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Molecular Mechanisms of FSH Muscular Dystrophy Pathogenesis

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Molecular mechanisms of FSH muscular dystrophy pathogenesis

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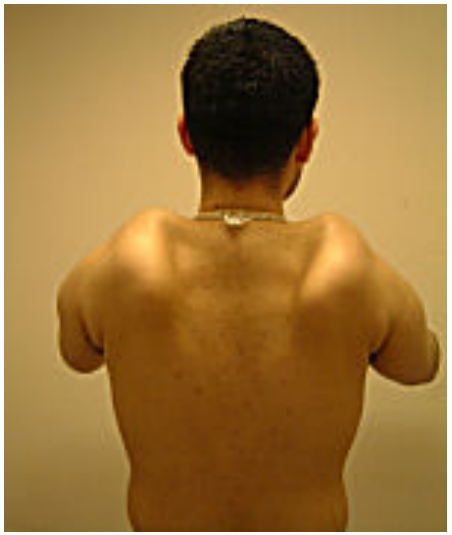
Facioscapulohumeral Muscular Dystrophy (FSHD)

Most prevalent muscular dystrophy afflicting children and adults (~1:7,000-15,000)

Autosomal dominant

Facio: refers to face
Scapulo: refers to shoulders
Humeral: refers to humerus (upper arm bone)



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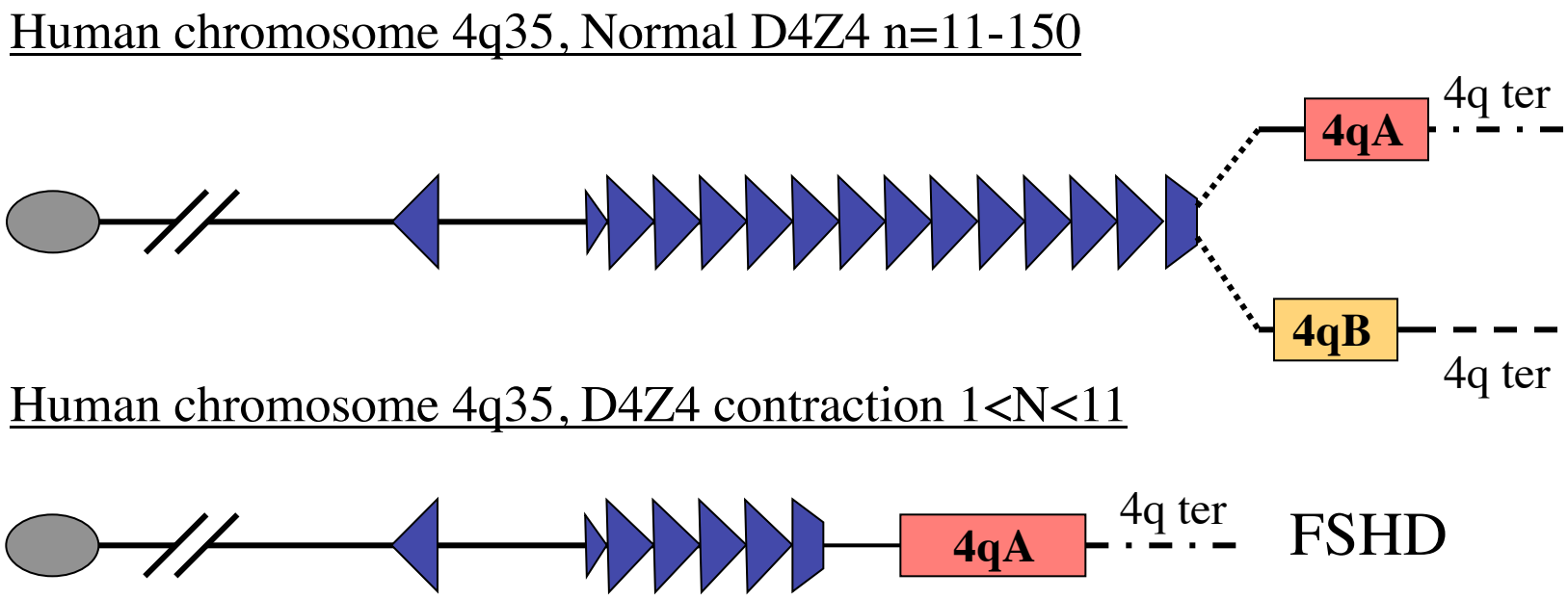
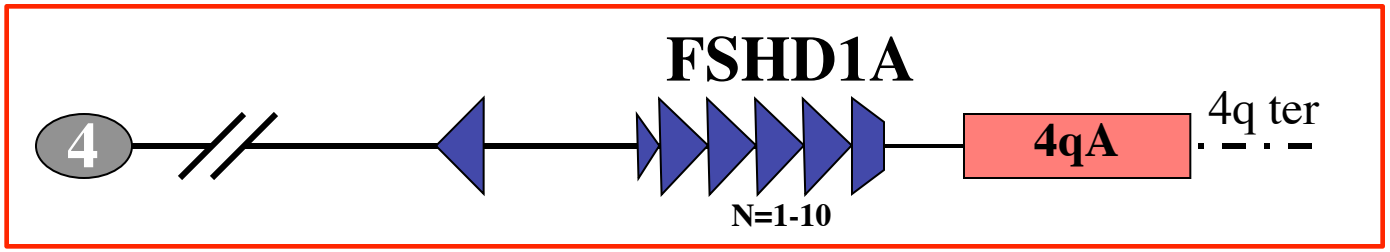
Winging on both sides in a patient with FSHD due to weakness of all the scapula stabilizing muscles

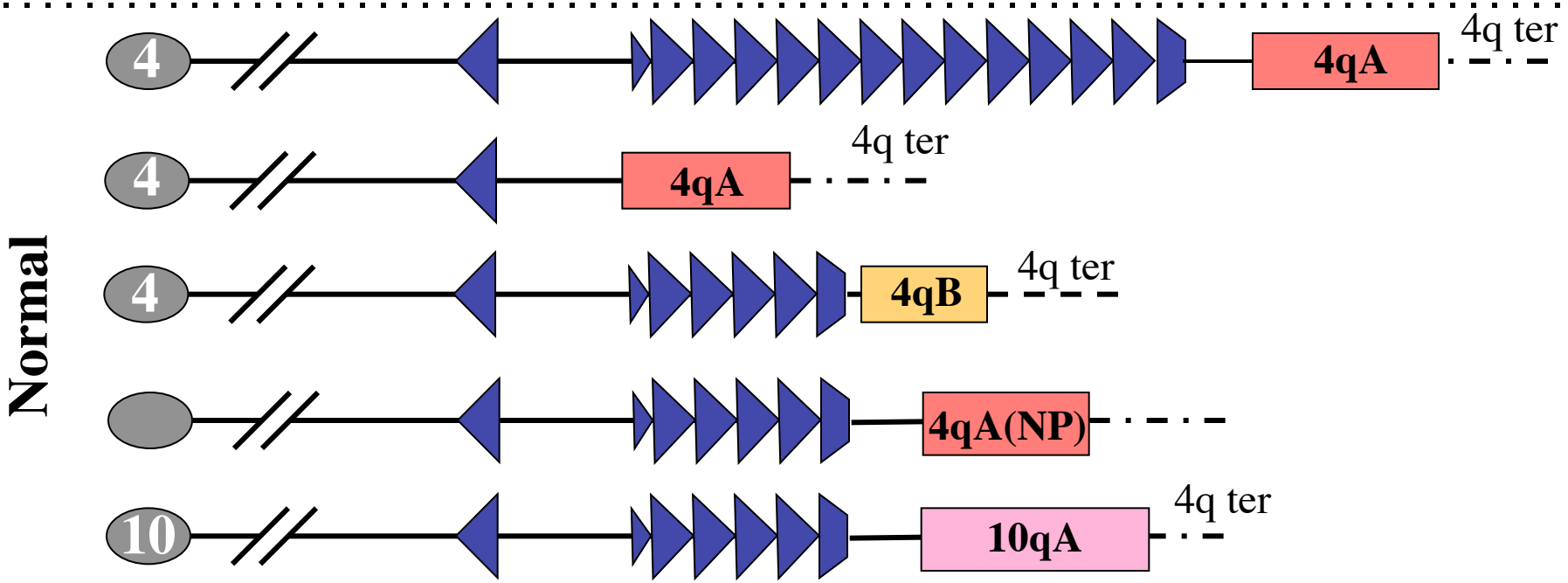
Great genetic and clinical heterogeneity
Each patient may differ in severity
Most patients exhibit symptoms by age 20
>50% of patients retain ability to walk

The FSHD1 genetic lesion is a deletion in a tandem repeat array at 4q35



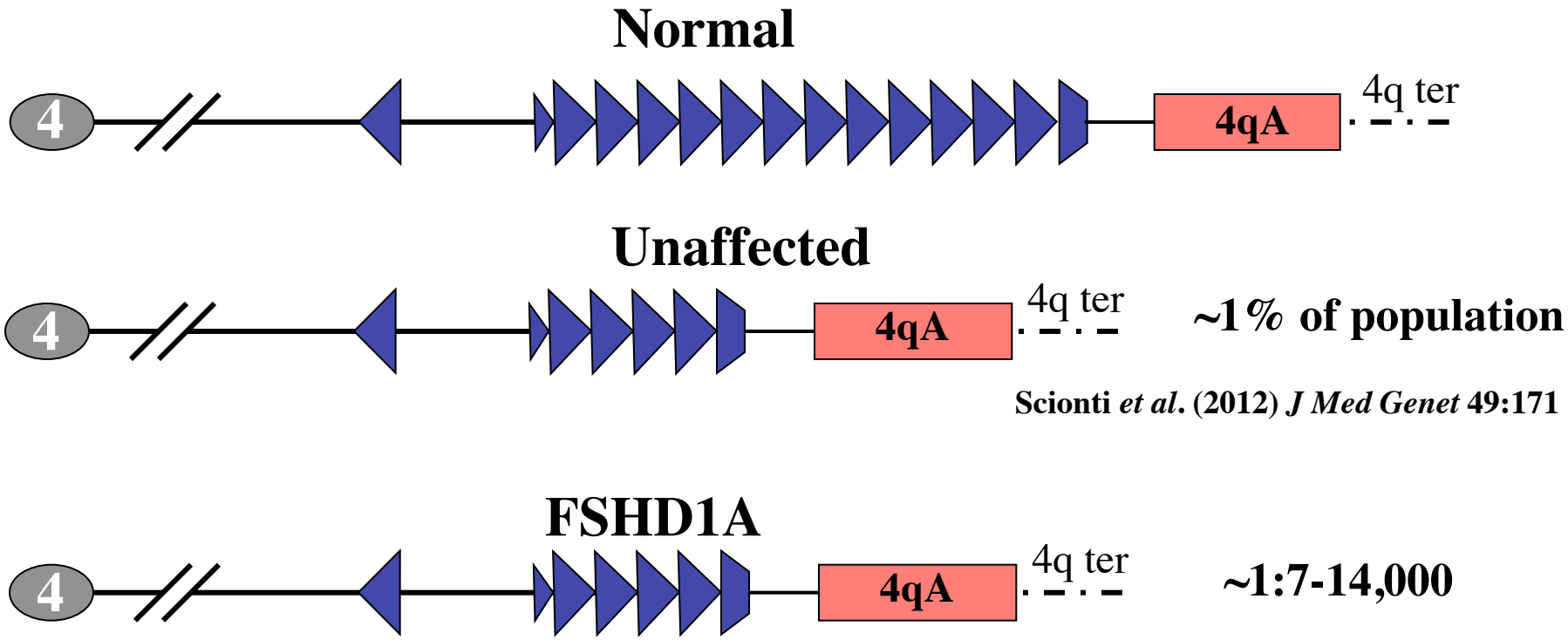
A pathogenic FSHD1 deletion is complex





- The deletion itself is not pathogenic
- The 4qA sub-telomere is permissive, not pathogenic
- The 4qB sub-telomere is not permissive
- Requires at least 1 D4Z4 repeat unit for FSHD
- Chromosome 10 arrays (devoid of chr. 4 D4Z4) are not linked to FSHD

