The (elusive) perfect mouse model

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MDF Drug Development Roundtable
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Outline

What do we want to model?
What is the utility of a mouse model?
What are the ideal features?
What do we have?
What are the pros and cons?
What do we want to model?

What is the utility of a mouse model?
What are the ideal features?
What do we have?
What are the pros and cons?
Two forms of Myotonic Dystrophy (DM)

- **autosomal dominant**
- **most common form of adult onset muscular dystrophy**
- **second most common form of muscular dystrophy**

- **DMPK (chrom. 19)**
  - type 1 (DM1)
  - \(\text{CTG})_{80 \text{ to } >2000}\)

- **CNBP (chrom. 3)**
  - type 2 (DM2)
  - \(\text{CCTG})_{75 \text{ to } >10,000}\)
Myotonic dystrophy is a multisystemic disease

- **Vision:** Cataracts, retinal damage
- **Bone:** Anomalies
- **Immune:** Hypogammaglobulinemia
- **Skin:** Pilomatrixomas
- **Respiratory System:** Breathing difficulties, aspiration, sleep apnea
- **Endocrine System:** Diabetes, low thyroid hormone levels
- **Reproductive System:** Low testosterone levels, testicular failure and gonadal atrophy in men. Weakened uterine muscle, pregnancy-related complications, and gynecological problems in women.
- **Cognitive Function:** Intellectual impairment, behavioral and psychological disorders, excessive daytime sleepiness
- **Cardiovascular System:** Heart condition abnormalities, arrhythmias, cardiomyopathy
- **Gastrointestinal Tract:** Swallowing issues, abdominal pain, irritable bowel syndrome, constipation/diarrhea, poor nutrition and weight loss, chronic infections
- **Muscle:** Weakness, wasting (atrophy), myotonia, pain

Therapeutics need to access and address pathology in multiple tissues

From: Myotonic Dystrophy Foundation http://www.myotonic.org/
Clinical data informs development of mouse models

Patient-Reported Impact of Symptoms in Myotonic Dystrophy Type 2 (PRISM-2).


Parent-reported multi-national study of the impact of congenital and childhood onset myotonic dystrophy

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Myotonic Dystrophy type 1 (DM1)

$DMPK$ gene

$(CTG)_{80\text{ to }2000}$

mRNA

$(CUG)_{\text{exp}}$

RNA gain-of-function

Davis et al. PNAS 94, 7388
Pathogenic effects of CUG)exp RNA

repeat-associated non-ATG translation
Pathogenic effects of CUG)exp RNA

- **MBNL1 & MBNL2 sequestration** (loss-of-function)
- **CELF1 protein induction** (gain-of-function)
- **disrupted developmental splicing**
- **misregulated translation**
- **mislocalized mRNA**
- **altered mRNA stability**
Extent of aberrant splicing for 20 events correlates with muscle weakness (TA dorsiflexion)

What is the utility of a mouse model?

1. Reproduce pathogenic mechanisms for studies to identify additional therapeutic targets

2. Model for productive preclinical testing
What are ideal features?

1. All affected tissues in one mouse model (CNS, heart, muscle, GI, etc.)
   • e.g., use DMPK to drive expression in correct tissues

2. Alternatively use clinical data to determine what promoters to use to express the CUGexp RNA
   • e.g., are GI symptoms due to autonomic nervous system or smooth muscle (or both?)

3. Straightforward mouse population maintenance and expansion

4. Goldilocks mouse: phenotype that is progressive, not too subtle and not too severe

5. Model adult and congenital DM1
What do we have: published DM1 models

HSA^{LR}

skeletal muscle specific (transgene)

multisystemic expression (transgene)
Seznec, H. et al. 2001. HMG. 10, 2717–2726

>1000 CTG repeats

Conditional, skeletal muscle or heart specific (transgene)

Conditional, skeletal muscle and heart expression (transgene)

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>1000 CTG repeats

DMWD
DMPK
SIX5

Conditional, skeletal muscle and heart expression (transgene)

1. Charles Thornton M.D., Univ. Rochester
2. 250 CTG repeats in the 3’ UTR of the human skeletal alpha actin gene
3. expressed only in skeletal muscle
4. used as homozygote for stronger phenotype
5. >1000 fold higher expression than endogenous DMPK

