

Awards for August 2010 Cycle

The Scientific Advisory Board (SAB) met on November 22, 2010, to review grant applications received for the August 2010 round of FSH Society grants funding. Below are: 1.) a listing of the funded projects by grant applicants along with project descriptions as submitted by grant applicants.

1. "Small Molecule Screen to Identify Inhibitors of DUX4-mediated Toxicity, Therapeutic Approach for FSHD

Darko Bosnakovski, D.V.M., Ph.D.

University "Goce Delcev" Stip

Faculty of Medical Sciences

Krste Misirkov bb, 2000 Stip

R. MACEDONIA

\$90,000 over 2 years

PROJECT SUMMARY

We and others have shown that DUX4 is toxic to different cell types, and induces FSHD-associated morphological and transcriptional changes in vitro. As a first step towards developing a targeted therapy for FSHD, we have taken advantage of conditional toxicity of DUX4-inducible myoblasts and we developed a small molecule screening platform for identifying inhibitors of DUX4. In our iC2C12-DUX4 inducible myoblasts, we incorporated full length of the last D4Z4 repeat, so prior its induction, we can not exclude that besides DUX4, some other products are not expressed (RNA, spliced proteins). Assay based on rapid cell death within 24 hours induced by high levels of DUX4 was used for high throughput screen of 200,000 chemicals, part of UT Southwestern HTS compound library. We identified more than 586 compounds with significant rescue ability (60 to >100% cell survival). To identify direct inhibitors, we have conducted serial follow up assays, including secondary screens to eliminate compounds which interfere with the rtTA/TRE inducible gene expression system, to distinguish anti oxidants, to confirm reversion of toxicity in other DUX4-expressing cell types. Several classes of compounds reverted toxicity indirectly, including antioxidants. After these secondary screens, we have narrowed down the list to 82 potentially direct DUX4 inhibitors. The goal of this proposal is to discover a chemical compound/s which efficiently inactivates the DUX4 protein and build on that discovery to develop a drug for a therapeutic approach to FSHD. To achieve this we will have to filter our current list (82 compounds) with additional secondary screens. Among them will be an analysis of MyoD expression and stability as well as cellular localization of the DUX4 protein (Aim 1). We reported that DUX4 is a potent inhibitor of MyoD expression. Therefore, a compound that will rescue MyoD expression in DUX4 induced cells is likely to be a therapeutically effective DUX4 inactivator. We assume that compounds which will be able to inactivate DUX4 in our iC2C12-DUX4 system most likely will be able to rescue FSHD myoblast phenotype. FSHD myoblasts were reported to have impaired differentiation, misregulation of myogenic transcription factors and increased susceptibility to oxidative stress. For that reason, as a functional in vitro study, we will test selected compounds for reversion of FSHD myoblast phenotype (Aim 2). Furthermore, we will test whether selected compounds exhibit their effect on inactivation of DUX4 protein or inhibition of DUX4 transcription or translation (Aim 2). At the end the most potent compound /s will be test for pharmacokinetic and pharmacodynamic properties (Aim 3). The aims of our proposed study target the most crucial topic and urgent needs of FSHD patients: specific and direct pharmacological therapy.

Aim 1. To narrow our focus to the most promising direct DUX4 inhibitors.

Aim 2. To evaluate effectiveness of DUX4 inhibition

Aim 3. To analyze pharmacokinetic and pharmacodynamic properties of the selected compounds.

+

2. “Defining the Tissue and Cell Specificity of the Human DUX4 promoter in Mice.”

Scott Harper, Ph.D.

Center for Gene Therapy
The Research Institute at Nationwide Children’s Hospital
The Ohio State University
Room WA3015
700 Children’s Drive
Columbus, OH 43205 USA
\$50,000 over 1 year

PROJECT SUMMARY

FSHD was formally classified in 1954, and the primary genetic defect, D4Z4 contraction, was identified in 1992, but the pathogenic mechanisms underlying the disease have only recently started to come into focus. One reason for the difficulties in understanding FSHD biology is the lack of a relevant animal model expressing FSHD-permissive D4Z4 arrays. Since animal models, particularly mice, are crucial tools for studying disease pathogenesis and developing potential therapeutics, the absence of an FSHD mouse model is a fundamental problem in the FSHD field. A major goal of the Harper lab is to generate an FSHD mouse model expressing a single FSHD-permissive human D4Z4 repeat, and to use this model to understand the role of the D4Z4-resident gene, DUX4, in FSHD pathogenesis, and develop RNAi therapeutics targeting DUX4. In preliminary data, supported by previous FSH Society Fellowships to the Harper Lab, we delivered DUX4 to mouse muscle using adenoassociated viral vectors (AAV). DUX4 over-expression in muscle caused myopathy, but DUX4 is generally toxic to many non-muscle cells as well. Thus, we hypothesized that if DUX4 over-expression is an underlying pathogenic event in FSHD, it must be preferentially expressed only in affected muscles. We therefore developed transgenic mice expressing the green fluorescent protein (GFP) gene from the human DUX4 promoter (DUX4p-GFP mice), to determine the tissue and cell specificity of DUX4. In preliminary studies, we observed gross GFP expression in the face, shoulder girdle, and limbs of three independent DUX4p-GFP mouse lines. In this proposal, we will more carefully define the developmental and cell-specific expression patterns of DUX4p-GFP mice, and develop an AAV vector to determine whether a viral-mediated vascular delivery approach can produce the same expression patterns. Ultimately, these studies will be important first steps toward developing an AAV-mediated D4Z4 mouse model.

