What we know about the biology and variability of Duchenne

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June 28, 2018
Disclosures

• Site principal investigator for PTC Therapeutics, Prosensa, Abeona Therapeutics, Akashi, and the NIH FOR-DMD study; site co-investigator for Sarepta

• Advisory boards for Sarepta, PTC, Audentes, Eli Lilly, 4D Therapeutics, and Dynacure
Dystrophinopathies:
Duchenne and Becker muscular dystrophies

- Duchenne muscular dystrophy (DMD):
  - Onset age 3-5
  - Pelvic girdle weakness
  - Tight heel cords
  - CK 50-100X normal
  - Loss of ambulation by age 12 (range 7-12)
  - Death by age 20 (historically)

- Becker muscular dystrophy (BMD):
  - Classic definition: loss of ambulation > age 12
  - Alternatively:
    - “intermediate muscular dystrophy” for loss of ambulation ages 12 through 15
    - BMD for loss of ambulation > age 15
  - Limb-girdle syndromes in adulthood
  - Muscle aches (myalgias)
  - Isolated cardiomyopathy
X-linked Recessive Inheritance

- 1/3 of cases occur de novo – that is, as a brand new mutation
Roberts, Genome Biology, 2001
DMD pathology

Absence of dystrophin

Activity induced damage

Influx of calcium

Hypercontraction
Mitochondrial damage
Activation of proteases

Necrosis

Inflammation and fibrosis

Loss of cell signaling

Oxidative stress
Nitrosylation

Loss of strength

Failure of regeneration

Dominick Wells,
Royal Veterinary College
Dystrophin Mutations

- Dystrophin gene (Xp21.1) is huge:
  - 2.4 million nucleotides
  - 79 exons and 8 promoters

- Large deletions (≥ 1 exon) account for ~65% of DMD/BMD patients

- ~5% have duplications
- ~15% of boys have nonsense mutations
- Remainder are frameshifting insertions/deletions, splice site mutations, missense mutations
Dystrophin mutations: Duchenne vs Becker

- **Size** of deletion does not correlate well with phenotype
- Best correlation is whether the deletion is “in-frame” or “out-of-frame”
- **In-frame deletions** are more likely to result in translation of a protein with partial function
  - (i.e., out-of-frame deletions are DMD ~90% of the time)
Roberts, Genome Biology, 2001
What accounts for patients who break the reading frame rule?

What accounts for variability among patients with DMD?
### Table 2. The Value of Mutational Reading Frame in Predicting a Phenotype of Duchenne Muscular Dystrophy

<table>
<thead>
<tr>
<th></th>
<th>DMD</th>
<th>I/BMD</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exonic deletions only</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Truncating (out-of-frame) mutations</td>
<td>254</td>
<td>32</td>
<td>88.8%</td>
<td>—</td>
<td>Positive predictive value</td>
<td>—</td>
</tr>
<tr>
<td>Non-truncating (in-frame) mutations</td>
<td>30</td>
<td>38</td>
<td>55.9%</td>
<td>—</td>
<td>Negative predictive value</td>
<td>—</td>
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<tr>
<td>Sensitivity</td>
<td>89.4%</td>
<td>—</td>
<td></td>
<td>54.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>—</td>
<td>44.4%</td>
<td></td>
<td>—</td>
<td></td>
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</tr>
<tr>
<td>All mutations</td>
<td></td>
<td></td>
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<tr>
<td>Truncating mutations</td>
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<td>79</td>
<td>86.8%</td>
<td>—</td>
<td>Positive predictive value</td>
<td>—</td>
</tr>
<tr>
<td>Non-truncating mutations</td>
<td>37</td>
<td>63</td>
<td>63.0%</td>
<td>—</td>
<td>Negative predictive value</td>
<td>—</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>93.3%</td>
<td>—</td>
<td></td>
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<tr>
<td>Specificity</td>
<td>—</td>
<td>44.4%</td>
<td></td>
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</tr>
</tbody>
</table>

45%-55% of BMD patients have out-of-frame mutations

Nonsense Mutations Do Not Always Predict DMD

- Mutations predicted as nonsense mutations may instead affect exon splice regulatory signals\(^1,2\)
  - This results in exclusion of exons
  - The remaining mRNA may be in-frame

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Distribution of BMD versus DMD nonsense mutations

p.Trp3X

In-frame exons (39) shaded
Out-of-frame (40) unshaded

patient count

exon

BMD
DMD
IMD

p = 0.004

DMD 176
I/BMD 26
A little bit of dystrophin goes a long way

- 10 year old boy
- Toe-walking at age 7 years
- Diagnosed at age 9 years
- Predicted nonsense mutation in exon 42

- 6 minute walk distance = 575 meters
  - 157% predicted for age matched DMD patients
  - Normal controls = 630 meters
- North Star Ambulatory Assessment Score = 30/34
A little bit of dystrophin goes a long way

3.2% of normal dystrophin level is sufficient to greatly modify the disease

c.6115A>T, p.Lys2039*
Other genes modify other pathways and can influence disease severity

- **Osteopontin** (*SPP1*)
  - Cytokine involved in immune cell migration and survival
  - Implicated in fibrosis through the TGF-beta pathway
- **Latent TGF-beta binding protein 4** (*LTBP4*)
  - Binds and sequesters TGFβ
  - Polymorphisms influence TGFβ activity
- **CD40**
  - Co-stimulatory protein involved in T helper cell polarization
  - Found on the surface of antigen-presenting cells
- **THBS1** (thrombospondin-1)
  - Upstream regulator of the TGFβ pathway
Takeaways
Takeaways

- DMD results from the absence of dystrophin
  - Mutations that allow a small amount of dystrophin can influence severity
- Not all nonsense mutations result in DMD
- We are learning about other genes that influence pathways important to disease progression
  - These may prove to be additional targets for interventional therapies
Questions?
Thank you!