6. molecular features
   • robust splicing abnormalities
   • CUGexp RNA foci with Mbnl co-localization
   • characteristic transcriptomic changes

7. phenotypic features
   • centralized nuclei
   • myotonia
   • age-dependent myopathy (centralized nuclei, fiber hypertrophy, ringed fibers, size variability)

Cons

1. limited to skeletal muscle expression
2. does not contain DMPK sequence
3. expression of CUGexp RNA very high compared to DM1 muscle
4. weak muscle wasting phenotype despite robust histopathology
1. Genvieve Gourdon, Inserm Paris, France
2. transgene containing 45 kb human genomic segment, >1000 CTG repeats
3. used as homozygote for stronger phenotype
4. expression:
   • heart (0.3x endogenous DMPK)
   • muscle (0.1x endogenous DMPK)
   • brain (3x endogenous DMPK)

Seznec, H. et al. 2001. HMG. 10, 2717–2726

DMSXL

DMWD

DMPK

SIX5

>1000 CTG repeats

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5. molecular features
   - weak splicing abnormalities muscle, heart, brain; lessen with aging in muscle and heart
   - RNA foci in muscle, heart, brain (neurons and glia)
   - Celf1 increased in brain

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6. phenotypic features
   - general
     - 60% mortality of HOM from HET matings before weaning
     - 50% size first month and 60-80% of wild type size at 2 months

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   - muscle
     - 30% reduced muscle fiber area in TA
     - grip strength reduced but not significant when standardized to muscle weight
     - weak and variable myotonia

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   - muscle
     - 30% reduced muscle fiber area in TA
     - grip strength reduced but not significant when standardized to muscle weight
     - weak and variable myotonia
   - heart:
     - normal ECG at baseline, enhanced sensitivity to sodium channel blocker flecainide in 8-month-old DMSXL mice
     - developed mild abnormal echo parameters by 8 months of age
     - abnormal gating properties of the sodium current in isolated cardiomyocytes
   - brain:
     - behavioral differences (anxiety)
     - spatial memory reduced

Seznec, H. et al. 2001. HMG. 10, 2717–2726
Cons

1. animals born small and “sick”; phenotypic features in heart and muscle are weak. Therefore difficult to assay for rescue of phenotype beyond assays for molecular rescue

2. definitely “sick” but concerns about whether all phenotypes represent DM1

3. transgene inserted into a protein coding gene for which model is homozygous knock out

4. potential somatic instability

Seznec, H. et al. 2001. HMG. 10, 2717–2726
DM5 and DM200

Conditional, skeletal muscle or heart specific (transgene)


1. Mani Mahadevan, Univ. Virginia
2. CTG5 used in most papers; CTG200 poorly expressed - used only in one recent paper as back up
3. heart and muscle phenotypes described
4. reversible pathology

Cons

1. CTG5 pathogenic without expansion; potentially other aspects of transgene are pathogenic
2. CTG200 only used in one publication as back up
3. Extremely high level of expression
Additional mouse models
MDF and the Wyck Foundation have entered into a one-year partnership with Dr. Cat Lutz and Jackson Laboratory (Bar Harbor, ME) to develop a new mouse model of myotonic dystrophy type 1 (DM1).
Tet-inducible expression of DMPK-CUG\textsubscript{960} RNA in heart or skeletal muscle

