High-Yield Muscular Dystrophy Pathology

Karra A. Jones, MD, PhD
Clinical Associate Professor of Pathology
Division of Neuropathology
The University of Iowa

@BrainIsThePath
@UIPathology

AMERICAN ASSOCIATION OF NEUROPATHELOGISTS

The University of Iowa
Disclosures

• I have the following relevant financial relationships to disclose
  – Consultant
    • Audentes Therapeutics/Astellas Gene Therapies
      (Work not related to the content of this talk)
Learning Objectives

• Examine common proteins involved in muscular dystrophies and recognize dystrophic histopathologic features
• Compare and contrast immunostaining patterns in Duchenne versus Becker muscular dystrophies
• Summarize the molecular genetic basis of dystroglycanopathies and the tools helpful for diagnosis
• Identify unique histopathologic findings of dysferlinopathies
Muscular dystrophies in 1 hour?

Common

Pitfalls to avoid

Tips for diagnosis

Unique

1. Dystrophinopathies
2. Emery-Dreifuss Muscular Dystrophy
3. Myotonic Dystrophy
4. Limb Girdle Dystrophies
5. Facioscapulohumeral Dystrophy and Scapuloperoneal Syndrome
6. Bethlem Myopathy
7. Oculopharyngeal Muscular Dystrophy
8. X-Linked Muscular Dystrophy
9. Distal Muscular Dystrophies
10. Myofibrillar Myopathies
11. Congenital Muscular Dystrophies
12. Cardiomyopathies Associated with Muscular Dystrophies
DYSTROPHY

dys = bad/faulty
trophe/trophia = nourishment

a wasting of body tissues, of genetic origin (as we now know), or due to inadequate or defective nutrition (what was originally postulated)
What are muscular dystrophies?

• **Myopathies** = disease characterized clinically by muscle weakness
  – Muscular dystrophies are a subset of myopathies characterized physiologically/pathologically by repeated cycles of myonecrosis and regeneration

• Genetic (mostly inherited) muscle disorders

• Gene alterations → protein alterations → muscle disease

• Proteins located in many myofiber compartments are involved in muscular dystrophies
  – Reticular lamina and basal lamina (a.k.a. basement membrane), sarcolemma (a.k.a. plasma membrane), subsarcolemma, cytoskeleton, myofibrils, nuclei, Golgi, lysosomes, etc.
How do we classify (and keep straight!) muscular dystrophies?

1. Age

<table>
<thead>
<tr>
<th>Type of MD</th>
<th>Age at onset</th>
<th>Age at death</th>
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</thead>
<tbody>
<tr>
<td>Becker</td>
<td>Birth, Childhood</td>
<td>Early childhood, Adolescence</td>
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<tr>
<td>Congenital</td>
<td></td>
<td>Childhood, Young adult, Adulthood</td>
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<tr>
<td>Distal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duchenne</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emery-Dreifuss</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facioscapulohumeral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limb girdle (Type 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limb girdle (Type 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myotonic (Congenital)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myotonic (Adult onset)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oculopharyngeal</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
How do we classify (and keep straight!) muscular dystrophies?

2. Pattern of inheritance

**X-linked inheritance**

- Duchenne and Becker MD
- Emery-Dreifuss MD

**Autosomal recessive muscular dystrophies:**
- LGMD
- Congenital MD
- Distal MD

**Autosomal dominant muscular dystrophies:**
- LGMD
- Facioscapulohumeral MD
- Distal MD
- Myotonic dystrophy
- Oculopharyngeal MD

How do we classify (and keep straight!) muscular dystrophies?

3. Muscle groups

A. Duchenne and Becker MD
B. Emery-Dreifuss MD
C. Limb girdle MD
D. Facioscapulohumeral MD
E. Distal MD
F. Oculopharyngeal MD
How do we classify (and keep straight!) muscular dystrophies?