A putatively pathogenic FSHD1 deletion shows very low penetrance

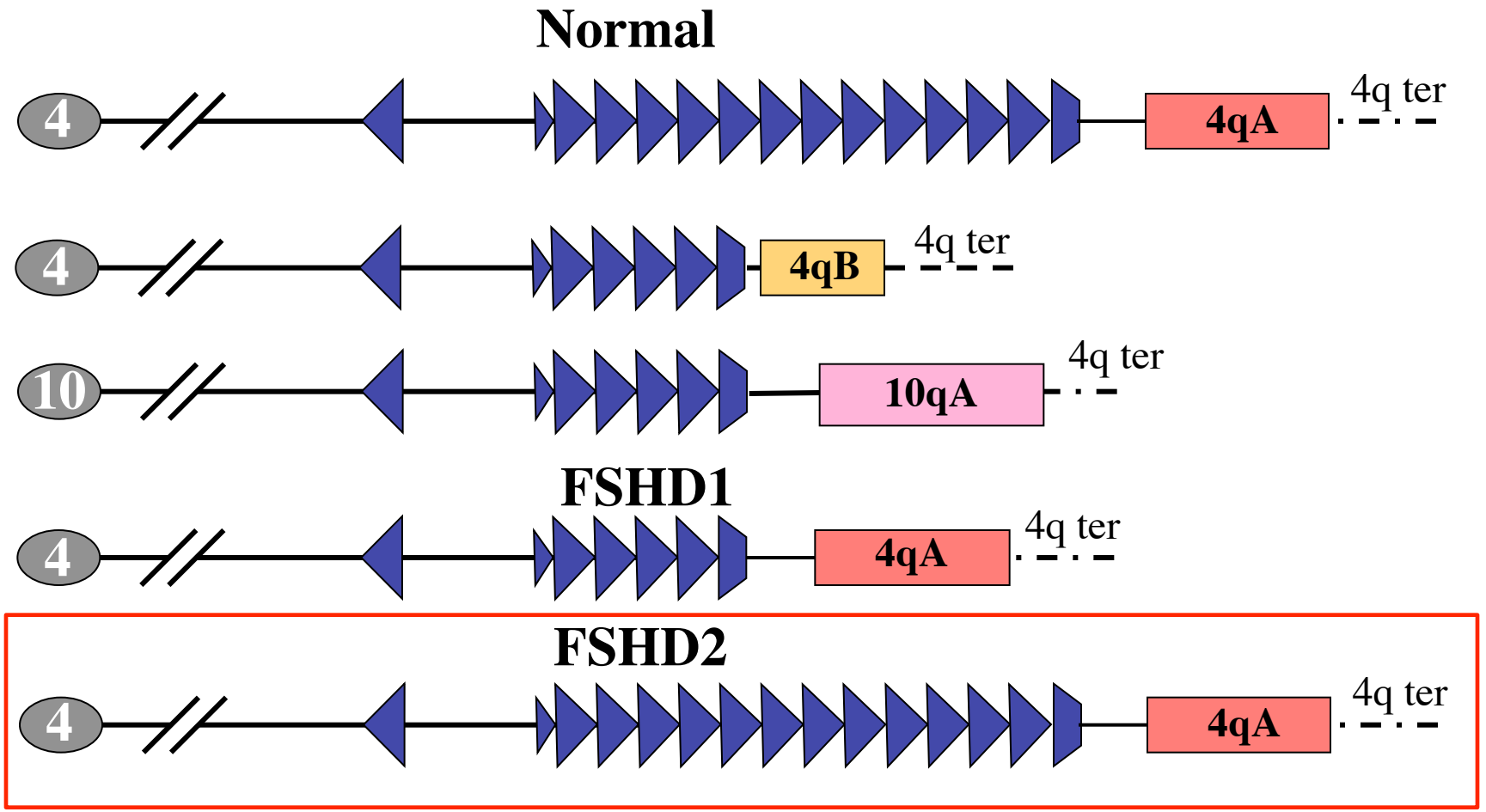


Scionti *et al*. (2012) *J Med Genet* 49:171

The deletion itself is not pathogenic

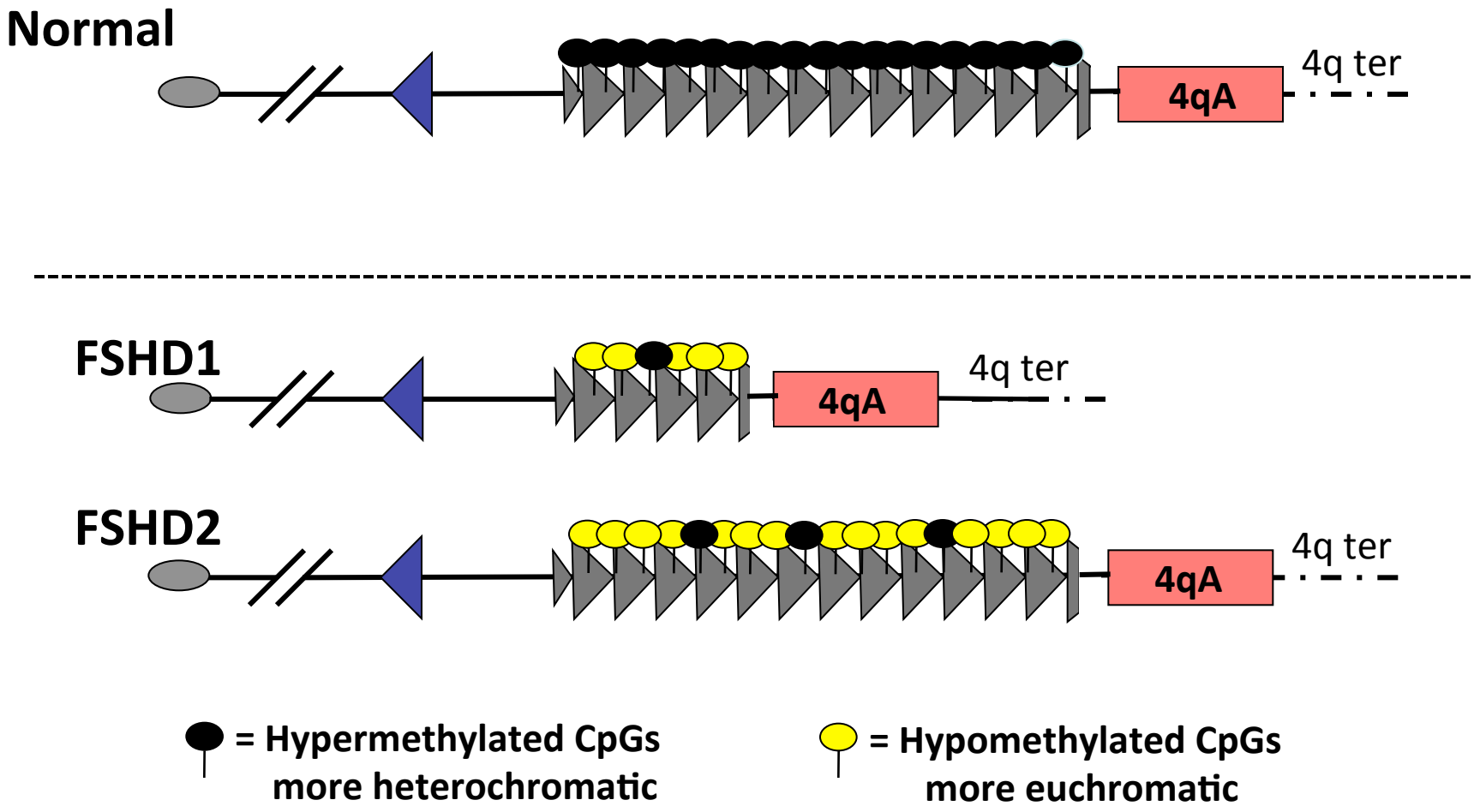
The 4qA sub-telomere is permissive, not pathogenic

FSHD2 is independent of the contraction



- The 4qA sub-telomere is required for FSHD1 and 2
- At least 1 D4Z4 is required for FSHD1 and 2

FSHD is linked to D4Z4, the A type subtelomere and the epigenetic status of the 4q35 D4Z4 repeat

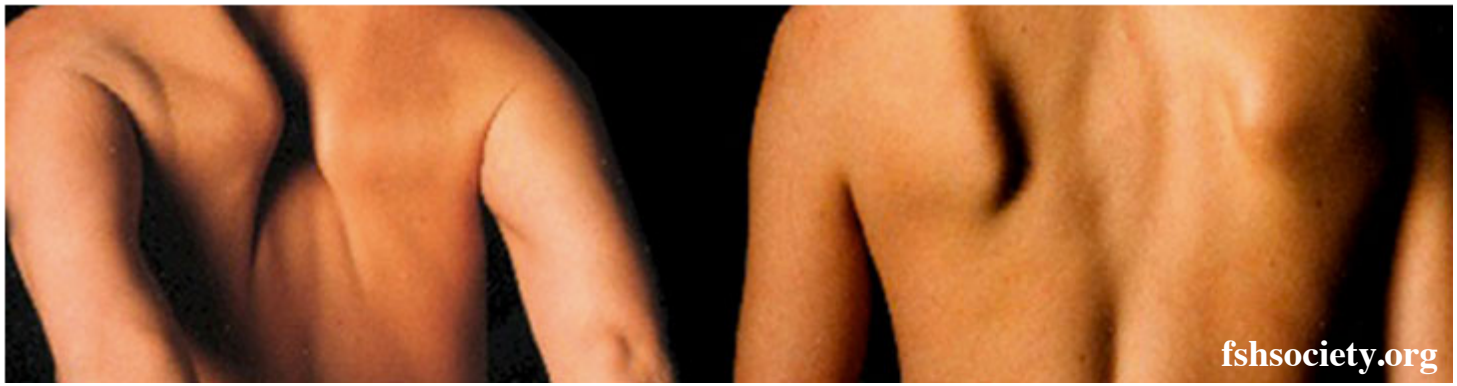


The 3 types of FSHD are linked by epigenetic dysregulation

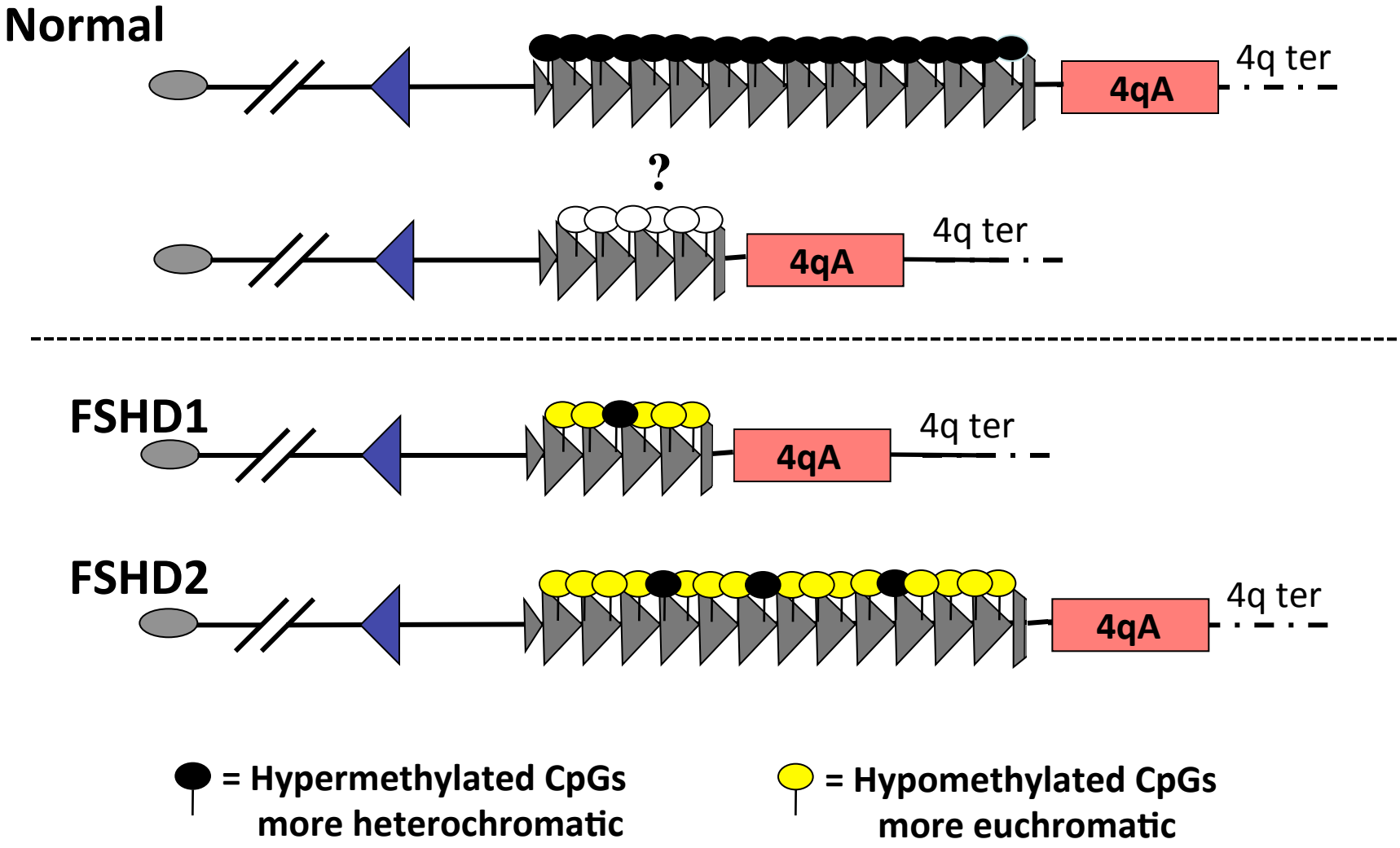
FSHD1: Dominant deletions at 4q35 D4Z4 array
Apparently low penetrance
DNA Hypomethylation of shortened 4q35

