

Why we think FSDH is treatable

Stephen J. Tapscott, MD, PhD
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Why we ~~think~~ know FSDH is treatable

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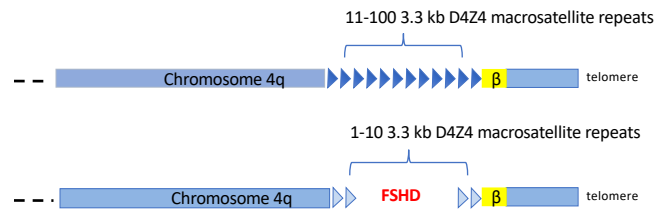
Mapping of facioscapulohumeral muscular dystrophy gene to chromosome 4q35-qter by multipoint linkage analysis and in situ hybridization.

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.

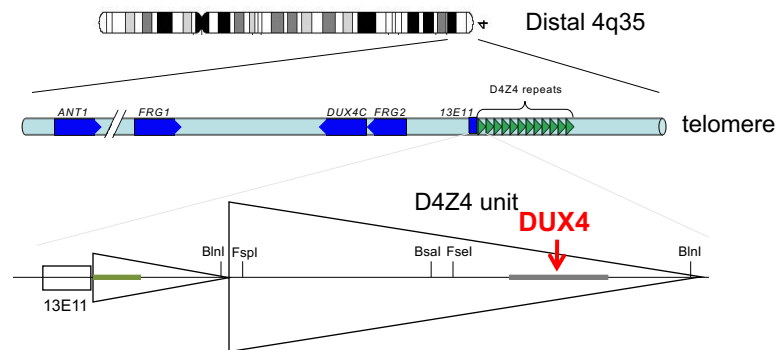


FSHD associated DNA rearrangements are due to deletions of integral copies of a 3.2 kb tandemly repeated unit.

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.

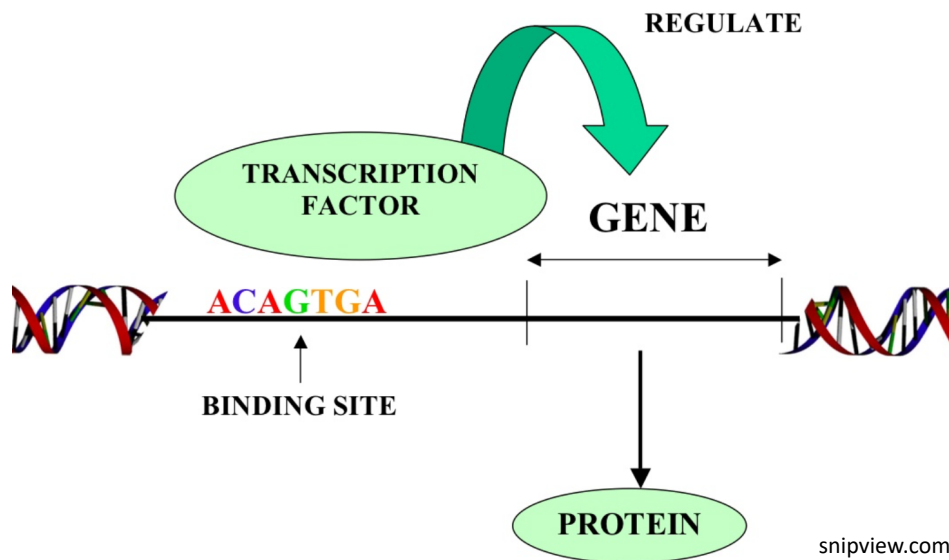


D4Z4 Macrosatellite Repeat

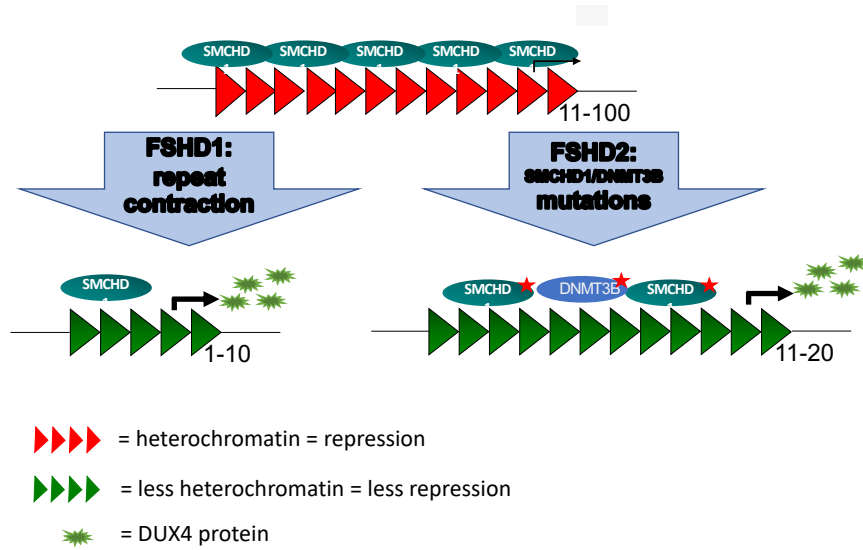


- D4Z4: 3.3 kilobase direct repeat
- Each repeat has a copy of the DUX4 gene
- DUX4 is a transcription factor

Legend: A transcription factor molecule binds to the DNA at its binding site, and thereby regulates the production of a protein from a gene.

