FSH Society
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Continuing to make progress in understanding and treating FSHD
Grant awards for August 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 fellow ship 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 August 2015 cycle.

Awards for August 2015 Cycle

The SAB reviewed grant applications and progress reports for the August 2015 round on December 15 and 16, 2015, the SAB had two meetings (sub-reviews) under Prof. Housman's

chairmanship and by January 25, 2016, the SAB reached a final ranking and majority consensus. By January 29, 2016, 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 grant applicants. For the August 31, 2015, round of grant applications twelve grant applications were received; eight were funded in the amount of $598,242.

1.

Jocelyn Eidahl [Scott Harper mentor], **Nationwide Children's Hospital**
Protein Chemistry and Protein-Protein Interactions of DUX4
One year extension request.
Amount Requested: US$70,000
Specific Aims

Autosomal dominant Facioscapulohumeral muscular dystrophy (FSHD) is the third most prevalent muscular dystrophy, affecting 1 in 20,000 individuals. FSHD was formally classified as a major form of muscular dystrophy in 1954, but the pathogenic events leading to the disease have only recently started coming into focus. Several studies now support an FSHD pathogenesis model involving aberrant expression of the primate specific DUX4 gene, which encodes a myotoxic transcription factor. The emergence of DUX4 represented a momentum shift in the FSHD field as it provided an important target for therapy design. Indeed, as FSHD is currently untreatable, developing effective therapies is a critical need in the field. An FSHD treatment should center on inhibiting DUX4, which could be accomplished by silencing the gene or transcript, or negating the toxic effects of the DUX4 protein. The overall objective of this study is to identify, characterize, and ultimately inhibit DUX4 protein modifications that may contribute to its toxic properties in FSHD muscle. Delineating how DUX4 protein function is regulated is an important unmet need in the field.

The DUX4 gene encodes a transcription factor that activates downstream toxic pathways, including apoptotic cascades. I hypothesized that post translational modification (PTM) may be one important mechanism affecting DUX4 protein function. PTMs play key roles in ligand binding affinity, subcellular localization and protein stability. My primary goal was to first identify whether DUX4 could be post translationally modified, then subsequently map DUX4 PTMs and determine their contribution to DUX4-induced toxicity. My aims are designed to define the role of DUX4 PTMs, which will allow us to potentially understand its protein function and regulation. By accomplishing this goal, we hope to establish a framework for therapeutic intervention designed to disrupt DUX4 modifications and prevent myotoxicity.

Aim 1: To define DUX4 post-translational modifications
The DUX4 transcription factor is associated with muscle pathology in FSHD and is toxic to numerous other non-muscle cell types. However, some cells and tissues seem to resist DUX4-associated damage, including the testes, where DUX4 is naturally expressed at high levels, as well as muscles of non-manifesting FSHD carriers. The mechanisms by which some cells resist DUX4-associated damage are unknown, but it is likely that DUX4-modifier genes may impact disease penetrance. Since PTMs can profoundly influence transcription factor activity, I hypothesized that the DUX4 protein may also be regulated by PTMs, and the enzymes that mediate these PTMs could therefore impact DUX4 toxicity. In preliminary studies, I found that the DUX4 protein is modified by methylation and phosphorylation using mass

spectrometric analysis of DUX4. In this aim, I will define the PTM signature of DUX4 in numerous cell types, including human myoblasts and primate testes, which endogenously express DUX4. Differences in the modification signature of DUX4 isolated from these cell types will provide insight about tissue-specific regulation of DUX4 protein, and may provide information about the differential toxicity of DUX4 in tissues or cells.

Aim 2: To examine the role of phosphorylation on DUX4 function

My preliminary results revealed phosphorylation of numerous DUX4 residues in both the N and C-terminal domains. In this aim, I propose to examine the effects of each phosphorylation event using mutagenesis to irreversibly mimic or ablate DUX4 phosphorylated residues. I will then determine the effects of these DUX4 mutants in vitro using several outcome measures that I have previously established in my preliminary studies. These include DUX4 DNA binding affinity, assessment of the impacts on key ligand interactions, DUX4 dimerization, cellular toxicity and gene target activation. This work will help establish an important first step toward developing therapies that could prevent DUX4-mediated toxicity, by differentially affecting the phosphorylation status of DUX4.

2.

