

# Opportunities for Therapeutic Intervention and Milestones for Success

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# FSHD: a disease of repeat-mediated repression

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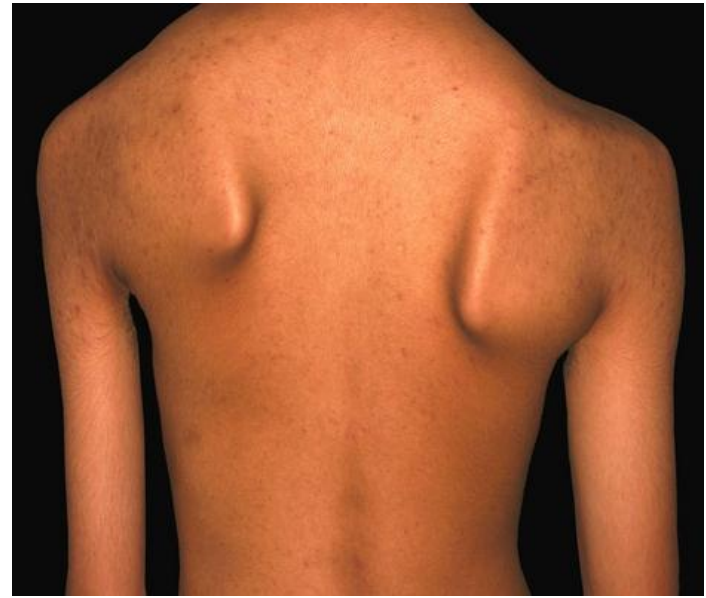
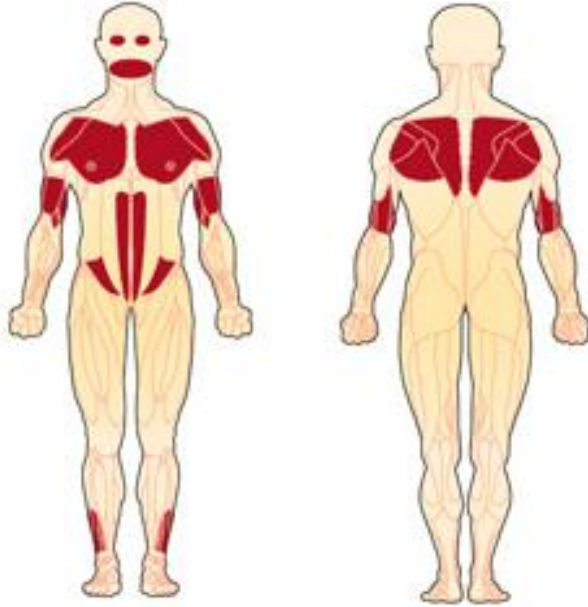
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# FSHD: Facioscapulohumeral muscular dystrophy

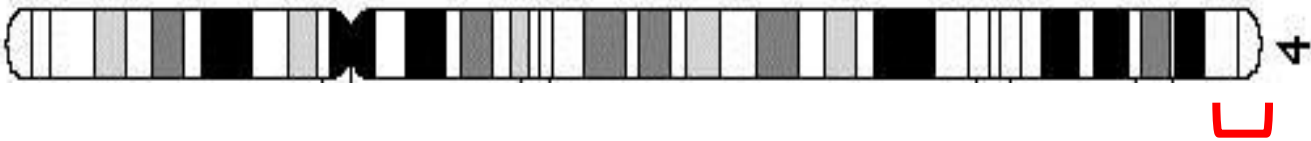


- Autosomal dominant
- Slowly progressive muscle weakness
- Onset in early adulthood
- ~1/20,000 prevalence

**Mapping of facioscapulohumeral muscular dystrophy gene to chromosome 4q35-qter by multipoint linkage analysis and in situ hybridization.**

Wijmenga C, Padberg GW, Moerer P, Wiegant , Liem L, Brouwer OF, Milner EC, Weber JL, van Ommen B, Sandkuyl LA, et al.