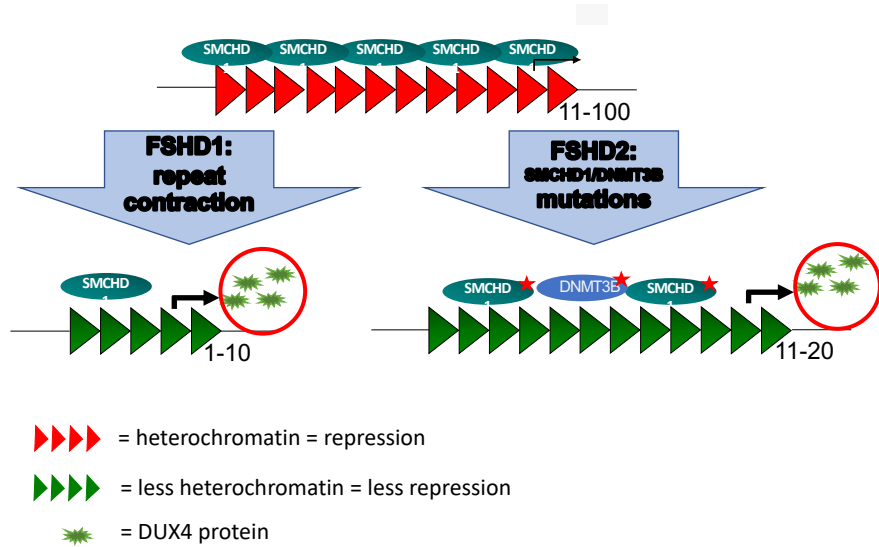


FSHD1 and FSHD2 Incomplete Repression of DUX4



Lemmers et al; Science 2010, Nat Genet 2012

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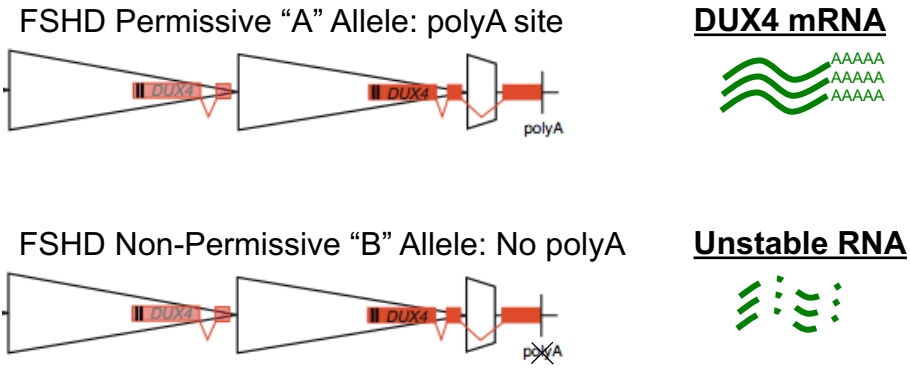
Elements supporting therapeutic development

- Clear genetics and mechanism
 - FSHD1: Small number of repeat units
 - FSHD2: Mutation in a repressor of the repeat units
 - Both result in the expression of the DUX4 gene in muscle cells
- Validated target with limited expression in adults
 - Prevent DUX4 expression in skeletal muscle
 - Prevent DUX4 from damaging skeletal muscle
- Preclinical models to find and test drugs prior to human trials
- Good ways to measure disease progression to determine efficacy
- International clinical trials infrastructure network
- Drugs to bring into clinical trials
- Organized and effective FSHD advocacy groups

