Continuing to make progress in understanding and treating FSHD
Grant awards for August 2014 and February 2014 grant application cycles

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 August 2014 and February 2014 cycles.

Awards for August 2014 Cycle

On December 8 and 11, 2014, the Scientific Advisory Board (SAB) of the FSH Society, chaired by David Housman, Ph.D., held two sub-reviews and a final SAB review. The SAB reviewed the grant applications and progress reports for the August 2014 round. By January 20, 2015, the FSH Society Board of Directors reviewed and approved the SAB recommendations for funding. Below is a list of the funded projects, including project descriptions as submitted by the applicants. For the August 31, 2014, round of grant applications eleven grant applications were received; five were funded in the amount of $485,840.

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Giancarlo Deidda, PhD
Institute of Cell Biology and Neurobiology National Research Council of Italy (CNR), Rome, Italy
Development of a new methylation assay for FSHD diagnosis
$ 56,000 for 18 months
03/01/2015-08/31/2016

PROJECT SUMMARY (Provided by Applicant)

FSHD is linked to chromosomal 4q35 region, that contains a D4Z4 array of up to 200 units. The most common form, autosomal dominant FSHD1, is caused by a contraction of the 4q D4Z4 array to less than 11 units, whereas FSHD2 is caused by reduced levels of functional SMCHD1 protein (Structural maintenance of chromosomes flexible hinge domain-containing 1). Although with different mechanisms, both genetic defects lead to DNA hypomethylation at D4Z4 on 4qter causing chromatin relaxation. This genomic modulation

provides a transcriptionally permissive chromatin environment that is associated with the expression of DUX4, the best candidate FSHD gene, enclosed within each D4Z4 unit. DUX4 expression requires also the presence of a polyadenylation signal (PAS) distal to the last D4Z4 unit, which stabilizes DUX transcript. There are two different allelic forms of the region distal to the D4Z4 array, A and B. Although a D4Z4 array followed by an A "Telomere" is also present on 10q, a functional PAS sequence has been identified almost exclusively on 4qA alleles. Ultimately, vast majority of FSHD1 and FSHD2 subjects show hypomethylation at D4Z4 region followed by an A allele containing a functional PAS.

Currently, FSHD diagnosis is based on the identification of shortened 4q arrays (FSHD1) or the presence of mutations in SMCHD1 (in FSHD2) and the assessment of the A/B genotype. In addition, methylation analysis of the proximal D4Z4 units is performed, using methylation sensitive restriction enzymes or by bisulfite sequencing of the overall D4Z4 units. Although hypomethylation is significantly associated with FSHD1 and FSHD2, it is not diagnostic per se because of the lack of information about the presence of permissive alleles (alleles that contains a polyadenylation signal – PAS) and of the interference of not-pathogenic arrays. For this reason, the diagnostic flowchart for FSHD considers hypomethylation as a secondary step to distinguish FSHD1 from FSHD2.

Our project aims to the introduction of a new assay that combines the different key features found in FSHD subjects. We propose methylation analysis of 10 CpGs within the 3' portion of the distal DUX4 copy (DUX4-fl), that is specifically expressed in muscles of FSHD patients. Despite the low complexity and the presence of repetitive elements in the region (pLAM), we were able to design PCR assays, on bisulfite treated DNA, that are specific for the presence of PAS sequence in the A allele.

Preliminary results in a subset of FSHD1, FSHD2 and Control subjects showed highly significant differences of methylation levels between affected and unaffected subjects in 8 out of 10 CpGs tested, strongly supporting the potential usefulness of this assay for FSHD diagnosis. Here, we propose:

- To develop additional assays to quantify the number of permissive alleles in order to assess whether different allelic combinations are relevant in the identification of diagnostic threshold;
- To analyze a large cohort of well genotyped FSHD patients and normal controls for precise evaluation of methylation threshold between affected and unaffected subjects;
- To assess specificity of this assay for FSHD disease by testing peripheral blood leukocytes DNA (PBLs) from individuals with unrelated muscular dystrophies;
- To analyze the prognostic potential of this assay by correlating methylation levels with different clinical severity scores;
- To study possible methylation differences distal to the D4Z4 array, between PBLs and muscle biopsies.

