Duchenne muscular dystrophy

X-linked recessive
1/5000 male births
Common muscular dystrophy
Resp/cardiac failure

Age (years) at death

Duchenne/Becker
All others
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

![Genetic Code Table]

<table>
<thead>
<tr>
<th>First Letter</th>
<th>Second Letter</th>
<th>Third Letter</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>TTT/TTA/TTG</td>
<td>T</td>
</tr>
<tr>
<td>C</td>
<td>CTT/CTC/CTA/CTG</td>
<td>C</td>
</tr>
<tr>
<td>A</td>
<td>ATT/ATC/ATA/ATG</td>
<td>A</td>
</tr>
<tr>
<td>G</td>
<td>GTT/GTC/GTA/GTG</td>
<td>G</td>
</tr>
</tbody>
</table>

- TTT/TTA/TTG: Phe/Leu
- CTT/CTC/CTA/CTG: Leu
- ATT/ATC/ATA/ATG: Ile/Met
- GTT/GTC/GTA/GTG: Val
- TAT/TAC/TAAG/TAAG: Tyr
- CAT/CAC/CAAC/CAAC: His
- AAT/ACC/ACAC/ACAC: Thr
- GAT/GAC/ACAC/ACAC: Asp
- TAC/TAG/TAAG/TAAG: Stop
- CAC/CAA/CAAC/CAAC: Gin
- AAC/AAA/AAAC/AAAC: Lys
- GAC/GAA/GAAC/GAAC: Glu
- GGT/GGC/GGAC/GGAC: Gly

- TGT/TGC/TC/TC: Cys
- CGT/CGC/GGAC/GGAC: Arg
- AGT/AGC/GGAC/GGAC: Ser
- GGT/GGC/GGAC/GGAC: Gly
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

Amino acid

Replacement of a single nucleotide.

Gln  Gln  Gln  Gln  Gln  Gln  Gln  Gln

Protein

Incorrect sequence causes shortening of protein.
Mutation types: missense rarely cause disease
Small insertions change reading frame

**Insertion mutation**

Original DNA code for an amino acid sequence.

DNA bases

- CATCATCATCATCATCATCATCATCATCATCATCAT

Amino acid

- Thr Ser Ser Ser Ser

Insertion of a single nucleotide.

Incorrect amino acid sequence, which may produce a malfunctioning protein.
Small deletions also change reading frame
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
DMD is X linked recessive: mostly males are affected and females are carriers. Determining DNA mutation critical for family.
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
<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>
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<tr>
<td><strong>Nonsense</strong></td>
<td>726</td>
<td>10</td>
</tr>
<tr>
<td>Missense</td>
<td>30</td>
<td>0.4</td>
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</table>
| **Mid-intronic mutations**     | 22     | 0.3 (may be higher)

*OFTEN NEED MUSCLE BIOPSY*  

Duchenne severity can depend on mutation in the $DMD$ gene: example from Duchenne Registry
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
Amount of dystrophin in muscle biopsy important
Duchenne can progress differently in different boys

LTBP4 Genotype Predicts Age of Ambulatory Loss in Duchenne Muscular Dystrophy

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Wendy M. King, PT,2 Alain Pestourie, MD,3 Julie M. Florence, DPT,4
Katherine D. Mathews, MD,7 Richard S. Finkel, MD,9 Kathryn J. Swoboda, MD,9
Edward Gappmaier, PhD,9 Michael T. Howard, PhD,10 John W. Day, MD, PhD,11
Craig McDonald, MD,11 Elizabeth M. McNally, MD, PhD,9 and Robert B. Weiss, PhD9 for
the United Dystrophinopathy Project

Objective: Duchenne muscular dystrophy (DMD) displays a clinical range that is not fully explained by the primary DMD mutations. Clinical, encoding/ micro-dystrophin (MDM) genes, was previously discovered in a genome-wide scan as a modifier of muscle mass and motor function. We sought to determine whether LTBP4 genotype influences DMD severity in a large patient cohort.

Results: Individuals homozygous for the LAMM LTBP4 haplotype remained ambulatory significantly longer than those heterozygous or homozygous for the LAMM haplotype. Glucocorticoid-treated patients who were LAMM heterozygous lost ambulation at 3.9 ± 3.3 years compared to 17.7 ± 2.1 years for treated VTTT heterozygotes or homozygotes. LAMM tandilex expression is correlated with transforming growth factor-β (TGF-β) signaling and phospho-SMAD expression.

Conclusion: LTBP4 genotype influences age at loss of ambulation, and should be considered in the management of DMD patients.
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

Cardiopulmonary Function in Duchenne
MTB-1 - MTB-1 mediated gene regulation
MYOBLAST TRANSPLANTATION - In Patients
NBD Peptide - Using NF-κB 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 Ezetromid (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)
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
- Loss of Standing
- Loss of Ambulation
- Loss of Self Feeding

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

Contemporary: with Steroids and Improved Cardiac Management
- Loss of Standing
- Loss of Ambulation
- Loss of Self Feeding
- 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
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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  – Ann Martin
  – Jenifer Lavigne
Questions?
Thank you!