Specific Aim 1: To define the developmental and cell-specific expression patterns of the human DUX4 promoter in mice. Mounting evidence supports the hypothesis that over-expression of the D4Z4-resident DUX4 gene is an underlying pathogenic event in FSHD. DUX4 is generally toxic to many cell types, and since FSHD is characterized by dystrophy of very specific muscle groups, we hypothesized that DUX4 is preferentially expressed only in affected muscles. Our newly generated DUX4p-GFP reporter mice grossly express GFP in areas that are preferentially affected in FSHD. In this Aim, we will perform a detailed characterization of GFP expression in our DUX4p-GFP mice. These results will help define the expected expression patterns of DUX4, and ultimately increase our understanding about the role of DUX4 FSHD pathogenesis.

Specific Aim 2: To develop an AAV vector-mediated DUX4p-GFP mouse model. Previous endeavors to generate D4Z4 or DUX4 FSHD mouse models using traditional transgenic approaches have been unsuccessful. Although the previous attempts are not published in peer-reviewed literature, the difficulties encountered in generating these models were discussed in abstracts and talks at various scientific meetings over the last several years, including at the FSH Society’s 2008 International Research

Consortium and Research Planning Meeting held in Philadelphia, Pennsylvania (http://www.fshsociety.org/assets/pdf/FSHD_ASHG_IRC2008_Philadelphia_11Nov_ProgramAndAbstract_s_proof.pdf). Vascular delivery of AAV vectors carrying FSHD-permissive D4Z4 repeats to adult mice may circumvent the early embryonic death or developmental defects arising from germline transmission of D4Z4 repeats using traditional methods. In this Aim, we will test the feasibility of using AAV vectors to drive D4Z4-specific expression patterns in mouse muscle using an AAV.DUX4p-GFP reporter vector.

+

3. “Identification of a Novel FSHD Biomarker [an unknown 50 kDa polypeptide highly expressed in FSHD samples]

Sun, Ph.D./Jones, Ph.D.

Boston Biomedical Research Institute

64 Grove Street

Watertown, MA 02472 USA

Partial funding for more preliminary data \$10,000 over 1 year

PROJECT SUMMARY

Screening FSHD patient-derived myoblasts, control myoblast, and muscle samples for expression changes at the proteomic level produced an unknown 50 kDa polypeptide highly expressed in FSHD samples compared to controls. Interestingly, this polypeptide is equally expressed in both normal and FSHD-patient derived myoblasts and early myotubes, however, unlike in control cells where its expression decreases, this unknown polypeptide remains highly expressed in differentiated muscle suggesting it is developmentally regulated and this regulation is disrupted in FSHD. This proposal will utilize standard biochemical techniques including column chromatography and mass spectrometry to purify and identify this 50 kDa putative FSHD biomarker. Subsequently, specific antibodies will be generated and characterized for further use to screen FSHD-derived cells to establish the universality of this biomarker. In addition, regardless of what its eventual identification turns out to be, identifying this protein will provide insight into FSHD pathophysiology, will be a useful FSHD biomarker, and may be one of the first proteins consistently and specifically upregulated in viable FSHD muscle. Therefore, generating specific and standardized antibodies to this protein will provide a useful resource for clinicians and basic FSHD researchers.

+

4. “Toward Therapeutics for FSHD: Understanding mRNA Processing”

Thomas A. Rando, M.D., Ph.D. / Antoine de Morree, Ph.D.

Department of Neurology and Neurological Sciences

Stanford University School of Medicine

Stanford Neurology Clinic

300 Pasteur Drive, Boswell A-301

Stanford, CA 94305 USA

\$100,000 over 2 years

Project is being matched dollar for dollar by the Stanford Office of Medical Development and Dr. Gary Steinberg, Stanford Institute for Neuro-Innovation and Translational Medicine (SINTN).

PROJECT SUMMARY

The pathogenesis of FSHD has remained a mystery despite remarkable advances in the understanding of the underlying genetics. It was determined in 1992 that patients with FSHD have

unusual contractions of a repeat element (so called D4Z4) at position 4q35 in the genome.¹ However, what has remained elusive until now is how those contractions, i.e. loss of genomic material, could lead to an autosomal dominant disease. Within each D4Z4 repeat is a sequence termed Dux4 that encodes a putative double homeobox gene. Studies of the protein product have demonstrated that Dux4 overexpression can interfere with muscle differentiation. Thus, much effort has gone into the exploration of how D4Z4 repeats could lead to a “toxic-gain-of-function” related to the Dux4 transcript and protein. To date, no hypothesis has withstood experimental scrutiny. For one thing, there are individuals with D4Z4 contractions that do not develop FSHD.