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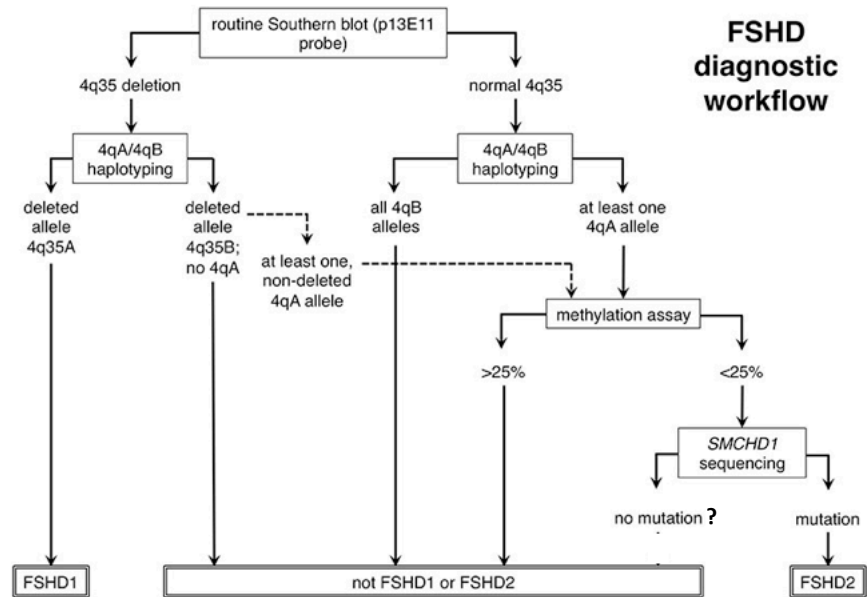
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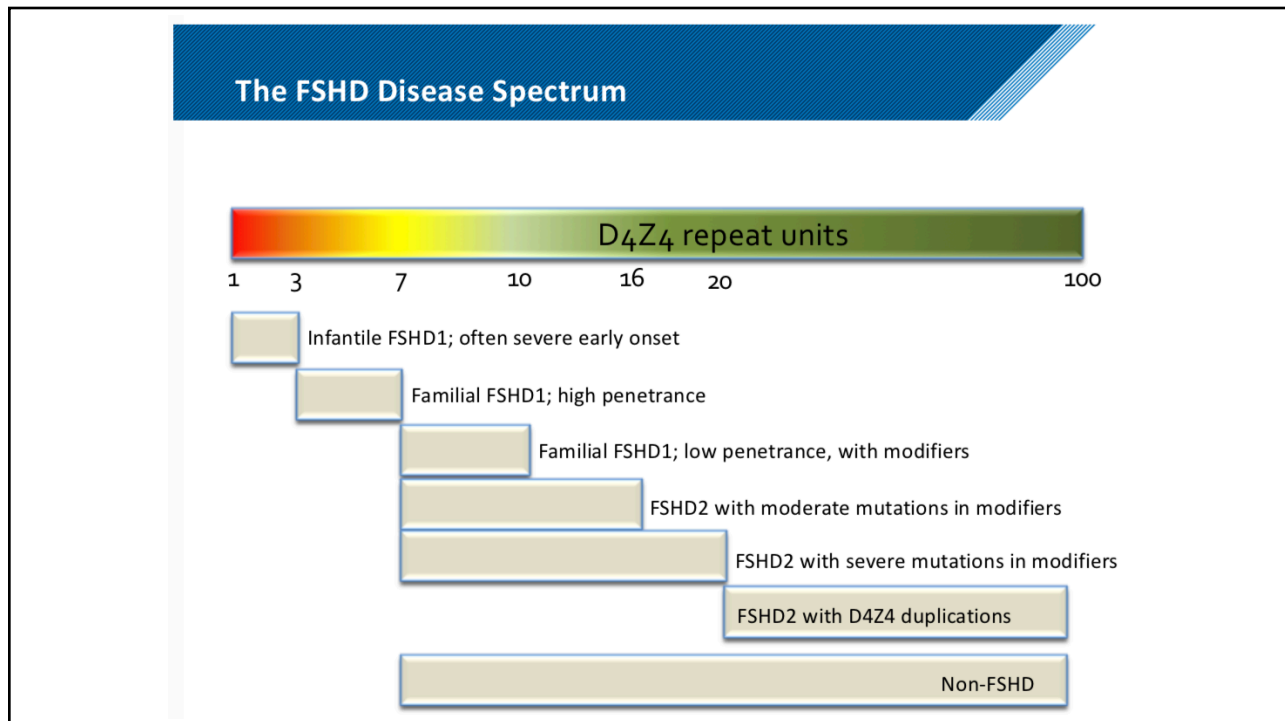
FSHD-permissive 4qA chromosomes have a DUX4 poly-A site



Lemmers et al, Science 2010

This decision tree diagram depicts how FSHD genetic testing is carried out. (Reference: University of Iowa) (modified SJT)





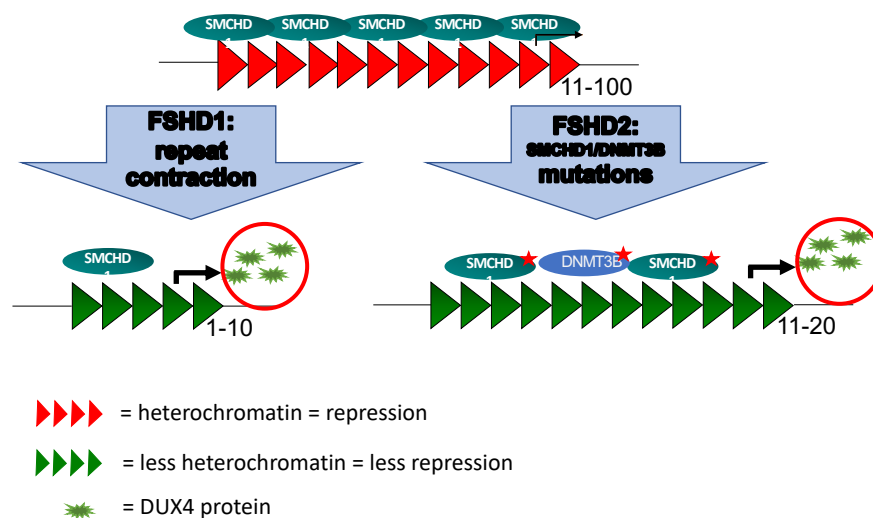
FSHD Genetics and Clinical Development

- Opportunities for genetic sub-classification
 - FSHD1
 - 10 repeats or fewer
 - FSHD2
 - SMCHD1, DNMT3B, or other mutation
 - FSHD1 and FSHD2
- Outstanding questions
 - Complex re-arrangements
 - Improved methods of genotyping

