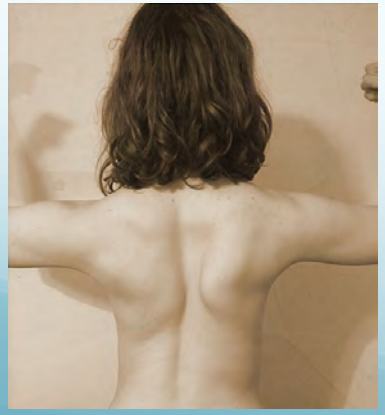
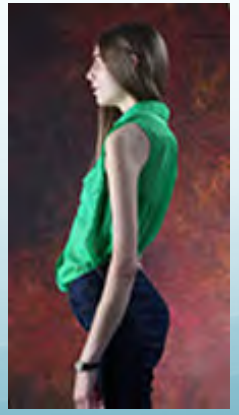
University of Nevada, Reno
School of Medicine
Department of Pharmacology

Emerging Treatment Strategies for FSHD

Peter L. Jones, Ph.D. and Takako I. Jones, Ph.D.
Co-Principal Investigators



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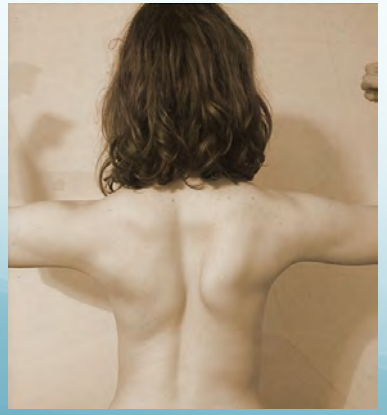
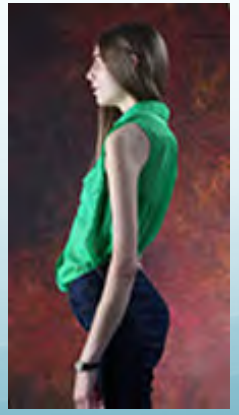
Disclosures:

Peter Jones and Takako Jones are listed as inventors on US patent applications for epigenetic diagnosis of FSHD, epigenetic therapeutic targets and CRISPR therapy for FSHD.

Peter Jones is on the SAB for Fulcrum Therapeutics and receives financial compensation






Rare Diseases as a group are not so rare
>90 Neuromuscular Diseases

~30 muscular dystrophies: progressive weakness and degeneration of the skeletal muscles that control movement.

Muscular dystrophy: 9 classes of disease

- **BMD (Becker)**
- **CMD (Congenital)**
- **DMD (Duchenne)**
- **DDM (Distal)**
- **EDMD (Emery-Dreifuss)**
- **FSHD (Facioscapulohumeral)**
- **LGMD (Limb-Girdle)**
- **MMD (Myotonic)**
- **OPMD (Oculopharyngeal)**

Aging can be considered a muscle disease

Big Picture Perspective

Research across the full spectrum of neuromuscular diseases leads to scientific and medical breakthroughs that accelerate treatments and cures. The power in this approach is that we can often apply learnings from one disease to progress in others to bring urgently-needed answers to affected patients and families.

Jones Lab expertise is epigenetics and developmental biology

Since 2003, our focus has been on FSHD, which we now know is an epigenetic-based disease

Epigenetics

"Treasure your exceptions."

Thomas Hunt Morgan

- **Non-Mendelian pattern of heritability**
- **Context-dependent sequence independent gene expression**
- **Can be influenced by the environment (diet, aging, etc…)**

Epigenetic differences can have profound long-term health consequences

Epialleles

A^{IAP} allele, methylated

Brown, normal

A^{IAP} allele, unmethylated

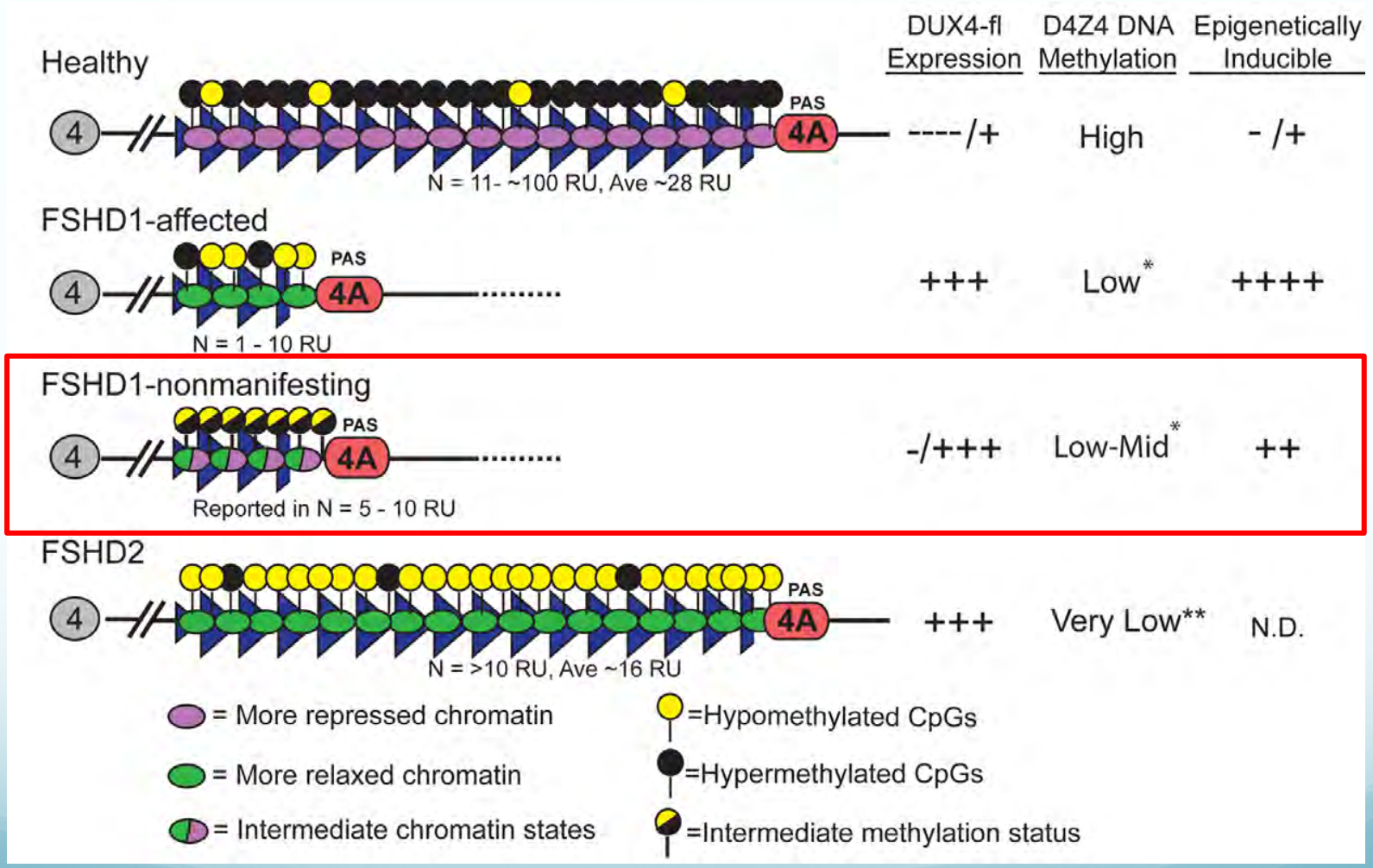
Yellow, obese, spontaneous tumors

Genetically identical
Epigenetically different

→ **Affects long-term health**
→ heritable?

