Understanding genetics, mutation and other details

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6/29/18
DMD is X linked recessive: mostly males are affected and females are carriers. Determining DNA mutation critical for family
Duchenne can progress differently in different boys

**Duchenne/Becker Genetic Modifier Study**

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**LTBP4 Genotype Predicts Age of Ambulatory Loss in Duchenne Muscular Dystrophy**

Kevin M. Flanigan, MD; Ermelinda Ceco, BS; Kay-Marie Lamar, BS; Yuuki Kaminoh, BS; Diane M. Dunn, BS; Jerry R. Mendell, MD; Wendy M. King, PT; Alix Pestonik, MD; Juliane M. Florence, DPT; Katherine D. Matthews, MD; Richard S. Finkel, MD; Kathryn J. Swoboda, MD; Eduard Gappmaier, PhD; Michael T. Howard, PhD; John W. Day, MD, PhD; Craig McDonald, MD; Elizabeth M. McNally, MD, PhD; and Robert B. Weiss, PhD for the United Dystrophinopathy Project

**Objective:** Duchenne muscular dystrophy (DMD) displays a clinical range that is not fully explained by the primary DMD mutations. CLIP, encoding a secreted growth factor binding protein 4, was previously discovered in a genome-wide scan as a modifier of murine muscular dystrophy. We sought to determine whether LTBP4 genotype influenced DMD severity in a large patient cohort.

**Results:** Individuals homozygous for the I4452_A4453 deletion had a mean age at loss of ambulation of 12.9 ± 3.3 years compared to 18.7 ± 2.1 years for I4452 homozygotes or heterozygotes. I4452/AS4453 haplotypes were associated with later age at loss of ambulation (p = 0.001). Former and current smokers had a lower age at loss of ambulation compared to non-smokers (p = 0.001). LTBP4 genotype was a predictor of age at loss of ambulation (p = 0.002). LTBP4 plays a role in extracellular matrix organization and may influence the age at loss of ambulation.

**Conclusions:** LTBP4 genotype influences age at loss of ambulation, and should be considered in the management of DMD patients.
Amount of dystrophin in muscle biopsy important.
Duchenne changes over time

Schematic Natural History of Duchenne Muscular Dystrophy
(Adapted from Bushby and Connor Clin Investig (Lond), 2011; McDonald et al. Muscle & Nerve 2013)

Prior to treatment 1960’s
- 5 Years
  - Loss of Standing
  - Loss of Ambulation
  - Loss of Self Feeding

- 9 Years
  - Loss of Standing
  - Loss of Ambulation
  - Loss of Self Feeding
  - Ventilation

Contemporary: with Steroids and Improved Cardiac Management
- 14 Years
  - Loss of Standing
  - Loss Ambulation
  - Loss of Self Feeding
  - Ventilation

- 20 Years
  - Loss of Ambulation
  - Ventilation

Steroids affect disease progression in DMD over the entire course of the disease prolonging clinically meaningful functions (time to loss of milestones)
The Duchenne Registry: online patient registry for Duchenne and Becker muscular dystrophy

• >3500 individuals in registry
• Newer format, multiple modules
• Behaviour and learning
• Bone
• Cardiac
• Corticosteroid (N=3044)
• Family history
• Genetic testing (N=1973)
• Muscle function (N=3383)
• Respiratory

Duchenne Registry: Example of Data use: deflazacort appears superior to prednisone
<table>
<thead>
<tr>
<th>Therapy</th>
<th>Probability of walking through age 12</th>
<th>N</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Current steroids alone</td>
<td>0.54</td>
<td>303</td>
<td>--------</td>
</tr>
<tr>
<td>+ Vitamin D</td>
<td>0.72</td>
<td>246</td>
<td>0.004</td>
</tr>
<tr>
<td>+ Calcium</td>
<td>0.68</td>
<td>207</td>
<td>0.07</td>
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<tr>
<td>+ Coenzyme Q10</td>
<td>0.74</td>
<td>155</td>
<td>0.007</td>
</tr>
<tr>
<td>+ Vitamin C</td>
<td>0.57</td>
<td>41</td>
<td>0.43</td>
</tr>
<tr>
<td>+ Vitamin E</td>
<td>0.73</td>
<td>28</td>
<td>0.06</td>
</tr>
<tr>
<td>+ Protandim</td>
<td>0.92</td>
<td>44</td>
<td>0.22</td>
</tr>
<tr>
<td>+ Creatine Monohydrate</td>
<td>0.75</td>
<td>33</td>
<td>0.10</td>
</tr>
<tr>
<td>+ Magnesium</td>
<td>0.63</td>
<td>21</td>
<td>0.75</td>
</tr>
<tr>
<td>+ Melatonin</td>
<td>0.58</td>
<td>21</td>
<td>0.39</td>
</tr>
<tr>
<td>+ L-Arginine</td>
<td>0.94</td>
<td>21</td>
<td>0.22</td>
</tr>
<tr>
<td>+ Green Tea Extract</td>
<td>0.75</td>
<td>17</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 2. Kaplan-Meier estimates of time to loss of ambulation. P values represent the log-rank
Do you need a muscle biopsy to diagnose Duchenne/Becker?