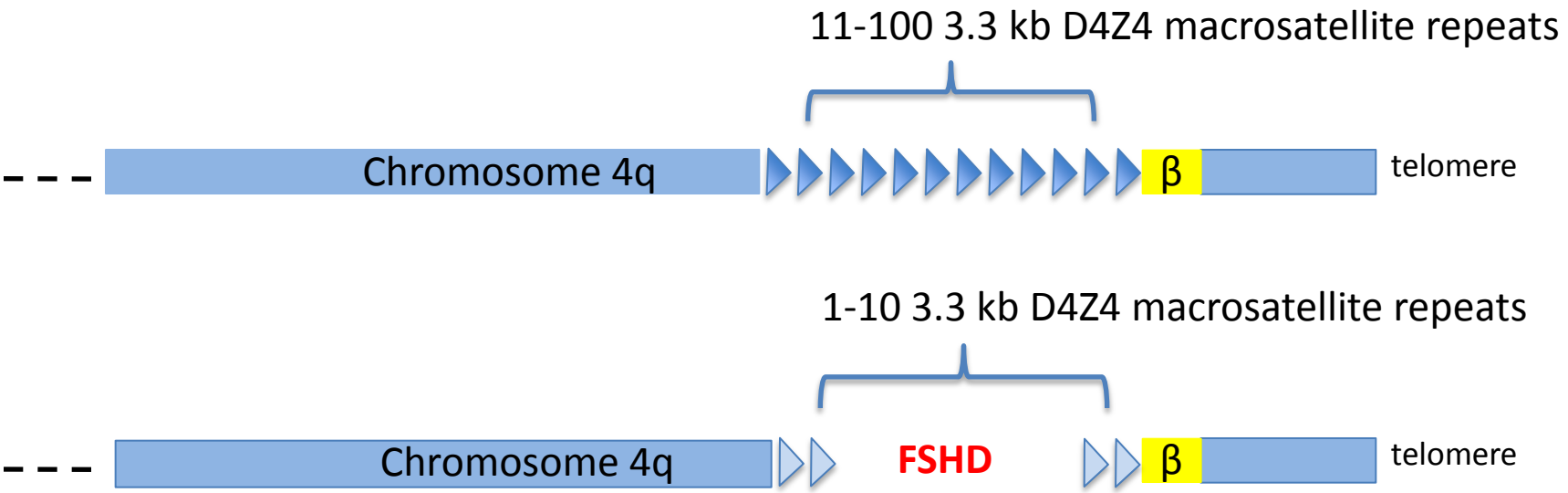
Genomics 1991; 9:570-5.