Waterland and Jirtle, *Mol Cell Biol*, 2003

All types of FSHD are linked to epigenetic status of 4q35 D4Z4



C. Himeda *et al* 2014 *Antiox Redox Sig*
T. Jones *et al*. 2015 *Clinical Epigenetics*

FSHD Therapeutic Development in 2003

FSHD gene?	**Unknown**
Pathogenic mechanism?	**Unknown**
Cellular models?	**Not significant**
Animal models?	**Non existent**

Treatments: Steroids, myostatin inhibition
Rationale: ~work for DMD, so why not

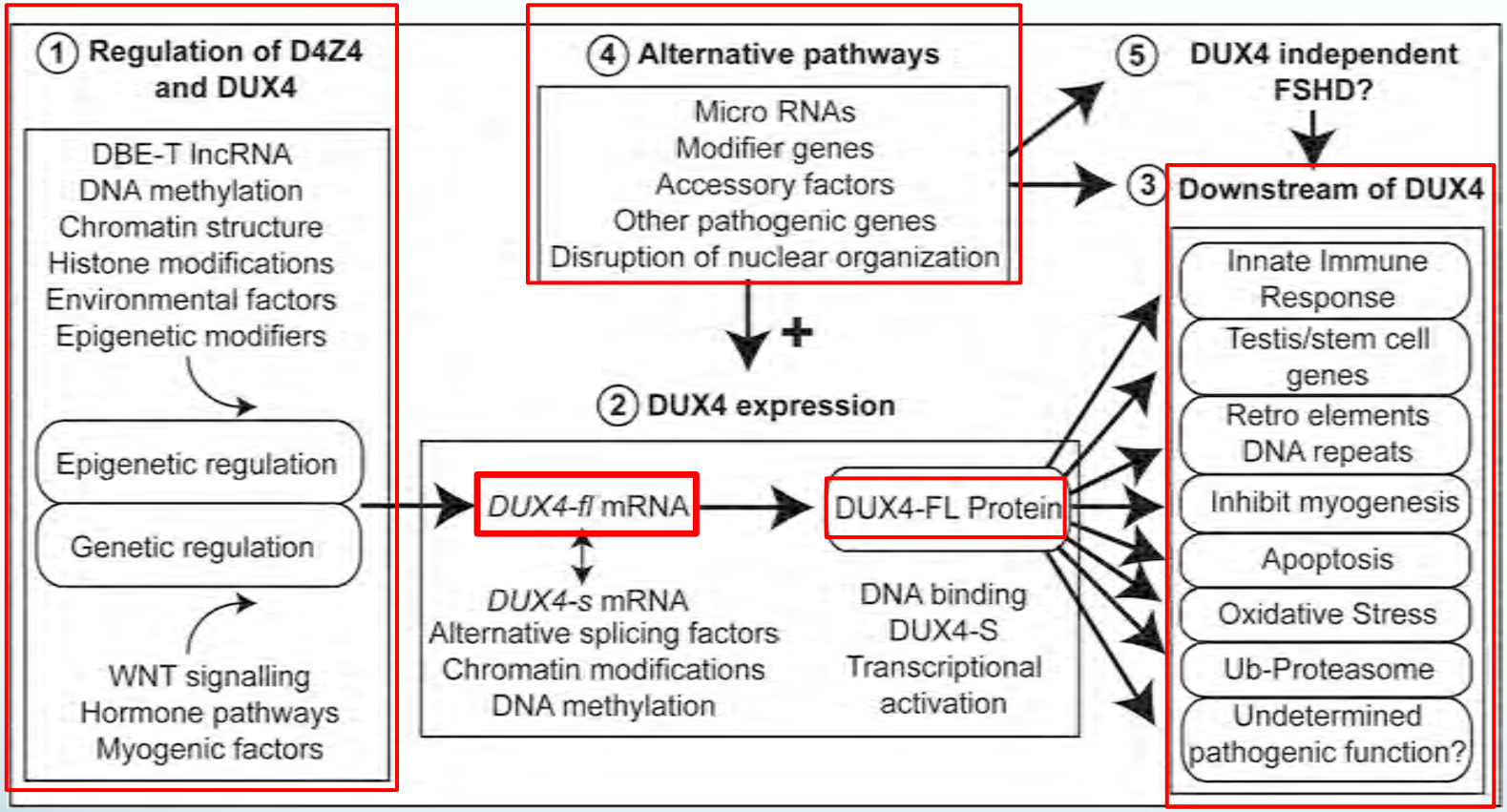
FSHD Therapeutic Development in 2017

FSHD gene?	**DUX4**
Pathogenic mechanism?	**Epigenetic dysregulation** **Still many possibilities**
Cellular models?	**Many**
Animal models?	**Mice, Fly, Zebrafish**

Treatments: **Myostatin inhibition, immune suppression**
Rationale: **FDA approved, basis in the biology**

FSHD in 2017

Many viable therapeutic approaches!



1,4 Small molecule inhibitors; CRISPR technology
→ prevent expression of *DUX4*

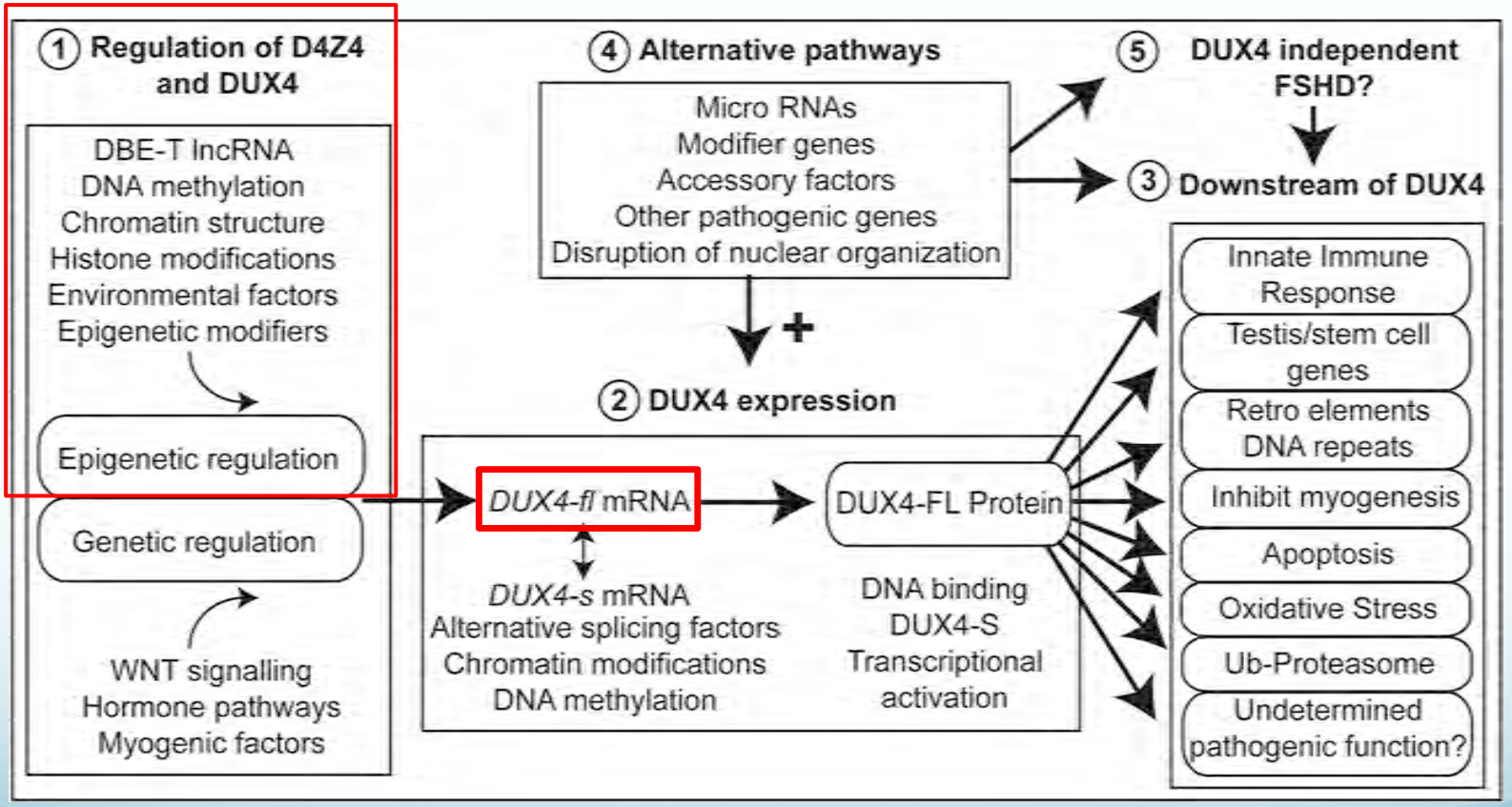
2 Anti-sense, morpholinos, PMOs, microRNAs
→ inactivate or destroy the DUX4-fl mRNA

