Continuing to make progress in understanding and treating FSHD
Grant awards for February 2015 and Ad hoc 2015 cycles

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 2015 cycle.

Awards for February 2015 Cycle

On June 16 & 22, 2015, the Scientific Advisory Board (SAB) of the FSH Society, chaired by David Housman, Ph.D., held two sub-reviews and a final SAB review. The SAB reviewed the grant applications and progress reports for the February 2015 round. By June 25, 2015, the FSH Society Board of Directors reviewed and approved the SAB recommendations for funding. Below is a list of the funded projects, including project descriptions as submitted by the applicants. For the February 28, 2015, round of grant applications eight grant applications were received; three were funded in the amount of $263,502 and a fourth approved at $30,000 contingent on matching funds.

+
Sachiko Homma, PhD / Jeff Boone Miller, PhD
Boston University, Boston, MA USA
Inhibited protein turnover and TDP-43 aggregation in FSHD pathogenesis
$68,920 1 year

PROJECT SUMMARY (provided by applicant)

The goals of our studies are to identify pathogenic mechanisms and develop new therapeutic strategies for facioscapulohumeral muscular dystrophy (FSHD). We discovered that full-length isoform of double homeobox protein DUX4 (DUX4-FL), but not DUX4-S (short isoform of DUX4), inhibits protein turnover and leads to abnormal ubiquitin expression and nuclear aggregation of TDP-43, one of the aggregation-prone and an RNA/DNA-binding proteins previously associated with amyotrophic lateral sclerosis (ALS) and inclusion body

myositis (IBM)(Homma et al., 2015). These phenotypes were not side effects of DUX4-FL-induced cell death and were enhanced when cell death was blocked by caspase inhibitors. These data suggest that DUX4-FL induces abnormal protein degradation, which can in turn lead to cytotoxicity. Importantly, the abnormal deposition of ubiquitinated protein and nuclear aggregation of TDP-43 were observed in DUX4-FL-expressing cells from its endogenous promoter as well as when exogenously expressed. Thus, DUX4-FL produced from its endogenous promoter is sufficient to promote pathogenesis, and our results identify inhibition of protein turnover and impaired proteostasis as potential pathological mechanisms in FSHD. We now propose to identify mechanisms that underlie the DUX4-FL-induced inhibition of protein turnover and promotion of abnormal protein aggregation. Under Specific Aim 1, we will identify the mechanisms by which DUX4-FL expression inhibits proteasome function. We will isolate proteasomes from DUX4- positive and -negative myotubes and the amount and activity of proteasome will be examined. If intrinsic proteasome activity and/or the amount are unchanged by DUX4-FL expression, then we will investigate indirect mechanisms by which ubiquitinated proteins could abnormally accumulate in DUX4-FL-positive myotubes. Under Specific Aim 2, we will examine FSHD muscle biopsies to identify if there are signs of dysfunction of protein degradation system. We will immunostain FSHD and control tissues with antibodies for ubiquitin, TDP-43 and other proteins that are associated with myopathies and protein aggregation diseases. The outcome of this study could use as clinical marker(s) for FSHD. Thus our proposed studies will provide valuable insights into the mechanisms of DUX4-FL induced pathology in FSHD that might share with some other myopathies and/or protein aggregation diseases. This new knowledge could develop potential new therapeutic strategies based on regulating proteasome activities and identify new clinical biomarker(s) for FSHD.

+

Yosuke Hiramuki, PhD / Stephen Tapscott, MD, PhD
Fred Hutchinson Cancer Research Center, Seattle, WA USA
Determining the effectiveness of increased SMCHD1 expression to suppress DUX4 in FSHD muscle cells and model mice
$101,132 for 2 years

PROJECT SUMMARY (provided by applicant)