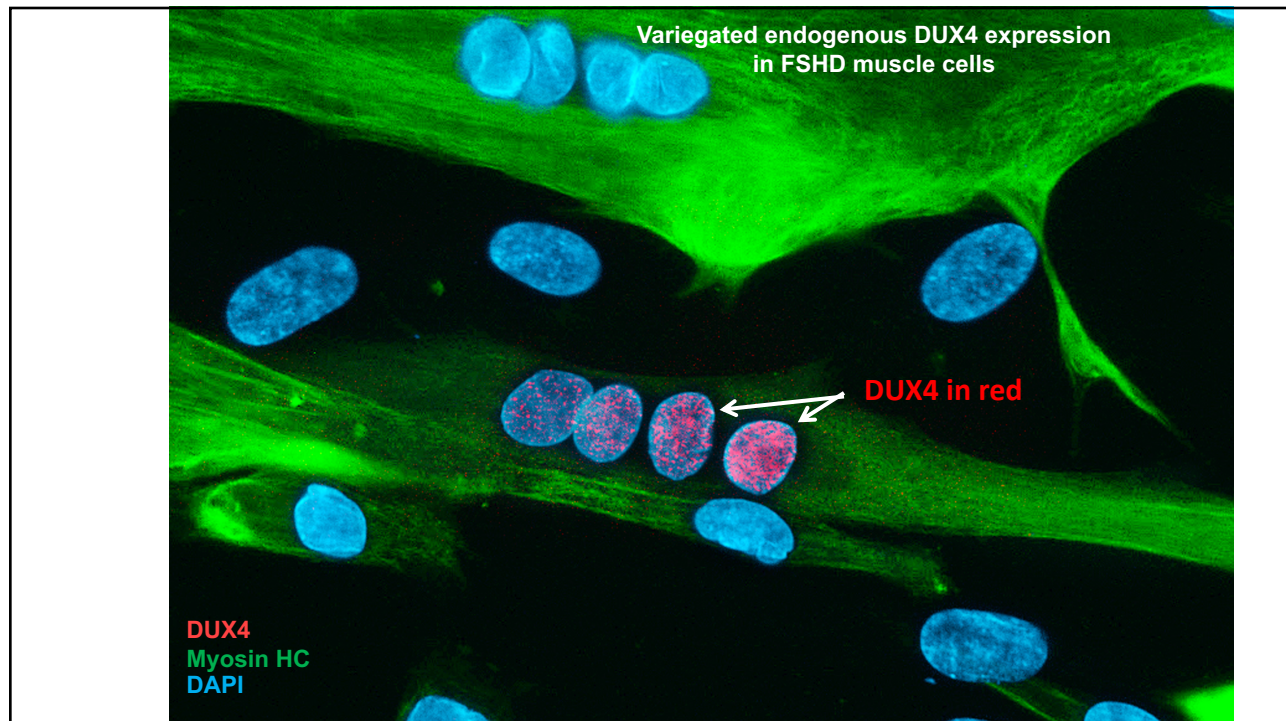
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FSHD1 and FSHD2 Incomplete Repression of DUX4

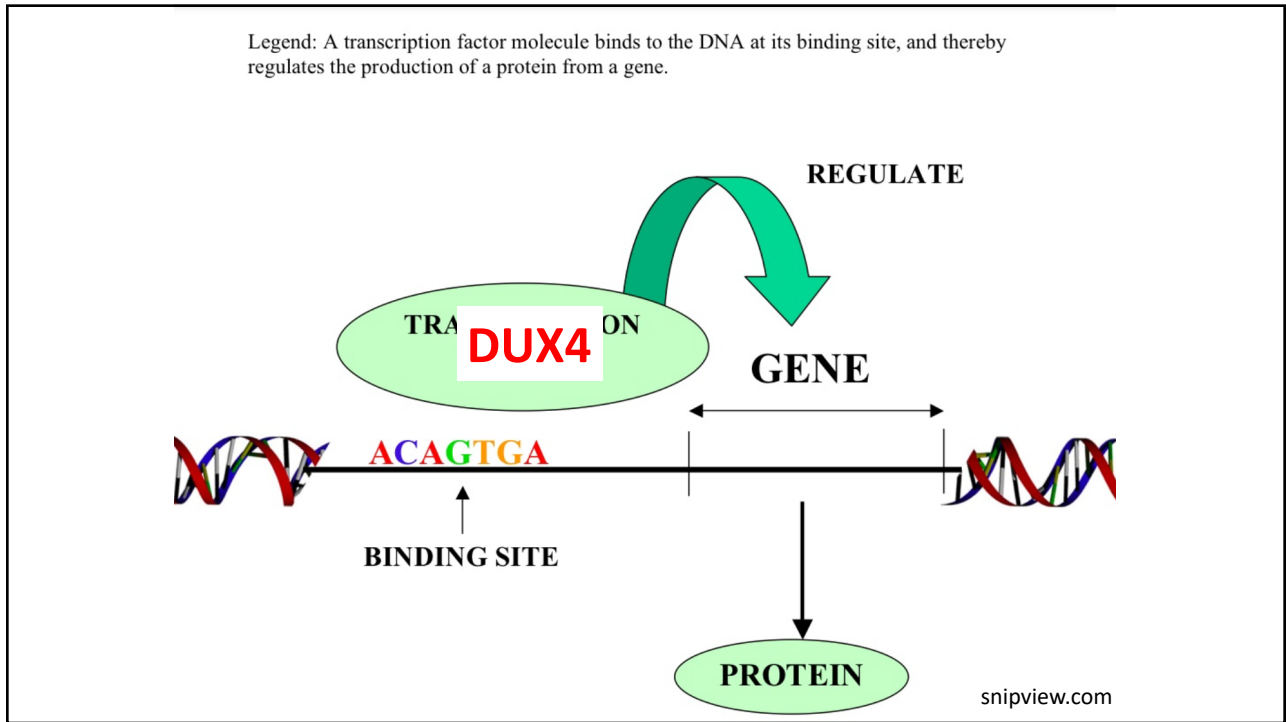
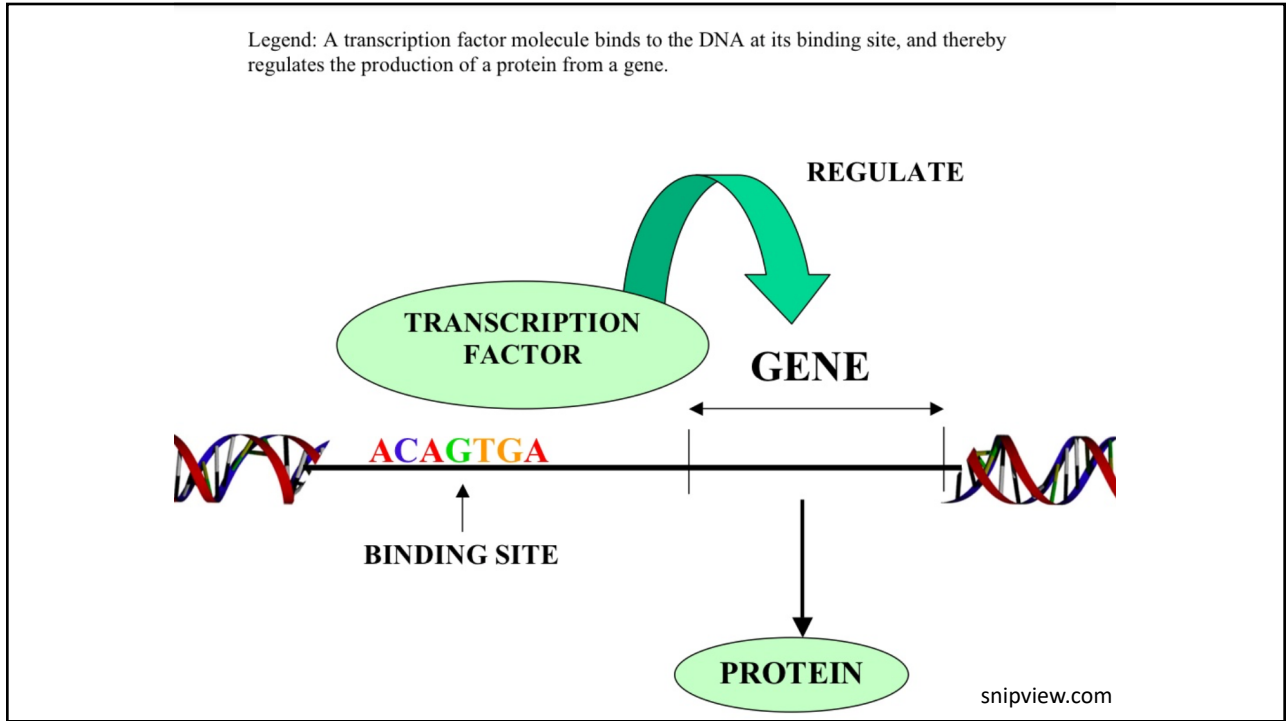


