Facioscapulohumeral dystrophy: New disease mechanisms & potentials for treatment

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Objectives

1. The genetics and pathophysiology underlying FSHD
2. MRI biomarkers for FSHD
3. Research on correlating MRI biomarkers with disease progression
Case
43-year-old gentleman with progressive arm atrophy for 16 years. At 27, he dislocated his shoulder while in the Army and while doing rehabilitation could not go higher than 15 pounds in weight. He then lost his right biceps. His right biceps kept shrinking.
In his early 20s, he noticed he could not blow up balloons. He could not seal down on balloons. He has problems using straws and now he has problems with duck calls. He is still able to whistle. He denies any swallowing difficulties or shortness of breath. He has some problems getting off of short chairs.

There is no family history of facial weakness or myopathy.
Cranial nerves

• Extraocular movements are intact.
• Eye closure and lip closure are slightly weak. Cheek puff is weak.
• Tongue is strong.
• Sternocleidomastoid are strong. Facial sensation is intact to light touch. Hearing is intact bilaterally.
Motor exam

Tone is normal.
There is weakness of biceps and atrophy. There may be some weakness of the deltoids. Triceps are intact. FDI and APB are intact.
What is FSHD?
• Third most common muscular dystrophy
  – prevalence of ~1:15,000-20,000
• Age of onset is variable from presentations at birth to late life
• Penetrance is high with 95% of patients manifesting weakness by age 20
• Classically, the disease presents with facial, proximal arm weakness with winged scapula followed by weakness of foot dorsiflexion and hip girdle muscles
• Asymmetric involvement is frequent
• Bulbar, extraocular, and respiratory muscles tend to be spared
• 20% of the patients wheelchair bound and 1% with respiratory weakness

Pastorello E, et al. (2011)
Tawil R, van der Maarel SM. (2006)
• Typical symptoms (facial or scapulo-humeral weakness) on presentation in ~70-85%
• No facial involvement in 6-18%
• Case reports of:
  – Facial-sparing scapular myopathy
  – Limb-girdle weakness
  – Late-onset distal myopathy after age 50
  – Asymmetric brachial weakness
  – Isolated axial weakness
  – Monomelic lower limb atrophy

Genetics of FSHD
Chr. 4

4q35

11-100 D4Z4 units

Normal allele

1-10 units

Normal allele

Other haplotypes

FSHD1

3.3 kb KpnI fragment

DUX4 mRNA

4A161

AAAAAA

AAAAA

AAAAA

AAAA

AAAA

Chromosome 4 Restriction Fragments

- Allele I (EcoRI): 70 kb
- Allele I (EcoRI/BlnI): 67 kb
- Allele II (EcoRI): 80 kb
- Allele II (EcoRI/BlnI): 77 kb

- Chromosome 10 EcoRI restriction fragments: 100 kb and 50 kb

- IMPRESSION: Restriction fragments consistent with normal 4q35

- EcoRI Normal: = [>38 kb]
- EcoRI/BlnI Normal: = [>35 kb]
Not FSHD?
Specific permissive haplotypes

10q26

No FSHD

> 11 D4Z4 units

1-10 units

Other haplotypes

No FSHD

4q35

No FSHD

4A161, 4A159, 4A168

No FSHD

FSHD

Lemmers et al, Science 2010
Decreased epigenetic suppression of D4Z4 causes FSHD

4q35

> 11 D4Z4 units

1-10 units

4A161

No FSHD

FSHD1

FSHD2

Heterochromatin

Less heterochromatin
DNA CpG methylation at FSHD locus

Control

FSHD1

FSHD2

Observed methylation

CpG methylation (%)

- Control (n=25A)
- FSHD1 (n=19A)

= CpG hypermethylated

= CpG hypomethylated

R Lemmers
Southern blot looking for hypomethylation of the D4Z4 array

- **BglII** and **FseI** enzymes are used.
- A 3.4 kb band is observed, indicating unmethylated regions.
- A 4.0 kb band is observed, indicating methylated regions.

Legend:
- **4.0kb**: methylated
- **3.4kb**: unmethylated
Methylation Southern

Patient has definite hypomethylation
And is likely candidate for SMCHD1 mutation

Dan Miller, Seattle Children’s Hospital
Mutation in SMCHD1 exon 19 (P810S)

Patient

Wild type

Dan Miller, Seattle Children’s Hospital
Patient has FSHD2 with mutation in SMCHD1!
SMCHD1

- Structural maintenance of chromosomes flexible hinge domain containing 1
- 18p11.32
- Involved in methylation of CpG islands with slow methylation kinetics in X inactivation
- 15/19 families with FSHD2 have heterozygous loss-of-function mutations

What are D4Z4s?
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 but not muscle**

<table>
<thead>
<tr>
<th>DUX4 Western blot</th>
<th>Healthy testis</th>
<th>Healthy muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Western Blot Image]</td>
<td>![Healthy Testis Image]</td>
<td>![Healthy Muscle Image]</td>
</tr>
</tbody>
</table>

Snider and Geng et al. *PLoS Genet*, 2010
**DUX4 mRNA** is detected in FSHD myoblast cultures but at extremely low abundance.