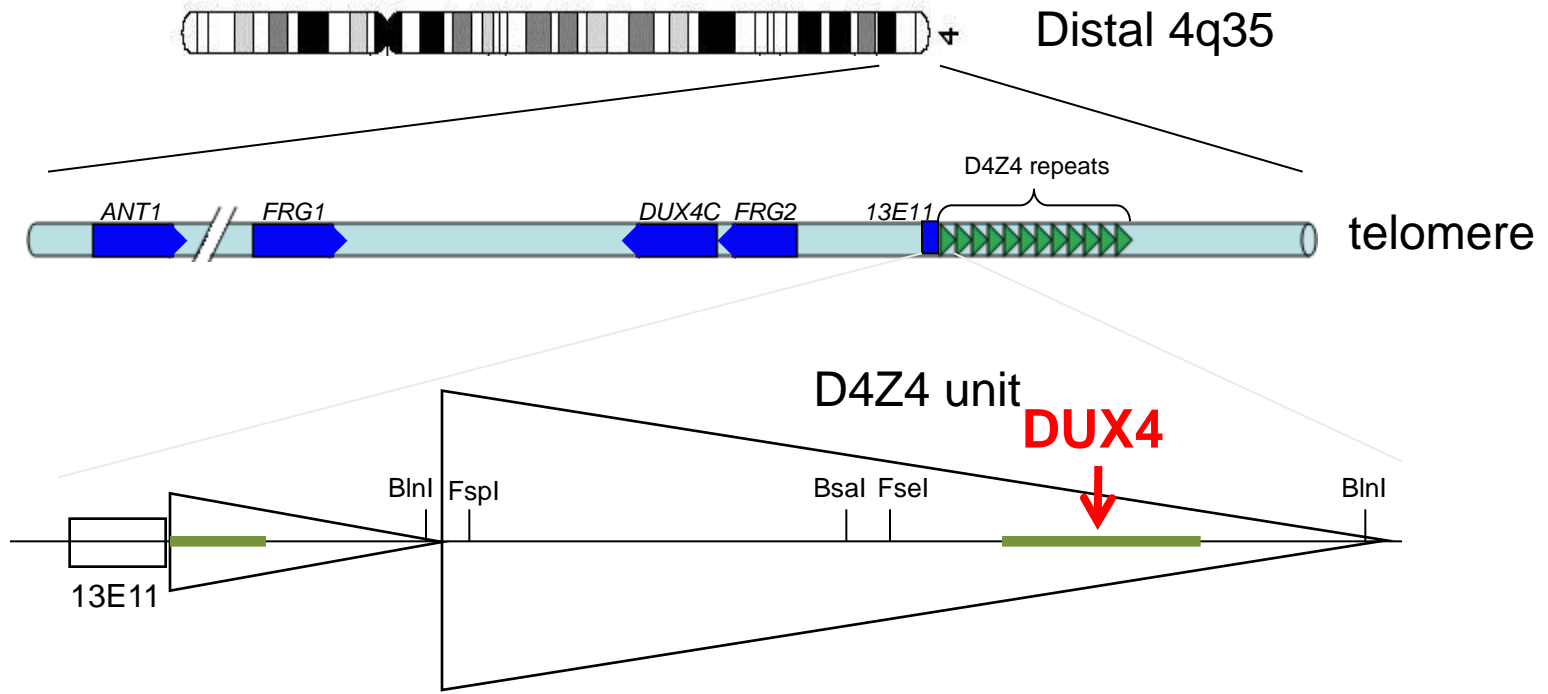
**FSHD Locus**

**FSHD associated DNA rearrangements are due to deletions of integral copies of a 3.2 kb tandemly repeated unit.**

Van Deutekom JC, Wijmenga C, van Tienhoven EA, Gruter AM, Hewitt JE, Padberg GW, van Ommen GJ, Hofker MH, Frants RR.  
Hum Mol Genet 1993; 2:2037-42.



