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**Continuing to make progress in understanding and treating FSHD**
*Grant awards for February 2014, August 2013 and February 2013*

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. We are very pleased to list the grantees funded in the February 2014 and August 2013, and February 2013 grant rounds.

## Awards for February 2014 Cycle

The FSH Society Scientific Advisory Board (SAB) met in June 2014 to review grant applications received for the February 2014 round of FSH Society grants funding. Below is a list of the funded projects, including project descriptions as submitted by grant applicant(s).

### 1. Novel Role for Reduced RNA Quality Control in FSHD Pathogenesis

Sujatha Jagannathan, Ph.D. / Stephen Tapscott, M.D., Ph.D.
Fred Hutchinson Cancer Research Center, Seattle, Washington USA
$116,725 over 2 years

Summary (Provided by Applicant): Facioscapulohumeral muscular dystrophy (FSHD) is a prevalent and currently untreatable myopathy. FSHD is caused by the misexpression of DUX4, a germline transcription factor, in post-mitotic muscle cells where it activates a germline transcription program and also induces expression of retroelements and repetitive sequences. Ectopic expression of DUX4 triggers cell death in a variety of cells including primary myoblasts and immortalized epithelial cells via an unknown mechanism. We recently discovered that DUX4 reduces the efficiency of a cytoprotective, RNA quality control pathway called the nonsense mediated RNA decay (NMD), thus stabilizing hundreds of aberrant RNAs. It is known that reduced NMD efficiency can affect cellular proteostasis due to expression of malfolded proteins, which can in turn lead to cytotoxicity through the unfolded protein response (UPR). Hence we hypothesized that DUX4-induced reduction in NMD efficiency leads to the stable expression and translation of aberrant RNAs, generating toxic proteins that cause cell death, possibly through UPR-mediated apoptosis. In Aim 1, we will identify the mechanism by which DUX4 expression reduces NMD efficiency. In Aim 2, we will determine the contribution of reduced NMD to DUX4-induced cytotoxicity and elucidate the downstream mechanisms responsible for this phenomenon. These studies will provide valuable insights into the mechanism of DUX4-induced cytotoxicity and uncover potential novel avenues for therapeutic intervention for FSHD.

### 2. BET Proteins as Therapeutic Targets in FSHD

Francis M. Sverdrup, Ph.D.
Center for World Health & Medicine, Saint Louis University, Saint Louis, Missouri USA
$51,425 over 1 year

Summary (Provided by Applicant): Promoting the appropriate epigenetic repression of DUX4 is a therapeutic strategy for FSHD that addresses the underlying mechanism of disease pathology. However, the molecular details of DUX4 de-repression are not completely understood and few specific targets amenable to small molecule drug intervention have been identified. We have used a chemical genetics approach to identify a key role for the bromodomain and extraterminal domain (BET) proteins in the epigenetic switch that activates DUX4. The experiments proposed here will extend these findings by confirming by genetic means the specific BET family member(s) involved in pathogenic DUX4 expression. This will be accomplished by a combination of RNAi technology and overexpression studies. In

addition, we will similarly determine the involvement of mediators of the BET pathway of transcriptional activation including the role of protein acetylation. We will aslo determine the functional effects of BET inhibitors (BETi) on FSHD muscle biology in vitro. A 24 h pulse of BETi results in a sustained decrease in expression of DUX4 and its downstream targets in cultured myotubes without long-term interference with muscle differentiation. These data demonstrate that the pharmacodynamics of DUX4 inhibition and undesirable effects on muscle cells are distinct. We propose to perform a more detailed analysis of the effects of BETi on FSHD myoblasts and myotubes by comprehensive gene expression and functional assays. In addition, we will assess protection of FSHD muscle cells from DUX4-induced apoptosis during myotube differentiation.

**3. FSHD Clinical Trials Network Proposal**

Rabi Tawil, M.D.
University of Rochester Medical Center, Rochester, New York USA
$25,000 over 1 year

Summary (Provided by Applicant): The discovery of a unifying hypothesis for the cause of FSHD means that, for the first time since the discovery of the genetic defect twenty years ago, it is possible to develop targeted treatments for FSHD. The next steps on the road to therapeutic development are: preclinical work to develop and test potential treatments, and the conduct of clinical trials to determine the efficacy of such treatments. A number of laboratories are actively investigating various therapeutic approaches to treat FSHD. In parallel to this research, it is vital that clinical investigators work to develop the tools necessary for the efficient conduct of future FSHD clinical trials.