Facioscapulohumeral dystrophy (FSHD) is a muscular dystrophy clinically characterized by progressive weakness and wasting of the facial, shoulder and upper arm muscles. The causative gene is DUX4 that resides in D4Z4 macrosatellite repeat unit varied between 11 and 100 on chromosome 4. Normally D4Z4 macrosatellite array is hypermethylation and DUX4 is not expressed in normal skeletal muscle but D4Z4 macrosatellite array is hypomethylation and DUX4 is expressed in FSHD skeletal muscle. FSHD has two types, FSHD1 and FSHD2, and both are phenotypically indistinguishable, whereas the cause to induce the abnormal DUX4 expression in skeletal muscle is distinguishable. FSHD1 is caused by contraction of the D4Z4 macrosatellite repeat unit (1-10), whereas FSHD2 is caused by mutations in SMCHD1. Each of FSHD1 and FSHD2 is furthermore divided into two classes. D4Z4 macrosatellite repeat size in FSHD1 (1-6 unit and 7-10 unit) is involved in disease severity. Mutations in SMCHD1 are grouped into haploinsufficient and dominant negative mutations. In addition, SMCHD1 modifies disease severity in families affected by FSHD1. However there is no study to test whether SMCHD1 has a possibility of the effective treatment for FSHD1 and FSHD2. This proposal builds on the hypothesis that SMCHD1 overexpression decreases aberrant DUX4 expression in FSHD1 and FSHD2. The outline of research plan to test this hypothesis is as follows.

Aim 1. Doxycycline inducible lentivirus-SMCHD1 in FSHD1 and FSHD2 muscle cells.

We will determine the effectiveness of increased SMCHD1 expression to suppress DUX4 in FSHD with different mutations using doxycycline inducible lentivirus-SMCHD1 (a and b). SMCHD1 is composed of exons 48 and has ATPase and Hinge domain. We will determine the critical region of SMCHD1 to suppress abnormal DUX4 in FSHD muscle cells using doxycycline inducible lentivirus-SMCHD1 that has short SMCHD1 coding sequence (c).

a. Ability to suppress DUX4 in FSHD1 with
   i) Slightly lower than normal repeat size (7-10 unit)
   ii) Severe contraction (1-6 unit)

b. Ability to suppress DUX4 in FSHD2 with
i) Haploinsufficient mutation
ii) Dominant negative mutation
c. Ability to suppress DUX4 in a range of SMCHD1 coding sequence in FSHD muscle cells.
i) Full length (exons 1-48)
ii) Short length (exons 1-10, 1-20, 1-30, 1-40)

Aim 2. rAAV6-SMCHD1 in FSHD muscle cells and D4Z4-2.5 mice.

We will determine the effectiveness of increased SMCHD1 expression to suppress DUX4 with recombinant adenoassociated virus 6 (rAAV6) - cytomegalovirus (CMV) - SMCHD1 in FSHD1 and FSHD2 muscle cells and D4Z4-2.5 mice (FSHD1 model mice). In addition, to distinguish the effect of SMCHD1 in whole body from muscle on suppression of DUX4 in FSHD, we will choose CMV promoter and enhancer/promoter regions of muscle creatine kinase and α-myosin heavy-chain (MHCK) genes and administrate rAAV6-CMV-SMCHD1 and rAAV6-MHCK-SMCHD1 into D4Z4-2.5 mice.

a. Ability to suppress DUX4 in FSHD1 and FSHD2 muscle cells using rAAV6-CMV-SMCHD1.
b. Ability to suppress DUX4 in D4Z4-2.5 mice using rAAV6-CMV-SMCHD1 and rAAV6-MHCK-SMCHD1.

In this application, Aim 1 will be the proof of principle experiments showing that higher SMCHD1 will be effective as a potential therapy and Aim 2 will develop a method for delivery that might eventually be suitable for pre-clinical or clinical studies based on rAAV6.

+

Eugenie Ansseau, PhD / with Frederique Coppee, PhD and Alexandra Belayew, PhD
University Mons, Mons, BELGIUM
Functional study of the DUX4 and DUX4c double homeodomain proteins in skeletal muscle
$93,450 for 1 year

PROJECT SUMMARY (provided by applicant)