3 Small molecule inhibitors
→ block downstream pathogenic effects of DUX4-FL protein (immune suppression; aTyr trial)

DUX4-independent approaches
→ Myostatin inhibition (Acceleron ACE-083 trial)

FSHD in 2017
Many viable therapeutic approaches!

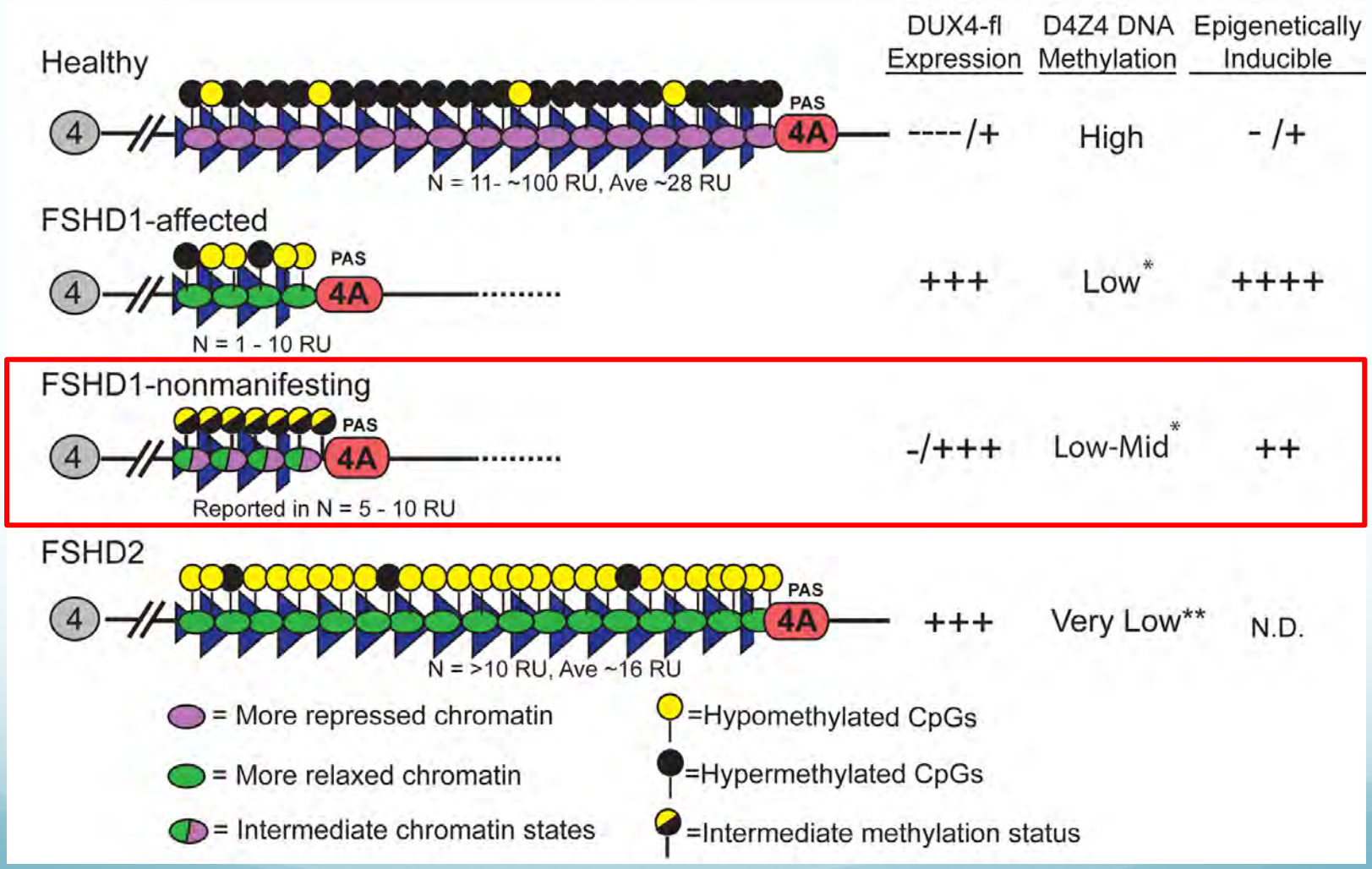
Jones Lab at UNRSOM



- **Small molecule epigenetic effectors**
- **CRISPR/dCas9 silencing**

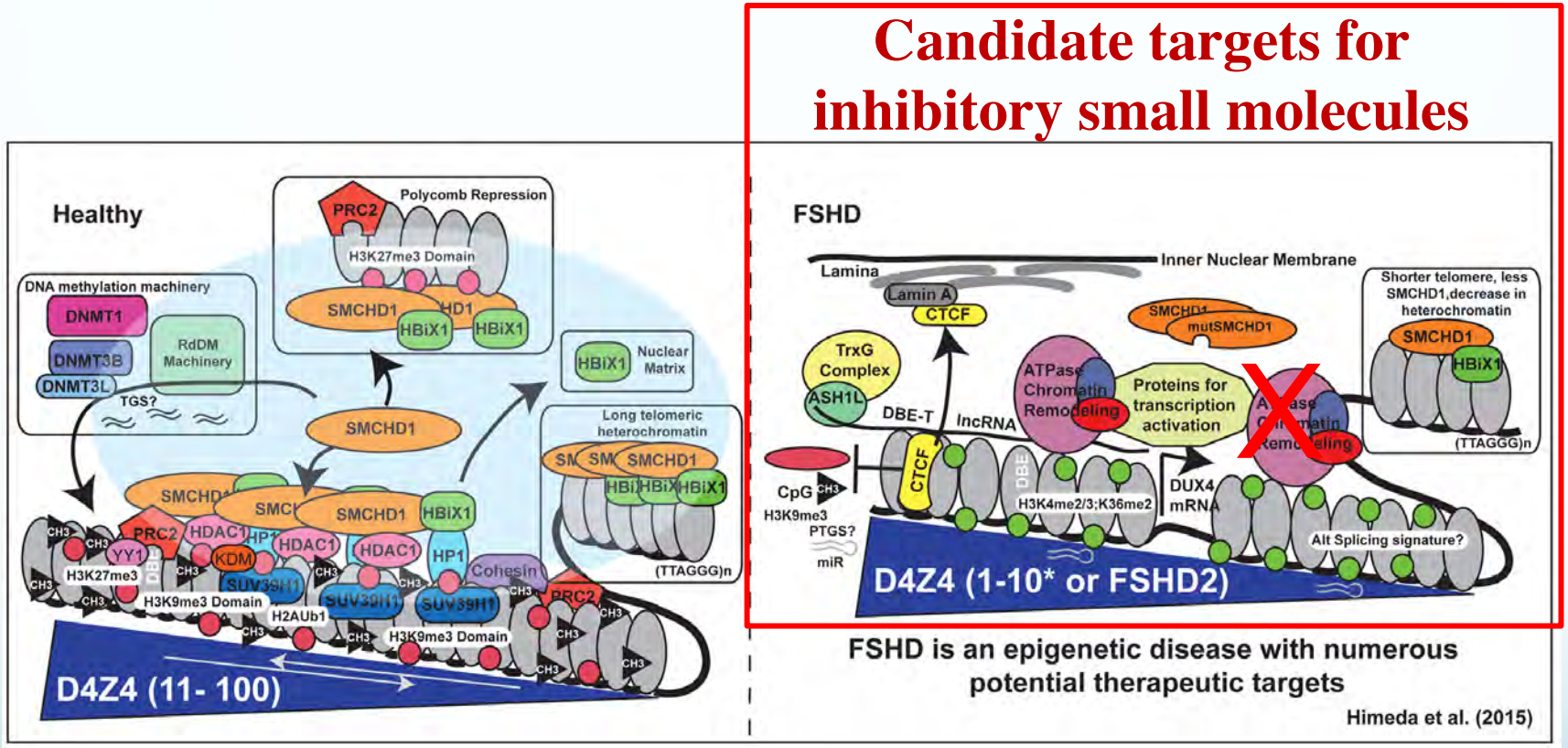
