Fulcrum Therapeutics

Facioscapulohumeral Dystrophy Key Opinion Leader Breakfast Forum

8:30 a.m. – 10:30 a.m.

November 7, 2019

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This presentation contains “forward-looking statements” within the meaning of the Private Securities Litigation Reform Act of 1995 that involve substantial risks and uncertainties, including statements regarding the development status of the Company’s product candidates, the timing of availability of clinical trial data and the Company’s ability to fund its operations with cash on hand . All statements, other than statements of historical facts, contained in this presentation, including statements regarding the Company’s strategy, future operations, future financial position, prospects, plans and objectives of management, are forward-looking statements. The words “anticipate,” “believe,” “continue,” “could,” “estimate,” “expect,” “intend,” “may,” “plan,” “potential,” “predict,” “project,” “should,” “target,” “will,” “would” and similar expressions are intended to identify forward-looking statements, although not all forward-looking statements contain these identifying words. Any forward-looking statements are based on management’s current expectations of future events and are subject to a number of risks and uncertainties that could cause actual results to differ materially and adversely from those set forth in, or implied by, such forward-looking statements. These risks and uncertainties include, but are not limited to, risks associated with Fulcrum’s ability to obtain and maintain necessary approvals from the FDA and other regulatory authorities; continue to advance its product candidates in clinical trials; replicate in later clinical trials positive results found in preclinical studies and early-stage clinical trials of losmapimod and its other product candidates; advance the development of its product candidates under the timelines it anticipates in current and future clinical trials; obtain, maintain or protect intellectual property rights related to its product candidates; manage expenses; and raise the substantial additional capital needed to achieve its business objectives. For a discussion of other risks and uncertainties, and other important factors, any of which could cause the Company’s actual results to differ from those contained in the forward-looking statements, see the “Risk Factors” section, as well as discussions of potential risks, uncertainties and other important factors, in the Company’s most recent filings with the Securities and Exchange Commission. In addition, the forward-looking statements included in this presentation represent the Company’s views as of the date hereof and should not be relied upon as representing the Company’s views as of any date subsequent to the date hereof. The Company anticipates that subsequent events and developments will cause the Company’s views to change. However, while the Company may elect to update these forward-looking statements at some point in the future, the Company specifically disclaims any obligation to do so.

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Agenda

8:30am	Welcome – **Robert J. Gould, Ph.D.,** *President and Chief Executive Officer, Fulcrum Therapeutics*
8:40am	Introduction to FSHD – **Kathryn Wagner, M.D., Ph.D.,** *Professor of Neurology and Neuroscience at Johns Hopkins School of Medicine and Director of the Center for Genetic Muscle Disorders Kennedy Krieger Institute*
9:00am	Biologic rationale and genetics – **Peter Jones, Ph.D.,** *Mick Hitchcock, Ph.D. Endowed Chair in Medical Biochemistry and Associate Professor of Pharmacology at University of Nevada, Reno School of Medicine*
9:15am	The role of DUX4 - **Peter Jones, Ph.D.**
9:30am	Overview of p38i – **Fran Sverdrup, Ph.D.,** *Associate Professor of Biochemistry and Molecular Biology at Saint Louis University, School of Medicine*
9:50am	Imaging and biopsy as an approach & clinical trial design implications – **Kathryn Wagner, M.D., Ph.D.**
10:10am	Q&A ***Please hold questions until the Q&A*

Fulcrum at a glance

Proprietary Product Engine

Fulcrum's product engine is designed to systematically address the root cause of many genetically defined diseases

Our Progress to Date

- Developed proprietary drug discovery platform
- Established patient-driven discovery & development process
- Ongoing **Phase 2 studies** in Facioscapulohumeral Muscular Dystrophy (FSHD)
- Initiated FTX-6058 **IND-enabling studies** for select hemoglobinopathies (Sickle Cell Disease and β-thalassemia)
- Demonstrated systematic target and therapeutic discovery potential

Integrated FSHD development strategy

Natural history & preparatory studies — *Ongoing*

Assessing disease progression and validating clinical endpoints

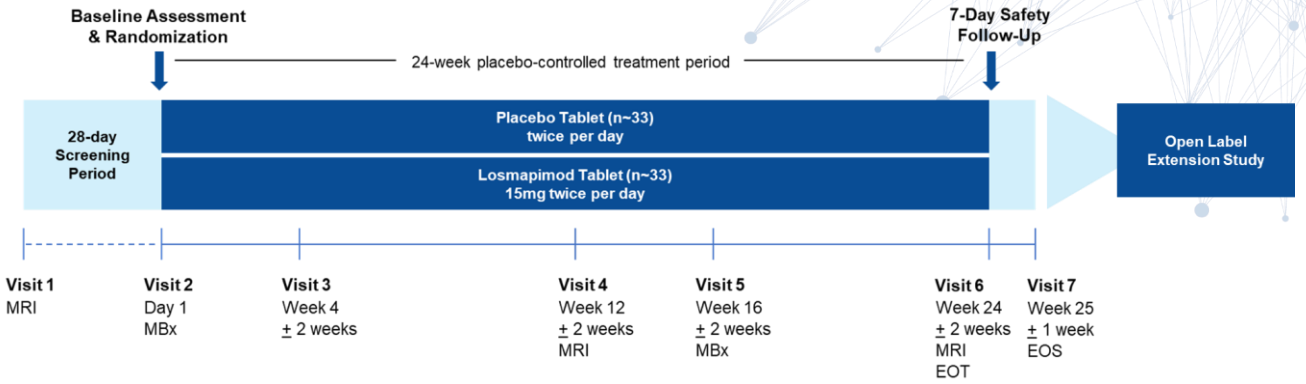
Phase 1 — *Enrollment complete – analysis ongoing*

Demonstrated losmapimod target engagement, muscle penetration, and safety in FSHD patients

Phase 2 Open Label Study (52 weeks) *with interim analyses* — *Ongoing*

Assessing molecular endpoint (DUX4 muscle biopsy), MRI disease measurement, clinical assessment of mobility, and PROs

Phase 2b (ReDUX4) *24 weeks dosing* → **Open Label Extension**



Day 1 & Week 16: Muscle Biopsy (MBx)
DUX4-driven gene expression in skeletal muscle needle biopsy

Visit 1, Week 12, Week 24: MRI
lean skeletal muscle volume; skeletal muscle fat fraction

Day 1, Weeks 4, 12, 16, 24:Clinical assessments
PK; safety; Reachable Work Space; FSHD-Timed Up & GO, Muscle function measures, dynamometry and Patient Reported Outcomes

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Introduction to FSHD

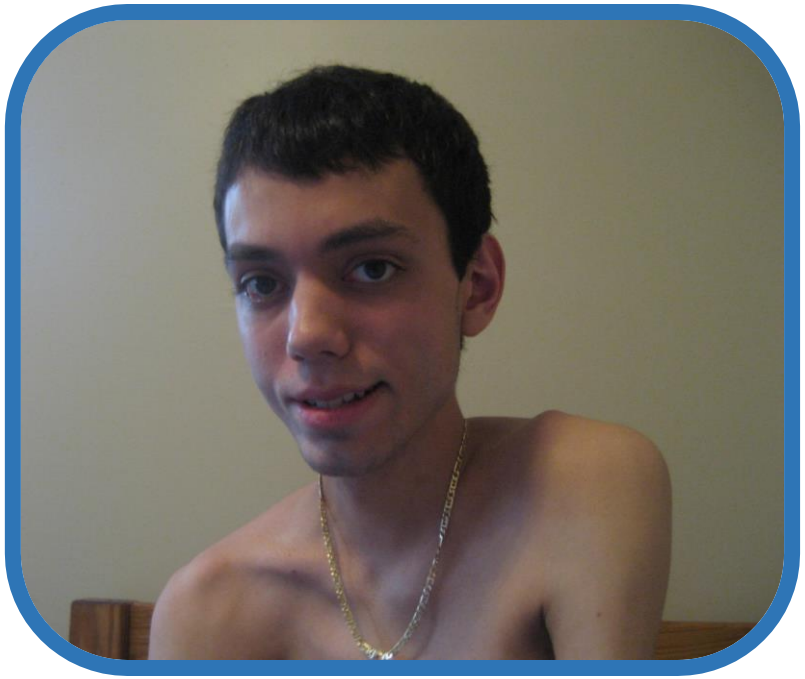
Kathryn Wagner, MD, PhD
Center for Genetic Muscle Disorders
Kennedy Krieger Institute
Johns Hopkins School of Medicine

Facioscapulohumeral muscular dystrophy (FSHD)

- Prevalence of ~1:8,000 - 1:20,000
- Autosomal dominant disorder of families
- Extremely disabling but not fatal

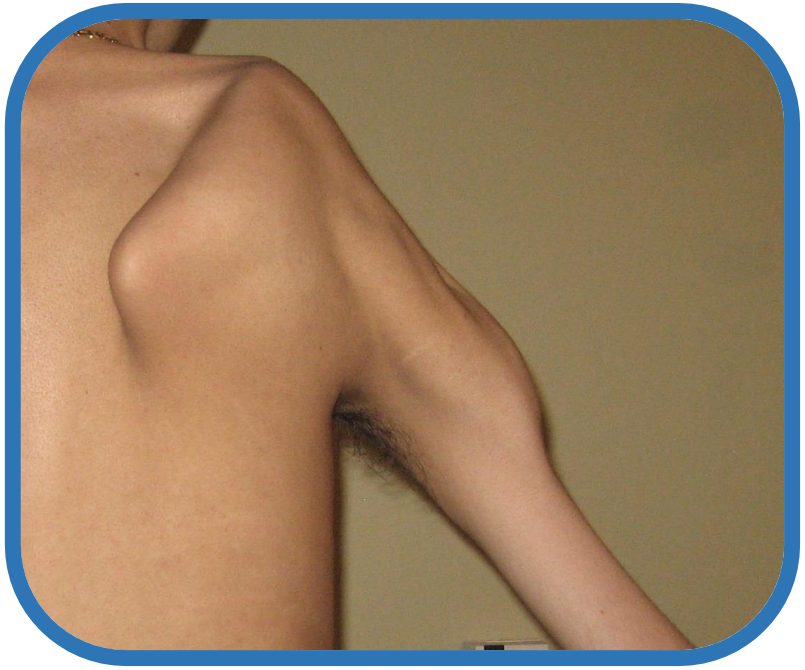
Where it gets its name

facio= face



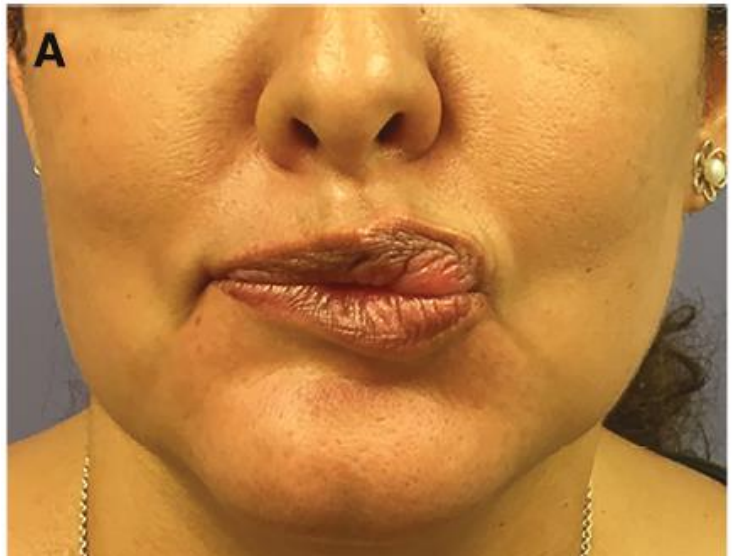
scapulo= muscles of scapular fixation

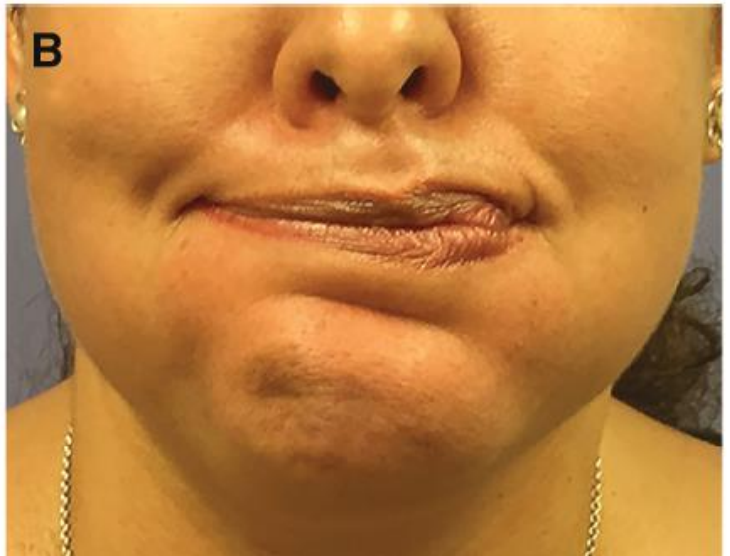
humeral= muscles overlying humerus



Clinical Presentation:

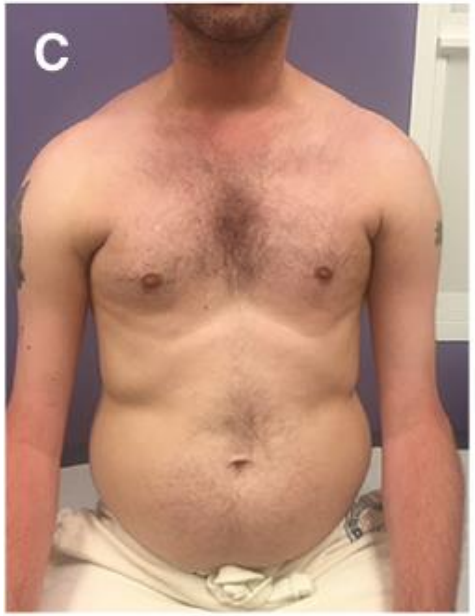
- Symptoms first noticed in muscles of face and scapular region
- Patients present with wide open eyes and have a history of sleeping with their eyes partially open
- They have an inability to pucker and may never learn to whistle
- They frequently have a transverse or asymmetric smile

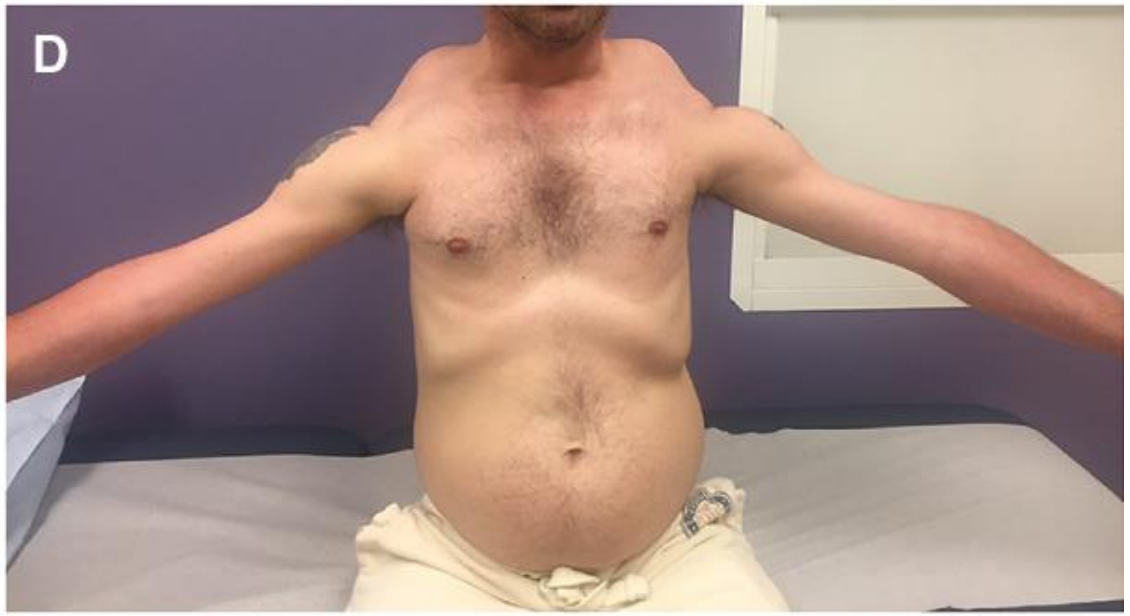




Clinical Presentation continued

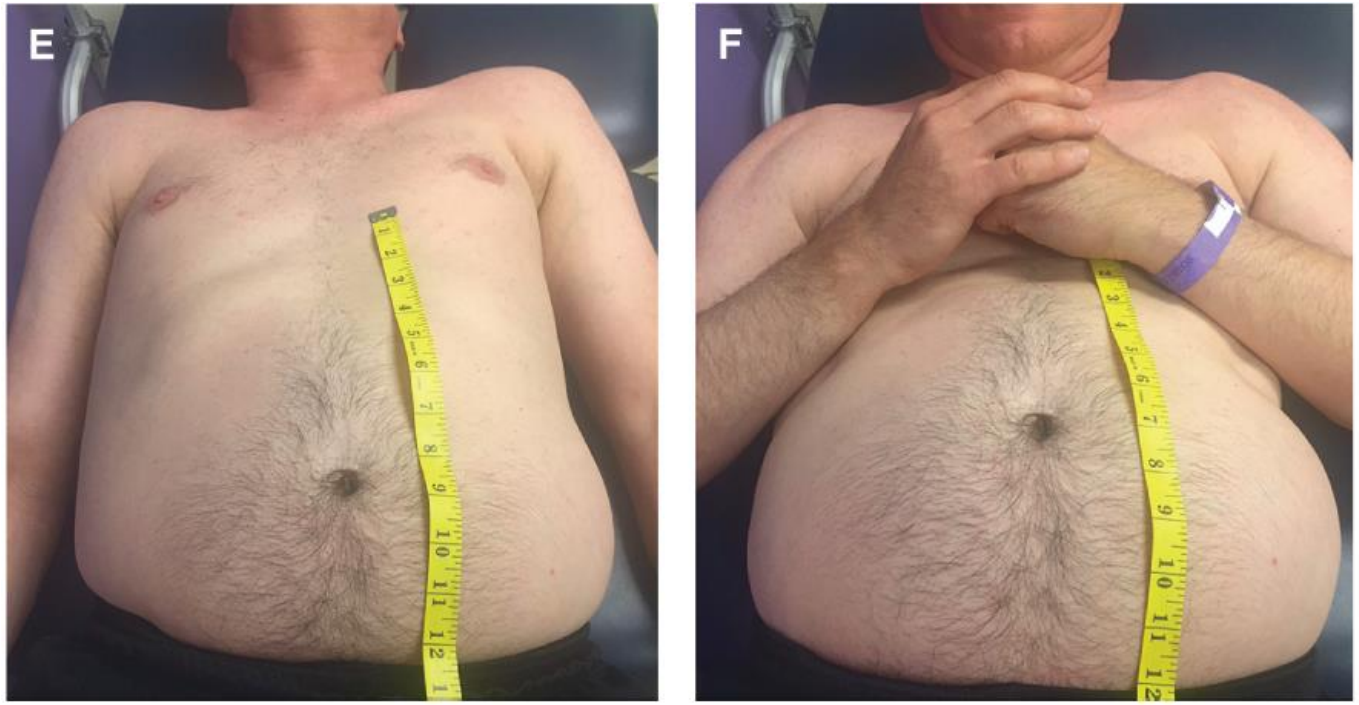
- Muscles of scapular fixation (rhomboids and serratus anterior) are weak
  - Medial border of scapula “wings”
  - Rostral border rises up: Poly-hill sign on arm abduction
  - Inability to slowly abduct or extend arms to 180 degrees
- Pectoralis weakness
  - Horizontal clavicles
  - Deep axillary creases





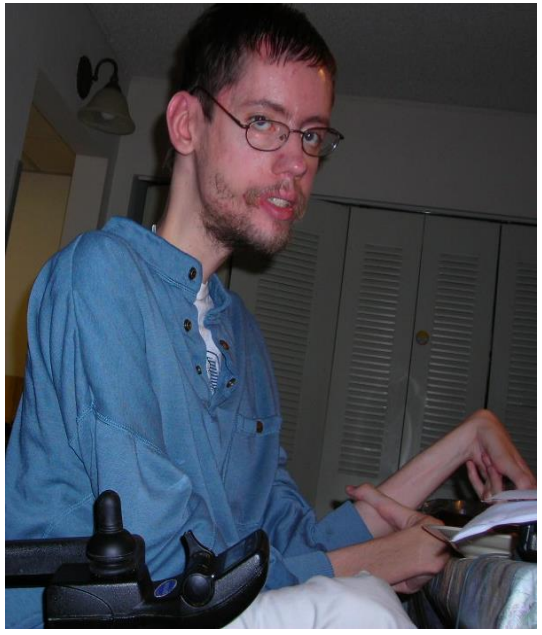
Clinical Presentation continued

- Biceps and Triceps are disproportionately involved compared to deltoid and forearm flexors
- Weakness of tibialis anterior results in footdrop
- Paraspinal and abdominal weakness leads to lordosis and protruberant abdomen.
- Umbilicus moves rostrally when the individual attempts to sit up: Beevor sign
- Eventually forearm flexors and extensors, knee flexors and extensors may become weak
- Weakness and wasting are frequently asymmetric



Clinical Presentation

- Two forms: FSHD1 and FSHD2 present similarly
- Onset and severity vary widely
- Most classic onset is teenage or early adult years
- Range from infantile onset to nonmanifesting carriers
- Correlation to size of allele

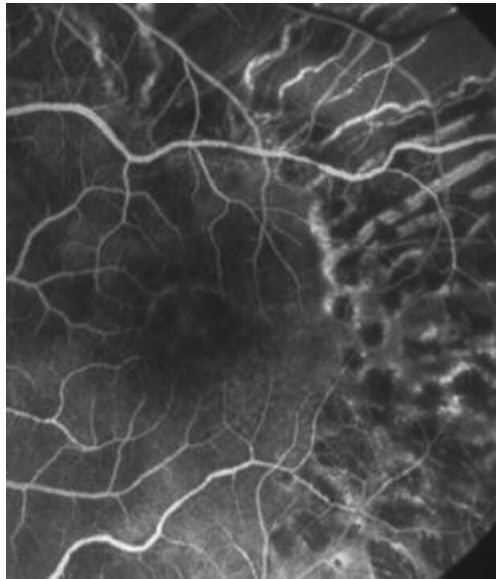


How does FSHD affect day to day life?

- Inability to communicate via facial expression
- Inability to do activities requiring upper arms including brushing hair, putting dishes on a shelf, shampooing
- Difficulty getting out of bed
- Tripping and falling
- 30% lose ambulation
- Chronic pain and fatigue

Associated Symptoms

- 30% of FSHD who are nonambulatory have respiratory involvement
- Cardiomyopathy not associated with FSHD
  - Conduction defects or arrhythmias might be more prevalent
- Retinal vasculopathy
  - 50% have mild retinal abnormalities such as telangiectasias or microaneuryms
  - 0.8% have vasculopathy with neovascularization, retinal detachment, neovascular glaucoma
  - Severe retinal vasculopathy associated with early-onset FSHD and large 4q35 deletions
- Hearing loss
  - High frequency hearing loss more common in early-onset FSHD
- Musculoskeletal pain
  - 88.6% of patients reported current pain



Diagnostic Evaluation

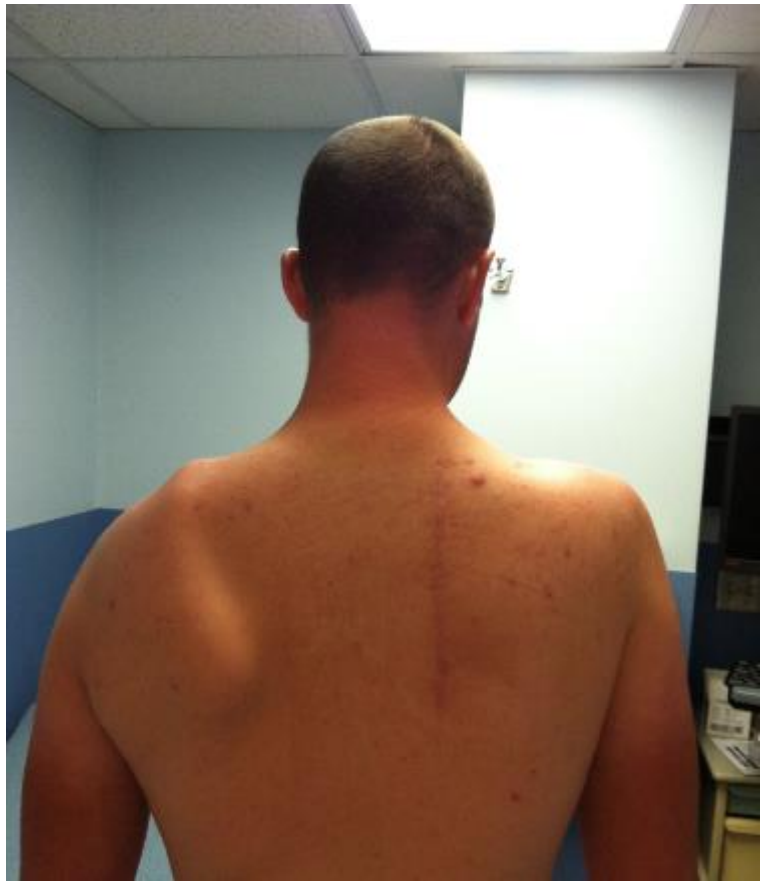
- Clinical presentation fairly distinct from other myopathies
- CK will be normal to mildly elevated
- EMG shows nonspecific myopathic features (small, polyphasic motor units) and occasional irritability (fibrillations and positive sharp waves)
- Muscle biopsy rarely indicated: Nonspecific myopathic features
- Genetic testing is commercially available, sensitive and specific

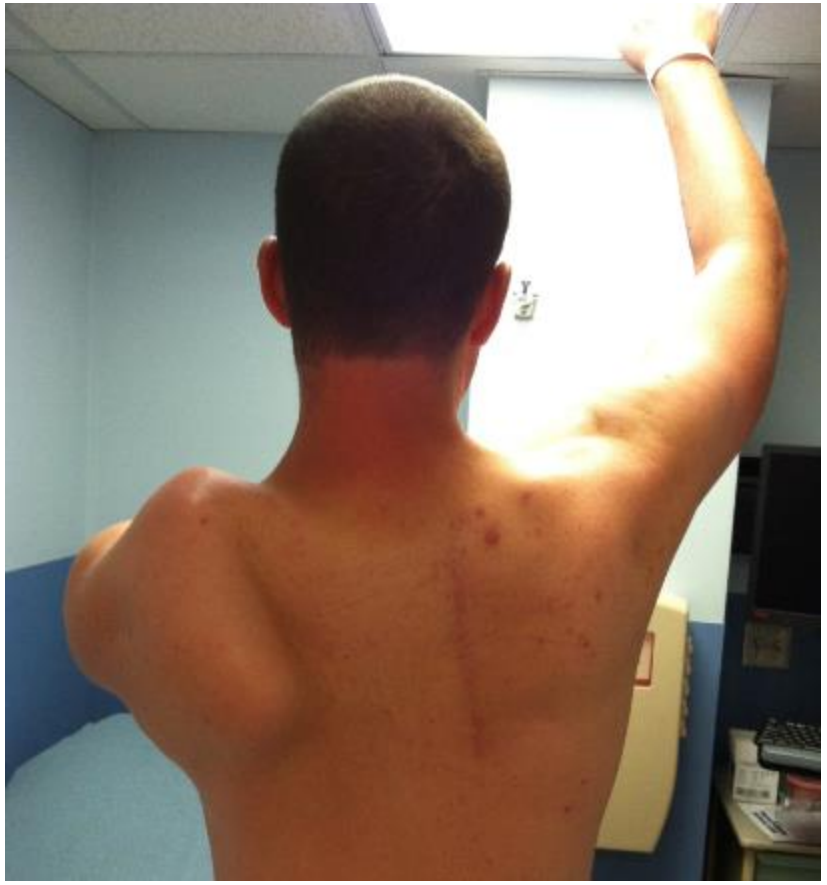
Current Management

- No accepted pharmacological treatments for progressive muscle weakness: Challenge managing the disease
- Dilated eye examination to r/o reversible retinal vascular disease
- All patients with early-onset FSHD screened for hearing loss
- Pulmonary function testing at baseline and annually for those with severe weakness, kyphoscoliosis, wheelchair dependence
  - Referral to sleep specialist when FVC<60% or excessive daytime somnolence, frequent nocturnal arousals or morning headaches
- Pain management
  - PT
  - NSAIDs
  - Antidepressants

Current Management continued

- Surgical scapular fixation
  - When can’t abduct to 90 degrees but good deltoid preservation
- Bracing
  - Ankle foot orthoses
- Exercise
  - Cycling 30 min/day, 3X/wk
- Annual DEXA
  - treatment of low bone density





Conclusions

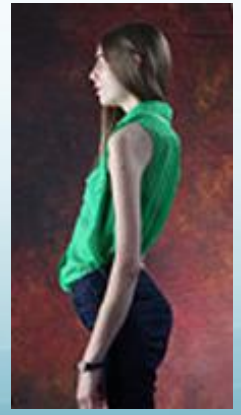
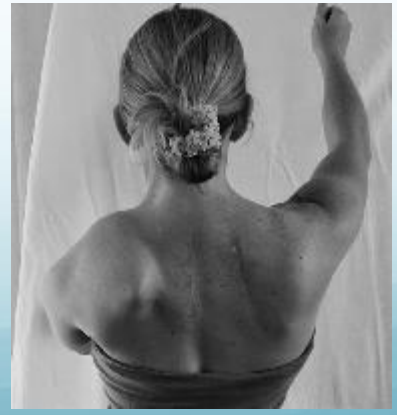
- FSHD is an autosomal dominant disorder that preferentially affects face, muscles of scapular fixation and arms
- Eventually, most skeletal muscles are affected
- FSHD is associated with rare extramuscular manifestations including retinal vasculopathy and hearing loss
- There are no treatments for the muscle weakness associated with FSHD

University of Nevada, Reno
School of Medicine
Department of Pharmacology

FSHD Genetics and Epigenetics

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



Epigenetics

“Treasure your exceptions.”