4. Genes/proteins and 5. Compartment of muscle involved

### A note on nomenclature

- LGMD nomenclature recently changed – European consensus
- Helpful to use the protein’s name:
  - e.g. dystroglycanopathies, dystrophinopathies, sarcoglycanopathies, dysferlinopathy, etc

<table>
<thead>
<tr>
<th>Old name</th>
<th>Gene</th>
<th>Proposed new nomenclature</th>
<th>Reason for exclusion</th>
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<tr>
<td>LGMD 1A</td>
<td>Myot</td>
<td>Myofibrillar myopathy</td>
<td>Distal weakness</td>
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<tr>
<td>LGMD 1B</td>
<td>LMNA</td>
<td>Emery–Dreifuss muscular dystrophy (EDMD)</td>
<td>High risk of cardiac arrhythmias; EDMD phenotype</td>
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<tr>
<td>LGMD 1C</td>
<td>CAV3</td>
<td>Rippling muscle disease</td>
<td>Main clinical features rippling muscle disease and myalgia</td>
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<tr>
<td>LGMD 1D</td>
<td>DNAJB6</td>
<td>LGMD D1 DNAJB6-related</td>
<td>True linkage</td>
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<tr>
<td>LGMD 1E</td>
<td>DES</td>
<td>Myofibrillar myopathy</td>
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<td>CAPN</td>
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<td>CAPN</td>
<td>LGMD D4 calpain3-related</td>
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<td>DYSF</td>
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<td>SGCG</td>
<td>LGMD R2 dysferlin-related</td>
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<td>LGMD 2D</td>
<td>SGCA</td>
<td>LGMD R3 α-sarcoglycan-related</td>
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<td>SGCβ</td>
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<td>SGCD</td>
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<td>TCAP</td>
<td>LGMD R7 teletolin-related</td>
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<td>LGMD R10 titin-related</td>
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<td>DAG1</td>
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<td>PLEC</td>
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<td>TRAPPC11</td>
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<td>GMPPB</td>
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<td>LGMD 2U</td>
<td>ISPD</td>
<td>LGMD R21 ISPD-related</td>
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<td>LGMD 2V</td>
<td>GAA</td>
<td>Pompe disease</td>
<td>False linkage</td>
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<tr>
<td>LGMD 2W</td>
<td>PINCH2</td>
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<td>Reported in one family</td>
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<td>LGMD 2X</td>
<td>BVES</td>
<td>BVES related myopathy</td>
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<td>LGMD 2Y</td>
<td>TOR1AIP1</td>
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<td>LGMD 2Z</td>
<td>POGLUT1</td>
<td>POGLUT1 related myopathy</td>
<td>Reported in one family</td>
</tr>
</tbody>
</table>

*Straub et al. Neuromuscul Disord. 2018. PMID: 30055862*
What do all muscular dystrophies have in common?

• **DYSTROPHIC PATHOLOGY!**  
  – Myonecrosis and regeneration

• Satellite cells are important
Dystrophic pathology = acute coagulative necrosis → myophagocytosis → satellite cell proliferation → regeneration

Pitfall - this pathology occurs in varying degrees of severity!
Basophilia = regeneration

Embryonic myosin heavy chain
Grouped regeneration is a clue!
Dystrophin-glycoprotein complex

- Dystrophin
  - Duchenne/Becker muscular dystrophy (DMD/BMD)
  - actin

- Sarcoglycan complex
  - LGMD2C/R5
  - LGMD2D/R3
  - LGMD2E/R4
  - LGMD2F/R6

- Merosin-deficient CMD (MDC1A)

- Laminin-α2 (merosin)

- FCMD, MEB, WWS
  - MDC1C, MDC1D

- MDC1C, MDC1D

- Basal lamina

- Extracellular
  - Intracellular

- Sarcolemma
Duchenne and Becker muscular dystrophies – *DMD* gene – X-linked recessive disorders

- **Duchenne MD**
  - Most common and prototypical MD
  - Translational reading frame lost
    - Out-of-frame deletions, duplications, mutations, rearrangements
    - *no* dystrophin protein expressed
  - Lose the ability to ambulate independently before the age of ~14
  - CK 10-50x normal
  - Proximal muscle weakness (LG pattern)

- **Becker MD**
  - Milder allelic variant of DMD
  - Translational reading frame maintained
    - In-frame deletions, duplications, or mutations
    - Reduction in amount, alteration in size, change in expression of protein
  - Maintain independent ambulation longer than DMD patients, but there is a continuum
Age in wheelchair full time

DMD  Intermediate  BMD

12 yrs  16 yrs

Amount of functional dystrophin
When are muscle biopsies done for the diagnosis of DMD/BMD?