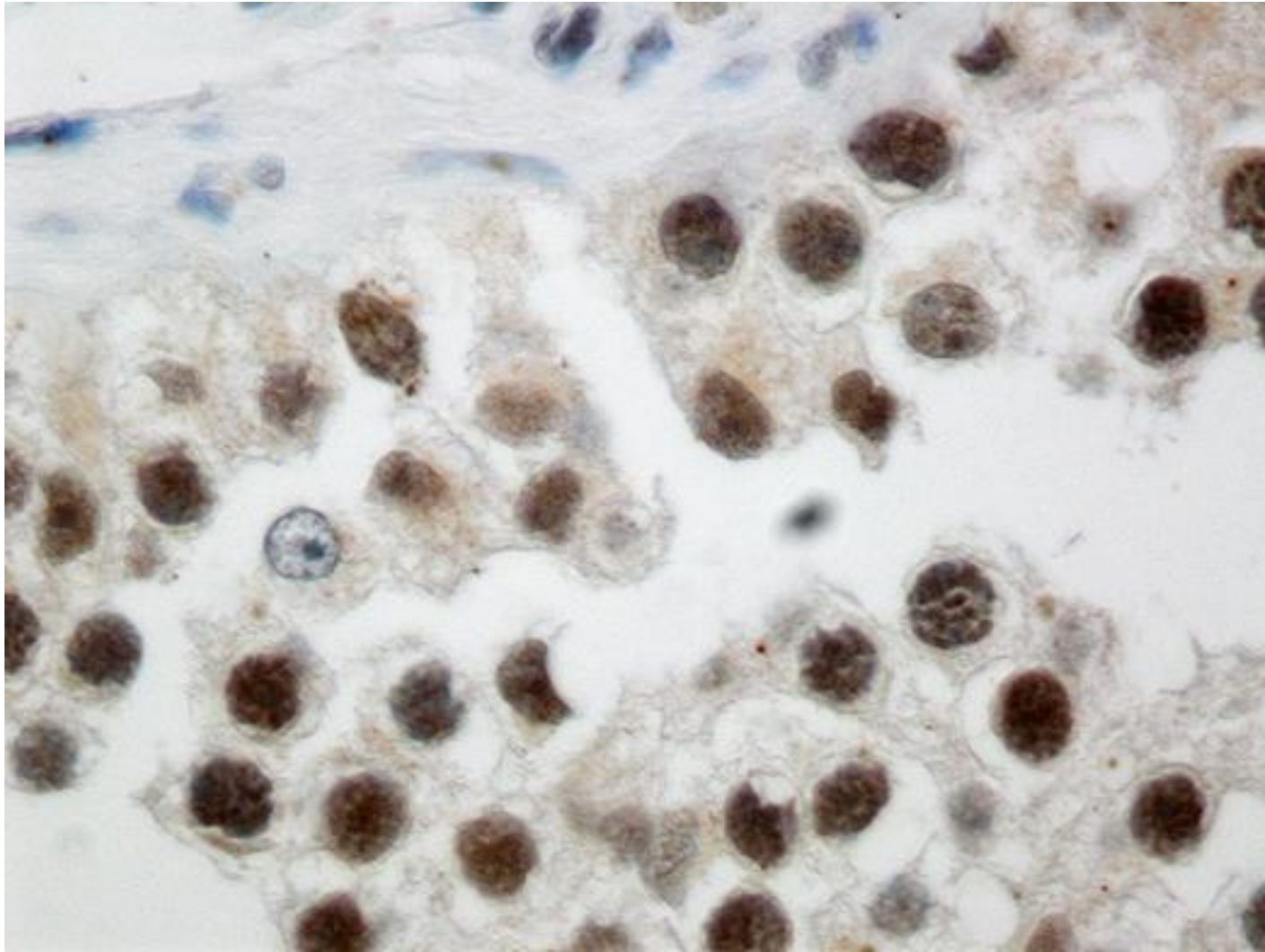
# D4Z4 Macrosatellite Repeat



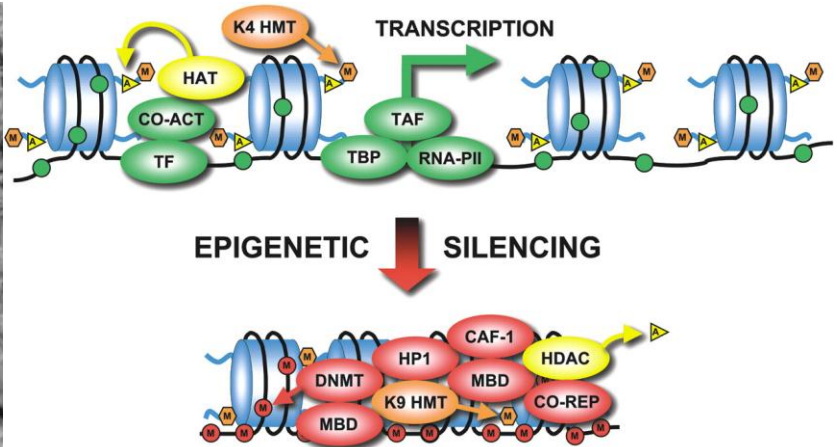
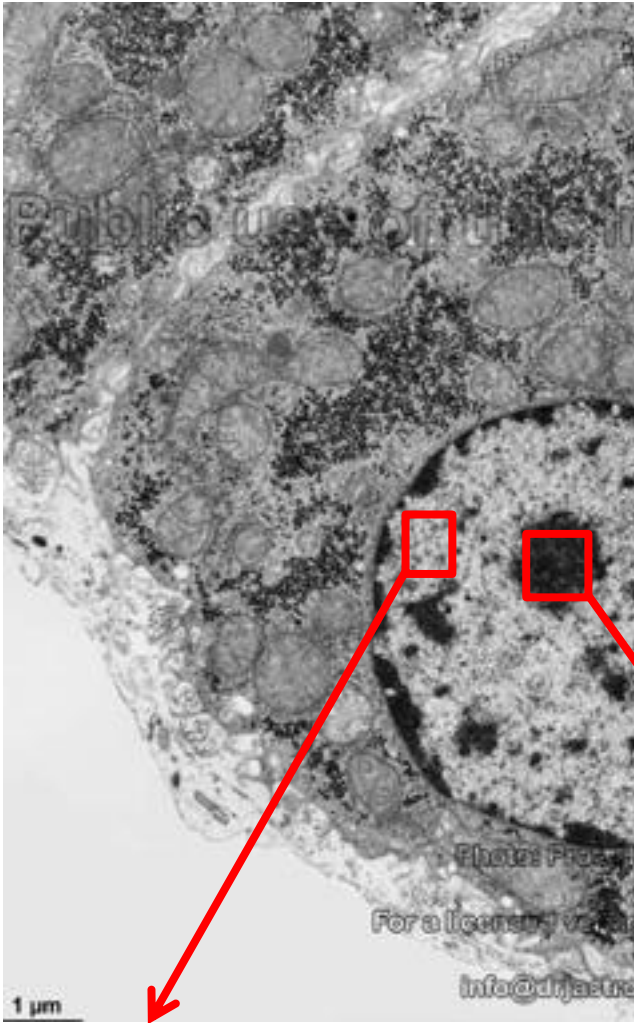
- D4Z4: 3.3 kilobase direct repeat
- Each repeat has a retrogene: DUX4, a double homeobox transcription factor

