

CONTACT:
FSH Society
Daniel Perez, CSO, PCo-founder FSH Society
450 Bedford Street, Lexington, Massachusetts 02420 USA
t: (781) 301-6650
e: daniel.perez@fshsociety.org



Continuing to make progress in understanding and treating FSHD

Grant awards for February 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 February 2018 round of grant applications, we received 11 applications. On May 24th and June 18th the FSH Society's Scientific Advisory Board (SAB) met to review grant applications for the February 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 September 25th, 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 February 2018 round of grant applications, we received eleven applications (seven new, three resubmission). Three were awarded; eight were rejected. Three were funded in the amount of US\$388,445. 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 February 2018 cycle.

February 2018 Cycle

1. Determining the therapeutic potential of pluripotent stem cell-derived myogenic progenitors in the iDUX4pA mouse model

Rita Perlingeiro, PhD

University of Minnesota, Minneapolis, Minnesota

\$99,998 total (\$49,999 annually x 2)

FSHS-22018-01

Project Summary [as submitted on application]

Facioscapulohumeral muscular dystrophy (FSHD) a genetically dominant progressive muscular dystrophy associated with derepression of the DUX4 gene. A major roadblock to FSHD basic research and therapeutic testing has been the lack of a suitable animal model, however the newly developed iDUX4pA mouse, which shows DUX4 expression and deterioration of skeletal muscle fibers, has now made possible the testing of therapies in an animal model. Here, we propose to study cell therapy in this model, using pluripotent stem cell-derived myogenic progenitors, delivered both locally into the tibialis anterior muscle, as well as systemically, through the circulation. These studies will establish the feasibility of cell therapy in FSHD.

Significance of this work for FSHD patients. There has been tremendous excitement for the therapeutic potential of skin reprogrammed induced pluripotent stem (iPS) cells in treating genetic diseases, such as FSHD. This grant application builds on Dr. Rita Perlingeiro's successful studies developing such cell therapies specifically in mouse models of Duchenne DMD and limb-girdle LGMD muscular dystrophy. Based on Dr. Rita Perlingeiro's extensive proof-of-concept findings, they are currently finalizing the optimization of manufacturing these cells under cGMP medical-grade conditions and performing preclinical studies for their future testing in a phase 1 safety trial. They have had a pre-IND [FDA investigational new drug] meeting (March 27th, 2017) and their goals are in line with the FDA.

The intent of this cell product is to replace diseased muscle with normal functional muscle fibers as well as muscle stem cells, which have the potential to provide long-term therapeutic effect in DMD, as well as other devastating types of muscular dystrophies, including FSHD. Because all of Dr. Rita Perlingeiro's work to date has been with recessive models involving membrane defects (mutations in the dystrophin glycoprotein complex, DGC, members, like DMD) it will be essential to understand how effectively cell replacement can address muscle damage due to the distinct mechanism underlying FSHD. Now that a mouse is available that can be induced to provide very low levels of DUX4, resulting in a slow decline in muscle over several months, it will be possible to evaluate the effectiveness of cell therapy in the context of such a relevant muscle damage mechanism. Because Dr. Rita Perlingeiro's preclinical work to date has been limited to DGC mutant mouse models, her current discussions with the FDA have included only DGC mutant diseases in their clinical plans. The work proposed in this grant will provide proof-of-principle for including FSHD in the pipeline for future clinical trials.

+

2. A decoy trapping DUX4 for the treatment of facioscapulohumeral muscular dystrophy

Virginie Mariot, PhD

UCL Great Ormond Street Institute of Child Health, London, United Kingdom

\$163,447 for 18 months

FSHS-22018-02

Project Summary [as submitted on application]

FSHD is one of the most common muscular dystrophies and so far there is no curative or preventive treatment. FSHD is characterized by a loss of repressive epigenetic marks within the D4Z4 array, leading to

chromatin relaxation and, when associated with a permissive chromosome 4, to the expression of the normally silenced DUX4 protein whose ORF is present in each D4Z4 repeat.

FSHD has the unique characteristic among muscular dystrophies to aberrantly express the DUX4 transcription factor which binds genomic DNA, inducing the mis-regulation of hundreds of genes. We took advantages of this specificity to develop an original therapeutic approach, based on a DNA decoy trapping the DUX4 protein, preventing its binding to genomic DNA and thereby blocking the aberrant activation of DUX4's transcriptional network.

The proof of principle for this therapeutic strategy has been already performed in vitro and in vivo. The aim of our grant is now to validate these results in the FLE_x ACTA MCM mice.