Successful clinical trials depend on several factors including: access to patients, a good understanding of the natural history of the disease, and reliable outcome measures that are sensitive to change. Optimal, accepted standard outcome measures will result in more effective and efficient clinical trials, significantly shorten the drug development process and result in more robust clinical trial data. The trial preparedness workshop recently held in Leiden developed and published a consensus approach to what is needed for clinical trial readiness for FSHD and sets forth the milestones necessary to accomplish this objective(1). The development and validation of outcome measures requires a prospective, longitudinal study with a substantial number of patients followed for at least one year. To achieve this goal it is important to coordinate the development and validation of clinical trial tools across multiple centers. To this end, this proposal seeks to establish an FSHD Clinical Trials Network. This network will be composed of academic research centers working collaboratively in developing, testing and validating clinical outcome measures and biomarkers. Establishing and validating a consensus for compatible outcome measures and biomarker assessment, both molecular and radiological, among network members is necessary for future multi-institutional FSHD therapeutic trials, and, just as important, for comparison of trials performed at different institutions. The existence of such a network significantly increases the likelihood that promising therapeutic interventions in FSHD come to clinical trials and that those trials will have a transparently meaningful outcome.

AIMS: The consensus plan from the Leiden in April 2013 meeting forms a roadmap for a future FSHD trial network. The collaboration between the University of Washington and the University of Rochester represents a regional effort to implement inter-institutional cooperation to develop meaningful outcome measures. This application seeks to broaden and expedite the development of outcome measures and to develop a larger FSHD Clinical Trials Network composed of academic sites with established expertise in FSHD and neuromuscular clinical trials. The long term objectives of this Network are to: l (Aim 1) optimize patient access to clinical trials by helping recruit patients to respective national registries and creating local/regional databases of patients interested in future clinical trials, and (Aim 2) develop and validate shared clinical, radiological, and molecular outcomes measures. These will be achieved by having the working groups established during the Leiden workshop, composed of representatives from each academic neuromuscular center (below), develop consensus approaches and cross-validation studies.

Centre de Référence des Maladies Neuromusculaires, Nice, France
King's College, London, UK
Leiden University, The Netherlands
Kennedy Krieger Institute, Baltimore
University of Rochester, Rochester, NY
University of Copenhagen, Copenhagen, Denmark
University of Washington, Seattle, WA
Newcastle University, Newcastle, UK
University of Niejmegen, Niejmegen, The Netherlands
Ohio State University, Columbus, OH
University of Iowa, Iowa City, IA
Catholic University School of Medicine, Rome, Italy

*Budget covers the cost of conference calls, administrative support and full cost of the in person meeting in Rochester, New York for a total of 23+ participants. FSH Society, FSHD Stichting (Netherlands) and FSHD Global (Australia) have agreed to each contribute one-third of the original budgeted costs of the workshop (total $75,000). FSH Society agreed, following the approval of the FSHD Stichting and FSHD Global Research foundation, to co-fund the original requested budget three ways the organization of the trial readiness workshop in Rochester, New York in the Spring of 2015.*

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**Awards for August 2013 Cycle**

The FSH Society Scientific Advisory Board (SAB) met in January 2014 to review grant applications received for the August 2013 round of FSH Society grants funding. Below is a list of the funded projects, including project descriptions as submitted by grant applicant(s).

