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FSH Society, 450 Bedford Street, Lexington, MA 02420, phone: (781) 275-7781, before the
United States Senate Appropriations Committee,
Subcommittee on Labor, Health and Human Services, Education and Related Agencies on the subject of
**\$21 million in FY2016 Appropriations for U.S. DHHS National Institutes of Health
NIH Research Programs on Facioscapulohumeral Muscular Dystrophy (FSHD) March 31, 2015**

Agency: National Institutes of Health (NIH)

Account: National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) and the National Institute of Neurological Disorders and Stroke (NINDS) and the Eunice Kennedy Shriver National, Institute of Child Health and Human Development (NICHD) and the National Human Genome Research Institute (NHGRI)

FY 2016 Report Language: The Committee strongly encourages the NIH to accelerate research efforts and significantly increase projects and funding on what may be the most common form of muscular dystrophy facioscapulohumeral muscular dystrophy (FSHD). The Committee hopes and recognizes that scientific opportunities and recent epigenetic breakthroughs in FSHD will help advance treatments and access to therapies for this and many other grave diseases.

Honorable Chairman Blunt and Ranking Member Murray, thank you for the opportunity to submit this testimony. Facioscapulohumeral muscular dystrophy (FSHD), is one of the most common adult muscular dystrophies with a prevalence of 1:8,000.¹ FSHD is a rare disease or an orphan disease (according to U.S. criteria it affects fewer than 200,000 people). For approximately 870,000 men, women, and children worldwide the major consequence of inheriting this genetic form of muscular dystrophy is a lifelong progressive loss of all skeletal muscles. FSHD predominantly initially affects muscles in the face, trunk and upper extremities. FSHD is a crippling and life shortening disease. It can affect multiple generations and entire families.

With FSHD there is a loss of muscle strength that ranges between one and four percent a year during a lifetime. In terms of functional impairment, 20 percent of FSHD-affected individuals over age fifty will require the use of a wheelchair. FSHD also has very specific non-muscular manifestations; hearing-loss, respiratory, cardiac (arrhythmias) and vision. 95% of individuals with FSHD have the FSHD1 (OMIM: 158900) genetic variation -- caused by the contraction of DNA macrosatellite repeat units, termed D4Z4 repeats, on chromosome 4, leading to the release of transcriptional repression of a retrogene (DUX4) believed to be associated with the cause of disease. Of the 5% of FSHD individuals remaining, 80% of those are the FSHD2 (OMIM: 158901) genetic variation -- caused by mutation in the structural maintenance of chromosomes hinge domain 1 (SMCHD1) gene on chromosome 18p that helps to maintain the repressed-state structure of the D4Z4 repeats on the long arm of chromosome 4; which when mutated cause unwanted toxic and inappropriate DUX4 gene/protein expression.

The National Institutes of Health (NIH) is the principal source of funding of research on FSHD currently at the \$7 million level. For nearly two decades, this Committee has supported the incremental growth in funding for FSHD research. I am pleased to report that this modest investment has produced remarkable scientific returns.

1. Congress has made a major difference. I have testified many times before Congress, approximately fifty. When I first testified, we did not know the genetic mechanism of this disease. Now we do. Now we can target it. When I first testified, we assumed that FSHD was a rare. Now we understand it to be the most prevalent forms of muscle disease, based on new ways of evaluating the disease clinically within families. Congress is responsible for this success, through its sustaining support of the NIH and the enactment of the Muscular Dystrophy CARE Act. We are aware that MD Care Act does not set the amount of spending on FSHD or the other dystrophies at the NIH and we recognize that funding levels are determined in the appropriations process and the numbers of grant applications received and funded by the NIH on FSHD. Even though it is a technically separate legislative process, the reauthorization of the MD Care Act does raise the visibility of all the muscular dystrophies which can be of help in the appropriations process – and we thank you for your support of the MD Care Act amendments 2014. Given these requisites there are additional efforts and pathways that Congress can request and the NIH can enact to increase the amount of research funding on FSHD in the NIH portfolio that neither increases the NIH budget required nor takes money from another area of research and achieves more efficiency out of a non-growing research budget.

2. Quantum leaps in our understanding of FSHD. The past four and a half years have seen remarkable contributions made by a very small but dedicated tribe of researchers funded by NIH and non-profits.