Lemmers et al; Science 2010, Nat Genet 2012



FSHD mutations result in DUX4 expression in skeletal muscle cells

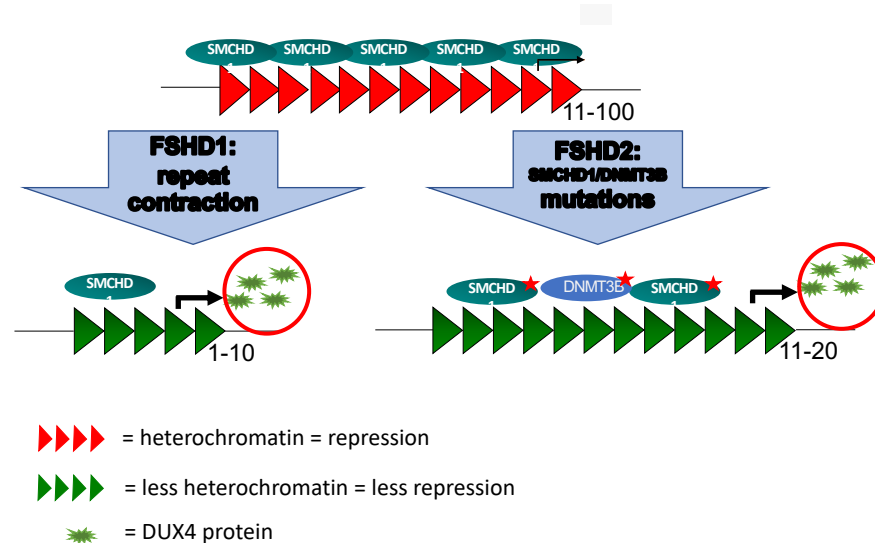
- The DUX4 gene can be made into RNA and protein
- What does DUX4 do?



What is DUX4 instructing the cell to do?

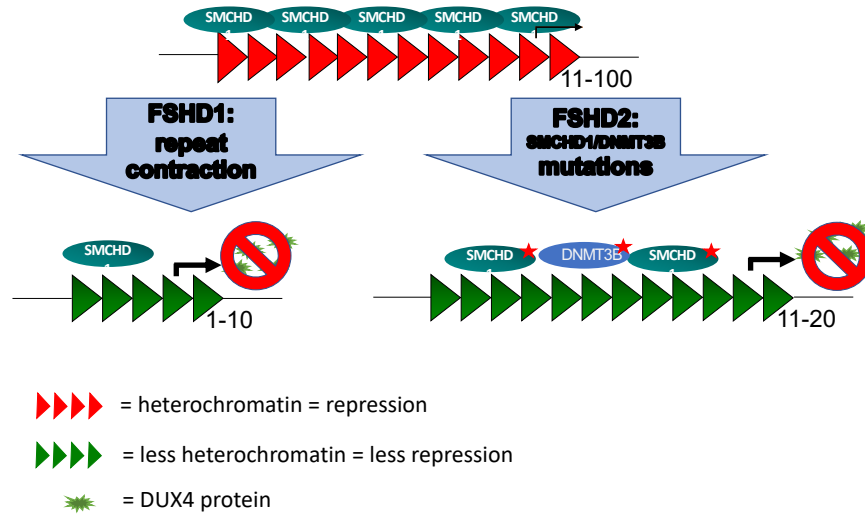
- DUX4 is normally expressed in the early stem cells of the embryo
- It normally turns on the first set of genes in the early embryo
- When mis-expressed in muscle, it tells the muscle cell to turn on the genes normally expressed in an early embryo

