Modeling FSHD in zebrafish

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Advantage of zebrafish

- Many eggs: 100-300 eggs / pair
- Rapid development: Embryos hatch in 72 hours
- Muscular dystrophic phenotype is easily detected by birefringence
- Small compounds penetrate into zebrafish embryos

![Image of zebrafish embryo with arrows indicating muscle degeneration.](image-url)
Final goal

Drug screening in zebrafish model of FSHD

Aim 1

How expression of DUX4 causes FSHD-like phenotype in zebrafish

Aim 2

To establish DUX4-transgenic fish
Chemical screens of Dystrophin Deficient Zebrafish for Functional Modifier
Chemical screening of small molecules using dystrophin null fish

**Purpose:**
Screening for effective chemicals to rescue the muscle phenotype

**Methods:**
1. Sapje and Sapje-like fish (heterozygous fish +/-)
   
   
   (+/-) X (+/-)
   
   ++  +-  -/
   
   25%  50%  25%

2. Chemical treatment with 2.4 µg/ml from 1 to 4 dpf
   
   Chemical library: Prestwick Collection  1120
   
   - Chemicals are already approved by the FDA for treating disease
   - The mechanism for drug action of the compounds is already known.

3. Birefringence assay
   
   At 4 dpf, all fish were examined by birefringence and the number of affected fish were counted.
   
   - 25%  non effective
   - less than 25%  effective for decreasing affected fish
Observation of muscle by Birefringence

Dissecting scope

Polarizing filter

Anesthetized fish

Polarizing filter

Wild type fish

Sapje fish (DMD model fish)
Examples of effective and ineffective chemicals
Examples of effective and ineffective chemicals

5 affected fish / 20 fish (25%)

1 affected fish / 20 fish (5%)

Non-effective

Effective
## Candidate chemicals from our screens

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<th>No.</th>
<th>Chemicalname</th>
<th>Chemicallibrary</th>
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<td>#1</td>
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<td>#13</td>
<td>Cerulenin</td>
<td>ICCBKnownBioactivesLibrary</td>
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<td>#14</td>
<td>9a,11b-ProstaglandinF2</td>
<td>ICCBKnownBioactivesLibrary</td>
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Affected Fish with Chemicals #1-7

Het (+/-) X Het (+/-)

Day 4 embryos -10 /cage

1. Treatment with individual chemicals (2.5 \( \mu \text{g/ml} \))
2. Non treatment
3. Wild type

Culture fish from day 4 to day 30 in triplicate

Survival fish for 30 days
- Number of surviving fish
- Genotyping
- IHC
Some chemicals increase the life span of dystrophin null fish

The number of surviving fish

dpf

#1 #2 #3 #4 #5 #6 #7 Non treatment Wild type
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How expression of DUX4 causes FSHD-like phenotype in zebrafish

Aim 2

To establish DUX4-transgenic fish
1. Cloning of human DUX4

2. Synthesize DUX4 mRNA \textit{in vitro}

3. Injection into zebrafish embryos

- pCS2(+) vector
- EcoRI
- XhoI
- Human DUX4
- V5

- DUX4-fl
  - 1359 nt
  - V5

- DUX4-s
  - 568 nt
  - V5

- HOX1mut
  - 1359 nt

- SP6

- 10, 0.5, 0.2, 0.1 pg mRNA per embryo
Only 1/1000 cells from FSHD patient express DUX4-fl

What will happen if we inject DUX4 less than 0.5 pg?
Day 4
0.5, 0.2, 0.1 pg / embryo

N = 150-200 embryos
- Low levels of DUX4-fl resulted in asymmetric abnormalities of the eyes, ears and fins in a dose-dependent manner.
- Along with muscular dystrophy, FSHD patients experience hearing loss and retinal vasculopathy.
- Is asymmetry caused by localization of DUX4-expressing cells to one side?
Birefringence was mildly affected in injected fish.

uninjected

DUX4-fl 0.2 pg

DUX4-fl 0.1 pg
Conclusions

- Very small amount of DUX4-fl (1 x 10⁵ molecules) caused abnormal phenotypes on the eyes, face, and fin muscles in zebrafish.
- DUX4-fl perturbed myogenesis of face and fin muscles in an asymmetrical manner.
- Zebrafish can start to model features of FSHD.
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How expression of DUX4 causes FSHD-like phenotype in zebrafish

Aim 2

To establish DUX4-transgenic fish
Only 1/1000 cells from FSHD patient express DUX4-fl

We need to express DUX4-fl in a small percent of cells.
Tamoxifen-inducible Cre-loxP system

Ubiquitous transgene expression and Cre-based recombination driven by the *ubiquitin* promoter in zebrafish

Christian Mosimann¹,²,³, Charles K. Kaufman⁴, Pulin Li¹,²,³, Emily K. Pugach¹,²,³, Owen J. Tamplin¹,²,³ and Leonard I. Zon¹,²,³,⁴,*

![Image of zebrafish embryos with EGFP and mCherry fluorescence]
A transgenic zebrafish model of FSHD

- Currently no widely accepted animal model of FSHD
- Regulating expression levels of DUX4 is key to creating a successful FSHD animal model
- We generated an inducible DUX4 transgenic zebrafish using a tamoxifen-controlled CreERT2-loxP system
- Enables regulation of the dosage and timing of DUX4 expression
Mosaic expression of DUX4 in our transgenic model mirrors low DUX4 expression in human cells

- Approximately 1 in 1000 myonuclei are DUX4 positive in primary human cultures
- Evidence of disorganized myofibers by day 3 of DUX4 induction in our transgenic model

Block et al. 2013 Hum Mol Gen
• Automated system to track movement of zebrafish larvae
• Enables high-throughput functional screening

**Does altered muscle structure in DUX4 fish affect its function?**

- DUX4-mCherry
- Mylz:EGFP

- DUX4-mCherry
- Mylz:EGFP
DUX4 transgenic fish significantly swim less than control in a 15 minute assay.
Conclusions

- Both our injection and transgenic model of DUX4 result in a muscular dystrophy phenotype
- Despite the primate-specific origins of DUX4, our zebrafish model is able to mimic FSHD patient phenotypes
- This suggests that misexpression of DUX4 results in a toxic disease pathway that is conserved across vertebrates
- We can use these fish to find important targets of DUX4
- Future work will be to use our transgenic model for drug screening
Acknowledgements

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FSH Society
Several fish lines with different promoters to test varying levels and tissue specific expression of DUX4

(1) z-ubi pro

(High™Middle, ubiquitous)

(2) z-myiz2 pro

(High expression, skeletal muscle)

(3) h-DUX4 pro200; cryaa: GM2

(Low expression, ?)

(4) z-hsp70l pro: DUX4-V5
cryaa: Cerulean

(Low expression at 28°C, ubiquitous)
(High expression at 42°C)
Transgenic induction of DUX4 is linked to a spectrum of abnormal birefringence phenotypes

Birefringence of DUX4 fish

Days post-fertilization

- Wildtype
- DUX4 ‘Abnormal’
- DUX4 ‘Mild’
- DUX4 ‘severe’
Day 2

mylz:EGFP

DUX4-mCherry

Day 3

Day 4

Day 5

Day 6

Day 7

Progressive myofiber degeneration in DUX4 fish
Parasagittal section of DUX4 fish show areas of compromised muscle architecture
In our model, we also see that fibers are likely to have multiple adjacent DUX4 positive nuclei. Stochastic DUX4 activation in nucleus can spread to adjacent nuclei, causing diffusion of the pathological phenotype in myotubes.