William Bateson “The Methods and Scope of Genetics” 1908

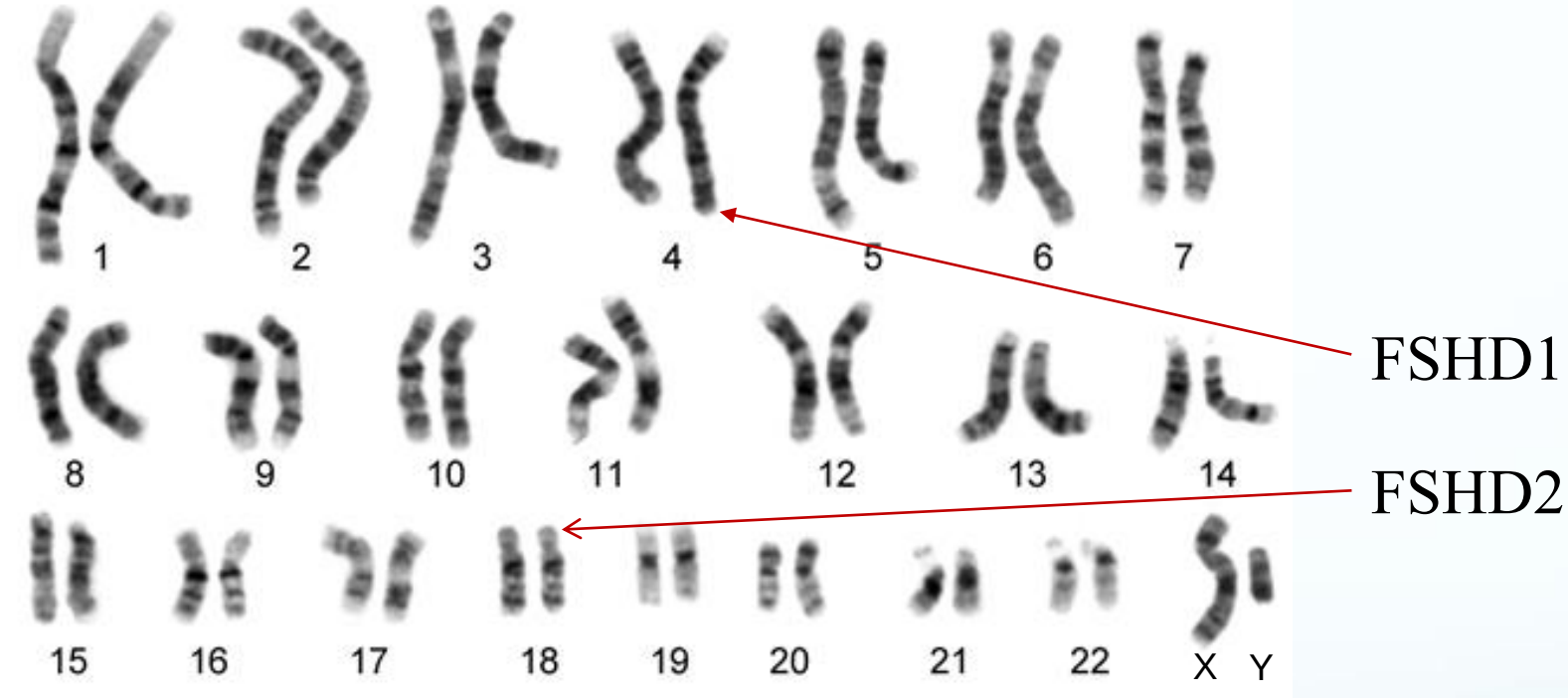
PEV

***In utero* diet**

X-inactivation

FSHD

FSHD is caused by genetic changes that lead to epigenetic changes at Chr 4q35



Human haploid genome has ~3,100,000,000 base pairs of DNA (GATCs)

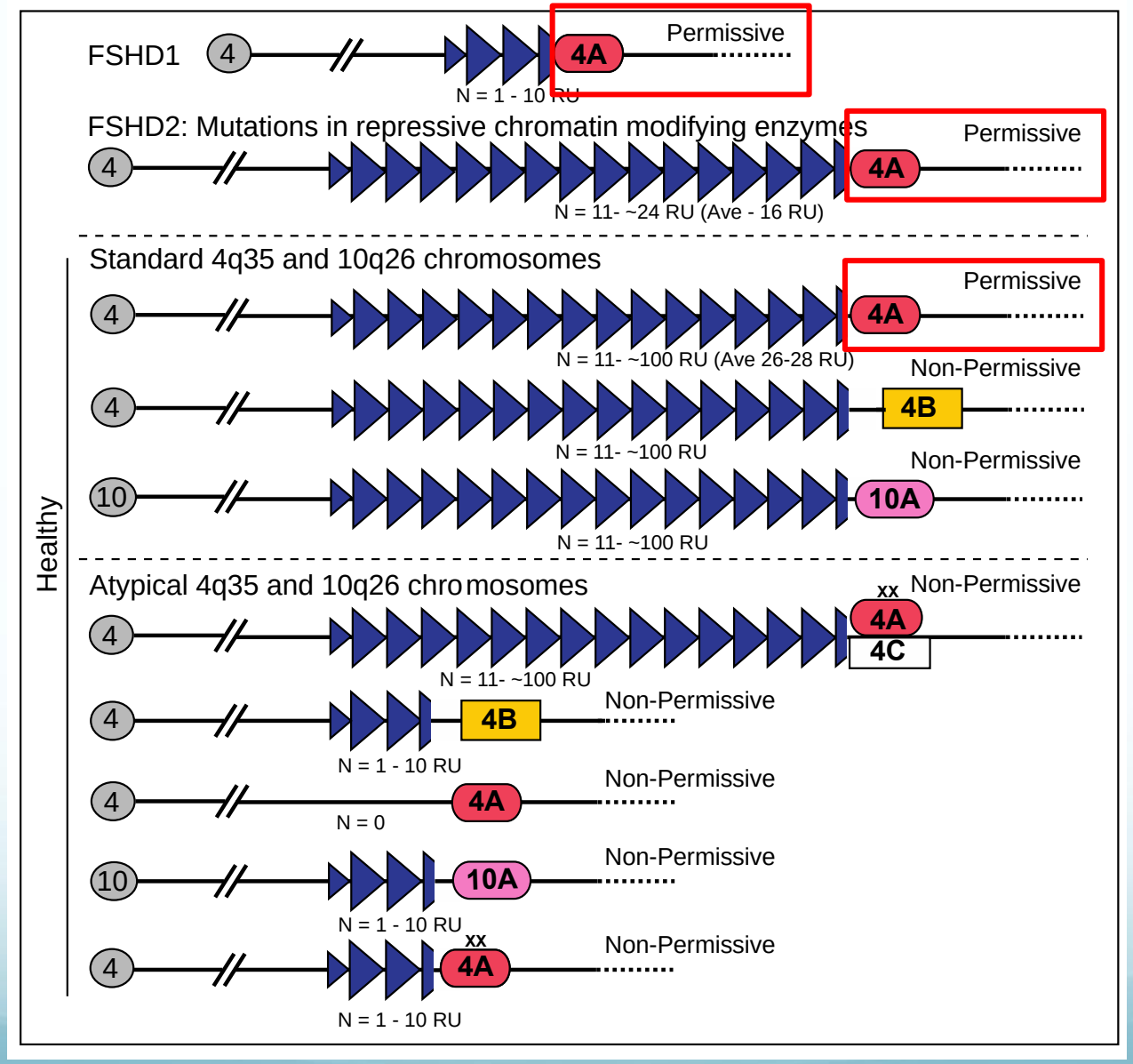
FSHD1 is caused by small deletions on Chr 4q

→ lead to epigenetic changes at Chr 4q

FSHD2 is usually caused my mutations on Chr 18p

→ lead to epigenetic changes at Chr 4q

FSHD genetics are complex



Himeda *et al.* (2014) *Antiox Redox Signaling*

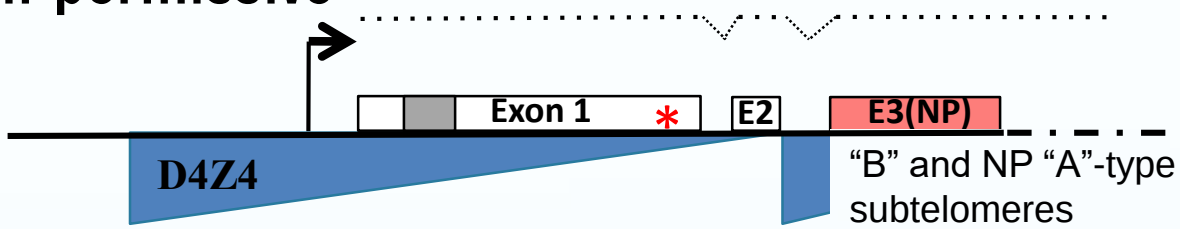
Sciencexpress Report 2010

A Unifying Genetic Model for Facioscapulohumeral Muscular Dystrophy

Richard J. L. F. Lemmers,[1] Patrick J. van der Vliet,[1] Rinse Klooster,[1] Sabrina Sacconi,[2] Pilar Camaño,[3,4] Johannes G. Dauwerse,[1] Lauren Snider,[5] Kirsten R. Straasheijm,[1] Gert Jan van Ommen,[1] George W. Padberg,[6] Daniel G. Miller,[7] Stephen J. Tapscott,[5] Rabi Tawil,[8] Rune R. Frants,[1] Silvère M. van der Maarel[1]*

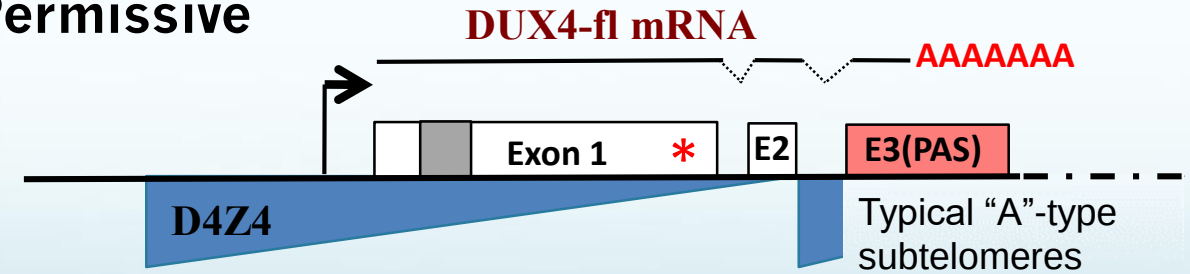
DUX4 genetics link all forms of FSHD

Non-permissive



-Unstable mRNA
-DUX4 protein not made
-Nontoxic

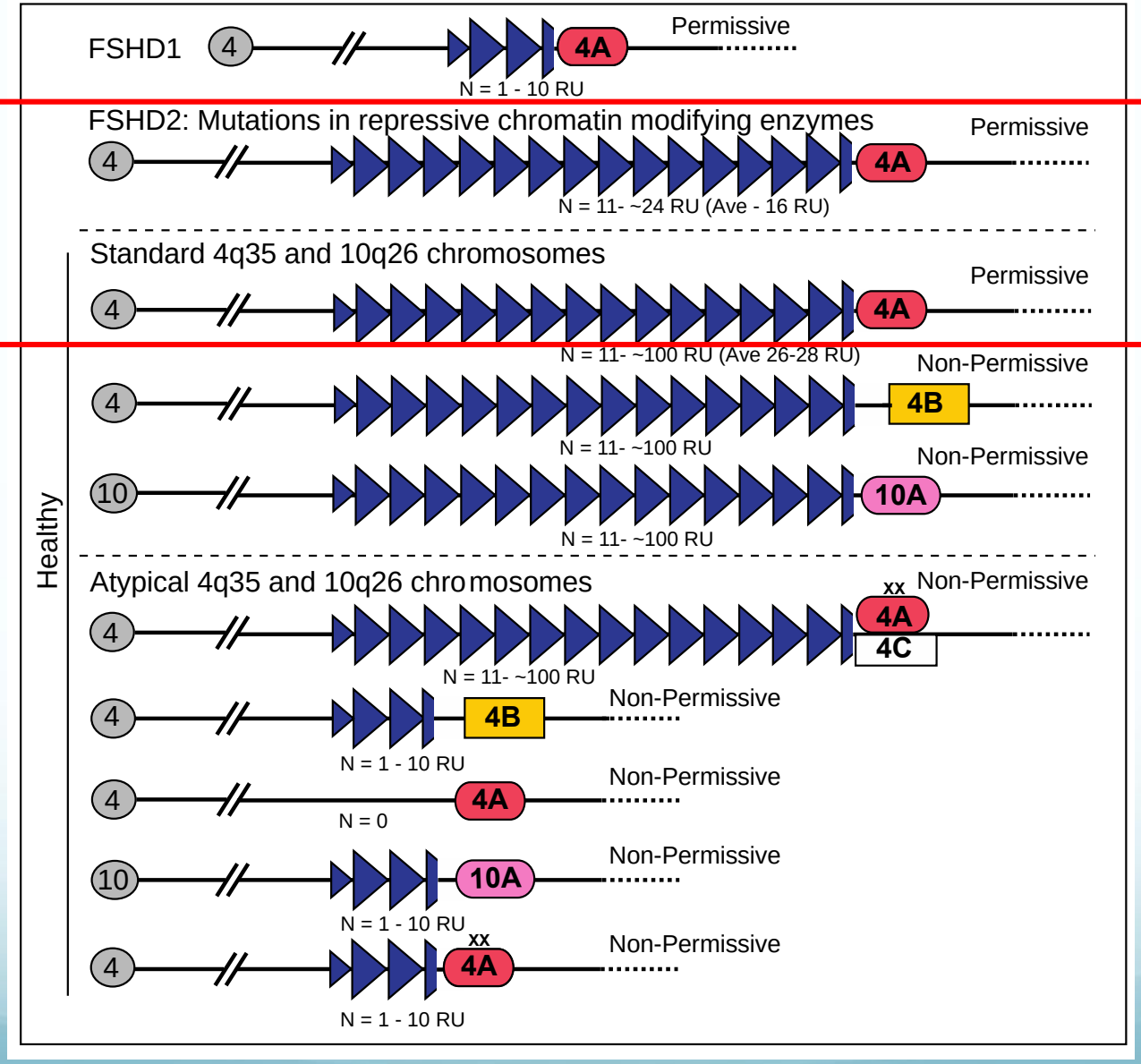
Permissive



-Stable poly A mRNA
-DUX4 protein produced
-Cytotoxic

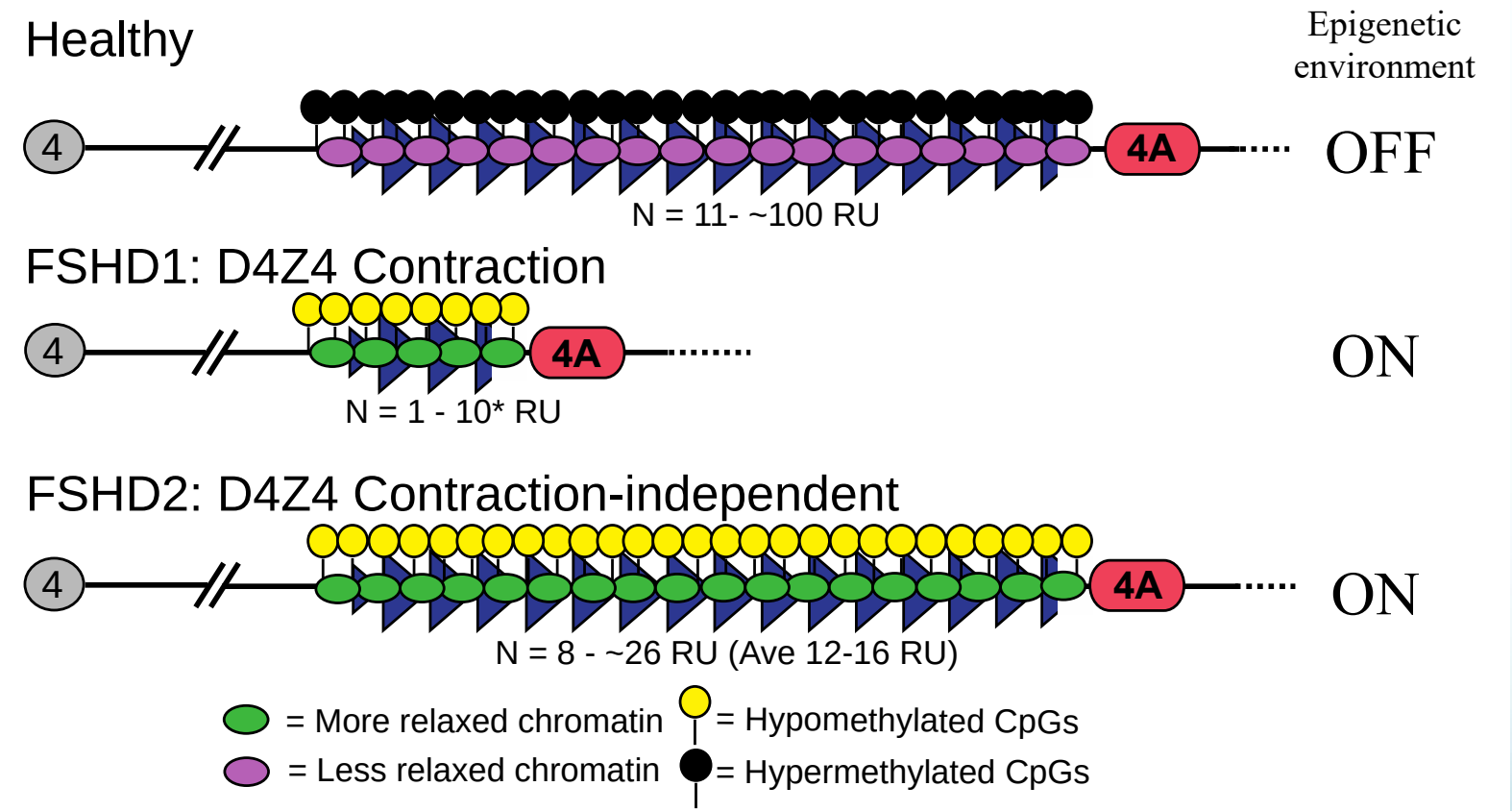
* = translation stop PAS = DUX4 mRNA polyadenylation site NP = non-permissive

FSHD genetics are complex



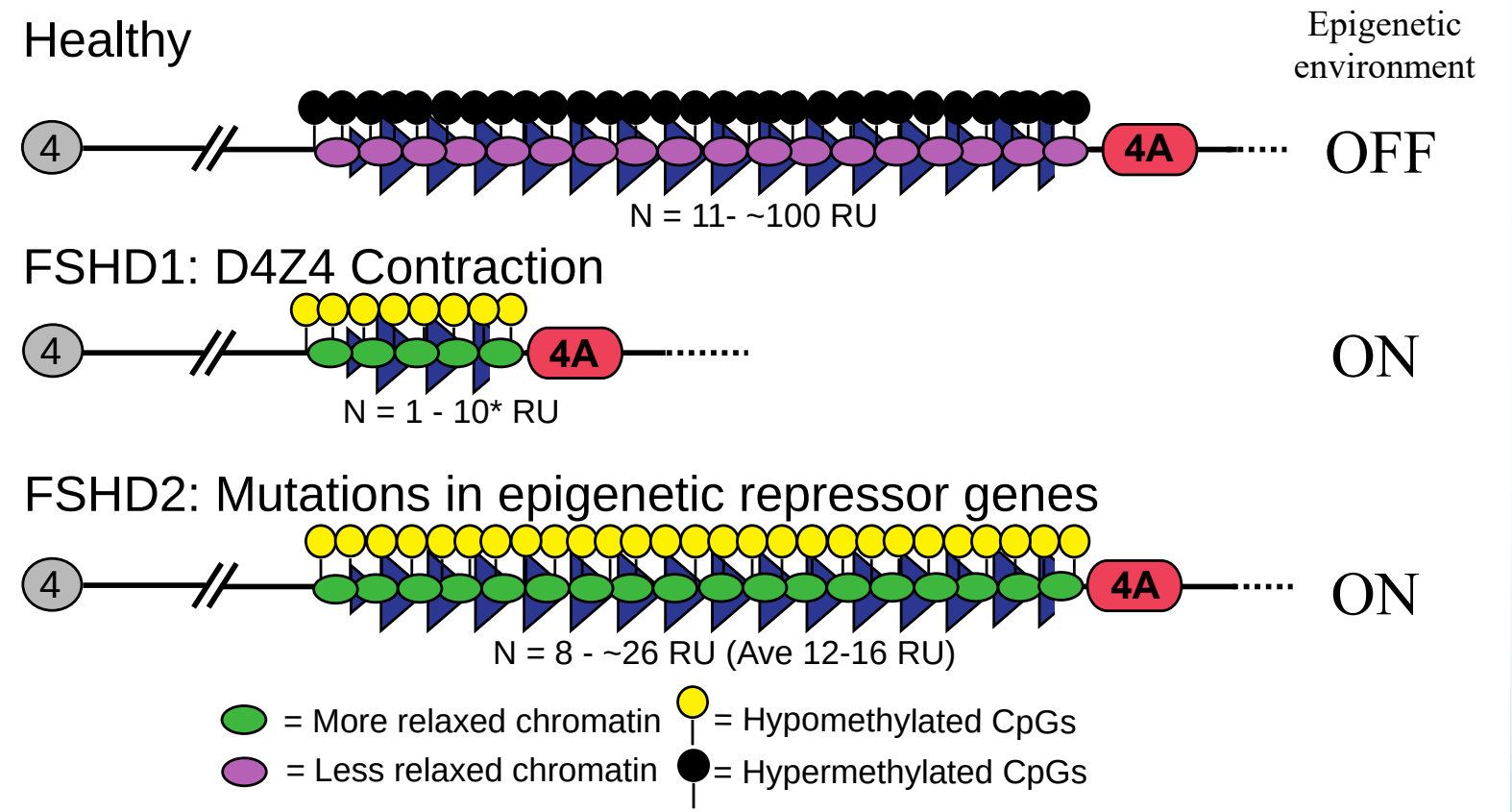
Himeda *et al.* (2014) *Antiox Redox Signaling*

Epigenetic dysregulation links all forms of FSHD



De Greef *et al.* (2009) *Human Mutation*

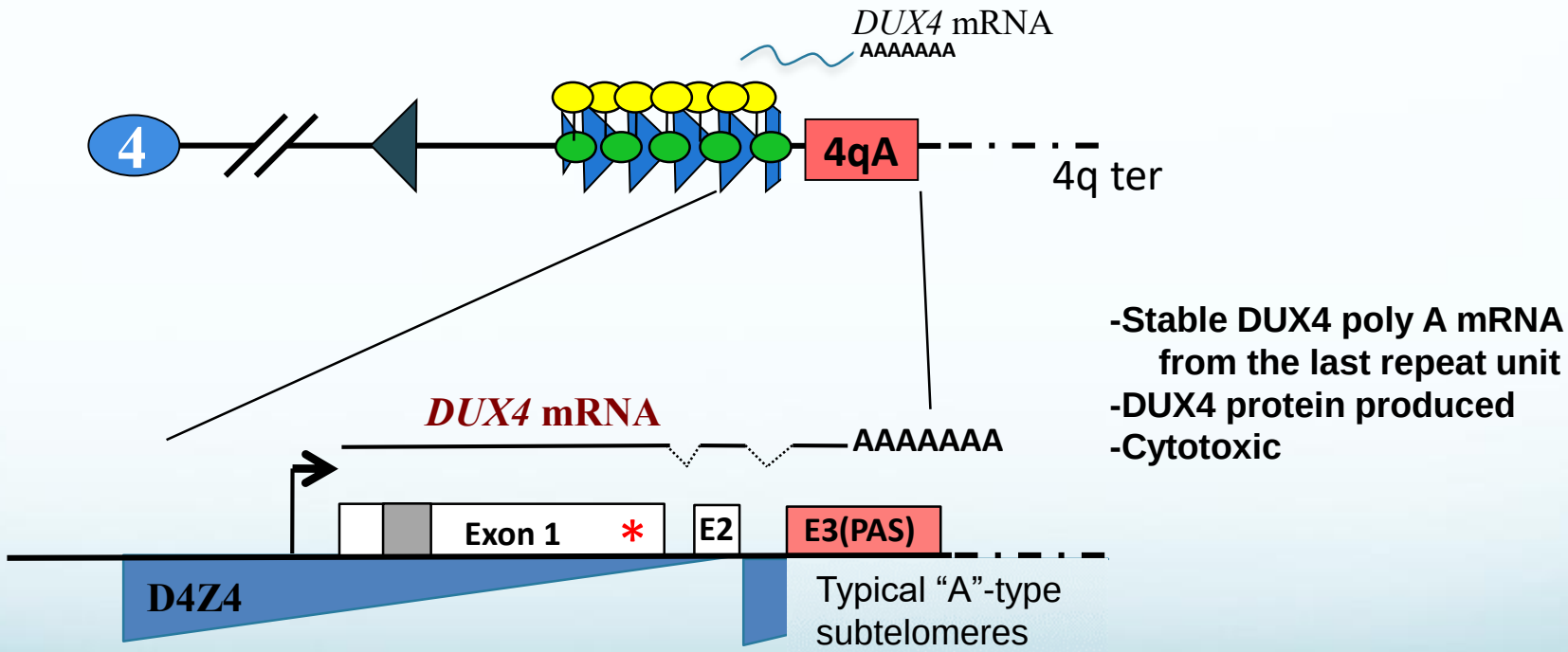
FSHD is an epigenetic disease with a genetic component



De Greef *et al.* (2009) *Human Mutation*

What are the consequences of dysregulated epigenetics in FSHD?

Aberrant epigenetics combined with DUX4-permissive genetics leads to expression of the *DUX4* gene resulting in FSHD



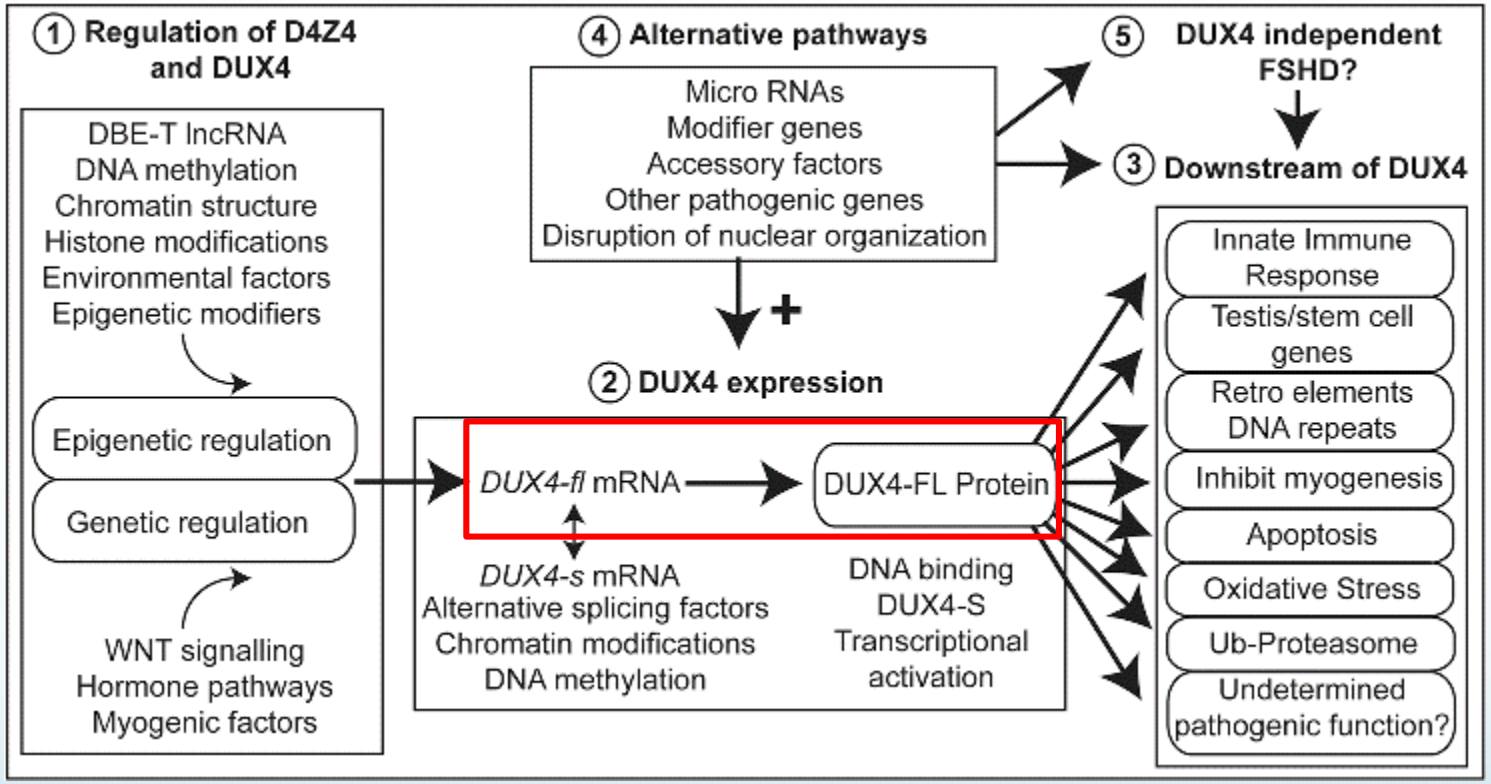
-Stable DUX4 poly A mRNA from the last repeat unit
-DUX4 protein produced
-Cytotoxic

* = translation stop PAS = DUX4 mRNA polyadenylation site

DUX4 encodes an early developmentally active transcription factor that is silent in healthy somatic cells

***DUX4* is expression is aberrantly increased in FSHD skeletal muscle**

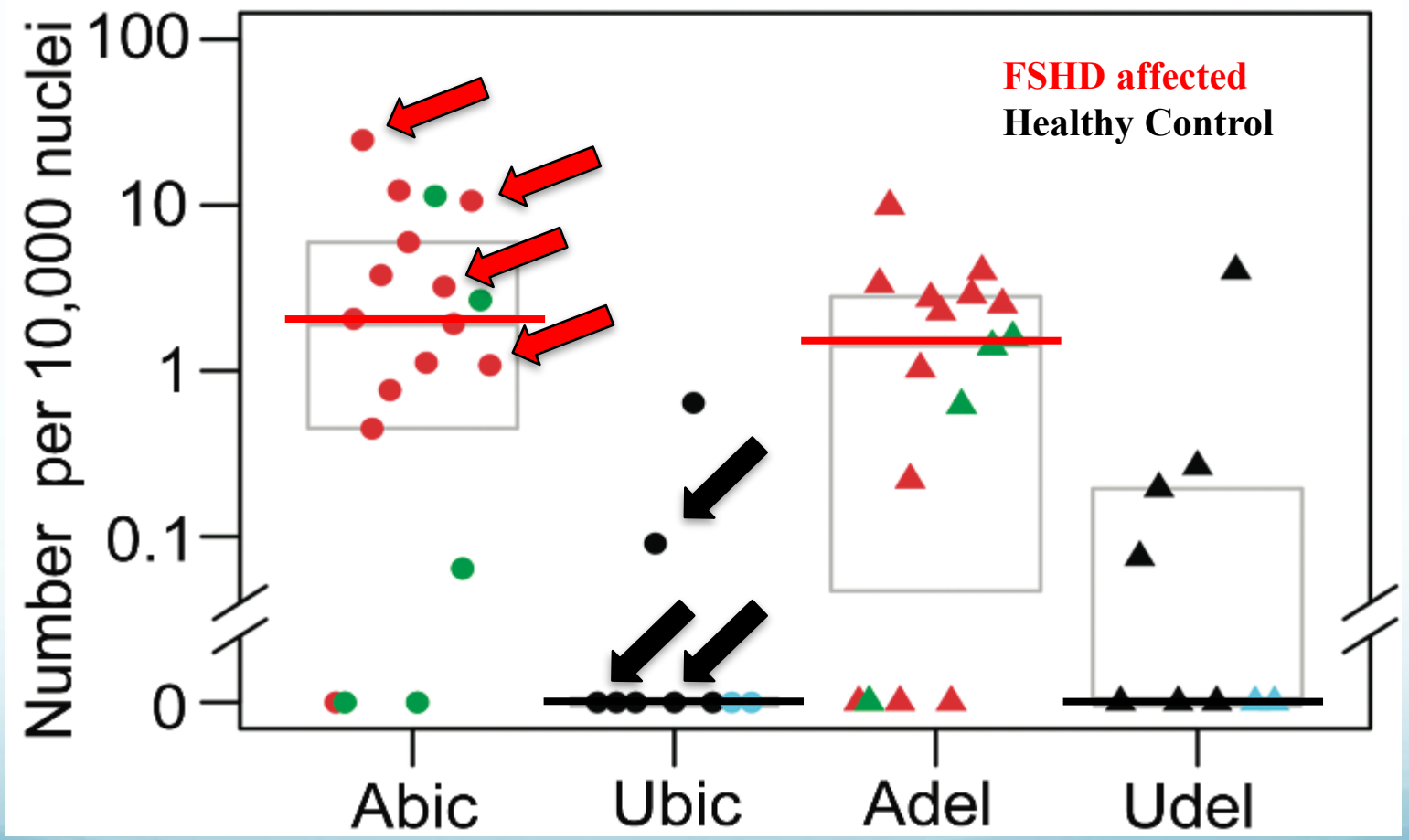
Pathogenic mechanisms of FSHD are dependent upon DUX4



Himeda *et al.* (2015) *Antiox Redox Signaling*

Quantitative model of DUX4-FL expression

DUX4-FL expression in <1% of nuclei of myogenic cells from FSHD subjects

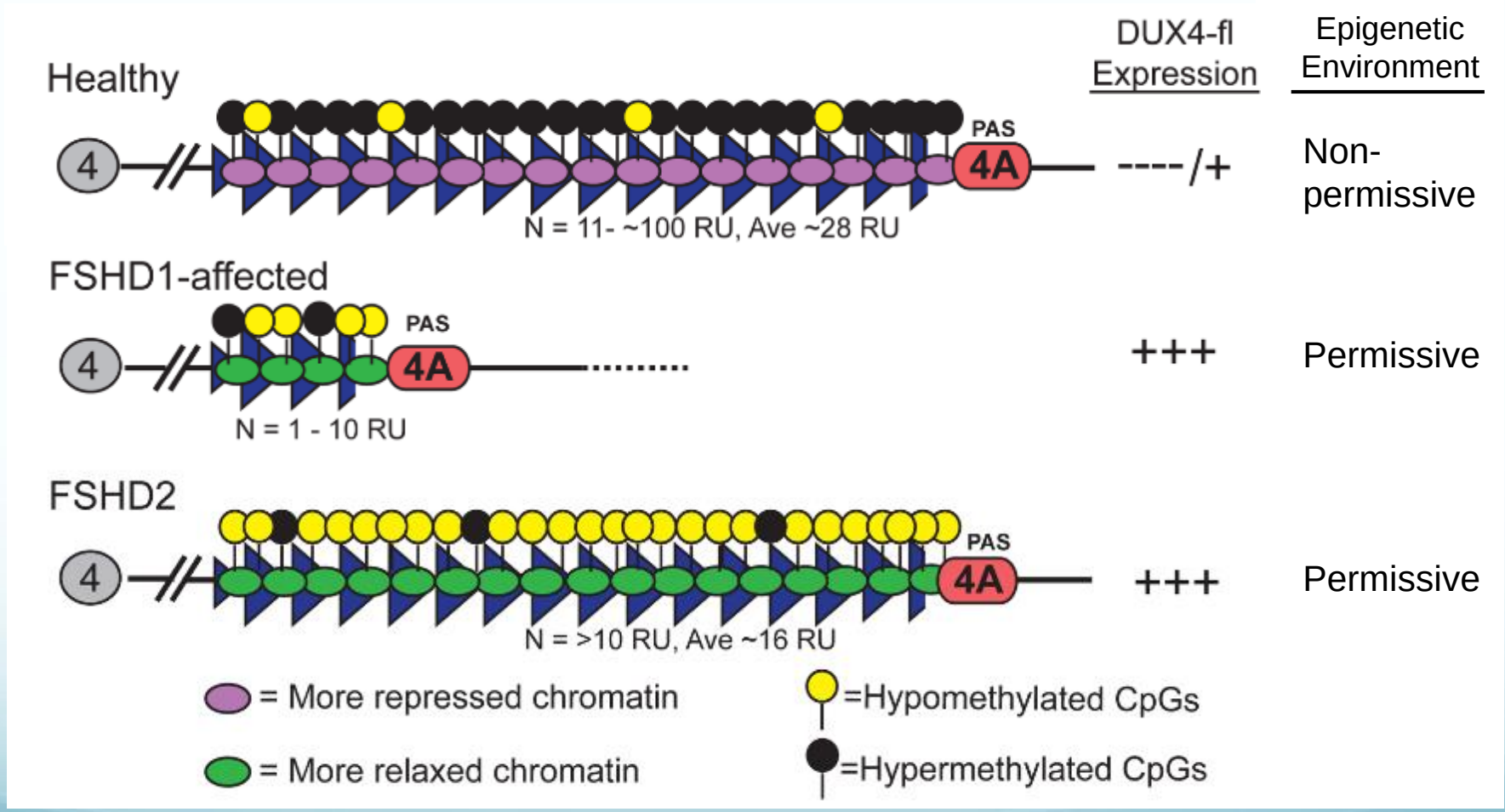


T. Jones *et al*. (2012) *HMG*

Aberrantly increased expression of the *DUX4* gene is the key pathogenic mechanism in FSHD

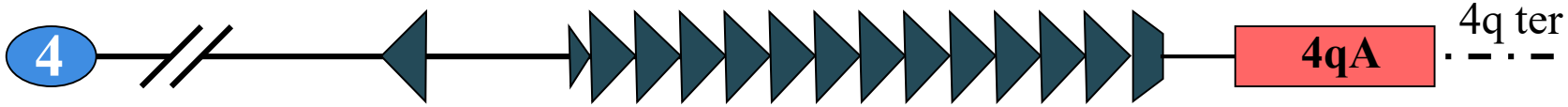
- FSHD is a pathogenic gain-of-function disease
- Most muscle diseases are loss of function
  → need to “fix” or ”replace” something
- FSHD → need to remove an unwanted protein

FSHD is an epigenetic disease with a genetic component

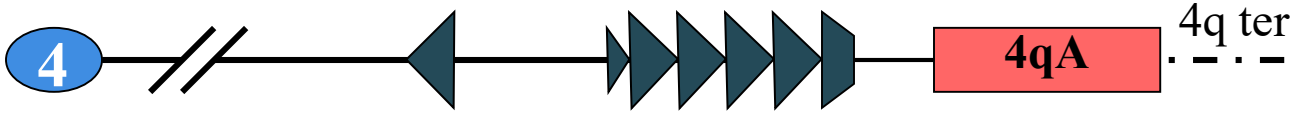


FSHD1 genetics fail to account for the large number of asymptomatic individuals

Healthy



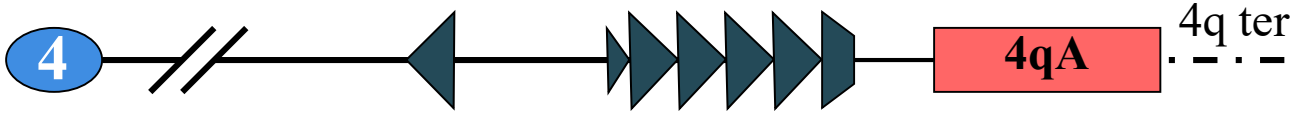
Asymptomatic: Genetically FSHD1, clinically unaffected



~1-3% of population

Scionti *et al.* (2012) *J Med Genet* 49:171
Ricci *et al.* (2013) *Brain* 136:3408

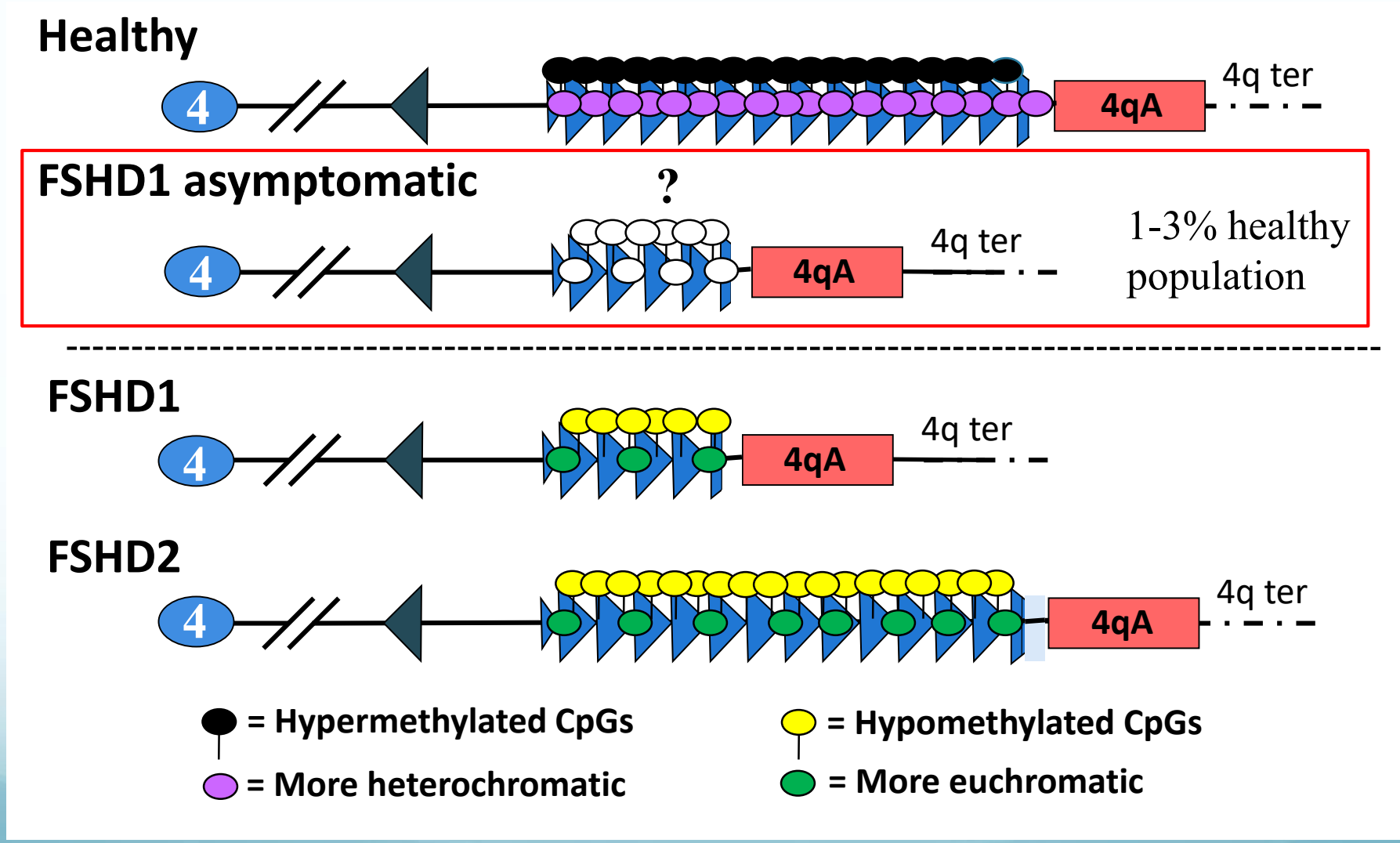
FSHD1



~1:7-14,000

The deletion itself is not pathogenic
The 4qA sub-telomere is permissive, not pathogenic
Existence of modifiers of disease severity

What is the epigenetic state of asymptomatic FSHD subjects?



