Gene therapy in Neuromuscular disease

Jakkrit Amornvit, M.D.
Outline

• Basic in Genetic Neuromuscular disorder
• Gene therapy approach
• Future direction
Basic in Genetic Neuromuscular disorder

• From “Gene” to “Protein”
Basic in Genetic Neuromuscular disorder

• Monogenic (Single Gene) disorder (most common)

• Mutation type
  • Change in number of gene sequence copies
  • Change in the nucleotide sequence of a gene
  • Repeat expansion or contraction
Basic in Genetic Neuromuscular disorder

Mutation type

- Change in number of gene sequence copies (exon or whole gene)
  - Deletion
  - Duplication
Basic in Genetic Neuromuscular disorder

Mutation type

• Change in the nucleotide sequence of a gene
  - Point mutation
  - Insertion
  - Deletions
Basic in Genetic Neuromuscular disorder

Mutation type
- Change in the nucleotide sequence of a gene

Original: THE FAT CAT SAT

Point mutation: THA FAT CAT SAT

Insertion: THE FTT ATC ATS AT

Deletion: THE FTC ATS AT
### Basic in Genetic Neuromuscular disorder

**Mutation type**
- Change in the nucleotide sequence of a gene

<table>
<thead>
<tr>
<th>Original:</th>
<th>THE FAT CAT SAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Point mutation:</td>
<td>THA FAT CAT SAT</td>
</tr>
<tr>
<td>Insertion:</td>
<td>THE FTT ATC ATS AT</td>
</tr>
<tr>
<td>Deletion:</td>
<td>THE FTC ATS AT</td>
</tr>
</tbody>
</table>

- Missense
- Nonsense
- Frameshift
- Premature stop codon
Dystrophinopathies

- Dystrophic changes
- Calf pseudohypertrophy
- Proximal weakness
  - Waddling gait
  - Gowers’ sign
- Ankle contracture
  - Toe walking
Dystrophinopathies: Clinical diagnosis

“Limb girdle muscle weakness + Calf pseudohypertrophy + Cardiomyopathy”

<table>
<thead>
<tr>
<th>Duchene muscular dystrophy (DMD)</th>
<th>Becker muscular dystrophy (BMD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset age 3-5 year</td>
<td>Phenotypic variability</td>
</tr>
<tr>
<td>Tight heel cords</td>
<td>• Limb-girdle syndromes</td>
</tr>
<tr>
<td>“Loss of ambulation age 12 year”</td>
<td>• Myalgia</td>
</tr>
<tr>
<td>CPK 50-100X normal</td>
<td>• Isolated cardiomyopathy</td>
</tr>
<tr>
<td></td>
<td>“loss of ambulation &gt; age 12”</td>
</tr>
</tbody>
</table>
Dystrophinopathies

• Mutation in Dystrophin gene (*DMD*): Xp21.1
  • 2.4 million base pairs
  • 79 exons and 8 promoters
### Dystrophinopathies

Large deletions (≥ 1 exon) account for 66% of DMD/BMD patients

#### TABLE I. Distribution of Mutations Among 68 Unselected Dystrophinopathy Probands From One Clinic

<table>
<thead>
<tr>
<th>Mutation type</th>
<th>DMD</th>
<th>BMD</th>
<th>Carrier</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1 exon deletion</td>
<td>32</td>
<td>13</td>
<td></td>
<td>45 (66%)</td>
</tr>
<tr>
<td>Nonsense</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>9 (13%)</td>
</tr>
<tr>
<td>Missense</td>
<td>1</td>
<td>2</td>
<td></td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Frameshift insertion or deletion</td>
<td>1</td>
<td></td>
<td>1</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>≥1 exon duplication</td>
<td>3</td>
<td>1</td>
<td></td>
<td>4 (6%)</td>
</tr>
<tr>
<td>No mutation detected</td>
<td>3</td>
<td>2</td>
<td></td>
<td>5 (7%)</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>21</td>
<td>2</td>
<td>68</td>
</tr>
</tbody>
</table>

DMD, Duchenne muscular dystrophy; BMD, Becker muscular dystrophy; carrier, manifesting carrier related to a deceased DMD patient.
Dystrophinopathies - Effects of deletion mutations

“Size of deletion does not correlate well with phenotype”

Original: THE BIG RED DOG RAN AND SAT

in-frame: THE BIG RED DOG RAN AND SAT
THE DOG RAN AND SAT
-> Becker Muscular dystrophy

out-of frame: THE BIG RED DOG RAN AND SAT
THE BIR EDD OGR ANA NDS AT
-> Duchene Muscular dystrophy
Outline