# DUX4 is abundantly expressed in healthy human testis

DUX4 IHC of testis



**Brown = DUX4 immunodection**



**Light = Genes On  
Out in the living room**

**Dark = Genes Off  
Stored in the attic**



**Stem Cell**

**Differentiated Cell**



**Stem Cell Genes**  
- On, Living Room

**Heart Genes**  
- Off, Attic

**Stem Cell Genes**  
- Off, Attic

**Heart Genes**  
- On, Living Room

**DNA Methylation**

**Locks the Attic Door**

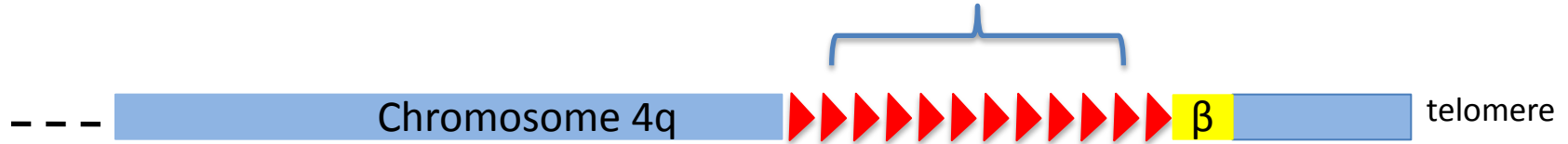
# **Facioscapulohumeral Dystrophy (FSHD):**