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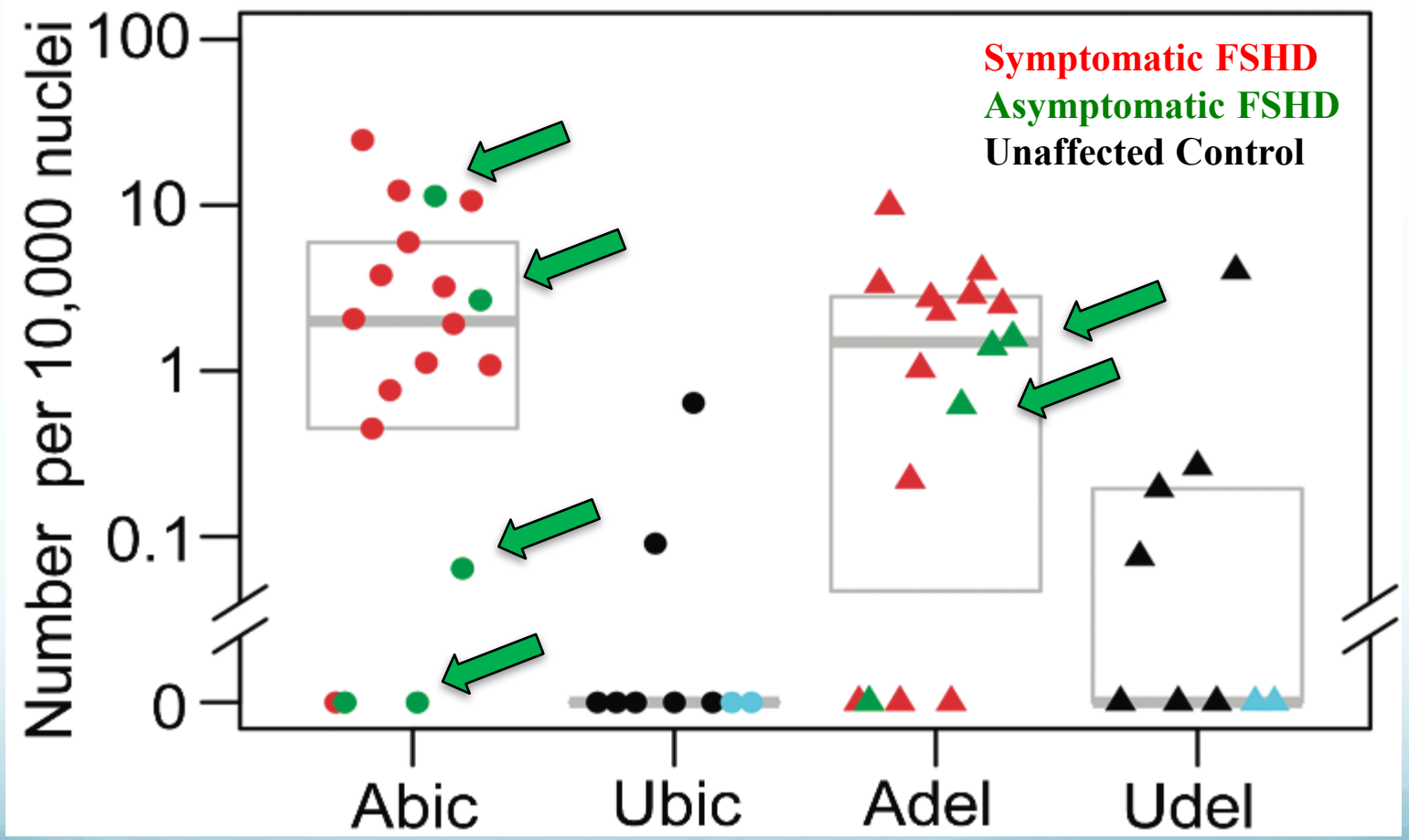
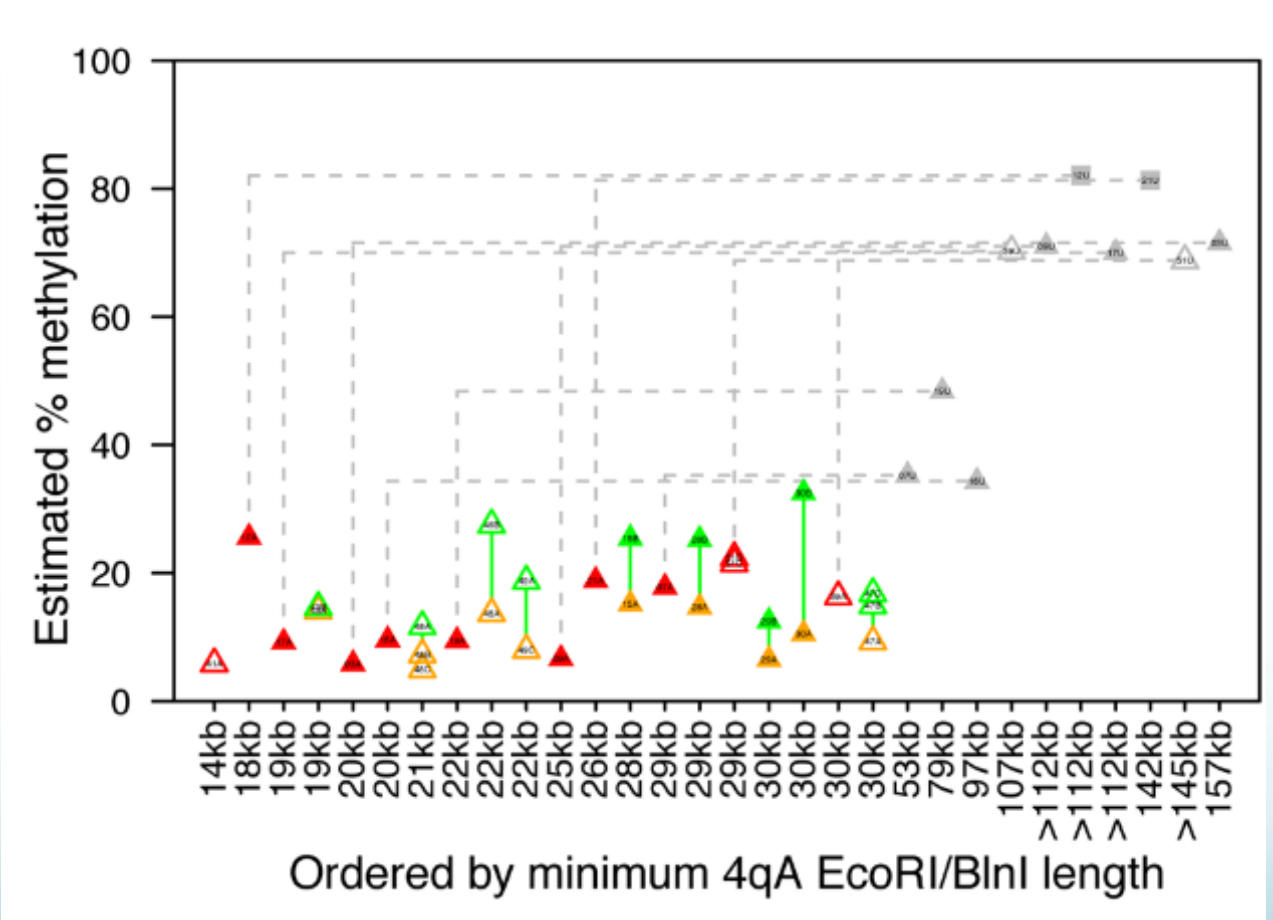


T. Jones *et al*. (2012) *HMG*

Asymptomatic FSHD1 subjects have an intermediate level of DNA methylation



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

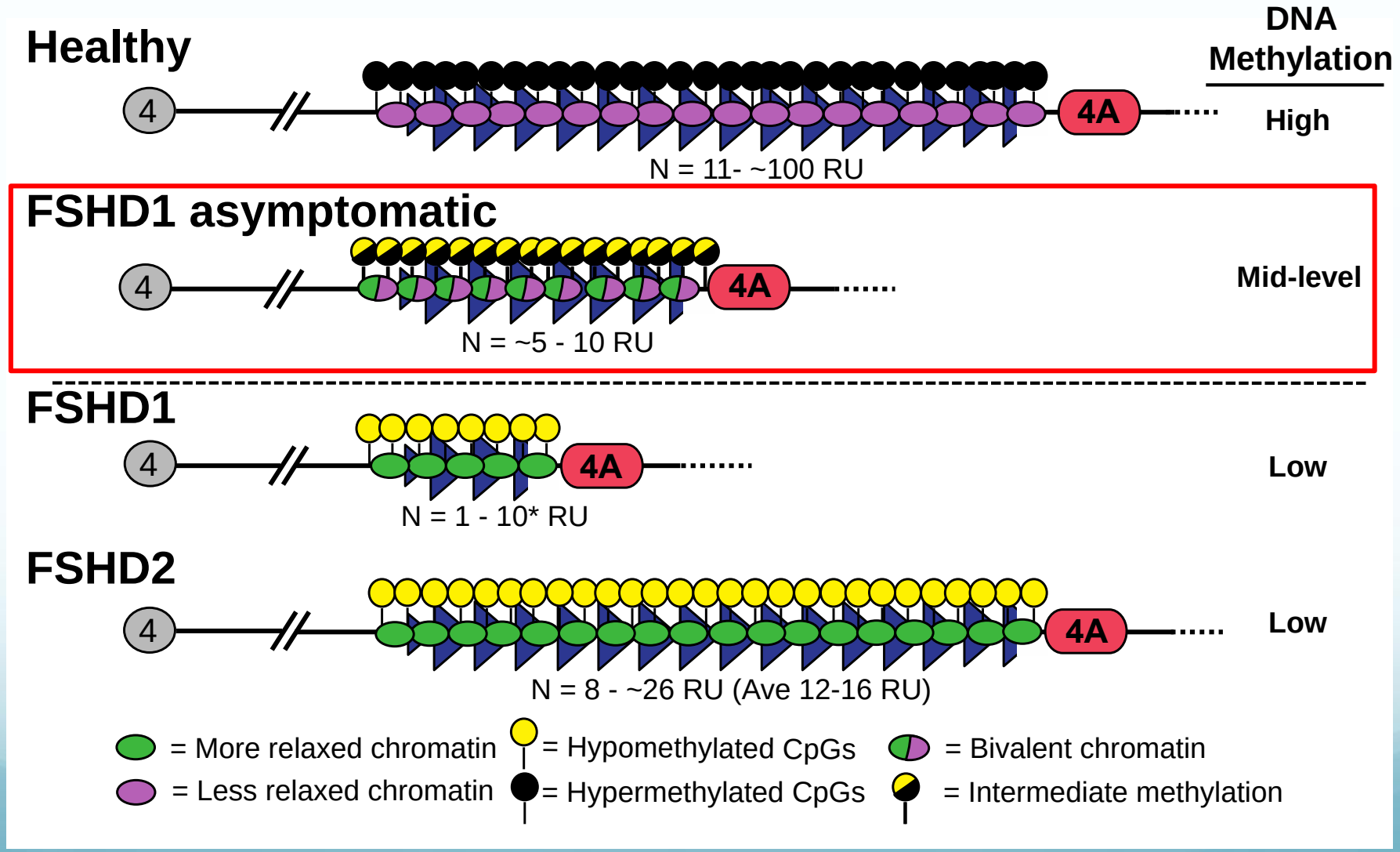
Asymptomatic FSHD1 subjects have an intermediate level of DNA methylation that is significantly higher than FSHD1-affected and significantly lower than healthy controls

Cohort	Manifesting	Nonmanifesting	*Eco*RI/*Bln*I	D4Z4 RU*
15	15.2%	25.4%	28kb	8
28	14.6%	25.2%	29kb	8
29	6.5%	12.5%	30kb	8.5
30	10.6%	32.6%	30kb	8.5
43	14.2%	15.5%	19kb	5
46	13.7%	27.6%	22kb	6
47	9.3%	14.9% & 16.9%	30kb	8.5
48	7.3% & 4.9%	11.7%	21kb	6
49	8.0%	18.8%	22kb	6

*Calculated as D4Z4 RU = (*Eco*RI/*Bln*I fragment kb - 2kb)/3.3

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

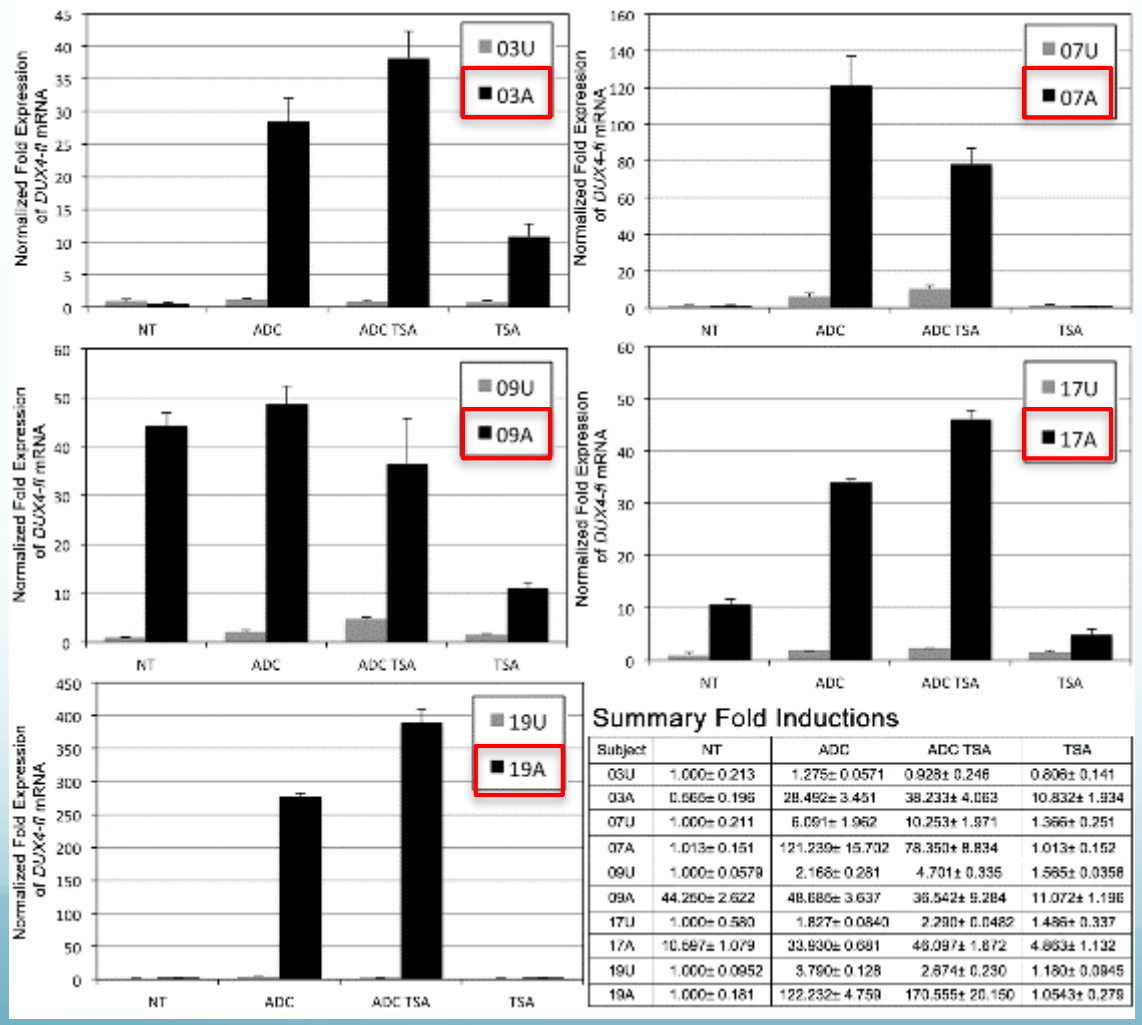
Epigenetic status correlates with FSHD disease presentation



What are the consequences of epigenetic dysregulation?

Do these small differences matter?

Epigenetic repression at the 4q35 D4Z4 array is very stable in healthy controls and epigenetically poised for expression in FSHD1



Summary Fold Inductions

Subject	NT	ADC	ADC TSA	TSA
03U	1.000± 0.213	1.275± 0.0571	0.928± 0.246	0.606± 0.141
03A	0.566± 0.196	28.462± 3.451	38.233± 4.063	10.832± 1.934
07U	1.000± 0.211	6.091± 1.962	10.253± 1.971	1.366± 0.251
07A	1.013± 0.151	121.239± 15.702	78.350± 8.834	1.013± 0.152
09U	1.000± 0.0579	2.168± 0.281	4.701± 0.335	1.585± 0.0358
09A	44.250± 2.622	48.686± 3.637	36.542± 9.284	11.072± 1.196
17U	1.000± 0.580	1.827± 0.0840	2.290± 0.0482	1.486± 0.337
17A	10.697± 1.079	33.930± 0.681	46.097± 1.872	4.863± 1.132
19U	1.000± 0.0952	3.790± 0.128	2.674± 0.230	1.180± 0.0845
19A	1.000± 0.181	122.232± 4.759	170.555± 20.150	1.0543± 0.278

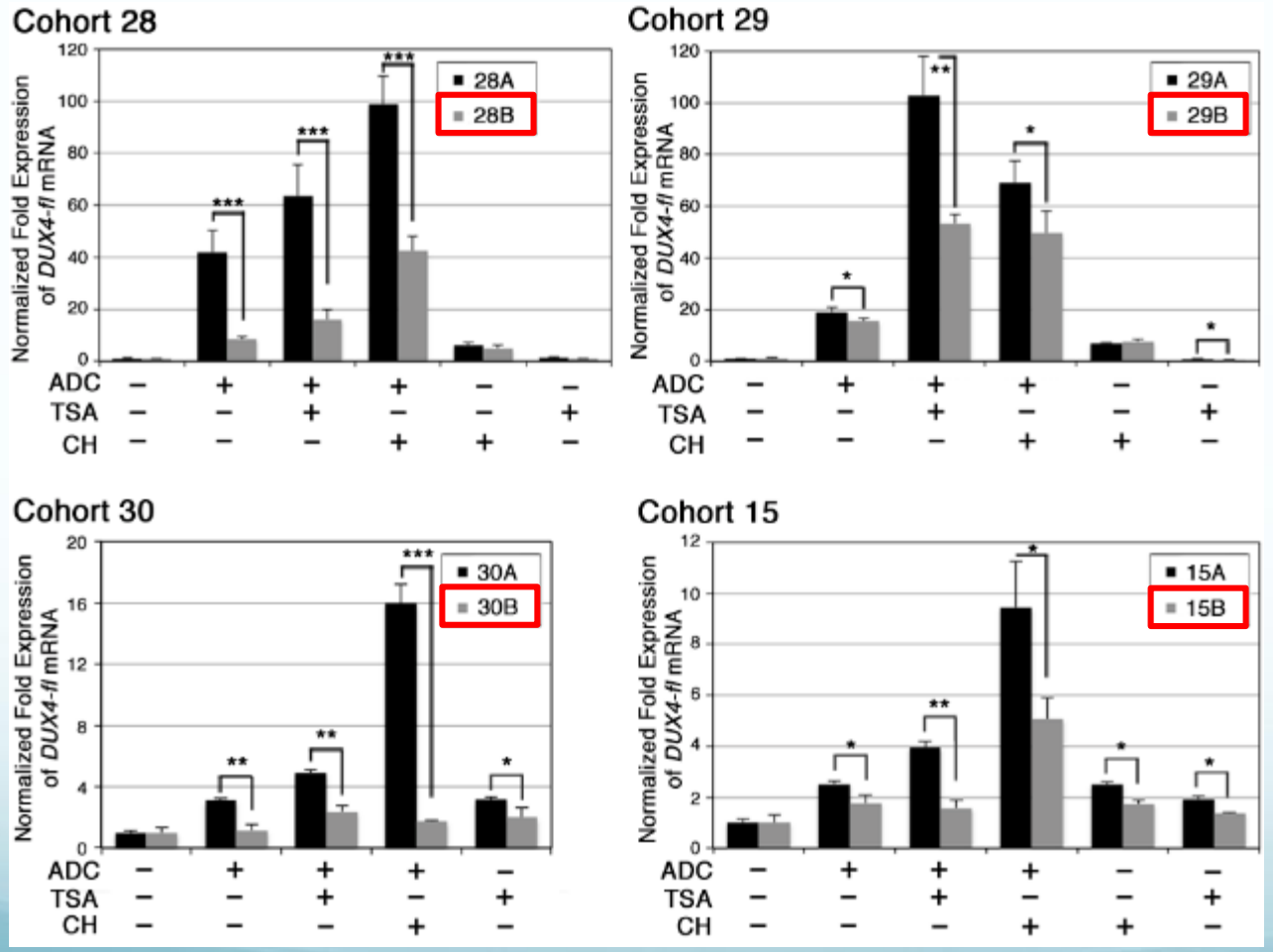
NT = not treated

ADC = Decitabine
inhibits DNA methylation
→ more euchromatic

TSA = Trichostatin A
HDAC inhibitor
→ favors histone acetylation
→ more euchromatic
→ can affect other proteins

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

Epigenetic repression at the 4q35 D4Z4 array is more stable in asymptomatic FSHD1 subjects compared with affected FSHD1 patients



NT = not treated

ADC = Decitabine
→ inhibits DNA methylation
→ more euchromatic

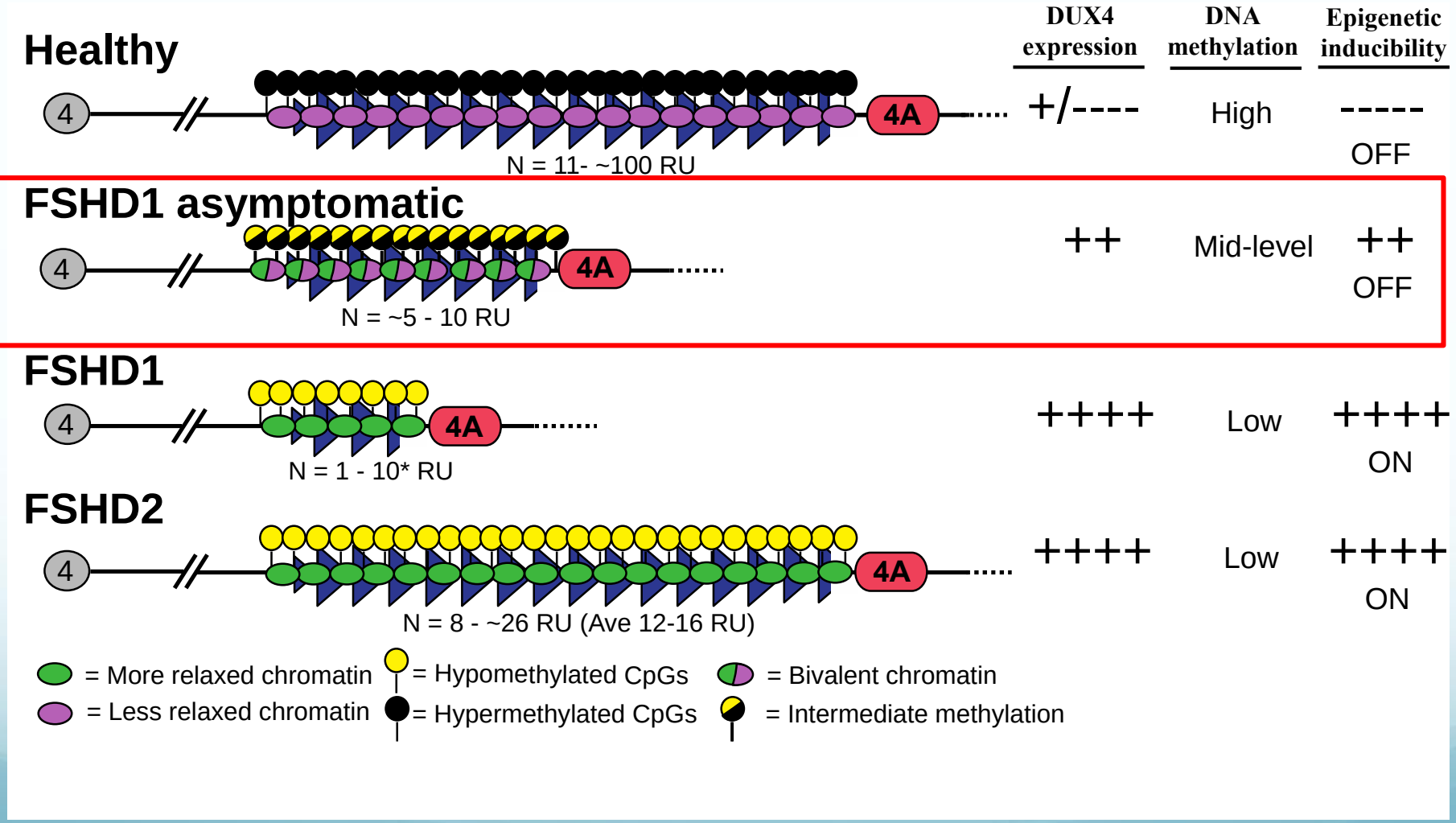
TSA = Trichostatin A
HDAC inhibitor
→ favors histone acetylation
→ more euchromatic
→ can affect other proteins

CH = Chaetocin
→ inhibits H3K9me3
→ more euchromatic

B = Non-manifesting

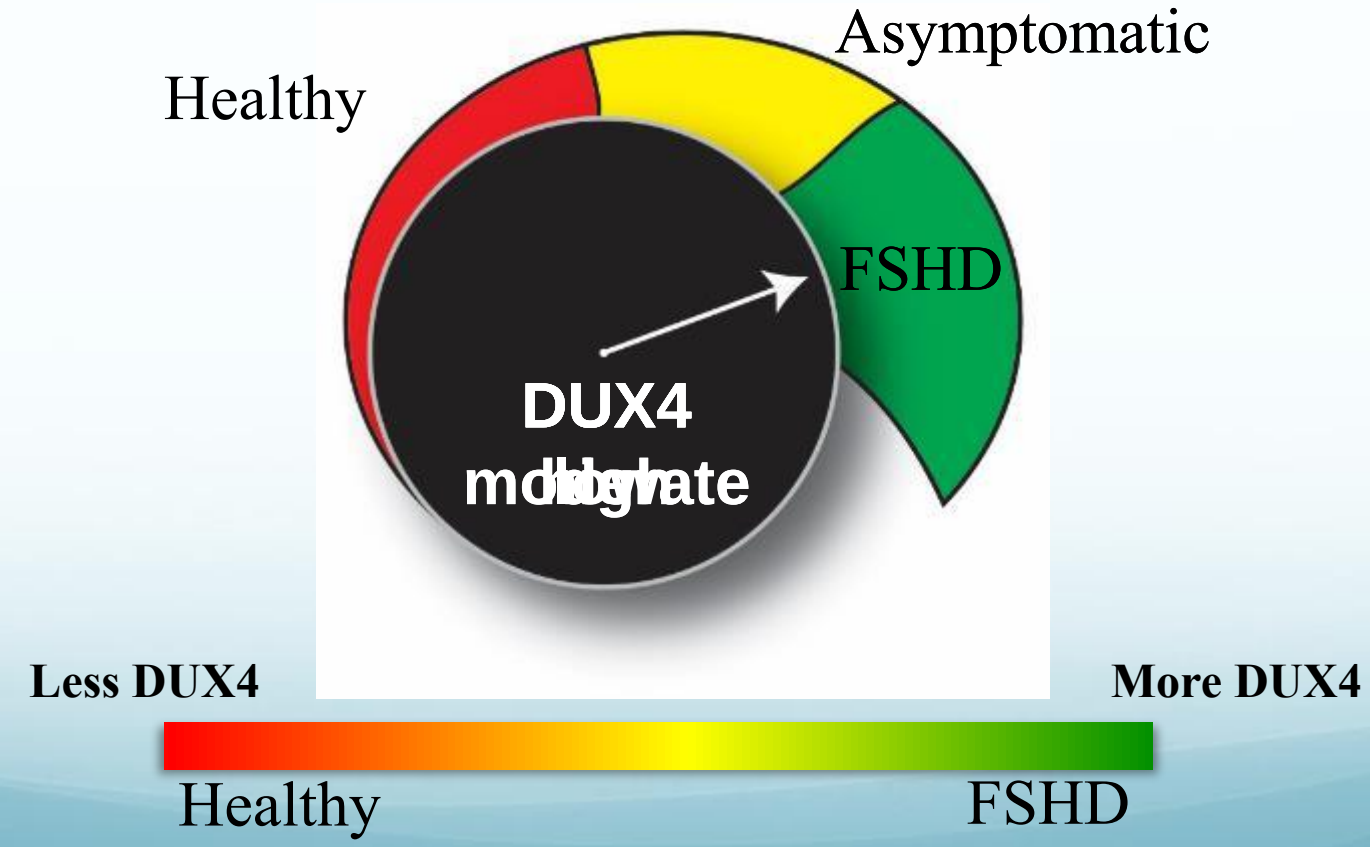
T Jones *et al.* 2015 *Clinical Epigenetics*

Asymptomatic FSHD1 subjects have an intermediate status



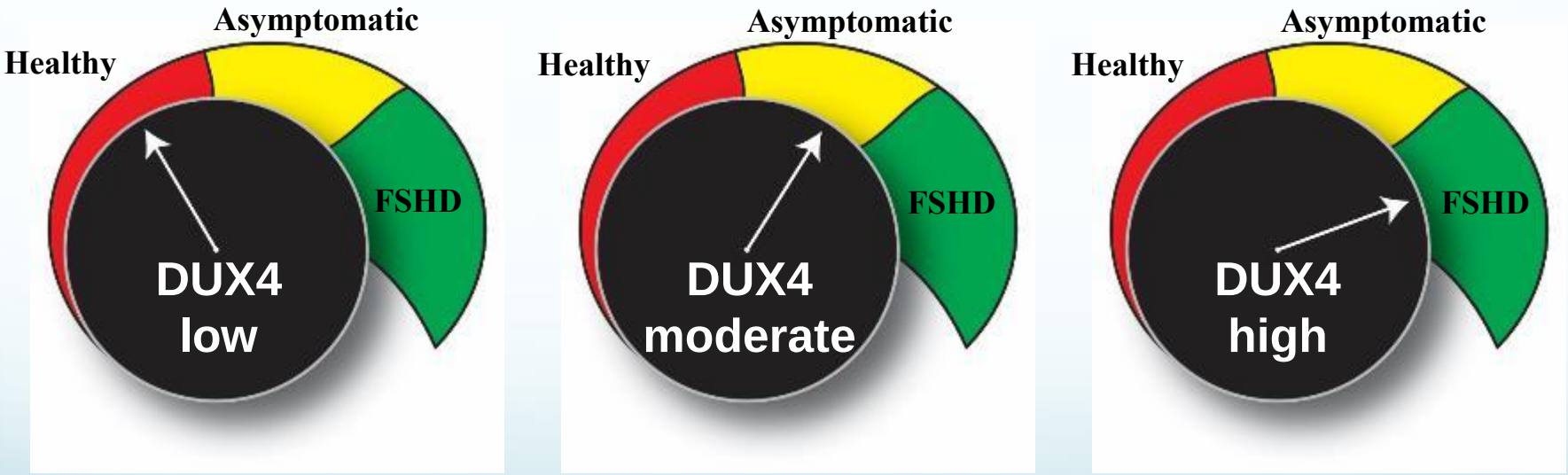
T Jones *et al.* 2015 *Clinical Epigenetics*

Small changes in epigenetic state and/or DUX4 expression levels have large clinical consequences

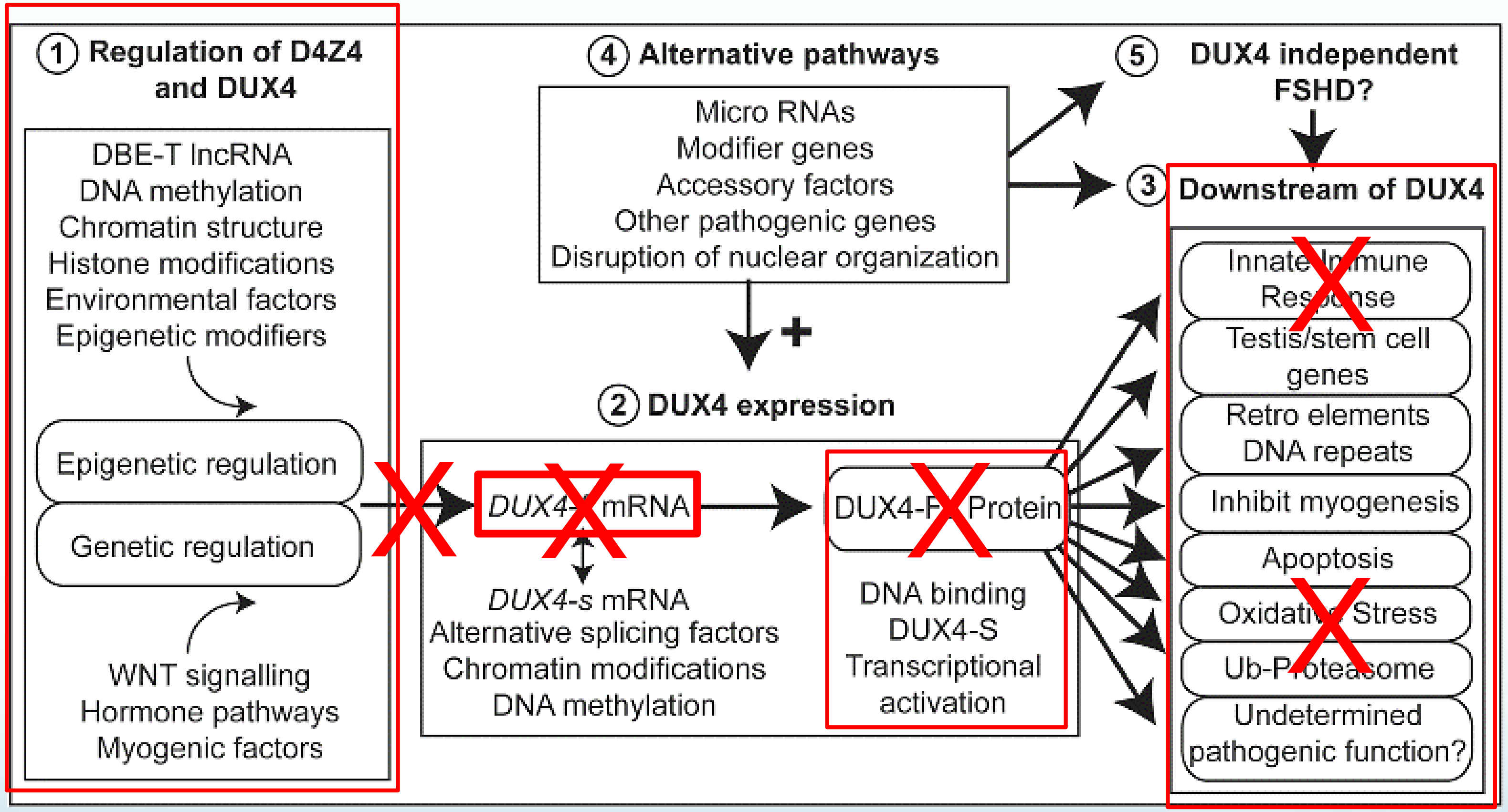


What does the epigenetic and DUX4 expression data tell us about FSHD therapies?

