Facioscapulohumeral Muscular Dystrophy: the road to disease-directed therapies

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Overview

- Clinical features of FSHD
- Natural history
- Advances in our genetic understanding of FSHD
- Revisiting clinical variability
- Drug development
- Conclusion
Clinical Features of FSHD
Facioscapulohumeral Muscular Dystrophy (FSHD)

- One of the most common muscular dystrophies
  - Prevalence 1:15,000 to 1:20,000, or ~ 21,000 in US
- Slowly progressive
- \textit{Facio} = face, \textit{Scapulo} = scapular girdle, \textit{Humeral} = upper arms
- Not life limiting but can lead to significant morbidity
- Diagnosis is based on characteristic clinical presentation and genetic testing
FSHD: Types 1 and 2

- Two genetically distinct forms
  - Clinically identical – though limited studies to date
- Type 1: ~95% due to deletion of repetitive element (D4Z4 region) on chromosome 4q35
  - Autosomal dominant inheritance, but up to 30% spontaneous
  - Normal >10 repeats, FSHD 1-10 repeats
  - Commercial testing D4Z4 fragment 10-38 kb (normal > 38 kb)
- Type 2: ~5% deletion-independent mechanism with decreased methylation in D4Z4 region on 4q35
  - ~80% associated with mutations in SMCHD1 on chromosome 18
  - Digenic inheritance
Patterns of Muscle Involvement

- Typically descending pattern
  - First affecting the face, scapular fixators, and arms (deltoid and forearm sparing initially)
  - Followed by distal lower extremity (e.g. tibialis anterior), quads and hamstrings
  - Pelvic girdle
- Can have marked axial and abdominal weakness
- Striking side to side asymmetry
- No or minimal contractures
- Pectus excavatum
Face:
- Decreased brow furrow,
- weakness of eye closure
- Transverse smile
- and inability to pucker
- No ptosis or weakness of extra-ocular muscles

Chest/Arms:
- Flattening of clavicle
- Shoulder may appear dropped, with prominent upper trapezius
- Pectoral wasting with prominent axillary crease
- Wasting of biceps and triceps with forearm sparing = “Popeye” appearance
- Protuberant abdomen

Shoulders:
- Both posterior and lateral winging of scapula can be seen

Assymetry of muscle involvement
Beevor’s Sign

Lift head off table
FSHD: Age at diagnosis

- Linear relationship between age at diagnosis and size of D4Z4 deletion
- Men show peak in diagnosis around 20 years of age, women dx a little older
- Traditionally penetrance >90% by 20 years of age
  - Recent studies suggest penetrance of motor impairment at 50 years of age 60->90% depending on size of deletion
- Infantile onset typically 1-3 repeats (<18 kb)

FSHD: Respiratory involvement follows weakness

- Restrictive respiratory pattern in ~10%
  - Associated with lower extremity/ pelvic girdle involvement
  - Severe paraspinal weakness
- May see abnormalities in expiratory function before FVC
- Around 1% of Dutch FSHD population req mechanical ventilation

FSHD: Cardiac Involvement

- No association with structural changes
  - No cardiomyopathy
- Cardiac arrhythmias ~ 5-10%?
  - Mainly atrial, SVT
- Typically not symptomatic
  - Most common symptom palpitations
- Severe cardiac conduction deficit or cardiomyopathy = revisit diagnosis
Extramuscular manifestations

- Retinal vascular changes
- Hearing changes
- CNS involvement?
  - Cognitive impairment reported in severely affected infantile form of FSHD

Retinal Vasculopathy

- Although retinal vascular changes can be seen in over half of patients (peripheral telangiectasias)
  - Symptomatic retinal vasculopathy (a condition known as Coats disease) is quite rare <1% (aneurysmal dilations, exudates, retinal detachment, blindness)
Coats Disease in FSHD

- Idiopathic Coats:
  - Unilateral
  - Mostly male
- In FSHD (survey of 14 national and international FSHD referral centers)
  - Often bilateral
  - Mostly women
  - Small D4Z4 residual fragments
  - Infantile onset presentation
- Who do we screen?
  - Important because there is a treatment

<table>
<thead>
<tr>
<th></th>
<th>FSHD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case No.</td>
<td>n=14</td>
<td></td>
</tr>
<tr>
<td>Age Coats</td>
<td>10 (1, 15)</td>
<td></td>
</tr>
<tr>
<td>FSHD Dx years</td>
<td>12 (5, 18)</td>
<td></td>
</tr>
<tr>
<td>D4Z4 Fragment Kb</td>
<td>13 (12, 13)</td>
<td></td>
</tr>
<tr>
<td>Gender Female</td>
<td>92.9%</td>
<td></td>
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<tr>
<td>Bilateral</td>
<td>64.3%</td>
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FSHD: Hearing Loss

- Older studies suggested high frequency hearing loss in up to 60% of patients; however, more recent studies suggest it may not be different than the general population.
  - Largely asymptomatic
- Symptomatic hearing loss ≈1-5%.
  - Typically infantile onset, more severe disease.
- One of the largest case series showed:
  - Age at hearing loss 0-7 years
  - D4Z4 fragment 1-3 units (10-18 kb)
- May affect language development if not detected early in childhood onset disease.

