The Importance of Genetics in Duchenne

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Dystrophin

No protein produced
The absence of dystrophin leads to a muscle cell that is damaged when it generates force.
Chromosomes are made of tightly wound DNA
All of our genes are coded in the DNA
Genetic Basis

The dystrophin gene

- X-chromosome location → males are affected; females are “carriers”, rarely manifesting
- 1 in ~5,000 boys, of all ethnic groups
- 2/3 of mothers are carriers
- Results in abnormal dystrophin protein
  - Absent/unstable protein = Duchenne Muscular Dystrophy
    - Out of frame deletion, nonsense (premature stop) codon, mutation in promoter or splice site
  - Truncated, but somewhat stable protein = Becker Muscular Dystrophy
    - in-frame deletion or mutation that causes aberrant in-frame splicing
The dystrophin gene codes for dystrophin protein in muscle and brain.
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What is Exon Skipping?

- DNA is organized into Exons and Introns
- When creating the RNA (transcription), only the Exons are included and the Introns are spliced out.
- The protein is made (translation) by inserting amino acids specified by a string of nucleotides, organized into sets of three (codons) that must be read in sets of three (reading frame).
- In DMD, most patients have deletions that cause the reading frame to be destroyed (out-of-frame).
- In many cases, skipping another exon can restore the reading frame (in-frame), allowing a truncated BMD-like protein to be made.
- Sarepta has developed an oligonucleotide that is approved and causes skipping of Exon 51, leading to in-frame mRNA production in a subset of patients.
Phase of DMD exons showing mono-skippable exons

Actin-binding domain

Rod domain

R1 - R14

H1 - H3

Dystroglycan binding site

CYS-rich

WW

ZZ

Dystrophin domains

C-ter

End of Rod domain
Phase of DMD exons showing deletion of exon 50

- Actin-binding domain
- Rod domain
- Dystroglycan binding site
- 3'UTR
Phase of DMD exons showing deletion of exon 50 and skipping of exon 51

DP427

Actin-binding

Rod domain

R8

Rod domain

R9

DP260

R10

R11

R12

DP140

R13

R14

HPRT

R15

R16

R17

R18

R19

H3

H4

End of Rod domain

WW

CYS-rich

Dystroglycan binding site

α1-syntrophin binding site

β1-syntrophin binding site

Dystrophin domains

mono-skippable exons
What is Nonsense Suppression?

• ~15% of DMD patients fail to make dystrophin because of a single base change that creates a premature stop codon, or nonsense mutation.

• There are drugs, such as Ataluren, that cause the nonsense mutation to be ignored some percentage of the time, allowing full-length dystrophin to be made.
Nonsense Suppression

Mutation-based, rather than Disease-based, Therapy
The current dystrophin-restoration therapies appear to slow but not halt disease progression. Furthermore, they only are applicable to a subset of the patients.

Nonsense suppression:
For atlauren, it appears that only small amounts of dystrophin are produced, which while beneficial, will not halt disease progression. Given that patients have different premature stop codons and differing amounts of nonsense-mediated decay, the amount of benefit would be expected to vary among eligible patients.

Oligo-mediated exon skipping:
Delivery of oligos will not be uniform across muscles and may vary over time. The dystrophin protein produced is truncated, and therefore not fully functional and will be somewhat unstable. Due to variability in the patient deletions, not all patients will produce the same truncated dystrophin. Thus while it may beneficial, exon skipping will not halt disease progression and the amount of benefit may vary greatly among eligible patients.
What is Gene Therapy?

• Inserting a new gene into a patient’s cells
• The new gene can be a replacement for the defective gene or it can be a different gene designed to offset the loss of the normal protein.
Gene therapy relies on modified viruses to get genes into cells.

AAV (adeno-associated virus) is the most effective gene delivery vehicle currently in the clinic.
How can we deliver genes to all of the muscle cells in the body?