Daniela Karina Jacquelin [Alberto Rosa mentor], Catholic University of Cordoba (UCC) / National Research Council from Argentina (CONICET)

Study of the coregulatory role of DUX4 on sex hormone nuclear receptors and the protective effect of sex hormones on DUX4-mediated cell toxicity

12/01/2015 to 11/30/2017

$120,000

Our laboratory, together with A. Belayew's laboratory, originally proposed that aberrant expression of DUX4 is harmful to cells, contributing to the pathogenesis of FSHD. We demonstrated that DUX4 is a nuclear protein, endogenously expressed in cultured FSHD myoblasts, pro-apoptotic and cytotoxic when expressed in transfected cells. We recently analyzed the DUX4 molecular domains contributing to its toxicity, subcellular transit and nuclear location. In these studies we recognized an LLXXL motif at the C-terminal region of DUX4, which is present in coregulators of nuclear hormone receptors (NRs). Preliminary studies from our laboratory showed that DUX4 is a coregulator of the progesterone NR. We also found that progesterone protects cultured cells from the toxic effect of DUX4. In this project we will study the role of DUX4 as a coregulator of NRs of sex hormones as well as the protective effect of sex hormones on the toxicity of DUX4. These studies are relevant to the understanding of the normal function of DUX4 as well as its pathogenic role in FSHD and the future rational approaches for the treatment of FSHD patients.

3.

Jeffrey Statland, Kansas University, Kansas City, KS USA

To determine the initial responsiveness to FSHD disease progression of a system of synchronized wireless motion sensors

$39,044 12 Months

Facioscapulohumeral muscular dystrophy (FSHD), one of the most prevalent forms of muscular dystrophy, typically affects muscles of the face, shoulder, and arms early in the disease process but can affect any skeletal muscle over time. Twenty percent of FSHD

patients over the age of 50 require the use of a wheelchair. Since molecular advances have identified potential therapeutic targets for future FSHD clinical trials, there is an urgent need to develop reliable and responsive outcome measures for FSHD treatments. Established FSHD outcome measures for evaluating changes in strength over time, manual muscle testing (MMT) and quantitative myometry (QMT), have been validated in a large natural history study, but fail to demonstrate disease progression in time periods less than one year. And it is unclear what the meaning of a small change in a combined strength score would mean to a patient. Functional tasks would seem to have more inherent meaning to patients, as they measure motor performance during everyday tasks, but are not sensitive to changes in FSHD in less than 3 years. Using such strength or functional outcome measures to show slowing of disease progression in early phase FSHD treatment trials will require large numbers of subjects and long treatment intervals which will significantly hinder the drug development process. This is problematic in a rare disease where access to patients is limited, especially if several therapeutic approaches are being considered. A more sensitive surrogate outcome measure for strength and overall disability status could significantly shorten the duration of early phase clinical trials and hasten the therapeutic discovery process. Measures of dynamic motion while performing functional motor tasks may be more sensitive than isometric strength measures to early changes in muscle function. A prior study using laboratory based motion analysis identified a subset of FSHD patients with abnormal motion parameters despite normal manual muscle testing. Whereas such measurements previously required dedicated motion laboratories, synchronized networks of portable wireless motion sensors make analysis of complex functional movements more accessible and practical in the clinical trial setting.

The long term goal of this research project is to establish a quantitative assessment tool to evaluate changes in mobility status of persons with FSHD. We will use a portable wireless motion analysis system to instrument a timed up and go, postural sway during quiet standing, and arm range of motion. We plan to build on an existing University of Kansas Medical Center Frontier's pilot grant which will identify the specific outcome metrics obtained with portable wireless motion sensors which are important for examination in persons with FSHD and determine the reliability and cross sectional validity of those metrics. In the present FSHD Society study, we propose to extend our current pilot study to add 6 and 12 month follow up visits. We will conduct a 12 month longitudinal study in 20 genetically confirmed and clinically affected FSHD participants (10 mild to moderately affected, and 10 moderate to severely affected) to determine the responsiveness of wireless motion analysis to disease progression in FSHD, determine the minimal detectible change and minimally clinically important change in these metrics, and use factor analysis to create summary scores (e.g. upper extremity, lower extremity) for future clinical trials.

4.