70-cycle nested RT-PCR

Snider and Geng et al. *PLoS Genet*, 2010
Variegated endogenous DUX4 expression in FSHD muscle cells

 Courtesy of Stephen Tapscott and Dan Miller
A Developmental Model of FSHD

• DUX4 retrogene expressed in germ-line
• Chromatin repression silences DUX4 in somatic tissues
• Inefficient somatic repression causes FSHD
  – FSHD1 contraction of repeat
  – FSHD2 mutation in SMCHD1
• Results in stochastic bursts of DUX4 in muscle
How can DUX4 cause disease?
Cultured FSHD muscle cells express DUX4-induced germline genes

PRAMEF1

ZSCAN4

RT-qPCR from skeletal muscle biopsy RNA

Geng and Yao, et al. Dev Cell, 2011
β-defensin 3 is induced

Geng and Yao, et al. Dev Cell, 2011
MRI biomarker of FSHD
T1 and STIR imaging

- T1 weighted: fat bright, muscle dark, edema dark
- Short tau inversion recovery (STIR): fat is suppressed (dark), muscle dark, free water bright (edema)
- T2 weighted: muscle dark, edema bright, fat bright

Dennis Shaw and Seth Friedman, Seattle Children’s Hospital
FSHD MRI

- 15 FSHD (9 females)
- 11 controls (4 females)
- 3T Philips MR assessment of calf muscles (quadrature coil)
- T1/STIR

Dennis Shaw and Seth Friedman, Seattle Children’s Hospital
66-year-old mother
66-year-old mother and son

66 mother

35 son

Dennis Shaw and Seth Friedman,
Seattle Children’s Hospital
Dennis Shaw and Seth Friedman, Seattle Children’s Hospital

69-year-old asymptomatic father

19-year-old daughter with scapular winging

19-year-old fraternal twin sister with minimal winging


Imaging results

• Heterogenous: discrete muscle involvement
• More than one apparent pattern (Gastrocnemius vs TA first/most involved)
• Fatty replacement: variable maintaining muscle compartment volume
• Edema: evident in some muscles
  – with and without fatty replacement
  – including some clinically asymptomatic

Dennis Shaw and Seth Friedman, Seattle Children’s Hospital
Longitudinal imaging study

• 9 subjects were followed up after ~2 years

Friedman, S., et al., Muscle and Nerve 2014
19-year-old daughter with scapular winging

19-year-old fraternal twin sister with minimal winging

69-year-old asymptomatic father

Dennis Shaw and Seth Friedman, Seattle Children’s Hospital
STIR progression

35-year-old son  
37-year-old

Dennis Shaw and Seth Friedman, Seattle Children’s Hospital
Longitudinal imaging study

- 6/9 stable including father and 2 fraternal twin daughters
- 3/9 progressed to fatty replacement
- Vast majority of muscles are stable, taking a long time for changes to occur

Friedman, S., et al., Muscle and Nerve 2014
Longitudinal imaging study

- ~25% of muscles that are STIR+ bright (very bright) progress to fatty infiltration
- Does not seem to be related to exercise/use
- STIR signal either persisted or if diminished was associated with increased fatty replacement
- Model?

Dennis Shaw and Seth Friedman, Seattle Children’s Hospital
Is STIR signal related to inflammation?

- 14/25 patients with STIR hyperintensities showed increased circulating CD8⁺pSTAT1⁺, CD8⁺T-bet⁺, and CD14⁺pSTAT1⁺ cells.
- 5 muscle biopsies of STIR⁺ muscles showed CD8⁺ T cells in endomysium and CD4⁺ T cells in perivascular space.
- Increased circulating IL12p40, IFNγ and TNFα cytokine levels.

Frisullo et al., J. Clin. Immunol., 2010
University of Washington-
University of Rochester
Wellstone Center
Hypothesis

Normal
T1 dark
STIR dark

Edema (inflammation?)
T1 dark
STIR bright

Fatty replacement
T1 bright
STIR +/-
• Use MRI to guide selective biopsies of involved and uninvolved muscle
• 18 patients at each sites
• Question: Is STIR signaling related to:
  – Inflammation?
  – Differences in DUX4 levels? Biomarker levels?
  – Differences in epigenetics between muscle groups? Methylation of D4Z4
Treatments?
• Decrease effect of neo-antigen presentation
• Decrease DUX4 expression
Summary
FSHD is due to contraction and inappropriate expression of DUX4.
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