FSHD2: SMCHD1 inactivating mutations
--ATPase chromatin remodeling protein
--Modifier of metastable epialleles
DNA Hypomethylation of 4q35 and 10q26 arrays

IFSHD: Infantile form of FSHD1 or FSHD2, much more severe
DNA Hypomethylation of FSHD1 or 2



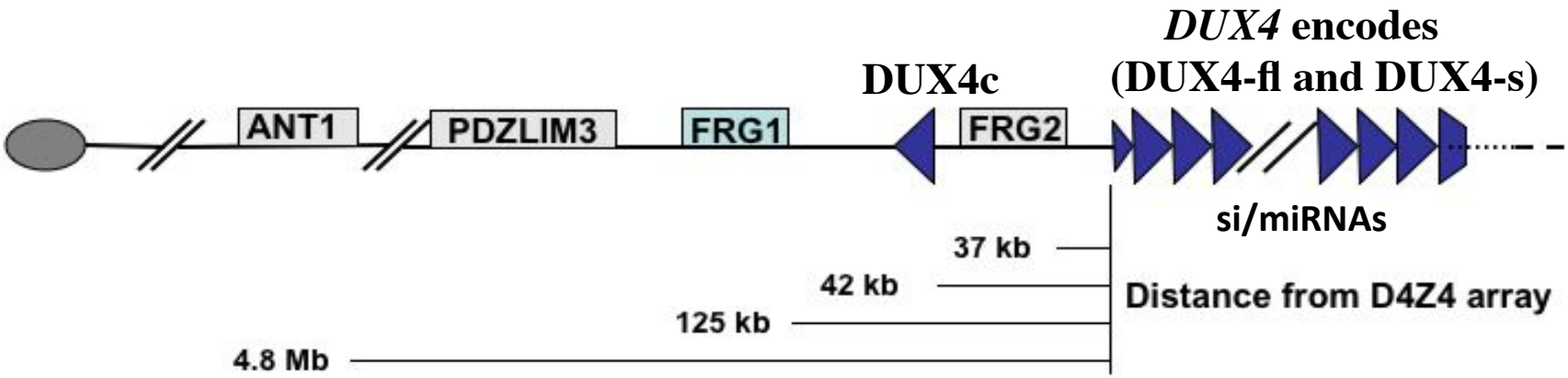
FSHD is linked to the A type subtelomere and the epigenetic status of the 4q35 D4Z4 repeat



FSHD results from an epigenetic-mediated dysregulation of gene repression

- DNA methylation
- Histone modifications
- Chromatin structure
- Long non-coding RNAs
- Nuclear organization
- High variability within the clinical population
  - Severity
  - Age of onset
  - Gene expression

Which gene(s) is responsible for FSHD pathology?



The FSHD causal gene:

1. Should be misexpressed in FSHD (Up or down)

→ mRNA, protein, cell type, developmental timing

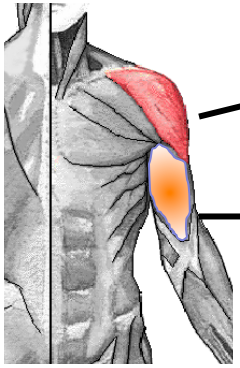
→ adversely affect skeletal muscle and potentially vasculature

2. Explain the 4qA linkage and under epigenetic repression

Both FRG1 and DUX4 produce phenotypes consistent with FSHD when overexpressed in animal models

Wellstone family cohorts of muscle biopsies and myogenic cell cultures

Subjects screened for FSHD clinically and genetically



Deltoid – Expect less pathology

Biceps – Expect more pathology

Genotyping (Iowa Wellstone)

Biopsies (KKI-JHU)

Cell Culture (UMMS)

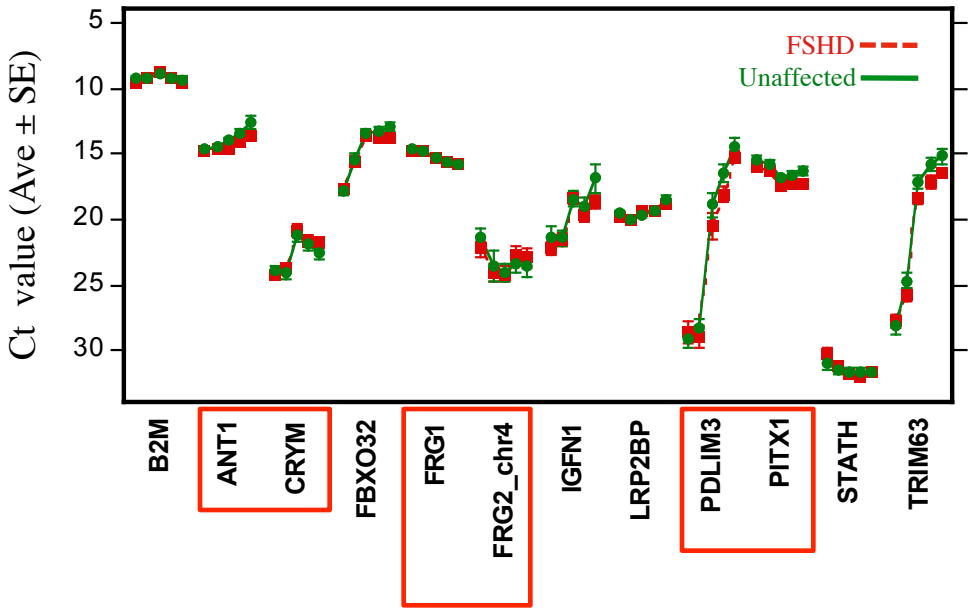
mRNA & protein analysis (CHB, UMMS)

Microsatellite analysis (CHB)

FSHD1 affected and genetically unaffected 1st degree relatives

Myogenic cultures from FSHD and unaffected first-degree relatives have similar patterns of gene expression during proliferation and myogenic differentiation

Normalization genes **FSHD Candidate genes**

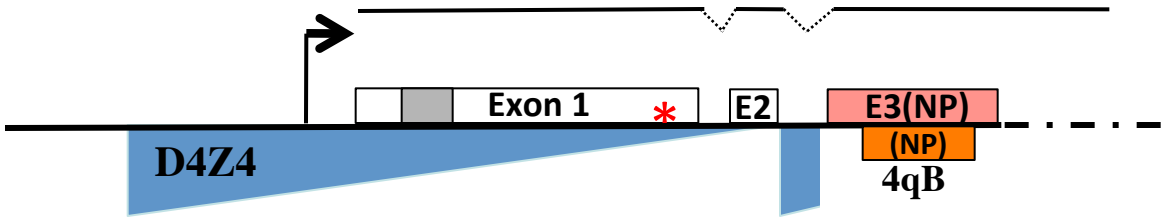


Homma *et al*. (2012) *EJHG*

"A Unifying Model for FSHD" based on DUX4

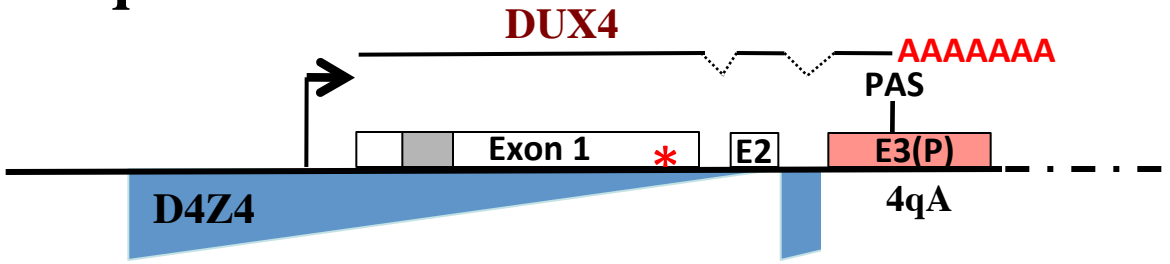
FSHD permissive 4qA subtelomeres encode a third exon containing a polyadenylation site that stabilizes the DUX4 mRNA

Chr 4qB or Chr 10qA or 10qB → Normal



-Unstable mRNA
-DUX4 protein not made
-Nontoxic

Chr 4qA → FSHD



-Stable poly A mRNA
-DUX4 protein produced
-DUX4 expression is exclusive to FSHD

PAS = polyadenylation site
* = translation stop
NP = non-permissive
P = permissive

Lemmers et al. (2010) *Science* 329:1650

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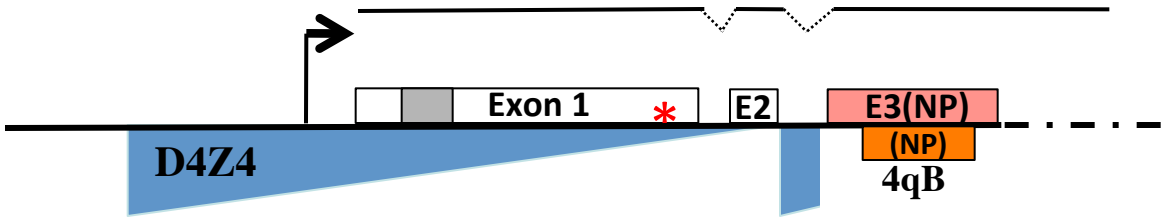

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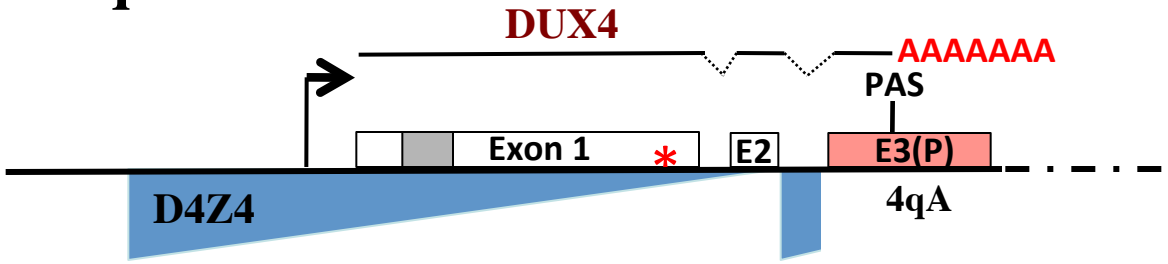