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Lionel Van Maldergem, MD, PhD
Université de Franche-Comté, Besançon, France, and
Björn Fischer-Zirnsak, PhD
Charité-Universitaetsmedizin, Berlin, Germany
Identification of the underlying genetic defect in a family with FSHD-like and optic atrophy phenotype
$8,000 for one year

PROJECT SUMMARY (Provided by Applicant)

Facioscapulohumeral muscular dystrophy (FSHD) is a disease group which can be subdivided into two groups (FSHD1, OMIM# 158900 and FSHD2, OMIM# 158901). All patients have in common a muscular dystrophy affecting the facial and the upper limb muscles. In both groups, additional features are known such as hearing loss and mild to moderate eye abnormalities. FSHD1 is inherited in an autosomal dominant fashion involving D4Z4 repeat on chromosome 4q35 [1]. The inheritance of FSHD2 is more complex following a digenetic model which includes heterozygous mutations in SMCHD1 which modulate the severity and an haplotype on chromosome 4 which is permissive for DUX4 [2].

Some years ago, a consanguineous family from Italy suffering from an FSHD-like phenotype came to our attention. Both parents were unaffected whereas all four children show a progressive phenotype. The patients show facial and upper limb muscular weakness which became more severe with age. A muscle biopsy from one proband was investigated and showed mild fibrosis, targetoid fibers and a neurogenic component with

increased abundancy of type one muscle fibers (JJ Martin, Antwerp). Beside muscular features, all affected individuals reported on eye abnormalities such as myopia in early infancy. A detailed investigation of the eye and the eye fundus revealed an atrophy of the optic nerve in all affected individuals, determining progressive blindness.

Furthermore, deep investigation of multiple serum and urine parameters showed an increased level of 3-methylglutaconic acid in the urine and in the serum samples analyzed. Additionally, creatine kinase values are also increase in all individuals tested.

The affected individuals from this exceptional family suffer from a disease which combines features known for FSHD and optic nerve atrophy. Furthermore, metabolic alterations which might point to mitochondrial dysfunction were identified which could lead to a better understanding of the affected gene product. Since unaffected parents were second-cousins, recurrence in sibship strongly suggested autosomal recessive inheritance.

So far, using various molecular genetic techniques we were not able to detect the causative genetic defect in our index family. Due to the fact that no deletions and causative coding mutations as well as alterations in regulatory regions around CCDC67 could be identified, it is very likely that a non-coding mutation is the cause of this disease. To identify the causative alteration, whole genome sequencing (WGS) is the method of choice. Raw data analysis and interpretation may be conducted in the Institute of Human Genetics at Charité (University of Berlin) where both the applicant fellow and his supervisor have acquired an international expertise Moreover, they collaborate tightly with strong bioinformatics group which is experienced in evaluation of large scale genetic data [3, 4]. We are planning to analyze whether larger genomic rearrangements such as an inversion or translocations are present which could explain for example the misexpression of CCDC67. Furthermore, mutations in other intergenic and potential regulatory structures will be investigated.

After the identification of the causative mutation, we plan to perform functional investigations in in vitro and in vivo models. We are experienced in cells culture driven investigations of genetic disease [5] and also the generation of mouse models using for example CRISPR/Cas is established in our lab and in the collaboration group at the Max-Planck Institute of Molecular genetics.

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Gabsang Lee, PhD, DVM
Johns Hopkins University, Baltimore, MD USA
Detailed transcriptional analysis of stage-specific early FSHD myogenesis
$70,977 for 1 year
01/01/2015-12/31/2015

PROJECT SUMMARY (Provided by Applicant)

The pathogenesis of facioscapulohumeral muscular dystrophy (FSHD) pathogenesis is complex and not yet fully understood. Recent detailed genetic studies have significantly increased our knowledge of this enigmatic and multifaceted disorder, suggesting the aberrant genetic events during very early myogenesis.