Recently, the group of van der Maarel reported in the journal *Science* their findings of the high resolution haplotype mapping of patients and unaffected individuals with D4Z4 contractions.² Their findings provide evidence that the disease develops in individuals who have BOTH a D4Z4 repeat contraction AND a specific sequence in the pLAM domain at the 3' end of the D4Z4 array (Figure 1). The D4Z4 repeat contraction results in “relaxed chromatin”, and allows the transcription of the Dux4 gene in the final D4Z4 repeat. However, it is the sequence in the pLAM domain that creates a site that is recognized by the cellular machinery allowing cleavage of the mRNA and the addition of a poly(A) tail. Without a poly(A) tail in the 3' untranslated region (3' UTR), transcripts are rapidly degraded and never translated into proteins.³ With these tails, transcripts are stabilized and appropriately localized in the cell, allowing for protein translation. In individuals who have D4Z4 contractions but a single base change in the distal sequence, the cell does not recognize it as a “polyadenylation signal” (PAS) site, no poly(A) tail is added to the 3' UTR of the transcript, the Dux4 transcript is unstable, no Dux4 protein is made, and the individuals are protected from getting the disease (Figure 1). Within this cascade are several opportunities, at least theoretically, to treat or even prevent FSHD in susceptible individuals. Any intervention that prevents the addition of the poly(A) tail to the Dux4 transcript is a potential therapeutic approach for FSHD.

These findings suggest a direct line to a novel therapeutic approach. The toxicity leading to FSHD depends on effective mRNA processing in which the Dux4 transcript is cleaved and modified by the addition of a poly(A) tail. If one of these processes could be blocked, then the mRNA would be destabilized and the FSHD genotype would yield a normal phenotype. Clearly, it is untenable to interfere with mRNA processing in general because of the toxicity to the cell. Therefore, understanding the mechanisms by which a cell can bypass a specific PAS site would suggest a mechanism for selectively blocking the PAS site in the pLAM domain in the Dux4 gene without generally affecting cellular mRNA processing. This would be an effective treatment for patients with FSHD.

+

5. “A multicenter collaborative study on the clinical features, expression profiling, and quality of life of pediatric facioscapulohumeral muscular dystrophy”

Jean Mah, M.D.

Alberta Children's Hospital

2888 Shaganappi Trail NW

Calgary, Alberta,

CANADA T3B 6A8

\$96,669, Year 1 \$ 51,434 & Year 2 \$ 45,235

Project is being co-funded by the FSHD Fund Muscular Dystrophy Canada FSHD Fund.

PROJECT SUMMARY

Facioscapulohumeral dystrophy (FSHD) is the third most common type of muscular dystrophy, with an estimated prevalence of 1 in 15,000 to 20,000 (Kissel, 1999) (Flanigan et al., 2001). It is an autosomal

dominant disorder due to a deletion within the D4Z4 repeat region located on the subtelomeric region of chromosome 4q35. FSHD causes progressive atrophy and frequently asymmetrical weakness involving the face, shoulder girdle, upper arm, abdominal, and lower limb muscles. Most affected individuals develop symptoms during their second or third decade, with 20% eventually become wheelchair dependent (Padberg, Lunt, Koch & Fardeau, 1991) (Zatz et al., 1998). Early childhood onset of FSHD may be associated with more severe weakness as well as extra-neuromuscular manifestations such as mental retardation, retinal vasculopathy, and sensorineural hearing loss (Jardine et al., 1994) (Klinge et al., 2006). Although the majority of cases of FSHD are inherited, about 20 - 30% of sporadic cases may occur as a result of spontaneous mutation or mosaicism (van der Maarel & Frants, 2005). Despite recent advances in the understanding of the molecular genetics of FSHD, the exact mechanism responsible for the disease remains unknown, and presently there is no cure (Tawil & Van Der Maarel, 2006) (van der Maarel, Frants & Padberg, 2007). As well, the prevalence, clinical variability, cross cultural presentation, and the psychosocial impact of FSHD on affected individuals constitute a significant public health concern. Emerging therapeutic trials will benefit from the availability of natural history data and reliable outcome measures (Rose & Tawil, 2004) (Tawil, 2008) for both children and adults with FSHD.

Purpose of Study

The main objectives of this study are: 1) to establish a standardized muscle testing protocol for use in children and youth with FSHD; 2) to describe the clinical phenotypes of pediatric onset FSHD; 3) to evaluate the impact of FSHD on health-related quality of life and disability across different age groups; and 4) to explore potential genetic modifiers of clinical phenotypes and disease progression in FSHD.