1. **Investigating effects of PARP1 inhibitors in DUX4 expression**
Yi-Wen Chen, D.V.M., Ph.D.
George Washington University & Children's National Medical Center, Washington DC, USA
$89,267 over 2 years

Summary (Provided by Applicant): Transcriptional de-repression of DUX4 gene due to epigenetic changes of the D4Z4 region is believed to cause FSHD. In our preliminary study, we identified that poly (ADP-ribose) polymerase 1 (PARP1) interacted with the promoter of the DUX4 gene. Interestingly, the interaction was only observed in the FSHD myoblasts but not control cells, suggesting that the interaction may be part of the disease mechanism of FSHD. PARP1 is a nuclear protein, which functions in various cellular processes and has been shown to play critical roles in regulating gene expression. Several studies showed that, at the promoter of a target gene, PARP1 binds to DNA methyltransferase 1 (DNMT1) and suppresses its function by poly-ADP ribosylation. As a consequence, expression of the target gene is de-repressed due to hypomethylation of its promoter region. Interestingly we identified that DNMT1 co-localized at the DUX4 promoter region in our preliminary study. In addition, FSHD myoblasts treated with PARP1 inhibitor showed reduced expression of ZSCAN4, a marker of DUX4 expression. Based on current knowledge and our preliminary data, we hypothesized that the interaction among the PARP1, DNMT1 and the DUX4 promoter contributes to the DNA hypomethylation of the region, and may further influence the expression of DUX4 in FSHD myoblasts. The goal of this study is to test one synthetic and one dietary PARP1 inhibitor for their effects on DUX4 expression and further investigate the involvement of PARP1 and DNMT1 in FSHD. In aim 1, we will determine the effects of PARP1 inhibitors on DUX4 expression and cell phenotypes of FSHD myoblasts. In aim 2, we will determine whether DNMT1 directly interacts with the DUX4 promoter region in FSHD myoblasts. In aim 3, we will determine whether PARP1 is a direct regulatory target of DUX4. The findings of the study will provide insights of the involvement of PARP1 in FSHD and have a direct impact on developing therapeutics for FSHD which does not have an effective treatment currently.

2. **Gene surgery using TALEN technology: a therapy for FSHD**
Julie Dumonceaux, Ph.D.
Institut de Myologie, University of Paris, U974 – Inserm, Paris, France
$117,500 over 1 year

Summary (Provided by Applicant): FacioScapuloHumeral Dystrophy (FSHD) is one of the most common myopathies and 2 loci of the disease have been characterized. The first one is located in the subtelomeric region of chromosome 4 and is mutated in 95% of FSHD patients (named FSHD1). This region is composed by a 3.3 kb tandemly repeated sequence named D4Z4. In the general population, the number of repeats varies from 11 to 150, whereas FSHD1 patients carry between 1 and 10 repeats. The second one is located in chromosome 18 and is mutated in 5% of the FSHD patients in whom mutations in the SMCHD1 gene have been found. Despite the different genetic origins of the disease, all patients are

phenotypically indistinguishable and share common molecular features, among them the expression of a protein named DUX4. DUX4 is a transcription factor encoding a potential homeobox protein which is highly toxic after overexpression by mis-regulating more than 500 genes. DUX4 ORF is present in each D4Z4 unit but only the most telomeric unit might be able to produce a DUX4 mRNA stabilized by the addition of the poly(A) tail induced by 4qA sequences downstream the D4Z4 array.

Because DUX4 is the common pathogenic target between FSHD1 and 2 patients, our goal is to perform gene editing using transcription activator-like effector nuclease (TALEN) and CRISPR/Cas9 technology to modify the FSHD locus and permanently inhibit DUX4 expression. We have chosen to develop 2 strategies: (i) to remove the entire D4Z4 array because individuals with such deletions exist and do not present muscular pathology and (ii) to mutate the DUX4 poly(A) signal since it has been shown that a single point mutation in this poly(A) sequence is sufficient to inhibit DUX4 mRNA expression by modifying its stability. Specific aims will include: (i) designing nucleases with the best activity and sequence-specificity and optimizing the genome engineering strategy (ii) select FSHD cells carrying D4Z4 and 4qA sequence modifications for DUX4 inbhibition (iii) testing the therapeutic benefit of D4Z4 genome engineering in appropriate cell culture and animal models by performing several phenotypic measures to assess the consequences of the targeted mutations of the D4Z4 array on FSHD hallmarks. There are a number of advantages to of our proposed approach over other therapeutic strategies currently under investigation for FSHD. There will be no need for repeated long-term administration of treatment since genome editing offers the possibility of permanent correction following transient nuclease activity for the lifetime of the modified cell and its progeny. The benefit of this as a clinical therapy in terms of cost, toxicological and immunological risk is obvious. Moreover, this approach would be useful for all FSHD cases, whatever the precise mutation/contraction involved.

