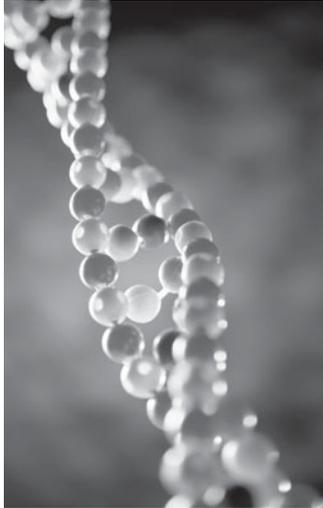
by **CHARIS HIMEDA, Ph.D.,** and **MARIETTA BARRO, Ph.D.**
Boston Biomedical Research Institute (BBRI), Watertown, Massachusetts

The Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD Research, which is funded by the National Institutes of Health and based at Boston Biomedical Research Institute (BBRI), focuses its research efforts on developing therapeutic strategies for the treatment of FSHD. A current focus of the Wellstone Center is the development of a mouse model to study the characteristics of FSHD muscle cells in the context of a live animal. This project is being conducted in the lab of Center Director Dr. Charles Emerson, and is led by Dr. Marietta Barro and Daniel Zuch with the help of Dr. Jennifer Chen and Dr. Kathryn Wagner.

FSHD is genetically linked to the loss of repetitive DNA sequences on chromosome 4. Although this genetic defect was identified over 20 years ago, the molecular basis of the disease is still unknown.

Because they reproduce disease mechanisms in the complex environment of a mammalian body, animal models are an invaluable aid to understanding the nature of human disorders and testing potential therapies. However, since the specific causative gene(s) linked to FSHD have not yet been identified, there is currently no established animal model of the disease.

To provide such a valuable tool, Dr. Barro and colleagues are developing a “humanized” mouse model by engrafting muscle stem cells from FSHD patients and their unaffected relatives into the muscles of live mice. Following engraftment, normal human muscle stem cells are able to form fibers in the host mouse that persist for months after transplantation. It is expected that transplanted FSHD muscle cells will display disease characteristics, allowing Wellstone



investigators to study the development of the disease in the context of a live animal. Recent evidence implicates the DUX4 gene as a strong candidate for FSHD, and the humanized mouse model will be used to investigate the hypothesis that inappropriate expression of DUX4 is a major cause of FSHD pathogenesis. Expression of the DUX4 gene will be evaluated during real-time muscle regeneration in live animals, and the consequences on muscle fiber maintenance and muscle stem cell renewal will be assessed.

The success of this project depends largely on the FSHD repository developed by the Wellstone Center, a collection of muscle biopsies and derived muscle cells from FSHD patients and their unaffected relatives. This repository, which represents the most comprehensive and well-controlled collection

of FSHD patient samples in the world, is coordinated by Dr. Wagner, Dr. Genila Bibat, Dr. Emerson, and Dr. Chen, and maintained at BBRI with technical assistance from Kendal Hanger. It allows researchers unique access to fresh muscle stem cells from cohorts of FSHD patients and their appropriate controls, i.e., first-degree family members. The Wellstone repository also provides an unprecedented opportunity to correlate FSHD progression with muscle biomarkers. As the genetic basis of this inherited disorder is just beginning to be elucidated, both the Wellstone repository and tools such as the humanized mouse model are helping researchers study the disease from a more clinically relevant vantage point and test novel intervention strategies designed to improve the quality of life for FSHD patients. **FSH Watch**

type-specific chromatin organization that is likely involved in the tissue-specific clinical manifestations of the disease.

Additional D4Z4 homologs are known to exist near centromeres on multiple chromosomes, but they are largely uncharacterized and their relationship to 4q D4Z4 was undetermined. Our more recent study revealed that unlike D4Z4 on chromosomes 4q (and 10q), there are high degrees of nucleotide variation in D4Z4 homologs on other chromosomes that frequently disrupt their DUX4 open reading frames (ORF). Although heterochromatin marks are also present on these homologs in normal cells, they remain unchanged in both FSHD1 and FSHD2 cells. The results indicate that the specific preservation of the DUX4 ORF and the unique epigenetic alterations are restricted to 4q and 10q D4Z4 repeat clusters, further highlighting their critical role in FSHD pathogenesis.

