CONTACT:
Daniel Perez, President & CEO
FSH Society, Inc.
64 Grove Street, Watertown, Massachusetts, 02472
t: (617) 658-7811
e: daniel.perez@fshsociety.org

Continuing to make progress in understanding and treating FSHD
Grant awards for February 2011 Cycle

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 cycle.
Awards for February 2011 Cycle

The FSH Society Scientific Advisory Board (SAB) met in June 2011 to review grant applications received for the February 2011 round of FSH Society grants funding. Below is a list of the funded projects, including project descriptions as submitted by grant applicants.

1. **Antisense strategies against DUX4 as therapeutic approaches for FSHD**
   
   Eugénie Ansseau, 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, β-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 and Song 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

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.

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

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 demonstrate 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 contain 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.

*FSH Society California Walk and Roll 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

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 and Song 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

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.
6. **Analysis of DUX4-fl expression**

Peter L. Jones, Ph.D.

Boston Biomedical Research Institute, Watertown, Massachusetts

$7,500 for 1 year

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 and Roll fellowship grant*