Identifying Patient-Specific Mutations in the *DMD* gene: Implications for Phenotype and Therapies

Kevin M. Flanigan, M.D.
Center for Gene Therapy, The Research Institute at Nationwide Children’s Hospital & Departments of Pediatrics and Neurology, The Ohio State University
Disclosures

- Clinical trial advisory boards:
  - PTC Therapeutics, Inc.
  - Prosensa, Inc.
  - AVI Therapeutics, Inc.

- Site investigator:
  - PTC Therapeutics
  - GSK

- Funding: NINDS
Outline

■ Molecular diagnosis of dystrophinopathies

■ Private mutations and new insights into Becker vs. Duchenne mutations
  □ Altered protein translation initiation
  □ Altered mRNA splicing
    ■ Nonsense-associated splicing and exon splice site strength

■ Relevance to therapeutic trials/approaches:
  □ Nonsense mutation readthrough
  □ Exon skipping
Dystrophinopathies: Clinical diagnosis

- **DMD:**
  - Onset age 3-5
  - Pelvic girdle weakness
  - Tight heel cords
  - CK 50-100X normal
  - Loss of ambulation by age 12
  - Mean age at death around 19 years
    - Dilated cardiomyopathy
    - Ventilatory insufficiency

- **BMD:**
  - Classic definition: loss of ambulation > age 12
  - Alternatively:
    - “intermediate muscular dystrophy” for loss of ambulation ages 12 through 15
    - BMD for loss of ambulation > age 15
  - Limb-girdle syndromes in adulthood
  - Myalgias
  - Isolated cardiomyopathy
Dystrophin Mutations

- Dystrophin gene is huge:
  - 2.2 million base pairs
  - 79 exons and 8 promoters

- Large deletions ($\geq 1$ exon) account for $\sim65\%$ of DMD/BMD patients
  - Detectable with $>98\%$ sensitivity by multiplex PCR

- $\sim5\%$ have duplications
- $\sim15\%$ of boys have premature stop codon mutations
- Remainder are frameshifting insertions/deletions, splice site mutations, missense mutations
Large cohorts expand genotype analysis

**Figure 1.** Distribution of large rearrangements and point mutations in Duchenne (DMD) and Becker (BMD) patients.

**Figure 3.** Frequency of different types of small lesions in DMD and BMD patients.

Tuffery-Giraud *et al*, Hum Mutat. 2009 Jun;30(6):934-45
Distribution of mutations in an unselected cohort
(Dent et al; AJMG, 2005 Apr 30;134(3):295-8)

<table>
<thead>
<tr>
<th>Mutation Type</th>
<th>DMD</th>
<th>BMD</th>
<th>Carrier</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>≥1 exon deletion</td>
<td>32</td>
<td>13</td>
<td></td>
<td>45 (66%)</td>
</tr>
<tr>
<td>Premature Stop</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>
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<tr>
<td><strong>Total</strong></td>
<td>45</td>
<td>21</td>
<td>2</td>
<td>68</td>
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</tbody>
</table>

Currently available methodology can detect 93%-96% of dystrophinopathy mutations from blood samples.

(Yan et al, Hum Mutat 2004; 23:203-204).
Duchenne vs Becker: the reading frame rule

- Size of deletion does not correlate well with phenotype

- In-frame deletions are more likely to result in translation of a protein with partial function
  - i.e., out-of-frame deletions are DMD ~90% of the time
Roberts, *Genome Biology*, 2001
Roberts, *Genome Biology*, 2001
DMD mutational analysis requires two steps:

- Copy number interrogation of all exons

and if negative

- Sequencing of the entire coding region
Multiplex Ligation-Dependent Probe Amplification (MLPA)

Sellner & Taylor, Hum Mutat. 2004 May;23(5):413-9
MLPA: exon copy number change
Duplication exons 18-19
Deletion exons 8-9

43410(F)
Array-CGH
(comparative genomic hybridization)

Hegde et al, Human Mutation 29(9),1091-1099, 2008
Direct sequencing of the *DMD* gene

- From genomic DNA
  - Single condition amplification/internal primer (SCAIP) sequencing
    - Scalable, single plate
  - Chip-based sequencing

- Sequencing of cDNA
  - From mRNA retrieved from archived muscle biopsies
  - Has the added benefit of detecting pseudoexon mutations
Mutational Spectrum of DMD Mutations in Dystrophinopathy Patients: Application of Modern Diagnostic Techniques to a Large Cohort