Based on these findings, we hypothesize that HP1-gamma and cohesin play a pivotal role in exerting a repressive effect at D4Z4. Over expression of

DUX4 within the D4Z4 repeat may also be explained by the loss of these heterochromatin marks. Recent studies indeed confirmed the critical role of cohesin in developmental gene regulation, further supporting our model and the significance of its loss at D4Z4 in FSHD. We are trying to understand how D4Z4 chromatin is altered in the two genetically distinct types of FSHD. Furthermore, it remains unclear how exactly DUX4 de-repression occurs, how it leads to the development of disease, and whether there are other genes that contribute to the disease phenotype. Active collaboration among FSHD researchers in the field will be essential for solving the mysteries of FSHD pathogenesis. Our common goal is to apply our research results to the design of effective therapeutic methods in the near future.

Work is made possible by a FSH Society Sanford Batkin & Helen Younger and David Younger research fellowship grant

NIH BBRI Wellstone MD CRC for FSHD notes recent accomplishments

Developing biomarkers for therapy of FSHD

by CHARLES P. EMERSON, JR., Ph.D., and ROBERT BLOCH, Ph.D.

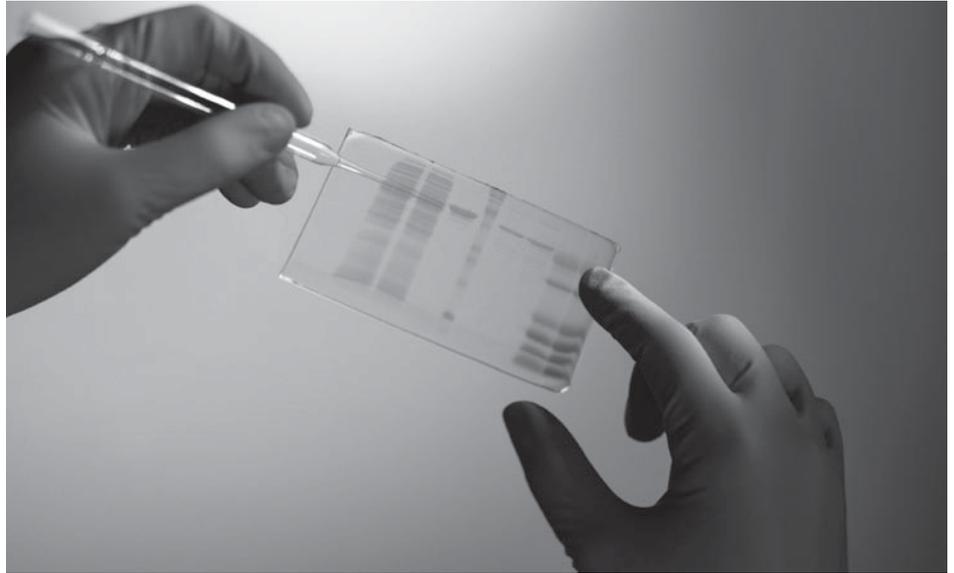
Boston Biomedical Research Institute, Watertown, Massachusetts and University of Maryland, Baltimore, Maryland

The NIH-funded Wellstone Muscular Dystrophy Cooperative Research Center for FSHD headquartered at Boston Biomedical Research Institute, Watertown, Massachusetts reports:

1. on the establishment of an unique cell repository of mortal and immortalized FSHD and control muscle cell lines from more than 60 individuals over 30 families, lead by Drs. Kathryn Wagner of Kennedy Krieger Institute, Baltimore, Maryland and Charles Emerson and Jennifer Chen at BBRI. Cells from eight family cohorts are now available for distribution worldwide to FSHD researchers. To find out how to obtain FSHD and control primary skeletal muscle cell strains and immortalized clonal cell lines for research please see <http://wellstone.bbri.org>. These cell lines are a unique resource to identify disease biomarkers for FSHD to better understand the pathology of FSHD and to monitor efficacy of drugs in future FSHD clinical trials, to develop cell models for drug screens to develop therapeutics for FSHD, and to generate humanized mouse models of FSHD for studies of disease pathology and drug testing (see accompanying article by Drs. Barro and Himeda).