- On August 19, 2010, American and Dutch researchers published a paper which dramatically expanded our understanding of the mechanism of FSHD.² A front page story in the *New York Times* quoted the NIH Director Dr. Francis Collins saying, "If we were thinking of a collection of the genome's greatest hits, this would go on the list."³ *FSHD patients carry specific single-nucleotide polymorphisms in the chromosomal region distal to the last D4Z4 repeat. This FSHD-predisposing configuration creates a canonical polyadenylation signal for transcripts derived from DUX4, a double homeobox gene of unknown function that straddles the last repeat unit and the adjacent sequence. Transfection studies revealed that DUX4 transcripts are efficiently polyadenylated and are more stable when expressed from permissive chromosomes. These findings suggest that FSHD arises through a toxic gain of function attributable to the stabilized distal DUX4 transcript.*²
- Two months later, another paper was published that made a second critical advance in determining the cause of FSHD. The research shows that FSHD is caused by the inefficient suppression of a gene that may be normally expressed only in early development.⁴ *The contraction of the D4Z4 repeat in FSHD results in a less efficient suppression of the full-length DUX4 mRNA [DUX4-fl] in skeletal muscle cells. Therefore, FSHD represents the first human disease to be associated with the incomplete developmental silencing of a retrogene array normally expressed early in development.*⁴
- On January 17, 2012, an international team of researchers based out of Seattle discovered a stabilized form of a normally suppressed gene called DUX4 affects many different germline genes, retroelements, and immune mediators; all potential targets.⁵ *We identify genes associated with germline and early stem cell development as targets of the DUX4 transcription factor, a leading candidate gene for FSHD. The genes regulated by DUX4 are reliably detected in FSHD muscle but not in controls, providing direct support for the model that misexpression of DUX4 is a causal factor for FSHD.*⁵
- Six months later, another high profile paper produced by a Senator Paul A. Wellstone Cooperative Research Center of the NIH (mandated by MD CARE Act), used sufficiently "powered" large collections of genetically matched FSHD cell lines generated by the NIH center that are both unique in scope and shared with all researchers worldwide, to improve on the Seattle group's finding by postulating that DUX4-fl expression is necessary but not sufficient by itself for FSHD muscle pathology.⁶ *We confirmed that stable DUX4-fl mRNA and protein were expressed in myogenic cells and muscle tissues derived from FSHD affected subjects, including several genetically diagnosed adult FSHD subjects yet to show clinical manifestations of the disease in the assayed muscles. In addition, we report DUX4-fl mRNA and protein expression in muscle biopsies and myogenic cells from genetically unaffected relatives of the FSHD subjects, although at a significantly lower frequency. These results establish that DUX4-fl expression per se is not sufficient for FSHD muscle pathology and indicate that quantitative modifiers of DUX4-fl expression and/or function and family genetic background are determinants of FSHD muscle disease progression.*⁶
- On July 13, 2012, a team of researchers from the United States, Netherlands and France identified mutations in a gene causing 80% of another form of FSHD called FSHD1B or FSHD2. This paper furthers our understanding of the molecular pathophysiology of FSHD. This work too was supported in part by a program project grant from NIH.⁷ *FSHD2 occurs in individuals who inherited both the SMCHD1 mutation and a normal-sized D4Z4 array on a chromosome 4 haplotype permissive for DUX4 expression. Reducing SMCHD1 levels in skeletal muscle results in D4Z4 contraction-independent DUX4 expression. Our study identifies SMCHD1 as an epigenetic modifier of the D4Z4 metastable epiallele and as a causal genetic determinant of FSHD2 and possibly other human diseases subject to epigenetic regulation.*⁷
- On September 25, 2014, researchers from United States, France, Spain, Netherlands and United Kingdom narrow the focus mechanistically opening the possibility of all types of FSHD having an epigenetic basis.⁸ *In FSHD1, for individuals with D4Z4 repeat arrays of 1-6 units, the clinical severity mainly depends on the size of the D4Z4 repeat. However, in individuals with arrays of 7-10 units, the clinical severity also depends on other factors that regulate D4Z4 methylation because affected individuals, but not non-penetrant mutation carriers, have a greater reduction of D4Z4 CpG methylation than can be expected based on the size of the pathogenic D4Z4 repeat array. In FSHD2, this epigenetic susceptibility depends on the nature of the SMCHD1 mutation in combination with D4Z4 repeat array size with dominant negative mutations being more deleterious than haploinsufficiency mutations. Our study thus identifies an epigenetic basis for the striking variability in onset and disease progression that is considered a clinical hallmark of FSHD.*⁸
- On March 29, 2015, different researchers involved with the NIH Senator Paul A. Wellstone Cooperative Research Center using its large collection of different FSHD patient samples and different techniques arrive at the same answer that there is an underlying principle of epigenetics defining asymptomatic or non-manifesting and playing a role in disease severity.⁹ *The epigenetic status of the distal 4qA D4Z4 repeat correlates with FSHD disease; FSHD-affected subjects have hypomethylation, healthy unaffected subjects have hypermethylation, and non-manifesting subjects have characteristically intermediate methylation. Thus, analysis of DNA methylation at the distal D4Z4 repeat could be used as a diagnostic indicator of developing clinical FSHD. In addition, the stability of epigenetic repression upstream of DUX4 expression is a key regulator of disease and a viable therapeutic target.*⁹

