Testimony of Daniel Paul Perez, Co-Founder & Director Emeritus and past Chairman, President & Chief Executive Officer, Chief Scientific Officer, FSHD Society before U.S. Senate Appropriations Subcommittee on Labor, HHS, Education and Related Agencies June 24, 2021

Honorable Chairwoman Murray, Ranking Member Blunt, and distinguished members of the Subcommittee, thank you for the opportunity to testify. We are requesting the FY2022 appropriation of an *amount* of **$33 million** for the *agency* U.S. DHHS National Institutes of Health (**NIH**) *program* on research specifically directed at facioscapulohumeral disease and facioscapulohumeral muscular dystrophy (*hereafter called* **FSHD**).

FSHD is a heritable disease and one of the most common neuromuscular disorders with a prevalence of 1:8,000.[1] It affects 934,000 children and adults of both sexes worldwide. FSHD is characterized by progressive loss of skeletal muscle strength that is asymmetric in pattern and widely variable. Muscle weakness typically starts at the face, shoulder girdle and upper arms, often progressing to the legs, torso and other muscles. In addition to affecting muscle it can bring with it respiratory failure and breathing issues[62], mild-profound hearing loss, eye problems and cardiac bundle blockage and arrhythmias[79]. FSHD causes significant disability and death according the U.S. Centers for Disease Control and Prevention (CDC), National Center on Birth Defects and Developmental Disabilities, Atlanta, Georgia and others.[80,81]

FSHD is associated with epigenetic changes on the tip of human chromosome 4q35 in the D4Z4 DNA macrosatellite repeat array region leading to an inappropriate gain of expression (function) of the D4Z4-embedded double homeobox 4 (DUX4) gene[2]. DUX4 is a transcription factor that kick starts the embryonic genome during the 2- to 8-cell stage of development[3-5]. Ectopic expression of DUX4 in skeletal muscle is associated with the disease and the disease's pathophysiology that leads to muscle death. DUX4 is never expressed in 'healthy' muscle. FSHD has had few clinical trials[6-10], and currently there is no cure or therapeutic option available to patients. DUX4 requires and needs to activate its direct transcriptional targets for DUX4-induced gene aberration and muscle toxicity[11-24]. The genetics of FSHD are so remarkable, that NIH Director Dr. Francis Collins said on the front page of the New York Times, "If we were thinking of a collection of the genome's greatest hits, this [FSHD] would go on the list."[78]

Blocking DUX4's DNA, DUX4's RNA or DUX4's protein ability to activate its targets has profound therapeutic relevance[25]. The FSHD scientific community has in recent years pioneered inroads to treating FSHD using the enormous potential of genomic sequencing, genomic medicine, gene editing and next generation diagnostics. Table 1 lists a dozen approaches detailed in thirty-eight proof-of-concept publications that molecular and genetic treatment approaches work in cellular and animal models for FSHD. All with the central paradigm of the reduction of: DUX4, DUX4 expression, DUX4 protein activity, or the effects of DUX4-mediated toxicity. Strategies include modulating DUX4 repressive pathways, targeting DUX4 mRNA, DUX4 protein, or cellular downstream effects of DUX4 expression. Simply unfathomable as to why NIH funding is this area is not increasing with the pace of discovery.

TABLE 1: Genetic Approaches with Potential to Treat FSHD

- Targeting the DUX4 gene itself by repression using CRISPR/dSaCas9 or CRISPR/dCas9-KRAB;
- Targeting and correcting the FSHD2 SMCHD1 gene mutation with CRISPR/Cas9;
- Knockdown and silencing of the DUX4 gene by going after DUX4 mRNA with antisense oligonucleotides and with RNA interference; U7-asDUX4 snRNAs;
- Targeting DUX4 protein expression using through DNA aptamers; proteins homologous to DUX4; and DNA decoys;
- Going after and controlling expression target downstream [post-expression] of DUX4;
- Going after genetic modifiers of DUX4 expression and DUX4-mediated toxicity between the DUX4 gene and DUX4 mRNA; G-quadruplexes (GQs); and
- Targeting proteins that perturb DUX4-mediated toxicity or secondary features of FSHD pathology.[26-63]

The clinical trials readiness priorities remain similar to last year's testimony. The FSHD scientific community has listed emphasis areas as: 1.) clinical trials readiness infrastructure and therapeutics; 2.) direct and surrogate

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biomarkers; 3.) genetic testing, genetics and epigenetics; 4.) imaging and outcome measures; and, 5.) registries and patient focused and reported outcomes.[73] The way to measuring disease progression and the effectiveness and safety of drugs remains deep and hard-going for industry, clinical partners and patients.