###

Awards for February 2010 Grant Cycle

The Scientific Advisory Board (SAB) met on June 21, 2010, to review grant applications received for the February 2010 round of FSH Society grants funding. Below are: 1.) a listing of the funded projects by grant applicants along with project descriptions as submitted by grant applicants.

1. "Epigenetic Abnormality in FSHD"

Weihua Zeng, Ph.D.

Mentor: Kyoko Yokomori, Ph.D.

University of California, Irvine, California

University of California, Irvine

Biological Chemistry

240D Med Sci I

Irvine, CA 92697-1700 USA

\$35,500 for one year

PROJECT SUMMARY

Based on our new data described above and our promising ongoing analyses, I would like to request an extension of the FSH Society Helen and David Younger Fellowship Grant. My specific aims for the next grant period are: 1) to continue the analysis of the genome-wide epigenetic changes linked to FSHD pathogenesis, 2) to characterize the genome-wide long-distance chromatin interaction changes in FSHD, and 3) to characterize the regulation and function of the candidate genes identified in Aims 1 and 2. I believe that my research is making steady progress towards deciphering the epigenetic abnormality

mechanism in FSHD, which should provide novel insight into the disease mechanism and thus potentially present new therapeutic strategies. Aim 1. Analysis of the genome-wide epigenetic changes linked to FSHD pathogenesis. 1) ChIP-sequencing In order to get a genome-wide pattern for these epigenetic markers, I am currently analyzing cohesin, HP1 γ , H3K9me3 and H3K27me3 ChIP sequencing in both normal and FSHD myoblasts to identify genomic regions where these factors are specifically enriched. Some of the patient and control myoblasts spontaneously lost MyoD expression, which negatively impacted my assays. I set up a routine expression and differentiation screening to ensure the quality of myoblasts used for the experiments. Thus, myoblasts used in these studies have been tested. I have received the sequencing data and have begun to analyze them. With an extension of funding, I should be able to complete this analysis, including the manual confirmation.

Aim 2: Genome-wide characterization of the long-distance chromatin interaction changes in FSHD by Hi-C and ChIA-PET assays

This is a continuation of the previous Aim 3. Based on our epigenetic studies and preliminary 3C data by us and others, alteration of higher-order chromatin organization in FSHD is likely to be an important molecular change underlying FSHD pathogenesis. Chromatin interactions have typically been examined by cytological and biochemical methods: colocalization in the nucleus by 3D-fluorescent in situ hybridization (3D-FISH), and chromatin conformation capture (3C), respectively. 3C entails chemical crosslinking of interacting chromatin domains followed by restriction enzyme digestion and intramolecular ligation of the crosslinked DNA fragments. Crosslinking is then reversed and the ligated chromatin domains are analyzed by PCR using specific DNA primers (Fig. 4). The frequency of interaction can be measured by the amount of PCR product. 3C can be done in combination with ChIP, which allows the enrichment of chromatin interactions that involve a particular protein (Fig. 4, ChIP-loop). Variations of 3C that allow screening of unknown interactions were developed (e.g., 4C and 5C). However, these techniques did not have the capability to analyze interactions genome-wide [25]. With the recent availability of deep sequencing technologies, it is now possible to directly sequence the 3C products in an unbiased manner. Two recent papers describe the techniques termed “Hi-C” and “Chromatin Interaction Analysis by Paired-End Tag sequencing (ChIA-PET)” that offer new approaches to study 3D chromatin organization in the nucleus [26, 27]. Hi-C (without immuno-enrichment for interactions involving a specific factor) requires enormous amounts of sequencing in order to analyze the data at a resolution higher than 1Mb [27], which is too costly and impractical. I will take two strategies: 1) Use a 4C method to PCR amplify those interactions that involve D4Z4 using primers designed based on my sequence analysis of D4Z4 homologs to distinguish 4q and 10q D4Z4 as opposed to D4Z4 homologs, and 2) perform a ChIA-PET-based high-throughput sequencing protocol to analyze genome-wide chromatin interactions involving HP1 γ , cohesin, or condensin II. The data from FSHD myoblasts will be compared to control myoblasts, which may link epigenetic changes at D4Z4 and gene expression alterations critical for FSHD pathogenesis.

Aim 3. Characterization of the regulation and function of the candidate genes identified in Aims 1 and 3. This is the next step that I plan to take once a candidate gene(s) is identified in Aims 1 and 2.