• Basic in Genetic Neuromuscular disorder

• Gene therapy approach

• Future direction
Gene Therapy in Neuromuscular disease

- Short synthetic nucleotide
- Virus-mediated gene therapy
- Genomic DNA editing/engineering
Short synthetic nucleotide

• Antisense oligonucleotide (ASOs)
  • Two ASO-mediated therapies have received approval from the US FDA
    - “Eteplirsen” for DMD with exon 51 deletion
    - “Nusinersen” for SMA1

• RNA interference (RNAi)
  • “Patisiran” for hereditary transthyretin amyloidosis
Short synthetic nucleotide

Antisense Oligonucleotides (ASO)
- Using nucleic acid to modulate gene expression
- “ASOs” – synthetic nucleic acid sequences that can bind to selected sequences of ribonucleic acid (RNA)

Mechanism of action to RNA
- Degradation
- Preventing the translation
- Altering the splicing of pre-mRNA
Mechanism of action

- Degradation
- Preventing the translation
- Altering the splicing of pre-mRNA
Effects of deletion mutations - Dystrophinopathies

“Size of deletion does not correlate well with phenotype”

Original: THE BIG RED DOG RAN AND SAT
out-of frame: THE BIG RED DOG RAN AND SAT
THE

-> Duchenne Muscular dystrophy

deletion of exons 49-50” (13% of DMD mutation)
Exon skipping approach in DMD

“DMD with deletion of exons 49-50” (13% of DMD mutation)

Eteplirsen (ASOs) skipping exon 51 enable production of functional dystrophin protein
Survival motor neuron gene (SMN)

Healthy individual
Survival motor neuron gene (SMN)
“Nusinersen” for Spinal muscular atrophy type 1

ASO bind to a regulatory sequence in intron 7

Stimulate SMN2 exon 7 inclusion

increased concentrations of SMN protein
Short synthetic nucleotide

RNA interference (RNAi)

- A natural cellular defense mechanism against RNA-based viruses in which foreign, double-stranded (viral) RNA molecules are identified and used as templates for the specific cleavage of complementary (viral) RNA
RNAi-based therapy

- Administering synthetic double-stranded RNA molecules to downregulate their target transcript (ie, the mRNA) and protein.
Hereditary transthyretin amyloidosis

Transthyretin (TTR) is a protein primarily made in the liver.

A genetic mutation in the TTR gene causes the TTR protein to form clusters known as amyloid deposits.

Amyloid deposits build up in different parts of the body, leading to symptoms of hATTR amyloidosis.
Patisiran (RNAi) for TTR

AM Rosser et al 2018
Gene Therapy

- Short synthetic nucleotide
- Virus-mediated gene therapy
- Genomic DNA editing/engineering
Gene Therapy Vectors

- Vectors = Gene delivery vehicles

- Viral
  - Adenovirus
  - Adeno-associated virus (AAV)
  - Lentivirus / Retrovirus

- Non-viral
  - Naked plasmid DNA
  - Liposome encapsulated plasmid DNA
Viral transduction
Gene Therapy – Viral Vectors

• Remove some viral genes required for viral replication

• Replace with therapeutic genes
# Vector comparison

<table>
<thead>
<tr>
<th>Feature</th>
<th>Adenovirus</th>
<th>AAV</th>
<th>Lenti</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size (nm)</td>
<td>70–100</td>
<td>20–25</td>
<td>100</td>
</tr>
<tr>
<td>Cloning capacity (kb)</td>
<td>35</td>
<td>4.7</td>
<td>9</td>
</tr>
<tr>
<td>Chromosome Integration</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cell Entry</td>
<td>Receptor mediated endocytosis</td>
<td>Receptor mediated endocytosis</td>
<td>Receptor binding, membrane fusion, nuclear entry rt DNA</td>
</tr>
<tr>
<td>Transgene Expression</td>
<td>Rapid, weeks to months</td>
<td>&gt;1 year</td>
<td>Long term correction</td>
</tr>
<tr>
<td>Oncolytic Potential</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Replication Comp. Vector in vivo</td>
<td>Low risk</td>
<td>Negligible risk</td>
<td>Possible risk</td>
</tr>
<tr>
<td>Infects quiescent cells</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Risk of Oncogene activation</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Considerations for Choosing Appropriated Vectors

- Target (Dividing vs Non-Dividing cells)
- Target (Tropism)
- Transgene size – Vector Capacity
- Method of Delivery
- Integration
- Immune response
AAV Vector Properties

Pros
• Efficient for gene transfer
• Non-integrating
  • Persists as an episome in cells for years
• Low immunogenicity
• Broad tropisms with about 6 serotypes widely used
• “Most promising gene therapy vector”