Julie Dumonceaux, Association Institut de myologie

Development of antisense oligonucleotide drugs as a therapeutic agents for FSHD

01/01/2016 to 06/30/2017

Amount Requested: US$94,606

Summary

Antisense oligonucleotides (AOs) are chemically modified single-stranded DNA, RNA or chemical analogue molecules which are able to modulate the expression of a specific targeted

gene. The advances in the development of antisense chemistries, in particular phosphorodiamidate morpholino oligomers (PMOs), have led to numerous studies investigating the therapeutic potential of antisense technology. AO-mediated exon skipping is currently one of the most promising therapeutic options for Duchenne muscular dystrophy (DMD). Importantly, BioMarin and Sarepta have announced that U.S. Food and Drug Administration (FDA) has accepted for review the submission of a New Drug Application (NDA) for drisapersen (2'OMePS PRO051) and eteplirsen (PMO AVI-4658) for the treatment of DMD.

The overall objective of out project is to suppress DUX4 expression and develop a therapeutic approach for FSHD based on AOs. We have chosen to target the 3' key elements of DUX4 mRNA and have already obtained robust results demonstrating the feasibility of such an approach. For the first time, we demonstrated in vitro that targeting a functional PAS can be an efficient therapeutic strategy for a genetic disease. We observed that targeting DUX4 3'key elements leads to an efficient extinction of DUX4, does not redirect polyadenylation and prevents aberrant expression of genes downstream of DUX4.

Our goal is now to (i) improve DUX4 mRNA extinction by developing sequence optimized AONs and to (ii) validate these AOs in vivo. Int the first aim, we will otpimize the sequence and chemistry of AO drugs targeting the 3' key elements of DUX4 mRNA. In the second aim, we will test the body-wide administration of the most active anti-FSHD AO drugs for delivery and effectiveness in animal models after the creation of a new mouse model carrying a reporter gene (LacZ) with the 3'UTR of DUX4 mRNA. Two strategies will be developed: the direct injection of vivo-PMO and the vectorization of the PMO in an AAV vector. In the first case, mice will be subjected to treatment regimens of intravenous systemic delivery of therapeutic optimised AOs in naked form or conjugated to cell-penetrating moieties (eg octaguanidine or CPPs). In the second case, AOs will be vectorized under the control of the U7 promoter as it has been done for exon skipping for DMD for instance. AAVs are now well known to be able to target the muscles in a whole body without toxic effects.

5.

Angela Lek [Louis Kunkel mentor], Boston Children's Hospital

A genome-wide CRISPR knock-out strategy to identify modifiers of FSHD

12/01/2015 to 11/30/2017

Amount Requested: US$156,000

Facioscapulohumeral dystrophy (FSHD) is a common but unique form of muscular dystrophy requiring multiple factors to create a 'permissive' state for disease manifestation. Over recent years, several genetic (DUX4) and epigenetic (hypo-methylation) factors have been linked to FSHD pathogenesis; however, it has become clear that the field has not elucidated all factors required for disease manifestation. Mounting clinical evidence suggests the existence of modifier genes with the capacity to regulate DUX4 transcript and/or protein function. Recent advances in genome-editing technologies proposed for use in this project now should enable us to uncover these remaining missing links. Through the systematic introduction of loss-of-function mutations into genomic DNA, we can interrogate the genome for answers that may explain the phenotypic variability between patients, as well as the non-penetrant effects of DUX4 in some individuals. In this project, we propose a targeted genome-scale knock-out

screen to identify genes that can reduce the phenotypic impact of DUX4 expression when inactivated. We hypothesize that there exists gene targets of DUX4 whose loss will render DUX4 unable to trigger a dysregulated cascade of gene expression, thus abrogating its toxicity. These candidates likely serve as genetic modifiers of FSHD, and will be readily identified by downstream sequencing and computational analysis for detection of CRISPR target genes enriched within these DUX4 'resistant' cell populations. This will allow the generation of a complete list of gene candidates with the potential to influence the pathogenic outcomes associated with DUX4 misexpression. Identified gene hits will be cross-referenced to our whole-genome sequencing data of nonmanifesting carriers to search for sequence variants that may enable us to narrow down promising candidates for functional follow up studies. Validation of candidate modifier genes will be performed in our established zebrafish model of FSHD for rescue of phenotype to confirm functional significance. Additionally, we will revert to our repository of FSHD patient cells to genome edit our candidate genes under these permissive allelic conditions, and subsequently measure changes in known FSHD biomarker expression. FSHD is a challenging disease whose remaining unanswered questions cannot be accomplished alone. Hence, our proposal involves a multi-institute collaboration, bringing together a wealth of patient resources (Wellstone Center), the latest in genomic technology (Broad Institute), and a well-established animal model of FSHD (Boston Children's Hospital). Not only will the identification of these modifier genes for DUX4 resistance provide valuable insights into FSHD disease pathogenesis, but they will also present as solid leads that can be directly targeted for therapeutic intervention in humans with FSHD