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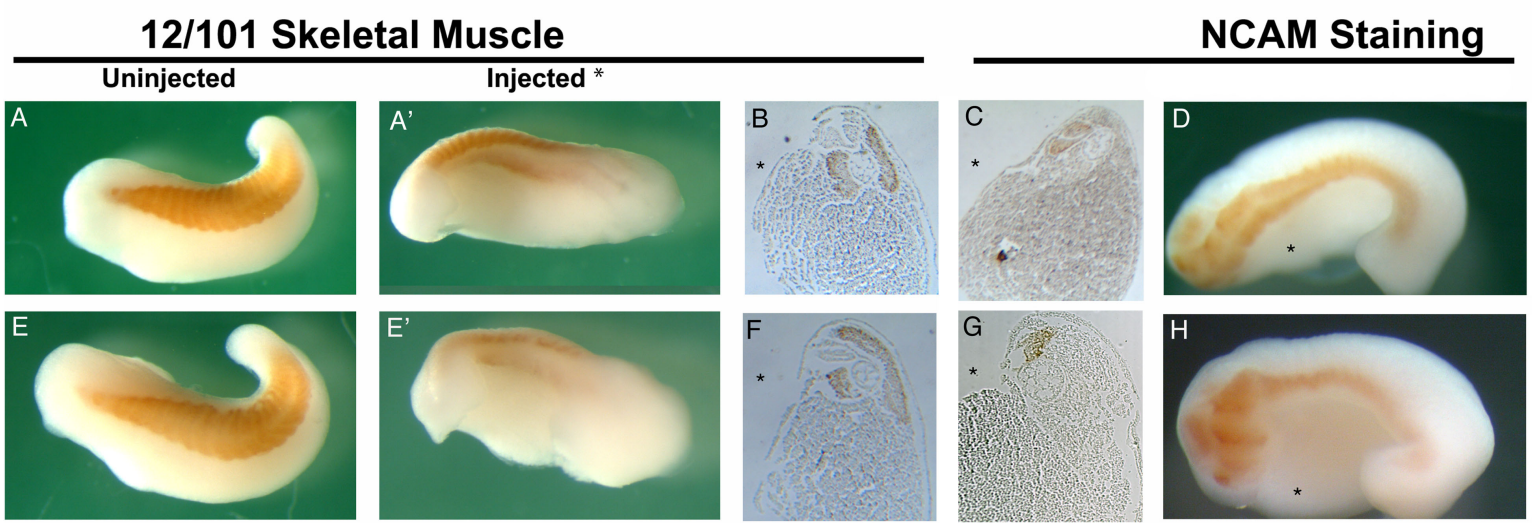
Lemmers et al. (2010) *Science* 329:1650

DUX4-FL expression leads to massive loss of developing myogenic cells

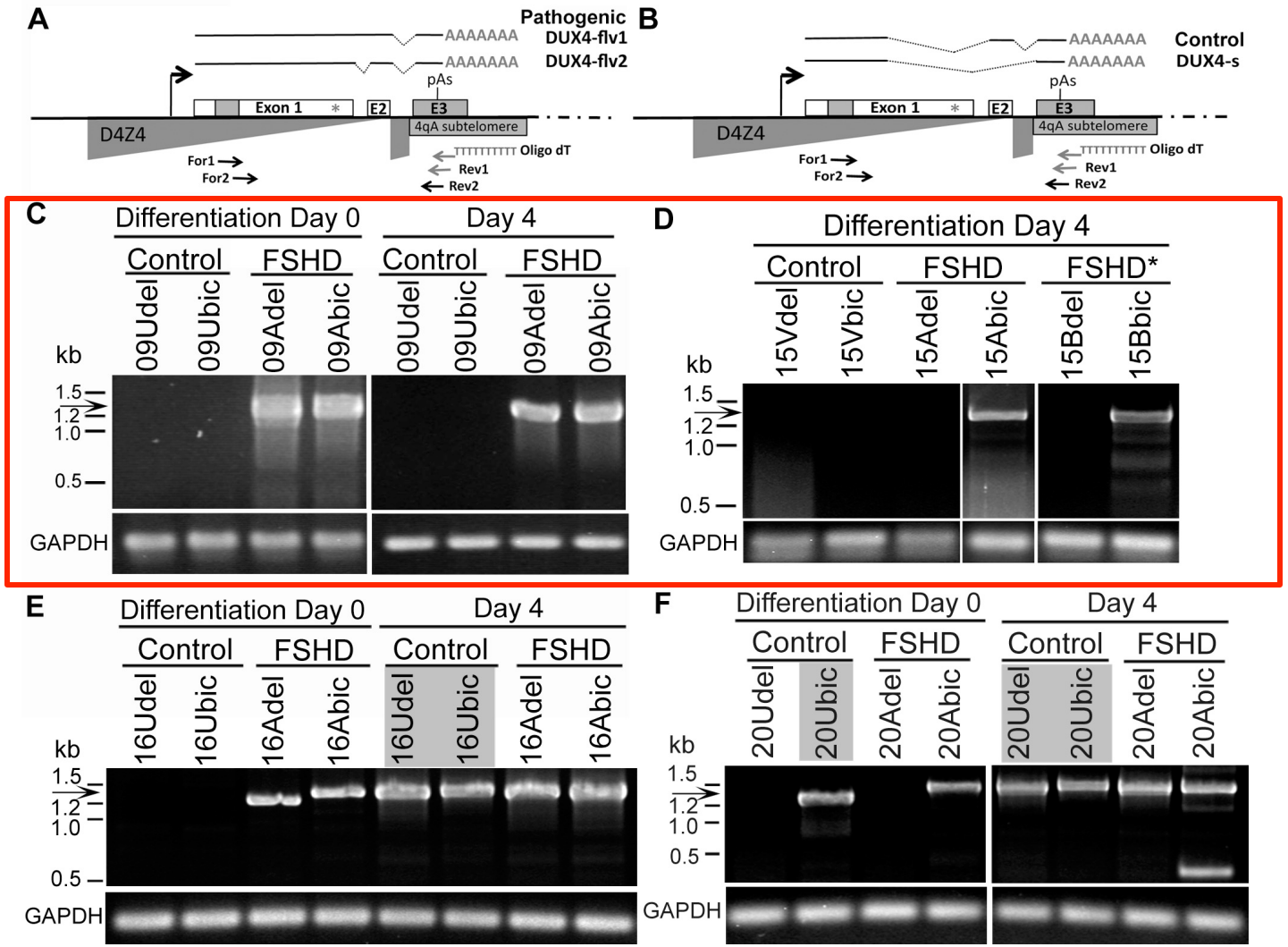
Xenopus embryos



Immunostaining for developing skeletal muscle

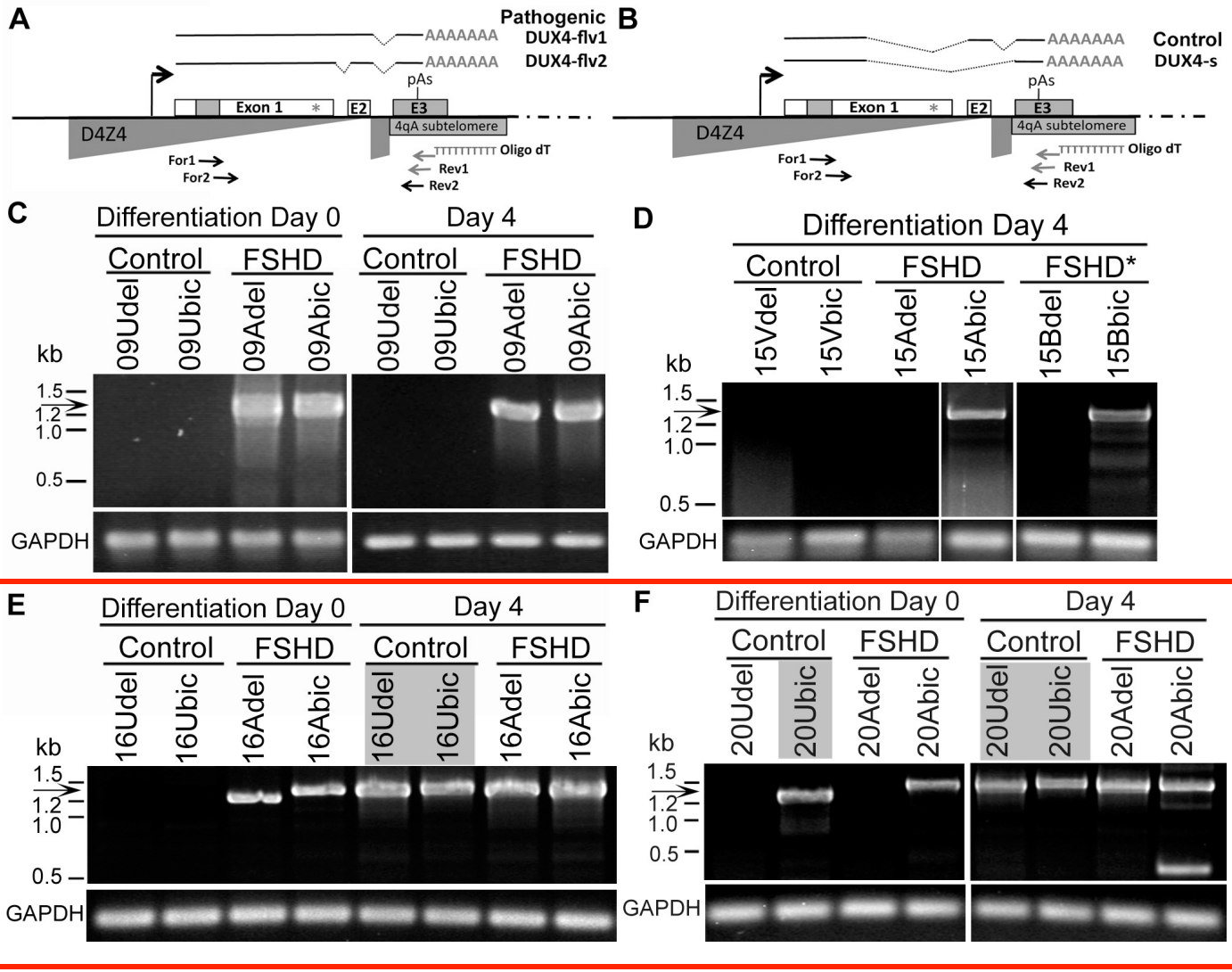
Wuebbles et al. (2010) *IJCEP*

DUX4-fl mRNA is expressed in FSHD1-derived myogenic cells



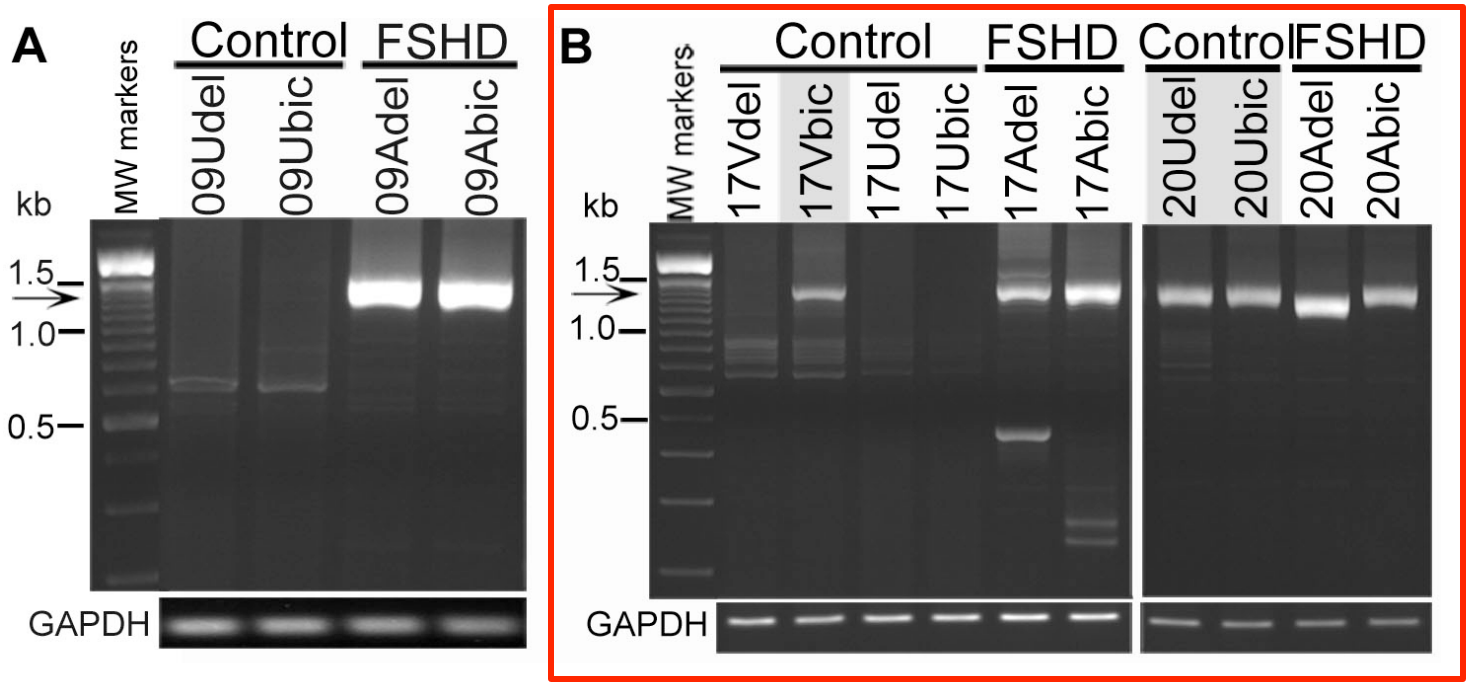
T. Jones *et al.* (2012) Hum. Mol. Genet 21:4419