Small changes in epigenetic state and/or DUX4 expression levels have large clinical consequences



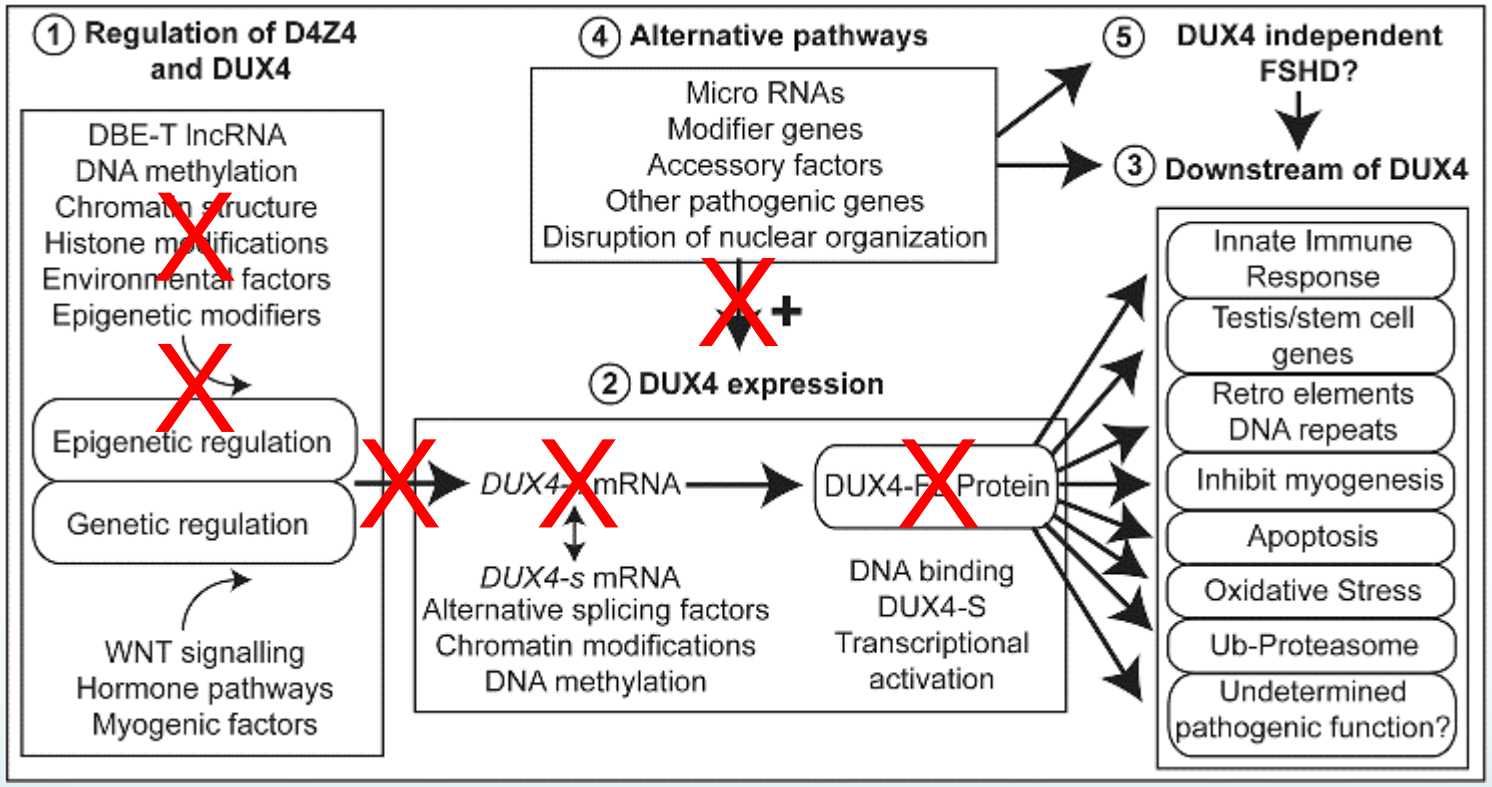
FSHD in 2019
Many viable therapeutic approaches



Drugs blocking DUX4 protein function (Icagen)

Reduction or elimination of DUX4 expression targets the key pathogenic mechanism

Any level of DUX4 reduction may have therapeutic benefit!



Small molecules targeting DUX4 regulation or function

*Morpholinos/PMOs/shRNAs & *miRNAs

*CRISPR-inhibition

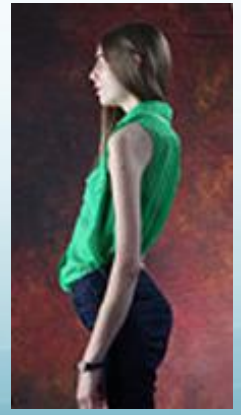
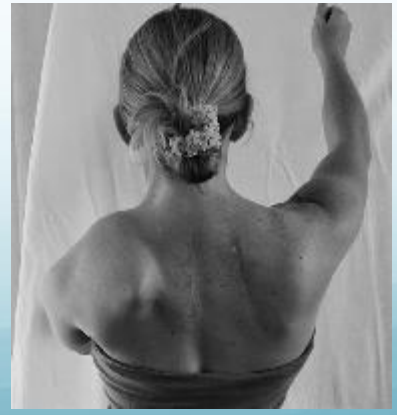
*delivery concerns

University of Nevada, Reno
School of Medicine
Department of Pharmacology

The Role of DUX4 in Development and Disease

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



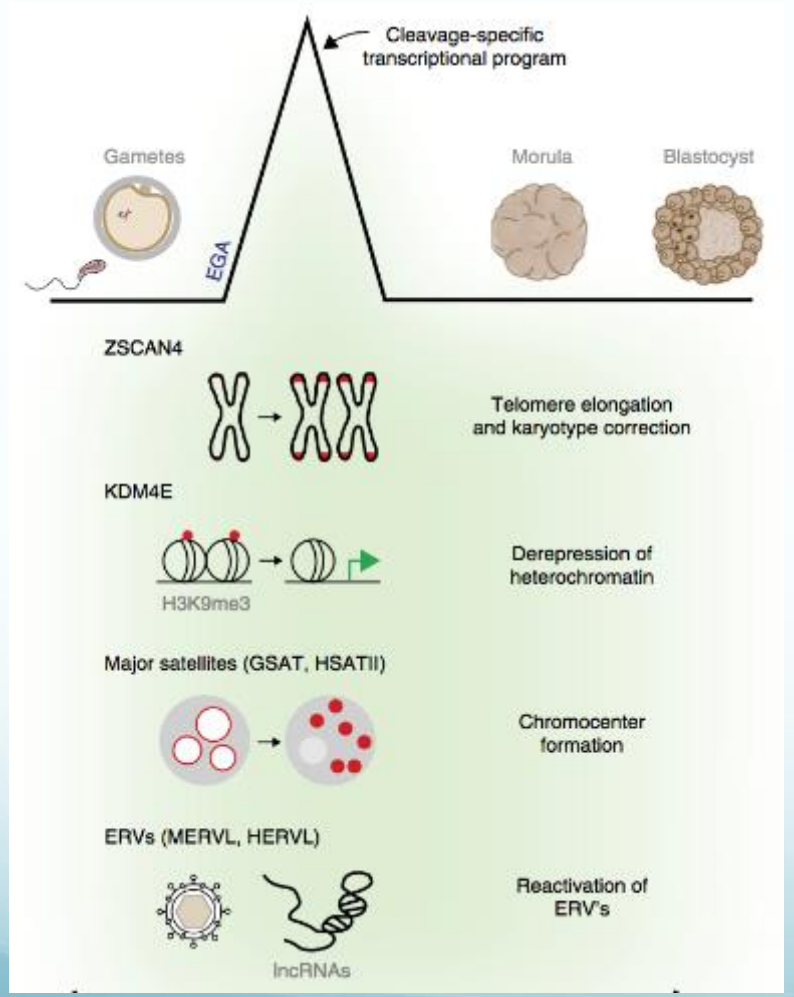
We now have a clear therapeutic target: DUX4

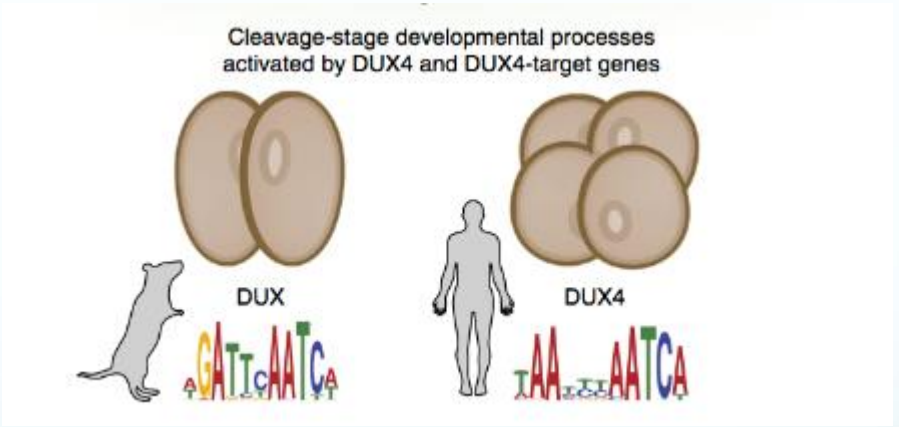
Produced by Bill Milling and Susan Egert
Directed by Arie Ohayon
Featuring Steven Blier and Kelli O'hara

DUX4 expression is a FSHD therapeutic target

- **Why do we have DUX4 and what does it do?**
- **How does it cause FSHD?**
- **What will reducing expression do outside of FSHD?**

DUX4 encodes an important developmental transcription factor

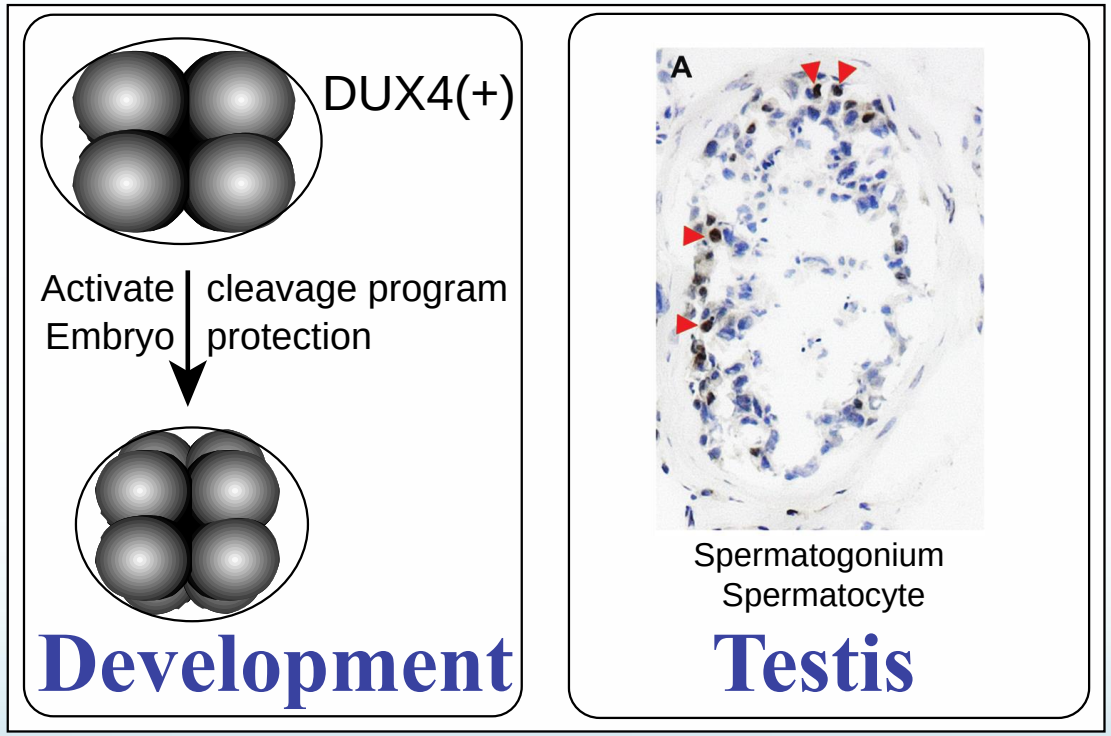




Conserved function in DUX gene family in mammals

Hendrickson *et al.* 2017 Nat Genetics

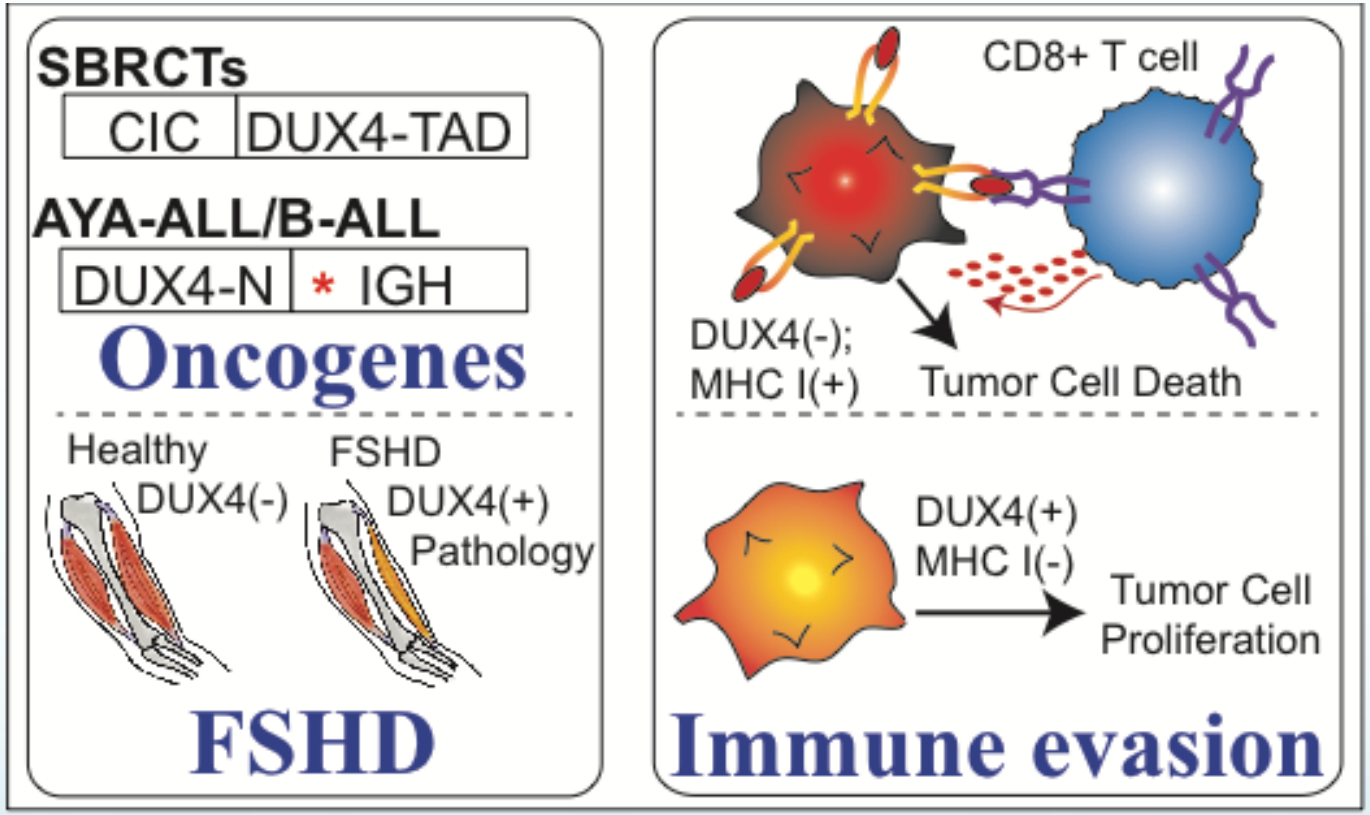
Healthy DUX4 expression is extremely limited



Hendrickson *et al*. 2017 Nat Genetics

Snider *et al.* 2010 PLoS Genetics

Aberrant DUX4 expression is pathogenic



Kawamura-Saito *et al*. 2006 Hum Mol Genetics
Wei *et al*. 2018 Cancer Discov
Yasuda *et al*. 2016 Nat Genetics
Lemmers *et al*. 2010 Science
Snider *et al*. 2010 PLoS Genetics

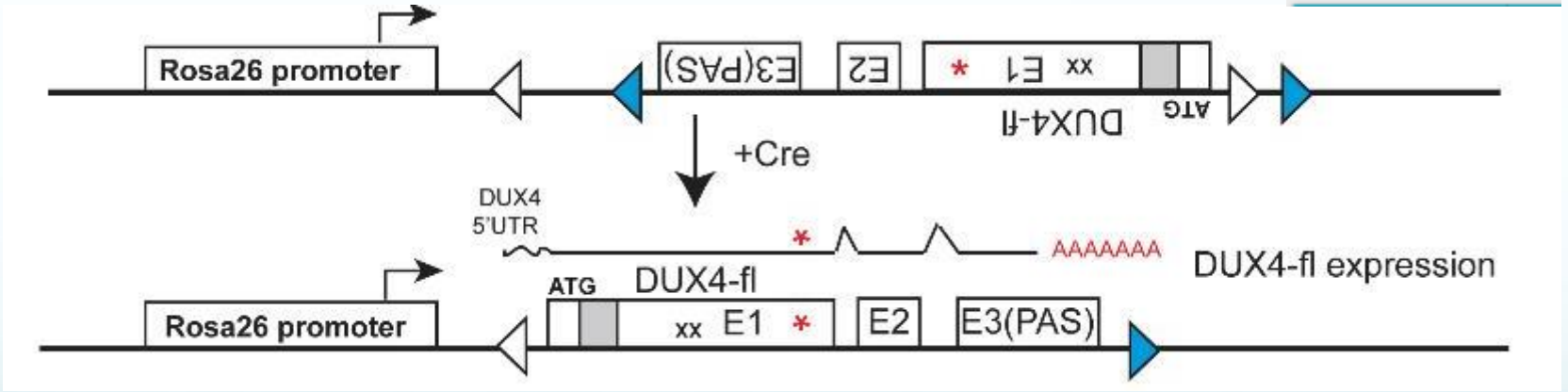
Chew *et al*. 2010 Dev Cell

What happens when DUX4 is misexpressed?

Generation of an FSHD-like mouse based on low level DUX4 expression

Takako Jones, PhD

FLExDUX4

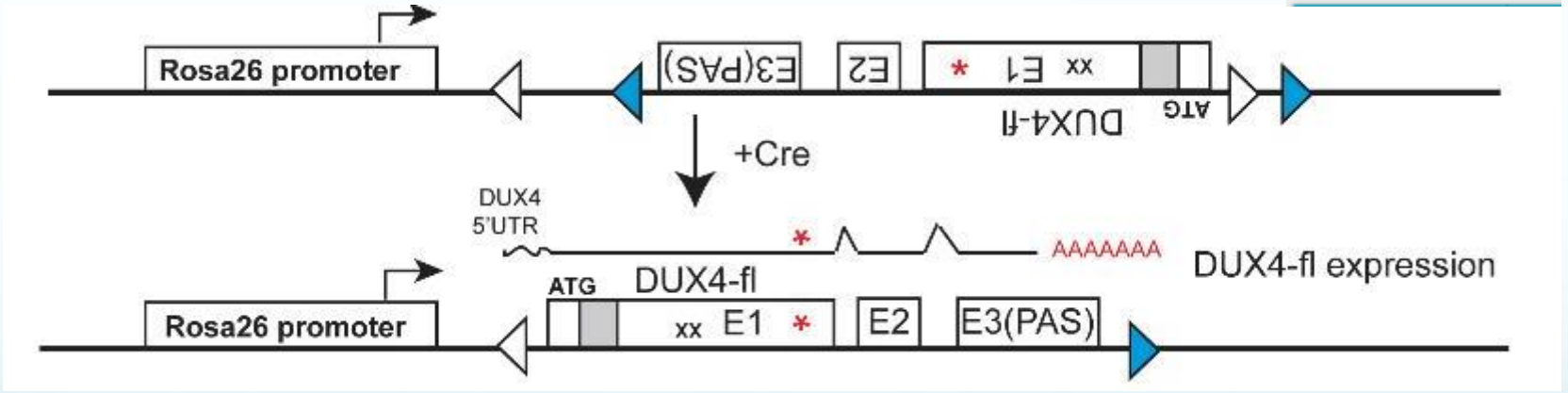


The *Rosa26* promoter ensures robust DUX4-fl expression in all cells that underwent cre-mediated inversion

The *FLExDUX4* mouse model allows for control of the timing and level of human DUX4 expression in muscles of an adult mouse

Takako Jones, PhD

FLExDUX4

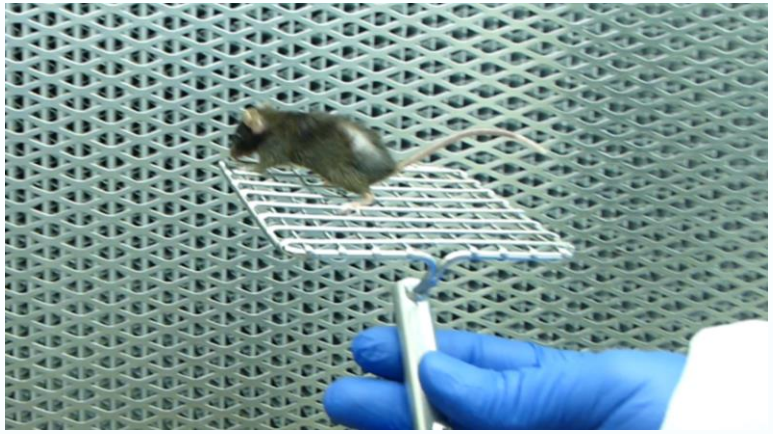


No DUX4 **More DUX4**

Healthy FSHD-like pathology

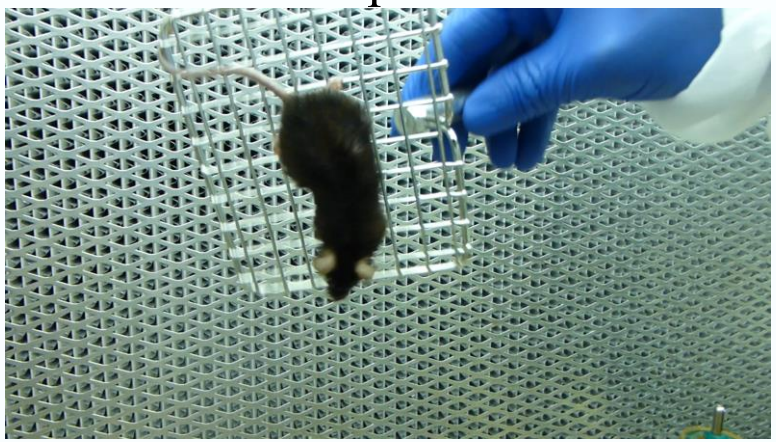
Induced expression of DUX4 in adult mice leads to an FSHD-like myopathy