2. on identification of biomarkers of myostatin inhibition, lead by Drs. Kathryn Wagner at KKI and Louis Kunkel at Children's Hospital, Boston, in collaboration with Acceleron Pharma. Myostatin is a natural regulator of muscle growth and leading drug target for improving muscle strength in patients with FSHD and other muscular dystrophies. The Wellstone study, published in *Physiological Genomics* ON January 25, 2011 [2011 Apr 27;43(8):398-407] "Gene expression profiling of skeletal muscles treated with a soluble activin type IIB receptor," investigated Acceleron's anti-myostatin drug, ACE-031 in mice utilizing genome-wide RNA expression array. Their findings identify a unique set of RNA biomarkers expressed in muscle in animals treated with drug. These biomarkers will be invaluable tools, providing a molecular signature for clinicians to conduct clinical trials to monitor the activities of anti-myostatin drugs from Acceleron and other pharmaceutical companies, enabling researchers to quickly evaluate whether patients with different muscular dystrophies including FSHD respond positively to drug treatment.

3. that Drs. Robert Bloch, University of Maryland, Baltimore, Maryland, and Jeffrey Boone Miller have developed a line of transgenic mice that express mu-crystallin specifically in skeletal muscle.



They are breeding the mice in a mixed genetic background with each other, and are continuing to do so to characterize their muscles, physiologically and morphologically. They are also that have been used as possible models of FSHD.

So far, they have only had a sufficient number of heterozygotes (mice that carry the transgene for mu-crystallin on only one of their chromosomes) to study, in the mixed genetic background. The heterozygotes have a phenotype, i.e., they are different from normal mice. In particular, the diameter of the muscle fibers in the tibialis anterior (TA) muscle is decreased, more of the fibers show evidence of increased degeneration and regeneration, and the muscles are weaker. Overall, these results suggest that the transgenic mice have muscle pathology, but it is still not clear if the pathology mimics that seen in FSHD. They must still examine the homozygotes (mice that carry the transgene on two chromosomes), which should express twice the amount of mu-crystallin. They hope to be able to begin these experiments soon and expect to observe more pronounced changes in these mice than in the heterozygotes.

They are of course hoping that the mice reproduce some of the key features of FSHD. If so, they will use them to begin to test some of the therapeutic approaches suggested for the treatment of the disease (e.g., myostatin inhibitors), in collaboration with members of the Wellstone Center. They will also use them to study the pathogenic process. Having additional strains of mice would be important for either plan to move forward. If we find that the mice we are already studying show FSHD-like pathology, they will go through another round of transgenesis to obtain additional strains with different levels of mu-crystallin.

Work made possible by a FSH Society Stuart Lai Mouse Model Development fellowship grant. [FSH Watch](#)

FSH SOCIETY: Organization & Mission

The FSH Society is a 501(c)(3) non-profit tax-exempt U.S. corporation organized in 1991 to fund, encourage and promote scientific and clinical research on FSHD. The FSH Society is a world leader in combating muscular dystrophy. It has provided three million dollars in seed grants to pioneering research worldwide and it has created an international collaborative network of patients and researchers. The Society relies entirely on private grants and donations.