3. **Protein chemistry and protein-protein interactions of DUX4**

Jocelyn Eidahl, Ph.D.
The Research Institute at Nationwide Children's Hospital, Columbus, Ohio USA
$70,000 over 1 year

Summary (Provided by Applicant): DUX4 has been identified as an underlying insult in FSHD, but the mechanisms by which DUX4 contributes to FSHD pathologies is unclear. Our central hypothesis is that the DUX4 transcription factor is involved in protein-protein interactions that influence its ability to induce toxicity in muscle cells and ultimately contribute to FSHD. The two DUX4 N-terminal homeodomains are responsible for its ability to bind specific sequences of DNA. C-terminal residues 160-424 of the DUX4 transactivation domain are essential for inducing toxicity in the muscle, however the mechanisms by which the C-terminal domain mediates DUX4 activity are unknown. We propose that the DUX4 C-terminal domain is involved in the recruitment of proteins that influence its ability to transactivate normal and toxic genes. Our preliminary data identified several candidate DUX4-interacting proteins. The goal of our proposed studies is to identify critical interactions between DUX4 and its candidate binding partners that we can therapeutically target to abolish the toxic effects of DUX4 in FSHD muscles. Our proposed aims will define

DUX4 protein binding partners and mechanisms, and delineate the influence of protein-protein interactions on the DUX4-associated pathogenic cascade. We plan to pursue the following two specific aims to test our hypothesis: Specific Aim 1: To define the binding partners and protein-protein interaction mechanisms of the DUX4 C-terminal domain. Specific Aim 2: To examine the functional significance of protein-protein interactions of DUX4 that are critical for DUX4 toxicity.

4. **Exploiting genome editing technology to modify and regulate the FSHD disease locus**
Michael Kyba, Ph.D.
Lillehei Heart Institute, University of Minnesota, Minneapolis, Minnesota USA
$125,000 over 1 year

Summary (Provided by Applicant): The recent discovery of DNA-binding factors whose sequence specificity is encoded by modular domains that recognize single bases (TALENs) or by a guide RNA (CRISPRs) have opened up tremendous new possibilities in genome editing. With early support from a 2 year ARRA grant, and now with continuing support of an NIH R01, we have developed a zinc finger nuclease (ZFN) that targets 4q35.2. We have used this tool to introduce a new telomere at this site in FSHD iPS cells, which effectively eliminates the genetic lesion. Individuals who lack 4qter on one allele are normal, and our targeted iPS cells that have lost the contracted D4Z4 element are similarly normal. This modification rids the cells of DUX4 mRNA expression and corrects a differentiation defect that we have identified in FSHD iPS cells.

We seek funding from the FSH Society to expand this research program (1) to include FSHD human embryonic stem cells (our NIH grant supports FSHD iPS cells), (2) into the exciting new area of CRISPR technology with more specific genetic reversion of the pathogenic 4qA161 allele, and (3) to test the hypothesis that epigenetic silencing can be introduced by targeting D4Z4 with engineered sequence-specific chromatin nucleators. Aim 1. To correct the FSHD locus in human embryonic stem cells bearing the FSHD mutation. Our work to date has shown that FSHD iPS cells express DUX4 mRNA and suffer from an impaired response to Pax7-induced skeletal muscle differentiation and that these phenotypes are reverted by genetic removal of the contracted D4Z4 array. These iPS cells were derived from myoblasts, therefore there is some question of whether these phenotypes represent an epigenetic memory of the pre-iPS cell type. It will therefore be essential to perform this genetic correction in FSHD human embryonic stem cells.

Aim 2. To design CRISPRs that target existing and novel sites at 4q35.2. The efficiency of targeted integration with our ZNF reagent is low, therefore we will test whether our existing genetic repair method can be made more efficient by CRISPER technology. We will also design and test CRISPERs targeting the pathogenic poly A signal, which may allow correction of the locus without elimination of the entire D4Z4 array.