>1 min suspended



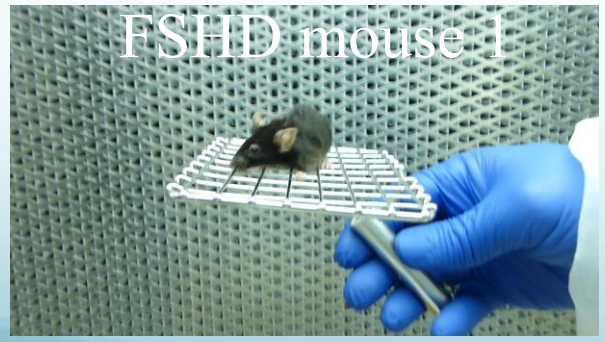
Healthy control

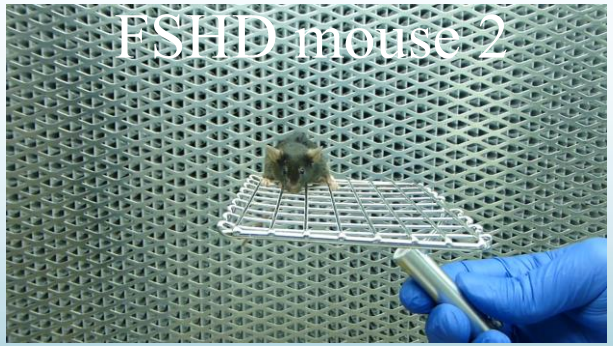
>1 min suspended

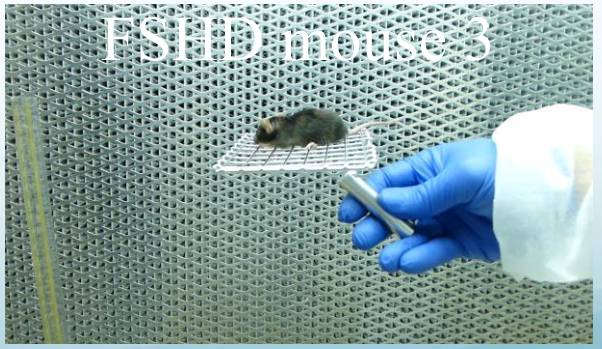


FLExDUX4 mouse before disease onset

<2 second suspended

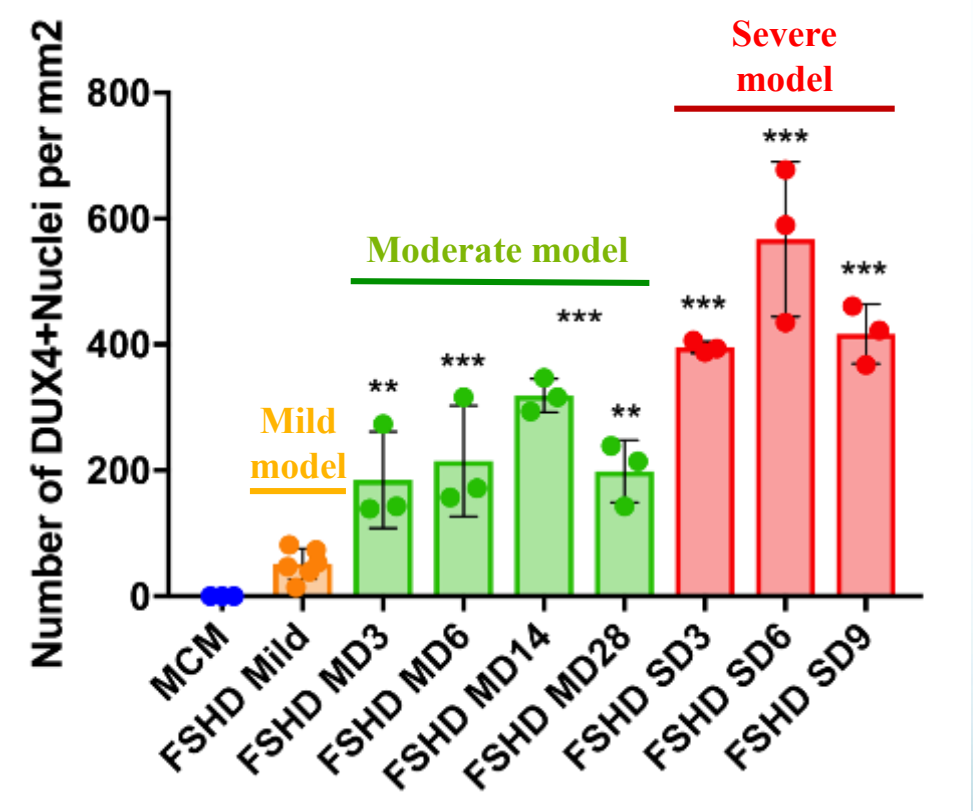




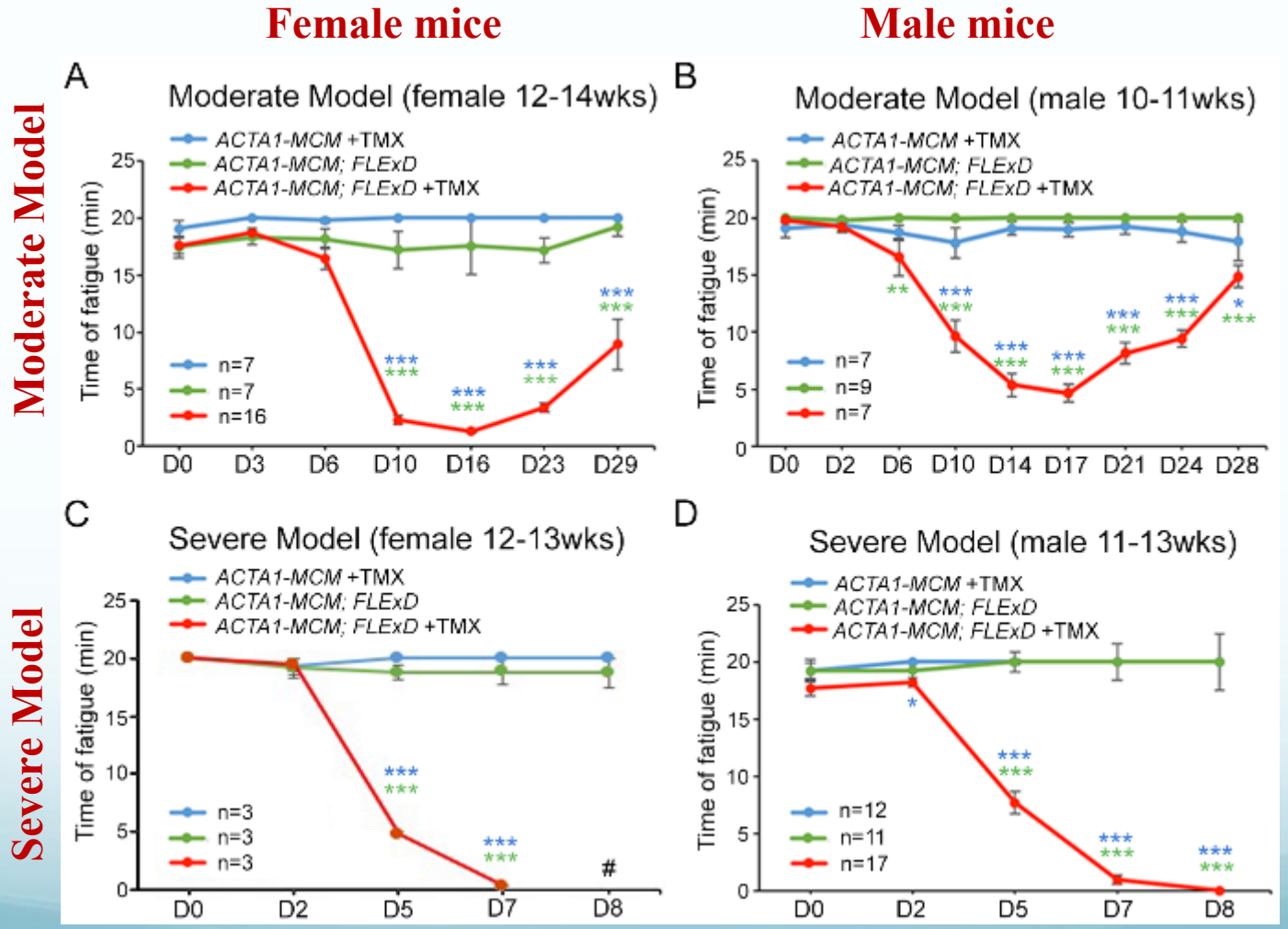


FLExDUX4 mice after induced DUX4 expression

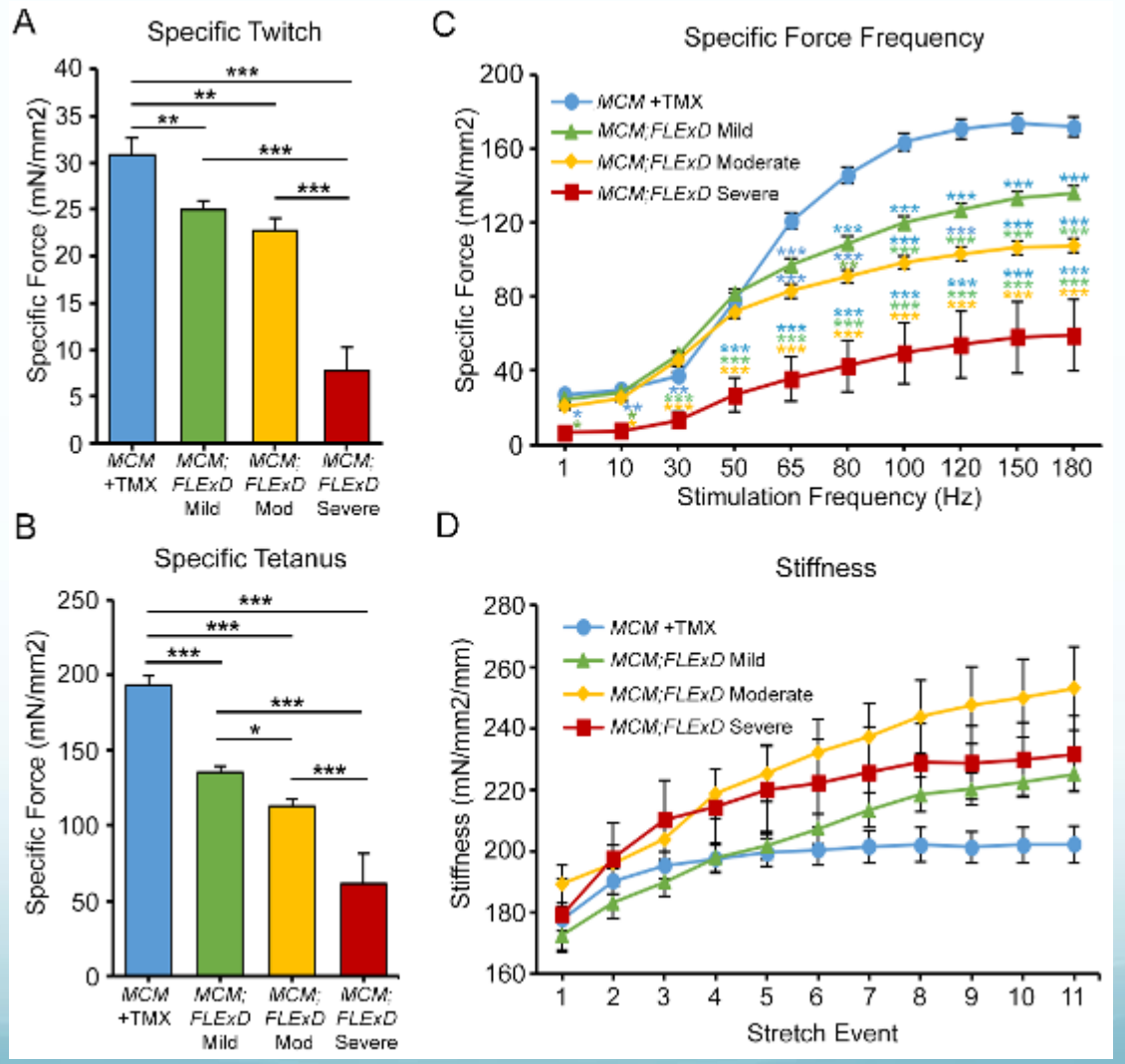
Small increases in DUX4-FL protein levels lead to increased severity of FSHD-like disease



As DUX4 expression increases, treadmill running fitness declines

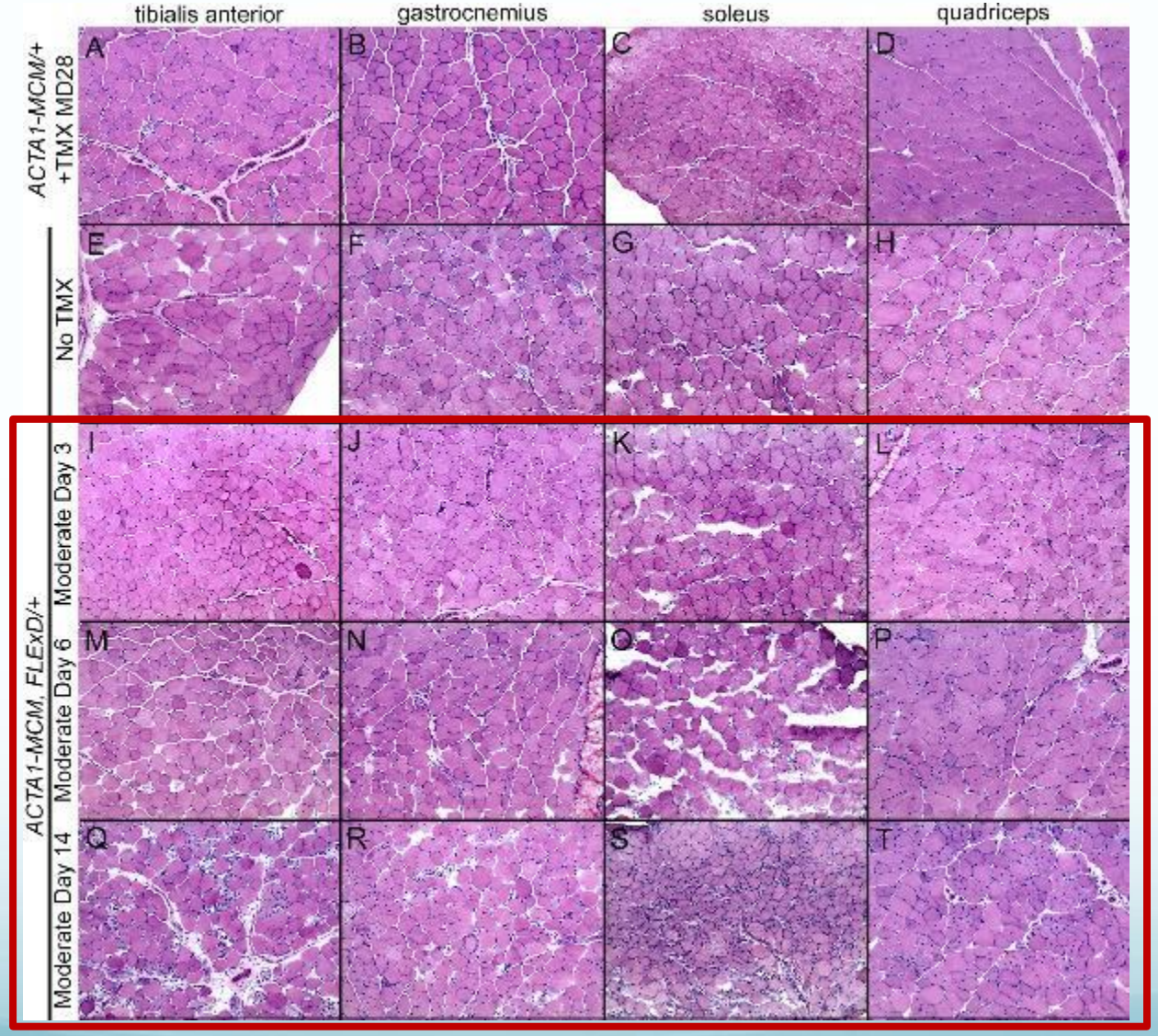


As DUX4 expression increases, skeletal muscles get weaker



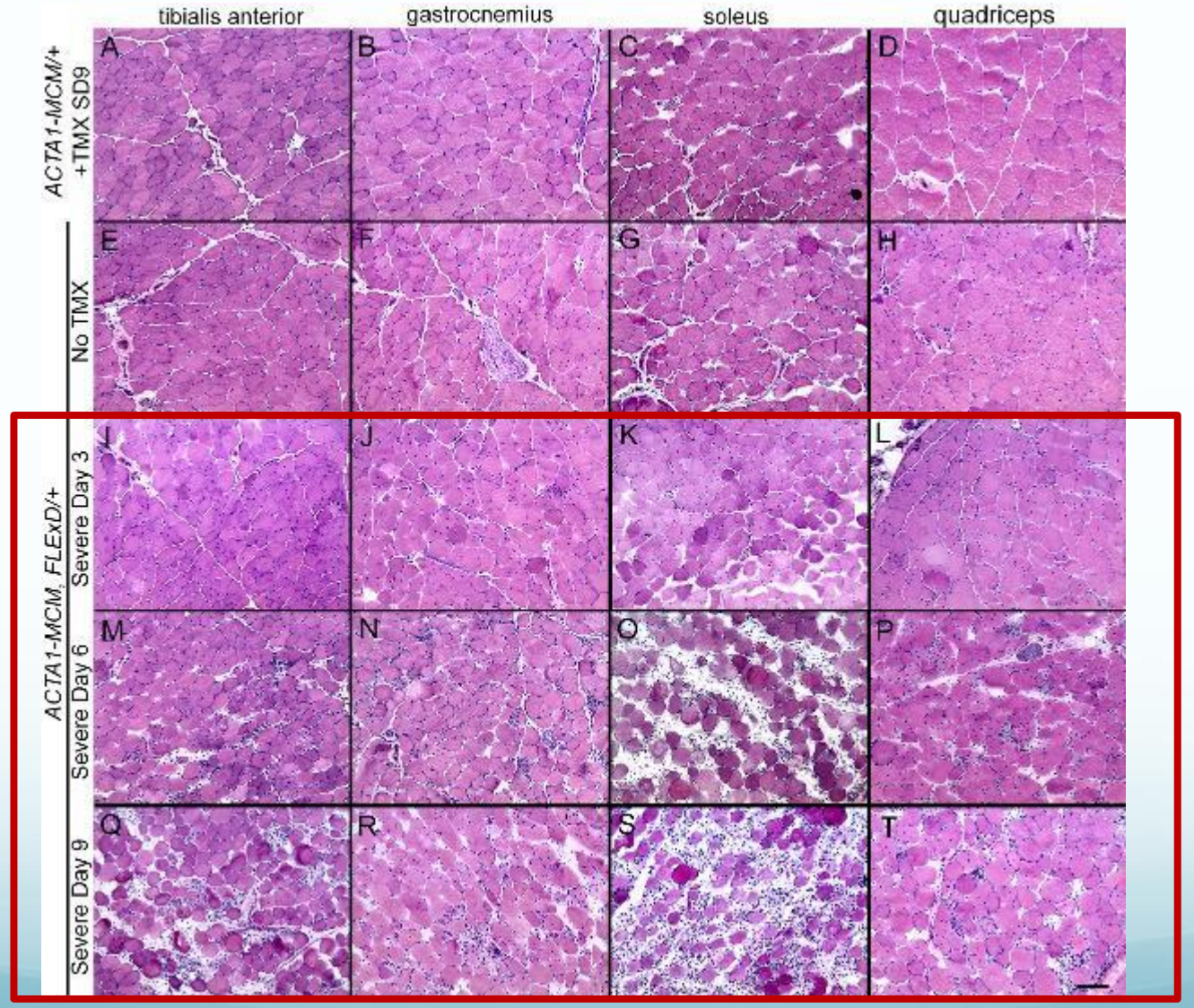
Increased DUX4 expression leads to increased muscle histopathology

Moderate Model



Increased DUX4 expression leads to increased muscle histopathology

Severe Model



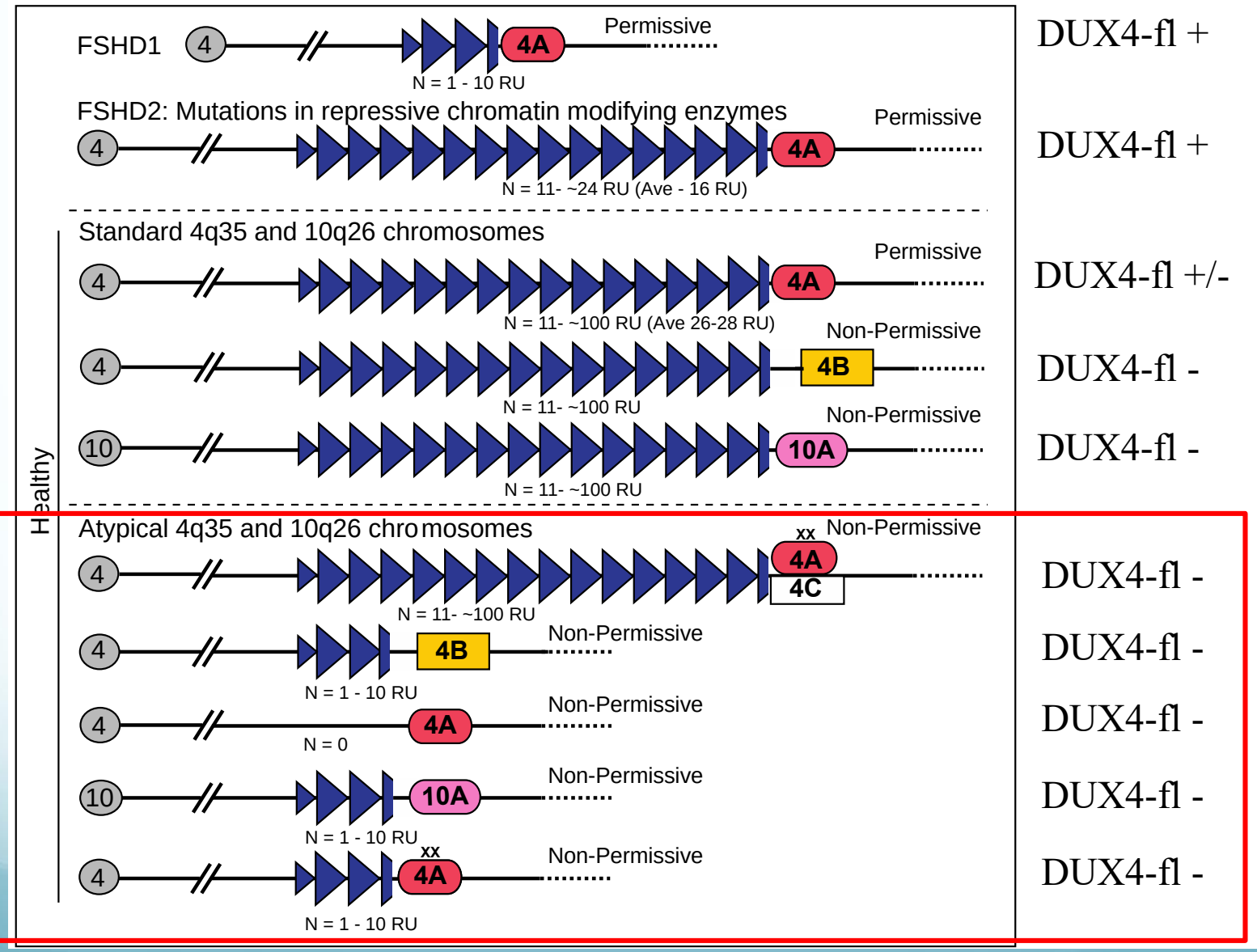
Dose dependent increases in DUX4 expression in skeletal muscle lead to:

- Decreased muscle function
- Decreased muscle strength
- Increased muscle histopatholgy

Do we need DUX4 expression as adults?

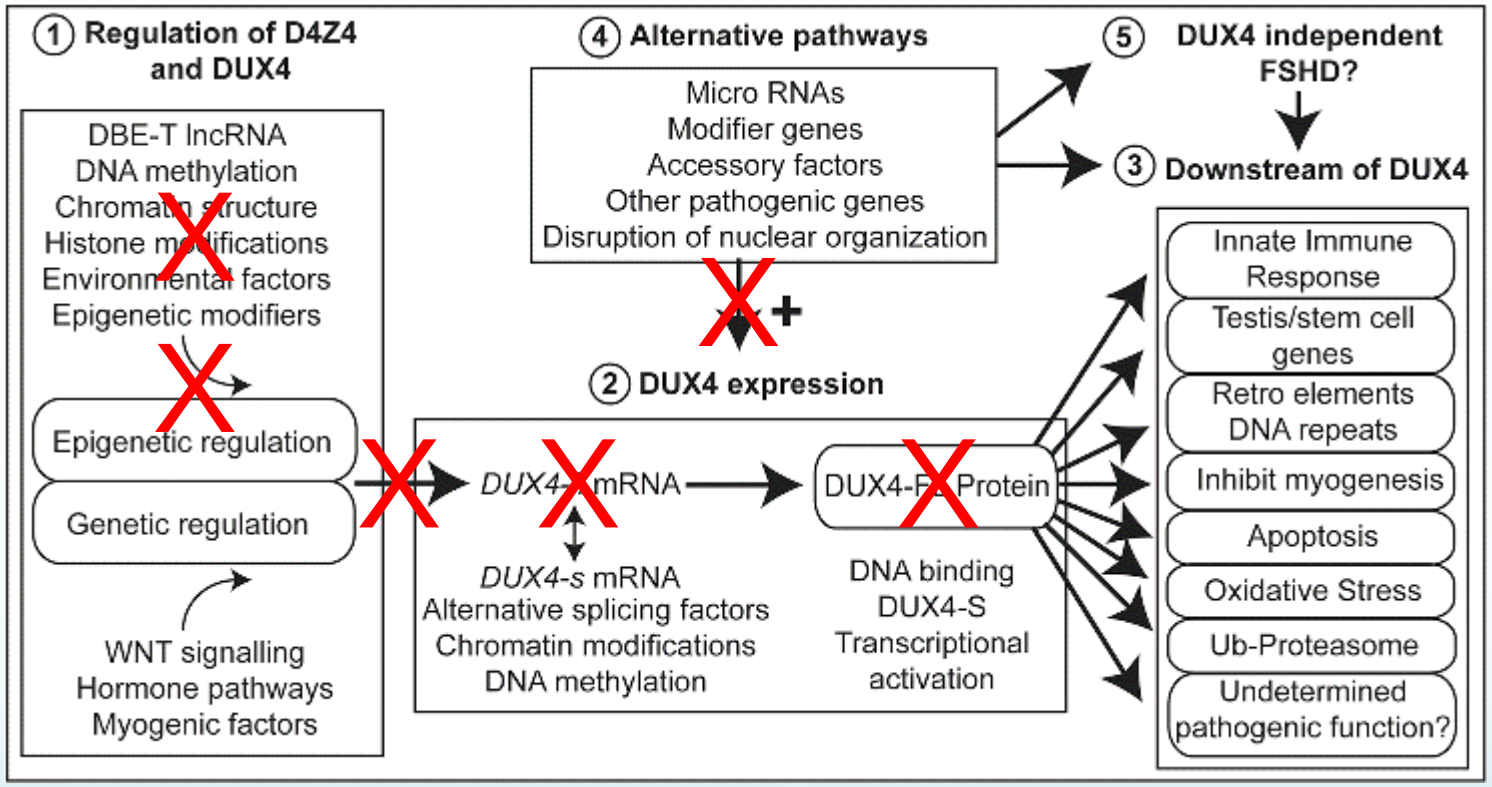
***25% of the population is “non-permissive” for the somatic DUX4 mRNA polyadenylation signal**

Half of the 4q chromosomes are non-permissive for DUX4



Reduction or elimination of DUX4 expression targets the key pathogenic mechanism

Any level of DUX4 reduction may have therapeutic benefit!



Small molecules targeting DUX4 regulation or function

*Morpholinos/PMOs/shRNAs & *miRNAs

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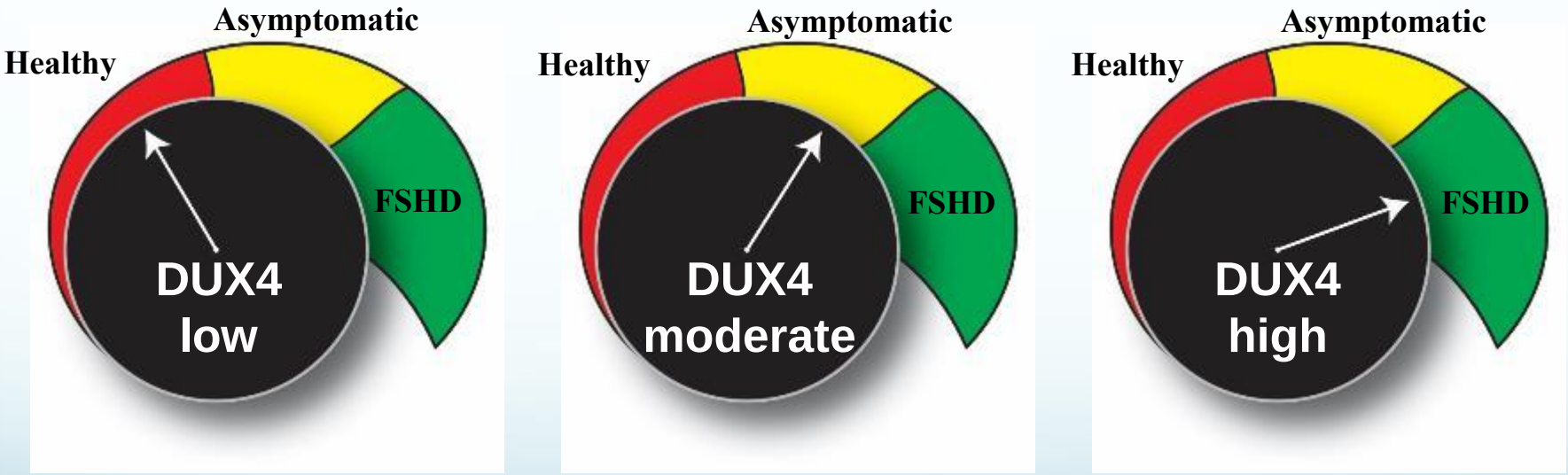


Questions?

Contact: peterjones@med.unr.edu
https://med.unr.edu/jones-lab

p38 inhibitors for FSHD: turning off DUX4

Fran Sverdrup, PhD
Department of Biochemistry and Molecular Biology
Saint Louis University
November 7, 2019

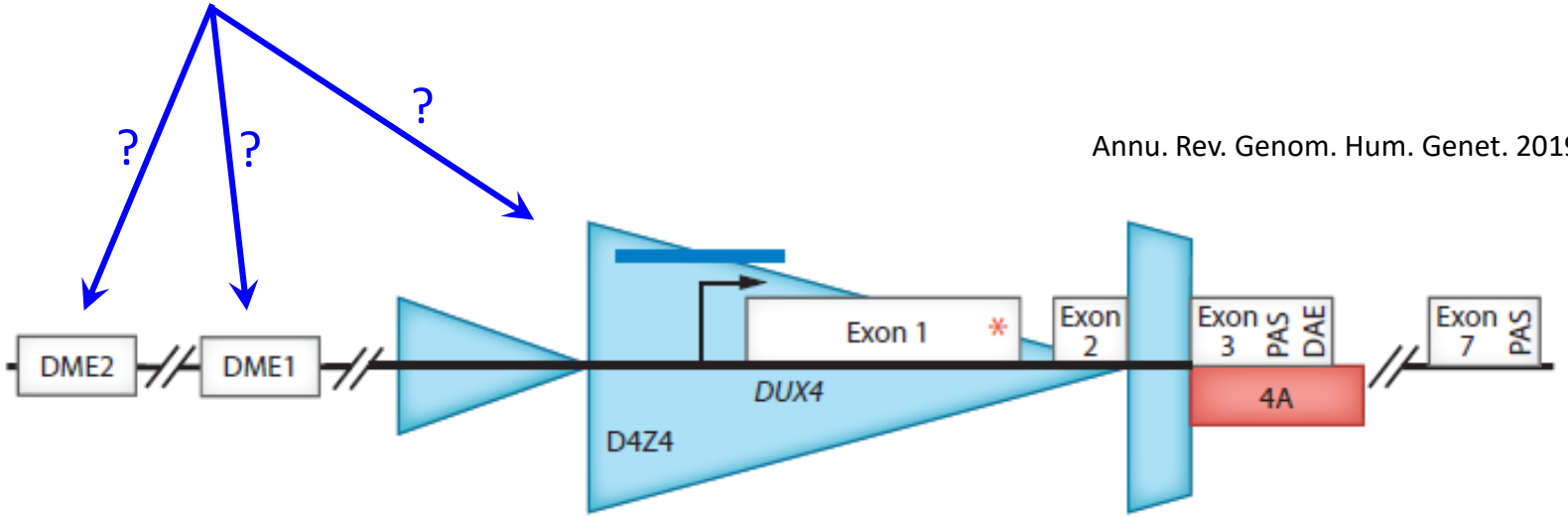
p38 inhibitors for FSHD: Turning off DUX4

- Therapeutic strategy: targeting DUX4 expression
  - Identification of p38 inhibitors
- p38: muscle biology
- Choice of losmapimod
- Example of losmapimod turning off DUX4 in xenograft mouse model
- Role of p38 in promoting DUX4 expression (work in progress)

Therapeutic strategy

Suppress transcription of DUX4 mRNA

Factors that promote transcription are not understood

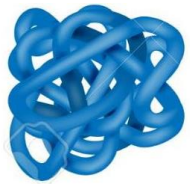


Annu. Rev. Genom. Hum. Genet. 2019. 20:8.1–8.27

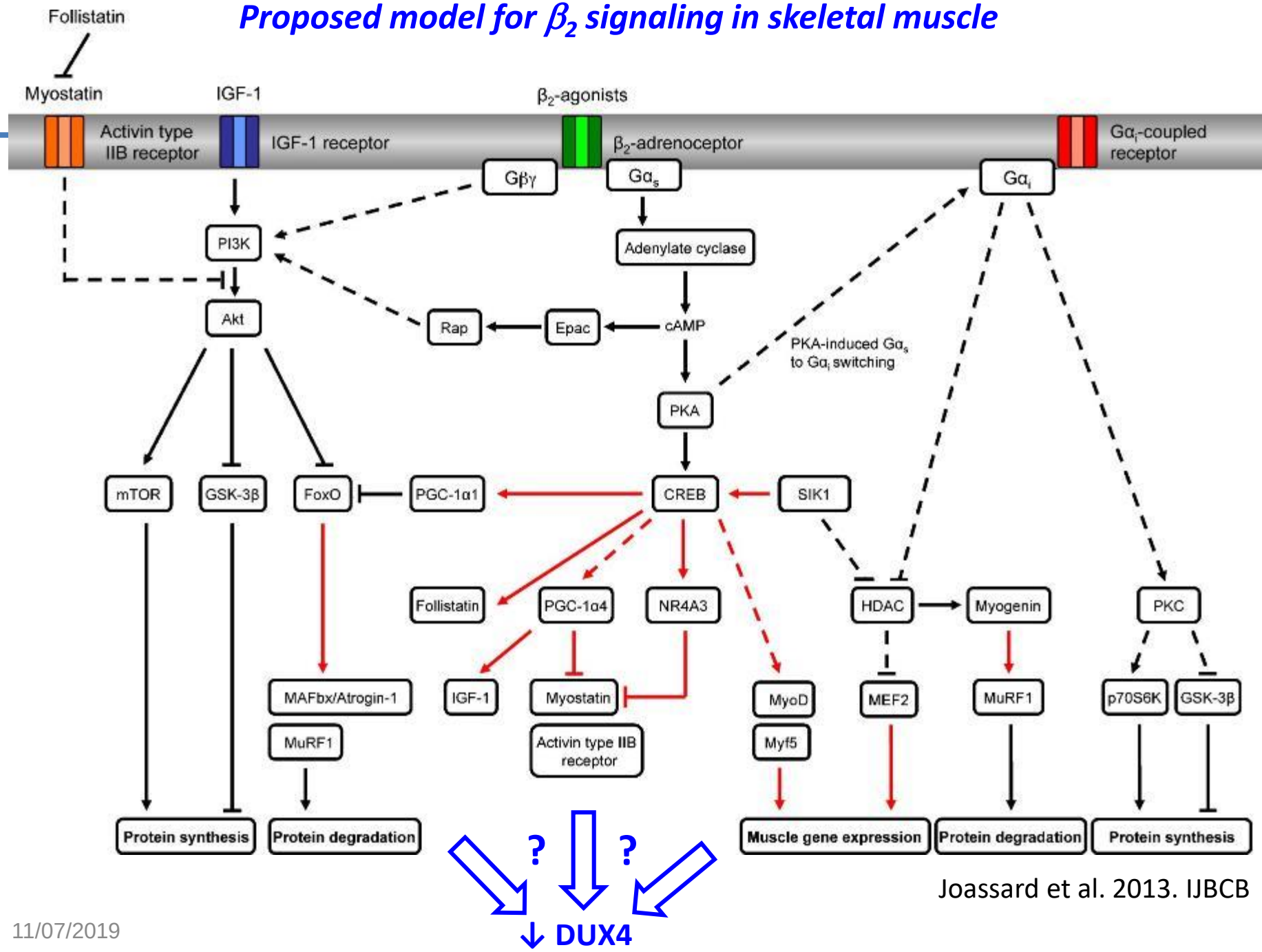
p38 inhibitors

DUX4 mRNA

DUX4 Protein

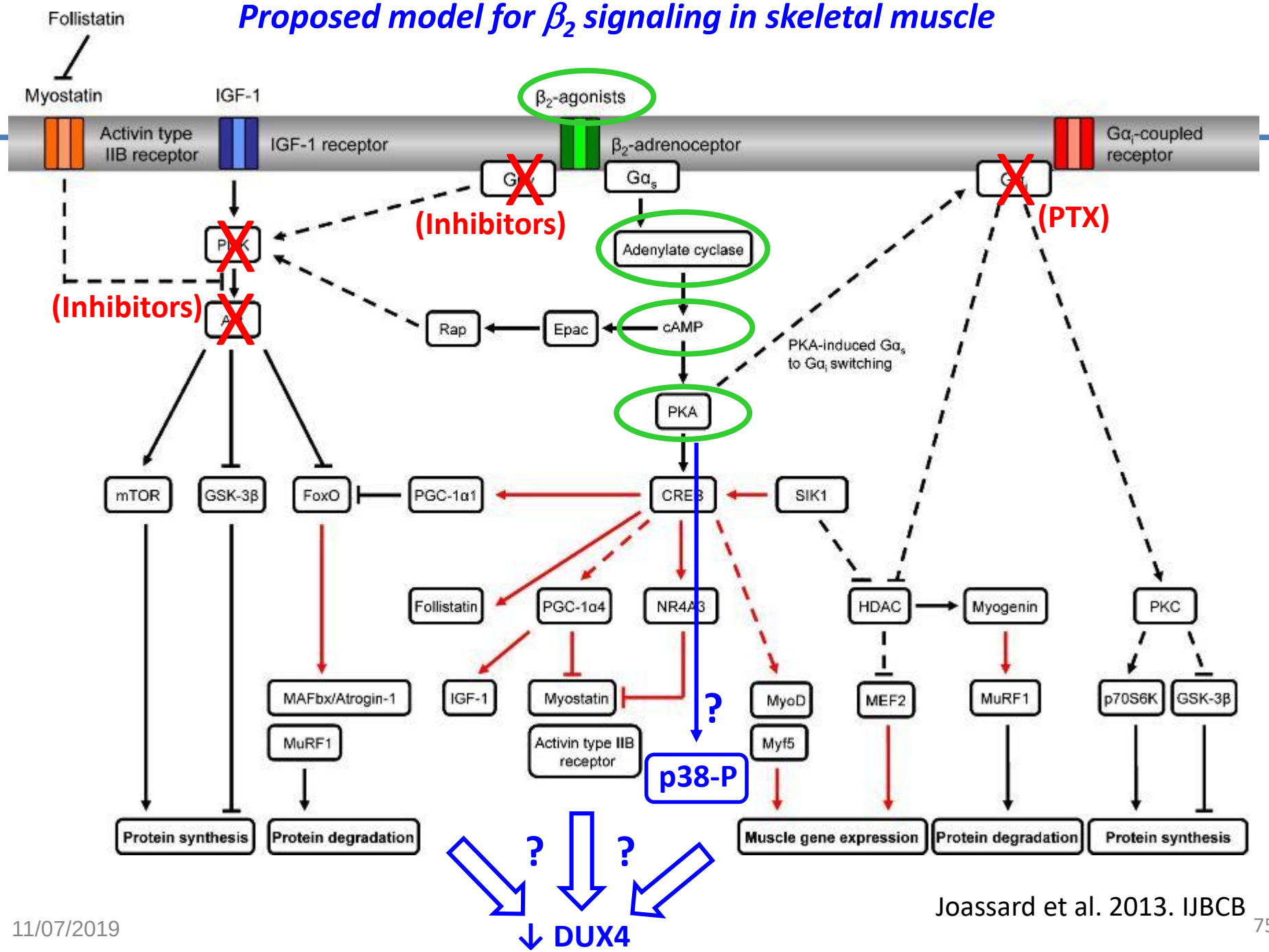


Proposed model for β_2 signaling in skeletal muscle



Joassard et al. 2013. IJBCB

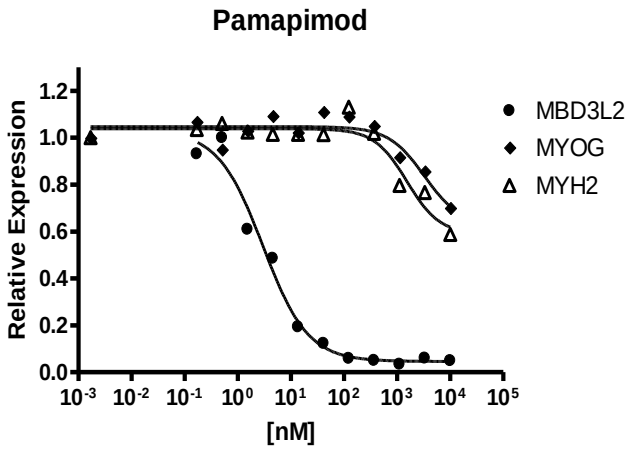
Proposed model for β_2 signaling in skeletal muscle