FSHD is an epigenetic disease

Can we therapeutically dial down DUX4 expression?



T Jones *et al*. 2015 *Clinical Epigenetics*

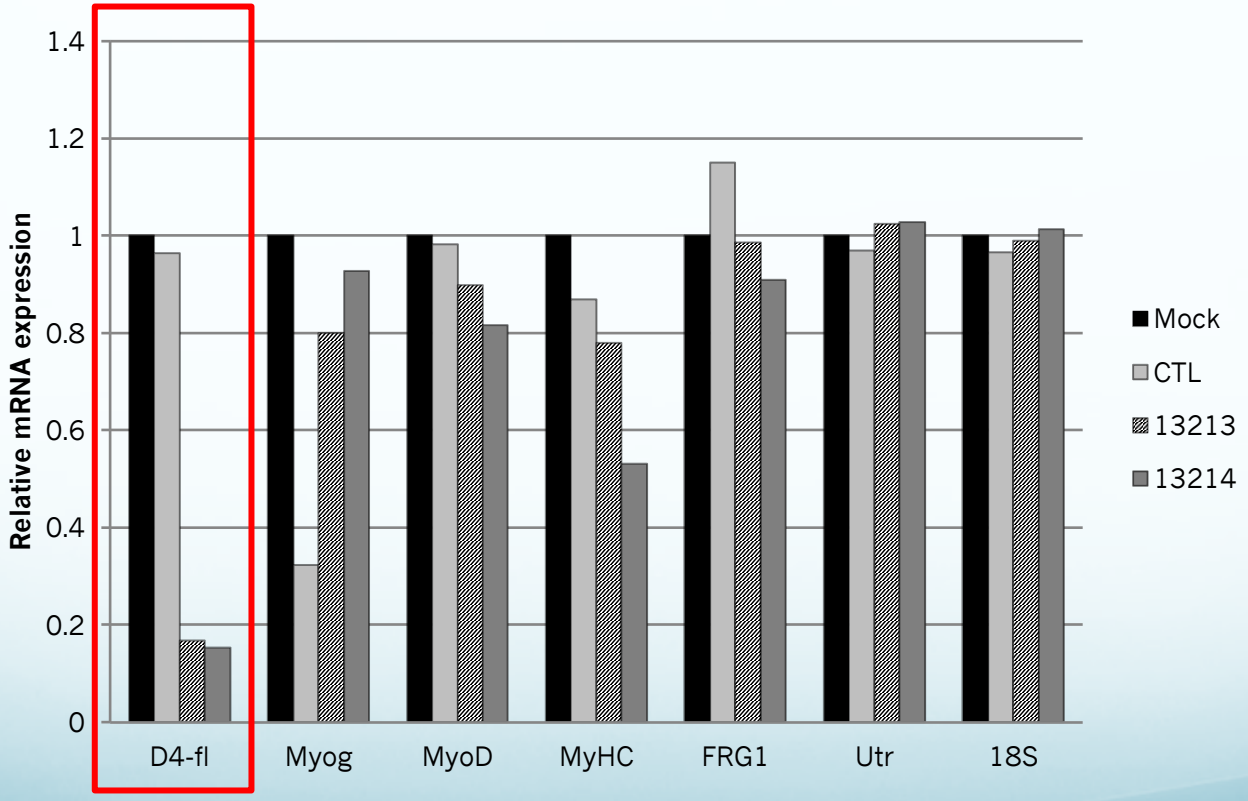
Epigenetic regulation at chrom 4q35 is distinct between healthy and FSHD



Asymptomatic ← ← ← ← ← ← ← FSHD

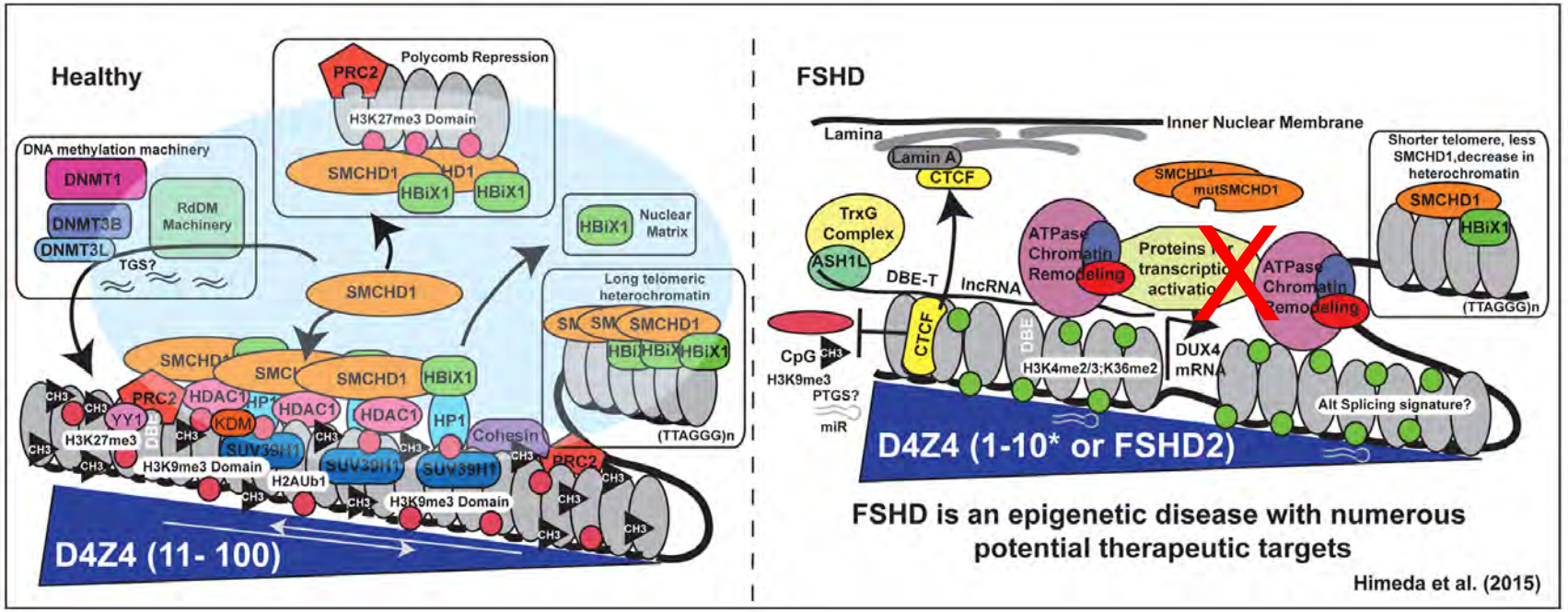
We have identified 3 strong candidates for targeted FSHD therapy