• ~95% of cases are diagnosed by genetic testing

• BUT
  – Sometimes genetic testing isn’t diagnostic
    • If only deletion/duplication testing is performed and not sequencing
    • If a variant of uncertain significance (VUS) is called in the DMD gene
  – Sometimes DMD/BMD isn’t suspected clinically
    • Older patients and manifesting female carriers
  – Sometimes the clinical presentation doesn’t line up with the suggested molecular changes
    • DMD variant predicted to give a severe DMD phenotype, but patient’s clinical presentation is more concerning for BMD
Classic DMD

Images courtesy of Steve Moore
Commonly used anti-dystrophin antibodies can miss some BMD patients, if the in-frame deletion has a minimal affect on protein expression.

Adapted from Anderson "Dystrophinopathies" (2002) by Yvonne Kobayashi

Other anti-dystrophin antibodies can be added to pick up deletions in “hot spot” regions.

Illustrated here are antibodies to exons 46 and 50.

Immunostaining for diagnosis of BMD

- BMD deletions
- BMD/DMD deletions
- nNOS anchor point
- “d” duplication

Adapted from Anderson “Dystrophinopathies” (2002) by Yvonne Kobayashi
Pitfall - when DYS1, DYS2, and DYS3 are not enough

- 55-year-old man with mild neck flexion and extension weakness and cardiac arrhythmias that began in his early 40s
- Clinical concern for myotonic dystrophy type 2
- Gene DX cardiomyopathy panel:
  - *DMD* VUS that over the course of being worked up was changed to a benign variant
- Whole exome sequencing:
  - *SYNE1* heterozygous VUS (AR or AD; Emery-Dreifuss muscular dystrophy, spinocerebellar ataxia)
  - *TTN* heterozygous VUS (AR and AD; LGMDR10/2J, myofibrillar myopathy-HMERF, tibial muscular dystrophy, core myopathy, centronuclear myopathy)
### Summary

<table>
<thead>
<tr>
<th>HGVS</th>
<th>NM_004006.2.c.7183G&gt;A (Click here to check variant at Mutalyzer)</th>
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<tbody>
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<td>Genomic location (GRCh38)</td>
<td>ChrX:31836735C&gt;T</td>
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<tr>
<td>Genomic location (GRCh37)</td>
<td>ChrX:31854852C&gt;T</td>
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<td>Mutation type</td>
<td>Point mutation</td>
</tr>
<tr>
<td>Exon number(s)</td>
<td>49</td>
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<tr>
<td>Domain(s)</td>
<td>Central rod domain: Repeat 19</td>
</tr>
<tr>
<td>Length of mutated sequence</td>
<td>1 nucleotide(s)</td>
</tr>
<tr>
<td>Predicted consequence</td>
<td>Missense p.(Ala2395Thr)</td>
</tr>
<tr>
<td>Therapies Available or In Development</td>
<td>Not currently - Please see 'Therapies' tab</td>
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</table>

### Splicing Motifs

<table>
<thead>
<tr>
<th>Motif</th>
<th>Scoring used</th>
<th>Type</th>
<th>Relative change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon Splice Enhancer (ESE)</td>
<td>Rescue-ESE</td>
<td>None</td>
<td>Disruption of ESE</td>
</tr>
<tr>
<td>ESEFinder</td>
<td></td>
<td></td>
<td>No motifs significantly changed</td>
</tr>
<tr>
<td>Exon Splice Silencer (ESS)</td>
<td>Fas-ESS-Hexamers</td>
<td>NA</td>
<td>Mutation creates a novel ESS motif</td>
</tr>
</tbody>
</table>

### In Silico Predictions

Changes to splice regulatory element(s) predicted
Please check the 'In Silico Predictions' for more details

### ClinVar

Variant not found in ClinVar via myVariant.info, however, please click here to search ClinVar directly
Dystrophin antibodies

DYS2 carboxy

Missense mutation Exon 49

- Exon 49
- Exons 48-50
- Exon 47
- Exon 46
- Exons 45-46
- Exons 43-45
- Exons 41-43
- Exons 26-30