A disease of inefficient epigenetic repression

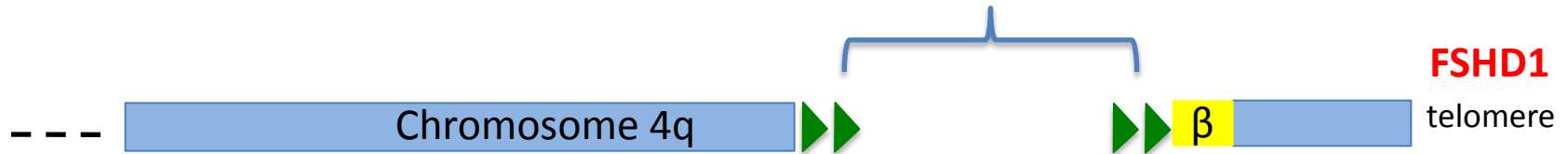
A faulty lock on the attic door

# Fewer D4Z4 repeats have less repressive heterochromatin

11-100 D4Z4 repeat units: heterochromatin



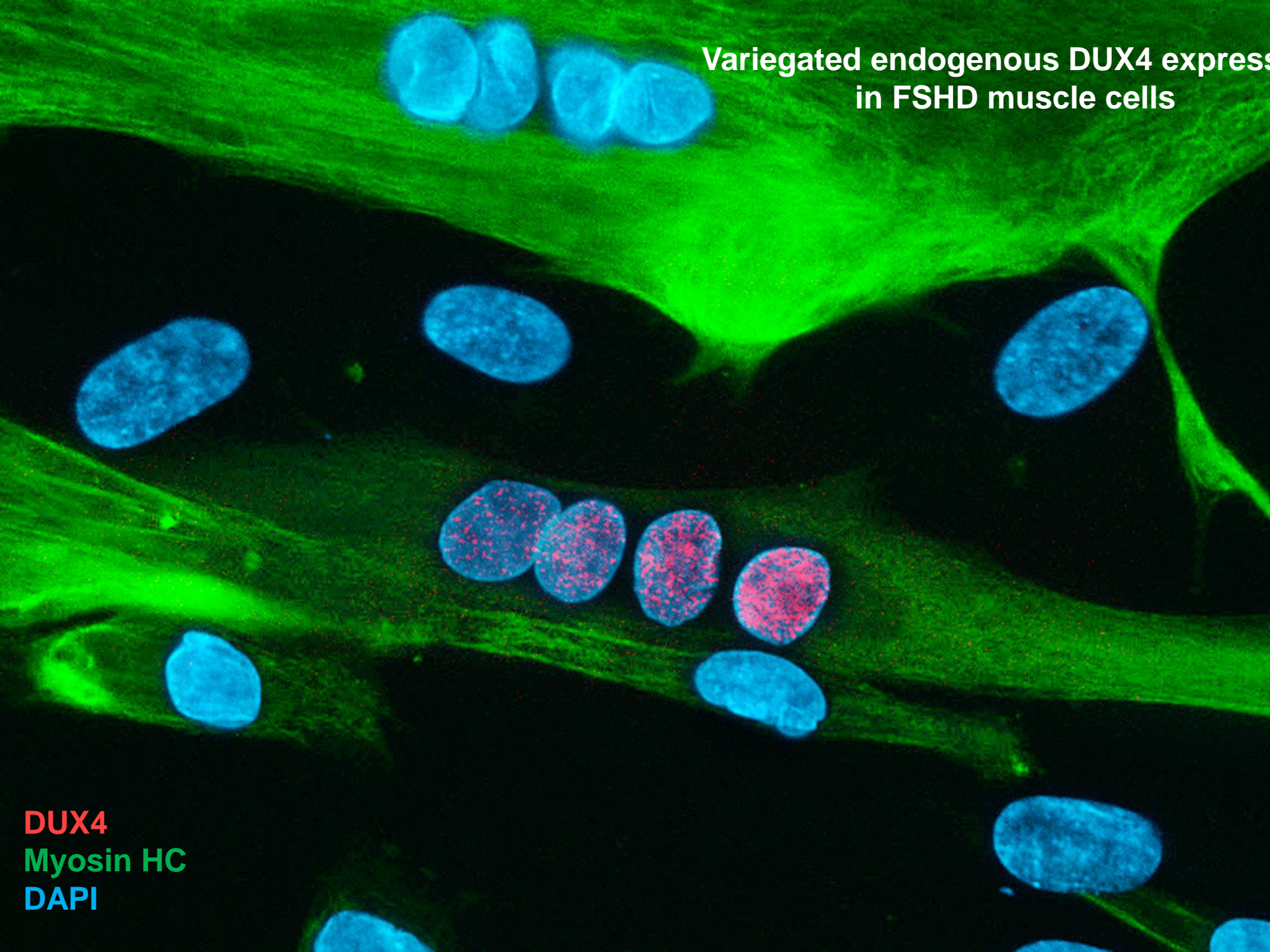
1-10 D4Z4 repeat units: less heterochromatic



▶▶▶▶ = heterochromatin (H3K9me3, H3K27me3, meCpG)

▶▶▶▶ = less heterochromatic (H3K4me3, less meCpG)