The establishment of human induced pluripotent stem cells (hiPSCs) ushered a new era in biomedicine and provide unprecedented opportunities for modeling human genetic diseases. The Lee lab has developed a novel strategy to direct the hiPSCs into myoblasts as well as gene-targeting approach with CRISPR/Cas9 system.

Here we propose to isolate pluripotent cells, somite cells and myoblast cells of FSHD-hiPSCs using the established techniques, followed by detailed transcriptional analysis. Our proposed studies will shed light on the FSHD pathogenesis in stage-specific manner during very early human myogenesis.

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Jun Udaka, MD, PhD
University of Massachusetts Medical School, Worcester, MA USA
Physiological Studies of Muscle Weakness in FSHD
$212,060 for 2 years
12/01/2014-11/30/2016

PROJECT SUMMARY (Provided by Applicant)

The pathophysiology of Facioscapulohumeral Dystrophy (FSHD) is poorly understood and understudied. This FSH Society fellowship award to Dr. Jun Udaka will support comprehensive and detailed physiological investigations of force generation and calcium signaling in skinned and permeabilized single fiber preparations ofFSHD and unaffected control muscle biopsies. Proposed studies address whether FSHD muscles are defective in: 1) troponin-mediated Ca2+ signaling for myofibrillar force generation, 2) sarcoplasmic reticulum (SR) calcium release, 3) myosin ATPase activity, and/or 4), contraction fatigue. Additional studies investigate the roles oftitin and extracellular matrix (ECM) in muscle fiber elasticity as contributing factors in FSHD disease pathology. Findings wiII identify muscle proteins and contractile processes that become dysfunctional during FSHD disease progression and reveal the underlying pathophysiology of FSHD muscle weakness to enable the development and evaluation of FSHD therapeutics in pre-clinical and clinical studies.

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Françoise Helmbacher, PhD
Centre National de la Recherche Scientifique, Marseille, France

Deciphering the contribution of FAT1-dependent phenotypes to FSHD symptoms and relevance for therapeutic design

$138,803 for 2 years
02/01/2015-01/31/2017

PROJECT SUMMARY *(Provided by Applicant)*

FSHD is a hereditary human muscular dystrophy affecting groups of muscles in the face and shoulder, characterized by the asymmetry of these muscle symptoms, and additional non-muscular symptoms including hearing loss and retinal vascular abnormalities. FSHD is caused in most cases by chromosomal abnormalities at 4q35, leading to excess production of a transcription factor, DUX4, thereby triggering a cascade of gene de-regulations. However, although necessary, DUX4 activation is not sufficient to trigger the symptoms on its own, implying the existence of disease modifiers necessary for the symptoms to appear. My team studies neuromuscular development and the pathologies resulting from alterations of these processes. We have recently started to work on Facioscapulohumeral muscular Dystrophy (FSHD), a hereditary human myopathy characterized by degeneration of muscles in the face and shoulder area, after having found that disruption of the Fat1 cadherin gene in mice caused muscular and non-muscular symptoms resembling those of FSHD, and shown that alterations of the FAT1 locus in humans, located near the FSHD critical region on chromosome 4q35, were associated with FSHD, identifying FAT1 as a modifier gene in FSHD and as a key player of muscular pathologies ([1] : Caruso, PLoS Genetics, 2013).