Usually ‘no’

Situations where it may be recommended

When mutation not clearly predictive of disease course
When no DNA mutation identified in DMD
Research of unusual and unexpected disease course:
   Out of frame mutation with more mild disease
   In frame mutation with more severe disease
   High variability in disease progression in a family

Needle biopsy viable option to open biopsy
2.5mb *DMD* gene in 79 exons encodes dystrophin protein
Reading frame of *DMD* 
Mutations in *DMD* causes Duchenne if out of frame and Becker MD if in frame (about 95% true)
Genetic Code translates DNA/RNA sequence into protein

<table>
<thead>
<tr>
<th>First Letter</th>
<th>Second Letter</th>
<th>Third Letter</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>TTT { Phe }</td>
<td>TTT</td>
</tr>
<tr>
<td></td>
<td>TTC { Leu }</td>
<td>TTC</td>
</tr>
<tr>
<td></td>
<td>TTA { Leu }</td>
<td>TTA</td>
</tr>
<tr>
<td></td>
<td>TTG { Leu }</td>
<td>TTG</td>
</tr>
<tr>
<td>C</td>
<td>CTT { Leu }</td>
<td>CTT</td>
</tr>
<tr>
<td></td>
<td>CCT { Pro }</td>
<td>CCT</td>
</tr>
<tr>
<td></td>
<td>CCA { Pro }</td>
<td>CCA</td>
</tr>
<tr>
<td></td>
<td>CGG { Pro }</td>
<td>CGG</td>
</tr>
<tr>
<td>A</td>
<td>ATT { Ile }</td>
<td>ATT</td>
</tr>
<tr>
<td></td>
<td>ATC { Met }</td>
<td>ATC</td>
</tr>
<tr>
<td></td>
<td>ATA { Met }</td>
<td>ATA</td>
</tr>
<tr>
<td></td>
<td>ATG</td>
<td>ATG</td>
</tr>
<tr>
<td>G</td>
<td>GTT { Val }</td>
<td>GTT</td>
</tr>
<tr>
<td></td>
<td>GTC { Val }</td>
<td>GTC</td>
</tr>
<tr>
<td></td>
<td>GTA { Val }</td>
<td>GTA</td>
</tr>
<tr>
<td></td>
<td>GTG { Val }</td>
<td>GTG</td>
</tr>
</tbody>
</table>

- Phe
- Leu
- Ser
- Tyr
- Stop
- Cys
- Stop
- Trp
- Pro
- His
- His
- Gin
- Arg
- Met
- Thr
- Asn
- Lys
- Asp
- Glu
- Glu
- Gly

Do not overlap this box with text. Video of presenter will display in this area for picture in picture. Please delete from Slide Master before presenting.
Gene structure is complex
Mutation types

Nonsense mutation

Original DNA code for an amino acid sequence.

DNA bases

C A G C A G C A G C A G C A G C A G C A G C A G C A G C A G

Amino acid

Replacement of a single nucleotide.

Protein

Incorrect sequence causes shortening of protein.
Mutation types: missense rarely cause disease

Missense mutation

Original DNA code for an amino acid sequence.

DNA bases

Original DNA code for an amino acid sequence.

Amino acid

Replacement of a single nucleotide.

Incorrect amino acid, which may produce a malfunctioning protein.
Small insertions change reading frame

Insertion mutation

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Original DNA code for an amino acid sequence.

DNA bases

\[ \text{CATCATCATCATCATCATCATCATCATCATCATCAT} \]

\[ \text{His His His His His His His His} \]

Amino acid

Insertion of a single nucleotide.

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 Incorrect amino acid sequence, which may produce a malfunctioning protein.
Small deletions also change reading frame

Deletion mutation

Original DNA code for an amino acid sequence.

DNA bases

His His His His His His His

Amino acid

Deletion of a single nucleotide.

CATCATCATCATCATCTCATCATCATCATCATC

His His His Leu Ile Ile Ile Ile

Incorrect amino acid sequence, which may produce a malfunctioning protein.
Splicing mutations disrupt reading frame and are usually in intron

- c.IVS12+1G>T (c.1482+1G>T)
- c.IVS12-1G>C (c.1483-1G>C)
Genetics 101: DNA encodes genes, they are transcribed to RNA, and RNA is translated into protein. The missing or mutant protein is the problem for genetic diseases. Over 5,000 genetic diseases found affecting 10’s of millions of people.

DMD gene is on X chromosome is mutated one in every 10,000 cell divisions, which is why Duchenne/Becker are among most common genetic diseases in humans.

