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Continuing to make progress in understanding and treating FSHD
Grant awards for August 2017 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.

On October 16 and November 20, 2017, the Scientific Advisory Board (SAB) of the FSH Society, chaired by David Housman, Ph.D., held its biannual review of grant applications. The SAB reviewed new grant applications, resubmitted grant applications, applications requesting continuations for the August 2017 round and progress reports. By December 19, 2017, 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. Below is a list of the funded projects, including project description as submitted by the applicant. For the August 31, 2017 round of grant applications, we received eleven applications (nine new, one resubmission) and one request for one-year extension on ongoing research projects. Six were awarded; five were rejected. Six were funded in the amount of US$690,894.

We are very pleased to list the projects and grantees funded in the August 2017 cycle.

August 2017 Cycle

1. Natural microRNAs as potential modifiers of DUX4 toxicity
Nizar Saad, Ph.D.
The Research Institute at Nationwide Children's Hospital, Columbus, Ohio USA
02/01/2018 - 01/31/2019
US$80,000 for 1 year
FSHS-82017-01 (a continuation of FSH Society Grant FSHS-22016-04)

Project Summary

Facioscapulohumeral dystrophy (FSHD) is a complicated disorder. After many decades of study, the FSHD research field now has focused on mis-expression of the DUX4 as a primary insult underlying the disease. DUX4 is toxic to muscle and numerous non-muscle cell types. FSHD symptoms are often variable from person to person, and there may be also variability in severity of symptoms, rate of progression and age at onset, even in families with several affected relatives. Asymmetry is often seen, where a person may have more muscle weakness on one side of the body versus the other. Although DUX4 is toxic, some cells and tissues seem to resist its damaging effects. We hypothesize that FSHD variability and the differential toxicity of DUX4 are linked; it is possible that the toxic effects of DUX4 may be reduced in cells or muscles that are spared in FSHD. However, the mechanisms by which some cells might resist DUX4 damage are unclear. In this proposal, I will investigate my hypothesis that natural microRNAs – which are produced normally in all human cells and help activate natural cellular gene silencing pathways - could reduce DUX4 expression, reduce its toxicity, and potentially slow FSHD progression. In Aim 1, I will continue my investigation of a single miRNA that we identified in a limited candidate screen. This miRNA binds the DUX4 transcript and reduces its translation into DUX4 protein, thereby decreasing its toxicity in cultured cells. This aim represents a proof-of-principle for our second aim, which is focused on identifying the full set of natural miRNAs (humans have 1,881 different ones known to date) that could potentially target DUX4. 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 natural FSHD modifiers, we propose they could still be used as potential therapeutics if they are capable of binding and reducing DUX4. Specifically, some drugs are known to increase the expression of specific natural microRNAs; thus it may be possible to use drugs to increase the expression of DUX4-targeted miRNAs, thereby reducing the expression of DUX4 so it is no longer toxic.

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2. Hypermorphic SMCHD1 variants
Jessica C. de Greef, Ph.D.
Leiden University Medical Center, Leiden, The Netherlands
07/01/2018 – 06/30/2021
US$171,000 for 3 years (US$ 57,000 per year)
FSHS-82017-02

Project Summary

The D4Z4 repeat array has heterochromatic features in most somatic tissues. As a result, probably due to repeat-mediated epigenetic repression, the transcription factor DUX4 is not, or rarely, expressed in somatic tissues. Individuals with facioscapulohumeral muscular dystrophy (FSHD) present with a partial failure of the epigenetic repression of the D4Z4 repeat array, resulting in DUX4 expression in a subset of muscle nuclei. This failure in epigenetic repression can be caused by contraction of the D4Z4 repeat array to 1-10 units (FSHD1) or by heterozygous mutations in the chromatin modifiers SMCHD1 and DNMT3B (FSHD2). These chromatin modifiers are necessary to establish or maintain the repressed chromatin structure of the D4Z4 repeat array in somatic cells. Our group previously generated a transgenic mouse model carrying a D4Z4 repeat array of 2.5 repeat units. These D4Z4-2.5 mice are a faithful model for some