DUX4-fl mRNA expression is not exclusive to FSHD1-derived myogenic cells



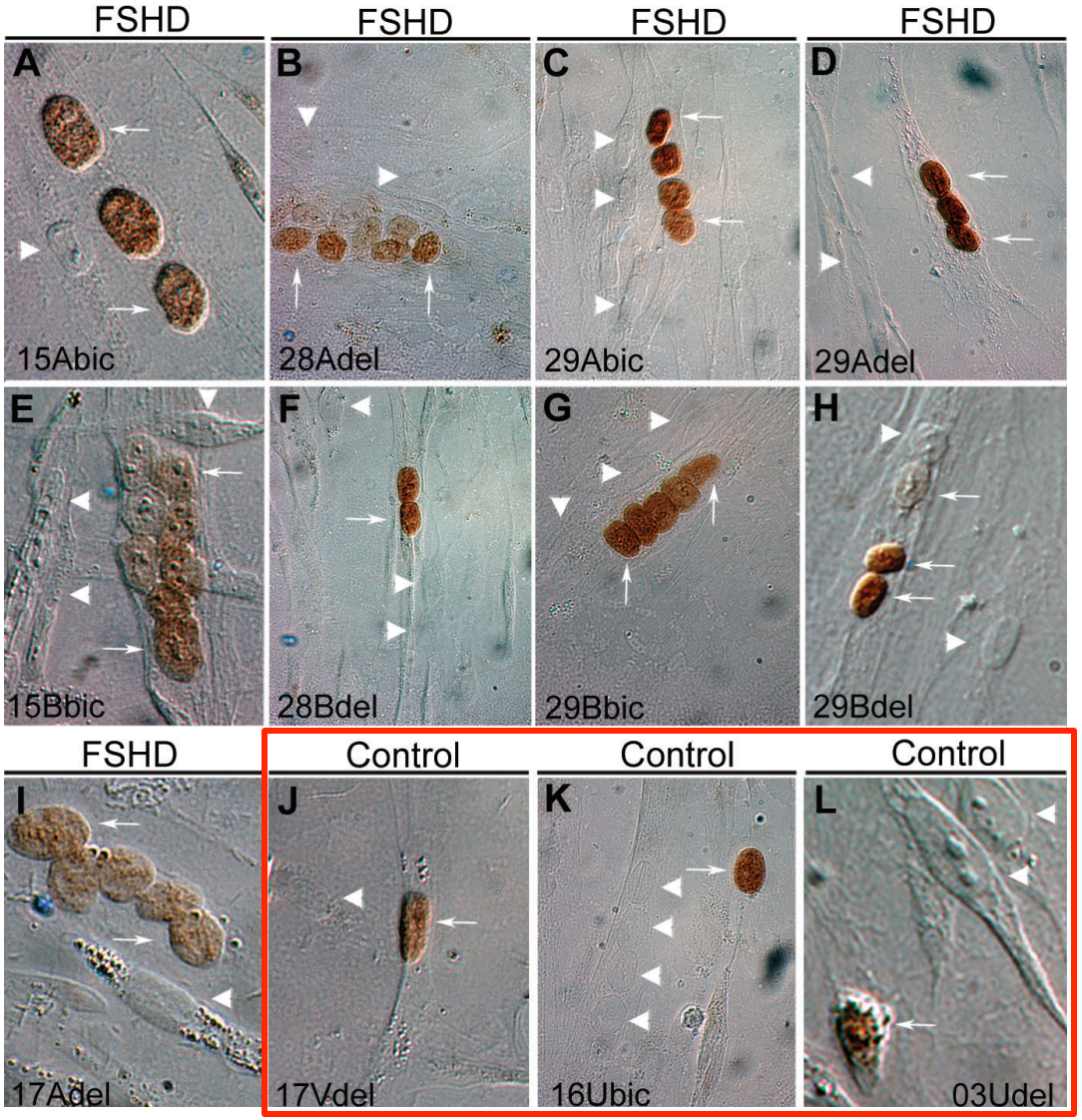
T. Jones *et al.* (2012) Hum. Mol. Genet 21:4419

DUX4-fl mRNA expression is expressed in both FSHD1-derived and unaffected muscle biopsies



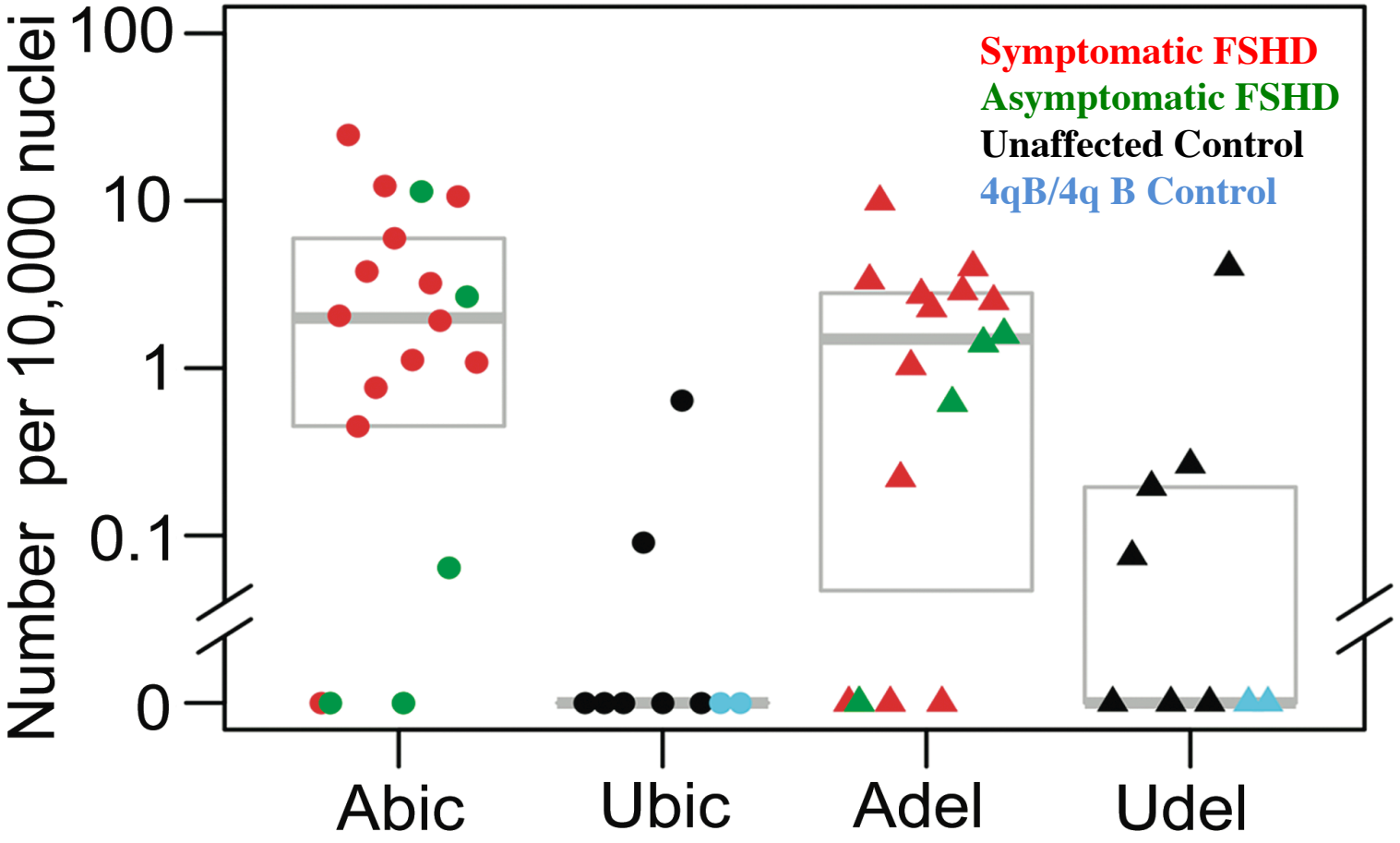
T. Jones *et al.* (2012) Hum. Mol. Genet 21:4419

Differentiated myogenic cells from genetically FSHD1 and control subjects express DUX4-FL protein



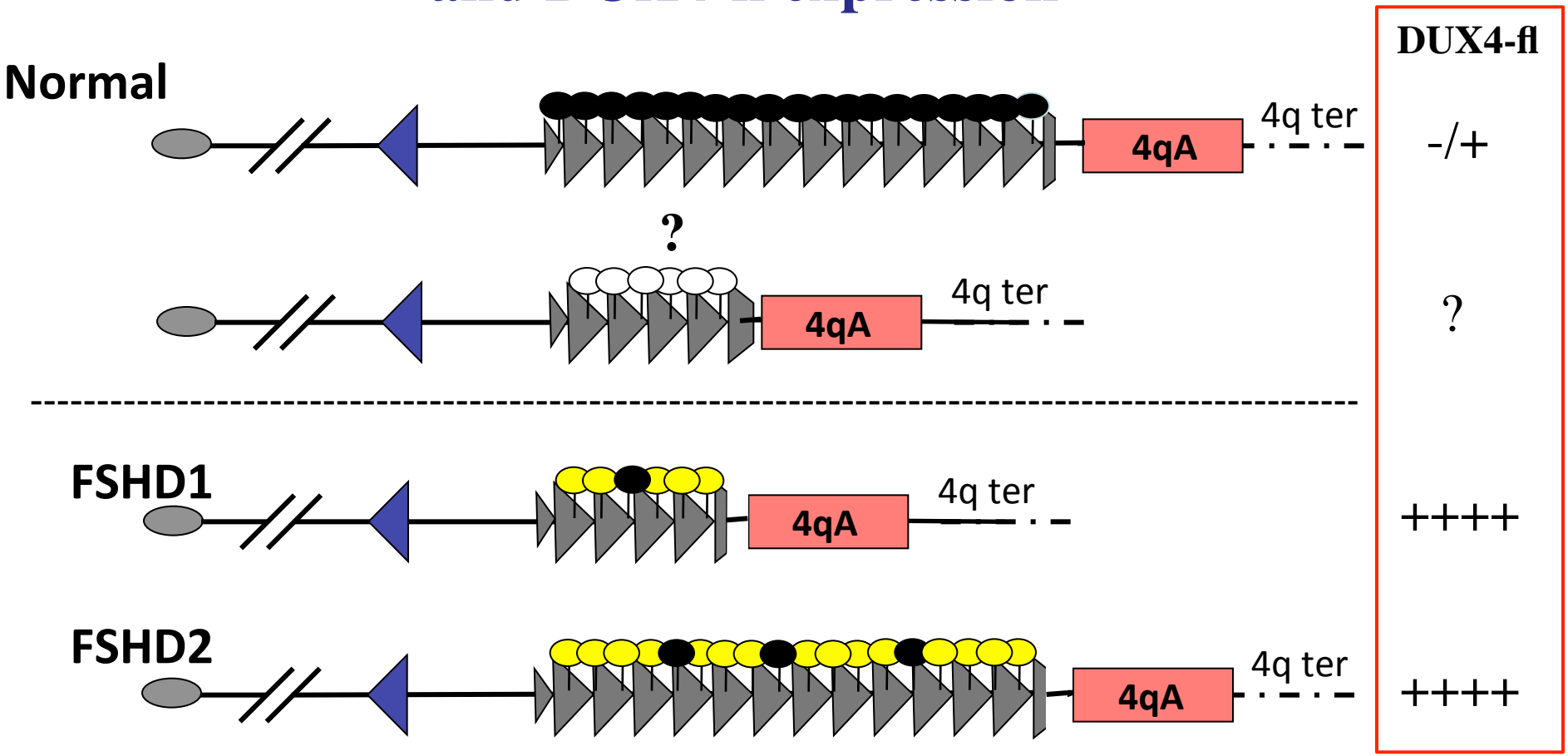
T. Jones *et al.* (2012) Hum. Mol. Genet 21:4419