Fat1 ablation in mice causes abnormalities in shape of selective groups of muscles and leads to regionalized muscle wasting at postnatal stages, the map of affected muscles being highly similar to the map of muscles affected in FSHD [1]. Such a possibility was investigated in collaboration with Pr. N. Levy and M. Bartoli, La Timone, Marseille, and J. Dumonceaux, Myology Institute, Paris. a) We found reduced FAT1 expression levels in muscles of fetal [1], but also adult ([2]: Mariot et al. submitted) FSHD cases; b) We identified human mutations in the FAT1 locus segregating with FSHD: i) Heterozygous deletions of a putative regulatory enhancer, predicted to cause tissue-specific depletion of FAT1, co-segregate with FSHD [1]; ii) heterozygous point mutations, either perturbing splicing of FAT1, or leading to deleterious aminoacid changes, were found in FSHD-like patients carrying neither 4q35 alterations nor mutations in SMCHD1 ([3]: Puppo et al., under revision, coll Bartoli/Levy). Thus, FAT1 is a compelling novel FSHD modifier gene, which tissue-specific loss-of-function is sufficient to recapitulate FSHD-like symptoms on its own, and which deregulation was found to co-occur with FSHD. This collaborative work was the objective of a network grant from FSHD Global for which I was the coordinator. In addition, part of this work was also supported by the FSH society through a grant to V. Mariot, working with J. Dumonceaux. My group first initiated work aimed to elucidate in which cell type FAT1 functions were relevant to FSHD-like phenotypes. Through cre/lox-mediated ablation of Fat1 functions in premigratory myoblasts (Pax3-cre), we showed that Fat1 is required in the myogenic lineage to control myoblast migration polarity [1]. We are currently studying the consequences in developing embryos and adult mice of ablating Fat1 in muscle, neurons and mesenchyme. This work has previously received support from the FSH society through a postdoctoral fellowship to Angela Zimmermann in my lab, and is being prepared for publication.

The present project aims to extend this work through following approaches: 1) Using CRISPR/Cas9 technology in ES cells, we plan to generate two mouse models carrying FAT1 alterations found in FSHD-like patients. We will select mutations that altered FAT1 splicing, as these could be corrected (in vitro) with antisense oligonucleotides (AON). Ultimately, we aim to evaluate the capacity of such splicing-correcting-AON to alleviate muscle symptoms in the resulting humanized mice. 2) We will evaluate the relative frequency of any genetic alteration occurring in the FAT1 locus among classical FSHD1 patients, with particular focus on patients with retinal vascular symptoms, also present in Fat1-deficient mice, to determine whether alteration of FAT1 expression occurs as a result of DUX4 expression, or synergizes with DUX4 expression to cause FSHD symptoms. Results of this project will help understanding to what extent the phenotypes caused by perturbations of FAT1 functions contribute to the appearance of FSHD symptoms, and will be instrumental to elaborate novel therapeutic strategies for FSHD patients.

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Awards for February 2014 Cycle

On June 20 & 23, 2014, the Scientific Advisory Board (SAB) of the FSH Society, chaired by David Housman, Ph.D., held two sub-reviews and a final SAB review. The SAB reviewed the grant applications and progress reports for the August 2014 round. By June 30, 2014, the FSH Society Board of Directors reviewed and approved the SAB recommendations for funding. Below is a list of the funded projects, including project descriptions as submitted by the applicants. For the February 28, 2014, round of grant applications six grants applications were received; two were funded in the amount of $168,150. An additional conference grant was approved by the SAB and Board of Directors in the amount of $25,000.

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Sujatha Jagannathan, Ph.D. / Stephen Tapscott, M.D., Ph.D.
Fred Hutchinson Cancer Research Center, Seattle, Washington USA
Novel Role for Reduced RNA Quality Control in FSHD Pathogenesis
$116,725 over 2 years

PROJECT 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.

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Francis M. Sverdrup, Ph.D.
Center for World Health & Medicine, Saint Louis University, Saint Louis, Missouri USA
BET Proteins as Therapeutic Targets in FSHD
$51,425 over 1 year

PROJECT SUMMARY

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.

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Rabi Tawil, MD
University of Rochester Medical Center, Rochester, New York USA
FSHD Clinical Trials Readiness Network Meeting Proposal
$25,000 over 1 year

PROJECT SUMMARY

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 Nijmegen, Nijmegen, 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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