The functional significance of the identified candidate gene(s) in Aims 1 and 2 whose chromatin, expression, and chromatin interaction are specifically altered in FSHD will be analyzed further. I will prioritize and focus initially on factors that may likely be upstream of the dystrophic pathways, such as transcription factors, or some structural genes whose critical function in skeletal muscle function may be obvious. I will simulate the expression change seen in FSHD cells in normal human myoblasts (by over-expression with expression plasmid transfection or depletion by small interfering RNA (siRNA) transfection). The effect on the expression of potential downstream genes and cell viability, proliferation, and differentiation will be examined and compared to the phenotypes of FSHD myoblasts to determine whether the candidate gene contributes to the FSHD cellular phenotype. A similar analysis was recently

done with Dux4 overexpression in mouse C2C12 cells [31]. If promising, our future plan (beyond the scope of the current project) will be to use a transgenic mouse strategy to recapitulate the expression change of the mouse homolog of the candidate gene in skeletal muscles to test whether it may lead to muscular dystrophy. If the dystrophic phenotype can be recreated in these mice, they can serve as a powerful and versatile disease model with which treatment strategies can be screened more freely than in patients. However, it will be important to analyze human myoblasts in parallel since signaling pathways are sometimes different between mice and humans.

###

Awards for August 2009 Cycle

The Scientific Advisory Board (SAB) met on September 30, 2009, to review grant applications received for the August 2009 round of FSH Society grants funding. Below are: 1.) a listing of the funded projects by grant applicants along with project descriptions as submitted by grant applicants.

1. "A ncRNA regulating the epigenetic switch at the basis of FSHD"

Daphne Cabianca, M.S.

Mentor: Davide Gabellini, Ph.D.

Division of Regenerative Medicine

San Raffaele Scientific Institute

2A3-Room 43

Via Olgettina 58

20132 Milano

ITALY

Awarded the FSH Society New York Music and Song Fellowship Grant

\$45,000 USD for 18 months

PROJECT SUMMARY

[Abstract Provided by Applicant]: Facioscapulohumeral muscular dystrophy (FSHD), the most common myopathy, is an autosomal dominant neuromuscular disorder characterized by progressive weakness and atrophy affecting specific muscle groups. FSHD is not due to a mutation within a protein-coding gene, but is caused by contraction of the 3.3 kb macrosatellite repeat D4Z4 in the subtelomeric region of chromosome 4q35. The mechanism through which contraction of D4Z4 repeats causes muscular dystrophy is currently not clear, but there is a general agreement that reduction of D4Z4 activates an epigenetic cascade leading to 4q35 chromatin re-organization and altered gene expression.

My preliminary results suggest that a non-protein coding RNA (ncRNA) transcribed proximally to D4Z4 regulates 4q35 gene expression in FSHD. Furthermore, I found that the trithorax protein Ash1 is recruited to the region selectively in FSHD patients and is involved in 4q35 gene de-repression. It is tempting to speculate that production of the ncRNA activates an epigenetic switch culminating with 4q35 gene de-repression in FSHD. An attractive hypothesis would be that transcription of the region proximal to D4Z4 plays a role in de-condensation of the 4q35 genomic region, setting the stage for activation of 4q35 genes and, most importantly, preventing re-repression of the region. Here, I propose to rigorously investigate the role played by the ncRNA in regulating the epigenetic state of D4Z4 and in 4q35 gene de-repression.

My specific aim is:

1. To elucidate the mechanism underlying control of gene expression at 4q35. Understanding the mechanism through which the ncRNA is inducing 4q35 gene de-repression in FSHD will generate novel insights into the biological role of ncRNAs in chromatin structure regulation in higher eukaryotes. Moreover, it will help to elucidate the molecular pathways that become altered in FSHD, provide useful molecular markers of FSHD and favor the identification of potential therapeutic targets.

+

2. "Molecular mechanisms involved in FSHD"

Julie Dumonceaux, Ph.D.

Gillian Butler Browne, Ph.D.

Thérapie des maladies du muscle strié / Institut de myologie

UMRS 974 _ UPMC Univ Paris 6 / U974 _ INSERM7215 _ CNRS _ AIM

47, boulevard Vincent Auriol

G.H. Pitié Salpêtrière-Batiment Babinski

75651 Paris Cedex 13

FRANCE

Awarded the FSH Society Delta Railroad Construction Company Fellowship Grant

\$37,800 USD for 1 year

PROJECT SUMMARY

[Abstract Provided by Applicant]: The global deregulation of muscle genes in facioscapulohumeral dystrophy (FSHD) is still poorly understood : despite the identification of a contraction in the D4Z4 repeats in the chromosome 4 shared by the patients, the molecular mechanisms responsible for the disease have not yet been resolved. Our aim is to increase our understanding of these mechanisms. We will focus our studies on the abnormal expression of miRNA and on DUX4 expression in FSHD myoblasts and myotubes in order to determine the transcriptional alterations mediated by the D4Z4 contraction observed in the FSHD patients.

