Genotype/Phenotype in DMD

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Duchenne muscular dystrophy

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

Age (years) at death

- Duchenne/Becker
- All others
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. The human genome is 3 billion bases long (GATC) and encoded within 23 pairs of chromosomes with a total of about 20,000 genes.

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 (High rate), which is why Duchenne/Becker are among most common genetic diseases in humans.
2.5mb DMD gene in 79 exons encodes dystrophin protein
DNA mutations 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

Nonsense mutations are like ‘out of frame’ mutations
Reading frame of *DMD*

Mutations in *DMD* causes Duchenne if out of frame and Becker MD if in frame (about 95% true)
In frame mutations can be severe
Out of frame mutations can be mild

Exon 45 deletion can be more mild

HOT SPOT

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.
<table>
<thead>
<tr>
<th>Mutation subgroup</th>
<th>N</th>
<th>%</th>
<th>Median age at LOA (years)</th>
<th>log rank P-value</th>
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</thead>
<tbody>
<tr>
<td>Exon 8 skippable</td>
<td>18</td>
<td>2.4</td>
<td>NA</td>
<td>&lt;0.01</td>
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<tr>
<td>Exon 44 skippable</td>
<td>74</td>
<td>9.7</td>
<td>20</td>
<td>0.04</td>
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<tr>
<td>Exon 45 skippable</td>
<td>70</td>
<td>9.1</td>
<td>13</td>
<td>0.80</td>
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<td>Exon 50 skippable</td>
<td>33</td>
<td>4.3</td>
<td>16</td>
<td>0.24</td>
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<tr>
<td>Exon 51 skippable</td>
<td>106</td>
<td>13.8</td>
<td>12</td>
<td>0.04</td>
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<tr>
<td>Exon 52 skippable</td>
<td>29</td>
<td>3.8</td>
<td>16</td>
<td>0.52</td>
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<tr>
<td>Exon 53 skippable</td>
<td>78</td>
<td>10.2</td>
<td>12</td>
<td>0.62</td>
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<tr>
<td>Exon 55 skippable</td>
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<td>0.24</td>
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<tr>
<td>Duplication</td>
<td>83</td>
<td>10.8</td>
<td>13</td>
<td>0.50</td>
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<tr>
<td>Nonsense</td>
<td>71</td>
<td>9.3</td>
<td>14</td>
<td>0.59</td>
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<td>All other exonic deletions</td>
<td>182</td>
<td>23.8</td>
<td>13</td>
<td>NA</td>
</tr>
</tbody>
</table>
Duchenne severity can depend on mutation in *DMD* gene: Duchenne Registry data

Wang R et al, Human Mutation, in press
Amount of dystrophin in muscle biopsy important
mRNA skipping in cultured myotubes can restore mRNA reading frame
Duchenne Genetic Modifier Study

Figure 1: Distribution of Age at Loss of Ambulation for steroid and non-steroid users with Duchenne (DuchenneConnect data, R. Wang)
5 million DNA variants in each person

Variant Filter (Whole Genome Trio: Parents Unaffected)

All
All variants identified in the proband

SM

Allele Frequency
ExAC Adjusted Alt Allele Fra <0.01
ExAC Adjusted Homozygous/Hemizygous count <10
UCLA internal control database (500) Homozygous/Hemizygous count <5
UK10K Twins Alt Allele counts <100
1kG Phase 3 Alt Allele counts <100
UDN UCLA Whole Genome unaffected parents (12) homozygous count <3 and heterozygous count <5

500K

De novo

Coding 0-2 variants
ExAC LoF Z-score >3
AD disorder gene <5 variants

intronic

intergenic

Homozygous/Hemizygous

Parents Heterozygous (Autosome)

Hemizygous (X chromosome)

Potential hemizygous (Autosome)

Coding <5 variants
Intronic ~300 variants
Intergenic

AR disorder gene ~30 variants

>=2 coding <5 genes
1 coding + >=1 intronic

Compound heterozygous

AR disorder gene <50 genes

+ RNA-seq
Takeaways
We need to understand naturally occurring differences

Different DMD gene mutations
• MORE NEEDLE BIOPSIES FOR CELLS/STUDY
  – Different other gene mutations/variants
    • Broader sequencing of the genome (modifiers)

We need to understand different environments
• Off Label drug use, differences in practices,
• nutricueticals, timing of steroids,
• dosing of steroids, type of steroids, etc
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  – Jenifer Lavigne

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Questions?
Thank you!