Induce at postnatal day 1 (through nursing doe) or adult (6-10 weeks old)
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Model</th>
<th>HSA&lt;sup&gt;LR&lt;/sup&gt;</th>
<th>DMSXL</th>
<th>CTG5</th>
<th>TRE-H muscle</th>
<th>TRE-H heart</th>
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<tr>
<td>muscle</td>
<td>Myotonia</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
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<tr>
<td>muscle</td>
<td>Histopathopathology</td>
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<td>Weakness (grip)</td>
<td>✓</td>
<td>✓#</td>
<td>✓</td>
<td>✓</td>
<td></td>
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<td>RNA foci</td>
<td>✓</td>
<td>✓</td>
<td>no</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>muscle</td>
<td>MBNL colocalization</td>
<td>✓</td>
<td>✓</td>
<td>no</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>muscle</td>
<td>Celf1 upregulation</td>
<td>inconsistent</td>
<td>?</td>
<td>✓**</td>
<td>✓@</td>
<td></td>
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<td>muscle</td>
<td>Mis-splicing</td>
<td>✓</td>
<td>mild, resolves</td>
<td>✓</td>
<td>✓</td>
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<td>stimulated</td>
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<td>heart</td>
<td>Abnormal echo</td>
<td>8 mo</td>
<td>✓</td>
<td>✓</td>
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<td></td>
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<tr>
<td>heart</td>
<td>RNA foci</td>
<td>✓</td>
<td>no</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>heart</td>
<td>MBNL colocalization</td>
<td>✓</td>
<td>no</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>heart</td>
<td>Celf1 upregulation</td>
<td>mild; 1 of 4</td>
<td>no</td>
<td>?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>heart</td>
<td>Mis-splicing</td>
<td>mild, resolves</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
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<tr>
<td>brain</td>
<td>RNA foci</td>
<td>✓</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>brain</td>
<td>MBNL colocalization</td>
<td>?</td>
<td>?</td>
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</tr>
<tr>
<td>brain</td>
<td>Celf1 upregulation</td>
<td>✓</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>brain</td>
<td>Mis-splicing</td>
<td>✓</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>brain</td>
<td>Functional abnormal.</td>
<td>✓</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* severe degeneration
** potentially secondary to severe degeneration
@ only by immunofluorescence; not detected by western.
# grip strength reduced but not significant when standardized to muscle weight
Should we consider other mammalian models?
Acknowledgements

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Muge Kuyumcu-Martinez, Ph.D.
Johanna Lee, Ph.D.
Donnie Bundman

The Cooper Lab
Early onset myotonic dystrophy

Parent-reported multi-national study of the impact of congenital and childhood onset myotonic dystrophy

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# Patient-scored symptoms in adult onset DM1

<table>
<thead>
<tr>
<th>Symptomatic themes</th>
<th>Total population</th>
<th>5: Problems with physical health&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Prevalence, %</td>
</tr>
<tr>
<td>Total no. of respondents</td>
<td>278</td>
<td>Relative impact on lives (SD)</td>
</tr>
<tr>
<td>Symptomatic themes</td>
<td></td>
<td>6: Limitations with mobility or walking</td>
</tr>
<tr>
<td>1: Problems with hands or arms</td>
<td></td>
<td>Prevalence, %</td>
</tr>
<tr>
<td>Prevalence, %</td>
<td>93.5</td>
<td>Relative impact on lives (SD)</td>
</tr>
<tr>
<td>Relative impact on lives (SD)</td>
<td>2.27 (1.22)</td>
<td>7: Inability to do activities</td>
</tr>
<tr>
<td>2: Fatigue</td>
<td></td>
<td>Prevalence, %</td>
</tr>
<tr>
<td>Prevalence, %</td>
<td>90.8</td>
<td>Relative impact on lives (SD)</td>
</tr>
<tr>
<td>Relative impact on lives (SD)</td>
<td>2.49 (1.22)</td>
<td>8: Gastrointestinal issues</td>
</tr>
<tr>
<td>3: Myotonia</td>
<td></td>
<td>Prevalence, %</td>
</tr>
<tr>
<td>Prevalence, %</td>
<td>90.3</td>
<td>Relative impact on lives (SD)</td>
</tr>
<tr>
<td>Relative impact on lives (SD)</td>
<td>2.09 (1.30)</td>
<td>9: Pain</td>
</tr>
<tr>
<td>4: Impaired sleep or daytime sleepiness</td>
<td></td>
<td>Prevalence, %</td>
</tr>
<tr>
<td>Prevalence, %</td>
<td>87.9</td>
<td>Relative impact on lives (SD)</td>
</tr>
<tr>
<td>Relative impact on lives (SD)</td>
<td>2.25 (1.31)</td>
<td>10: Problems with vision, hearing, or smell</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prevalence, %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Relative impact on lives (SD)</td>
</tr>
</tbody>
</table>