Variegated endogenous DUX4 expression  
in FSHD muscle cells



DUX4  
Myosin HC  
DAPI

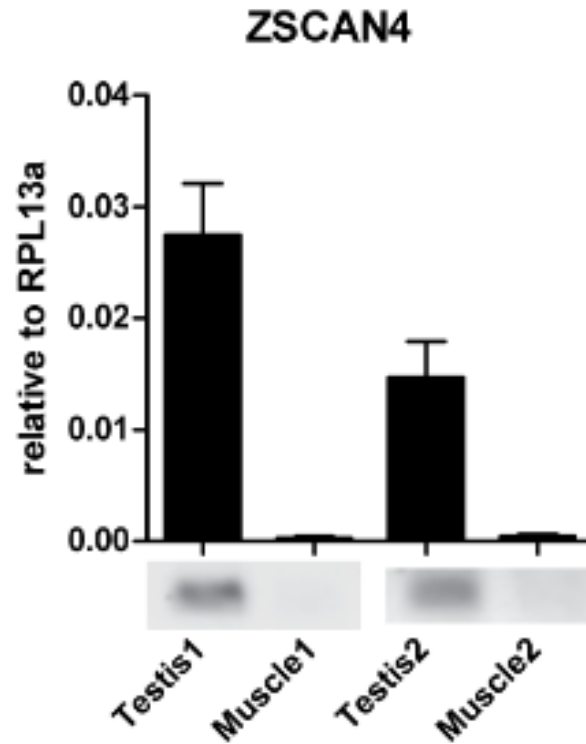
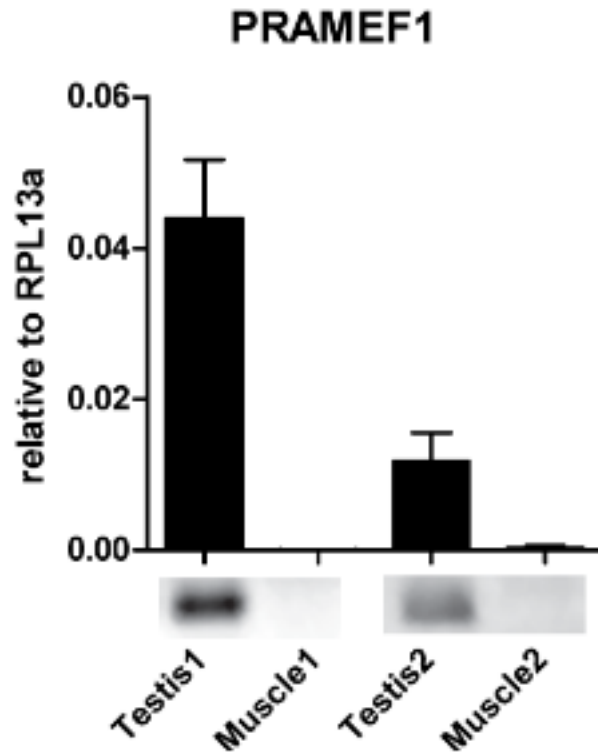
# A Developmental Model of FSHD

- DUX4 is expressed in the testis germ-line
  - Possible role in stem cell biology
- DUX4 is repressed (moved to the attic) in muscle
  - Repeat-mediated silencing
- Inefficient repression causes FSHD
  - Fewer repeats = less efficient repression
  - Faulty lock (e.g., SMCHD1 in FSHD2)
- Results in occasional bursts of DUX4 in muscle

# DUX4 is a transcription factor

- DUX4 can “turn-on” other genes
  - When DUX4 comes out of the attic it brings a lot of genes with it!
- Turns on germline genes in skeletal muscle
  - Tells the muscle to become a germline cell

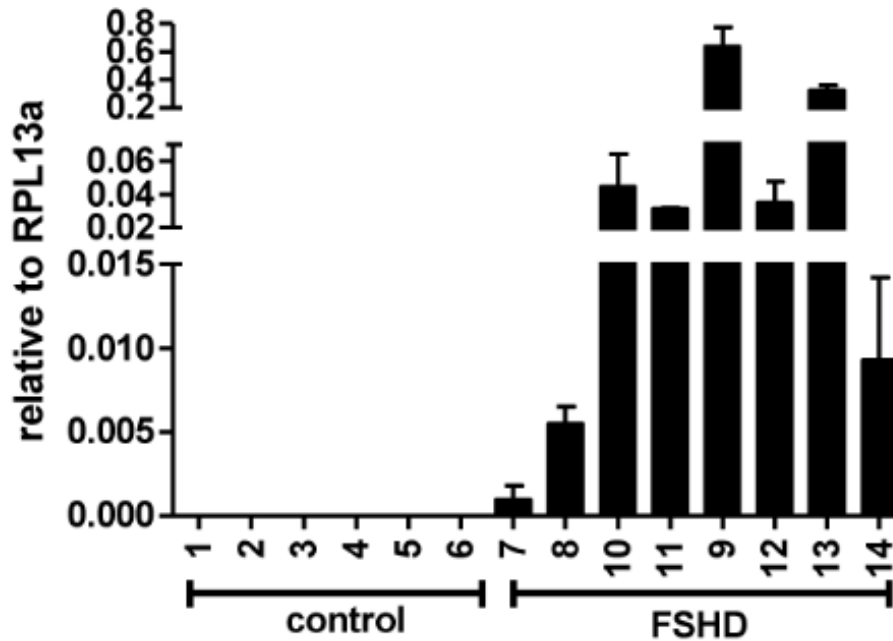
# DUX4 target genes are normally present in testis and absent in skeletal muscle