DUX4-FL expression in myogenic cells from FSHD affected unaffected subjects suggests a quantitative model of pathogenesis



T. Jones *et al.* (2012) Hum. Mol. Genet 21:4419

FSHD is linked to the permissive 4qA subtelomere, the epigenetic status of the 4q35 D4Z4 repeat and DUX4-fl expression



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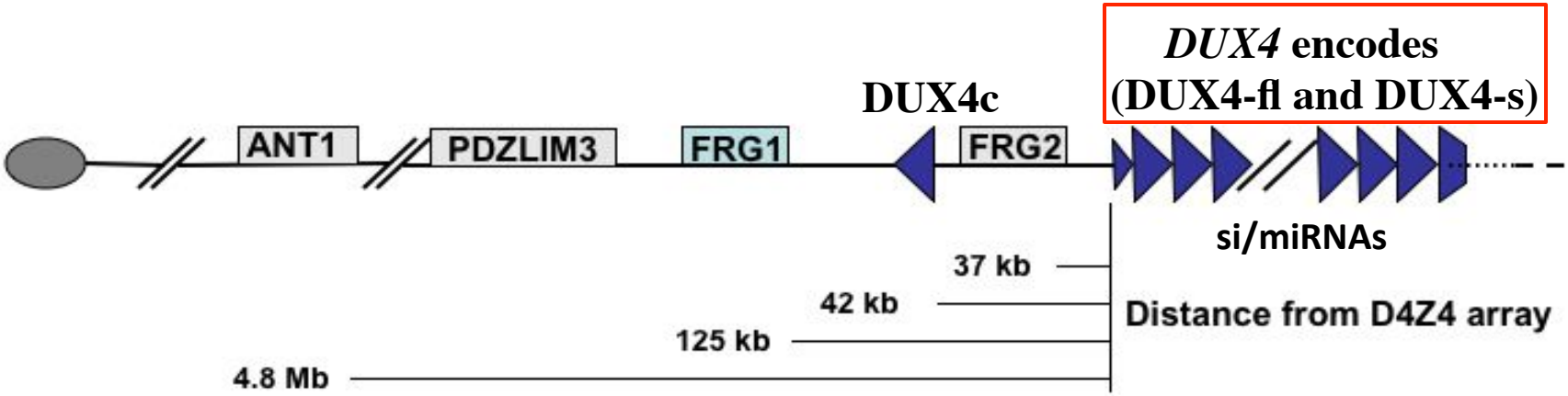


The FSHD causal gene:

1. Should be misexpressed in FSHD (Up or down)

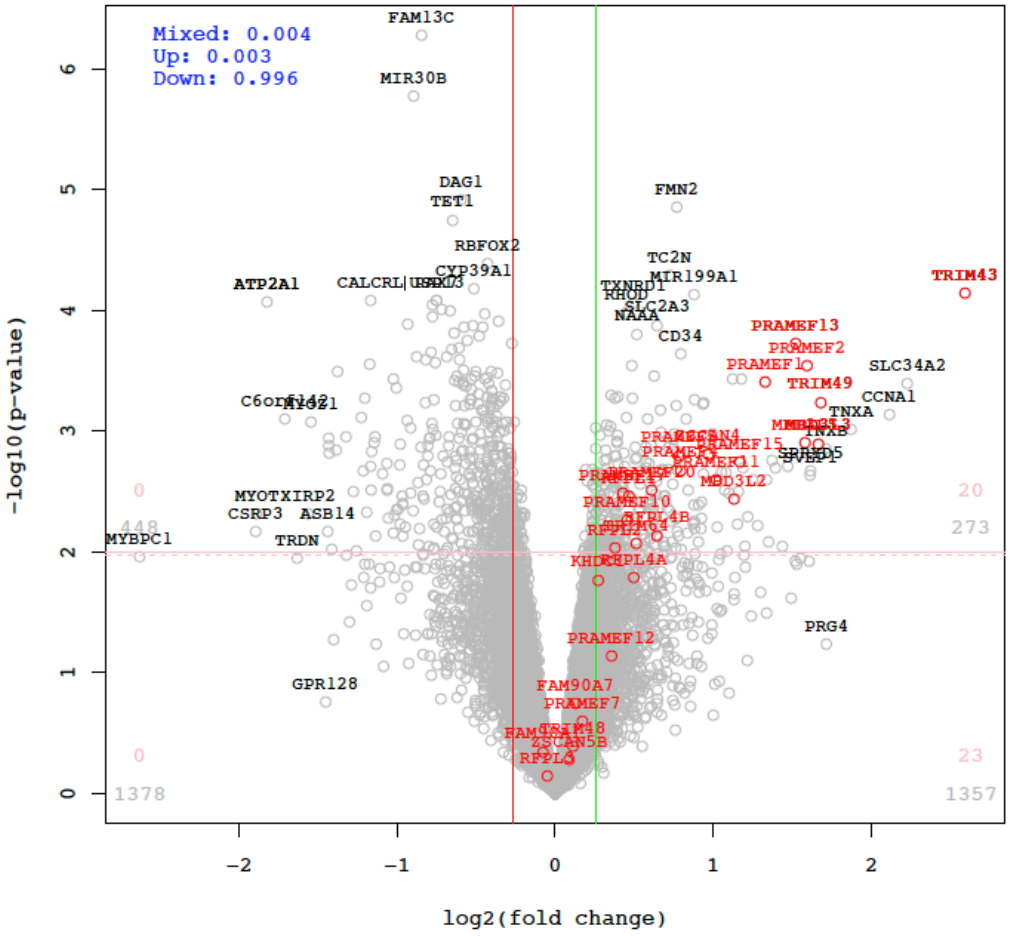
→ mRNA, protein, cell type, developmental timing

→ adversely affect skeletal muscle and potentially vasculature

2. Explain the 4qA linkage and under epigenetic repression

DUX4 fulfills these criteria

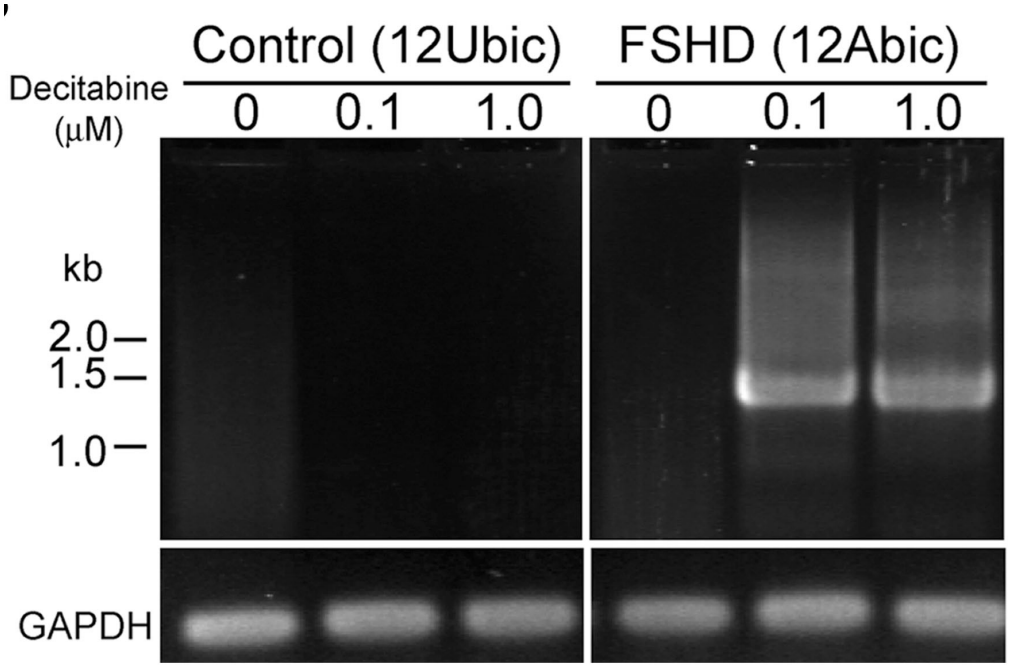
Many DUX4-FL responsive genes are upregulated in FSHD myotubes (biomarkers)



Oliver King, *et al.* (2012)

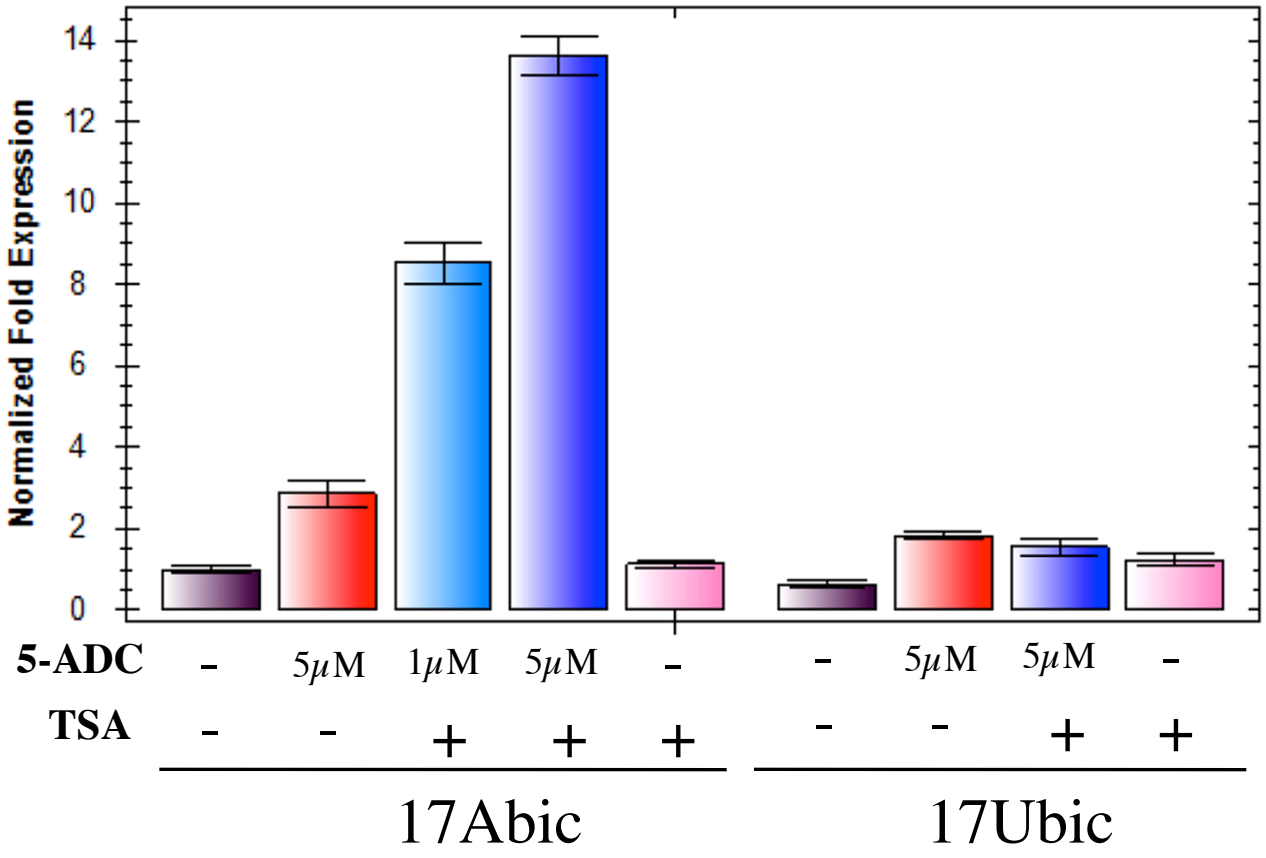
Increased DUX4-fl expression appears necessary* but alone is not sufficient for FSHD