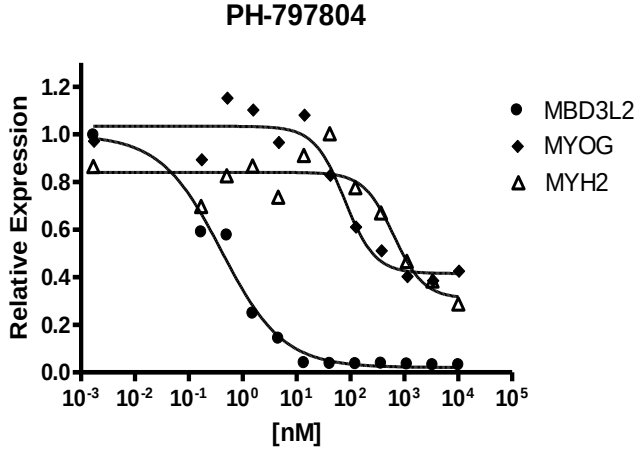


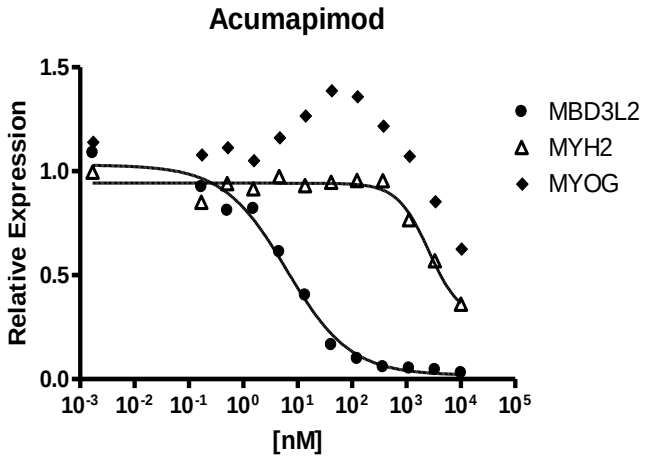
Joassard et al. 2013. IJBCB

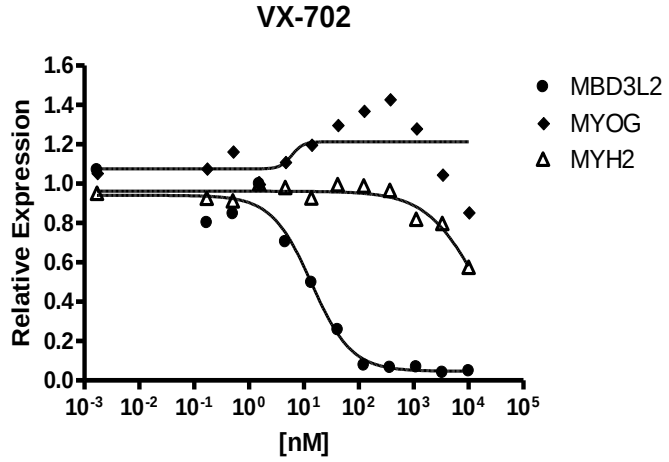
FSHD drug targets

Clinically advanced p38 inhibitors suppress DUX4 expression





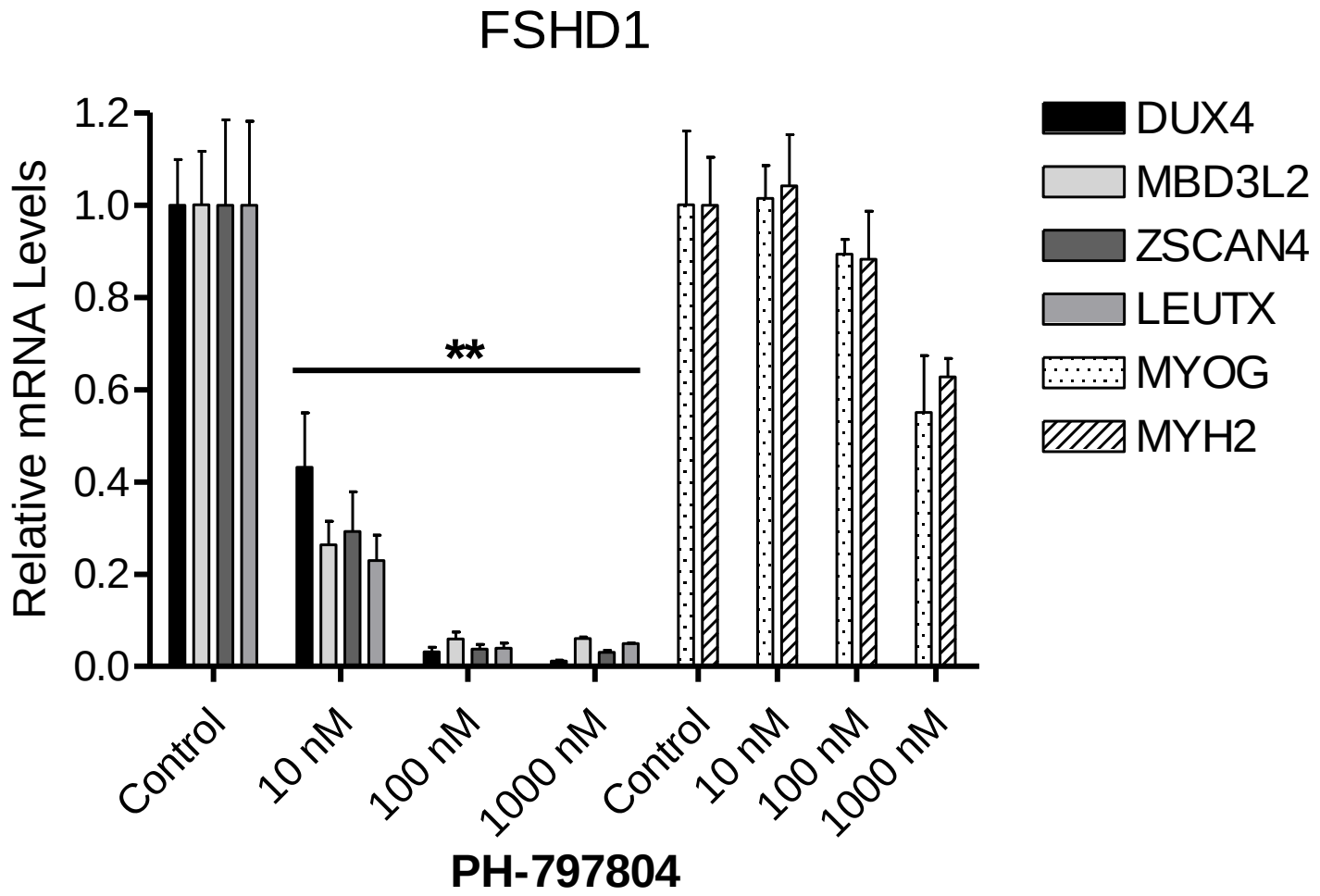




- p38α/β inhibitors suppress DUX4 at levels that do not inhibit myogenesis

FSHD drug targets

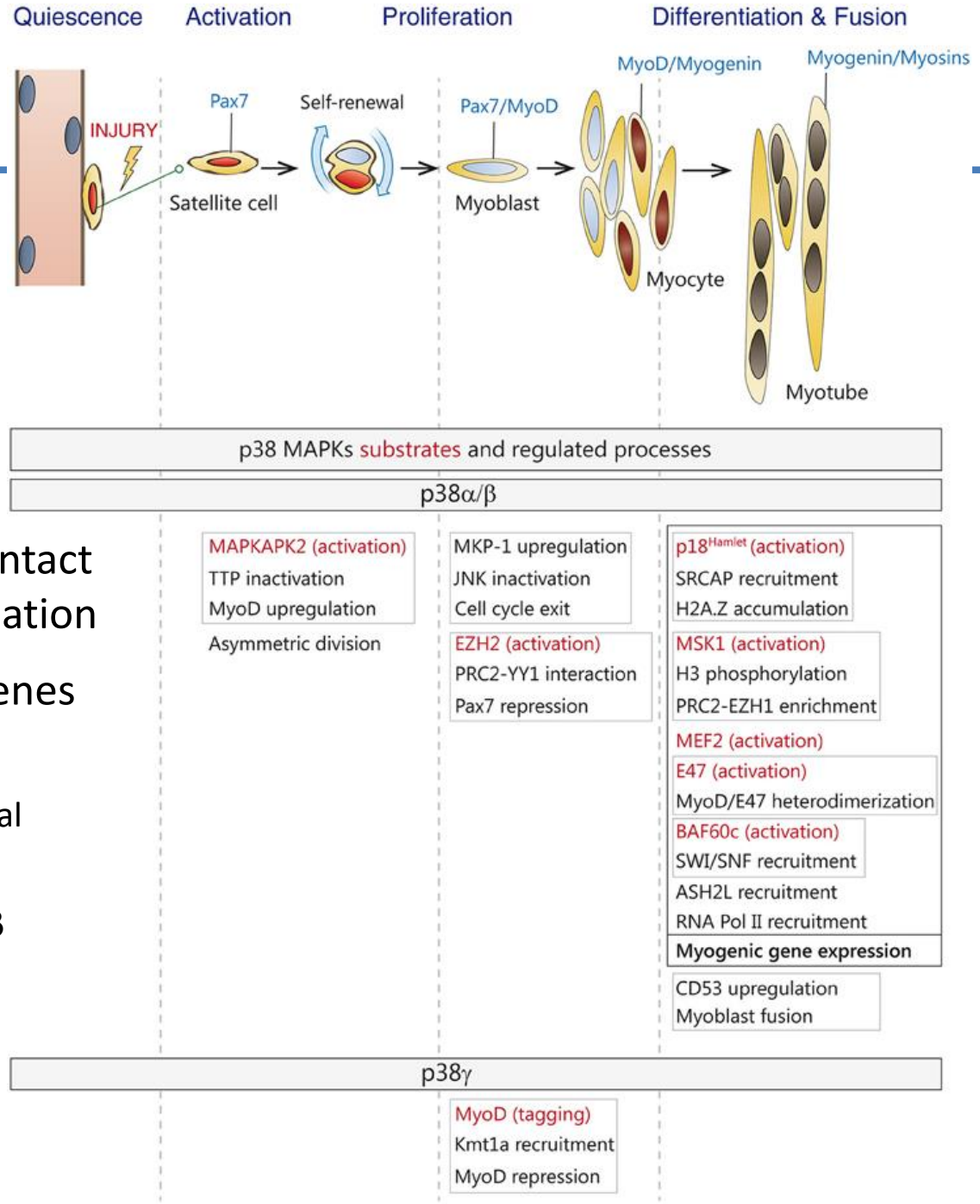
Clinically advanced p38 inhibitors suppress DUX4 expression



- p38α/β inhibitors suppress DUX4 at levels that do not inhibit myogenesis

p38 MAP kinases

Role in muscle biology



- p38α, p38β and p38γ isoforms expressed in skeletal muscle
- p38α/β activated by cell-cell contact during normal muscle differentiation
- p38α regulates large number genes during muscle differentiation
  - Many activities involved in temporal order of events
  - 5-fold higher expression than p38β

p38

Therapeutic potential

- Knock out mice:
  - p38α: (muscle-specific)
    - NOT DIRECTLY PATHOGENIC: Delayed myofiber growth and maturation, hyperproliferation of progenitors
    - reduced pathology in Mdx- and Sgcd-null dystrophic mice
  - p38β: no muscle phenotype

- In vivo p38 inhibition (inhibitors targeting p38α/β)
  - Improved self-renewal of satellite cells in aged muscles
  - Reduced pathology in Sgcd-null dystrophic mice

- p38 inhibition is valid therapeutic strategy
  - Suppress DUX4
  - Maintain muscle health
  - Potentially enhance muscle progenitor pools (satellite cells/myoblasts)

Clinically Advanced p38 Inhibitors

Inhibitor	Mechanism/ Selectivity	Indications, Phs
PH-797804	p38α, 4X > p38β	RA, COPD, Pain Phs II
Losmapimod (GW856553)	p38α/β	Cardiovascular (ACS, MI) Phs III, MDD, COPD, Phs II
Dilmapimod (SB-681323)	p38	COPD Phs I, RA, ACS, LI Phs II
VX-702	p38α, 14X > p38β	RA, Phs II
ARRY-371797	p38	LMNA-Cardiomyopathy, Phs III
Pamapimod (RO4402257)	p38α, 34X > p38β	RA Phs II
Acumapimod (BCT197)	p38α/β	COPD Phs II
Pexmetinib (ARRY-614)	p38/Tie2	Myelodysplastic syndrome, Phs I
Ralimetinib (LY2228820)	p38α/β JNK2, JNK3 > JNK1	Advanced cancer, Phs II
Talmapimod (SCIO 469)	p38α, 10X > p38β	RA, Myelodyplastic syndrome, Phs II
BMS-582949	P38α, 5X > p38β	Atherosclerosis, RA, Phs II
TAK-715	p38α, 28X > p38β	
Neflamapimod (VX-745)	p38α, 22X > p38β	Alzheimer's
Doramapimod (BIRB 796)	p38α/β/γ/δ	Phs II

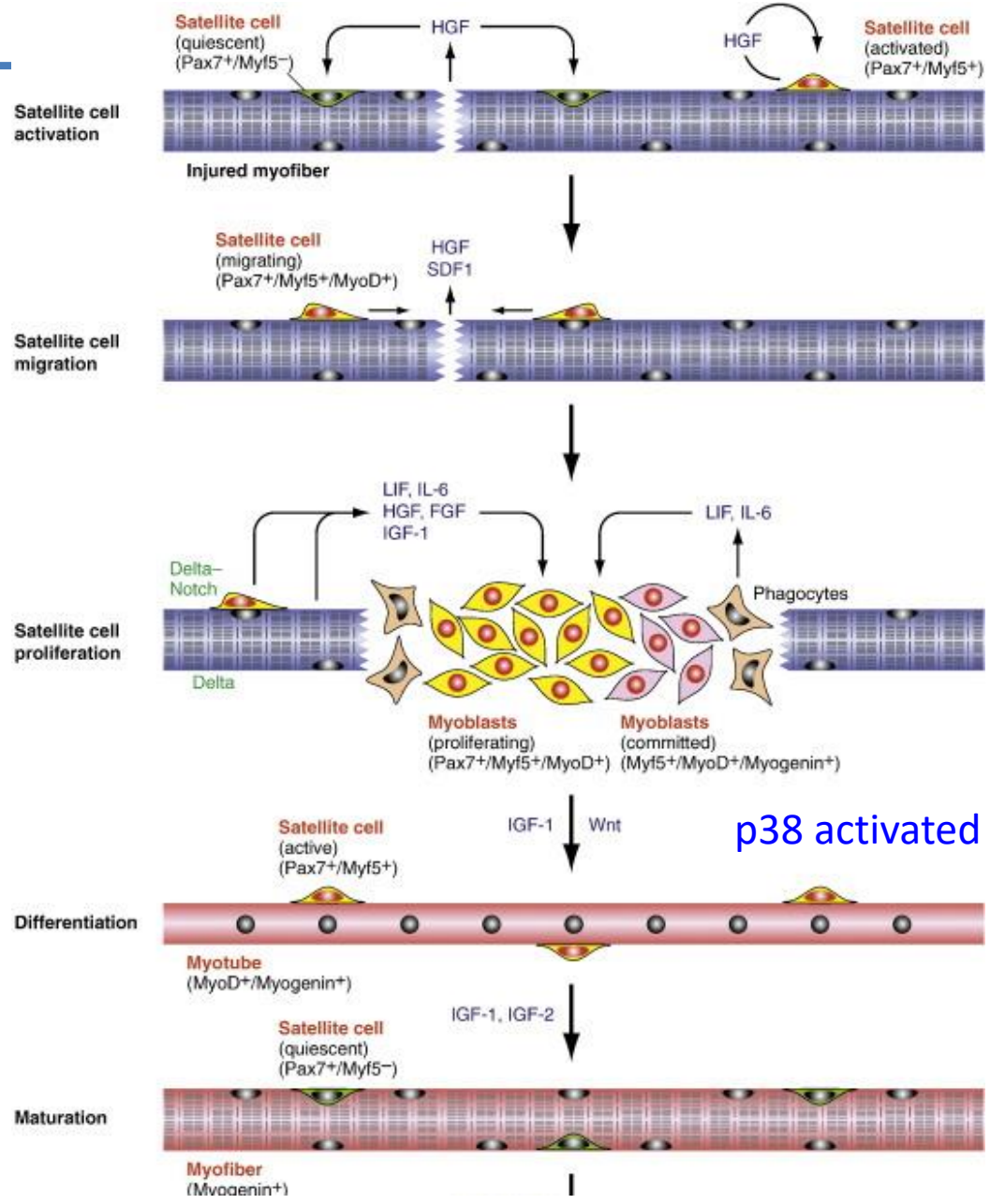
Failure in the clinic for efficacy in intended indications leaves many potential drug candidates to repurpose for FSHD!

RANK by:

- Efficacy in mouse model
- Safety profile/experience

Muscle Regeneration in vivo

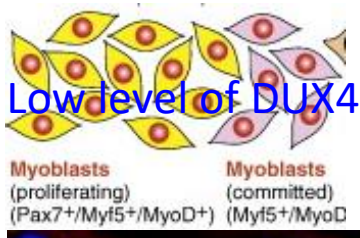
borrowed from: Endo, T. Bone. 2015. 80:2-15.



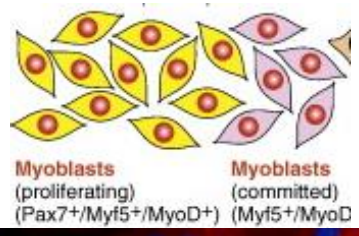
p38 activated

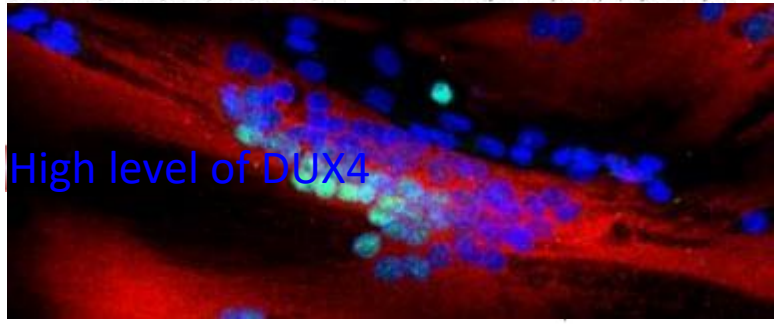
Cell culture model

Mouse xenograft model

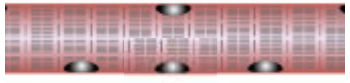


Low level of DUX4





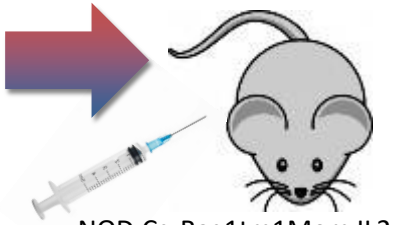
High level of DUX4



Xenograft model of FSHD

Human Epigenetic Regulation of DUX4

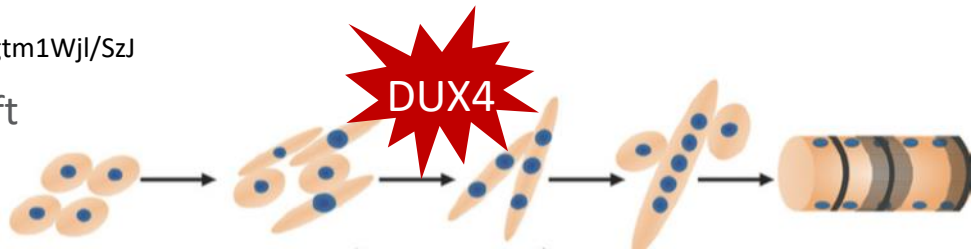
Human FSHD cells



NOD.Cg-Rag1tm1Mom IL2rgtm1Wjl/SzJ

FSHD xenograft

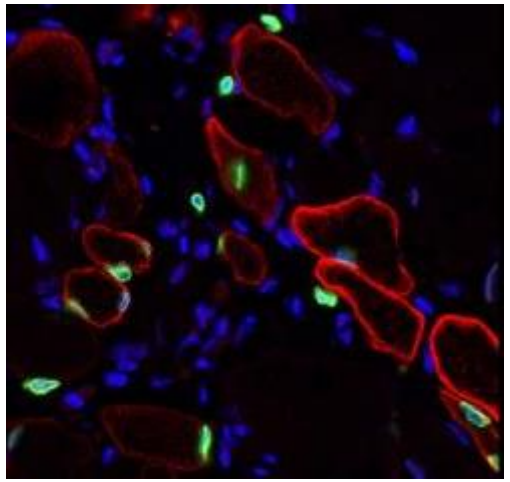
+/- Drug



Proliferating myoblasts
MyoD

Differentiating myocytes
Myogenin

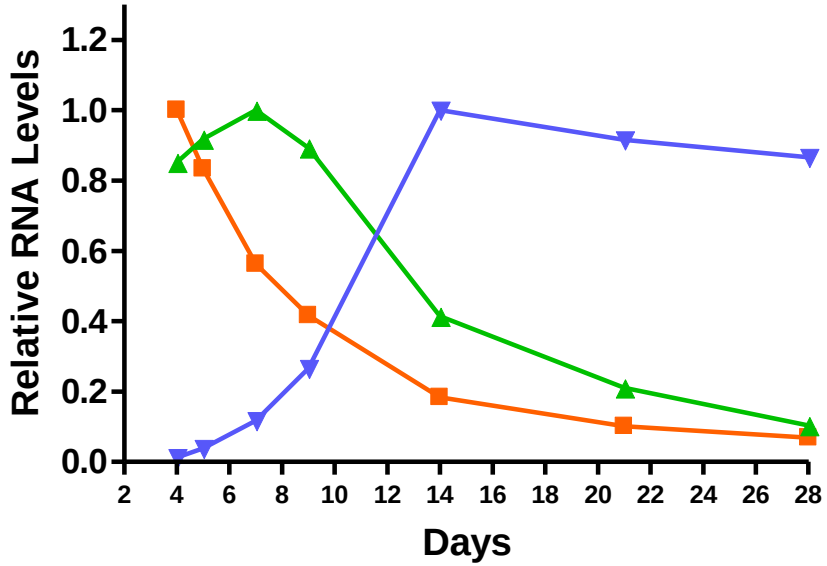
Myofibers
Myosin heavy chain



1) Measure inhibition of DUX4 and downstream targets (4 day xeno)
2) Measure improved survival and muscle differentiation (14 day xeno)

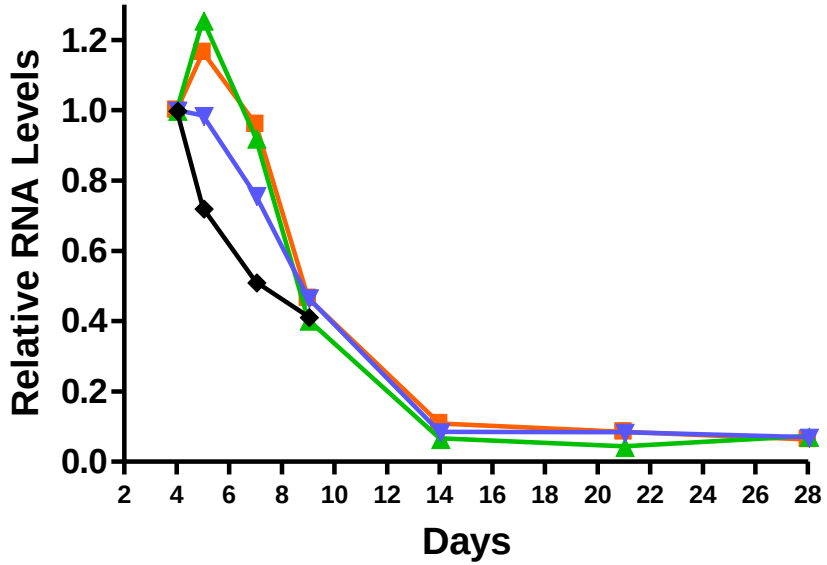
Xenograft model of FSHD

4 week profiling of gene expression



n = 6 animals per time point

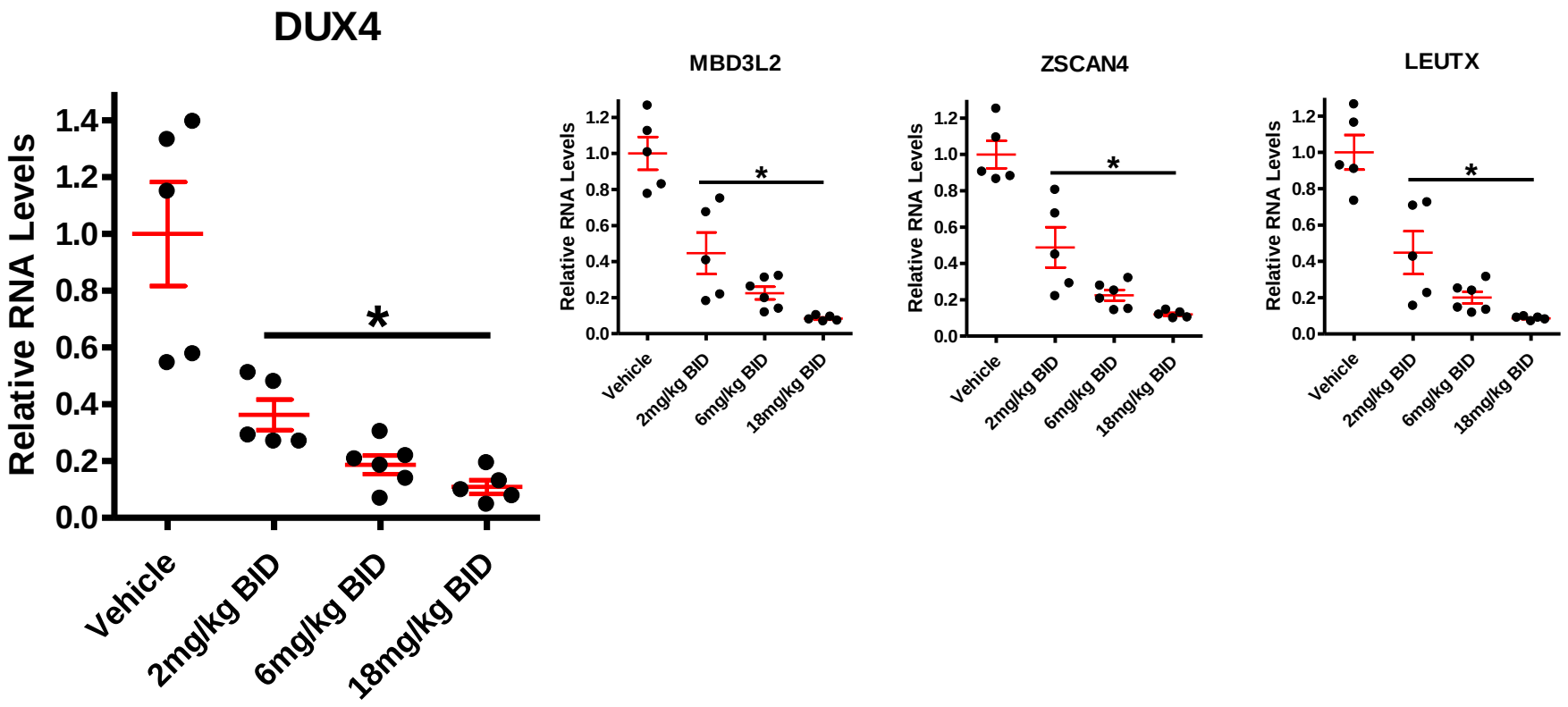
- MYOG (early differentiation) peaks on or before day 4
- MYH3 (regeneration) peaks ~day 7
- MYH2 (late differentiation) peaks ~day 14



- DUX4 peaks around day 4
- DUX4 targets peak ~day 5

Xenograft model of FSHD

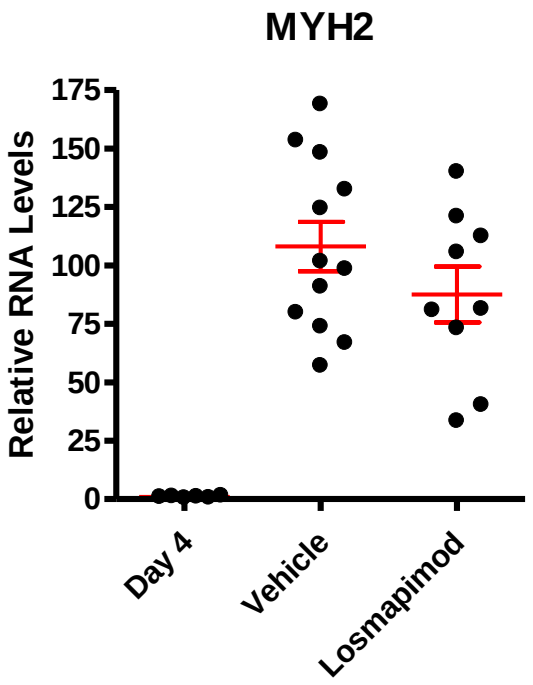
Losmapimod suppress DUX4 in human FSHD cells transplanted to mice

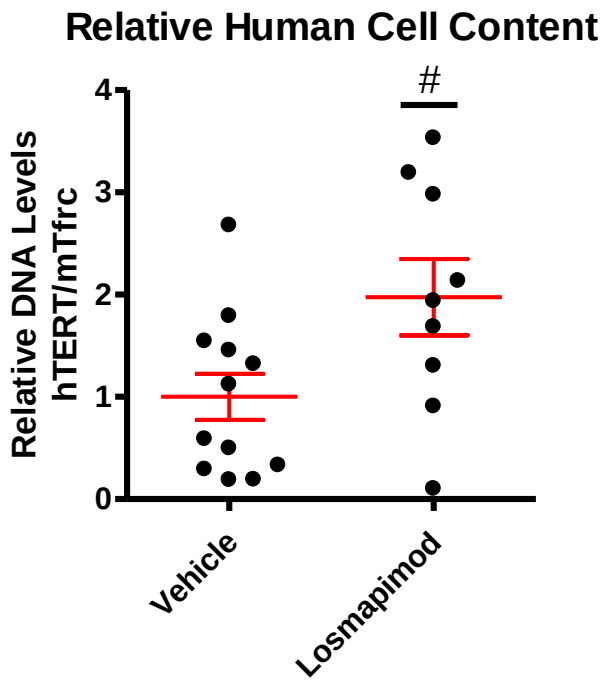


- Losmapimod reduces DUX4 expression by 80-90%
- These doses produce drug levels in mice that are similar to drug levels achieved in humans

Xenograft model of FSHD

Losmapimod 14-day dosing (6 mpk BID)



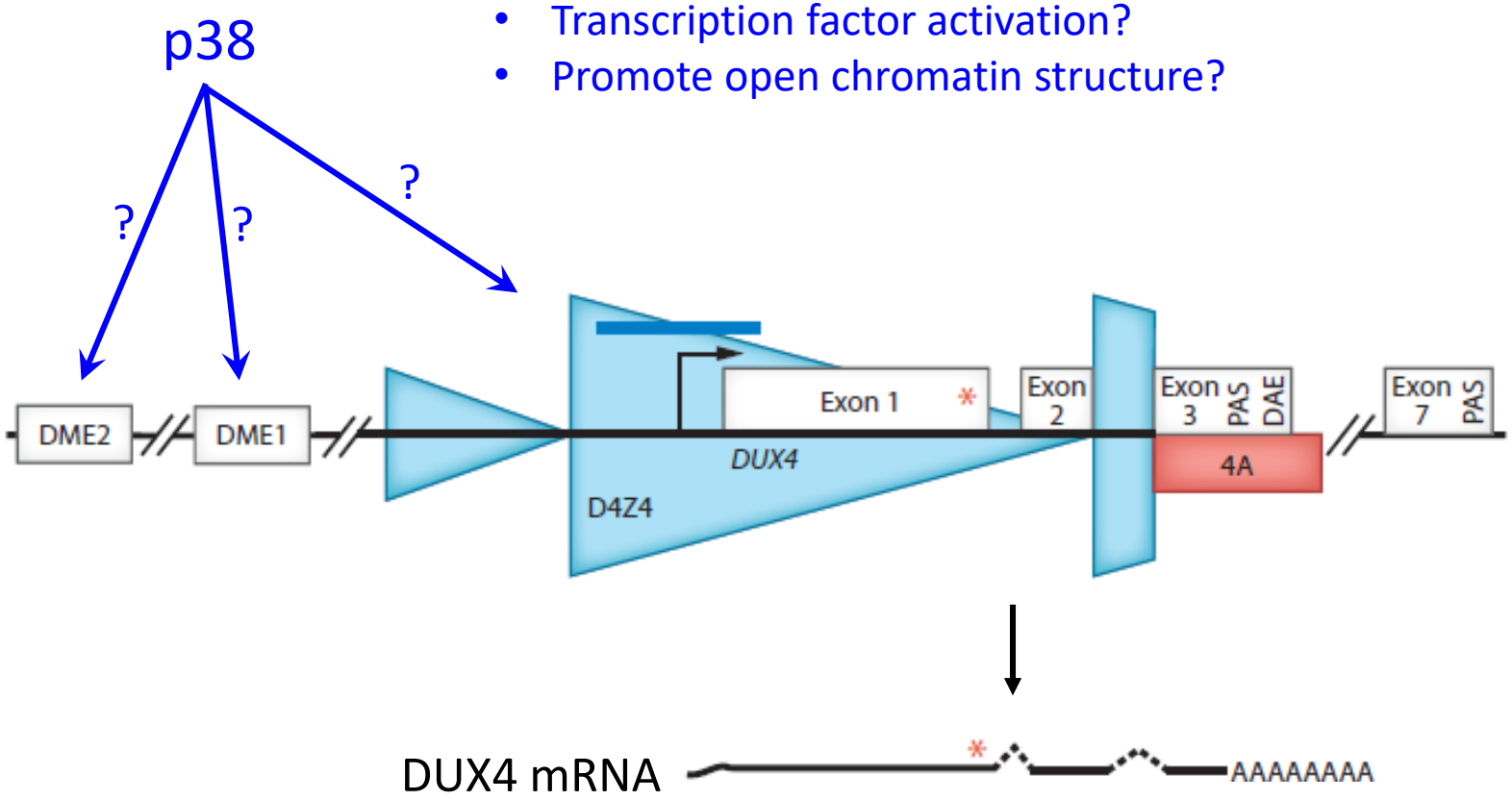


- Human cells differentiate to mature myofibers with treatment (MYH2)
- Increase in human cell content suggests treatment may protect human cells from DUX4 toxicity

Mechanism

How does p38 turn on DUX4?

