New Developments in CRISPR/Cas9 to Correct Duchenne Muscular Dystrophy

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UT Southwestern Medical Center
Duchenne muscular dystrophy (DMD)

- 1 in 5,000 boys (~300,000 worldwide)
The Central Dogma of Molecular Biology

DNA → mRNA → Protein

Transcription

Translation

DNA mRNA Protein
Dystrophin and muscular dystrophy

- The dystrophin gene on the x chromosome

- The largest gene
- ~2.6 million bases
- > 3,000 human mutations
Mutations that cause Duchenne muscular dystrophy

The Dystrophin gene on the x chromosome

- Exon deletions: 60-70%
- Point mutations: 25-35%
- Duplications: 5%

The image shows a chromosomal representation with exons labeled and a pie chart indicating the percentage of mutations.
Inheritance of a dystrophin gene mutation causes DMD
Dystrophin stabilizes muscle membranes

Dystrophin beneath the muscle membrane
Dystrophin protein and gene

Cytoskeleton binding

Membrane binding

1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16  17  18  19  20

21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40

41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79
Mutational "hotspots" in the Dystrophin gene
The many therapies for DMD

- Microdystrophin Gene Therapy
- Gene editing CRISPR/Cas9
- Oligo Exon Skipping
- Steroids
- Calcium Regulators
- Muscle growth and protection (Myostatin, Utrophin, Follistatin Modulation)
- Stem cells & Exosomes
- Anti-Fibrotics
Correction of genetic disorders by genome editing

CRISPR gene editing

Correct disease-causing mutations.
How CRISPR gene editing works

1. **GUIDE RNA** binds to the DNA sequence to be cut.
2. **CAS9** enzyme binds to the guide RNA and cuts the DNA at the specified location.
3. Double-stranded cut occurs at the mutated gene.
4. **EDITED GENE** is created by repairing the DNA at the cut site.
Correction of a Dystrophin mutation by CRISPR editing

Gene

mRNA

Protein

Dystrophin
Gene editing of human DMD muscle cells

DMD patient and controls

Blood sample → Reprogramming → Induced pluripotent stem cells (iPSCs)

Cas9 guide RNA → Editing → Corrected iPSCs

Differentiation → Corrected cardiomyocytes
Editing a human multi-exon deletion

DMD Patient

47 48 49 50 51 52 53

Deleted in DMD

Exon skipping

Cas9

DMD cardiomyocytes

Corrected DMD cardiomyocytes

Dystrophin negative

Dystrophin positive
Editing of a human exon 44 deletion

Normal cardiomyocytes

DMD cardiomyocytes

Corrected DMD cardiomyocytes
Editing of a human exon 44 deletion

Δ44 DMD → 42 43 44 45 46 → 42 43 46

Mutant gene → Edited gene

Normal DMD Corrected

kD

250

150

100

Dystrophin

Vinculin
Correction of a point mutation by gene editing

DMD cardiomyocytes

Dystrophin negative

Corrected DMD cardiomyocytes

Dystrophin positive
Creation of a mouse with Exon 50 deletion

Normal muscle

Dystrophic muscle

Dystrophin
Viral delivery of gene editing machinery

Adeno-Associated Virus (AAV)

- Harmless virus
- AAV9 is highly specific for muscle
Rescue of ΔExon 50 mice by systemic SingleCut CRISPR/Cas9 delivery

Normal

ΔExon 50

ΔExon 50 + AAV9-Cas9

4 weeks after Systemic delivery

Tibialis anterior muscle
Restoration of dystrophin in ΔExon 50 mice by Systemic CRISPR/Cas9 delivery: 4 weeks

<table>
<thead>
<tr>
<th></th>
<th>Tibialis anterior</th>
<th>Triceps</th>
<th>Diaphragm</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal tissue with dystrophin expression</td>
<td>Normal tissue with normal dystrophin expression</td>
<td>Normal tissue with normal dystrophin expression</td>
<td>Normal tissue with normal dystrophin expression</td>
</tr>
<tr>
<td>Dystrophic ΔExon 50</td>
<td>Low dystrophin expression</td>
<td>Low dystrophin expression</td>
<td>Low dystrophin expression</td>
<td>Low dystrophin expression</td>
</tr>
<tr>
<td>ΔExon 50 + AAV9-Cas9</td>
<td>Normal tissue with high dystrophin expression</td>
<td>Normal tissue with high dystrophin expression</td>
<td>Normal tissue with high dystrophin expression</td>
<td>Normal tissue with high dystrophin expression</td>
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</tbody>
</table>
Restoration of dystrophin in ΔExon 50 mice by Systemic CRISPR/Cas9 delivery: 8 weeks

<table>
<thead>
<tr>
<th>Tibialis anterior</th>
<th>Triceps</th>
<th>Diaphragm</th>
<th>Heart</th>
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</thead>
<tbody>
<tr>
<td>WT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dystrophic ΔExon 50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔExon 50 + AAV9-Cas9</td>
<td></td>
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</tr>
</tbody>
</table>
Restoration of Dystrophin by systemic delivery of CRISPR