FSHD-derived myoblasts are epigenetically poised to express DUX4-fl



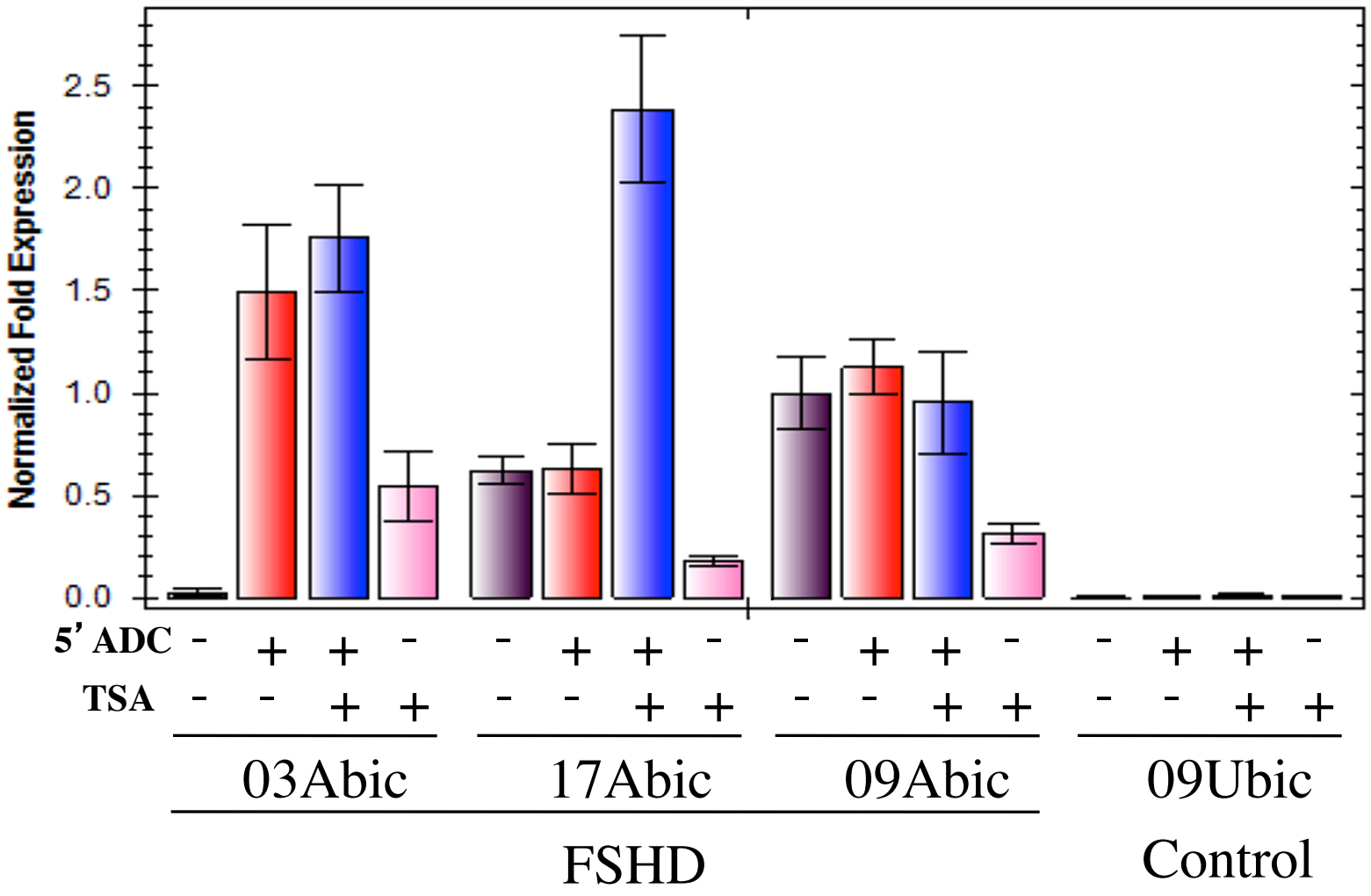
Decitabine treatment leads to DNA demethylation

FSHD derived myogenic cells are epigenetically poised to express DUX4-fl mRNA



DUX4-fl qRT-PCR Analysis

FSHD subjects show individual variability in the stability of DUX4-fl epigenetic repression

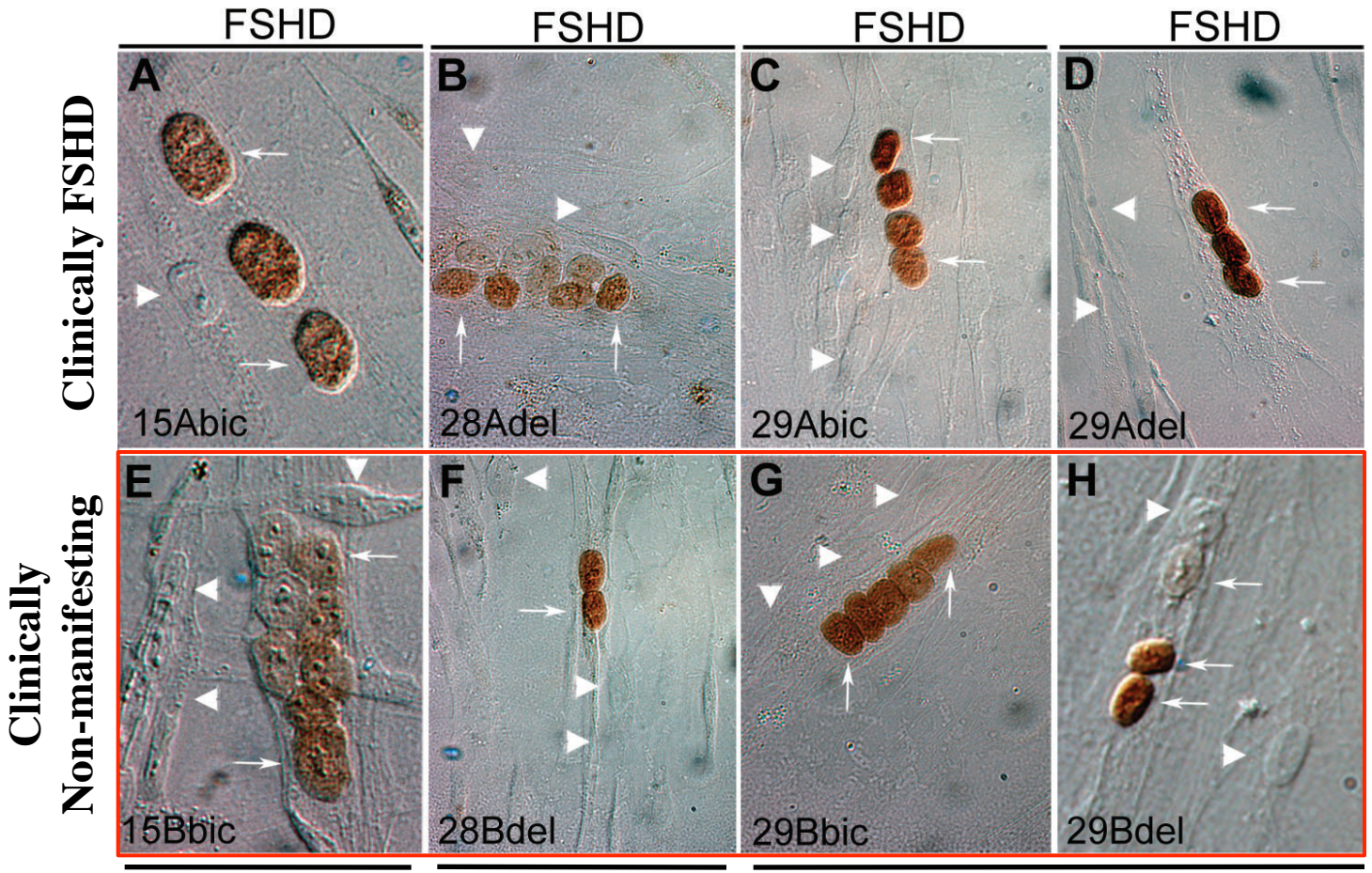


The 4q35 D4Z4 in FSHD exists as differentially metastable epialleles among affected subjects

→ epigenetically poised for DUX4 expression

The 4q35 D4Z4 in normal subjects exhibits stable epigenetic repression

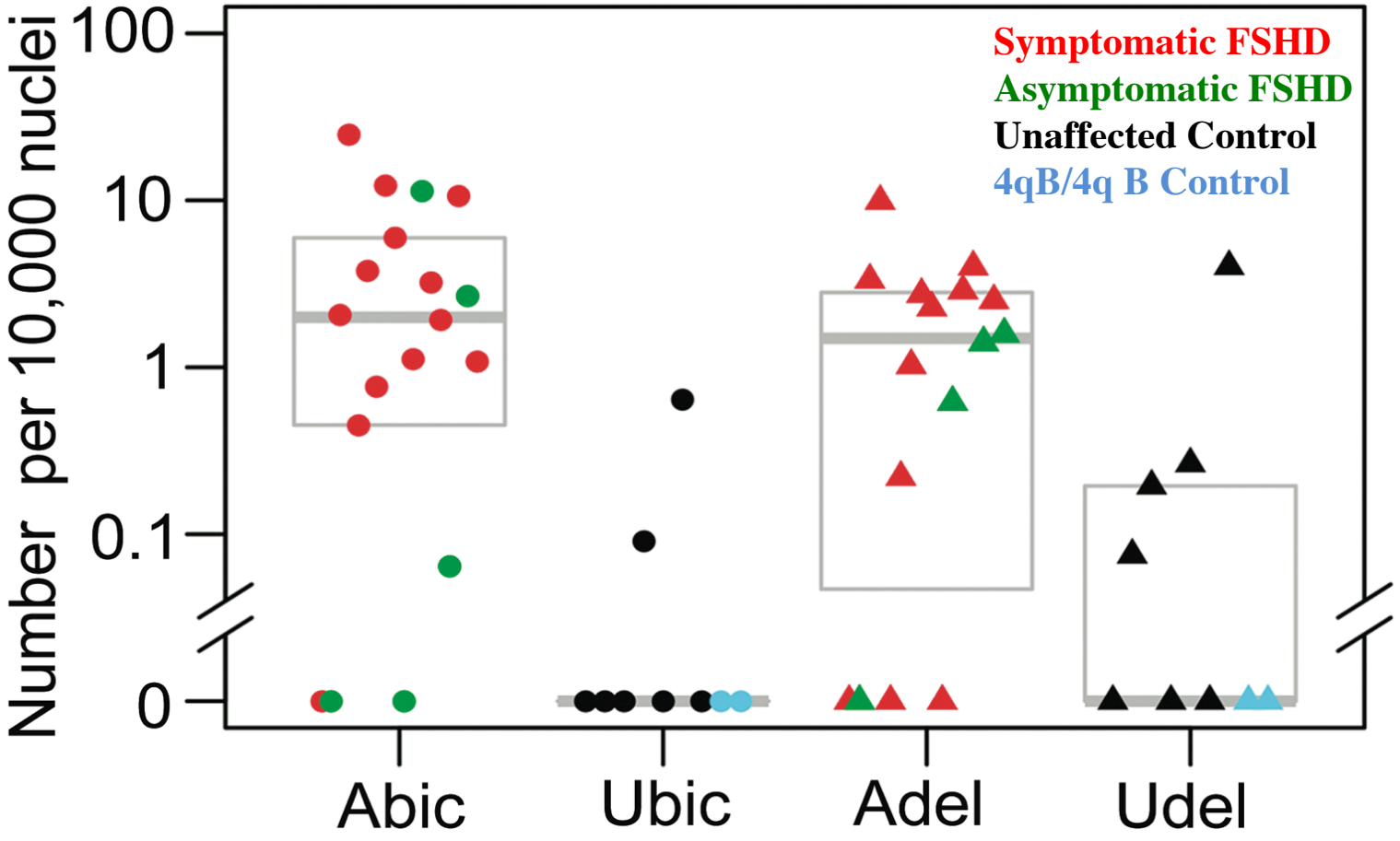
Differentiated myogenic cells from genetically FSHD1 but clinically non-manifesting subjects express DUX4-FL protein