Infantile Onset Disease

- Uncommon (5-8%?)
- Almost entirely associated with 1-3 residual D4Z4 units (10-18 kb)
  - Separate disease or spectrum of disease
- Symptomatic extra-muscular manifestations almost exclusively in this group
- Rather than gradation based on deletion size
  - Almost appears to be ‘threshold’ effect
Natural History: Data from a large US Registry of FSHD Patients
Registry

- Limited data about progression of functional impairment in FSHD
- 313 genetically confirmed and clinically affected FSHD1 participants
  - An average of 6 years of follow up
- Mean age 51.5 years, range 9-91 years
- Slightly more women (52%)
- Geographically distributed across the US
- Largely Caucasian (>90%)
- Mostly well educated (>60% some college or beyond)
# Disease Characteristics

<table>
<thead>
<tr>
<th>Disease Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age initial symptom (SD)</td>
<td>21.1 (15.0)</td>
</tr>
<tr>
<td>Age diagnosed (SD)</td>
<td>31.3 (17.3)</td>
</tr>
<tr>
<td>D4Z4 contraction (kb)</td>
<td>24.8 (5.7)</td>
</tr>
<tr>
<td>Facial weakness (%)</td>
<td>282 (90.1%)</td>
</tr>
<tr>
<td>Scapular weakness (%)</td>
<td>303 (96.8%)</td>
</tr>
</tbody>
</table>

### Functional Burden

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Dry or irritated eyes (%)</td>
<td>152 (48.6%)</td>
</tr>
<tr>
<td>Difficulty whistling or drinking through a straw (%)</td>
<td>188 (60.1%)</td>
</tr>
<tr>
<td>Difficulty raising arms above shoulder height (%)</td>
<td>228 (72.8%)</td>
</tr>
<tr>
<td>Difficulty getting out of a chair (specific maneuvers or assistance) (%)</td>
<td>108 (34.5%)</td>
</tr>
</tbody>
</table>
WC Use by Decade and D4Z4 Deletion

Wheelchair Use Anytime

Decade

Prevalence

6 Year Risk

Average D4Z4 Contraction (kb)

Average D4Z4 Contraction

Wheelchair Use Anytime

Decade

Prevalence

6 Year Risk

Average D4Z4 Contraction (kb)
# Linear Relationship to Age for Other Assistive Devices

<table>
<thead>
<tr>
<th>Device</th>
<th>Age at First Use (SD)</th>
</tr>
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<tbody>
<tr>
<td>Ankle Foot Orthotic (SD) n=91</td>
<td>40.2 (15.2)</td>
</tr>
<tr>
<td>Ankle Knee Orthotic (SD) n=48</td>
<td>43.2 (14.6)</td>
</tr>
<tr>
<td>Cane (SD) n=124</td>
<td>49.1 (14.1)</td>
</tr>
<tr>
<td>Walker (SD) n=79</td>
<td>56.8 (15.5)</td>
</tr>
</tbody>
</table>
Registry Summary

- Although approximately 24% of a general population of FSHD patients may start using a WC over 6 years (10% become WC bound)
  - This risk is not distributed evenly across the population
  - With higher risk in patients with a small number of residual D4Z4 units, and older patients
- Unless we can find other markers to determine who is most at risk
  - The ability to use time to event outcomes, or WC use as endpoint in study will be limited due to the long time needed for such studies
Natural History: outcomes

- What have we learned about the Natural History of FSHD as measured by standard outcomes?
- Natural history study 3 year prospective longitudinal study (1997) n=81

How Do We Measure Strength

- Quantitative myometry (QMT) and manual muscle testing (MMT): use standardized positions for isometric strength testing
- Both have excellent reliability (QMT ICC 0.86-0.99, MMT kappa 0.81-0.98)
- Both have concurrent validity to disease severity scales, and other functional measures

- QMT can be normalized for gender, age and height to yield % predicted normal, or SD off normal (z-score)
- Both have combined scores that can be used to follow disease progression over time

Personius et al. (1994) Phys Ther 74: 253-63
Natural History Combined Scores

- Followed subjects at 6 months intervals for 3 years
- Responsiveness to disease progression: Effect size (change/SD) at one year 0.3
- Most responsive to disease progression: compared to functional measures, functional grades, and muscle mass
Extension of Natural History

- Extending natural history in 15 subjects who subsequently enrolled in albuterol trial
  - Confirmed slow but steady loss of strength over 2-7 years follow up
- Meta-analysis using data from 3 clinical trials confirmed variability estimates for QMT and MMT from natural history study

A. Slope (±SE): -0.29 (±0.08)
  95% CI (-0.46, -0.11)

The number of subjects needed to show a difference in strength depends on how big an effect you think you’re going to see:

- For example to show half of progression would need ~160 patients per treatment arm.
- On the other hand for an effect twice as large would only need ~40 per treatment arm.