The effects of DUX4 over-expression will be analyzed in normal and immuno-deficient mice after transduction of the whole muscle by a DUX4 coding AAV vector. The DUX4 gene has been cloned under the control of a tetracycline dependent promoter (collaboration with Alexandra Belayew). This system allows us to control the expression level of DUX4 mRNA and to stop the DUX4 over-expression at any time. Our preliminary results shows that after a massive over-expression of DUX4, the majority of the muscle fibers have a centrally located nuclei, suggesting a toxicity of the transgene. We will now confirm this result on more mice and determine if this DUX4 over-expression induces a miss-regulation of other genes (FRG1, p21, PITX1, etc.) or of miRNAs. We will also modulate the expression of DUX4 to a lower and less toxic level.

Using immortalized FSHD myoblasts we have observed that some miRNAs are miss-regulated in these FSHD compared to DMD or control clones. We would like to confirm these results using a unique and rare material: immortalized clones of myoblasts generated from mosaic human muscle biopsies which will allow us to eliminate the extremely high inter individual variations classically observed among FSHD patients. Many clones have been isolated and have been sent to our collaborators Silvere Van der Maarel who provided the muscle biopsies and Stephen Tapscott. We will also receive from Nicolas Levy a skin biopsy from 2 highly interesting cases: identical twins of 32 years old, of whom one is totally asymptomatic whereas the other one is in a wheelchair. Both of them carry the same deletion: a southern blot of the peripheral lymphocytes has revealed that they both carry 2 D4Z4 units. We would like to understand how 2 identical twins have a totally different phenotype. We will immortalized the skin

fibroblasts, transduced them using a lentivirus encoding MyoD under the control of an inducible promoter and analyse the expression of DUX4, FRG1, p21 etc in these cells. Moreover, all the immortalized clones we will generate (from the mosaic patients, as well as from the twins) will be injected into regenerating tibialis anterior muscles of immuno-deficient mice to analyse their fusion potential and the mRNA and miRNA mis-regulation in an in vivo context.

This work will contribute to a better understanding of the molecular mechanisms leading to FSHD. Some therapeutic targets as well as some bio-markers of the disease may be identified which would be essential for a cure.

+

3. "Investigating DUX4 Structure, Function, Expression Using Rational Mutagenesis and the Human DUX4 Promoter"

Scott Harper, Ph.D.

Center for Gene Therapy

The Research Institute at Nationwide Children's Hospital

The Ohio State University

Room WA3015

700 Children's Drive

Columbus, OH 43205 USA

Awarded an FSH Society [Cape Cod Walk 'n' Roll Fund, Connors/Jacobs Families Research Fund, and Kelly Family Research Fund] Research Fellowship Grant

\$40,000 USD, 1 year

PROJECT SUMMARY

[Abstract Provided by Applicant]: The pathogenic mechanisms underlying facioscapulohumeral muscular dystrophy (FSHD) are unclear. We hypothesize that DUX4 over-expression in muscle contributes to FSHD development. In our pilot study which was partially funded by FSH Society in 2008 (FSHD-LCT-002; \$10,000), we showed the first in vivo evidence that DUX4 caused apoptosis and phenotypes associated with muscular dystrophy in zebrafish and mice. In a follow-up study, also funded by the FSH Society in 2009 (FSHS-JJFR-001; \$40,000), we began to define the biochemical function of the DUX4 protein as it related to apoptosis and muscle toxicity, using rational mutagenesis. We have so far demonstrated that DUX4-mediated cell death and dystrophy were dependent upon its ability to bind DNA, and presumably transactivate downstream pro-apoptotic cascades. These results suggested that DUX4 toxicity is related to increased activity of natural DUX4 function and not simply to non-specific effects caused by over-loading cells with excessive protein.

Specific Aim 1: To define DUX4 domains necessary for stimulating apoptosis and muscle toxicity in vitro. In our preliminary work, we over-expressed DUX4 in mouse muscle using adeno-associated viral vectors (AAV). In parallel experiments, we also generated DUX4-expressing zebrafish embryos. Our data support that DUX4 induced apoptosis in vitro and caused dystrophic phenotypes in two different animal models (mice and zebrafish) in vivo. To gain a better understanding of DUX4 structure and function, we rationally mutagenized 8 predicted DUX4 functional domains or residues to investigate the functional effects of these changes on DUX4-induced apoptosis. In our most definitive data to date, we showed that DNA binding by the DUX4 homeodomain 1 (HOX1) is required to induce cell death in vitro and dystrophy-associated phenotypes in vivo. In this Aim, we will continue to investigate DUX4 structure function relationships pertaining to DUX4 pro-apoptotic activity in vitro using DUX4 mutants we have already generated or new constructs that we will generate. These studies will be an important step toward

understanding DUX4 structure and function relationships as they pertain to stimulation of apoptosis and muscle toxicity.