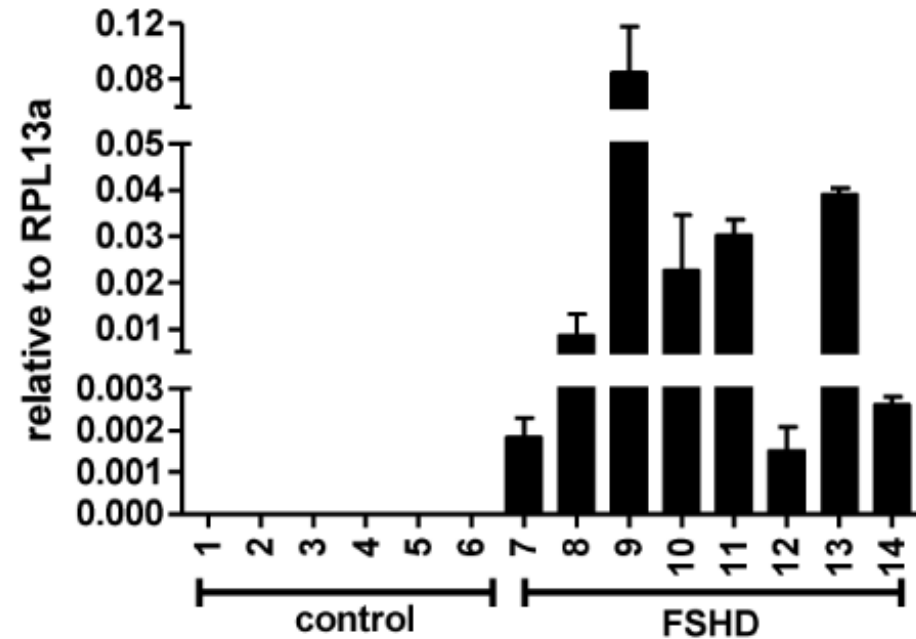
RPL13a 

# FSHD skeletal muscle biopsies express DUX4-induced germline genes

## PRAMEF1



## ZSCAN4



RT-qPCR from skeletal muscle biopsy RNA



# Candidate Mechanisms for FSHD

- **Activation of a germline program muscle cells**
  - Confusion causes death and dysfunction
- **Immune response to germline proteins**
  - FSHD cells express Cancer Testis Antigens
- **DUX4 genes can suppress muscle repair**
  - Defensin protein blocks new muscle formation
- **DUX4 re-activates virus-like elements in the genome**
- **And more ....**

# Therapeutic Opportunities

- **Suppress DUX4 mRNA expression**
    - General enhancement of chromatin repression
    - Targeted enhancement of D4Z4 chromatin repression
      - RNA-mediated (lncRNA, small si-RNA)
      - SMCHD1 pathway (Repeats, X-inactivation, retrotransposons, ....?)
  - **Decrease DUX4 mRNA stability/translation/splicing/pA**
    - sh-, si-, mi, or mo-RNA; small molecule inhibitors
  - **Block DUX4 protein activity**
    - Target protein interactions
- 
- **Interfere with pathological mechanism(s)**
    - Cell autonomous
      - Apoptosis, atrophy, splicing abnormalities, etc
    - Non-autonomous
      - CTA and immune response; DEFB103 and regeneration

# Preclinical Models

- Cultured FSHD muscle cells
- Mouse with human DUX4 genomic region
- Mouse with DUX4 expression
- Human-to-mouse muscle transplants
- Human DUX4 expressed in zebrafish
- Model organisms

# Identifying Candidate Therapies

- Screen existing chemical compounds
  - FDA approved compounds
  - Clinical candidate compounds
  - Diverse libraries
- Rational development of new drugs
  - Targeting a specific protein/RNA
    - Small molecule drugs and siRNAs
- Lifestyle, diet, exercise
- Immunomodulation?

# Milestones for Success

- **Halt or reverse disease progression**
  - Slowly progressive disease
    - Requires long-term study
    - Large numbers of participants
  - Natural history studies and FSHD registries
- **Demonstration of drug activity**
  - DUX4 mRNA or regulated genes
  - Immune response or regeneration
- **Biological response**
  - MRI or serum markers of muscle damage

# How long will it take?

- Within a few years if ... ?
  - FDA approved drug
  - Repurposed drug candidate
  - Developing class of drugs
- Within a decade if ... ?
  - New drug development
  - Progressively more effective drugs

# When will we start?

- We have, thanks to you.
  - Consensus model of disease
  - Candidate biomarkers
  - Clinical history studies
  - Multiple efforts at drug development

# Thanks to the groups providing funding