Background: Power and Sample Size

Clinical Summary

- FSHD is a slowly progressive disease
- Can affect people from birth through old age
- Considerable variability between individuals and even within single families from generation to generation
- But patients with the smallest number of D4Z4 units appear to have more severe disease
  - Increased freq of extra-muscular manifestations
Advances in our Genetic Understanding of FSHD: FSHD is a epigenetic disease
FSHD1: Deletion of repeated units in D4Z4 region

FSHD associated DNA rearrangements are due to deletions of integral copies of a 3.2 kb tandemly repeated unit.
FSHD1: Genetic Lesion

- At least one unit of D4Z4 required for disease
  - Monosomy of 4q does not cause disease
- One open reading frame within D4Z4 repeats codes DUX4, a putative retrogene

FSHD: A problem with gene regulation, “the on/off switch”

- D4Z4 region normally highly methylated => not transcriptionally active
- Loss of D4Z4 repeats => decreased methylation => opening up of the chromosome
Deletion D4Z4 repeats => Decreased methylation => opening of chromatin structure

Raised possibility gene contained in D4Z4 repeat, DUX4, could be expressed

But FSHD only occurs with certain polymorphisms
FSHD1: Model of Disease

• DUX4 mRNA lacks a stabilizing polyA tail
• A SNP results in viable polyA sequence
• PolyA tails crucial for stable mRNA
• Allows the production of a stable DUX4 mRNA and protein
Support for FSHD1 Model

- Permissive alleles found in FSHD patient samples
- Muscle culture from FSHD patients express DUX4 but normal controls do not

FSHD2: Genetic defect

- FSHD2 represents ~5% of patients with FSHD and is clinically identical to FSHD1
- Normal number of repeats on 4q35 (>10) but profound hypomethylation on both copies of 4q35
- This leads to opening of the chromatin structure (more permissive to transcription)
- Like FSHD1, at least one 4q35 allele is A variant with the permissive poly(A) sequence
- Like FSHD1, muscle cultures from FSHD2 patients show bursts of DUX4 expression
Exome sequencing of DNA from families with FSHD2

- 14 individuals from 7 independent families
- Findings confirmed in 12 additional families

About 80% had sequence variants in the SMCHD1 gene on chromosome 18

- Missense mutations, out-of-frame deletions and splice-site mutations => loss of function
SMCHD1 Mutations Cause FSHD2?

- Structural Maintainance of Chromosomal Hinge Domain 1 (SMCHD1):
  - Regulates chromatin repression
  - Smchd1 protein is necessary for hypermethylation and is required for X-chromosome inactivation

- Mutations result in abnormal SMCHD1 mRNA and lower SMCHD1 protein in cell lines from individuals with FSHD2 => decreased methylation

- RNAi knockdown of SMCHD1 in cell culture results in stochastic DUX4 expression, similar to that seen in both FSHD1 and 2
FSHD2 is a Digenic disease

= permissive 4qA allele (# of units)

= SMCHD1 mutation
FSHD Unified Model: a normally silenced gene gets ‘turned on’
What is DUX4?

- DUX4
  - DUX4 (Double Homeobox 4) is a transcription factor normally expressed in the human germline but not in somatic cells
  - In the male germline, the spermatogonia and primary spermatocytes express DUX4, whereas the more mature spermatids do not
  - Role of DUX4 in normal germline biology is not known
Effects of DUX4 Expression

- DUX4 activates the expression of many genes that are normally expressed in the germline including:
  - cancer testis antigens
  - genes involved in protein degradation and muscle atrophy
  - genes involved in the innate immune system

DUCT4 Downstream Effects

- Possible pathologic mechanisms
  - Induction of mitotic program in a post mitotic cell resulting in apoptosis
  - Immune response to germline antigens
    - Germline is immune privileged
    - Expression of these proteins in somatic cell may induce and immunologic response

- Evidence that DUCT4 expression:
  - Highly toxic causing apoptotic cell death
  - Interferes with myogenic differentiation
  - Makes cells more susceptible to oxidative stress