- Normal
- Dystrophic \( \triangle \text{Exon 50} \)
- \( \triangle \text{Exon 50} + \) AAV9-Cas9

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Dystrophic ( \triangle \text{Exon 50} )</th>
<th>( \triangle \text{Exon 50} + ) AAV9-Cas9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dystrophin</td>
<td><img src="image" alt="Dystrophin Band" /></td>
<td><img src="image" alt="Dystrophin Band" /></td>
<td><img src="image" alt="Dystrophin Band" /></td>
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<tr>
<td>250</td>
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<td><img src="image" alt="Dystrophin Band" /></td>
<td><img src="image" alt="Dystrophin Band" /></td>
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<tr>
<td>Vinculin</td>
<td><img src="image" alt="Vinculin Band" /></td>
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<td><img src="image" alt="Vinculin Band" /></td>
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<tr>
<td>150</td>
<td><img src="image" alt="Vinculin Band" /></td>
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</tr>
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</table>

> 80%
Enhanced grip strength in ΔExon 50 mice following systemic CRISPR/Cas9 delivery: 4 weeks
A vision for gene editing in DMD

DMD Patients

 Phenotyping & Mutations Identified

DMD-iPSC Myoediting

Corrected DMD-iPS Muscle Cells

Optimized Myoediting

DMD Editing In Vivo

Scale up

Safety

DMD Myoediting
Dystrophin correction in DMD ΔExon50 dogs

8 weeks post systemic injection
Dystrophin correction in muscles of DMD ΔExon50 dogs

<table>
<thead>
<tr>
<th></th>
<th>Heart</th>
<th>Diaphragm</th>
<th>Cranial Tibialis</th>
<th>Biceps</th>
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<tbody>
<tr>
<td></td>
<td>WT</td>
<td>ΔEx50</td>
<td>ΔEx50-AAV9</td>
<td>WT</td>
</tr>
<tr>
<td>Dystrophin</td>
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<tr>
<td></td>
<td>92%</td>
<td>58%</td>
<td>70%</td>
<td>64%</td>
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</table>

8 weeks post systemic injection

The top 12 exons for restoring dystrophin expression by exon skipping
The next exon deletions being edited

<table>
<thead>
<tr>
<th>Exon With Gene Defect</th>
<th>Treatable Duchenne Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔEx50 49-51-52-53</td>
<td>13.1%</td>
</tr>
<tr>
<td>ΔEx44 42-43-45-46</td>
<td>11.9%</td>
</tr>
<tr>
<td>ΔEx52 50-51-53-54</td>
<td>7.7%</td>
</tr>
<tr>
<td>ΔEx43 41-42-44-45</td>
<td>6.2%</td>
</tr>
<tr>
<td>ΔEx45 43-44-46-47</td>
<td>4.3%</td>
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</tbody>
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Fixable DMD mutations

Nonsense mutations

Out of frame deletions

Duplications

Pseudoexons
The many therapies for DMD

- Microdystrophin Gene Therapy
- Gene editing CRISPR/Cas9
- Oligo Exon Skipping
- Steroids
- Calcium Regulators
- Muscle growth and protection (Myostatin, Utrophin, Follistatin Modulation)
- Stem cells & Exosomes
- Anti-Fibrotics
Single Cut CRISPR gene editing versus micro-dystrophin

<table>
<thead>
<tr>
<th></th>
<th>Protein size</th>
<th>Actin-binding</th>
<th>Rod domain</th>
<th>WW CYS CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dystrophin</td>
<td>3684</td>
<td>H1, H2, H3, H4</td>
<td></td>
<td></td>
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<tr>
<td>Dystrophin ΔEx51</td>
<td>3606</td>
<td>H1, H2, H3, H4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micro-Dystrophin</td>
<td>1001</td>
<td>H1, H2, H4</td>
<td></td>
<td>WW CYS</td>
</tr>
</tbody>
</table>

78aa Ex51
Different therapies for DMD

- **CRISPR**: Removes mutation from DNA
- **Microdystrophin gene therapy**: Provides shortened dystrophin from a virus
- **Oligonucleotide exon skipping**: Temporarily bypasses mutation in RNA
  Requires life-long treatment

**DNA** → **Transcription** → **mRNA** → **Translation** → **Protein**
A vision for gene editing in DMD

**Distinguishing features:**
- Editing of disease-causing mutations
- Minimal modification of the gene
- One-time treatment, in principle
- Reaches skeletal muscles and heart
- Normal expression of repaired gene

**Future questions:**
- Long term durability
- Off-target mutations
- Immune response
A vision for gene editing in DMD

- DMD Patients
- Phenotyping & Mutations Identified
- DMD-iPSC Myoediting
- Corrected DMD-iPS Muscle Cells
- Scale up
- Safety
- DMD Editing In Vivo
- DMD Myoediting

Exonics Therapeutics
Distinguishing features of gene editing

- Correction of disease-causing mutations
- One-time treatment
- Potentially reaches all affected cell types
- Potential to correct up to 80% of DMD mutations