Many of these researchers have started their efforts in FSHD with seed funding from the FSH Society and have received continued support from the FSH Society, the NIH, and the Muscular Dystrophy Association and other partners. In simpler terms, the above research shows that our own genes within us are being inappropriately expressed in tissue at a time and place where they do not normally reside or function by a confluence of events in a variety of ways giving rise to the decay and destruction of skeletal muscle; and we begin to focus on the very narrow stretch of DNA down to the nucleotide level in an area adjacent to the toxic gene inappropriately turned on so-named DUX4-fl. You might think of it as the opposite of cancer rather than runaway genes causing unbridled cell

division; runaway genes are causing unbridled cell death. What is fascinating is, though one has all the requisites to have FSHD (e.g. the presence of a chromosome 4qA containing a DUX4 polyadenylation signal; and either a truncation of D4Z4; or a SMCHD1 mutation with D4Z4 repeat array with array sizes at the lower end of the normal repeat size spectrum) there are modifiers that allow a person to have a severe course of disease whilst other genetically tested positive relatives are spared of disease symptoms e.g. methylation. We can see clearly now that the stability of epigenetic repression by the region just upstream of DUX4 gene on the very last distal D4Z4 repeat, regardless of which route DUX-fl was stabilized and presented FSHD1, FSHD2, FSHD3, etc., is a key regulator that can be modified perhaps via its methylation level/status. We can see clearly that FSHD2 modifies FSHD1 in individuals who carry both mutations presenting even more severe disease. Even more remarkably, we know of and we have compounds and techniques to modify and target modifiers and expression of DUX-fl, and still the FSHD research and clinical enterprise is starved for federal funding from NIH! In 2014, the FSH Society funded projects to silence the DUX4 gene using leading-edge genome-editing technologies (CRSPR/Cas9, TALEN), helped support efforts in development efforts and models to test anti-sense oligonucleotide (ASO) and morpholino and we aided the development of animal models and a novel method that we believe will revolutionize FSHD diagnostics. We are thrilled that our grantees and colleagues have data that proves that DUX4-fl and cascading events can be turned off.

3. We must keep moving forward. In October the FSH Society held its annual FSHD International Research Consortium meeting in San Diego, California. The meeting was funded in part by the NIH NICHD University of Massachusetts Medical School Wellstone center for FSHD. Nearly 80 researchers from around the world gathered to present latest data and discuss research strategies. There was considerable progress achieved when comparing to the 2013 agenda. The discussion agenda focused on being prepared for intervention development and clinical readiness. To keep the discussion focused, we followed the path: Genetics > Mechanisms and targets > Models > Patients. For each area, an expert moderator was nominated. The priorities stated for 2015, at the October 18, 2014, FSH Society FSHD IRC meetings can be found at: <http://www.fshsociety.org/international-research-consortium/>

Additionally, on March 17th, 2015, the FSH Society presented to the federal advisory committee mandated by the MD CARE Act called the Muscular Dystrophy Coordinating Committee (MDCC) its concern about the small number of NIH grants and that much greater funding is required to address the most pressing challenges for FSHD research, including research on the following topics:

- Mechanisms of DUX4 toxicity
- More molecular, imaging and functional markers of disease progression
- Modifiers of disease: genetic, chemical, and lifestyle
- Preclinical models validated to represent aspects of FSHD pathophysiology
- Better animal models based on low expression of DUX4 as seen in patients
- Mechanisms of pathology in patients' muscles
- Normal functions of DUX4 in tissues other than muscle
- Methods of administering anti-DUX4 agents to muscle
- Muscle regeneration capacity in FSHD muscles
- Large animal models (monkey, marmoset)
- Biomarkers that can indicate impact of therapeutic agents.

We need to be prepared for this new era in the science of FSHD. Many leading experts are now turning to work on FSHD because it represents the potential for great discoveries, insights into stem cells, transcriptional processes, new ways of thinking about disease of epigenetic etiology, and for treating diseases with epigenetic origin.

4. NIH Funding for Muscular Dystrophy. Mr. Chairman, these major advances in scientific understanding and epidemiological surveillance are not free. They come at a cost. Since Congress passed the MD CARE Act in 2001, research funding at NIH for muscular dystrophy has increased 4-fold (from \$21M). While FSHD research funding has increased 14-fold (from \$0.5M) during this period, the level of funding is still anemic and, for FSHD, has been astonishingly flat for the past seven years.