FSHD is a most prevalent muscular dystrophy. FSHD affects men, women and children. It occurs with a frequency of 1/14,000 in the population and may be higher due to misdiagnosed cases. The majority of cases of FSHD are caused by a genetic defect or deletion on chromosome 4. FSHD is genetically autosomal dominant meaning that a child of either sex has a fifty percent chance of inheriting the genetic defect from an affected parent. Thirty percent of new FSHD patients have no prior family history and are a result of a congenital spontaneous genetic mutation. Once present, FSHD is genetically transmissible. Genetic diagnostic and prenatal diagnostic tests are available as well as pre-implantation genetic diagnosis (PGD).

FSHD causes a progressive loss of all skeletal muscle, with weakness usually noticeable starting with facial, scapular/back and upper arm muscles and progressing to all skeletal muscles. The prognosis includes both a loss of muscular strength that limits personal and occupational activities of most FSHD individuals and a loss of mobility in perhaps twenty percent of the cases. The early facial involvement is a hallmark of FSHD. The age of onset is variable as is the eventual extent and degree of muscle loss, but noticeable muscle weaknesses are usually present by the age of twenty in ninety-five percent of patients. Hearing loss and retinal abnormalities associated with FSHD have been reported, but the frequency of these effects and their relationship, if any, to the causative gene for the muscle defect are uncertain.

- ▶ The FSH Society was created because of a need for a comprehensive resource for FSHD individuals and families. Purposes of the organization are:
 - to encourage and promote scientific and clinical research and development on the causes, alleviation of suffering, treatment and cure of FSHD;
 - to support such research and development through solicitation of grants and contributions from individuals, private foundations, the pharmaceutical industry and others to support such research and development;
 - to make grants and awards to qualified applicants so that such applicants may accomplish such research and development;
 - to accumulate, disseminate and encourage the exchange of information about FSHD, including educating the general public, relevant governmental bodies, and the medical and scientific professions about the existence, diagnosis and treatment of FSHD;
 - to actively cooperate with related organizations and foster communication among all interested parties;
 - to serve as a resource for individuals and families with FSHD, represent them and advocate on their behalf.

The Society invites contact from any interested individuals, families, physicians, caregivers, charitable organizations, government agencies, industry, scientific researchers and academic institutions. Any inquiries regarding membership, charitable donations, purposes and goals or other issues pertaining to the Society and FSHD, should be addressed to the FSH Society Watertown office.

FSH SOCIETY RECEIVES CHARITY NAVIGATOR'S HIGHEST 4-STAR RATING

The FSH Society has earned Charity Navigator's "third consecutive 4-star rating for its ability to efficiently manage and grow its finances. Only 13% of the charities we [Charity Navigator] rate have received at least 3 consecutive 4-star evaluations, indicating that FSH Society consistently executes its mission in a fiscally responsible way, and outperforms most other charities in America. According to Charity Navigator, "This 'exceptional' designation from Charity Navigator differentiates FSH Society from its peers and demonstrates to the public it is worthy of their trust." See <http://www.charitynavigator.org> or www.fshsociety.org for direct link.



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Go to www.facebook.com and sign up. It is free and easy. Search on FSH Society and join the discussion. You can also join FSH Society Yahoo! Groups forum, online since the 1990's and with tens of thousands of searchable posts. Bookmark them and come back often. You can also access the FSH Society FaceBook page and Yahoo! Groups by going to www.fshsociety.org clicking on the "Community & Reference" menu tab at top of page and then selecting "Online Community" in the left hand vertical navigation menu.

HAVE YOU MADE A GIFT TO THE SOCIETY IN 2011?

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

COMBINED FEDERAL CAMPAIGN (CFC), 2011 CAMPAIGN

The FSH Society has been approved by the Office of Personnel Management for the 2011 campaign. The CFC is the world's largest and most successful annual workplace charity campaign, with more than 300 CFC campaigns throughout the country and internationally to help to raise millions of dollars each year. Pledges made by Federal civilian, postal and military donors during the campaign season (September 1st to December 15th) support eligible non-profit organizations that provide health and human service benefits throughout the world. The FSH Society's identification number is 10239.

