Variable Dystrophin Content within Myofibers, between Myofibers, and between Regions of Muscles:

Determining the Level of Dystrophin that makes a Clinical Impact in BMD and Manifesting Carriers of DMD

Eric Hoffman
Children’s National Medical Center
Washington DC
Dystrophin in drug development

• Parallel independent effort of Charley’s Fund to address many similar questions

  • Will ‘riff off’ their themes with review of literature supporting answers to three questions

  • What does the literature say about the link between dystrophin expression and clinical outcomes?
  • What questions in general need to be answered for dystrophin to be validated as a surrogate endpoint?
  • Which might be real issues, but are not so critical that they can't be solved on more of a rolling basis?
What does the literature say about the link between dystrophin expression and clinical outcomes?

- Dystrophin loss of function is the cause of all dystrophinopathies – traditional names for groups
  - **Duchenne**
    - Little or no dystrophin (<3%)
  - **Female manifesting carriers of DMD**
    - Varying amounts of normal dystrophin
      - **Key variable**: % of normal vs abnormal genes ‘active’
  - **Becker**
    - Varying amounts of abnormal dystrophin
      - **Key variable**: Functionality of the protein (how abnormal?)
Dystrophin – clinical correlations

Duchenne

• 103 biopsies.
• Duchenne (n=40)
  • Loss of function
  • <3%

• Becker/Intermediate
  • Partial loss of function
  • More variable

<table>
<thead>
<tr>
<th>Dystrophin Data</th>
<th>Duchenne’s Dystrophy</th>
<th>Intermediate Dystrophy (Outliers)</th>
<th>Becker’s Dystrophy</th>
<th>Other Neuromuscular Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal size; 60–100% normal levels</td>
<td>1 (2.6)*</td>
<td>—</td>
<td>4 (22.2)*</td>
<td>38 (95.0)</td>
</tr>
<tr>
<td>Abnormal size; 40–100% normal levels</td>
<td>—</td>
<td>1 (14.3)</td>
<td>12 (66.6)*</td>
<td>2 (5.0)*</td>
</tr>
<tr>
<td>Normal size; 3–60% normal levels</td>
<td>1 (2.6)</td>
<td>4 (57.1)</td>
<td>1 (5.5)</td>
<td>—</td>
</tr>
<tr>
<td>Abnormal size; 3–40% normal levels</td>
<td>1 (2.6)</td>
<td>1 (14.3)</td>
<td>—</td>
<td>—</td>
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<tr>
<td>&lt;3% Normal level, or undetectable</td>
<td>35 (92.1)</td>
<td>1 (14.3)</td>
<td>1 (5.5)*</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>7</td>
<td>18</td>
<td>40</td>
</tr>
</tbody>
</table>

*The patients from whom these specimens were obtained were considered to represent unusual cases (see clinical histories in Methods).
Dystrophin-clinical correlations

Female carriers

- Two girls with phenotype intermediate between those of Duchenne's and Becker's dystrophy.
  - balanced X-chromosome translocation
    - 5 percent of normal
    - DMD-like
  - second girl had no detectable structural abnormality of her X chromosomes
    - Proximal weakness at the age of 5 years, ambulatory at age 12
    - 20 percent of normal
Dystrophin blots:
• 4 blots/patient
• vs. 2 Normal controls
• 8 measurements/patient
• Pre-flashed films; <5 s exp