Kevin M. Flanigan,1–4⁎ Diane M. Dunn,1 Andrew von Niederhausern,1 Payam Soltanzadeh,1 Eduard Gappmaier,1 Michael T. Howard,1 Jacinda B. Sampson,2 Jerry R. Mendell,5 Cheryl Wall,5 Wendy M. King,5 Alan Pestronk,6,7 Julaine M. Florence,6 Anne M. Connolly,6 Katherine D. Mathews,8 Carrie M. Stephan,8 Karla S. Laubenthal,1,8 Brenda L. Wong,9,10 Paula J. Morehart,10 Amy Meyer,10 Richard S. Finkel,11,12 Carsten G. Bonnemann,11,12 Livija Medne,11 John W. Day,13 Joline C. Dalton,13 Marcia K. Margolis,13 Veronica J. Hinton,14 the United Dystrophinopathy Project Consortium,1 and Robert B. Weiss1

1Departments of Human Genetics, University of Utah School of Medicine, Salt Lake City, Utah; 2Department of Neurology, University of Utah School of Medicine, Salt Lake City, Utah; 3Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah; 4Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah; 5The Research Institute of Nationwide Children’s Hospital and Ohio State University, Columbus, Ohio; 6Department of Neurology, Washington University at St. Louis, St. Louis, Missouri; 7Department of Pathology, Washington University at St. Louis, St. Louis, Missouri; 8Department of Pediatrics, University of Iowa, Iowa City, Iowa; 9Department of Pediatrics, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio; 10Department of Neurology, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio; 11Department of Neurology, The Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania; 12Departments of Neurology and Pediatrics, The University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; 13Department of Neurology, University of Minnesota, Minneapolis, Minnesota; 14Columbia-Presbyterian Hospital, New York, New York

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1,111 mutation-positive patients
Subexonic mutations are distributed throughout the *DMD* gene

**Figure 1.** Exon distribution of nonsense, splice, and small insertion/deletion (indel) mutations by exon in unrelated kindreds. Exons containing CpG codons are marked by asterisks (exon 70 contains three CpG codons).

*Hum Mutat.* 2009 Dec;30(12):1657-66
The reading frame rule updated

<table>
<thead>
<tr>
<th>Table 2. The Value of Mutational Reading Frame in Predicting a Phenotype of Duchenne Muscular Dystrophy</th>
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<tr>
<td>DMD</td>
</tr>
<tr>
<td>Exonic deletions only</td>
</tr>
<tr>
<td>Truncating (out-of-frame) mutations</td>
</tr>
<tr>
<td>Non-truncating (in-frame) mutations</td>
</tr>
<tr>
<td>Sensitivity</td>
</tr>
<tr>
<td>Specificity</td>
</tr>
<tr>
<td>All mutations</td>
</tr>
<tr>
<td>Truncating mutations</td>
</tr>
<tr>
<td>Non-truncating mutations</td>
</tr>
<tr>
<td>Sensitivity</td>
</tr>
<tr>
<td>Specificity</td>
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Hum Mutat. 2009 Dec;30(12):1657-66
<table>
<thead>
<tr>
<th>MUTATION CLASS</th>
<th>DMD</th>
<th>IMD</th>
<th>BMD</th>
<th>Unknown (B/DMD)</th>
<th>Manifesting Carrier&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Carrier (all phenotypes)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>DELETION</td>
<td>283</td>
<td>15</td>
<td>55</td>
<td>107</td>
<td>3</td>
<td>14</td>
<td>477</td>
<td>42.9%</td>
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<tr>
<td>in</td>
<td>30</td>
<td>2</td>
<td>36</td>
<td>17</td>
<td>1</td>
<td>2</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>out</td>
<td>243</td>
<td>13</td>
<td>18</td>
<td>88</td>
<td>1</td>
<td>12</td>
<td>375</td>
<td></td>
</tr>
<tr>
<td>other</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>STOP</td>
<td>176</td>
<td>4</td>
<td>30</td>
<td>46</td>
<td>4</td>
<td>34</td>
<td>294</td>
<td>26.5%</td>
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<tr>
<td>UGA</td>
<td>60</td>
<td>1</td>
<td>13</td>
<td>20</td>
<td>3</td>
<td>15</td>
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<td>UAG</td>
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<td>0</td>
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<td>13</td>
<td>0</td>
<td>4</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>UAA</td>
<td>45</td>
<td>3</td>
<td>6</td>
<td>13</td>
<td>1</td>
<td>15</td>
<td>83</td>
<td></td>
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<tr>
<td>SUBEXONIC</td>
<td>70</td>
<td>0</td>
<td>10</td>
<td>32</td>
<td>1</td>
<td>14</td>
<td>127</td>
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<tr>
<td>FS Ins</td>
<td>22</td>
<td>0</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>6</td>
<td>37</td>
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<tr>
<td>FS</td>
<td>46</td>
<td>0</td>
<td>4</td>
<td>23</td>
<td>0</td>
<td>8</td>
<td>81</td>
<td></td>
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<tr>
<td>FS Ins/Del</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>5</td>
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</tr>
<tr>
<td>in-frame deletion</td>
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<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
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<td>4</td>
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<tr>
<td>DUPLICATION</td>
<td>87</td>
<td>7</td>
<td>10</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>122</td>
<td>11.0%</td>
</tr>
<tr>
<td>SPLICE</td>
<td>22</td>
<td>3</td>
<td>7</td>
<td>18</td>
<td>2</td>
<td>12</td>
<td>64</td>
<td>5.8%</td>
</tr>
<tr>
<td>MISSENSE</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>1.4%</td>
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<tr>
<td>PSEUDOEXON</td>
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<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
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<td>0.5%</td>
</tr>
<tr>
<td>POTENTIAL</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>0.5%</td>
</tr>
<tr>
<td>OTHER</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>TOTAL MUTATIONS</td>
<td>642</td>
<td>32</td>
<td>120</td>
<td>220</td>
<td>15</td>
<td>82</td>
<td>1111</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Hum Mutat. 2009 Dec;30(12):1657-66
Nonsense-associate phenotypes among 1111 dystrophinopathy patients