FSHD1 and FSHD2 Inefficient Repeat-Dependent DUX4 Silencing

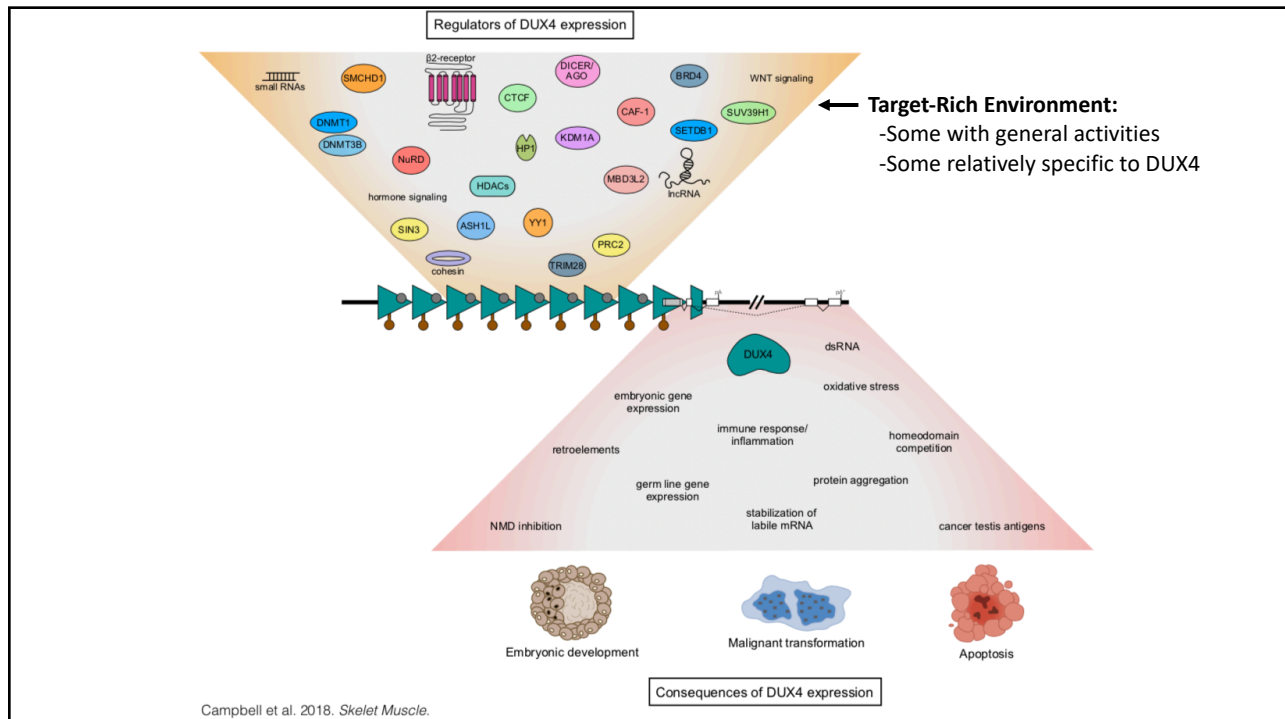


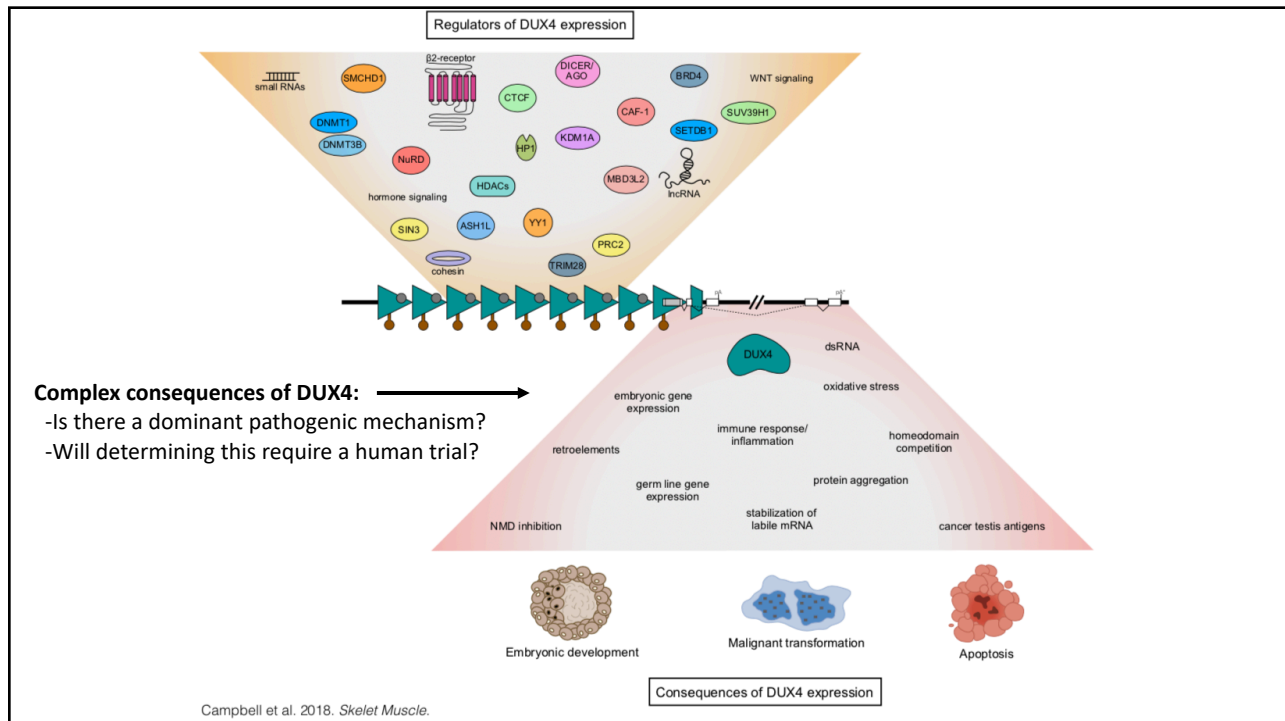
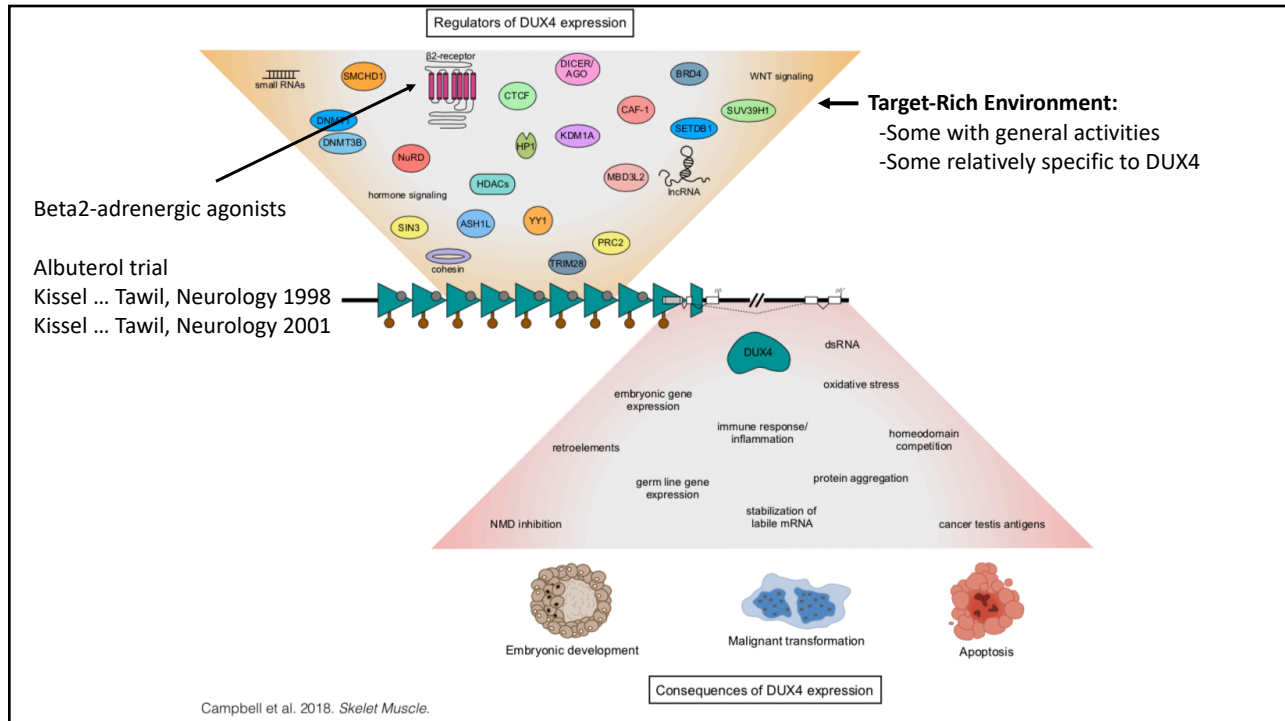
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Drug development opportunities

- Screen for drugs that inhibit DUX4 expression/activity
 - Repurpose existing drugs
 - Screen chemical libraries, e.g., chromatin modulators
- Screen for engagement of specific targets
 - DUX4 or upstream regulator or downstream effector
- General effectors
 - Immune-modulators
 - Muscle regeneration or hypertrophy

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Problems with FSHD Preclinical models

- Human DUX4 has diverged from the non-primate DUX genes
 - This makes it difficult to use mice as a simple model for FSHD
- Incomplete knowledge of pathophysiology
 - Single dominant pathway?
 - Additive consequences of multiple pathways?

Strengths of FSHD Preclinical Models

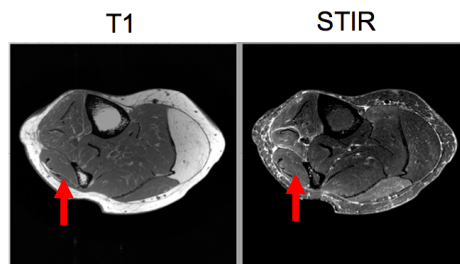
- Mouse models for regulation of DUX4 expression
 - Human DUX4 gene region inserted into the mouse genome
 - Short array (D4Z4-2.5 mice), long array (D4Z4-12.5 mice)
- Mouse models for targeting the DUX4 mRNA or protein
 - Virus (AAV) delivered DUX4
 - Inducible DUX4 inserted into the mouse genome
- Models for downstream consequences of DUX4?
 - Cell Toxicity
 - Mouse
 - Zebrafish
 - Other?
- Human muscle cell cultures

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Normal – T2 STIR (inflammation) – T1 (fatty replacement)