% normal vs DMD genes
• Quantitated muscle
• Quantitated blood

N=19 patients
<table>
<thead>
<tr>
<th>Pt</th>
<th>Inheritance of dystrophin gene mutations</th>
<th>Karyotype</th>
<th>Age at biopsy</th>
<th>Clinical severity</th>
<th>Dystrophin immunoblotting (% of normal)</th>
<th>% of active X in blood (height)</th>
<th>% of active X in muscle (height)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P</td>
<td>46,X,X,+d0(X) t(X;?)q11(?)</td>
<td>2</td>
<td>Severe</td>
<td>18 ± 5</td>
<td>33%</td>
<td>55</td>
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<tr>
<td>2</td>
<td>M-F</td>
<td>46,X,q1q25-qter</td>
<td>4</td>
<td>Intermediate</td>
<td>3 ± 3</td>
<td>100%</td>
<td>75</td>
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<tr>
<td>3</td>
<td>P</td>
<td>46,X,t(X;12)</td>
<td>5</td>
<td>Mild</td>
<td>21 ± 5</td>
<td>24%</td>
<td>45</td>
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<tr>
<td>4</td>
<td>P</td>
<td>46,X,t(X;3)</td>
<td>6</td>
<td>Intermediate</td>
<td>4 ± 3</td>
<td>75%</td>
<td>90</td>
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<tr>
<td>5</td>
<td>M</td>
<td>46,XX</td>
<td>3</td>
<td>Mild</td>
<td>40 ± 15</td>
<td>37%</td>
<td>55</td>
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<tr>
<td>6</td>
<td>P</td>
<td>46,XX</td>
<td>4</td>
<td>Mild</td>
<td>32 ± 4</td>
<td>12%</td>
<td>70</td>
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<tr>
<td>7</td>
<td>M</td>
<td>—</td>
<td>8</td>
<td>Mild</td>
<td>24 ± 4</td>
<td>16%</td>
<td>75</td>
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<tr>
<td>8</td>
<td>M</td>
<td>46,XX</td>
<td>9</td>
<td>Severe</td>
<td>5 ± 3</td>
<td>60%</td>
<td>90</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>46,XX</td>
<td>10</td>
<td>Mild</td>
<td>3 ± 2</td>
<td>66%</td>
<td>80</td>
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<tr>
<td>10</td>
<td>P</td>
<td>46,XX</td>
<td>10</td>
<td>Intermediate</td>
<td>16 ± 9</td>
<td>56%</td>
<td>100</td>
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<td>11</td>
<td>P</td>
<td>46,XX</td>
<td>12</td>
<td>DMD-like</td>
<td>48 ± 10</td>
<td>21%</td>
<td>65</td>
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<tr>
<td>12</td>
<td>—</td>
<td>46,XX</td>
<td>20</td>
<td>Severe</td>
<td>76 ± 23</td>
<td>30%</td>
<td>60</td>
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<tr>
<td>13</td>
<td>P</td>
<td>46,XX</td>
<td>29</td>
<td>Severe</td>
<td>70 ± 12</td>
<td>17%</td>
<td>15</td>
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<tr>
<td>14</td>
<td>M-F</td>
<td>46,XX</td>
<td>47</td>
<td>Intermediate</td>
<td>54 ± 15</td>
<td>28%</td>
<td>75</td>
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</tbody>
</table>

**CV**

With 8 dystrophin immunoblot measures/sample

**Average CVs – range**

- **HLOQ (>20%)**: 30% range: 12%-37%
- **LLOQ (<20%)**: 65% range: 33% - 100%

**FDA guidance – CVs**

- **< 15% (at HLOQ)**
- **< 20% (at LLOQ)**
Determine % normal genes

Use blood as ‘initiation point’

Use muscle as ‘set point’

Correlate with Dystrophin production
Evidence for failure of dystrophin production in dystrophin-competent myonuclei

In older, more dystrophic muscle –
- 30% less dystrophin from normal dystrophin genes
- The gene is there, but dystrophin protein is not
Normal dystrophin: How much is enough?

Biochemical normalization
• Dystrophin diffusion in myofiber
• 2-fold increase
• Why CKs decrease

Genetic normalization
• Degen of (-) regen by (+)
• 3-fold increase
• Why manifesting carriers can improve

Failure of dystrophin production in end stage muscle
• About 30% less dystrophin than expect

How much is enough?
• Obvious sampling error
• In older more severe muscle, only dystrophin-positive myofibers still remain
• Given caveats, dystrophin seems like most any other protein
  • Threshold <5% severe, >20% normal
Becker muscular dystrophy


97 patients (54 BMD): dystrophin-clinical correlations

Duchenne dystrophy
LoA ~11 years; dystrophin quantity less than 3% of normal

Severe Becker dystrophy
LoA 13 to 20 years; dystrophin 3% to 10%

Moderate/mild Becker dystrophy
LoA >20 years; dystrophin quantity greater than or equal to 20%
Exploring the Molecular Basis for Variability among Patients with Becker Muscular Dystrophy: Dystrophin Gene and Protein Studies

Alan H. Beggs,* Eric P. Hoffman,* † Judith R. Snyder,* Kiichi Arahata,§ Linda Specht,† Frederic Shapiro,‡ Corrado Angelini,‖ Hideo Sugita,§ and Louis M. Kunkel*  