The Double homeobox (DUX) genes map in 3.3-kb repeated elements that constitute a family with hundreds of members dispersed into the human genome. Even if the evolutionary conservation of their sequences argues in favor of a functionality, they were long considered as pseudogenes and thus poorly studied. However, several DUX genes are expressed in healthy muscle cells. Our group has characterized the DUX4 gene that causes FSHD and the homologous DUX4c gene (both located at 4q35). Both encoded proteins are highly similar transcription factors and only differ in the carboxyl-terminal region. There is no known orthologue of these genes in rodent. However, mouse Duxbl, a paralogue sharing similarities with DUX4 and DUX4c, was previously shown to play roles during myogenesis. DUX4c is expressed in healthy muscle and induced in FSHD and Duchenne muscular dystrophy (DMD). Our previous data suggested a role for DUX4c in normal human muscle regeneration and its activation (as in FSHD) could impact muscle regeneration in several myopathies. Deciphering the function of unstudied human muscle proteins should increase our understanding of physiological and pathological mechanisms of the skeletal muscle.

The present project stems from our identification of putative and validated protein partners that hint to unexpected cytoplasmic functions for the DUX4/DUX4c proteins in myofibril organization and mRNA translation control. Moreover, in addition to the endogenous DUX4c nuclear localization in myoblasts, we found a cytoplasmic localization of this protein in differentiating myoblast at particular times. We also observed DUX4 cytoplasmic translocation during myoblast differentiation following its overexpression. As a lot of identified partners are identical for DUX4 and DUX4c, the pathological increase of DUX4/DUX4c proteins in FSHD muscle cells could titrate out some partners and interfere with the normal DUX4c function in muscle and would contribute to explain why this tissue is particularly sensitive to pathological DUX4 expression (one of the FSH Society research priorities for 2015).

To further investigate these observations, we would

(1) monitor DUX4/DUX4c protein trafficking in live muscle cells (time-lapse microscope) by using fluorescent ligands to label DUX4/DUX4c expressed as HaloTag fusion proteins and by using different nuclear export/import inhibitors;

(2) produce additional antibodies specifically targeting DUX4c

(3) validate DUX4/DUX4c interactions with partners that play major roles in myofibril organization or mRNA translation using in situ proximal ligation assays;

(4) compare the interactions of selected partners and DUX4/4c in healthy and FSHD differentiating myoblasts

(5) map the specific DUX4/DUX4c peptidic domains that interact with validated partners using DUX4/DUX4c specific or deletion mutants and HaloTag pull down;

The latter will be used to screen a phage display library with the mapped interaction domains to select peptides that suppress or decrease the interaction with DUX4/DUX4c of partners involved in toxic pathways. The biological significance of the DUX-partner interaction will then be evaluated by introducing the blocking peptide in FSHD muscle cell cultures and analysing the resulting phenotype. This approach might present a therapeutic interest in FSHD by blocking DUX4/DUX4c toxic functions.

We expect this project will help (i) to define new functions for DUX4 and the poorly studied DUX4c, (ii) to discover their putative interactions through shared partners (iii) to bring new light on the mechanisms of DUX4 toxicity in FSHD muscle and (iv) to propose new therapeutic strategies preventing protein/protein interactions.

+

Jonathan Lonsdale, PhD
National Disease Research Interchange (NDRI), Philadelphia, PA USA
FSH Society-NDRI Tissue Procurement Project
$265,835 for 3.25 years -- was recommended for one year instead of three; and in addition one-third of the one year at $30,000 contingent on co-funding from other FSHD funding organizations. Fund if other FSHD research non-profits and FSHD Champions will co-fund.

PROJECT SUMMARY (provided by applicant)

In response to a request from the FSH Society, NDRI proposes to develop and implement a resource to recover surgical and post mortem human bio-specimens and distribute them to approved investigators. This resource will utilize NDRI's experience, expertise and established systems to expand and enhance the type, number and quality of human tissues available to the FSH research community. lt is proposed that NDRI's Private Donor Program will collaborate with FSH to recover and distribute tissues from patients who participate in the FSH Registry and who have provided consent for the recovery of tissues and organs for research. In addition to providing all resources required to recover tissues post mortem and from surgical procedures, NDRI will provide informational materials to the FSH Society for distribution to potential registry participants, as well as IRB-approved templates for obtaining informed consent from patients and authorization to donate from family decision makers.