DYS1 exons 26-30

DYS3 exons 10-12

Patient control
Dystrophinopathy take-home points

• Very common
• You will see unexpected dystrophinopathy biopsies despite advances in genetic testing
  – Can include much older patients and female manifesting carriers
• Classic DMD characterized by total loss of dystrophin protein expression with revertant fibers, utrophin expression, and nNOS loss
• While DYS1, DYS2, and DYS3 are good screening antibodies, you will miss a high percentage of BMD cases
DYSTROGLYCANOPATHIES
Duchenne/Becker muscular dystrophy (DMD/BMD)

Dystrophin-glycoprotein complex

Dystrophin

extracellular
sarcolemma
intracellular

LGMD2C/R5

LGMD2D/R3

LGMD2E/R4

LGMD2F/R6

LGMD2I/R9 and many others

Dystroglycan complex

fascia
sarcolemma

FCMD, MEB, WWS
MDC1C, MDC1D

Merosin-deficient CMD (MDC1A)

laminin-α2 (merosin)

CMD (MDC1A)

basal lamina

Dystrophies with CNS involvement

actin

slid courtesy of Steve Moore
glycosylation of alpha-dystroglycan

ER
- TRAPPC11 (ER trafficking)
- GMPPB
- GDP-Man
- DPM1, DPM2, DPM3
- DOLK
- B3GALNT2
- POMGNT2
- POMT1/2
- POMK
- Man-P
- galactosylation
- GlcNAc

Golgi
- FGTN
- FKRP
- B4GAT1
- POMK
- POMT1/2
- CTP
- CRPPA (ISPD)
- CDP-ribitol
- phospho-ribitol
- xylose glucuronate
- LARGE
- laminin binding glycan domain (matriglycan)

α-dystroglycan

slide modified from original by Toby Willer
FKRP Phenotypic Variability

**Severity**

- **Walker-Warburg Syndrome (WWS)**
  - Met1Val (homozygous)\(^1\)
  - Cys318Tyr (homozygous)\(^2\)

- **Muscle-Eye-Brain Disease (MEB)**
  - Tyr307Asn (homozygous)\(^2\)

- **Congenital Muscular Dystrophy (MDC1C)**
  - Ile478Thr (homozygous)\(^3\)
  - Pro315Thr (homozygous)\(^3\)
  - Ser221Arg (homozygous)\(^3\)
  - Asn463Asp (homozygous)\(^5\)

- **Limb-Girdle Muscular Dystrophy (LGMD2I/R9)**
  - Leu276Ile (homozygous – common mutation)\(^4\)
  - Arg339Leu (homozygous)\(^4\)

**Age of onset**

Common dystroglycanopathy pathology

- 26-year-old man with 6 years of progressive proximal muscle weakness
- CK >5000 U/L
- DMD deletion/duplication testing and sequencing normal.
Dystroglycanopathies have a LOT of phenotypic variability!

Genetic testing revealed homozygous variants in *FKRP (c.826C>A) – the “common mutation”*
Glycosylated alpha-dystroglycan (IIH6) in peripheral nerve
Western blot
Dystroglycanopathies take-home points

• Widely variable clinical and pathologic findings ranging from mild to severe
  – Even within the same gene!
• Pathophysiology involves abnormal glycosylation of alpha-dystroglycan
  – To diagnose you need to look at a glycosylation specific antibody
  – Pitfall - both alpha-DG protein and beta-DG protein staining will be mostly, if not entirely, normal
• Western blotting can help in cases with difficult to interpret immunostaining
DYSFERLINOPATHY
Limb-Girdle Muscular Dystrophies (LGMD)

- LGMD R6/2F
- LGMD R4/2E
- LGMD R3/2D
- LGMD R5/2C

Congenital Muscular Dystrophies (CMD)

- merosin-deficient CMD (MDC1A)
- FCMD, MEB, WWS, and milder CMDs
- LGMD R9/2I and several other LGMDs
- LGMD R5/2C
- LGMD R3/2D
- LGMD R4/2E
- LGMD R6/2F

Dystrophin

- Dysferlin
- LGMD R2/2B
- Miyoshi myopathy

Endomysial extracellular matrix

- collagen VI
- Ullrich CMD (UCMD)
- basal lamina
- sarcolemma

Dysferlin

- dystrophin
- sarcoglycan complex
- dystroglycan
- LGMD R1/2A

Duchenne/Becker Muscular Dystrophy (DMD/BMD)