Aim 3. To use engineered sequence-specific DNA-binding tools to target a chromatin nucleation complex to D4Z4. While most enthusiasm about TALENs and CRISPRs has been around their ability to target a nuclease to introduce double strand breaks in DNA, they can also be used to target other proteins to DNA. Because FSHD is caused by inappropriate relaxation of D4Z4 chromatin on the contracted allele, we will attempt to reestablish

heterochromatin at this site by fusing an engineered D4Z4- specific DNA-binding domain to proteins involved in nucleating heterochromatin. These studies take advantage of and leverage an existing research program in genome editing of FSHD iPS cells, and will provide the field with valuable new tools to study the pathogenesis of FSHD, and to develop cell therapies based on corrected, isogenic, iPS cells.

5. **Microdialysis for the study of inflammatory features in Facioscapulohumeral muscular dystrophy**

Giorgio Tasca, M.D.
Institute of Neurology Catholic University School of Medicine, Rome, Italy
$70,000 over 1 year

Summary (Provided by Applicant): In the last years, most of the efforts in the research on Facioscapulohumeral Muscular Dystrophy (FSHD), the third most common form of muscular dystrophy, have been focused on the characterization of the non-conventional genetic mechanism activated by pathogenic D4Z4 repeat contractions that underlies the disease.

In our study, we will make use of microdialysis with high cut-off membranes, a technique that has never been applied to the study of skeletal muscle, with the aim of elucidating the pathogenetic mechanisms downstream the genetic lesion, with a particular focus on the poorly clarified inflammatory aspects.

In the view of a translational research where information coming from clinical imaging is merged with molecular data, we will perform a comparison between the microenvironment of affected muscles in early disease stages (STIR hyperintense and T1-weighted normal signal on muscle MRI, i.e. muscles showing oedema changes but not yet or only partially replaced by fat tissue) and the microenvironment of apparently unaffected muscles (normal T1-weighted and STIR signal on muscle MRI), in FSHD patients. Muscle microdialysates will be analysed using xMAP technology (multi-analyte profiling beads) to compare the levels of inflammatory cytokines, chemokines and growth factors in the two conditions and to characterize the pattern of inflammation and mediators involved.
This will allow a better understanding of the role of the inflammatory process in the disease, the identification of biomarkers of disease activity at single muscle level and, finally, the acquisition of information useful for the development of a targeted anti-inflammatory therapy. In the future, the high cut-off muscle microdialysis protocol could be used for molecular monitoring and eventually drug administration in neuromuscular disorders.

6. **Dynamic mapping of perturbed signaling underlying FSHD**

Peter S. Zammit, Ph.D.
King's College London, London, England
$137,798 over 1 year to 18 months

Summary (Provided by Applicant): Facioscapulohumeral muscular dystrophy (FSHD) is the third most common myopathy worldwide, but its prevalence may have been underestimated, with it being the commonest muscular dystrophy in Europe. FSHD is an adult-onset, autosomal dominant disorder characterised by wasting of facial muscles and upper body

musculature. Disease can progress to affect muscles of the lower extremities and can severely impair quality of life. Over 95% of FSHD cases are caused by contraction of the D4Z4 microsatellite repeat on the subtelomeric region of chromosome 4. In unaffected individuals the D4Z4 repeat region comprises 11-100 D4Z4 units, but in FSHD patients the number is reduced to less than 11. At least one D4Z4 unit is required to cause FSHD however, and only when inherited with a specific polymorphism on the distal end of chromosome 4 (4qA161). Each D4Z4 unit contains an open reading frame for the double homeobox 4 (DUX4) gene, with the 4qA161 polymorphism providing a polyadenylation signal for DUX4 transcripts generated by the last D4Z4 unit. This permissive chromosomal configuration generates stable DUX4 transcripts and it is likely that FSHD is caused by a toxic gain-of-function of elevated levels of DUX4.

Little is known about the function of DUX4 and the challenge is now to elucidate how elevated levels of DUX4 cause muscular dystrophy. DUX4 is a putative transcription factor; its N-terminus contains two homeodomains with high similarity to the homeodomains of the transcription factor PAX7, and the C-terminus is an activator of transcription. DUX4 induces apoptosis, with lower expression levels decreasing MyoD and affecting myoblast function and differentiation: important as myoblasts and myotube formation are compromised in FSHD.