FSHD ADVOCACY EFFORTS ON THE U.S. PRESIDENT'S MUSCULAR DYSTROPHY COORDINATING COMMITTEE

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Centers for Disease Control [CDC].

For more information on the MDCC plans, staff, rosters, meeting agendas and minutes please go to www.fshsociety.org click on Research tab at top, then click on FSHD Research Plans/U.S. NIH MDCC in left hand navigation or see <http://www.fshsociety.org/pages/res/PNIHMDCC.html>. **FSH Watch**

CREATING A MULTICENTER COLLABORATIVE STUDY ON THE CLINICAL FEATURES, EXPRESSION PROFILING, AND QUALITY OF LIFE OF INFANTILE ONSET FSHD

... from page 12

Utilization of the Cooperative International Neuromuscular Research Group (CINRG) study sites will provide an ideal network of dedicated researchers to study the clinical features of infantile onset FSHD. In addition, collaboration with local and international experts in the fields of pediatric ophthalmology, audiology, speech, and neuropsychology is included in the study plan to help define and raise awareness about the extra-neuromuscular manifestations of infantile FSHD. The success of this single visit cross-sectional study will generate data to lay the groundwork to establish a larger prospective cohort for determination of the natural history of FSHD and the clinical outcome parameters for potential therapeutic trials.

To fulfill the above study objectives, 50 participants with infantile onset FSHD will need to be recruited from participating CINRG sites across the United States as well as other centers in Canada, Europe, Japan, and Australia. The participants may be adults or children having a confirmed diagnosis of infantile onset FSHD. If you would like more information about the infantile FSHD study and/or to find the nearest CINRG participating sites, please contact Robert Casper, Study Project Manager (RCasper@cnmcresearch.org) or visit the CINRG website at www.cinrgresearch.org for further information. **FSH Watch**

A POSSIBLE APPROACH FOR TREATING FSHD WITH RNAi THERAPEUTICS

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sh1FRG1 doses

"In conclusion, we have shown that we can prevent disease progression with systemic, AAV6-mediated FRG1 RNAi performed following disease onset. This work exemplifies the power and specificity of RNAi in a widespread tissue in a living animal and offers a potential route to clinical application and treatment for individuals who are already showing symptoms of disease. The efficient, stable, long-term therapeutic reduction of pathological signs in the FRG1 mouse suggests the added potential clinical benefit of efficacy with limited dosing. This therapy allowed significant improvement of disease and could potentially be translated to human patients. The knowledge gained through these studies could facilitate the development of new therapies to treat FSHD and other diseases." **FSH Watch**

FSHD International Research Consortium meeting is scheduled

The FSH Society FSHD International Research Consortium workshop will be held on Monday and Tuesday, November 7-8, 2011, at the Boston Biomedical Research Institute (BBRI), in Watertown, Massachusetts. Silvere van der Maarel, Ph.D. (Leiden University Medical Center and Fields Center for FSHD, Leiden, Netherlands) and Robert H. Brown, Jr., M.D., D.Phil. (The University of Massachusetts, Worcester, Massachusetts, USA) will be the scientific and clinical co-Chairs respectively, and Daniel Perez (FSH Society) will serve as scientific/organizational chair. Dr. Charles Emerson, Jr., Director, of BBRI and co-Director of the NIH NICHD BBRI Sen. Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD, has graciously offered to host the meeting at the headquarters of BBRI.

Monday will be a full day of research presentations and discussions and Tuesday will be a half day of research planning. The Society annually organizes, sponsors, and funds this meeting. There are no registration fees for professionals working on FSHD. Other co-sponsors to date are NIH NICHD BBRI Sen. Paul D. Wellstone Muscular Dystrophy Cooperative Research Center for FSHD. This will be an important time for the expected 100 FSHD research and clinical community to convene and network.



FSHD researchers, clinicians, patients and funding agencies gather from around the world to discuss the latest developments in FSHD at the 2010 FSH Society research workshop

INTERNATIONAL FSH CONSORTIUM OUTLINES RESEARCH PRIORITIES FOR FSHD

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systems. It becomes even more important to have a solid model and dataset to test.