- calpain-3 (cytoplasmic/sarcomeric)

Merosin-deficient CMD (MDC1A)

- laminin-α2 (merosin)
- FCMD, MEB, WWS, and milder CMDs
- LGMD R9/2I and several other LGMDs

Sarcoglycan complex

- sarcolemma
- LGMD R1/2A

LGMD R3/2D

- LGMD R4/2E
- LGMD R5/2C

Actin

- nNOS
- LGMD R1/2A
Dysferlinopathy – *DYSF* gene – autosomal recessive

- Protein localized to the sarcolemma
  - Important for membrane repair
- Clinical phenotype variable
  - LGMD 2B/R2
  - Miyoshi myopathy (distal)
- Unique histopathologic findings for a muscular dystrophy
Pitfall – nonspecific sarcoplasmic staining
Dysferlin (Hamlet) and calpain-3 (12A2) Western blot

- dysferlin
- calpain-3 (94kd)
- calpain-3 degradation products

Image and WB by Steve Moore and Mary Cox
Dysferlinopathy and inflammation

• Multiple papers have reported perivascular or endomysial inflammation as being a recurrent finding in dysferlinopathy biopsies
Inflammation OK, but how much?

Dysferlinopathy and complement

Dysferlinopathy

IBM

Complement C5b-9

- Negative
- Focal
- Diffuse

DYSF, BMD, CAPN3, DM, IBM

Anoctaminopathy (ANO5) and laminopathy (LMNA) share features with dysferlinopathy

- ANO5 and LMNA disease can also show complement C5b-9 deposition along myofibers (Iowa experience)
- ANO5 has also been reported to show amyloid deposition

Liewluck et al. Eur J Neurol. 2013. PMID: 23663589
Dysferlinopathy take-home points

• Proximal or distal phenotypes
• Total loss of dysferlin expression is diagnostic of disease
  – Loss of sarcolemmal positivity with increased sarcoplasmic positivity is NONSPECIFIC and can be a pitfall
• While inflammation can be seen in dysferlinopathies, it is seen at a level comparable to other MDs and less than expected in myositis
• Complement C5b-9 myofiber deposition is very common in dysferlinopathies and uncommon in other MDs
  – Also a shared feature with ANO5 and LMNA disease
• Amyloid deposition is a unique feature of dysferlinopathies
  – Also shared with ANO5 disease
Unknown case:

- 72-year-old man
- Multi-year history of gradually progressive proximal muscle weakness
- CK elevated while on statin, taken off statin, and CK remained elevated at >10,000 U/L
- Clinical concern for an immune-mediated necrotizing myopathy or myositis
Unknown case – dystrophin immunostaining

Control

DYS1 equivalent exons 27

DYS2 equivalent carboxy terminus

DYS3 equivalent exons 10-12

Patient
Unknown case – dystrophin immunostaining

Control

- exon 50
- exons 48-50
- exon 47
- exon 46
- exon 43

Patient

- exon 50
- exons 48-50
- exon 47
- exon 46
- exon 43

Area of suspected DMD variant
Case solved – strong implications for the patient and his family

- Genetic testing revealed deletion of exons 45-48

**LOVD exonic deletions/duplications reading-frame checker**

The predictions are based on direct translation of the mRNA, which is generated by deletion / insertion (duplication). Confirmation on RNA level (experimental evidence), this prediction does not provide certainty and cannot be used with changes on RNA level. For example, on RNA-level more exons might be missing because signals required for yielding newly recognized exons incorporated in the mRNA.

Currently viewing gene/transcript: **DMD / NM_004006.2**

<table>
<thead>
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<th>Deletion or Duplication</th>
<th>From exon</th>
<th>To exon</th>
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<tbody>
<tr>
<td>Deletion</td>
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<td>48</td>
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</table>

Deleting exon 45 to exon 48 leads to... an IN-FRAME deletion.

According to the DMD_NM_004006.2 reference sequence in the LOVD database, the HGVS notation of this deletion is: ex45ex48del -> c.6439-7_7098+7del -> c.(6439+1_6439-1)_7098+1_7099-1]del

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Questions?
Useful references