Specific Aim 2: To define DUX4 domains necessary for stimulating apoptosis and muscle toxicity in vivo using the human DUX4 promoter. DUX4 has been detected in human FSHD patient muscle biopsies, but its normal expression pattern in humans is unknown. Our preliminary data support that DUX4 induces apoptosis and phenotypes associated with muscular dystrophy in two different animal models, suggesting it contributes to FSHD development. For our preliminary studies, we expressed DUX4 using vectors containing the ubiquitously active CMV promoter or the engineered, muscle-specific MHCK7 promoter. Both of these promoters have well-characterized expression patterns, but it is unknown whether their cell-type and developmental specificity overlaps with that of natural DUX4. In our previous fellowship, we proposed to express DUX4 or mutant DUX4 constructs in zebrafish using the engineered muscle-specific MHCK7 promoter. Here, we have modified this expression strategy to more faithfully model DUX4 expression. We hypothesize that in vivo expression of DUX4 from its own promoter will produce phenotypes associated with muscular dystrophy in vivo. In this Aim, we will first investigate the developmental and cell-type expression patterns of the human DUX4 promoter by generating eGFPreporter zebrafish. We will then investigate DUX4 structure-function relationships pertaining to DUX4-induced muscular dystrophy in vivo by expressing wild type and mutant human DUX4 constructs from the DUX4 promoter in zebrafish. This work will help us understand temporal and cell-type specificity of DUX4 expression, and ultimately better define a potential role for DUX4 in FSHD pathogenesis.

+

4. "FSHD iPS cells: bioinformatics support"

Michael Kyba, Ph.D.

Minnesota Medical Foundation, University of Minnesota

Lillehei Heart Institute and Department of Pediatrics

University of Minnesota

Minneapolis, MN 55455 USA

Awarded the FSH Society New York Music and Song Fellowship Grant

\$45,000 USD, 1 year

PROJECT SUMMARY

Dr. Kyba's research in Minnesota uses the recent scientific discovery of reprogramming somatic cells from adults into an embryonic stem (ES)-like state called induced pluripotent stem cells [iPS cells] to create an opportunity to model human diseases in a new way. Reverse engineering adult stem cells into embryonic stem cells creates an opportunity whose potential is greatest for diseases such as FSHD whose early regenerative and developmental processes are hard to study using tissue samples of mature differentiated adult muscle and whose mechanisms are currently unknown.

Achieving a Link between the FSHD the Genetic Mutation and Molecular Mechanism that causes FSHD muscles to be weak. FSH Society funds are facilitating the use and study of the first iPS cell lines developed carrying the FSHD mutation. iPS cell lines aid studies to watch and observe the epigenetics of the affected FSHD chromosome in muscle development and myogenic differentiation. Researchers are probing the epigenetics and the associated function of the FSHD associated D4Z4 repeat through each developmental point in the differentiation hierarchy (pluripotent cells, mesoderm, myogenic stem cells, myogenic progenitors, and myotubes) looking for changes that distinguish normal unaffected muscle from that of muscle affected by FSHD.

Using induced pluripotent cells [iPS cells] derived from FSHD patient cells, researchers aim to:

- 1.) link the genetic mutation and molecular mechanism to provide a roadmap to a rational pharmacological intervention, and,
- 2.) test the feasibility of genetic cure of FSHD in cells for the purpose of developing an autologous cell therapy.

FSH Society is funding these studies to address what we believe are the three key roadblocks:

- 1.) understanding the chromatin mechanics of the 4q35.2 locus,
- 2.) understanding the myogenic defect in FSHD, and,
- 3.) testing strategies to genetically repair the FSHD causing genetic defect on chromosome 4.

[Abstract Provided by Applicant]: Facioscapulohumeral muscular dystrophy (FSHO) is a genetically dominant progressive myopathy affecting approximately 25,000 individuals in the United States. It is the third most common muscular dystrophy by incidence with a prevalence near or surpassing Duchenne's. The DNA lesion associated with this disease is a contraction within a series of 3.3 kb repeats - (D4Z4 repeats) near the telomere of 4q. It is not understood how this contraction results in disease, however it appears to modify the chromatin configuration of 4q35.2 and this has been proposed to lead to de-repression of nearby genes. There is currently no animal model bearing the actual FSHD mutation (D4Z4 contraction), and the lack of a suitable model system to study the effects of this mutation has severely hampered progress in understanding FSHD.

In an effort to shed light on the disease mechanism and to speed a potential cell therapy, we have recently derived induced pluripotent cells [iPS cells] from myoblast cultures taken from FSHD patients and controls. The overall goal of this research program is to take advantage of the unique tool represented by pluripotent FSHD-affected cells to accelerate our path towards a molecular understanding of this disease. To address this goal, we will combine in vitro differentiation of iPS cells with assays for chromatin status and gene expression at 4q35.2. We will use a combination of high throughput, in some cases whole genome assays, which will generate a large quantity of bioinformatics data. Funding is requested to support a bioinformatics specialist to generate chromatin maps based on this data, which will allow us to pinpoint where in the genome, and at what stage in development, and in what developmental lineages, chromatin changes take place in FSHD. This data will provide a critical and currently missing link between the genetic damage which ultimately causes FSHD (the D4Z4 repeat array contraction) and the eventual myopathic phenotype.