Serendipitously, new NextGen genomic sequencing and diagnostic technologies, as well as gene-targeted therapeutic approaches have emerged that will be game changing for FSHD patients and families. Understanding one's disease or condition is key for both mental and physical health. This can also aid with family and life planning decisions. With certainty many barriers to matching FSHD disease severity to outcome measures would rapidly fall. We could better align drug and therapeutic modalities with proper phenotypic/genotypic silos of FSHD based on repeat unit, methylation ranges and other requisites for FSHD. The current testing approach in the US, albeit excellent, has created a drag on the momentum towards clinical trials. With therapies on the way, identifying asymptomatic carriers and those that will decades later have later onset or mild symptoms, will allow us to then halt the disease in its early formative stages.[64,66-69,72]

Recently in 2021, two excellent papers were published on FSHD and DUX4. Both were outstanding – one was using Oxford Nanopore long read sequencing of direct-RNA to locate DUX4 gene targets and the other was a careful study of DUX4 expression in its endogenous [native] form versus the more common recombinant [created] form used in the laboratory.[70,71] As I read, I asked myself of each: "does this tell us anything more about what DUX4's function is? No. How DUX4 works? Nada. Or how DUX4 causes FSHD pathophysiology? Nothing at all. How and if DUX4 itself is toxic to skeletal muscle? Zilch. If all research using FSHD transgenic cells an animals is simply result of an artifact? Not sure now." Both papers yield the same thought: though DUX4 is the prime therapeutic target -- we know next to nothing about it. It is still a complete black box; yet the central focus for FSHD therapy. Questions and areas of research interest emerge from these publications and allied considerations; flowing fast -- each one hypothesis worthy of several NIH grants. "Is DUX4 cytotoxicity pathogenic in vivo? How does expression of DUX4 lead to muscle loss? What is the role of non-muscle cells in FSHD pathology? Can muscle pathology be stopped once it has started (as visualized via MRI images) or is it too late? How is DUX4 bursting regulated in vivo? What other cell types express DUX4 in FSHD and/or healthy individuals? Does the DUX4 mRNA play a nuclear role in FSHD? Are there noncoding RNA roles for DUX4? Are DUX4 induced protein aggregates cause or consequence for FSHD? Does autoimmunity play a role in FSHD? Are there other DUX4-dependent therapeutic targets?" NIH should certainly encourage proposals here. New data/information generated on the basic mechanism of DUX4 and how it causes muscle disease has the potential to focus the design of future clinical trials on muscles and measurements that will increase the rigor of the design and decrease the number of individuals necessary for initial tests of drug activity. It is absolutely necessary to increase our resolution, clarity and understanding of what DUX4 is and what it does to muscle in FSHD. The gains in this area will effectively unpin or untether FSHD from the difficulty category of "slowly progressing neuromuscular diseases remaining recalcitrant" to timely ascertainment that a clinical intervention can work.

Your Subcommittee and **Congress in partnership with NIH, patients and scientists have made truly outstanding progress in understanding and treating the nine major types of muscular dystrophy through the** Muscular Dystrophy Community Assistance, Research and Education Amendments of 2001 (MD-CARE Act, Public Law 107-84). Since passing the MD CARE Act in 2001, NIH funding for FSHD has not kept up pace with scientific opportunities listed herein. The NIH is the principal worldwide source of funding of research on FSHD. Currently active projects are $16.554 million FY2022 (current actual 23June2021), a 21% portion of the estimated $80 million spent on all muscular dystrophies. (source: NIH Research Portfolio Online Reporting Tools (RePORT) keyword 'FSHD or facioscapulohumeral or landouzy-dejerine').

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, a=actual)

Fiscal Year	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**
All MD ($ millions)	$83	$86	$75	$75	$76	$78	$77	$79	$81	$81	$83	$88e	80e
FSHD ($ millions)	$5	$6	$6	$5	$5	$7	$8	$9	$11	$11	$10	$11e	$10e
FSHD (% total MD)	6%	7%	8%	7%	7%	9%	10%	11%	14%	14%	12%	13%	13%

We request for FY2022, a doubling of the NIH FSHD research portfolio to $33 million. At this moment in time, FSHD needs an infusion of NIH grants both submitted and funded. NIH needs to increase funding by adding exploratory / developmental research grants (parent R21) and research project grants (parent R01) in areas outlined by experts both in

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this testimony and in the *2015 DHHS NIH MD Plan*.[77] NIH can issue targeted funding announcements covering FSHD. These efforts will help NIH receive more grant applications. This is NIH's wheelhouse and forte without a doubt.

Madam Chairman, this is my sixty-second testimony before the U.S. Congress' Appropriations Subcommittee on this matter. My FSHD is a strong fort; it has lasted my lifetime of fifty-nine years. That is a long time to live with a disease of this burden.[80] I hope with your help and action to be able to outlive my disease. I need your help, my friends and fellow FSHD patients and families need your help. Please implore NIH to double funding on FSHD and kindly remember that our lives matter. Madam Chairman, thank you again for your help and efforts.

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