*Neurology 79, 348–357 (2012)*

2. hemizygous DMSXL RNA is 10 fold LOWER than endogenous DMPK in muscle tissue which is 1-20 copies per cell

3. homozygous HSALR RNA 1000 fold higher than endogenous DMPK in muscle tissue which is 1-20 copies per cell

4. DMPK mRNA in human skeletal muscle is same in DM1 and normal and equal expanded and non expanded alleles; muscle tissue which is 1-20 copies per cell and each foci in cell culture is one to a few RNA molecules

---

I have not used these mice so this is from the literature but those who have worked with them can speak up

45 kb genomic segment

low expression in muscle

WHAT IS EXPRESSION IN HEART

HIGH EXPRESSION IN FRONTAL CORTEX

transgene is inserted into an endogenous gene; the impact needs to be evaluated

HOM 50% size “during” first month and 60-80% at 2 months

60% mortality of HOM from HET matings before weaning then only 5%

lower fasting levels of IGFBP3 and insulin

muscle fibers 31% reduction in area in TA, no difference fiber number

weak splicing changes in heart and muscle lessen over time

myotonia weak, variable and only with needle insertion

grip strength reduced but not sig with normalized to body weight

brain – foci through out in neurons and glia region specific differences

brain – mild splicing changes but revert to fetal, elevated Celf1 and 2 and increased PO4 of Celf1

brain – increased anxiety open field test but only first minute

brain – increased anxiety indicated by better buried marbles

brain – spatial memory Morris water maze – reduced

brain - electrophysiological profiling of DMSXL hippocampus - no major deficits in basal transmission

brain - the repeat expansion may affect (directly or indirectly) a limited number of synaptic targets

---


2. antisense transcripts, mild splicing defects, muscle affected and motor performance

3. DMSXL mRNA 1/3 endog in muscle and 3x in frontal cortex

**Figure 2.** *DMPK* expression profiles. Expression of the human *DMPK* transgene was studied in various hemizygous DMSXL tissues (A) and muscles (B), in parallel with the endogenous *Dmpk* mouse gene (C and D) (n = 3). (E) Expression of *DMPK* in human tissues. Dia., Diaphragm; Stero., Sternomastoid; Quadri, Quadriceps; TA, Tibialis Anterior; Gastroc. Gastrocnemius. (a.u.): arbitrary units. (F) Expression of *DMPK* in hemizygous (Hemi.) and homozygous (Homo.) DMSXL tissues. Data are presented as means ± standard deviation. doi:10.1371/journal.pgen.1003043.g002

Figure 3. Expression of sense and antisense DMPK transcripts. (A–B) DMPK antisense expression profile in 4-month-old DMSXL homozygotes (n = 3) and human control adult tissues (commercial panel) using amplicon B′ located upstream the CAG repeat. (C–D) Comparison of DMPK sense and antisense transcript levels in 4-month-old DMSXL homozygotes (n = 3) and human control tissues. (E–F) Comparison of antisense transcript levels measured in 5′ (before) and in 3′ (after) of the CAG repeat using amplicons B′ and C′ in DMSXL and control human tissues. H, heart; TA, tibialis anterior; Dia, diaphragm; FC, Frontal Cortex; Hemi, Hemizygous; Homo, Homozygous. Data are presented as means ± standard deviation in arbitrary units (a.u.).

doi:10.1371/journal.pgen.1003043.g003
Experimental Approach

Induce at postnatal day 1 (through nursing doe) or adult (6-10 weeks old)

- dox diet (2 or 6 g/kg dox chow)
- RNA foci
- Alternative splicing changes
- Histology
- Muscle wasting

- RNA foci
- Alternative splicing changes
- Heart size
- Cardiac Function

hDMPK e11-15

960-interrupted repeats
Dose-response CUGexp RNA expression in DM1 heart model

<table>
<thead>
<tr>
<th>Dose (g/kg)</th>
<th>2 weeks</th>
<th>2 months bitransgenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td></td>
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<tr>
<td>0.2</td>
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<td>0.5</td>
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</tr>
<tr>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td></td>
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</tr>
<tr>
<td>6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td>Fed dox chow</td>
<td></td>
</tr>
</tbody>
</table>