+

5. "DUX4-mediated control of Pitx1 gene expression"

Alberto Rosa, M.D., Ph.D.

Laboratory of Cellular and Molecular Biology

Fundacion Allende

CONICET (National Research Council Argentina)

Hipólito Yrigoyen 384 - 8vo Piso

5000 – Córdoba

Argentina

Awarded the FSH Society Tango Fellowship Grant for Research in Argentina

\$3,649.85 USD, 1 year

PROJECT SUMMARY

[Abstract Provided by Applicant]: A small research project request to identify specific amino acids from H1/H2 homeodomains controlling the expression of the Pitx1 gene. Hypothesis: Specific amino acids from the DUX4 H1/H2 homeodomains bind the promoter region of the gene Pitx1 to control its expression. Rationale: DUX4 wild type binds the sequence CCGATGCTGTCTTCTAATTAGT-TTGGACCC, located at the promoter region of the gene Pitx1. Homeobox motifs bind a core motif TAAT present in the promoter region of their specific target genes. In this project we will identify amino acids from H1 and/or H2 motifs participating in the control of Pitx1 gene expression mediated by DUX4.

+

6. "Genome-wide analysis of FRG1-mediated splicing defects in FSHD and iFSHD"

Yi Xing, Ph.D.

University of Iowa

Department of Internal Medicine

3294 CBRB, 285 Newton Road

Iowa City, Iowa 52242 USA

Awarded the FSH Society Aubrie Lee Family Research Grant for Infantile FSHD & Fire Island Fellowship

\$39,998 USD for 1 year

PROJECT SUMMARY

Researchers at the University of Iowa (UI) will lead an investigation on infantile facioscapulohumeral muscular dystrophy, or iFSHD, thanks to a grant from the FSH Society, Inc.

Led by Yi Xing, Ph.D., UI assistant professor of internal medicine, biomedical engineering, and biostatistics, the team will use cutting-edge genomic technologies, including exon array chips and ultra-deep mRNA-sequencing, to identify RNA splicing differences among healthy people and people with FSHD or the infantile form of FSHD. RNA splicing differences affect how the genetic code is assembled and translated, and these differences can end up creating defective messenger RNAs or proteins.

This research opportunity comes, in part, as a result of the iFSHD clinic that Katherine Mathews, M.D., ran in conjunction with the FSH Society patient meeting at the University of Iowa in July 2008 and the data collected at that time from iFSHD patients. Xing is collaborating with Dr. Mathews, UI professor of pediatrics and neurology, who has collected skin fibroblast cells from FSHD patients, iFSHD patients and healthy controls made available through the efforts of the University of Iowa NIH-Funded Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center cell core run by Steven Moore, M.D., Ph.D., and the efforts of the FSH Society in recruiting patient donors.

[Abstract Provided by Applicant]: The goal of this project is to systematically examine FRG1-mediated splicing defects in FSHD and infantile FSHD (iFSHD). FRG1 is a component of the spliceosome and several lines of evidence suggest its involvement in RNA processing. It is proposed that due to the deletion of a transcriptional silencer within D4Z4, FRG1 is overexpressed in the skeletal muscle of FSHD patients. Transgenic mice selectively overexpressing FRG1 in skeletal muscle develop phenotypes that resemble FSHD in human patients. Moreover, in skeletal muscle of FRG1 transgenic mice and FSHD patients, aberrant splicing of two muscle-expressed genes (Tnnt3, Mtmr1) was observed. However, the global impact of FRG1 overexpression on splicing, as well as its role in FSHD pathogenesis, remains poorly understood, and other models of pathogenesis are also attractive. We plan to test the hypothesis that overexpression of FRG1 in FSHD patients will result in global disruption of pre-mRNA splicing. Further, if this hypothesis is correct, we would predict that the severe clinical phenotype of iFSHD is

correlated with more profound and widespread transcriptome dysregulation at the level of splicing than is seen in milder, adult onset FSHD.

To test these hypotheses, we will utilize a high-density Affymetrix exon array (HJAY array), with 4.6 million probes for 315,137 exons and 260,488 exon junctions in the human genome to identify aberrant splicing events in FSHD and iFSHD. Our collaborator Katherine Mathews has collected skin fibroblast cells from FSHD patients, iFSHD patients and healthy controls and these are available through the Iowa Wellstone Center Core B, run by Steven Moore. After MyoD-induced myodifferentiation of fibroblasts, we will extract RNA and use exon arrays to identify splicing differences between healthy controls, FSHD patients and iFSHD patients. We intend to examine at least four individuals from each group. Following exon array analysis, candidate disease specific splicing events will be validated by RT-PCR/qPCR. We will also test the hypothesis that disease specific aberrant splicing events preferentially impact genes important for muscle or other organs known to be affected in FSHD/iFSHD. Together, results from this project will lead to improved understanding of FSHD/iFSHD pathophysiology, and reveal novel disease markers and therapeutic targets.