6.
Alec DeSimone [Charles Emerson mentor], UMass Medical School
Investigation of 4-methylumbelliferone as a C1QBP-targeting FSHD therapeutic
03/01/2016 to 02/28/2018
Amount Requested: US$150,000
Summary
Development of FSHD has been linked to the de-repression of the DUX4 gene in the skeletal muscle of affected individuals. However, individuals have been identified who express DUX4 in muscle biopsies, but who do not manifest any clinical signs of the disease. Thus, while derepression of DUX4 may be necessary for the FSHD phenotype, it is not sufficient. This suggests that there may be other factors that modify the FSHD disease phenotype. We used a proteomic approach to screen for DUX4-interacting proteins that may act as disease modifiers and identified the multifunctional C1QBP as one such candidate. C1QBP is known to regulate several of the molecular pathways that are affected by DUX4 expression, including gene expression, oxidative stress, and apoptosis. In our preliminary results we have found C1QBP is dynamically regulated in myogenic cells. It is localized primarily to ribbon-like structures outside of the nucleus in myoblasts, but appears to relocate to the nuclear periphery when they are allowed to fuse into myotubes. Expression of DUX4 leads to increased C1QBP concentration in the nucleus, supporting the hypothesis that DUX4 and C1QBP form functional interactions. Importantly, C1QBP is known to bind to the intra- and extra- cellular signaling molecule hyaluronic acid (HA), which can regulate its phosphorylation state. We have found that that decreasing intracellular HA by treating cells with 4- methylumbelliferone (4MU), an inhibitor of HA synthesis, results in a sharp decline in DUX4-target gene

expression. This raises the possibility that it could serve as an FSHD therapeutic. 4MU is of particular interest for development as a drug because it is already being used in Europe to treat biliary dyskinesia, and has had its short-term safety established in several studies. It is also being investigated in both cell culture and animal models as a treatment for specific cancers.

In this project we will evaluate the ability of 4MU to serve as an FSHD therapeutic and investigate its mechanism of action. We have observed that 4MU inhibits the expression of DUX4-target genes, both in myotubes and when DUX4 is overexpressed in myoblasts. We have hypothesized that this is a result of the loss of HA altering the post- translational modifications of C1QBP, which would interfere with its ability to interact with DUX4 and act as a transcriptional cofactor. This could take the form of preventing C1QBP from binding to DUX4 directly, altering the subcellular localization of C1QBP, or by causing changes in C1QBP stability. We will perform studies to evaluate each of these possibilities. Finally, we will use an FSHD mouse xenograft model, established in our lab, to conduct dose-escalation studies to determine if 4MU treatment can inhibit DUX4-target gene expression in vivo. This will better evaluate 4MU as a potential FSHD therapeutic.

7.
Tracy Zhang, Kennedy Krieger Institute
To cover the remaining months of graduate student Yuanfan "Tracy" Zhang in the Wagner lab
12/01/2015 to 04/30/2016
$21,592
Funds are being requested from the FSH Society to cover the remaining months of graduate student Yuanfan "Tracy" Zhang in the Wagner lab. Tracy is a 5th year Cellular and Molecular Medicine graduate student who works exclusively on FSHD. Her thesis work is to establish, validate and use a novel model of FSHD. She established and validated the human skeletal muscle xenograft for FSHD which she published as a first author in Human Molecular Genetics (Zhang et al., Hum Mol Gen 2014, 23: 3180-3188). She is now using the model to show proof-of-concept of antisense oligonucleotide knockdown of DUX4-fl in FSHD. While this work is generally supported by the FSHD Wellstone at UMMS, Tracy is no longer supported by the Wellstone and funds are being requested to cover her salary and benefits to finish this project.

8.
Scott Harper, Nationwide Children's Hospital
Characterization of a Tamoxifen inducible DUX4 knockin mouse
3-6 month bridge funding
Amount Requested: US$25,000
Specific Aims
We will submit formal grant applications to foundations, including MDA and the FSH Society, and are considering seeking some industry funding. However, this will take time (several months), and we no longer have discretionary funds to support the mouse colony. We want to expand, characterize, and publish this model as soon as possible, and we are seeking bridge funding for this purpose. It is our goal and priority to make this model available to anyone in the field who w ants it, as soon as is practicable.

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