Why this work is significant and vitally important. Few laboratories (including Drs. Mariot's and Dumonceaux's) have proposed therapeutic approaches for FSHD but no one can predict whether these approaches will be successful in the Human. It is therefore important to continue to develop new strategies. This application uses a "decoy" approach, which represents a new way of thinking, a new conceptual approach in the neuromuscular field. Unlike antisense oligonucleotides (ASO/AO) or siRNA which target DUX4 messenger (mRNA) prior to the creation of the DUX4 protein, the decoy mechanism of action is to trap the DUX4 protein post-RNA translation. It is therefore independent of the nucleus that produces DUX4 mRNA (which can be one out of 1000 nuclei), but the decoy will sequester the DUX4 protein during its cellular journey wherever it occurs. This decoy strategy may be highly powerful as shown by the preliminary data.

+

3. The Role of Estrogen Receptors in FSHD-1 Mechanism

Anna Pakula, PhD

Boston Children's Hospital, Boston, Massachusetts

\$125,000 for one year

FSHS-22018-03

Project Summary [as submitted on application]

Facioscapulohumeral muscular dystrophy-1 (FSHD-1) is the most prevalent muscular dystrophy affecting 1:8000, both children and adults. FSHD is progressive and leads to death of skeletal muscle cells in the facial, scapular, trunk and lower extremities muscles, resulting in muscle weakening, decrease of the lifespan and quality of life. The disease is caused by partial deletion of 4q35 D4Z4 subtelomeric repeat that causes the activation of the DUX4 locus. DUX4 encodes a homeodomain transcription factor (TF) that is expressed in gonads and in early embryonic stages but then is silenced. Aberrant expression of DUX4 during later stages of embryo development causes gene misregulation and manifestation of symptoms. Zebrafish (*Danio rerio*) is a very instrumental vertebrate model for studying genetic diseases, since it allows the performance of biochemical treatments and protein overexpression in early stages of development. In our lab, we have developed an injection zebrafish model of FSHD by overexpressing DUX4 in early stage embryos. These fish embryos developed phenotypic features that resemble FSHD manifestation in humans (Mitsuhashi, 2013). This zebrafish model could be used for further studies of the molecular mechanisms of the disease as well as screens for potential therapeutic molecules. This study is predicated on the several clinical observations of sex differences of manifestation of FSHD and our ChIP-seq preliminary data: in men, the symptoms are more severe than in women. A recent study (Teveroni, 2017) had provided a hint for the mechanism of this effect: Dux4 functioning is influenced by estrogen signaling. However, the detailed mechanism of this influence remains to be elucidated. In current study, we will analyze the role of estrogen signaling in the FSHD phenotype development in the zebrafish disease model. We aim to find the mechanism of this influence and to confirm the hypothesis, based on our preliminary ChIP-Seq results for zebrafish misexpression of DUX4, that estrogen receptorlike protein is

recruiting DUX4 to certain target sites and that its absence or inhibition causes DUX4 to occupy other sites that lead to a more severe phenotype.

Significance of this work for FSHD patients. Recently there has been significant effort put into characterizing FSHD on the genetic level. FSHD individuals with shorter D4Z4 repeats are reported to be more severely affected, however there is still an unsolved conundrum on different disease manifestation in women and men. Sexual dimorphism in FSHD has been studied among American, Brazilian, Italian and Dutch FSHD patients. Clinical (e.g. MRI) and neurological data revealed that in these populations men manifest the disease earlier in their life and are more severely affected than women. The underlying mechanism explaining these noticeable sex differences in disease severity remains yet unsolved and will be the goal of these studies.

In the Pakula/Kunkel lab they have generated a DUX4 zebrafish FSHD1 model (by injecting a very small amount of DUX4 mRNA into zebrafish embryos) that mirrors the FSHD. By performing analysis of DUX4 binding sites they have discovered that, at 12 hours of embryo development, Estrogen Receptor-like (ER-like) interacts with DUX4. The advantage of their model is that DUX4 and ER interaction can be detected at the very early stages of disease development, which is not feasible in human. By investigating that interaction further, they aim to understand in more details the disease initiation and progression.

Why this proposal is important -- using the zebrafish model they would like to unravel genetic mechanism of hormone involvement on the disease severity at early stages of disease development which they believe may help to discover further treatment for FSHD. This will help further help us understand the different disease protective factors and possible therapeutic avenues in women and men.

+

++