++

Awards for 2015 ad hoc cycle

On March 21, 2015, the Scientific Advisory Board (SAB) of the FSH Society, chaired by David Housman, Ph.D., reviewed a request for second year extension of a research project. The SAB reviewed the grant application and progress report. By March 27, 2015, the FSH Society Board of Directors reviewed and approved the SAB recommendations for funding. Below is a list of the funded projects, including project descriptions as submitted by the applicant(s). For the ad hoc round of grant applications one application was received; one was funded in the amount of $125,000.

+

Michael Kyba, PhD
Lillehei Heart Institute, University of Minnesota, Minneapolis, Minnesota USA
Exploiting genome editing technology to modify and regulate the FSHD disease locus
$125,000 over 1 year
04/01/2015 – 03/30/2016

PROJECT SUMMARY (provided by applicant)

The recent discovery of DNA-binding factors whose sequence specificity is encoded by modular domains that recognize single bases (TALENs) or by a guide RNA (CRISPRs) have opened up tremendous new possibilities in genome editing. With early support from a 2 year ARRA grant, and now with continuing support of an NIH R01, we have developed a zinc finger nuclease (ZFN) that targets 4q35.2. We have used this tool to introduce a new telomere at this site in FSHD iPS cells, which effectively eliminates the genetic lesion. Individuals who lack 4qter on one allele are normal, and our targeted iPS cells that have lost the contracted D4Z4 element are similarly normal. This modification rids the cells of DUX4 mRNA expression and corrects a differentiation defect that we have identified in FSHD iPS cells.

We seek funding from the FSH Society to expand this research program (1) to include FSHD human embryonic stem cells (our NIH grant supports iPS cells), (2) into the exciting new area of CRISPR technology to attempt more specific genetic reversion of the pathogenic 4qA161 allele, and (3) to test the hypothesis that epigenetic modifications can be introduced by targeting D4Z4 with engineered sequence-specific chromatin nucleators.

Aim 1. To correct the FSHD locus in human embryonic stem cells bearing the FSHD mutation. Our work to date has shown that FSHD iPS cells express DUX4 mRNA and suffer from an impaired response to Pax7-induced skeletal muscle differentiation and that these phenotypes are reverted by genetic removal of the contracted D4Z4 array. These iPS cells were derived from myoblasts, therefore there is some question of whether these phenotypes represent an epigenetic memory of the pre-iPS cell type. It will therefore be essential to perform this genetic correction in FSHD human embryonic stem cells.

Aim 2. To design CRISPRs that target existing and novel sites at 4q35.2. The efficiency of targeted integration with our ZNF reagent is low, therefore we will test whether our existing genetic repair method can be made more efficient by CRISPER technology. We will also design and test CRISPERs targeting the pathogenic poly A signal, which may allow correction of the locus without elimination of the entire D4Z4 array.

Aim 3. To use engineered sequence-specific DNA-binding tools to target a chromatin nucleation complex to D4Z4. While most enthusiasm around the discovery of TALENs and CRISPRs has been around their ability to target a nuclease to introduce double strand breaks in DNA, they can also be used to target other proteins to DNA. Because FSHD is caused by inappropriate relaxation of D4Z4 chromatin on the contracted allele, we will attempt to reestablish heterochromatin at this site by fusing an engineered D4Z4- specific DNA-binding domain to proteins involved in nucleating heterochromatin. These studies take advantage of and leverage an existing research program in genome editing of FSHD iPS cells, and will provide the field with valuable new tools to study the pathogenesis of FSHD, and to develop cell therapies based on corrected, isogenic, iPS cells.

Dr. Kyba's project is jointly funded through the Society as a result of collaboration and partnership with The FSHD Canada Foundation, Calgary, Alberta.

(In January 2014, the Scientific Advisory Board (SAB) of the FSH Society, chaired by David Housman, Ph.D., held three sub-reviews co-chaired by members of the SAB and a final SAB review. The SAB along with several ad-hoc expert reviewers reviewed the grant applications and progress reports for the August 2013 round. On February 6, 2014, the FSH Society Board of Directors reviewed and approved the SAB recommendations for Dr. Kyba's initial funding request which was only funded for one of two years pending progress. Subsequently Dr. Kyba requested a second year of continued funding which was approved by the FSH Society's SAB on March 21, 2015 and by its Board of Directors on March 27, 2015.)

++