9. Epigenetics/genetics. There is the need to further evaluate FSHD1, FSHD2 and phenocopies using the genetic approach. Resolve and establish genes involved in the pathophysiology of FSHD (DUX4, FRG1, FRG2 and other 4q35 loci, PitX1, Pax3, Pax7 and other impacted distributed loci, related cascades and pathways, etc.)

10. Clinical trials readiness. There is a need to revisit what we know about the pathological progression of FSHD.

Learn more about the group at: <http://www.fshsociety.org/assets/pdf/IRCWorkshop2010WorkingConsensusOfPrioritiesGalley.pdf>. **FSH Watch**

JOURNEY TOWARD DEVELOPING A DRUG FOR FSHD

... from page 1

and Jane Hewitt, believed that something inside D4Z4, rather than on its vicinity, could be an initial trigger for FSHD. Simultaneously, we worked on developing a cell-based system where we could study the mechanisms of DUX4 and screen for its inhibitors, and on creating a FSHD animal model, a DUX4 inducible mouse. We developed an in vitro system which allowed controlled DUX4 induction in myoblasts. By using it we showed that DUX4 can provoke FSHD-related cellular changes. When expressed at high levels, DUX4 is ubiquitously toxic for most of the cell types including muscle progenitor cells and it also increases cell susceptibility to oxidative stress by mis-regulating genes involved with buffering the redox system in the cells. At low levels DUX4 interferes with myogenic regulatory factors MyoD and Myf5 and it impairs myogenic differentiation.

Support for the prediction that DUX4 is involved in the disease came from the great effort from several groups that identified DUX4 at RNA and protein levels exclusively in the myoblast and muscle biopsy samples from FSHD patients³⁴⁵⁶. The recent publication described the crucial mutation in β -satellite sequence on 4q16.1 haplotype that provides stable DUX4 polyadenylation signaling reveals DUX4 as a key component in the pathogenesis of FSHD⁷. Thus, we have the gene (DUX4), we know something of its downstream targets (myogenic regulators, redox buffering system, caspases, p53, pitx1) and we have a system in which we can screen for different therapeutic strategies (our DUX4 inducible cell lines).

Designing a drug specific to target the cause of the disease is one of the best approaches to control or cure it. To develop a drug means to identify a compound with the ability to specifically block or regulate the pathological process while remaining safe for the rest of the organism. In order to find a specific drug to FSHD, I formed a collaborative initiative with Dr. Kyba and a multidisciplinary group consisting of molecular biologists, chemists and pharmacists from the University of Goce Delcev, the University of Minnesota and the UTSW Medical Center. The members of this group are

fortunate to be independently funded from various sources including the FSHD Society, MDA and NIH.

The main strategy is to discover a chemical compound which specifically binds to DUX4 and inactivates it. This potential interaction may cause DUX4 to become unstable and easily degradable; it could modify DUX4 structure that impairs its mobility in the cell or its binding ability to the target genes. To identify DUX4 inhibitors we screened more than 200,000 different compounds from the chemical library at UTSW Medical Center for their ability to protect the cells from DUX4 toxicity. The chemical compounds were added on DUX4 induced myoblasts and their effect was analyzed 24 hours later. If the compounds have rescue ability, then the DUX4 expressing myoblast will survive. Based on this assay we selected 586 compounds (0.3% of the library) with significant DUX4 rescue effect. Several compounds on the list, however, indirectly support cell survival by affecting downstream targets of DUX4. Chemical and structural analyses of the lead compounds followed by additional screenings for reverting oxidative stress induced cell death revealed that the majority (more than 70%) of the selected compounds are antioxidants. Since the redox system is affected in DUX4 expressing cells and in the FSHD myoblast, antioxidants would provide a beneficial effect to them. Therefore, we concluded that antioxidants are beneficial, but are not specific inhibitors of DUX4. Based on those assays, however, we cannot exclude that among the antioxidants there are no direct DUX4 inhibitors. To choose the most potent inhibitor we are conducting additional screenings using our 586 lead compounds. Some of the screenings are designed to distinguish whether the rescue is cell specific. Thus we generated DUX4 inducible fibroblast and human embryonic stem cell lines. If the compounds specifically blocked DUX4, then the rescue effect will be seen in different cell types as well. Our results suggest that more than 60% of the compounds with rescue effect in myoblasts have the same effect in fibroblast. Another screening that we are working on aims