- Transcription factor activation?
- Promote open chromatin structure?



Annu. Rev. Genom. Hum. Genet. 2019. 20:8.1–8.27

Summary

p38 inhibition turns off DUX4

- Clinically advanced p38i suppress DUX4 (and downstream target genes) without inhibiting muscle differentiation
  - Pharmacological and genetic depletion suggest viable therapeutic approach
  - Phs II/III p38 inhibitors are attractive drug candidates for FSHD
- Losmapimod suppresses DUX4 in mouse xenograft model at dosing levels that are relevant to human
- Losmapimod stands out as candidate p38 inhibitor for FSHD
  - Published human PK, muscle exposure in mice (internal), xenograft efficacy
  - Safety profile in large number of patients
- How p38 inhibitors suppress DUX4 currently under investigation
  - Potentially 2 or more mechanism

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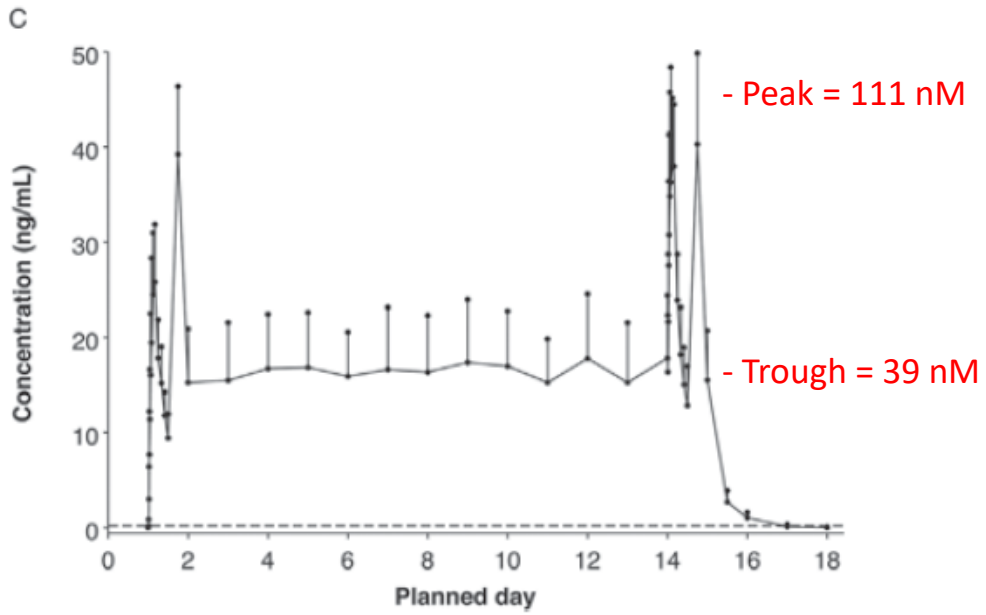
Ultragenyx Pharmaceutical, Inc.
Muscular Dystrophy Association
Chris Carrino Foundation

Losmapimod

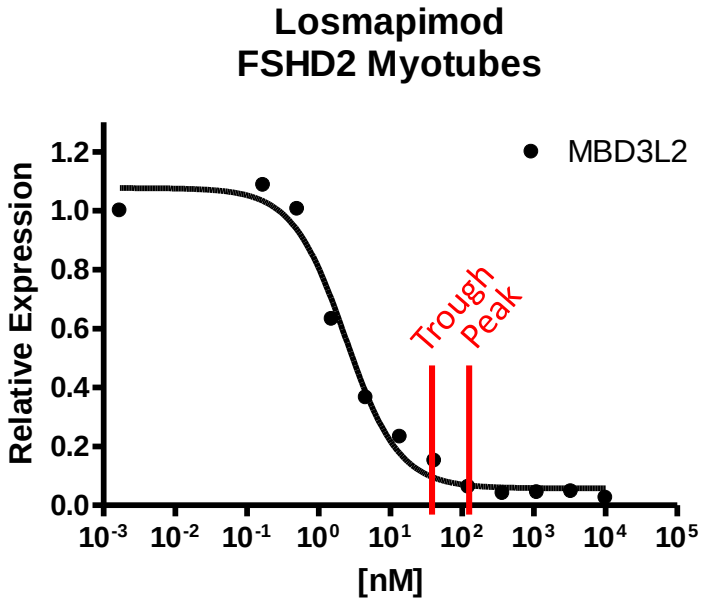
Clinical Pharmacokinetics

Clin Pharmacol Drug Dev. 2015; 4(4):262-9.

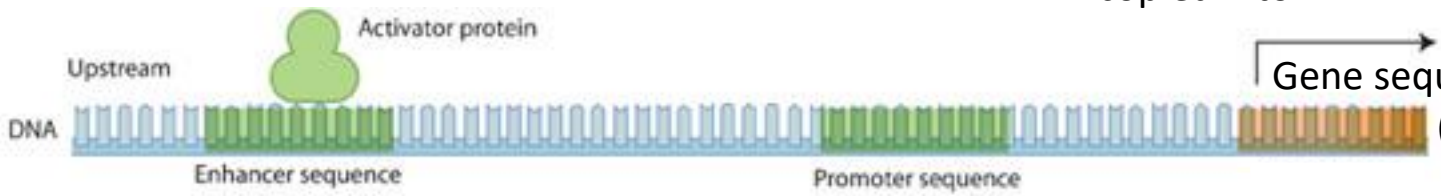


- Multi dose PK in Japan, PK consistent with single dose studies in US
- 7.5 mg BID

- Clinical dosing maintains narrow range of plasma levels
- Trough at FSHD myotube IC_{90}
- Trough above 14-day xenograph (23 nM)
- Muscle tissue levels approximately equal to plasma levels in mice

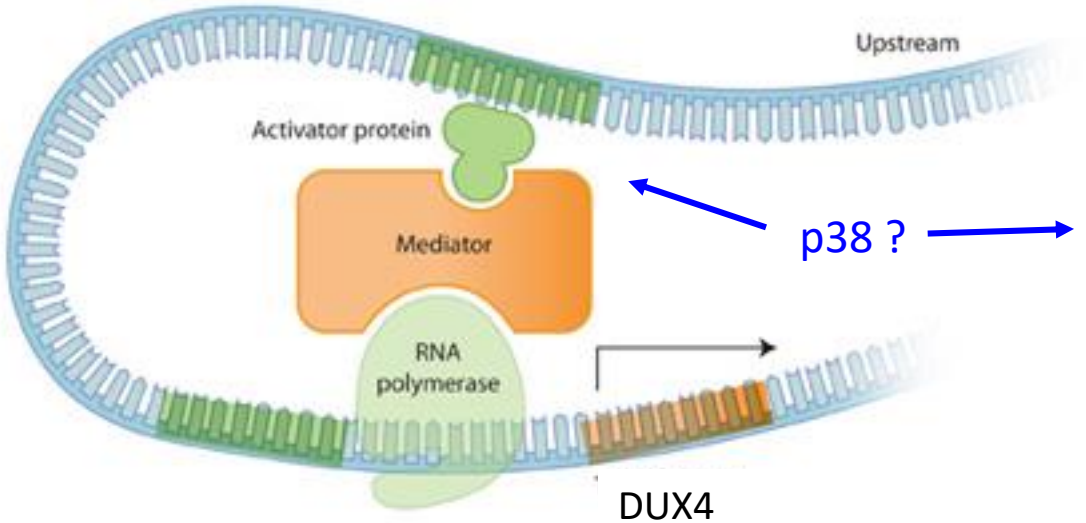


Start site on DNA where gene is copied into RNA when “turned on”



Gene sequence on DNA (e.g. DUX4)

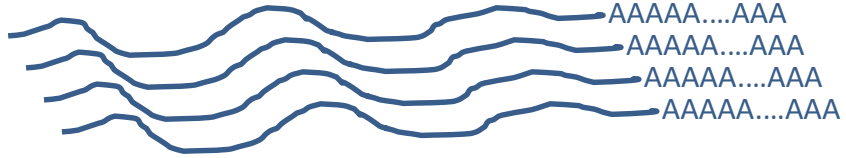
Transcriptional activation



p38 ?

“Open” or de-condensed chromatin facilitates this process

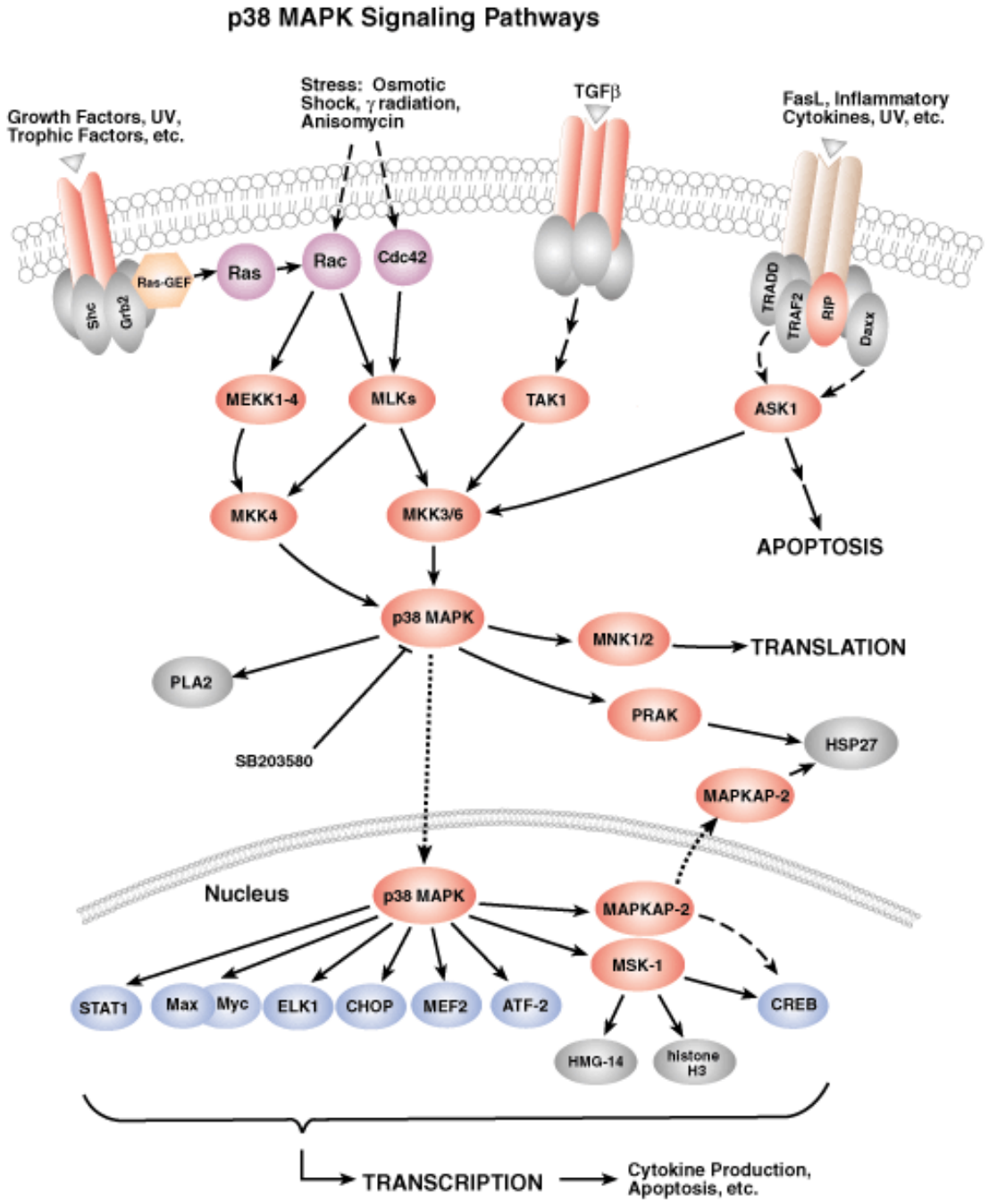
DUX4



RNA copies of DUX4 gene sequence (DUX4 mRNA)

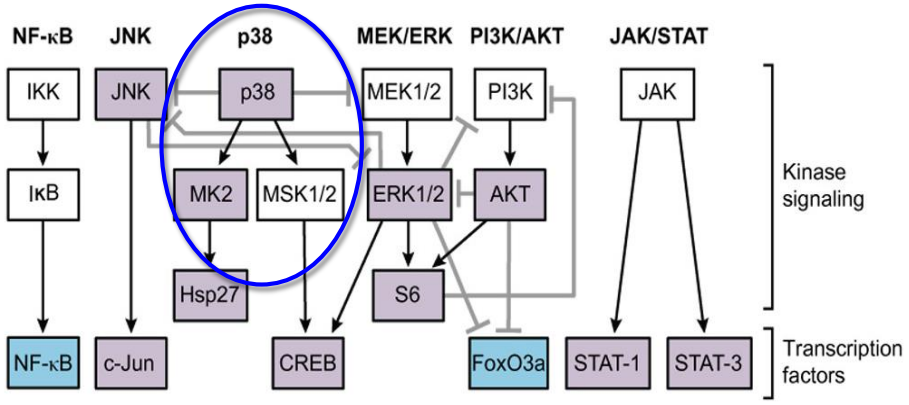
p38 MAP kinases

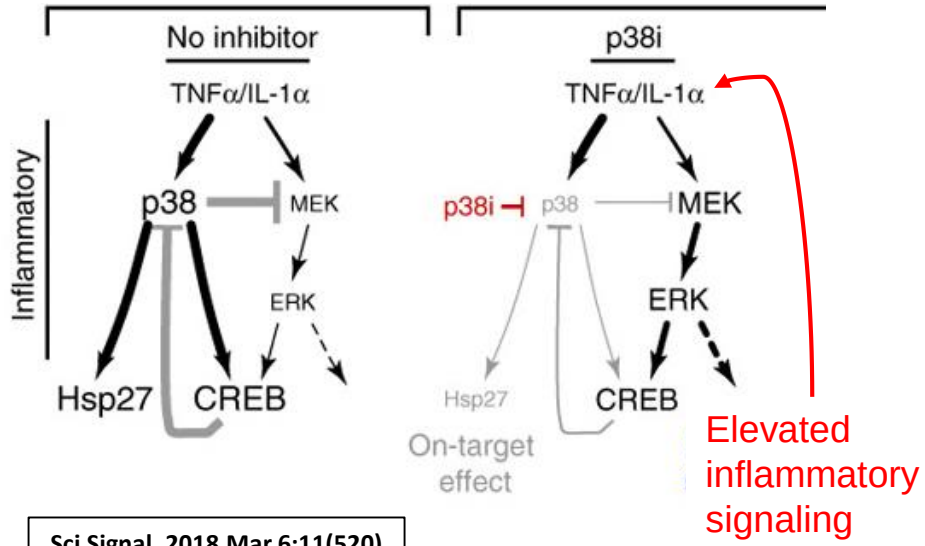
Inflammatory signaling



http://www.ufrgs.br/imunovet/molecular_immunology/p38map.html

p38 pathway for inflammation is different than in muscle





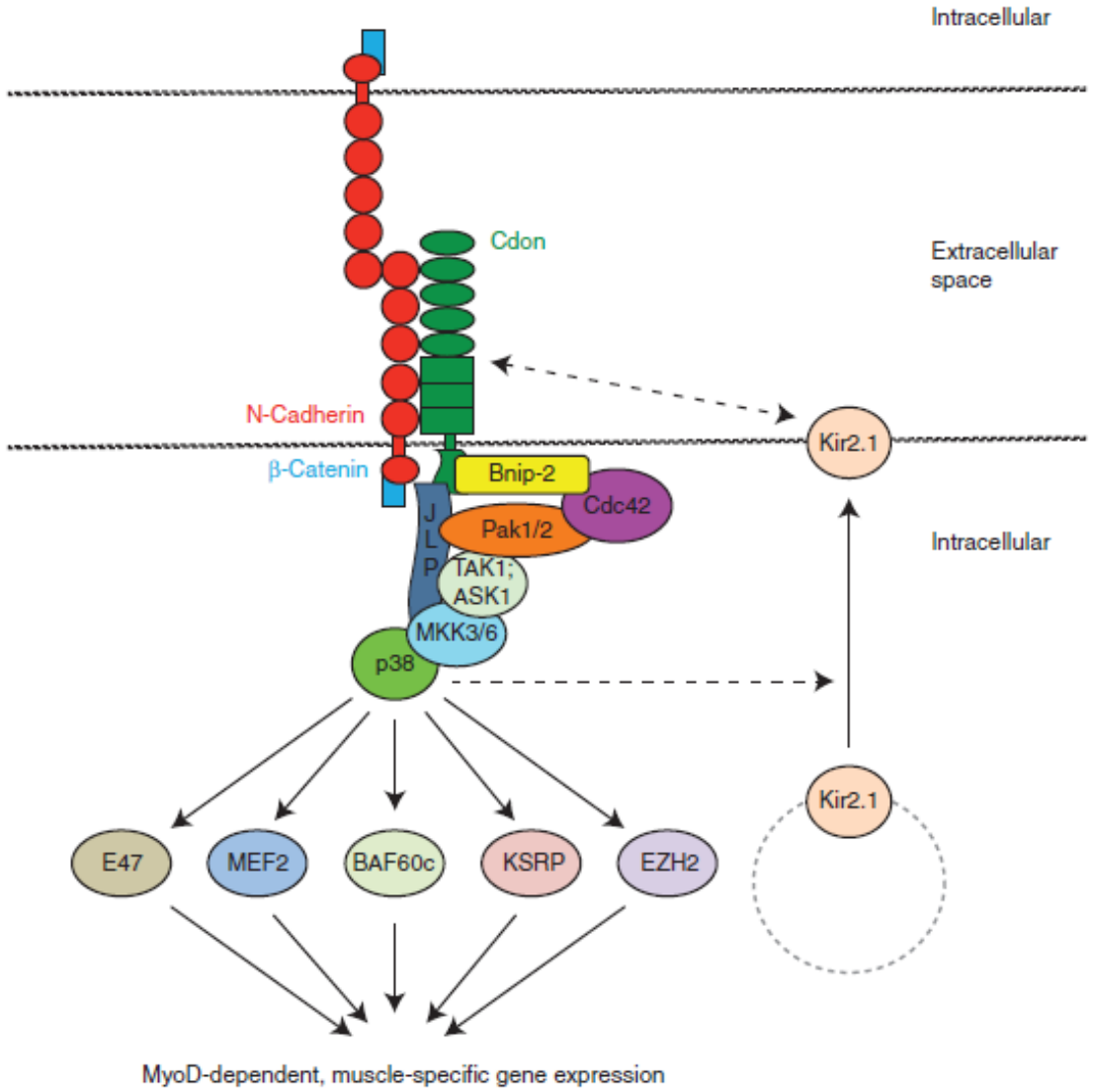
Sci Signal. 2018 Mar 6;11(520)

- In rheumatoid arthritis synovial fibroblasts (SF), p38 activated by inflammatory stimuli
  - In muscle, p38 activated by normal differentiation program
- In SF, MK2 and MSK1/2 are key p38 targets that promotes inflammation
  - DUX4 expression not mediated by MK2 or MSK1/2
- In SF, inflammatory environment promotes alternate MEK/ERK signaling
  - Inflammatory cytokines do not stimulate DUX4 expression
  - DUX4 expression not mediated by MEK/ERK

No indication that p38i would lose efficacy for suppressing DUX4

p38 kinase

Activated by muscle differentiation



- Cell-cell contact activates p38 during the normal differentiation process
- NOT inflammatory signaling

Cold Spring Harb Perspect Biol. 2017 Feb 1;9(2).

Imaging and Biopsy: Clinical Trial Design Implications

Kathryn Wagner, MD, PhD
Center for Genetic Muscle Disorders
Kennedy Krieger Institute
Johns Hopkins School of Medicine

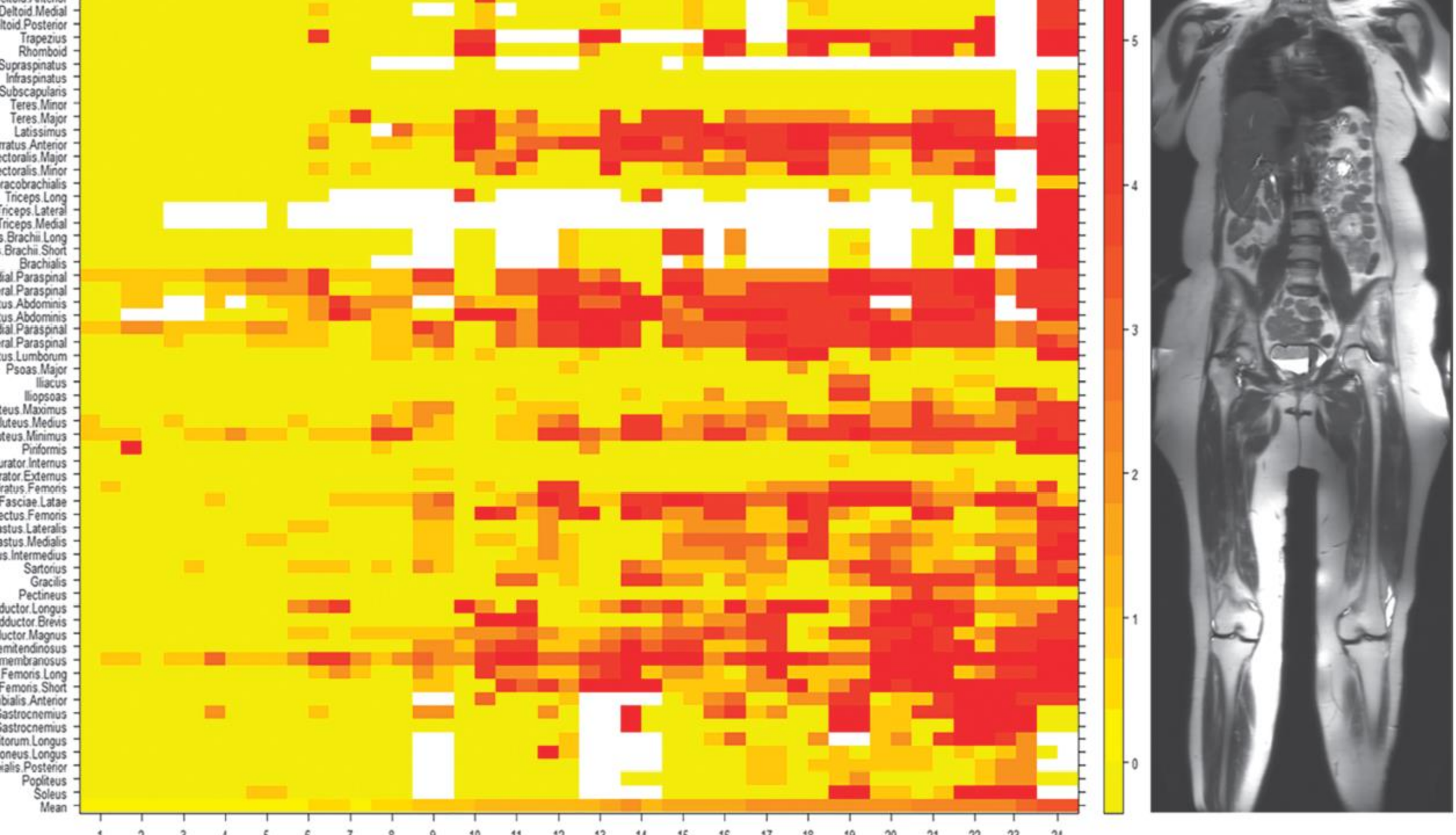
FSHD Challenges and Opportunities

- Challenges
  - Slowly progressing disorder
  - Disease heterogeneity
  - No established regulatory pathway

- Opportunities
  - Common rare disease
  - Highly motivated patient population
  - Engaged, experienced investigator community
  - Little competition (unlike DMD)

MRI

- Standard MRI sequence can identify healthy muscle, acute intramuscular inflammation and infiltration of fat and fibrosis
- Patterns of muscle involvement differentiate various genetic myopathies
- Distinctive pattern of muscle involvement in FSHD but not used as diagnostic due to superior specificity of genetic diagnosis
- Powerful clinical outcome measure
  - Noninvasive
  - Nonirradiating
  - Independent of patient effort, daily clinical variability and learning effects
  - Can be performed on most patients irrespective of disease severity
  - Sensitive to small increments of change
  - Repeatable measurements


Deltoid.Medial
Deltoid.Posterior
Trapezius
Rhomboid
Supraspinatus
Infraspinatus
Subscapularis
Teres.Minor
Teres.Major
Latissimus
Serratus.Anterior
Pectoralis.Major
Pectoralis.Minior
Coracobrachialis
Triceps.Long
Triceps.Lateral
Triceps.Medial
Brachii.Long
Brachii.Short
Brachialis
Lateral.Paraspinal
Lateral.Paraspinal
Rectus.Abdominis
Rectus.Abdominis
Medial.Paraspinal
Lateral.Paraspinal
Quadratus.Lumborum
Psoas.Major
Iliacus
Iliopsoas
Gluteus.Maximus
Gluteus.Medius
Gluteus.Minimus
Piriformis
Obturator.Internus
Obturator.Externus
Quadratus.Femoris
Tensor.Fasciae.Latae
Rectus.Femoris
Vastus.Lateralis
Vastus.Medialis
Vastus.Intermedius
Sartorius
Gracilis
Pectineus
Adductor.Longus
Adductor.Brevis
Adductor.Magnus
Semitendinosus
Semimembranosus
Biceps.Femoris.Long
Biceps.Femoris.Short
Tibialis.Anterior
Gastrocnemius
Gastrocnemius
Extensorum.Longus
Peroneus.Longus
Tibialis.Posterior
Popliteus
Soleus
Mean
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3
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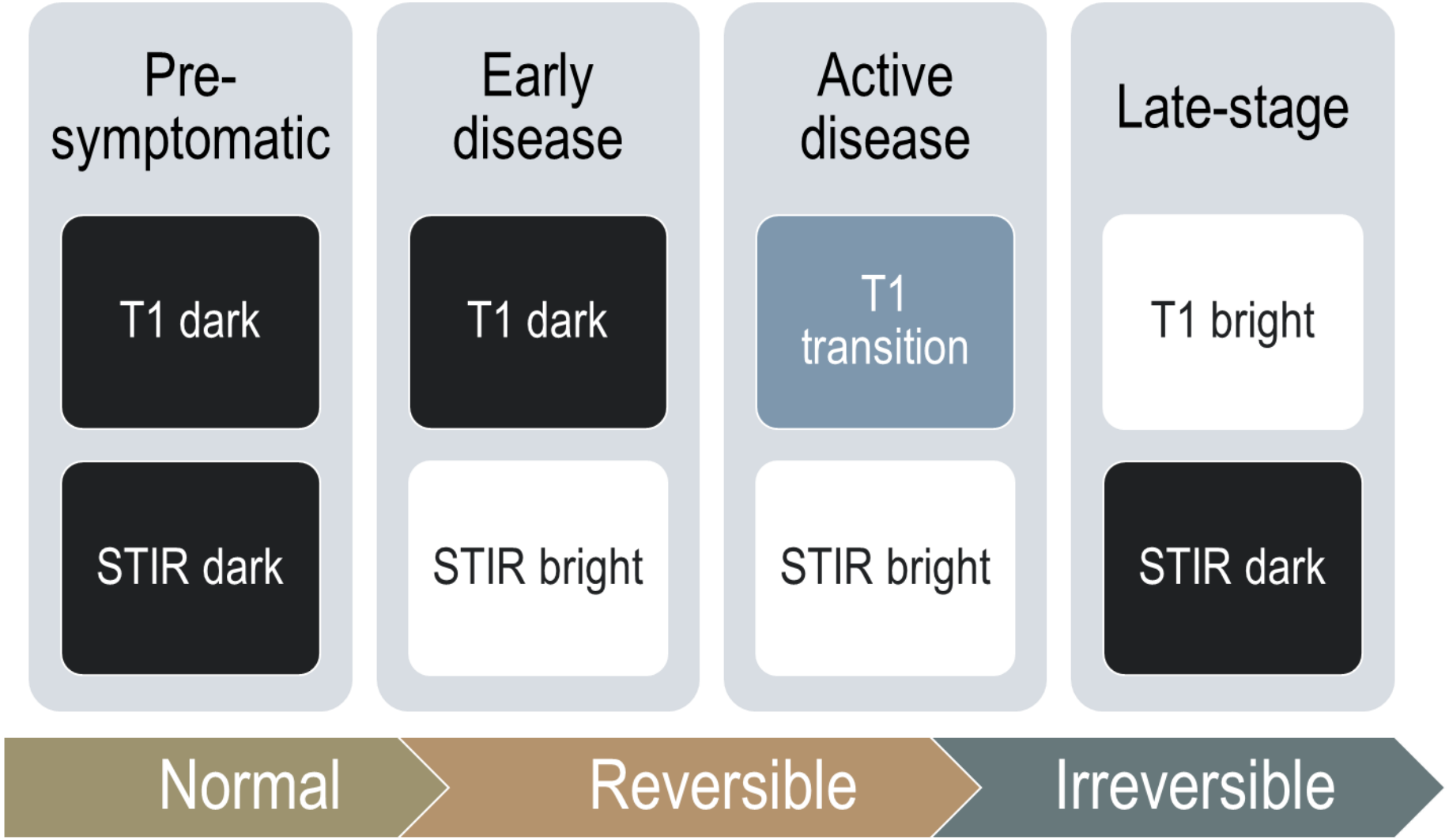
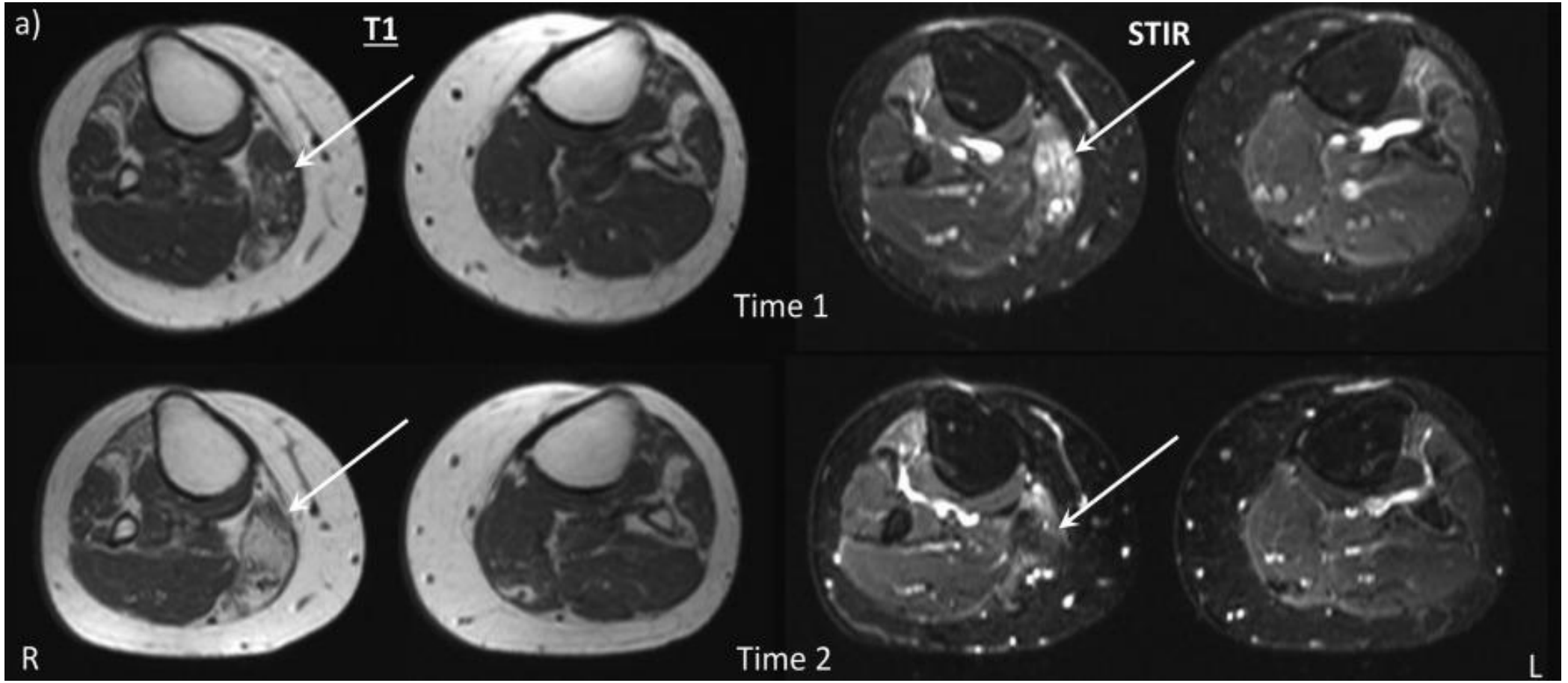
MRI

- Longitudinal studies confirm slowly progressive disorder
- Fatty infiltration does not progress over 6.9 to 13.8 months of follow up
- Fatty infiltration seen as T1-weighted hyperintensity
- Hyperintensity on STIR (Short-T1 Inversion Recovery) sequences correlate with edema and cellular inflammation
- STIR positivity may precede fatty replacement of T1-weight hyperintensity

