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Continuing to make progress in understanding and treating FSHD
Grant awards for August 2018 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.

For the August 2018 round of grant applications, we received 6 applications. On December 19, 2018 the FSH Society's Scientific Advisory Board (SAB) met to review grant applications for the August 2018 round of applications. The SAB made recommendations, gave guidance and indicated if additional information was needed or if action needed to be taken. The SAB gave a ranking by majority consensus. By January 23, 2019, the FSH Society Board of Directors reviewed and approved the FSH Society's SAB, the Society's Science, Technology and Research (STaR), and, Finance Committees' recommendations for funding. For the August 2018 round of grant applications, we received six applications (four new, one resubmission, one request for extension). Three were awarded; three were rejected. Three were funded in the amount of US\$332,906. Below is a list of the funded projects, including project description as submitted by the applicant. We are very pleased to list the projects and grantees funded in the August 2018 cycle.

August 2018 Cycle

Investigating the molecular consequences of reduced NMD in FSHD skeletal muscle myoblasts

Michael Dyle, PhD

Laboratory of Sujatha Jagannathan, PhD, University of Colorado Denver | Anschutz Medical Campus

01/01/2019 – 12/31/2020

\$ 115,937 for 2 years

FSHS-82018-01

Project Summary [as submitted on application]

Facioscapulohumeral muscular dystrophy (FSHD) is a progressive neuromuscular disease that diminishes the quality of life for hundreds of thousands of people throughout the world. Current evidence indicates that FSHD is caused by mis-expression of the DUX4 transcription factor in skeletal muscle fibers, which leads to skeletal muscle cell death and weakness. One important consequence of skeletal muscle DUX4 expression is the downregulation of a conserved RNA quality control pathway, called nonsense mediated RNA decay (NMD). Compromised NMD in FSHD skeletal muscle cells results in increased levels of aberrant mRNAs that contain premature translation termination codons (PTCs) and endogenous mRNAs that encode stress-inducible, pro-apoptotic factors. In the studies proposed here, we will 1) determine whether PTC-containing mRNAs are translated to potentially deleterious truncated proteins, and 2) whether rescuing NMD function is capable of preventing cell death by suppressing levels of pro-apoptotic factors. Thus, the studies proposed here will further elucidate the mechanisms by which reduced RNA quality control contributes to skeletal muscle deterioration in FSHD. Importantly, these studies may inform the development of novel biomarkers or pharmacologic therapies in FSHD.

Significance: A project proposed by a young promising post-doctoral fellow focused on providing insight into the molecular underpinnings of FSHD, which may lead to the development of novel diagnostic biomarkers and therapeutic strategies. Done by determining the consequences of diminished RNA quality control in FSHD skeletal muscle. Previous studies by this lab have found that DUX4 expression in skeletal muscle leads to severe perturbation of an evolutionarily conserved RNA quality control pathway: nonsense mediated decay (NMD). In healthy skeletal muscle, NMD plays a beneficial role in surveying and eliminating aberrant RNA molecules, as well as suppressing levels of stress response proteins that can cause cell death. Their preliminary data indicate that perturbed NMD in FSHD skeletal muscle leads to increased levels of aberrant RNAs, hyperactivation of cell stress response pathways, and muscle cell death. These findings reveal that diminished RNA quality control is a pivotal event that contributes to skeletal muscle deterioration in FSHD. Project might help clarify if rescuing NMD function can slow or prevent skeletal muscle deterioration in FSHD.

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2. Optimizing gapmer therapy for facioscapulohumeral muscular dystrophy

Yi-Wen Chen, DVM, PhD

Children's National Health System, Washington, DC

03/01/2019 – 02/29/2020

US\$ 125,969 for one year

FSHS-82018-02

Project Summary [as submitted on application]

Antisense oligonucleotide (AON) therapy shows promise for treating an array of disorders, however, several issues associated with AONs affect its applications, including 1) difficult in systemic drug delivery because these AONs could not easily cross the lipid bilayer of cells; 2) harmful off-target effects and toxicities; 3) low stability due to degradation by intracellular and extracellular nucleases.; and 4) immune

responses via toll-like receptors. The 2'-O-methoxyethyl (2'MOE) and locked nucleic acids (LNAs) modification are two widely used chemistries for designing gapmer-type antisense oligonucleotides, which overcome many of the issues associated with AONs. LNAs have the 2',4'-methylene bridge and 2'MOEs have a simple methoxyethyl substituent attached to the 2' oxygen. These modifications have been shown to enhance target binding affinity, specificity, and resistance to degradation by nucleases. The LNA gapmers provide a stronger affinity in comparison to many other modifications; therefore it is possible to design shorter gapmers for the same efficacy. This design will also increase the uptake by gymnosperms in the absence of any carriers or conjugation. Compared to LNA gapmers, 2'MOE gapmers may be less potent; however, 2'MOE gapmers are likely to be safer than LNA gapmers due to less off target effect (the 2'MOE gapmers are slightly longer than the LNA gapmers). Considering the pros and cons, we propose to examine and compare the LNA and 2'MOE gapmers that target the same region of the DUX4. We have designed three 2'MOE gapmers and conducted in vitro studies. We showed superior knockdown efficiency in vitro when tested in immortalized FSHD myoblasts. Given the properties mentioned above and their proven track record in the clinic, it would be favourable to design 2'MOE gapmers for FSHD treatment if the 2'MOE gapmers have a similar potency in vivo and less toxicity. In the proposed studies, we will study the efficacy and safety of the 2'MOE gapmers in vivo and compare to the LNA gapmers. We are currently characterizing the LNA gapmers using both in vitro and in vivo model, which is supported by the FSH society. In this proposal, we would like to request support for purchasing 2'MOE gapmers and conduct in vivo efficacy studies, the data will be compared with the data from our LNA studies. The findings will allow us identify the lead compound for treatment development for FSHD. In the Aim 1, we will systemically delivery the 2'MOE gapmers to the FLEXDUX4 mice and determine the efficacy of the treatments. In Aim 2, we will evaluate off-targets, immunogenicity and toxicities and compare the data to the LNA gapmer data. The proposed studies will allow prioritizing and identify the most promising gapmers for drug development.