Table 2

<table>
<thead>
<tr>
<th>Exons Deleted or Duplicated</th>
<th>No. of Patients</th>
<th>Predicted Size&lt;sup&gt;a&lt;/sup&gt; (kD)</th>
<th>Observed Size&lt;sup&gt;a&lt;/sup&gt; (kD)</th>
<th>Average Quantity&lt;sup&gt;a&lt;/sup&gt; (%)</th>
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</thead>
<tbody>
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<td>del 2–7 ..................</td>
<td>1</td>
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<td>388</td>
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<td>382</td>
<td>380 ± 4</td>
<td>44 ± 17</td>
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<td>del 45–48 ...............</td>
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<td>375</td>
<td>377 ± 5</td>
<td>53 ± 32</td>
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<td>371</td>
<td>377 ± 5</td>
<td>30 ± 16</td>
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<tr>
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<td>3</td>
<td>345</td>
<td>367 ± 6</td>
<td>70 ± 36</td>
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<tr>
<td>del 48 ...................</td>
<td>1</td>
<td>393</td>
<td>400</td>
<td>100</td>
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<td>45 ± 7</td>
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<tr>
<td>del 48–51 ................</td>
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<td>90</td>
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<td>dup 2–7 ...................</td>
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<td>421</td>
<td>420 ± 0</td>
<td>40 ± 14</td>
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<tr>
<td>dup 13–42 ................</td>
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<td>580</td>
<td>600</td>
<td>70</td>
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<tr>
<td>dup 14–18 ................</td>
<td>2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>427</td>
<td>420 ± 0</td>
<td>15 ± 7</td>
</tr>
</tbody>
</table>

68 BMD patients studied DNA, biopsy, clinical correlations
Dystrophin levels and clinical severity in Becker muscular dystrophy patients

J C van den Bergen,¹ B H Wokke,¹ A A Janson,² S G van Duinen,³ M A Hulsker,⁴ H B Ginjaar,⁵ J C van Deutekom,² A Aartsma-Rus,⁴ H E Kan,⁶ J J Verschuuren¹
Looking at only ex45-47 deletion patients (17%-80%)
- Age – strength – fat in muscle well correlated
- Dystrophin % is not well correlated
Threshold effect for abnormal dystrophin: <10% - Severe disease

• “Although we found no relation between (high) dystrophin levels and disease severity”

• “Our four patients with dystrophin levels **below 10%**”
  - low MVIC sum scores
  - early onset of symptoms

• “This finding implies a **threshold effect**, which is confirmed by several previous clinical studies suggesting that dystrophin levels below 10% are indicative of a more severe disease course. (*references 12, 14, 16*)”
Based on what we know from BMD, animal models, etc., what assertions should we feel comfortable making, and what major questions still need to be answered?

• **Assertions:**
  
  • Normal dystrophin – manifesting female carriers
    • 0-3% dystrophin = Duchenne muscular dystrophy (in a girl)
    • 3-5% dystrophin = severe disease
    • 10-20% = mild disease
    • >20% = asymptomatic
  
  • Abnormal dystrophin – Becker muscular dystrophy (all dystrophin replacement strategies)
    • 0-3% = Duchenne muscular dystrophy
    • 3-15% = Severe Becker muscular dystrophy (LoA 16-20 yrs)
    • >15% = mild/moderate/asymptomatic Becker muscular dystrophy

• What major questions still need to be answered?
• What questions in general need to be answered for dystrophin to be validated as a surrogate endpoint?
• Which are true "roadblocks" or hurdles that would be a mistake not to solve/answer before a drug were to be granted approval off a dystrophin surrogate?

• Studies need replicates, determine CVs, report these
• Options going forward: CVs of methods
  • Bend the rules for relatively poor CVs of immunoblot or immunostaining
  • Qualification of mass spec (CVs in range)

Blots: 8 dystrophin immunoblots
Average CVs – range

<table>
<thead>
<tr>
<th></th>
<th>Mass spec</th>
<th>FDA guidance – CVs</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLOQ (&gt;20%): 30%</td>
<td>6% HLOQ</td>
<td>&lt; 15% HLOQ</td>
</tr>
<tr>
<td>LLOQ (&lt;20%): 65%</td>
<td>22% LLOQ</td>
<td>&lt; 20% LLOQ</td>
</tr>
</tbody>
</table>
• Which might be real issues, but are not so critical that they can't be solved on more of a rolling basis?

• **Acknowledgement of sampling error**
  • Muscle is largest organ system of the body
  • A 0.1 g biopsy is a tiny sampling
  • There is substantial variation within myofibers, between myofibers and between muscles

• Change discussion from
  • **Improved sensitivity for clinically insignificant levels of dystrophin**
  • To
  • **Accurate detection of variable but clinically significant amounts in a subset of patients.**
  • **Pharmacodynamic marker**

• **Research:** Determine why dystrophin is not being made by some normal nuclei, and what this may teach about
  • Variability in dystrophin in Becker
  • Variability in dystrophin in exon skipping