features of FSHD1 since these mice also fail to epigenetically repress DUX4 in somatic cells, leading to the presence of DUX4 protein in sporadic myonuclei.
SMCHD1 encodes a well-conserved protein, but its function is largely unknown. Studies in mice suggest that Smchd1 has roles in the establishment and/or maintenance of DNA methylation, in X chromosome inactivation, and in the regulation of several imprinted and clustered genes. Our group has an ongoing collaboration with Dr. Marnie Blewitt (The Walter and Eliza Hall Institute of Medical Research, Australia), who was involved in an N-ethyl-N-nitrosourea (ENU) mutagenesis screen to identify modifiers of epigenetic reprogramming. Apart from the well-known Smchd1 loss-of-function mutant Smchd1^{MommeD1}, she identified a missense Smchd1 variant, which we now call the Smchd1^{Fresia} variant, which may act as a hypermorphic variant. This is an exciting finding as it suggests that naturally occurring SMCHD1 variants might exist that protect muscle from expressing DUX4.
In this project I will test the hypothesis that specific SMCHD1 variants either increase SMCHD1 activity or lead to increased SMCHD1 expression with consequences for the chromatin structure of the D4Z4 repeat array and for DUX4 expression. In Specific Aim 1, I will determine the functional consequences of the Smchd1^{Fresia} variant at the chromatin and expression level of the D4Z4 repeat array *in vivo* using our transgenic D4Z4- 2.5 mice. In Specific Aim 2, I will determine the effect of the Smchd1^{Fresia} variant and five SMCHD1 variants that may act as hypermorphic alleles in muscle cell cultures. In Specific Aim 3, I will search for novel potential hypermorphic SMCHD1 variants in our extensive and well characterized biorepository.

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3. Stryka-001 treatment in the FSHD-like mouse model
Ryan Wuebbles, Ph.D.
Takako Jones, Ph.D.
University of Nevada, Reno School of Medicine, Reno, Nevada USA
01/01/2018 - 12/3l/2018
US$$190,000 for 1 year
FSHS-82017-03

Project Summary
Facioscapulohumeral Dystrophy (FSHD) is a human specific dominant genetic disease caused by the contraction of a D4Z4 repeat array at chromosome 4q35 and a permissive epigenetic environment. These genetic and epigenetic circumstances lead to muscular dystrophy in patients with highly variable rates of muscle group penetration and progression. Recent advances in the FSHD field have shown that each D4Z4 repeat contains a gene called DUX4, and that only the most distal repeat in the contracted array is capable of producing translatable pathogenic transcripts, called DUX4-fl. Recently, Dr. Peter Jones was able to generate the first viable and fertile line of inducible DUX4-fl transgenic mice, referred to as FLExDux4+/-; ACTA-MCM+/-. This mouse model is now being leveraged by us and other labs to examine novel drugs that may have efficacy for FSHD patients. In Dr. Dean Burkin's lab we have previously performed a large-scale drug screen to identify small molecule enhancers of ITGA7, the gene encoding alpha7 lntegrin. In studying these "hit" compounds in other muscular dystrophy mouse models, we observed that a select agent gave us a large improvement in muscle regeneration along with the expected increase in alpha7 lntegrin. This regeneration occurred in the absence of telomere length shortening. We have gone on to perform preliminary treatments in the FLExDux4+/-; ACTA-MCM+/- mouse model, and while we see no decrease in DUX4-fl target gene expression or activity, we do find a large increase in ex vivo muscle force production. We hypothesize that Stryka-001 treatment of the tamoxifen treated FLExDux4+/-:ACTA-MCM+/- FSHD-like mouse model will improve muscle regeneration and recovery after DUX4-fl induced muscle insult. If successful, this technology will have immediate treatment implications for FSHD patients and will be extremely useful in combination with other upcoming therapeutic interventions targeting DUX4-fl.

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4. Characterization of a novel inhibitor of DUX4 activity
Davide Gabellini, Ph.D.
Fondazione Centro San Raffaele, Milan, Italy
02/01/2018 - 01/31/2019
US$85,000 for 1 year
FSHS-82017-04

Project Summary

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most prevalent neuromuscular disorders. Due to incomplete understanding of its molecular pathogenesis, no treatment is currently available. The disease is caused by aberrant expression of the double homeobox 4 (DUX4) gene encoding for a transcription activator normally silent in skeletal muscle. In FSHD, ectopic DUX4 expression activates a pro-apoptotic transcriptional program leading to muscle cell loss and degeneration. While blocking DUX4-induced toxicity would be a plausible therapeutic option, the mechanism through which DUX4 triggers cell death is poorly understood and no regulator of DUX4 activity is currently known. Hence, the identification of factors able to block DUX4-activated toxicity is crucial when considering future drug design.

We identified a novel molecule able to block DUX4 activity.

We plan to address the following questions:

1. Which are the molecular determinants of DUX4-inhibitor interaction?
2. Can the inhibitor be used for therapeutic purposes?

Our project will provide a better understanding of DUX4 mechanism of action and how its toxic activity could be blocked for the treatment of FSHD.