Significance: Antisense oligonucleotide (AON) therapy shows promise for treating FSHD, however, several issues arise with AONs including 1) difficulty in systemic drug delivery; 2) harmful off-target effects and toxicities; 3) low stability due to degradation; and 4) immune responses. The 2'-O-methoxyethyl (2'MOE) and locked nucleic acids (LNAs) modification are two chemistries for designing gapmer-type antisense oligonucleotides, which overcome issues associated with AONs. Previous support by the FSH Society helped this lab in developing an effective antisense oligonucleotide (AON) strategy to target DUX4 and reduce its expression. The LNA gapmer under investigation was able to effectively knockdown DUX4 both in cell culture and mice. In the FSHD mouse model generated by Dr. Peter Jones' group, Chen showed functional recovery of muscle strength after systemic delivery of the LNA gapmers. In this proposal, they will compare the LNA gapmers to 2'MOE gapmers which are targeting the same target sequences of the DUX4. The 2'MOE was recently approved for treating spinal muscular atrophy by the FDA. It is considered less potent but safer than LNA. In an in vitro experiment done by collaborator, Dr Yokota, he showed that the 2'MOE gapmers targeting the same DUX4 region effectively knocked down the DUX4 transcripts. In this proposal Chen will compare the in vivo efficacy and the safety of the 2'MOE gapmers to the LNA gapmers. The goal is to carefully characterize and identify the compound that will be moved forward for drug development.

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3. Identification of natural human DUX4-targeted miRNAs and development of a novel DUX4-targeted miRNA-based gene therapy for FSHD

Nizar Y. Saad, PhD

Postdoctoral fellow, the Harper lab

Center for Gene Therapy, Nationwide Children's Hospital

03/01/2019 – 02/29/2020

\$ 91,000 for one year

Project Summary [as submitted on application]

FSHD presentation is non-uniform, and there may be extreme variability in severity of symptoms, rate of progression and age at onset, even in families with several affected relatives. Similarly, asymmetrical weakness is common. It has been hypothesized that this non-uniformity of presentation might be due to the regulation of DUX4 expression by yet undetermined factors. Although some of the genes that modify DUX4 gene expression are already known (e.g., SMCHD1, DNMT3B), overall the regulation of DUX4 gene expression is still relatively unclear, and genes that directly target DUX4 mRNA have not been identified. We think that DUX4 gene expression modifiers might influence DUX4 toxicity and FSHD disease penetrance. In previous proposals to the FSH Society, our central hypothesis was that some endogenous microRNAs (miRNAs) could target the DUX4 transcript, thereby reducing DUX4 expression and toxicity. During the past two years, we have been investigating this hypothesis. In particular, we have been investigating the action of a long non-coding RNA (H19) and its miRNA by-product (miR-675) against DUX4. So far, my recent investigation provides the first proof for H19 and miR-675 reducing DUX4 expression and toxicity, which paves the way to develop new therapeutic approaches by targeting or using natural miRNAs such as miR-675. More specifically, my aim here is to expand our pipeline of DUX4-targeted miRNA-based gene therapy for FSHD by using miR-675 as a new miRNA-based gene therapy candidate. This proof-of-principle also supports the identification of the full set of natural DUX4-targeted miRNAs that would represent a set of potential miRNA therapeutics or drug targets. Our project has two aims. The first aim focuses on identifying the full set of natural miRNA that could target DUX4. In this aim, we would ideally like to tie one or multiple natural miRNAs into FSHD disease progression, but even if we are unable to find evidence for miRNAs acting as DUX4 modifiers, we propose they could still be used as potential therapeutics. The second aim focuses on performing a pilot study to develop a DUX4-targeted miR-675-based gene therapy for FSHD.

Specific Aim 1: To functionally identify every natural human miRNA capable of targeting DUX4 in vitro.
Specific Aim 2: To develop a DUX4-targeted miR-675-based gene therapy for FSHD.

Significance: Dr. Saad has with FSH Society funding for past two years been investigating endogenous microRNAs (miRNAs) that could target the DUX4 transcript, thereby reducing DUX4 expression. So far, he found that H19 and miR-675 reduce DUX4 expression and toxicity. Drs. Harper/Saad seek to develop new therapeutic approaches by targeting or using natural miRNAs such as miR-675. Project aims to create DUX4-targeted miRNA-based gene therapy for FSHD by using miR-675 as a new miRNA-based gene therapy candidate. First by functionally identify every natural human miRNA capable of targeting DUX4 in cell culture. Then by developing a DUX4-targeted miR-675-based gene therapy for FSHD.

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