Theory of disease progression in FSHD

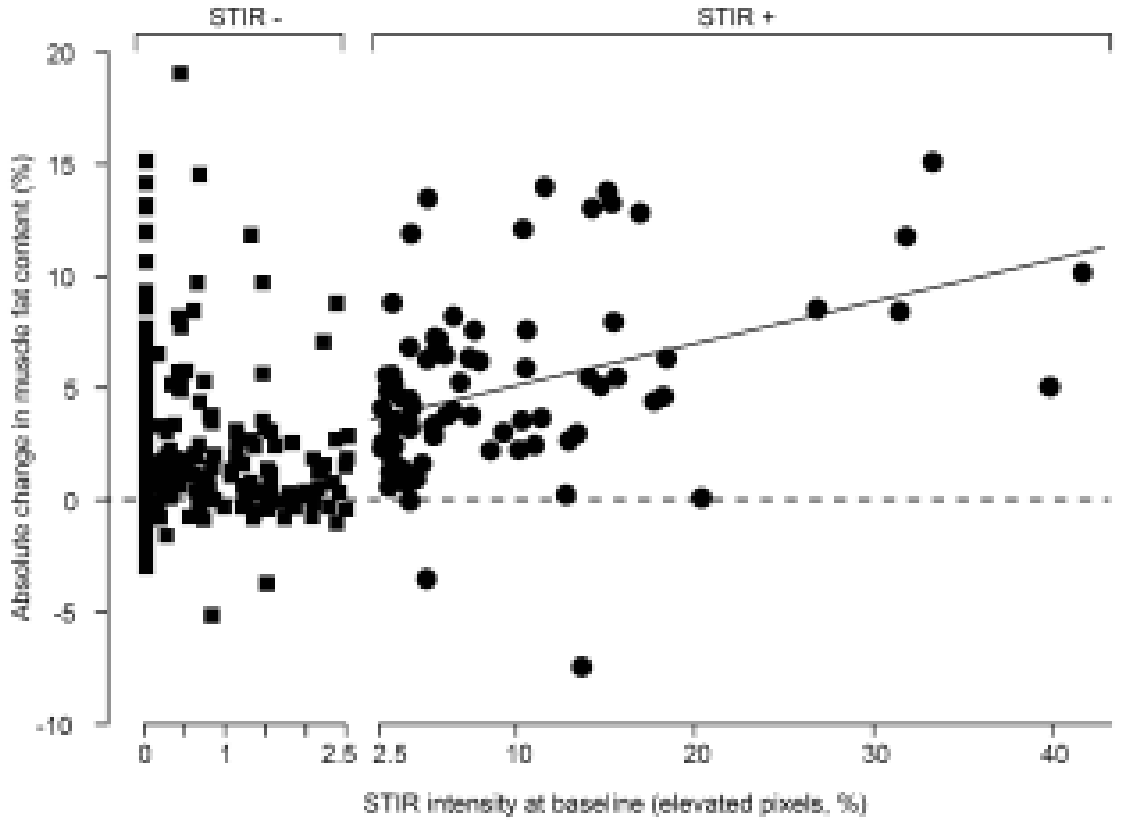


STIR Hyperintensity and Fat infiltration



Ferguson, *Muscle and Nerve*, 2017

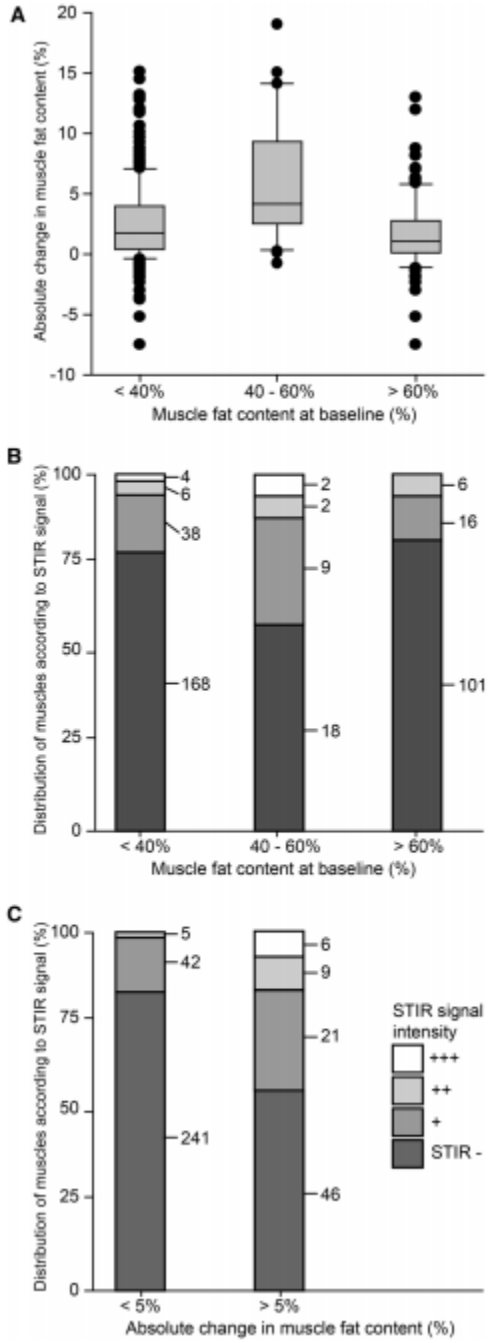
Relationship between STIR+ and fat



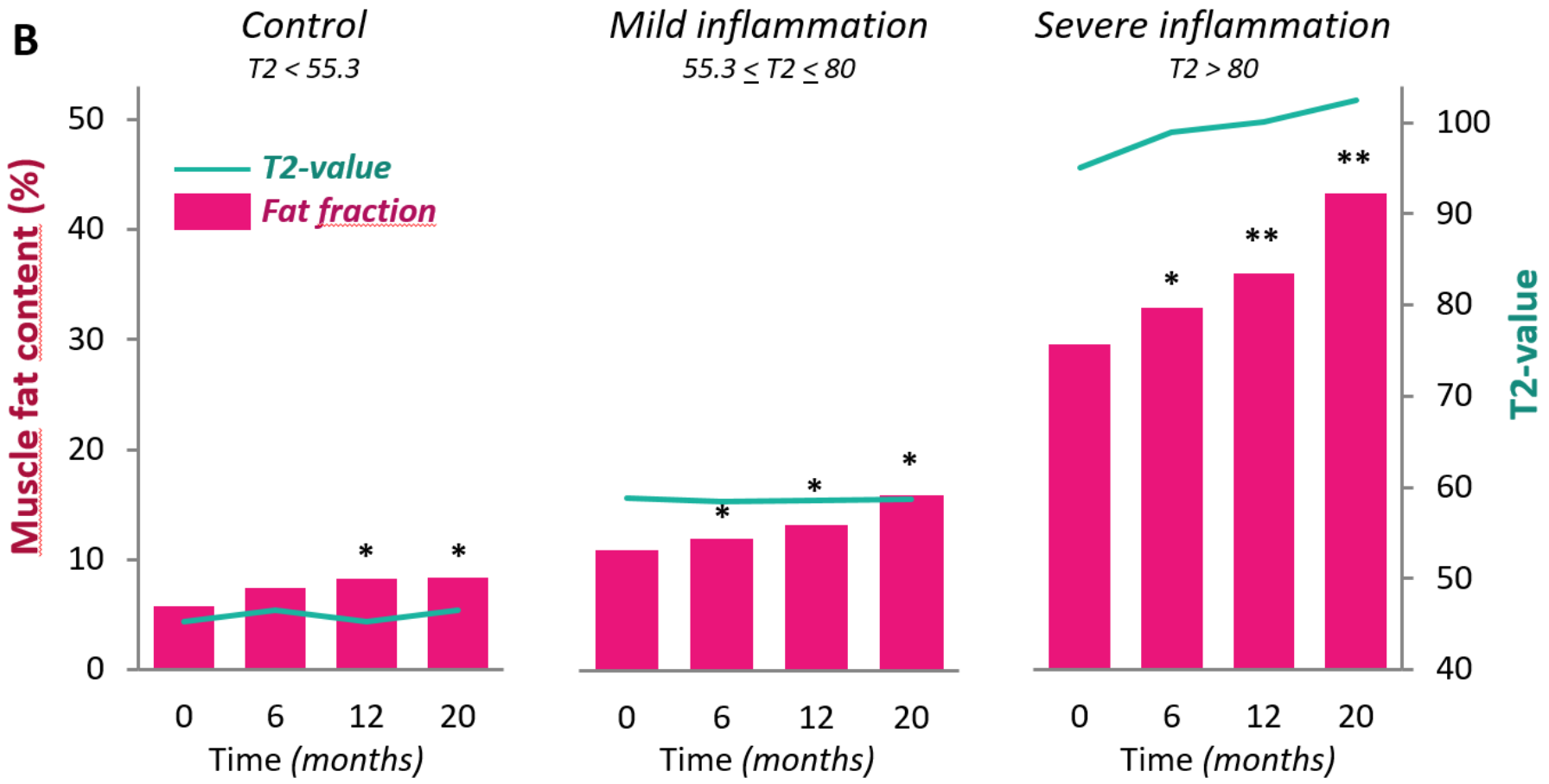
Dahlqvist et al., J Neurol 2019

Fat replacement progression

- Intermediate fat replaced muscles had greatest change over time
- Highest percentage of STIR+ muscles were among intermediate fat replaced muscles
- Stir+ muscle had a faster progression of fat replacement

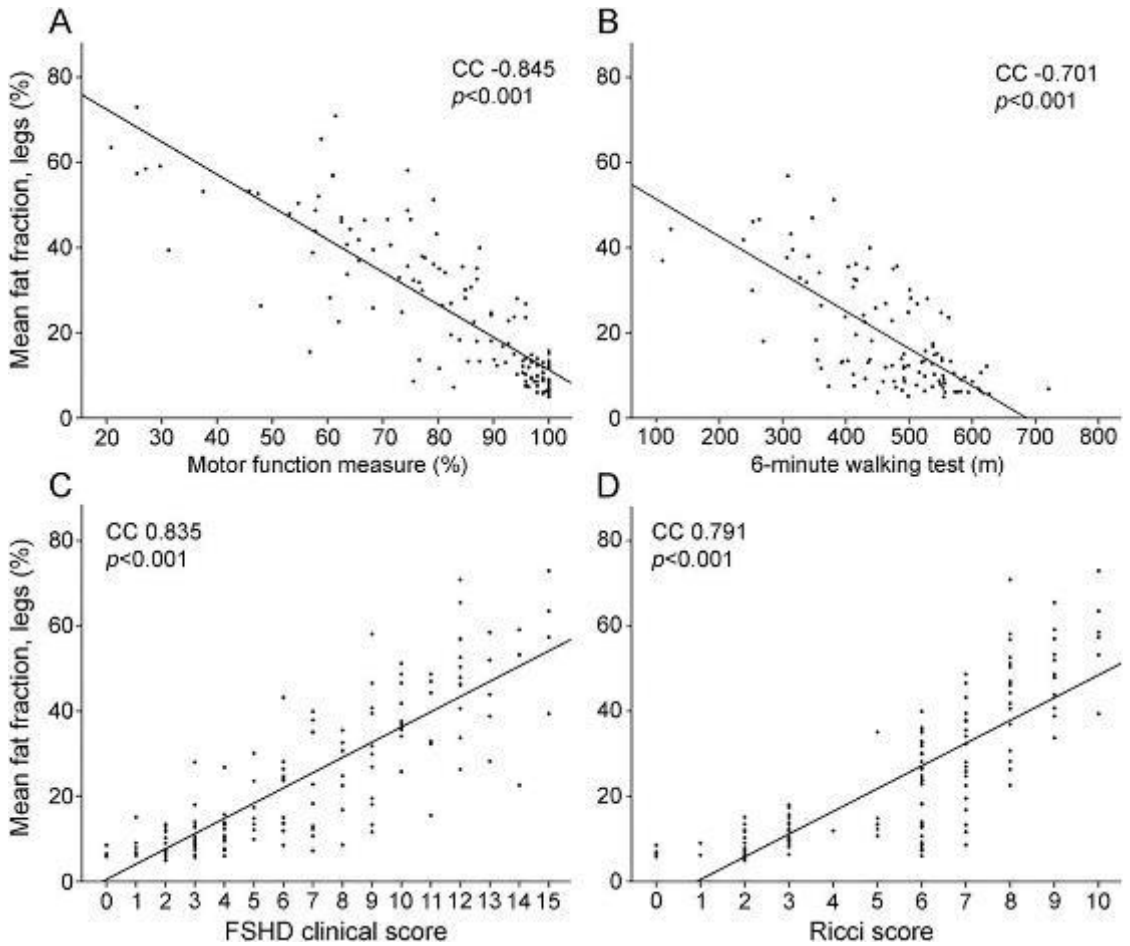


More severe progression with greater T2 signal abnormalities



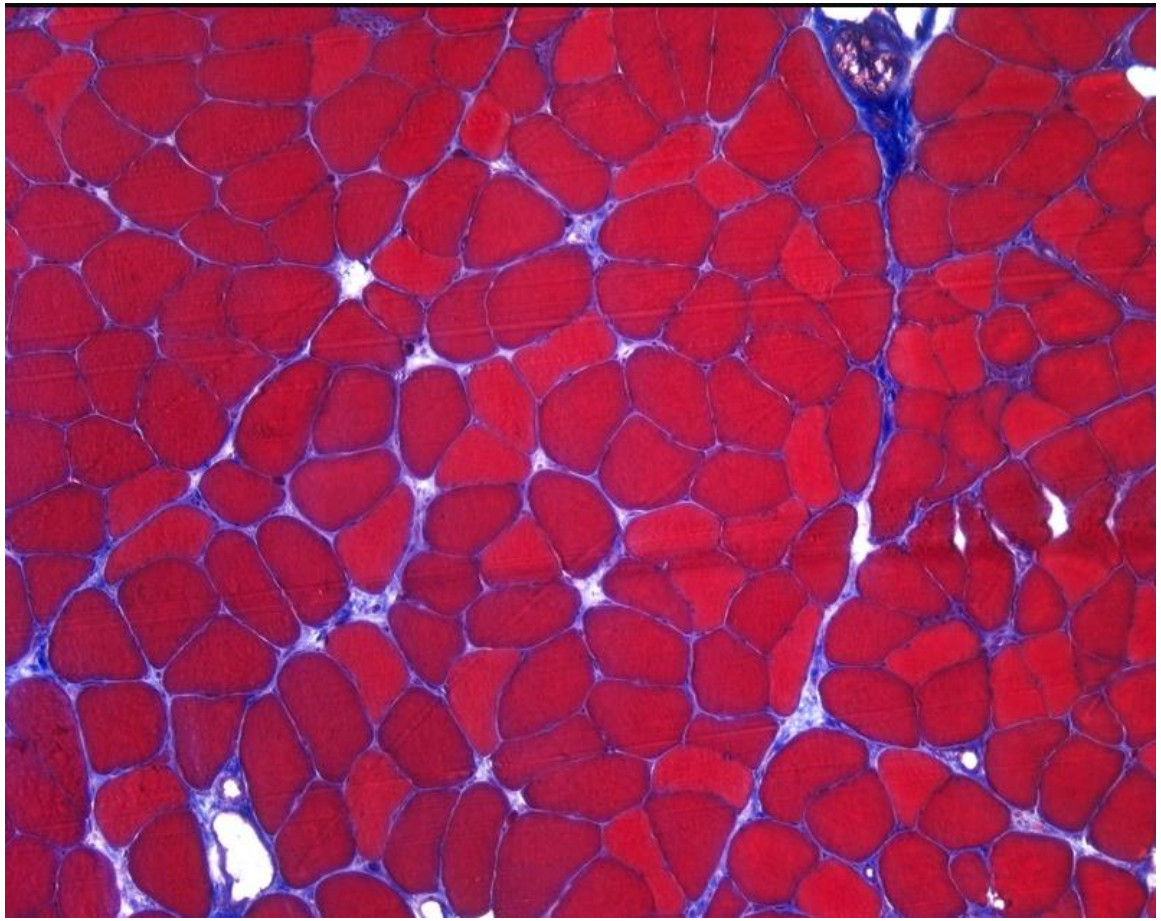
Vissing et al., WMS, 2019

Good correlation of fat fraction to function

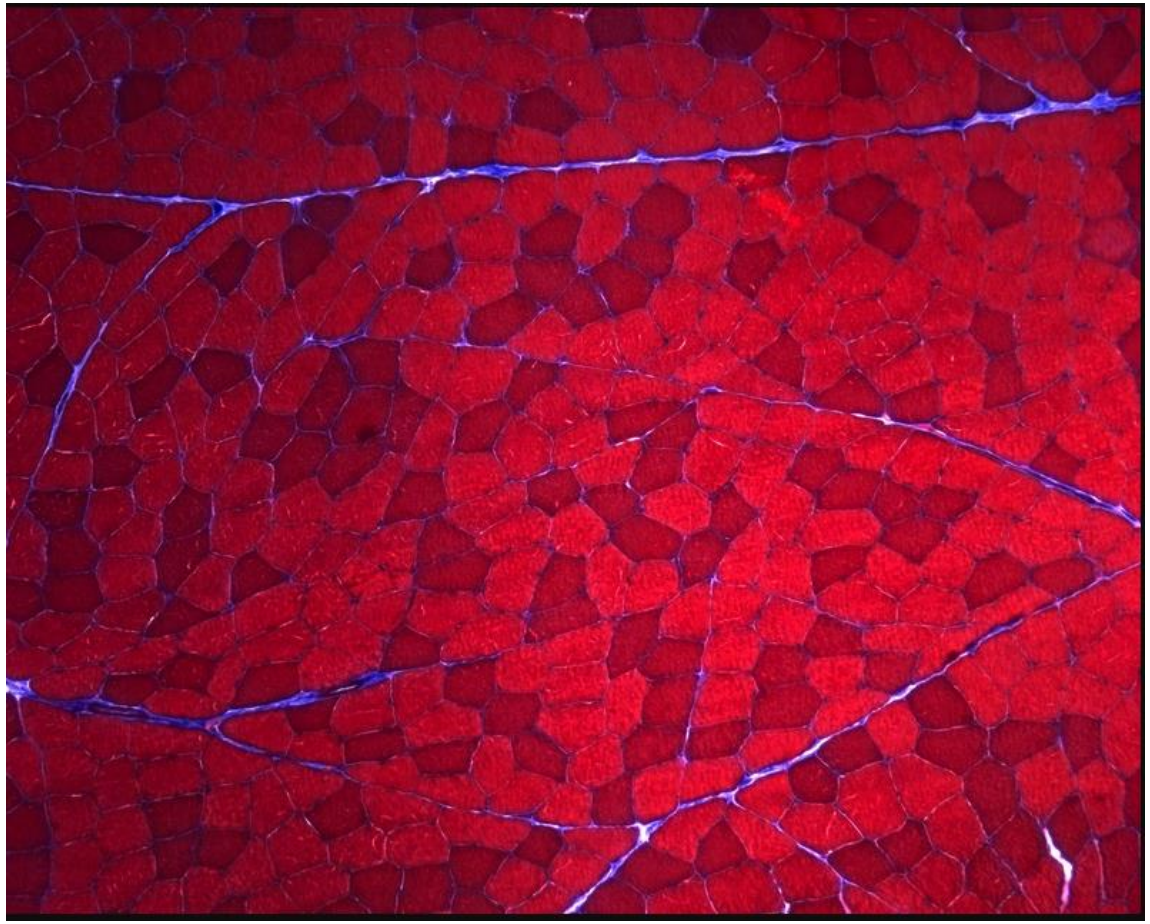


Mul, *Neurology*, 2017

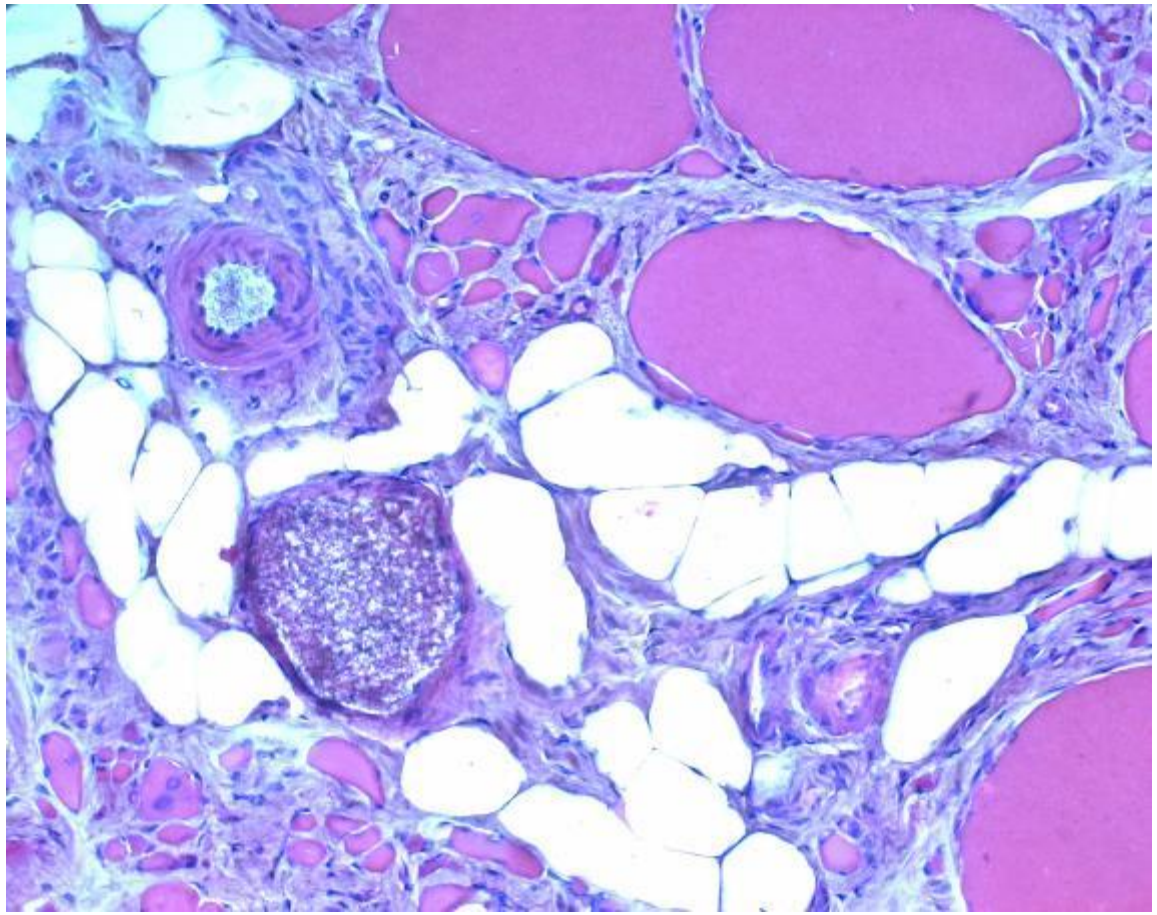
Muscle biopsy

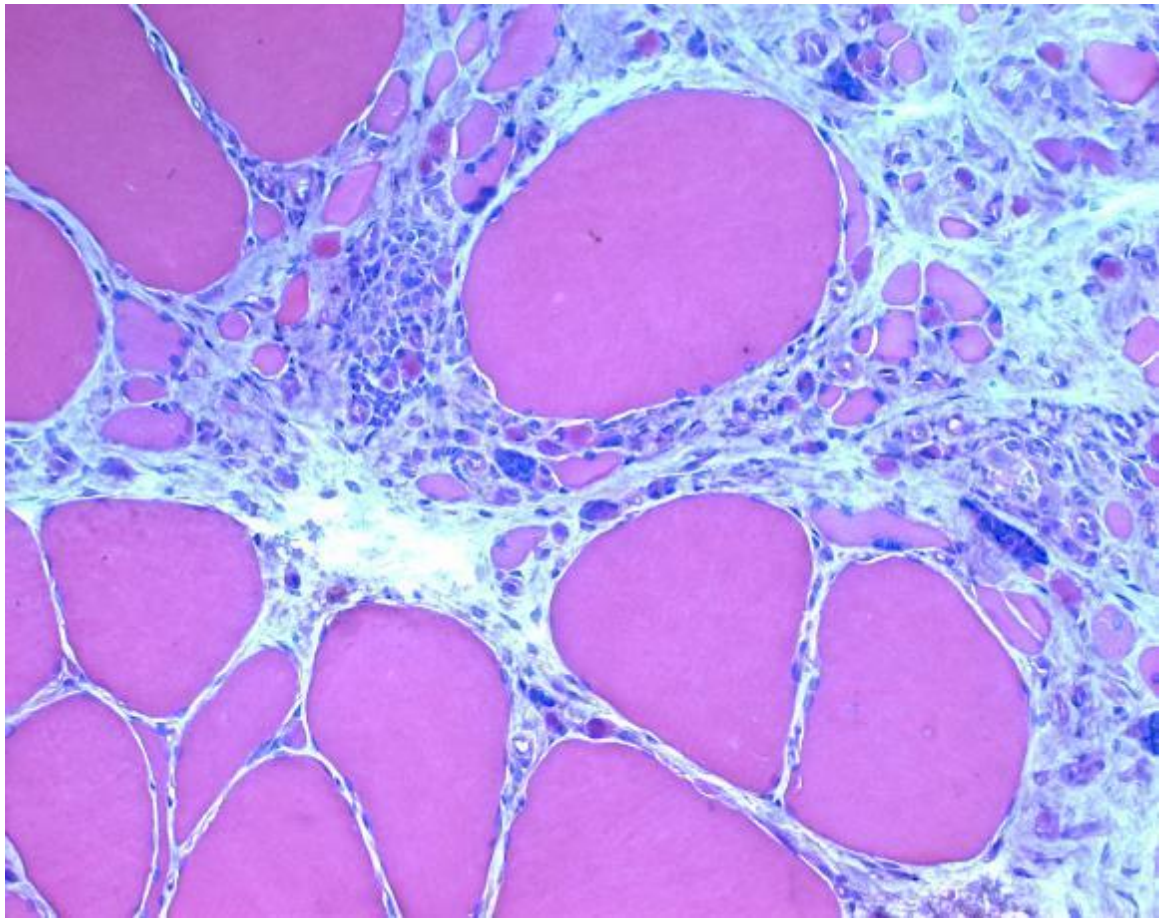


FSHD



Control

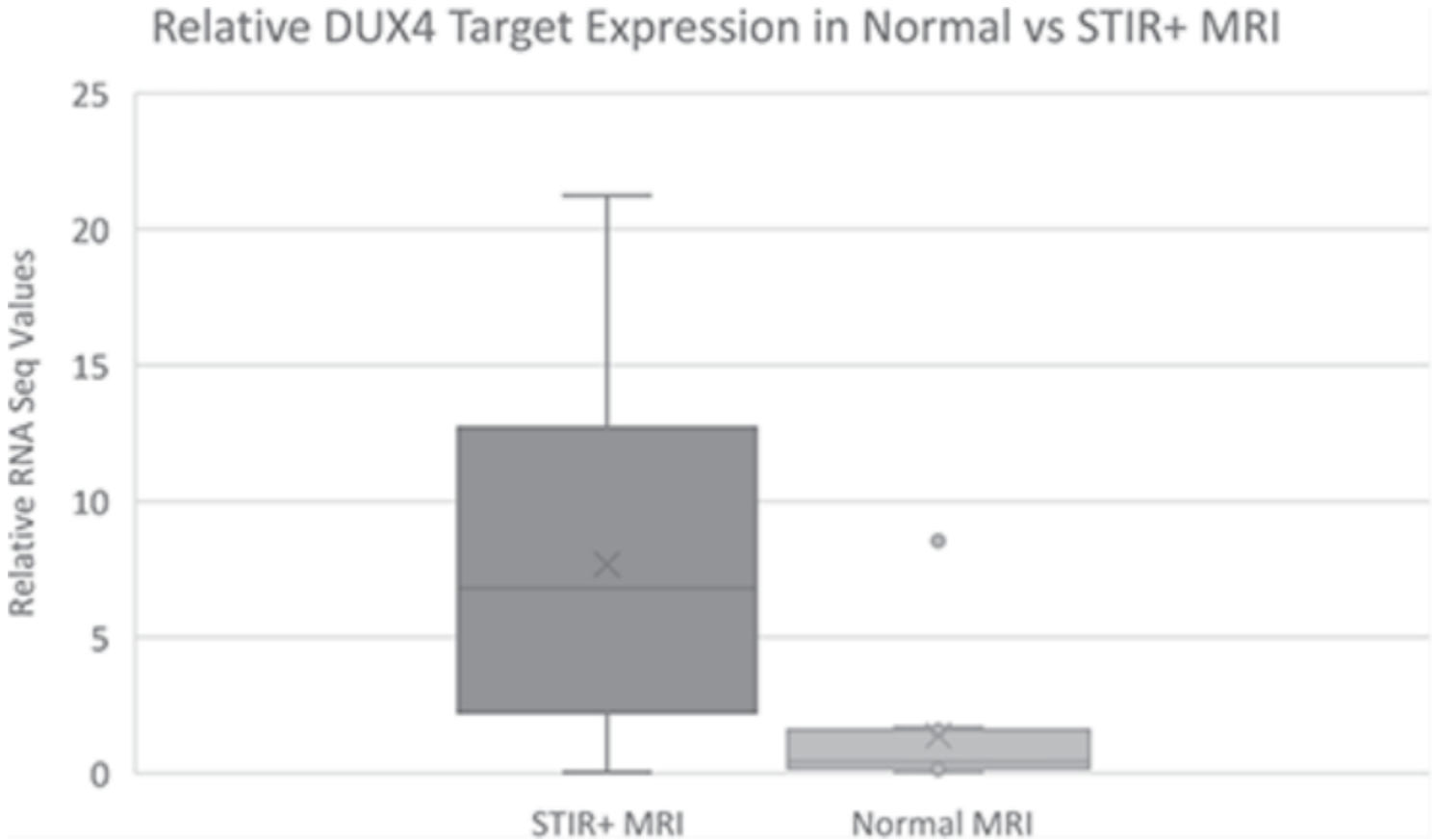




Pathological features

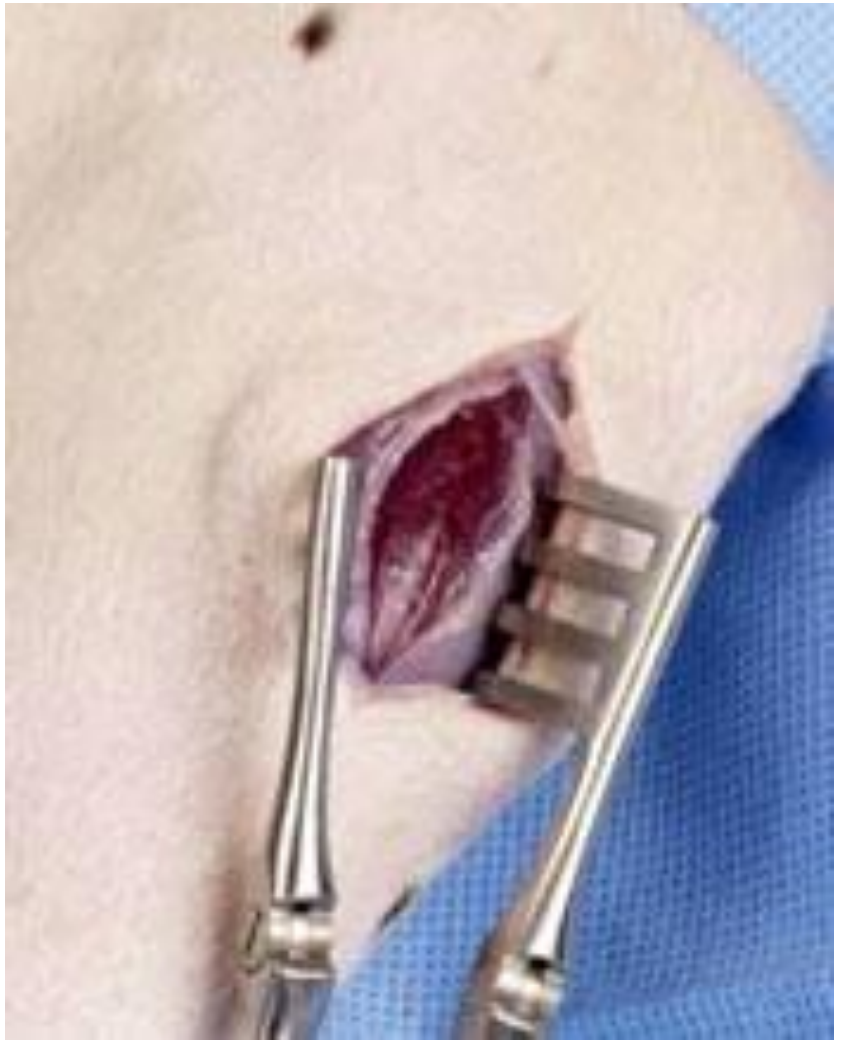
	FSHD		Control		p-value
	Mean	Std. Dev.	Mean	Std. Dev.	
Int. Nuclei (%)	0.028339	0.035156	0.015608	0.016162	0.03514
Split Fibers (%)	0.001185	0.002627	0.000385	0.00118	0.073456
Necrotic Fibers (%)	0.001356	0.002417	3.35E-05	0.000167	0.000334
Regenerating Fibers (%)	0.004628	0.010109	0.00016742	0.00059641	0.0125897
Atrophic Fibers (%)	0.023216	0.061632	0.002	0.002832	0.018824
Area of Fibrosis (%)	0.1	0.0396	0.07	0.0229	0.0415

Image guided biopsy in FSHD



Wang, *Human Molecular Genetics*, 2018

Open muscle biopsy



http://vetneuromuscular.ucsd.edu/

Needle muscle biopsy

Needle muscle biopsy

Open vs Fine Needle Biopsy

OPEN	FINE NEEDLE
Requires OR	Can be done in clinic
Leaves incision scar	Puncture scar or none
Nerves, vessels, muscle integrity well visualized	Blind
Well tolerated in most	Well tolerated in all
Hundreds of micrograms	Tens of micrograms
Hundreds of fibers	Few fibers
Well-oriented fibers for histology	Misaligned fibers not suitable for histology
$$$$$	$
Best for when histology needed	Best for when only RNA/protein analysis needed or when serial evaluations needed

Other clinical outcome measures

- ReSOLVE natural history study: 160 FSHD subjects across multiple US and EU sites
- Goal to identify clinical outcomes that are more responsive to change over shorter periods of time for drug development trials
- Reachable workspace (Hatch et al., Neuromuscular Disord 2019: ~8% decline per year in upper quadrants)
- EIM
- FSHD-COM
- FSHD-HI

Conclusions

- Muscle progresses from healthy muscle to fatty infiltration
- Muscle inflammation may act as a trigger for this process
- Muscle inflammation can be visualized by STIR positivity
- DUX4 expression has been linked to STIR+ muscles
- DUX4 and DUX4 biomarkers can be assessed by needle muscle biopsy
- For a very slowly progressive disease MRI and biopsy are good outcome measures