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5. Interplay between myogenesis and the immune system in FSHD pathology
Peter Steven Zammit, Ph.D.
Maryna Panamarova, Ph.D.
King’s College London, London, England
04/08/2018 – 04/07/2019
US$99,894 for 1 year
FSHS-82017-05

Project Summary

Facioscapulohumeral muscular dystrophy (FSHD) is a common muscle wasting diseases, caused by a combination of genetic and epigenetic abnormalities in the D4Z4 marcosatellite repeat array in the subtelomere of chromosome 4 at 4q35. The most common form, FSHD1, is linked to contraction of D4Z4 array from the 11-100 repeats in unaffected individuals, to less than 10. In the other 5% of cases (FSHD2), the D4Z4 region is un-contracted. Both forms are associated with epigenetic changes to the region such as DNA hypomethylation and loss of heterochromatic histone marks, which renders the region permissive to transcription. If such a hypomethylated D4Z4 array is present on a permissive 4qA allele supplying a polyA signal, a stabilised transcript from the terminal D4Z4 repeat for a transcription factor called DUX4 is made. When ectopically expressed in skeletal muscle, DUX4 disrupts the transcriptional networks of muscle cells and has a cytotoxic effect. However, molecular drivers of FSHD pathology remain poorly understood.

Upon injury, healthy muscle, in cooperation with the immune system, activates a complex repair program that involves activation of muscle-progenitor (satellite) cells that proliferate and differentiate to repair damage. These processes in FSHD are mis-regulated, which leads to an abnormal inflammatory response, ineffective repair and myofibre atrophy. Understanding the failure of FSHD muscle to activate effectively the muscle repair program could be important in developing novel therapeutic strategies.

In work partially funded by the FSH Society Shack Family and Friends research grant FSHS-82013-06), we have recently completed an extensive RNA-seq transcriptomics analysis of myogenic differentiation of immortalised and primary myoblasts isolated from FSHD patients alongside matched controls (Banerji C.R.S, Panamarova M., Hebaishi H., White R.B., Relaix F., Severini S. and Zammit P.S. (2017). PAX7 target genes are globally repressed in FSHD skeletal muscle. Nature Communications 8: 2152 (10.1038/s41467-017-01200-4). Multivariate regression analysis revealed 180 genes strongly associated with FSHD in every dataset analysed. Gene Set Enrichment analysis of these 180 genes revealed that the target genes of a transcription factor central to macrophage-coordinated muscle repair were significantly repressed in all FSHD cell lines. However, the effects of suppression of this transcription factor on muscle repair in FSHD, is currently unknown.

This research aims to determine the role of this transcription factor in FSHD, which could help augment muscle repair in FSHD to ameliorate muscle wasting. An overarching aim of this project is to better understand the interplay between muscle repair and the immune system in FSHD.

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6. Biomarker identification by high-resolution proteomic approach in facioscapulohumeral muscular dystrophy (FSHD)
Giorgio Tasca, M.D., Ph.D.
Università Cattolica del Sacro Cuore, Rome, Italy
05/03/2018 – 04/30/2019
US$65,000 for 1 year
FSHS-82017-06

Project Summary

To move forward in clinical trial readiness in FSHD, the identification of biomarkers of activity and progression is required to help assessing the efficacy of a treatment in a slowly progressing disease. Selective and targeted approaches are advisable in this disorder, and a correlation of molecular findings with other measures of disease activity and progression is needed to reduce the variability of the results. We developed an original approach that combines muscle imaging, microdialysis and proteomic analysis to identify and track the pathological processes taking place in single muscles. This approach, which consists in the proteomic analysis of interstitial fluid obtained from muscles with different MRI features (i.e., normal muscles vs. muscles showing signs of early involvement) in the same FSHD patients and controls, allows the contextualization of the molecular results in the frame of the comprehensive and sensitive assessment provided by MRI. Preliminary evidences on already collected samples support the feasibility of the analysis. After the discovery phase, we also plan to develop a sensitive and robust proteomic workflow to verify and enhance sensitivity of detection and quantification of proteins/peptides. Accurate masses of targeted peptides from proteins that were differentially expressed in the microdialysates will be analyzed in high-resolution LC-PRM (Liquid Chromatography-Parallel Reaction Monitoring)-mass spectrometry (MS) analysis mode, which is considered the method of choice for the verification step in biomarker discovery using MS. Proteomic protocols will be developed and tested for biomarker discovery also in serum of the same FSHD patients that underwent microdialysis.

The results of our study could provide information valuable for the discovery and characterization of novel tissue and circulating biomarkers with a comprehensive approach, as well as preliminary evidence for the application of an innovative technique in FSHD and potentially other neuromuscular disorders. Getting further insights into disease pathophysiology through the identification of biochemical pathways dysregulated in FSHD muscles could help in the development of new targeted therapies.

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