Cons
• Limit capacity about 4.7 kb
## AAV trophism

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Serotype(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>AAV8, AAV9</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>AAV1, AAV6, AAV5, AAV8, AAV9</td>
</tr>
<tr>
<td>CNS</td>
<td>AAV1, AAV4, AAV5</td>
</tr>
<tr>
<td>Retinal epithelium</td>
<td>AAV4, AAV5</td>
</tr>
<tr>
<td>Photoreceptor cells</td>
<td>AAV5</td>
</tr>
<tr>
<td>Lung</td>
<td>AAV9</td>
</tr>
<tr>
<td>Heart</td>
<td>AAV8, AAV9</td>
</tr>
<tr>
<td>Pancreas</td>
<td>AAV8</td>
</tr>
<tr>
<td>Kidney</td>
<td>AAV2</td>
</tr>
<tr>
<td>Drug/compound</td>
<td>Serotype</td>
</tr>
<tr>
<td>---------------</td>
<td>----------</td>
</tr>
<tr>
<td>rAAV2.5-CMV-minidystrophin (d3990)</td>
<td>rAAV2.5</td>
</tr>
<tr>
<td>rAAVrh74.MCK7.microdystrophin</td>
<td>rAAVrh74</td>
</tr>
<tr>
<td>rAAVrh74.MHCK7.microdystrophin</td>
<td>rAAVrh74</td>
</tr>
<tr>
<td>rAAV1.CMV.huFollistatin344</td>
<td>rAAV1</td>
</tr>
<tr>
<td>rAAVrh74.MCK.GLALT2</td>
<td>rAAVrh74</td>
</tr>
<tr>
<td>scAAV9.CB.hSMN</td>
<td>scAAV9</td>
</tr>
<tr>
<td>rAAV8-hMTM1</td>
<td>rAAV8</td>
</tr>
</tbody>
</table>
Gene Therapy

- Short synthetic nucleotide
- Virus-mediated gene therapy
- Genomic DNA editing/engineering
Genomic editing

CRISPR/Cas9

• CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)–Cas9 (CRISPR-Associated Protein 9)

• คำอธิบาย
CRISPR/Cas9 – Mediated Genomic Editing

Diagram:
- CRISPR-Cas9
- Target sequence
- PAM site
- sgRNA

- Nonhomologous end joining
- Homology-directed repair
- Insertion or deletion
- Repair in any phase of the cell cycle
- Precise correction
- Repair in late S and G2 phases of the cell cycle
In vivo gene editing in dystrophic mouse muscle and muscle stem cells

Mohammadsharif Tabebordbar, Kerxian Zhu, Jason K. W. Cheng, Wei Leong Chew, Jeffrey J. Widrick, Winston X. Yan, Claire Maesner, Elizabeth Y. Wu, Ru Xiao, F. Ann Ran, Le Cong, Feng Zhang, Luk H. Vandenberghhe, George M. Church, Amy J. Wagers

CRISPR-mediated Genome Editing Restores Dystrophin Expression and Function in mdx Mice

Li Xu, Ki Ho Park, Lixia Zhao, Jing Xu, Mona El Refaey, Yandi Gao, Hua Zhu, Jianjie Ma and Renzhi Han

1Department of Surgery, Davis Heart and Lung Research Institute, Biomedical Sciences Graduate Program, Biophysics Graduate Program, The Ohio State University Wexner Medical Center, Columbus, Ohio, United States
CRISPR/Cas9 – “Exon skipping”

Duchene muscular dystrophy

- Exon 1
- Exon 2
- Exon 3

Editing NHEJ

Degraded mRNA

Functional protein
CRISPR/Cas9 – “Mutation correction”

Spinal muscular atrophy (SMA)
CRISPR/Cas9 for “Repeat expansion disease”

Familial ALS

Myotonic dystrophy
Outline

• Basic in Genetic Neuromuscular disorder
• Gene therapy approach
• Future direction
Future direction

• AAV-mediated antisense oligonucleotide therapy
• AAV-mediated RNAi therapy
• AAV-mediated CRISPR/Cas9 therapy
Future direction

• Surrogates AAV-gene therapy
  • strategies do not correct the genetic cause of the disease, but they could be used as adjuvant therapies
Surrogates AAV-gene therapy

• rAAVrh74.MCK.GALGT2 gene therapy
  • overexpression of GALGT2 enzyme - responsible for glycosylation of α-dystroglycan in skeletal muscle
  • GALGT2 overexpression leads to an increment of dystroglycan-binding proteins, protecting dystrophic muscle from injury and inhibiting the development of muscular dystrophy

• Follistatin by AAV-gene therapy
  • Follistatin is a muscle growth-stimulating protein that acts by inhibiting the myostatin pathway and inducing muscle hypertrophy