Many gene expression studies have been performed on FSHD, and other muscular dystrophies, but these have not been thoroughly analysed by the latest bioinformatic techniques. To address this, we developed a novel differential network methodology, designed to identify perturbed signaling pathways in disease networks from just such expression data. Using this novel mathematical methodology, we performed a meta-analysis of multiple publicly available gene expression data sets from FSHD muscle biopsies. We then removed changes associated with muscle wasting, aging, atrophy and inflammation following meta-analysis of appropriate data sets. This integrated output is a high-confidence unified network of pathway changes explaining FSHD pathomechanisms. Interrogation of our network has revealed many promising drug targets and candidate therapeutics.

Here, we wish to extend and refine this analysis to identify pathways in our FSHD network that lead to compromised muscle repair and regeneration. To address this, we will collect and analyse by RNA-seq, a high-frequency time course of genome wide gene expression during myogenic differentiation in FSHD. Using mathematical methodologies with optimised network theoretic tools on this gene expression dataset, will reveal molecular mechanisms of myogenesis in FSHD. We will also perform high-frequency time-course RNA-seq on myogenic cells ectopically expressing DUX4, with the aim of identifying primary DUX4 transcriptional-mediated gene expression changes. This will order our FSHD network to identify causal signaling events in FSHD, further eliminating non-specific general adaptations to muscle wasting, inflammation, disuse etc. It will also better identify pathways in the network directly linked to DUX4. Together these results will identify key pathway targets, modification of which should help restore muscle regeneration in FSHD, reversing muscle weakness and wasting. Our ultimate aim is to gain knowledge on FSHD myogenesis and inform the design of therapies for FSHD.

There are four objectives:

1. Obtain the first high-frequency time course of genome-wide gene expression during myogenic differentiation in FSHD and control human myoblasts.

2. Obtain a high-frequency time course of genome-wide gene expression in myoblasts expressing ectopic DUX4.

3. Apply our mathematical methodologies with optimised network theoretic tools for analysis of time course gene expression datasets generated.

4. Validate a selection of identified targets for rapid translational studies as potential therapeutics for FSHD.

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**Awards for February 2013 Cycle**

The FSH Society Scientific Advisory Board (SAB) met in May 2013 to review grant applications received for the February 2013 round of FSH Society grants funding. Below is a list of the funded projects, including project descriptions as submitted by grant applicant(s).

1. **Pilot Study of Electrical Impedance Myography in Facioscapulohumeral Muscular Dystrophy**
Jeffrey Statland, M.D.
University of Rochester, New York
$48,909 over 1 year to 18 months

Summary (Provided by Applicant): Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common forms of muscular dystrophy with an estimated prevalence between 1: 15,000 and 1:20,000. The clinical spectrum of disease severity is wide, and the regional distribution of muscle weakness, as well as the pattern of progression, is unique. The molecular defect in FSHD on chromosome 4q35 was described in 1992 but the molecular pathophysiology remained unknown until recently. A unifying model has now emerged proposing the aberrant reactivation of the DUX4 gene - resulting in a toxic gain of function- in the pathophysiology of FSHD. This FSHD model has provided, for the first time, therapeutic targets for FSHD, and it is expected that several potential therapeutic interventions will emerge in the coming years. Because of these recent discoveries, there is an urgent need to develop the tools necessary to effectively and efficiently conduct therapeutic trials in FSHD. There are currently two validated, commonly utilized outcome measures in FSHD (manual muscle testing and quantitative myometry) both of which are based on direct strength testing. Our prior natural history study showed a small but significant change using both techniques at 1 year. The responsiveness to disease progression for both measures is considered small. Consequently, trials utilizing these measures require large sample sizes and long durations. Here we plan to test the reliability, validity and responsiveness to change of electrical impedance myography (ElM). EIM is a fast, non-invasive technique for quantifying