Example: Epigenetic Regulator PT-2



Design small molecule inhibitors to reverse the epigenetic state

Targeted repression of key epigenetic regulators reduces pathogenic *DUX4* expression

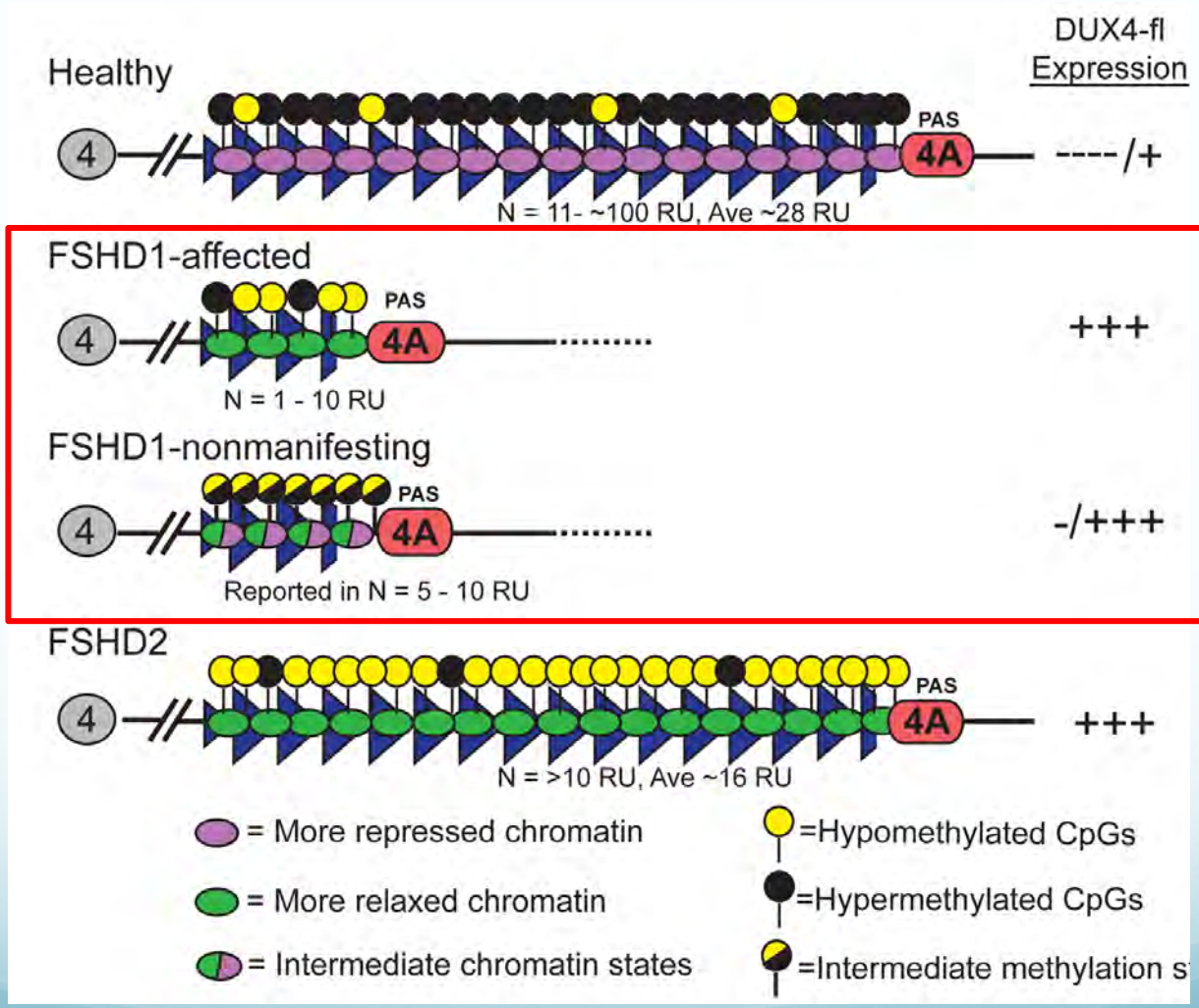


Asymptomatic ← **FSHD**

Epigenetic drugs are a viable therapeutic approach to FSHD

FSHD is gain-of-function

Can we therapeutically dial down DUX4 expression using CRISPR?



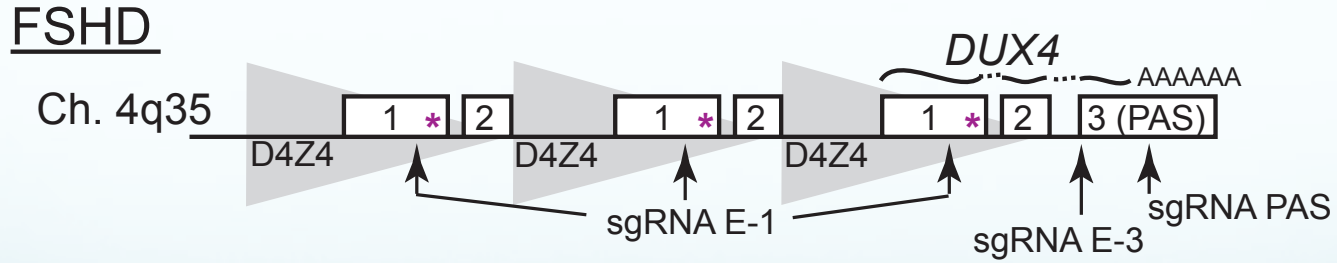
T Jones *et al*. 2015 *Clinical Epigenetics*

CRISPR-mediated "genome editing"
Powerful, controversial, scary?

