DM2
RESEARCH UPDATE

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Outline

- **Pathogenesis**
- **Modifier Genes**
- **Management**
- **Molecular Therapy**
- **Take Home Message**
Spliceopathy does not fully explain the multisystemic phenotype thus additional mechanisms may be involved.
the phenotypic differences between DM1 and DM2 can be explained by other cellular and molecular pathways involved besides the shared toxic-RNA gain of function hypothesized.
**Alternative splicing**

**DM1 vs DM2**

- Differences in muscle gene expression and splicing: in particular, the aberrant splicing isoform of **TNNT3** is twice as frequent in DM2 compared to DM1

**MOREOVER**

Different protein expression pattern in the highly atrophic fibers has been found between DM1 and DM2

**Table 3. Immunohistochemistry results of highly atrophic fibers**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Gene</th>
<th>DM2</th>
<th>DM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>MyHC-IIa</td>
<td>MYH2</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>MyHC-beta</td>
<td>MYH7</td>
<td>(+)</td>
<td>+++</td>
</tr>
<tr>
<td>MyHC-pn</td>
<td>MYH8</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>MyHC-emb</td>
<td>MYH3</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>fTnT</td>
<td>TNNT3</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>NCAM</td>
<td>NCAM1</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Myogenin</td>
<td>MYOG</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Vimentin</td>
<td>VIM</td>
<td>(+)</td>
<td>+</td>
</tr>
</tbody>
</table>

Protein expression: (+), in <1% of highly atrophic fibers; +, in 1–10%; ++, in 10–50%; ++++, in >75%. The results indicate how many fibers of the highly atrophic fibers pool expressed each given antigen in DM2 (n = 20) and DM1 (n = 5) muscle biopsies.
In DM2 muscle biopsies 273 alternative spliced exons in 218 genes were identified. Many of these splicing events had been previously described as deregulated either in DM1 or DM2 or both. A subset of alternative splicing events were validated by qPCR in biceps brachii biopsies from 19 DM2 and 15 CTR age and sex matched patients.
Alternative splicing

Perfetti et al., 2014

The affected genes are involved in numerous pathways and networks important for muscle physio-pathology, suggesting that the identified variants may contribute to DM2 pathogenesis.

of particular interest
Skeletal and Muscular Disorders
Neurological Diseases
Cell Death and Survival
Cellular Development
Calcium signaling
Cardiac Arrhythmia
both in DM1 and DM2
it is clear that MBNL1 is depleted from nucleoplasm through recruitment into ribonuclear inclusions even when clinical symptoms and muscle alterations are very mild

CUGBP1 overexpression has been clearly demonstrated in DM1 but not in DM2 muscle conflicting data have been reported on the expression of CUGBP1 in DM2 human skeletal muscle
CUGBP1 is overexpressed in DM1 muscle biopsies however the increase is evident only in “classic” DM1 form where CUGBP1 overexpression is accompanied by a parallel increase of the amount of phosphorylated isoform.

In DM2 muscle biopsies a slight increase of the CUGBP1 protein levels is observed not related to an increase of protein phosphorylation.

CUGBP1 seems to play a role in classic DM1 more evidently than in DM2.
ZNF9/CNBP protein levels are significantly reduced in DM2 muscle biopsies compared to DM1 and non-diseased biopsies.

ZNF9/CNBP expression might play a role in phenotypic differences between DM1 and DM2.
MiRNA profiling identified 20 miRNAs significantly modulated in DM2 muscle compared to CTR. Validation by more sensitive and specific qPCR assays identified 11 deregulated miRNAs. MiRNA score allowed to discriminate DM2 patients from CTR with a good sensitivity and specificity.

miR-193b-3p, miR-208a and miR-381 showed a similar significant modulation also in DM1 patients.
a repeat expansion mutation can produce potentially toxic RNA and protein products expressed through a combination of:

- bidirectional transcription
- ATG-initiated translation
- repeat associated non-ATG (RAN) translation

RAN translation of the expanded repeat results in the expression of up to six distinct RAN proteins.
In DM1 mouse model polyGln nuclear aggregates
- Cardiac myocytes
- Leukocytes
- Myoblasts
- Skeletal muscle

RAN translation has been demonstrated also in DM2
A terta-repeat expansion protein is produced
poly-Leu-Pro-Ala-Cys (LPAC)

DM2 brain poly-Leu-Pro-Ala-Cys (LPAC) nuclear aggregates in:
- Neurons
- Astrocytes
- Glia of frontal cortex
- Hippocampus
- Basal ganglia

Non-ATG-initiated translation directed by microsatellite expansions
Zu et al., 2010
DM myoblasts have lower proliferative capability than control myoblasts and reach *in vitro* senescence earlier than controls.

**However** differently from DM1, the p16 pathway is not responsible for the premature growth arrest observed in DM2 myoblasts which stop dividing with telomeres shorter than controls.

These data could explain the different histological alterations observed between DM1 and DM2 skeletal muscle as for example the selective type 2 fiber atrophy present in DM2 muscle.
Myotonia

In DM2 patients:

- usually is less severe than in DM1 patients
- sometimes may be difficult to reveal even with EMG

however

in several DM2 patients it can be very severe

in a cohort of 45 genetically confirmed DM2 patients 4/45 patients (8.89%) presented a severe or early onset myotonia.

The genetic analysis of *CLCN1* and *SCN4A* revealed that

- 2 patients showed a recessive mutation in *CLCN1* gene
- 2 patients showed a mutation in *SCN4A* gene
Cardani et al., 2012

A 15-year-old DM2 patient and her mother were studied to further investigate the unusually young onset in this DM2 family.

