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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)

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

Human chromosome 4q35, Normal D4Z4 n=11-150

Human chromosome 4q35, D4Z4 contraction 1<N<11

FSHD
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

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.

- Normal
- FSHD1
- FSHD2

- Black circles = Hypermethylated CpGs
- Yellow circles = Hypomethylated CpGs

More heterochromatic
More euchromatic
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
Subjects screened for FSHD clinically and genetically

Genotyping (Iowa Wellstone)

Cell Culture (UMMS)

Biopsies (KKI-JHU)

Microsatellite analysis (CHB)

mRNA & protein analysis (CHB, UMMS)

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.
“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
“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

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Chr 4qA → FSHD

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

PAS = polyadenylation site
NP = non-permissive
P = permissive

DUX4

PAS

Exon 1

E2

E3(P)

E3(NP)

D4Z4

4qB

4qA

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

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

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

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

Differentiated myogenic cells from genetically FSHD1 and control subjects express DUX4-FL protein.
DUX4-FL expression in myogenic cells from FSHD affected unaffected subjects suggests a quantitative model of pathogenesis.

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

Normal

FSHD1

FSHD2

DUX4-fl

-/+

++++

++++

= Hypermethylated CpGs
more heterochromatic

= Hypomethylated CpGs
more euchromatic
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

**DUX4** fulfills these criteria

**DUX4** encodes (**DUX4-fl** and **DUX4-s**)
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

**Graph**

- **X-axis**: 5-ADC, TSA, 17Abic, 17Ubic
- **Y-axis**: Normalized Fold Expression

- Treatment conditions: 5µM, 1µM, 5µM, -

**Legend**

- **5-ADC**
- **TSA**
- **17Abic**
- **17Ubic**

**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

Clinically FSHD

Clinically Non-manifesting

Non-manifesting

15B = 69 yr old vs 15A = 66 yr old

28B = 68 yr old vs 28A = 44 yr old

29B = 70 yr old vs 29A = 39 yr old

T. Jones et al. (2012)
Symptomatic FSHD
Asymptomatic FSHD
Unaffected Control
4qB/4q B Control

[Graph showing 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**

Normal

FSHD1

FSHD2

- = Hypermethylated CpGs
  more heterochromatic

- = Hypomethylated CpGs
  more euchromatic
Multiple therapeutic targets for FSHD

1. Prevent induction
2. Knockdown DUX4-fl
3. Block or reduce pathogenic drivers

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
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Downstream effectors:
- Inflammatory response
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- Gene regulation
- Genetic modifiers

1. Prevent induction
2. Knockdown DUX4-fl
3. Block or reduce pathogenic drivers

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

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