Non-manifesting	15B = 69 yr old	28B = 68 yr old	29B = 70 yr old
	vs	vs	vs
Affected	15A = 66 yr old	28A = 44 yr old	29A = 39 yr old

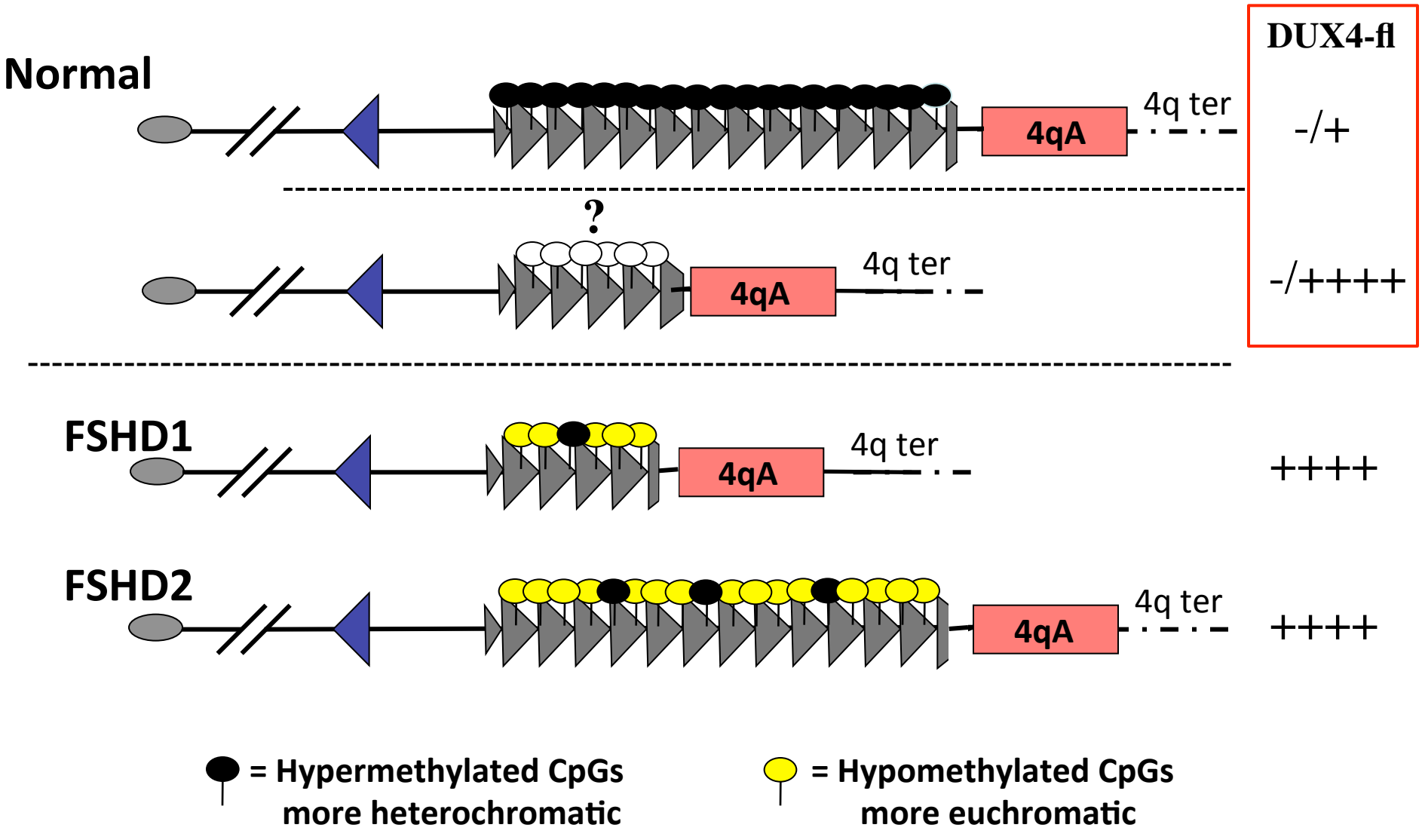
T. Jones *et al.* (2012)

DUX4-FL expression in myogenic cells from FSHD1 subjects that show no clinical manifestation of the disease suggests modifiers of disease

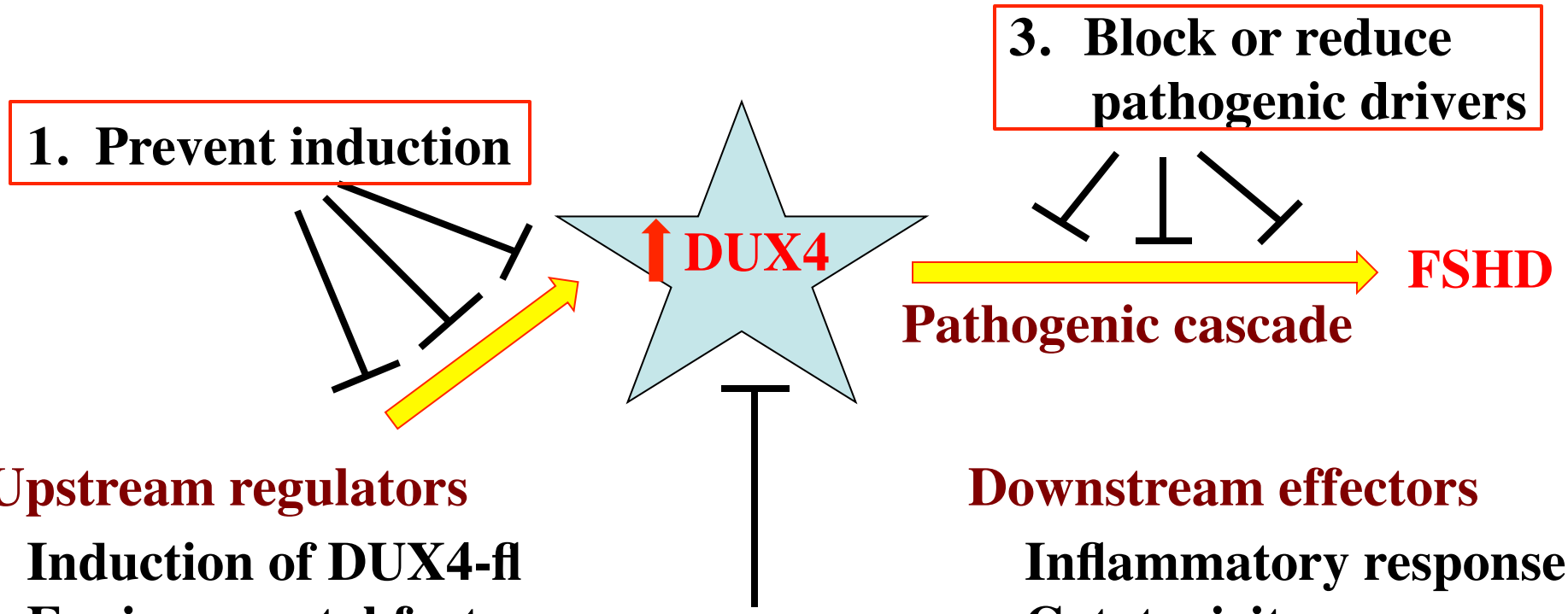


T. Jones *et al.* (2012)

DUX4 expression alone is not necessarily causal for FSHD



Multiple therapeutic targets for FSHD



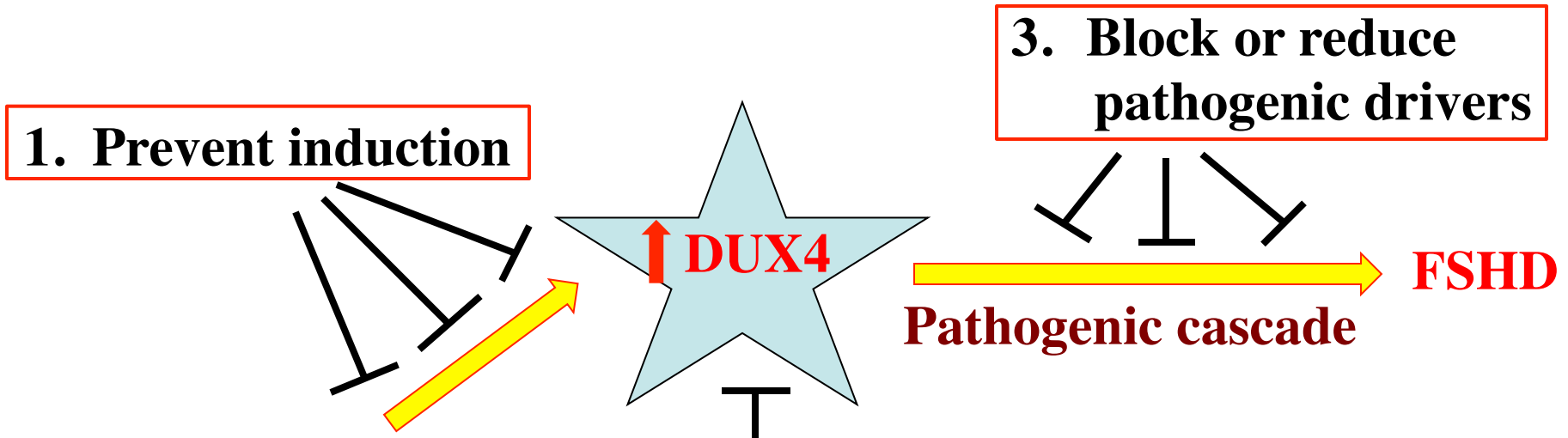
Upstream regulators

- Induction of DUX4-fl
- Environmental factors
- Family background
- Genetic modifiers

Downstream effectors

- Inflammatory response
- Cytotoxicity
- Gene regulation
- Genetic modifiers

Multiple therapeutic targets for FSHD



Upstream regulators

- Induction of DUX4-fl
- Environmental factors
- Family background
- Genetic modifiers

2. Knockdown DUX4-fl

Downstream effectors

- Inflammatory response
- Cytotoxicity
- Gene regulation
- Genetic modifiers

Key to therapy may lie with identifying the disease modifiers

Analysis of DUX4 mRNA and protein expression in muscles and myogenic cells from FSHD subjects and unaffected relatives

At University of Massachusetts Medical School (and formerly BBRI)

Takako Jones*, Jennifer Chen*, Oliver King, Charles P. Emerson Jr., and Peter L. Jones

At Kennedy Krieger Institute and Johns Hopkins University

Kathryn R. Wagner

At Children's Hospital – Boston and Harvard Medical School

Fedik Rahimov and Louis M. Kunkel

At Boston University School of Medicine (and formerly BBRI)

Sachiko Homma, Mary Lou Beerman and Jeffrey Boone Miller

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University of Massachusetts Medical School

&

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Acknowledgements

Takako Jones

Charis Himeda

Celine Debarnot

NIAMS National Institute of Arthritis and Musculoskeletal and Skin Diseases

MDA Muscular Dystrophy Association Fighting Muscle Disease

FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY fsh SOCIETY

AFM Association Française contre les Myopathies