The human genome is 3 billion bases long (GATC) and encoded within 23 pairs of chromosomes with a total of about 20,000 genes.
DNA mutations can predict disease severity

“Reading frame rule: about 95% accurate”

Out of frame
Large deletions (about 68%, most in region from exon 44-56 region hotspot)
  - Deletion of exon 46-51,
  - Deletion of exon 45-50
Large Duplications (exon 2) (about 10%, most in early part of gene exons 2-8)
  - Duplication exon 2
  - Duplication of exons 3-7

Many small mutations
Like Nonsense mutations are like ‘out of frame’
Mutations
# Types of mutation in DMD

<table>
<thead>
<tr>
<th>Mutation Types</th>
<th>Number</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large deletions (≥ 1 exon)</td>
<td>4,894</td>
<td>68</td>
</tr>
<tr>
<td>Large duplications (≥ 1 exon)</td>
<td>784</td>
<td>11</td>
</tr>
<tr>
<td>Small mutations</td>
<td>1,445</td>
<td>20</td>
</tr>
<tr>
<td>Small deletions (&lt;1 exon)</td>
<td>358</td>
<td>5</td>
</tr>
<tr>
<td>Small insertions (&lt;1 exon)</td>
<td>132</td>
<td>2</td>
</tr>
<tr>
<td>Splice sites (&lt;10 bp from exon)</td>
<td>199</td>
<td>3</td>
</tr>
<tr>
<td>Point mutations</td>
<td>756</td>
<td>11</td>
</tr>
<tr>
<td><strong>Nonsense</strong></td>
<td>726</td>
<td>10</td>
</tr>
<tr>
<td>Missense</td>
<td>30</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Mid-intronic mutations</strong></td>
<td>22</td>
<td>0.3 (may be higher) OFTEN NEED MUSCLE BIOPSY</td>
</tr>
</tbody>
</table>

Duchenne severity can depend on mutation in \textit{DMD} gene: example from Duchenne Registry

Wang R et al, Human Mutation, in press
Figure 1: Distribution of Age at Loss of Ambulation for steroid and non-steroid users with Duchenne (DuchenneConnect data, R. Wang)
Dozens of therapeutic approaches in trials for Duchenne

- FOR-DMD - Finding the Optimal steroid
  Dystrophy
- GALGT2 Gene Therapy - Viral gene therapy for Duchenne
- GENE TRANSFER OF MICRO-DYSTrophy
  Gene Transfer Trial of rAAVrh74.MCK
- Genetic Modifiers - Genetic Modifiers
  Dystrophy
- GIVINOSTAT (ITF2357) - A Histone D
  treatment of Duchenne
- HOPE - Heart/Emphysema ProgEs
- HT-100 - Akashi's Phase 1/2 Clinical F
  ImagingDMD - Magnetic Resonance I
  Dystrophy
- RTC13 Read-Through Compound - Development of a drug that corrects nonsense mutations in patients with Duchenne
- SPIRONOLACTONE and EPLERENONE - Therapeutic Potential for Aldosterone Inhibition in Duchenne
- SRP-4045/SRP-4053 (ESSENCE/4045-301) - A 96-week, Double-Blind, Placebo-Controlled, Multi-Center Study to Evaluate the Efficacy and Safety of SRP-4045 and SRP-4053 in Patients with Duchenne
- STEROIDS IN YOUNG BOYS - Historically Controlled Trial of Corticosteroids in Young Boys with Duchenne
- STRENGTH TRAINING - Development of a Strength Training Protocol in Duchenne
- TADALAFIL - A Randomized, Double-Blind, Placebo-Controlled, Phase 3 Trial of Tadalafil in Duchenne Muscular Dystrophy

Cardiopulmonary Function in Duchenne

- MTB-1 - MTB-1 mediated gene regulation
  Patients
- MYOB Last TRANSPLANTATION - N

WELLSTONE MRI/MRS BIOMARKERS - Failed Regeneration in the
Muscular Dystrophies

- NID Peptide - Using NF-kB blockers to Decrease Inflammation and Improve Muscle Function in Duchenne
- NS-065/NCNP-01 for exon 53 skipping - Safety and Dose Finding Study of NS-065/NCNP-01 in Boys With Duchenne
- PF-06252616 - Pfizer's Myostatin Inhibitor
- PhaseOut DMD - A 48-week Phase 2 Clinical Study to Assess the Activity and Safety of Utrophin Modulation with Ezutromid (Formerly Known as SMT C1100) in Ambulatory Boys with Duchenne
- RAXONE® - Phase 3 Study Assessing the Efficacy, Safety and Tolerability of Idebenone in Patients with Duchenne Muscular Dystrophy Receiving Glucocorticoid steroids (SIDEROS)
Summary

Knowing DMD mutation is important
DMD gene mutations help predict severity
Needle biopsy may be helpful
We do not yet know all genes that modify DMD
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Questions?
Thank you!