- The age at onset was earlier in the daughter than in the mother.
- The daughter’s clinical, histopathological and biomolecular findings did not show greater severity than those observed in her mother.

**HOWEVER**

Daughter presented handgrip myotonia at the age of 14 years.


The co-segregation of DM2 with a recessive CLCN1 mutation provided the explanation for the unusual clinical findings.
A 26 year old patient complaining of hand cramps and difficulty relaxing her hands after activity was evaluated.

Genetic testing was positive:
- for DM2 (2650 CCTG repeat)
- for a variant c.215C>T (p.Pro72Leu) in the SCN4A gene

The variation affects the cytoplasmic N-terminus domain of Nav1.4, where mutations have never been reported.

Electrophysiological studies of the P72L variant:

If CLCN1 screening is negative, this case supports for screening SCN4A mutations in DM2 patients with atypical cases with severe myotonia.

increase cell excitability
# Myotonic dystrophies management

**DM1**

| Brain                  | Psychological, educational, and counseling evaluations as needed  
|------------------------|---------------------------------------------------------------------  
|                        | Structural imaging as required                                      ।  
|                        | Routinely assess for sleep disturbances and respiratory insufficiency |  
| Heart                  | Yearly electrocardiograms                                            ।  
|                        | Cardiology consultation for symptomatic patients and long-term follow-up care |  
| Respiratory            | Serial monitoring of sitting and supine respiratory function; including forced vital capacity |  
|                        | Polysomnography and pulmonary medicine consultation as required |  
| Anesthesia             | Before elective surgery, have anesthesia consultation and pulmonary medicine evaluation |  
|                        | ECG reviewed by cardiology consult                                  ।  
|                        | Discuss known risks and any previous anesthesia related problems    |  

**DM2**

| Brain                  | Psychological, educational, and other counseling treatment and services |  
|------------------------|------------------------------------------------------------------------  
|                        | CNS medications (for example, stimulants) as necessary under close supervision of care providers |  
| Heart                  | Prompt pacemaker placement as needed                                    |  
|                        | Yearly immunizations                                                    |  
|                        | Noninvasive or invasive ventilation as required                          |  
|                        | Serial evaluation by pulmonary medicine and sleep consultation as required |  
| Respiratory            | Use of regional anesthesia over general when appropriate                |  
|                        | Use of non-depolarizing muscle relaxants                                 |  
|                        | Reduce use of opioids                                                   |  
| Anesthesia             | In general anesthesia, protection of the airway and minimizing aspiration, careful cardiac monitoring, and extensive postoperative monitoring (at least 24 hours) |
# Myotonic dystrophies management

## DM1

**Hypersomnia and fatigue**
- Polysomnograms
- Metabolic and endocrine screens
- Psychological, educational, and sleep consultant evaluations

**Endocrine**
- Symptomatic assessment of testosterone deficiency
- Yearly lipid profile, thyroid screening, diabetes screening
- Monitor sleep disturbances

**Gastrointestinal**
- Occupational and physical therapy consultation (dysphagia)
- Metabolic and endocrine screens
- Dietician, gastrointestinal consultations
- Careful assessment of bloating and signs of pseudo-obstruction

**Pregnancy**
- Obtain obstetrician and genetic consultation prior to pregnancy as appropriate
- Discuss possible complications
- Coordinate monitoring of pregnancy with other care providers, including a neonatal pediatric specialist
- Closely monitor respiratory function during the third trimester

## DM2

- Use of continuous positive airway pressure (CPAP) or bilevel positive airway pressure (BiPAP)
- Use of CNS stimulants

- Hormone replacement as required
- Dietary intervention
- Medications for lipid and glucose control as needed
- Treatment for sleep disturbances as required

- Gastroesophageal reflux may be treated with avoidance of late-night meals, elevation of the head of the bed, and medications
- Constipation, diarrhea, abdominal pain, and bloating may be treated with modifying the diet to small, low-fat meals
- Surgery as appropriate for gall bladder disease
- Use of cholestyramine may help alleviate diarrhea
- Antibiotics may be helpful in treating dyspepsia and other gastrointestinal complaints

- During delivery, monitor mother’s ECG
- Use regional anesthesia
- Notify consultants of mother’s status and request urgent evaluations as necessary
Most of the molecules identified resulted to be toxic in cellular assays

Selective inhibition of MBNL1–CCUG interaction by small molecules toward potential therapeutic agents for myotonic dystrophy type 2 (DM2)
Chun-Ho Wong, Yuan Fu, Sreenivasa Rao Ramisetty, Anne M. Baranger* and Steven C. Zimmerman*
2011

Small Molecules that Target the Toxic RNA in Myotonic Dystrophy Type 2
Lien Nguyen, JuYeong Lee, Chun-Ho Wong, and Steven C. Zimmerman*[a]
2014

Structure of the Myotonic Dystrophy Type 2 RNA and Designed Small Molecules That Reduce Toxicity
Jessica L. Childs-Disney*, Ilyas Yildirim*, HaeJeung Park*, Jeremy R. Lohman*, Lirui Guan*, Tuan Tran*, Partha Sarkar*, George C. Schatz*, and Matthew D. Disney*
2014

RNA level: small molecules
Take home message
The enormous advances in the understanding of the molecular pathogenesis of DM1 and DM2 has revealed pathways of molecular pathogenesis more complex than previously appreciated. However, the basis for the differences between DM1 and DM2 has not been clarified at the molecular level, important for the development of effective therapies.
Thanks to.....

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.....and to patients and their families