Not the whole story

CRISPR is much more than genome “editing”

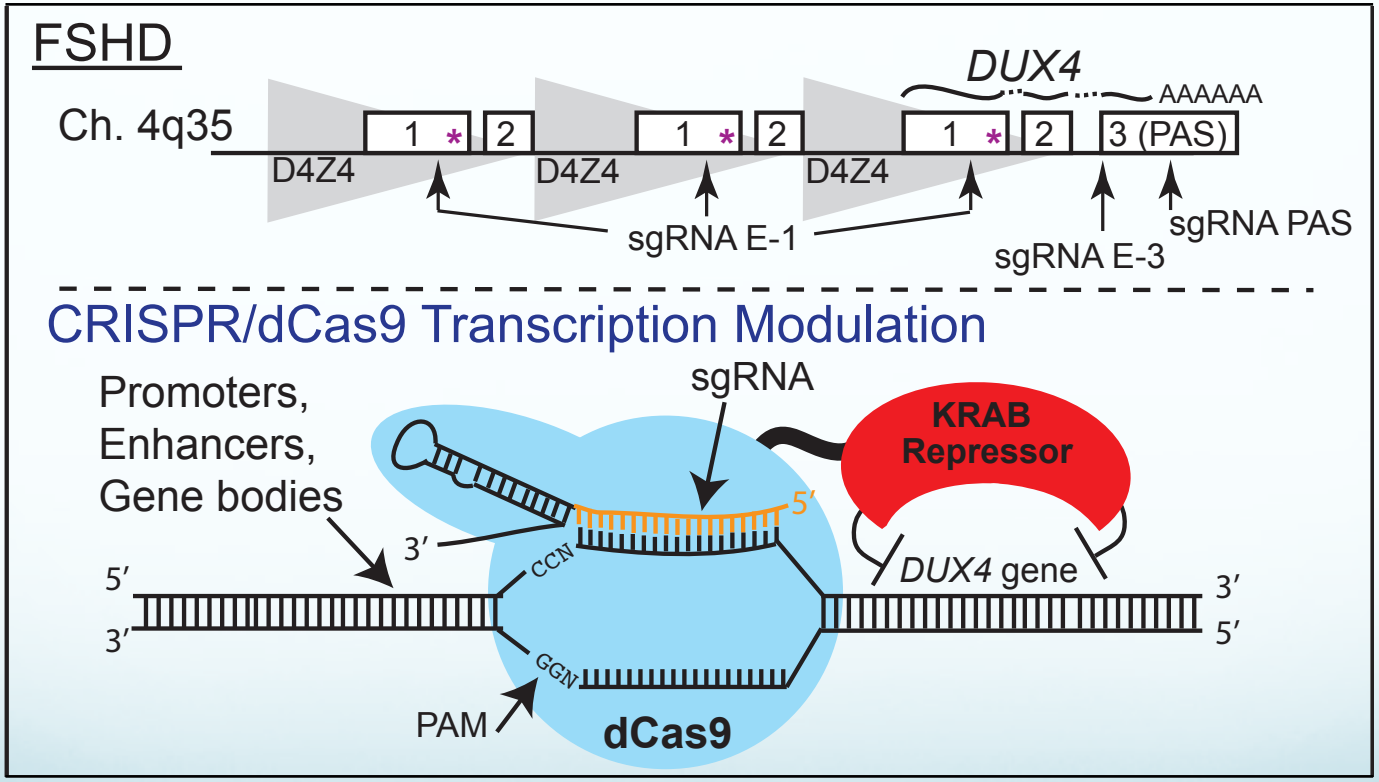
CRISPR/Cas technology is essentially a simple and more efficient way to specifically target the genome of any organism



Sequence-specific genome targeting

CRISPR/dCas9 in FSHD therapeutic development

Efficient genome targeting of a transcriptional repressor



Himeda *et al*. (2015) *Mol. Therapy*
Himeda *et al*. (2016) *Trends Pharmacol*.

Proof-of-principle CRISPR "cure" for FSHD

original article

Mol Ther. 2016 Mar;24(3):527-35. doi: 10.1038/mt.2015.200. Epub 2015 Nov 3.

CRISPR/dCas9-mediated Transcriptional Inhibition Ameliorates the Epigenetic Dysregulation at D4Z4 and Represses *DUX4-fl* in FSH Muscular Dystrophy

Charis L Himeda[1], Takako I Jones[1] and Peter L Jones[1,2]

[1]*The Department of Cell and Developmental Biology, University of Massachusetts Medical School, Worcester, Massachusetts, USA;* [2]*The Department of Neurology, University of Massachusetts Medical School, Worcester, Massachusetts, USA*

The Washington Post

Innovations

How CRISPR could lead to a cure for muscular dystrophy

By Dominic Basulto November 19

BOSTON BUSINESS JOURNAL

BIOFLASH

UMass researchers achieve several 'firsts' in new use for CRISPR/Cas9

Nov 16, 2015, 11:31am EST

INDUSTRIES & TAGS Technology, Health Care, Biotech, Pharmaceuticals

MOTHERBOARD Watch Machines Discoveries Space Futures Gaming Earth

CRISPR Technique Could 'Turn Off' Muscular Dystrophy Gene, Study Says

November 13, 2015 // 05:00 AM EST

Written by MELISSA CRONIN CONTRIBUTOR

How Controversial Gene Editing Could Lead To Groundbreaking Cures

This technology may change the way we think of some of the world's most challenging diseases.

Lila Shapiro
Senior Staff Reporter, The Huffington Post

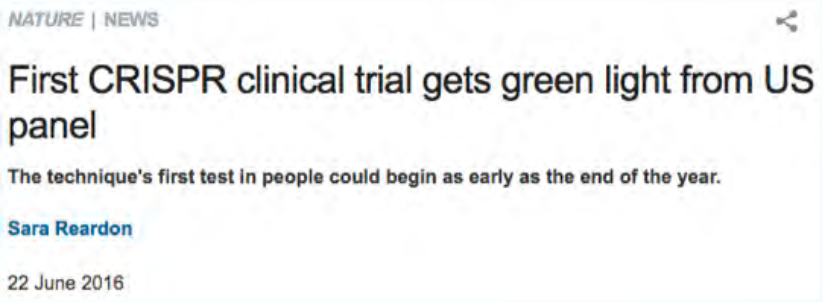
11/28/2015 07:31 am ET

Gregory Adams via Getty Images

Could CRISPR really become an FSHD therapeutic?

editas MEDICINE

CRISPR THERAPEUTICS


NATURE | NEWS

First CRISPR clinical trial gets green light from US panel

The technique's first test in people could begin as early as the end of the year.