- Muscle comprises 50-60% of body tissue so the delivery must be “systemic” (carried by the bloodstream to all the muscles, and heart).
- The AAV serotypes being used for muscle, such as AAV9, can escape the circulation and go into virtually all cells.
- The gene is only expressed in muscle because of the use of a muscle-specific promoter that allows the synthetic gene to be expressed in muscle and heart, but not other tissues.
AAV Gene Therapy for DMD

1) Express a micro-dystrophin
   - Express a truncated piece of dystrophin designed to be highly functional and stable

2) Use CRISPR/Cas9 gene editing to create a BMD gene (exon removal)
   - Use gene editing to remove DNA causing an exon to be removed from the RNA (similar to oligo-induced skipping, but a permanent change in the DNA)
CRISPR/Cas9 gene editing

- Cut and replace with new piece of DNA
- Cut and seal up with out replacement
CRISPR/Cas9 gene editing

Flag = guide RNA

Scissors = Cas9, cuts DNA

• Currently, removal of DNA without correction is being attempted using AAV delivery of Cas9 and a guide RNA

• This is much more efficient than correcting the gene, and can be used to create an in-frame, truncated dystrophin RNA.

• New approaches may be able to correct single base pair mutations with high efficiency.
Micro-Dystrophin Gene Therapy

• Replacement of the DMD gene using AAV delivery.

• The DMD gene needs to be reduced in size to be carried by AAV, and thus codes for a truncated dystrophin that is designed to be stable.

• After 20+ years of development, trials are now starting and underway.
The dystrophin coding sequence is too large to fit into AAV. By removing the least critical parts of the dystrophin gene, a truncated gene can fit into an AAV virus for delivery throughout the body.
Recent AAV Gene Therapy Successes

• Leber's congenital amaurosis (immune-privileged space)
• Hemophilia/Factor IX (mild immune suppression allowed liver delivery in adults)
• Type I SMA (mild immune suppression allowed systemic AAV delivery in infants)
Micro/Mini Dystrophin Gene Therapy

• Not dependent on type of patient mutation: may work for all patients. (Some patients are excluded from the first trials because they have deleted regions of dystrophin gene that are present in the µdystrophin, and thus could have an immune response to the dystrophin.)

• Prior exposure to AAV (antibodies against AAV) may prevent this therapy from being administered.

• Not permanent, but may last ≥ 10 years.

• Fibrosis interferes with virus delivery, so likely will be more efficient in the youngest boys.
Factors that are important for determining how well the dystrophin protein that is produced will function

1) Retention of actin-binding and binding to the membrane dystrophin complex

2) Protein folding into a stable protein (determined by which parts of the molecule are retained)

2) Other regions of dystrophin have additional functions that are of unknown importance
Limitations for Dystrophin Gene Therapy (Common to Micro-Dystrophin and CRISPR/Cas9)

- A bigger dystrophin is not always better.
  - **Micro-Dystrophin**: While micro-dystrophins are smaller than many of the dystrophins created by exon skipping, they have been designed to be stable.
  - **CRISPR/Cas9**: Some of the dystrophins created by exon skipping using CRISPR/Cas9 will be unstable, and thus not as effective as micro-dystrophin. Others may be quite stable and effective. This is why there are BMD patients who are as severe as DMD and others who are mild, and even a few who show little sign of disease.
Some In-Frame Deletions Lead to Mild BMD and Some Lead to Severe BMD

- Dystrophin repeats are triple helical bundles of amino acids
- Deletions that are in-frame can disrupt the folding of the bundles and make the protein unstable
- Exon boundaries do not correspond to repeat boundaries
Micro-Dystrophins for AAV Trials

Full-Length
Xiao Xiao
Nationwide (Chamberlain)
Solid (Chamberlain)
Limitations for Dystrophin Gene Therapy (Common to Micro-Dystrophin and CRISPR/Cas9)