Fed dox chow
RNA foci are observed in CUG\textsubscript{960} hearts and lost upon doxycycline removal.
DM1 splicing events are misregulated and reversible in CUG<sub>960</sub> heart

Control 6 wk dox, n = 4
CUG<sub>960</sub> 14 wk dox, n = 3
CUG<sub>960</sub> 14 wk dox/6 wk off, n = 4
*p < 0.05
6 g/kg dox induced PN1
CUG\textsubscript{960} hearts are enlarged compared with controls

* p < 0.05
Control n = 6
CUG\textsubscript{960} + dox n = 10
CUG\textsubscript{960} - dox n = 4
6 g/kg dox induced PN1
CUG$_{960}$ mice show abnormal cardiac function

- ECG abnormalities
- 17 of 19 CUG$_{960}$ mice abnormal…
- …but 8 of 16 control mice also abnormal
- addressing issues with background

Control n = 16
CUG$_{960}$ n = 19
*p < 0.05
6 g/kg dox induced PN1
Experimental Approach

- dox diet (2 or 6 g/kg dox chow)
- RNA foci
- Alternative splicing changes
- Histology
- Muscle wasting
- RNA foci
- Alternative splicing changes
- Heart size
- Cardiac Function

Induce at postnatal day 1 (through nursing doe) or adult (6-10 weeks old)
Expression of CUG$_{960}$ transgene is $>30x$ greater than DMPK expression in human skeletal muscle.

- CUG$_{960}$ + dox n = 4
- Control + dox n = 2
- Human DM1 n = 4
- Human Normal n = 3
$\text{CUG}_{960}$ skeletal muscles contain RNA foci
Splicing events misregulated in DM1 are misregulated in CUG\textsubscript{960} muscle

- **Control + dox, n = 3**
- **CUG\textsubscript{960} + dox, n = 3**
- **HSA\textsuperscript{LR}, n = 2**

*p < 0.05*
Severity of skeletal muscle histopathology is increased in CUG$_{960}$ mice at four months.
Muscle wasting is observed in CUG$_{960}$ mice 12 weeks after induction at PN1.
Summary of the skeletal muscle model

- CUG\textsubscript{960} transgene is expressed at levels 30-50 x human tissue
- Splicing effects are mild while wasting is robust

Plans

- Determine whether turning off CUG\textsubscript{960} RNA reverses muscle wasting
- Use transcriptome and signaling assays to identify changes relevant to mechanism of muscle wasting
- Use rescue as the assay to test mechanisms of muscle wasting
  - replace Mbnl1 and Mbnl2
  - deplete Celf1
### Comparison of expanded repeat mouse models

<table>
<thead>
<tr>
<th>Model</th>
<th>HSA&lt;sup&gt;LR&lt;/sup&gt;</th>
<th>DMSXL</th>
<th>CTG5</th>
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<tbody>
<tr>
<td>Myotonia</td>
<td>✔</td>
<td>✔</td>
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<tr>
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<tr>
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<td>RNA foci accumulation</td>
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<tr>
<td>Increased Celf1 levels</td>
<td>Inconsistent reports</td>
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<td>✔</td>
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<tr>
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Ginny Morriss, Ph.D.
## Comparison of expanded repeat models

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</tbody>
</table>
Tet-inducible expression of DMPK-CUG\textsubscript{960} RNA in heart or skeletal muscle
Experimental Approach

- dox diet (2 g/kg dox chow)
- RNA foci
- Mild alternative splicing changes
- Histopathology
- Muscle wasting
- RNA foci
- Strong alternative splicing changes
- altered ECG
- altered echocardiography

Induce at postnatal day 1 (through nursing doe) or adult (6-10 weeks old)
Modeling MBNL and CELF effects in mice

MBNL1 & MBNL2 sequestration (loss-of-function)

CELF1 protein induction (gain-of-function)

MBNL1 & MBNL2 knock out mice

CELF1 overexpression in transgenic mice
Modeling DM1 in mice

the RNA is the primary cause