Sara Reardon

22 June 2016

exonics THERAPEUTICS

Intellia THERAPEUTICS

Three companies have had recent IPOs

Other companies still privately held

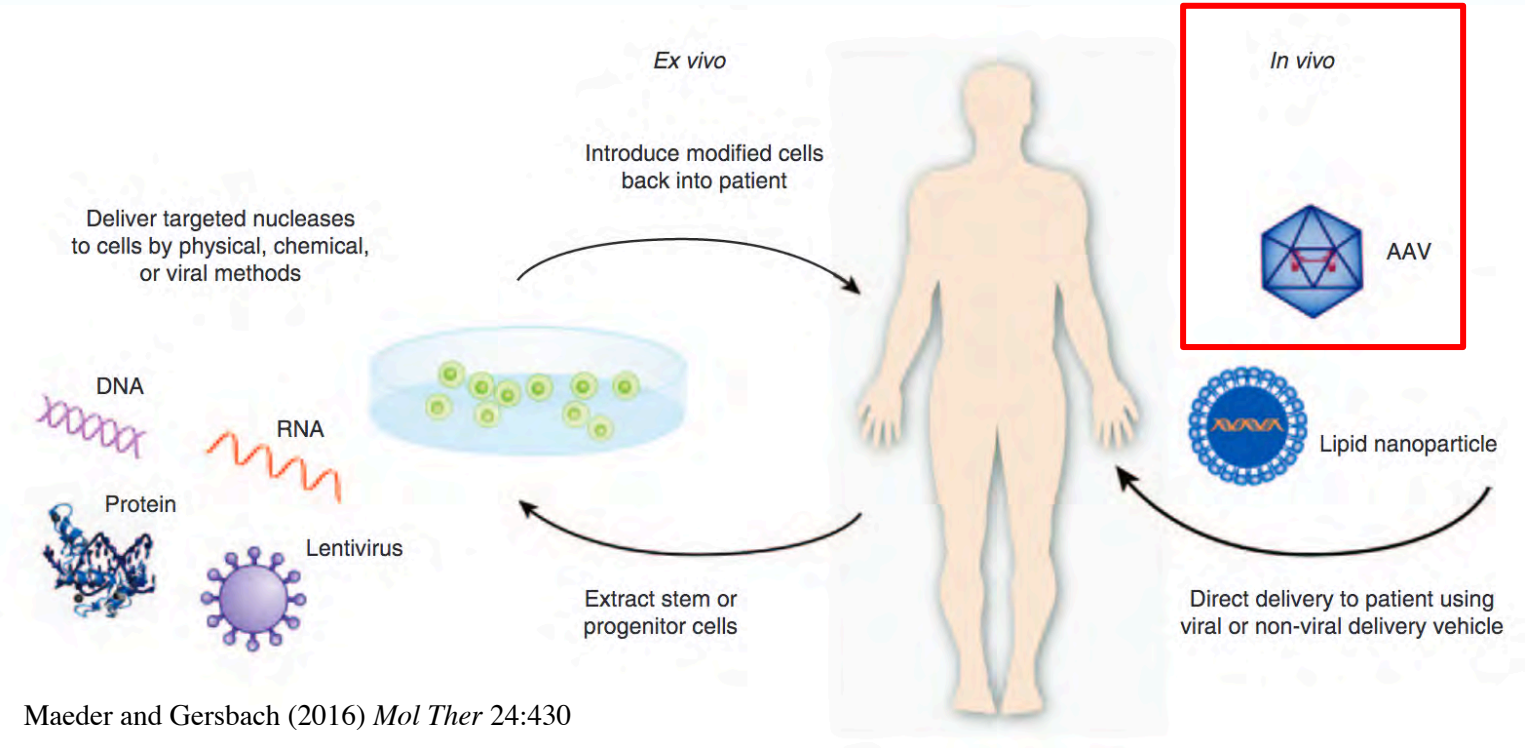
Projected market of $5.5 billion by 2021

Patent* is being contested: UC-Berkeley vs the Broad Institute*

New CRISPR and CRISPR-like systems being discovered

Therapeutic delivery of CRISPR/Cas *in vivo* is challenging

FSHD is a skeletal muscle disease

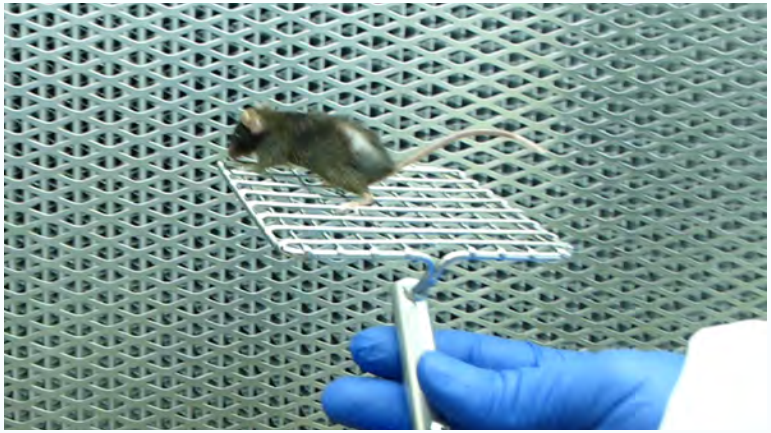


Maeder and Gersbach (2016) *Mol Ther* 24:430

Need an animal model → pathogenic gene is primate-specific

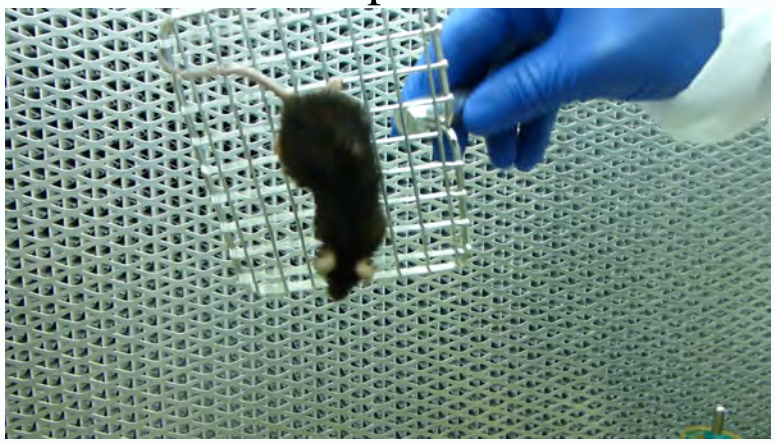
Inserted the human *DUX4* gene into the mouse genome to generate FSHD-like mice with a readily assayable phenotype