• Immune response against AAV (capsid).
  – This can be overcome if immune suppression is given prior to virus administration, but to date re-administration of AAV is difficult and may require development of new approaches or alternative AAV serotypes.
  – Prior exposure to AAV may prevent this therapy from being administered. Pre-existing immunity (~20% of older boys) means that virus uptake by muscle will be blocked by circulating antibodies. We may be able to remove them by plasmaphoresis and then administer AAV.
• Immune response to transgene.
  – For micro-dystrophin, if a region is deleted in the patient that is present in the micro-dystrophin, there may be an immune response to the µdystrophin. There may be approaches that lead to tolerance, but this has not been explored.
  – For CRISPR/Cas9, Cas9 is a bacterial protein and will provoke an immune response if expressed continuously. Strategies need to be put in place to inactivate its expression after it has excised DNA. As with exon skipping in general, there is the possibility of expressing dystrophin regions that will cause an immune response, but it may not
Micro-dystrophin for Nationwide Trial

- Micro-dys can potentially treat all DMD boys, but boys with mutations between exons 18 and 58 are not likely to have an immune response against the Nationwide micro-dystrophin.

- MHCK7 promoter enables expression in heart and skeletal muscle
CRISPR/Cas9

- Can permanently remove 1 or more exons. Will be able to be applied to a large number of mutations.
- Multiple strategies can be used to approach editing of the DMD gene.
- Furthest from trials.
- Issues are delivery and potential off target effects.
- Very new technology and safety needs to be established.
Gene Therapy – Impact of Age of Children

• Why dose younger?
  – Muscles are more intact.
  – Fibrosis is a barrier to virus delivery.
  – Likelihood of an immune response is lower
  – The downside is that the muscles are rapidly growing and may dilute out the dystrophin requiring another administration of virus.

• Is there a difference between CRISPR and micro-dystrophin gene therapy with regards to the age of the child?
  – No. The issues are all about AAV delivery, not the payload.
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<th>Microdystrophin</th>
<th>CRISPR/Cas9</th>
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<tr>
<td>Delivery</td>
<td>AAV</td>
<td>AAV</td>
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<tr>
<td>Protein Produced</td>
<td>Micro/mini dystrophin</td>
<td>Permanent cut to Dystrophin Gene to produce exon skipping</td>
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<td>Route</td>
<td>IV</td>
<td>IV</td>
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<td>Expected Result wrt % dystrophin expression</td>
<td>Stable in cell nucleus, small/stable version of dystrophin expressed at high levels</td>
<td>Modifies some % of cellular DNA allowing exon skipping; BMD protein expressed</td>
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<tr>
<td>Immune response</td>
<td>Potential immune response to viral vector</td>
<td>Potential immune response to viral vector</td>
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<tr>
<td>Unknowns</td>
<td>Potential immune response to microdystrophin in some patients</td>
<td>Likely immune response to Cas9 (bacterial protein) unless expression is inactivated</td>
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<td>Safety</td>
<td>Immune response</td>
<td>Immune response and off target effects</td>
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<td>Persistence</td>
<td>10+ years??????</td>
<td>If satellite cells are not modified, then persistence will depend on the dystrophin that is created.</td>
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<td>Re-treatment?</td>
<td>Eventually necessary and will need new technology</td>
<td>Will be necessary if satellite cells are not infected and will need new technology.</td>
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Importance of Sequencing the Dystrophin Gene for all DMD Patients

- Confirm that the mutation is in the dystrophin gene
- Specific mutations make a patient eligible for exon skipping or nonsense suppression therapies
- Potential for immune response against dystrophin regions can be identified
Free genetic testing, interpretation, & counseling for people with Duchenne or Becker muscular dystrophy.
Criteria for Free Testing:

- Symptomatic/suspected diagnosis of Duchenne or Becker
- Any financial barrier
- Legal resident of US or Canada

Available for...

- First time testing, regardless of age
- Repeat testing for those who need it

All testing performed at:

EGL Genetics | Eurofins Clinical Diagnostics
Over 800 applications and over 600 patients tested through the Decode Duchenne program!