Advances in Genetics: Questions

- DUX4 is expressed in high level infrequently in FSHD 1 and 2: 1:1000 myonuclei = ‘bursts of expression’
  - What causes DUX4 burst of expression, and when in development does it occur
  - How does this lead to disease => downstream mechanisms
  - How to explain unusual pattern of muscle involvement, and marked asymmetry
  - Why involvement of retinal vessels and hearing
- Do patients with FSHD2 have additional non-neuromuscular manifestations?
- 20% of patients with FSHD2 have no SMCHD1 mutation
  - Other modifier genes?
Revisiting Clinical Variability
Decreased Methylation

A

<table>
<thead>
<tr>
<th></th>
<th>4q</th>
<th>10q</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL (SMCHD1 +/+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSHD1 (SMCHD1 +/+)</td>
<td></td>
<td></td>
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<tr>
<td>FSHD2 (SMCHD1 +/-)</td>
<td></td>
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B

Human Molecular Genetics

- P=1.35E-47
- P=3.80E-22
- P=9.96E-24

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Predicted Methylation Based on d4Z4 Repeat Number

- Predict methylation based on number of D4Z4 units
- For FSHD2 some other factor beyond number of D4Z4 units decreasing methylation
  - E.g. mutation SMCHD1
FSHD1: mutation vs clinical severity

- Linear relationship between D4Z4 units and severity for 1-6 units
  - Where methylation predicted by number of units
- But 7-10 units not linear relationship
  - Methylation more negative than would be predicted by mutation alone

FSHD1: Epigenetic Disease

- 1-6 Repeats
  - Association between size of residual repeat and clinical severity, and between size of repeat and methylation – largely driven by size of deletion
  - 1-3 units = factor related to size and not methylation?
    - Increased freq of extra-muscular manifestations not seen in FSHD2

- 7-10 repeats
  - Less severe disease
  - In Japan do not get FSHD
  - Penetrance related to methylation levels = some other factor, environmental or genetic?

Genetic Modifiers? SMCHD1

- Three families with very mild FSHD1 with borderline D4Z4 contraction (9 repeats)
- Four individuals with unusually severe disease
  - All have a mutation in SMCHD1 and D4Z4 methylation levels comparable to FSHD2 patients

How does the disease progress?

- Is it there from birth?
  - Ultrastructurally non affected muscles look normal
- When you talk to patients they say
  - Periods of rapid decline, followed by plateaus
- Discrepancy with what we see when we look at populations
  - Slow but steady loss of strength over time
Does MRI Reveal Something About Disease Progression?

- ~20-40% of patients show STIR positivity in ultra structurally preserved muscles
- In one study, muscle biopsies of STIR positive muscles (n=5) all showed inflammation
  - Subsequent analysis showed increased expression of DUX4 downstream targets in 2/4
- Relationship between DUX4 induction of genes involved in innate immunity and inflammation seen on MRI?

Identifying Muscles at Risk

- The ability to identify muscles at risk for progressing would be important for clinical trials
  - Especially in disease where overall strength only declines about 3-5%/year
- Can help reduce variability and hasten drug development
Drug Development
AON: Splice site SMCHD1 mutations

RNAi: Interference RNA

Downstream Effects

Immune Suppression

Antioxidants

Block apoptosis

Methylate DNA?

Small molecules

FSHD1 / FSHD2

AON: targeting poly a tail

RNAi: Interference RNA

DUX4 mRNA
FSHD Therapeutics: where things stand

- Targeted approaches to FSHD now possible:
  - Multiple patent applications for technologies designed to knock down DUX4 expression
  - GSK is looking at small molecules that interfere with DUX4 function
  - POC similar approaches in other NM diseases
- Multiple animal models and cell lines in development
  - Possibility for high throughput drug screening
  - For preclinical drug development
Clinical Trials

- Immunomodulator (tRNA-synthetase)
  - aTyr = in dose escalating safety studies
- Improve muscle bulk or function
  - Myostatin inhibitor, SARM = safety/POC study planned
  - Other agents?
Conclusion

- Recent advances have elucidated a unified genetic model for FSHD1 and 2
- Identifies potential disease-directed therapeutic targets
  - DUX4 or downstream targets of DUX4 activity
- The slow disease progression and individual to individual variability present major challenges when developing outcomes for future trials
  - Identifying markers of disease activity to help stratify patients will be key
- International cooperation and standardization of procedures will be necessary
  - To understand the role of genetics or environment on disease
Thanks

- Organizations
  - FSH Society
  - Frontiers KL2 Program
  - KUMC
    - Richard Barohn, MD – mentor
    - Mazen Dimachkie, MD – collaborator
    - Laura Herbelin, Ayla McCalley, Melissa Currence – coordinators

- URMC
  - Rabi Tawil, MD – mentor
  - Chad Heatwole, MD – collaborator

- LUMC – the Netherlands
  - Silvere van der Maarel - collaborator

- Fred Hutchinson Cancer Center – Seattle
  - Stephen Tapscott - collaborator