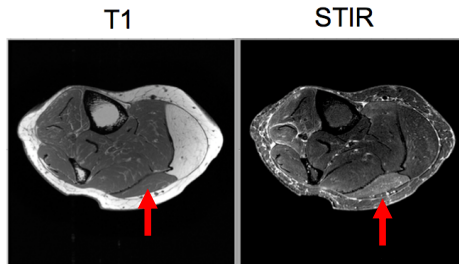
- T1 weighted: fat bright, muscle dark, edema dark
- STIR: Fat is suppressed (dark), muscle dark, “edema”/free water bright



Dennis Shaw and Seth Friedman
Seattle Children's Hospital

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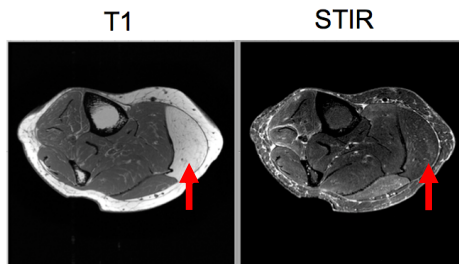
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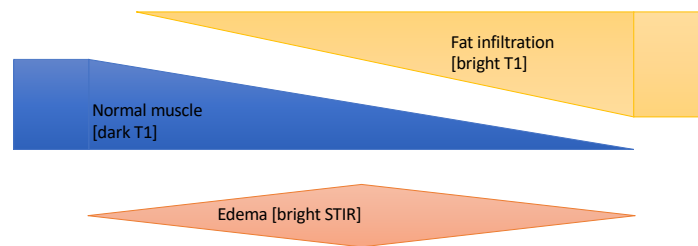
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Model for MRI progression:



What do we want to know?

- What are the molecular correlates of the abnormal MRI signal in FSHD? Does STIR correlate with:
 - DUX4-target gene expression levels
 - Inflammation or other pathology
- Can MRI provide a measure of disease activity/progression?

Wellstone Study Design

U. Washington, U. Rochester, U. Kansas

- Enroll FSHD individuals
- MRI informed needle muscle biopsy
 - T2-STIR positive if accessible
 - Muscle with normal MRI signal if T2-STIR muscle not biopsy accessible
- Biological Samples
 - Muscle for histology and RNA-seq
- Functional assessment

Molecular correlates of MRI in FSHD

- MRI normal muscles
 - Near normal pathology
 - very low DUX4 expression levels
- MRI STIR+ and/or T1+ muscles
 - More active pathology and inflammation
 - Higher expression of DUX4 and its regulated genes
- Nearly all FSHD muscles
 - Increased fibrosis
 - Signs of chronic inflammation

Wang ... Tawil, Hum Molec Genet 2018

Model for disease progression in FSHD

- MRI normal muscles have mild underlying pathology
 - Very low or no detectable DUX4 expression
 - Complement activation and deposition, inflammatory genes
- T2-STIR positive muscles
 - Increased DUX4 expression
 - Increased pathology and inflammation
- Possible model:
 - Initially very low levels of DUX4 expression
 - Progression associated with:
 - Areas of higher DUX4 expression
 - T2 STIR+ progressing to T1 and fatty infiltration

Outstanding questions

- Can MRI or DUX4 target gene expression be used to assess response to therapeutic interventions?
- Can the gene signatures of MRI normal FSHD muscle be used as a measure of disease activity?
- Will these correlate with functional outcomes?
- Can we identify a reliable serum biomarker?

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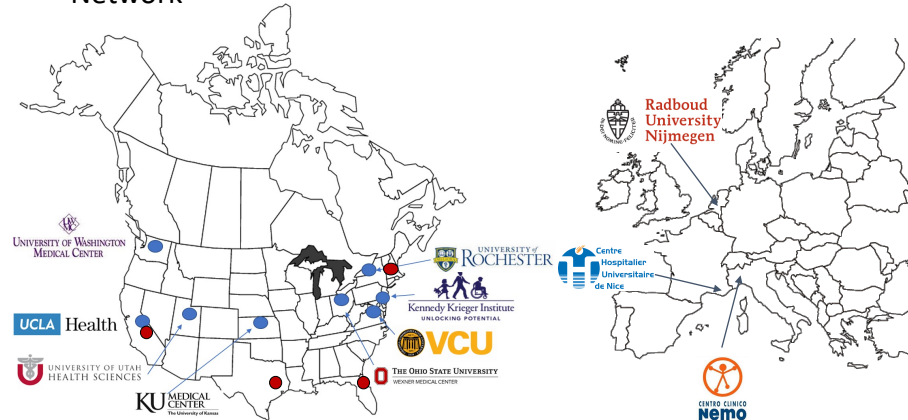
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Clinical Trials Readiness

- 2013 (Leiden) and 2015 (Rochester) International Trial Readiness Workshops
- Development of objective and subjective functional outcome measures
- NIH U01 supported national Clinical Trials Network 2016
 - Seven initial sites
 - Four expansion sites
- Addition of three European sites 2018-2019
- Active in natural history, biomarker studies, clinical studies and trials
- Funded by NIH, FSH Society, Friends of FSH Research, MDA, AFM, FSH Global, Stichting FSHD, private funding, pharma funding

FSH Clinical Trial Research Network (CTRN)

- **Goal:** To expedite the development of new therapies for facioscapulohumeral muscular dystrophy (FSHD) by maintaining and expanding a core FSHD Clinical Trial Research Network



43

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Clinical trials in FSHD

- aTyr Pharma
 - Resolaris, an immune modulator
- Acceleron Pharma
 - ACE-083, locally delivered promoter of muscle growth
- Fulcrum Therapeutics
 - Losmopamod, p38 signaling inhibitor developed by GSK
- And more on the way ...

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Thanks to the NIH and our taxpayer supported research



And thanks to you and the FSHD community!