FSHD Research Dollars (in millions) & FSHD as a Percentage of Total NIH Muscular Dystrophy Funding

Sources: NIH/OD Budget Office & NIH OCPL & NIH RePORT RCDC (e = estimate)

Fiscal Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015e	2016e
All MD (\$ millions)	\$38.7	\$39.5	\$39.9	\$47.2	\$56	\$83	\$86	\$75	\$75	\$76	\$78	\$79	\$81
FSHD (\$ millions)	\$2.2	\$2.0	\$1.7	\$3	\$3	\$5	\$6	\$6	\$5	\$5	\$7	\$7	\$7
FSHD (% total MD)	6%	5%	4%	5%	5%	6%	7%	8%	7%	7%	9%	9%	9%

Despite the great success of the past four and a half years in the science of FSHD brought about by Congress we are concerned that under the current funding environment that new research projects will not be funded or existing programs will not be renewed. We are already seeing this play out with some of the larger program projects in FSHD. We have conveyed to the NIH leadership at the Office of the Director, NIAMS, NINDS, NICHD, NHLBI and the Executive Secretary of the MDCC our grave concern that FSHD research is way too under-represented in the NIH portfolio and needs a proactive effort on the part of NIH. At the March 17, 2015, MDCC meeting we re-iterated to Alan E. Gutmacher, MD., Director, NICHD and Chair of the MDCC and all MDCC members that we are fully supportive of his efforts and the Action Plan for Muscular Dystrophy; while at the same time we requested that NIH redress the imbalance of funding in the muscular dystrophy portfolio by fostering opportunities for multidisciplinary research on FSHD commensurate with its prevalence and disease burden. The future action plan and NIH activity should address this issue head-on. We are stunned if not baffled that while on one hand, five years ago, NIH Director Dr. Francis Collins said "If we were thinking of a collection of the genome's greatest hits, this [FSHD] would go on the list"³ that -- on the other hand, the National Human Genome Research Institute (NHGRI) has only one R01 on FSHD!

In the last year alone, incredible opportunities for public, private and non-profit entities engaged in FSHD research and clinical research have emerged. Oddly these discoveries clearly belonging to the leading edge of human genetics and our understanding of the epigenome and treating epigenetic diseases are sitting idle at NIH. NIH needs to see through the thick fog of flat funding and austerity and maximize research funding by capitalizing on the low hanging fruit that FSHD presents as a gateway to treating human epigenetic disease. There are 26 active projects NIH-wide as of March 12, 2015, according to the NIH Research Portfolio Online Reporting Tools (RePORT) <http://report.nih.gov> within these 26 are: one F32 training grant, 6 R21, 12 R01, 1 P01, 2 U01, and 2 U54. NHGRI has only one R01. NHLBI has none. NICHD has no R21, no R01. In last 25 years, NIH has funded 76 grants in FSHD including only three training grants, 18 R21, and 25 R01. Remarkably, no grants ever on FSHD from key institutes studying heart, lung, blood, hearing, and vision.

While all muscular dystrophy research funding at NIH increased from \$39.9 million in 2006 to \$81 million; FSHD increased from \$1.7 million to \$7 million. The economy of scale is so vastly different in particular for FSHD, being equally devastating and burdensome as the disease receiving the most funding in this category called muscular dystrophy, and though it functions in the exact same U.S. federal research infrastructure. **We request for FY2016, a tripling of the NIH FSHD research portfolio to \$21 million or a level of approximately 25% of the total estimated muscular dystrophy funding at NIH.** This will allow an expansion of basic research awards, expansion of post-doctoral and clinical training fellowships, dedicated centers to design and conduct clinical trials on FSHD and more U.S. DHHS NIH Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Centers.

We are aware of the great pressures on the federal budget, but NIH can easily help increase its portfolio on FSHD given the breakneck speed of discovery in FSHD. These are easy ways for NIH to convey to researchers that it has a revised plan and an interest in funding research in FSHD. There are no quotas on peer-reviewed research above pay line at the NIH, and NIH can help by issuing written announcements that efforts invested in writing FSHD grant applications will be met with interest. This is the time to fully and expeditiously exploit the advances in the best scientific opportunities for which the American taxpayer has paid. Thank you for this opportunity to testify before your committee.

Footnotes:

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4. Snider, L., Geng, L.N., Lemmers, R.J., Kyba, M., Ware, C.B., Nelson, A.M., Tawil, R., Filippova, G.N., van der Maarel, S.M., Tapscott, S.J., and Miller, D.G. (2010). Facioscapulohumeral dystrophy: incomplete suppression of a retrotransposed gene. *PLoS Genet*. 6, e1001181
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7. Lemmers, RJ, et al, Digenic inheritance of an SMCHD1 mutation and an FSHD-permissive D4Z4 allele causes facioscapulohumeral muscular dystrophy type 2. *Nat Genet*. 2012 Dec;44(12):1370-4. Epub 2012 Nov 11
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