muscle structure, which may prove to be sensitive to disease progression in FSHD and predict future changes in motor strength or function. In addition EIM makes it possible to test muscles classically involved in FSHD not amenable to strength testing: including facial, abdominal, and paraspinal muscles. We plan to recruit 20 participants with FSHD for 2 visits over 6 months of follow up to test the reliability, validity and initial responsiveness to change of EIM in FSHD. Our current FSH Society-supported project (FSHS-82012-02: Evaluation of an FSHD-specific patient reported outcome measure and disease specific functional rating scale) gives us a unique opportunity to add ElM to our current protocol: EIM will be a valuable structural correlate for our ongoing study, and our existing study will provide the necessary 'context' to interpret changes on EIM. By combining recruitment with our existing FSH Society funded project we can minimize the costs and patient burden required to evaluate multiple outcomes. We expect that this proposal will provide preliminary data on the utility, reliability, and ease of administration of EIM. Data from this proposal will be used to fund a definitive validation study of EIM in FSHD. It is of vital importance for the FSHD research community that development of outcome measures parallels advancements in molecular pathophysiology and drug development. ElM represent valuable quantitative measure of muscle structure that is portable, easy to obtain, and relatively inexpensive, and a potential valuable addition to the FSHD clinical trial toolkit.

2. **Development of a novel ChIP-based diagnostic assay for FSHD**

Kyoko Yokomori, D.V.M., Ph.D. / Shohei Koide, Ph.D.
University of California, Irvine, California & University of Chicago, Chicago, Illinois
$40,000 over 1 year

Summary (Provided by Applicant): The long-term goal of the proposed project is to develop an accurate and robust diagnosis for FSHD. Although FSHD is reported to have a one in 20,000 incidence, there is great concern that the actual number of affected individuals is significantly higher due to undiagnosed cases (with a likely incidence of 1/7,000). Proper diagnosis depends initially on recognition of clinical signs and symptoms and differentiation of FSHD cases from other muscular dystrophies. Molecular studies have been used to reinforce the clinical impression. The primary approach has been through detection of 4qD4Z4 repeat contraction by pulsed-field gel electrophoresis (PFGE) following restriction digestion. However, this method cannot identify phenotypic FSHD (with no repeat contraction), and certain band patterns can prove difficult to interpret. More recently, DNA hypomethylation at the D4Z4 locus was also found to serve as a diagnostic marker. However, severe DNA hypomethylation was also found in the ICF syndrome cells, and thus is not FSHD-specific. Therefore, we have urgent need for a better diagnostic technology.

We will combine our complementary expertise in FSHD biology and in antibody engineering, respectively, to develop a new diagnostic method. The Yokomori group previously found a specific change in histone modification (histone H3 lysine 9 trimethylation (H3K9me3)) at the D4Z4 repeat sequences that is detected in both FSHD1 and FSHD2 patient cells. Importantly, this change is highly specific for FSHD, and is seen also in patient lymphoblasts from blood samples. Thus, in this project, we plan to test the possibility that ChIP can be used to detect the loss of H3K9me3 in patient chromatin as a diagnostic method

for FSHD. We plan to use peripheral blood mononucleocytes (PBMCs) from patient blood samples that can be obtained significantly less invasively (and less painfully) than standard muscle biopsy samples. Detection of H3K9me3 loss will be assessed by chromatin immunoprecipitation (ChIP) analysis. A fundamental problem in extending this potentially transformative finding to diagnosis is that the poor quality of commercially available H3K9me3 antibodies, which complicates and sometimes even mislead evaluation. Remarkably, the Koide group has recently developed a recombinant antibody that is equivalent, or even superior, to the best commercial antibody to the H3K9me3 mark. Because the Koide antibody is a recombinant protein produced from a defined DNA sequence, fundamentally eliminating poor quality and lot-to-lot variability inherent to current commercial antibodies. The antibody will thus enable us to standardize the ChIP assay. In this proposal, the two groups will join forces and establish an accurate and robust diagnostic method for FSHD. We will assess the specificity of our protocol by testing blood samples from healthy members of patients' families, from patients of different ages and disease severities, and from individuals with unrelated muscular dystrophies or unrelated diseases. We believe that the project is highly interdisciplinary, innovative and translational, and it will provide an important immediate basis for the development of a novel diagnostic test for FSHD.

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