to analyze the level of alternation of DUX4 gene targets in the rescued cells. For example, stress related genes such as MyoD, Myf5, p21 and caspases are known to be mis-regulated by DUX4. If the compound inactivates the DUX4 protein, then the expression of those downstream genes should be unchanged. In the assay we isolate RNA from cells that are treated by compounds and analyze their gene expression profile. The last ongoing approach is based on the trafficking of DUX4 protein. We are developing an assay suitable for a large scale study for tracking the mobility and the stability of DUX4 prior to interaction with the lead compounds.

We believe that our strategy involving the laborious follow up assay will lead to the identification of a direct DUX4 inhibitor which will be suitable for development into a drug for FSHD.

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Human embryonic stem cell (hESC) and induced-pluripotent stem cell (iPSC) tissue donation for research

The RENEW Biobank is located at the Center for Human Embryonic Stem Cell Research and Education (hESC) within the Stanford University Institute for Stem Cell Biology and Regenerative Medicine. The Center focuses on four areas of primary importance in stem cells research: human embryology, germ cell development, reprogramming cells, and directed differentiation of stem cells to other cell types. By using human embryonic stem cells (hESCs) and alternative pluripotent cells such as induced-pluripotent stem cells (iPSCs) as a model, it seeks to understand how pluripotent cells generate distinct fates during human development. At the same time, it is setting the stage to translate these findings into novel therapeutics.

Research on in vitro fertilization and

the human embryo prior to implantation is necessary if scientists are to understand human development and treat a wide range of human disorders including infertility. Advances in human embryonic stem cell (hESC) research may someday lead to new treatments for adult diseases such as muscular dystrophy, spinal cord injuries or Alzheimer's disease.

If you are interested in donating IVF materials for research, or are a clinician who wants to offer your patients disposition options, please contact:

Angie Morey, Research Manager
The RENEW Biobank
650-721-2259 Telephone
650-498-4320 Fax
amorey@gmail.com



Save the Date!

October 1, 2011

Third Annual Walk 'n' Roll for FSH Muscular Dystrophy
Harwich Bike Trail
Harwich Community Center – 100 Oak Street
Harwich, Massachusetts



October 9, 2011

Second Annual Southern California Celebrity Walk 'n' Roll for FSH Muscular Dystrophy
October 9, 2011

Heritage Park
14301 Yale Avenue
Irvine, California 92604

This year we have taken the event to new heights. Celebrities will accompany us around the lovely park lagoon and mingle with guests who have purchased the VIP Lunch sponsored by Dave & Buster's, Irvine. There will be music, a silent auction, raffles, prizes and more.

Help Raise Funds to "Unlock the Code"
Two Events to Benefit the FSH Society

To register, sign-up or support the events please go to the home page of www.fshsociety.org and click on 'Fundraisers' tab at right. Even if you cannot attend in person, please consider making a contribution to support FSHD research!

► Are you clinically diagnosed with FSHD but have tested negative with genetic testing?

FSHD1B (FSHD2) patients sought by research community

Researchers are very interested in identifying FSHD patients who are clinically diagnosed as having FSHD and who have genetically tested negative for the 4q35 FSHD deletion. If you are one of these individuals, the FSH Society would like to put you in contact with researchers. Please contact Daniel Perez at the Society by sending an e-mail with your address, phone and best way to reach you to daniel.perez@fshsociety.org with "non4q35" in the subject line or call 617-658-7811.

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