>1 min suspended



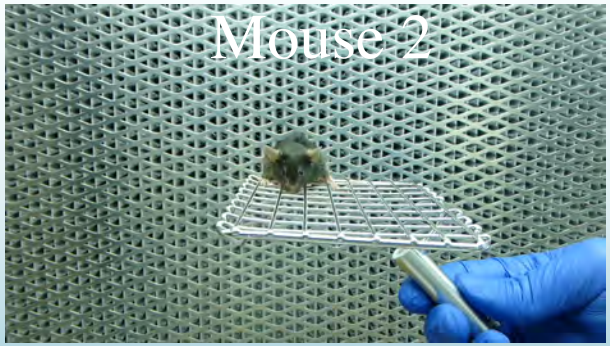
***FLEx/+* (control)**

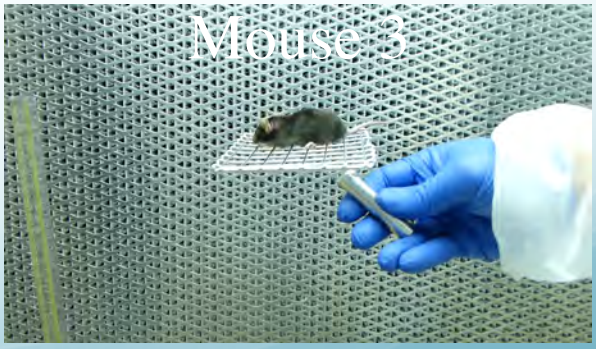
>1 min suspended



***FLEx/+, ACTA1 MCM* (no DUX4)**

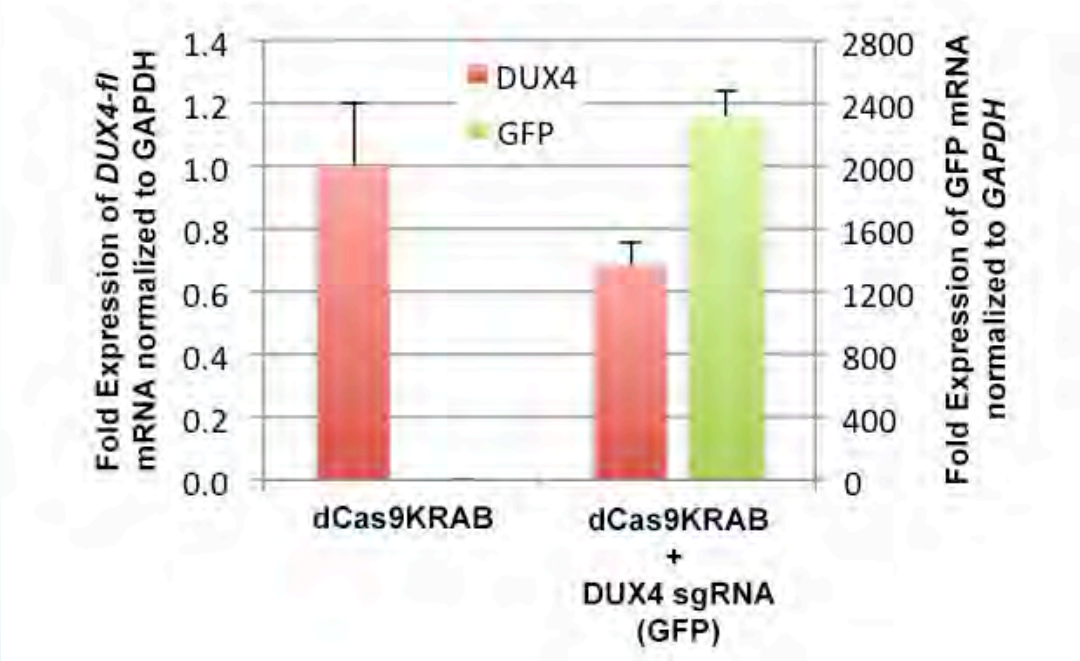
<2 second suspended





***FLEx/+, ACTA1 MCM* (DUX4 induced)**

In vivo delivery of AAV9-dCas9-KRAB + AAV9-sgRNA leads to significant DUX4 knockdown

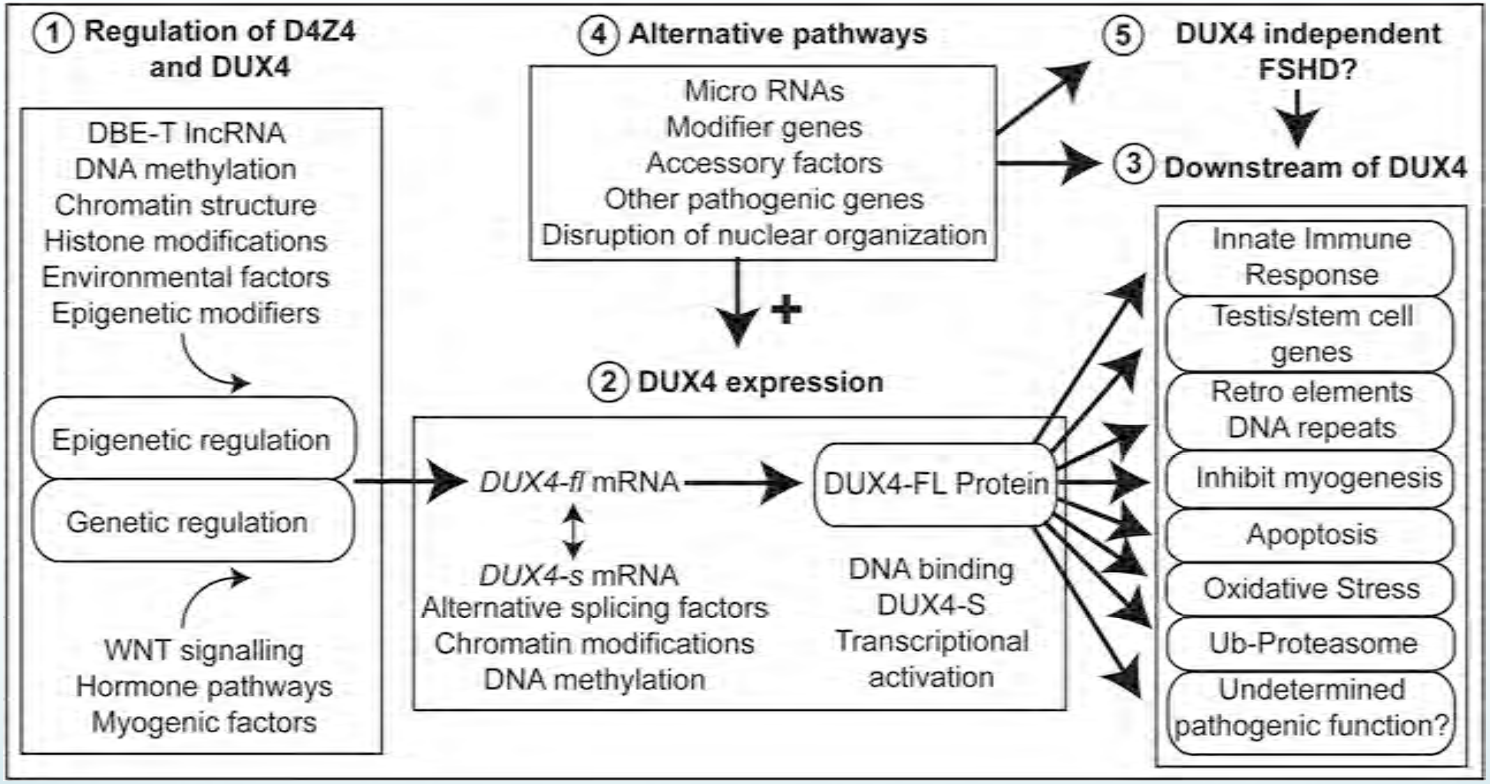


AAV9 delivery results in 30% decrease in TA muscle

Enough to be therapeutic?

Only need to dial back expression from affected to asymptomatic

Our recent increased understanding of FSHD pathogenic mechanisms has led to the development of numerous therapeutic approaches and tools



CRISPRi/dCas9-KRAB; CRISPR/Cas9; Myostatin inhibition
Morpholinos/PMOs/shRNAs; miRNAs; Anti-inflammatory
Small molecules targeting epigenetic regulators; more…

The FSHD field will be translating discoveries to the clinic and the future is bright

Steven Blier: Concert pianist, Professor at Julliard, FSHD patient, and friend
Kelli O'Hara: Tony award winning actress and advocate for FSHD

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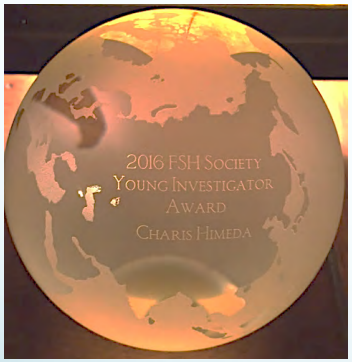
Acknowledgements

Mick Hitchcock, PhD, Endowed Chair in Medical Biochemistry



Charis Himeda, PhD Takako Jones, PhD

NIAMS
National Institute of Arthritis and Musculoskeletal and Skin Diseases

Eunice Kennedy Shriver
NICHD
National Institute of Child Health & Human Development

A Voice for FSHD
The Chris Carrino Foundation for FSHD

AFM
Association Française contre les Myopathies

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FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY

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