- 210 definitively phenotyped

- DMD = 176 → 84%
- IMD = 4
- BMD = 30 → 16%
BMD from “DMD” (nonsense) mutations: Potential molecular mechanisms of phenotypic amelioration

- Nonsense mutation readthrough
- Altered translational initiation (in exon 1 point mutations)
- Nonsense mutations and exon skipping
Readthrough efficiency does not clearly predict phenotype

Howard MT et al, Ann Neurol 2004; 55(3) 422-426
BMD from “DMD” (nonsense) mutations: Potential molecular mechanisms of phenotypic amelioration

- Nonsense mutation readthrough
- Altered translational initiation (in exon 1 point mutations)
- Nonsense mutations and exon skipping
Pt. 42790

c.9G>A; Trp3X (DMD)

• 65 year old man
  – onset of proximal weakness at age 20
  – wheelchair age 62.

• The proband’s brother (examined at age 58)
  – denied symptoms
  – only minimal-to-mild pelvic girdle weakness upon examination.
p.Trp3X BMD Pedigrees

Proband = 62 yr. old male, mild BMD, still ambulatory
Utah

Family 1

Family 2

Proband = 3.5 yr. old male, incidental to elevated CK levels,
Younger brother CK = 5080 iu/L
Michigan

Concordant resequencing haplotypes across the DMD gene
Family 3: patient 43800; Kansas City, Missouri presented at age 7 years with bilateral calf pain and elevated serum CK level (8,000-24,000 iu/L). Muscle biopsy showed degenerating and regenerating fibers and reduced N-terminal, rod, and C-terminal dystrophin antibody staining.

Family 4: patient 43676; also from Kansas City incidentally found at age 4 years to have an elevated serum CK level (4558 iu/L and 14,559 iu/L on separate occasions).

Family 5: patient 43831; Milwaukee, Wisconsin presented in childhood with myalgias but no weakness; an elevated serum CK led to a muscle biopsy with decreased amino-terminal and rod domain dystrophin antibody staining.

Family 6: patient 43889; Philadelphia, PA presented at age 13 years for evaluation of "hyperCKemia" found incidentally during an evaluation of short stature. His serum CK ranged from 5877 iu/L when active to 712 iu/L when more sedentary. Maternal grandfather (73 yrs) also Trp3X.
Trp3X is a founder allele in the *DMD* gene

- Associated with childhood hyperCKemia, with or without myoglobinuria
- Compatible with no significant weakness at age 70
- No effect on reproductive fitness
Trp3X

WT

GAPDH

WT dystrophin
Trp3X dystrophin

Gurvich et al, 2009; Hum Mutat 30:633-40
Alternate initiation from at least two exon six ATG sites contributes to the amelioration of the disease phenotype.

May be a general mechanism of rescue for early (exon 1-5) mutations.
BMD from “DMD” (nonsense) mutations: Potential molecular mechanisms of phenotypic amelioration

- Nonsense mutation readthrough

- Altered translational initiation (in exon 1 point mutations)

- Nonsense mutations and exon skipping
Nonsense mutation associated splicing alteration causing BMD

- Fajkusova et al (2001)
- Disset et al (2006): Creation of a splice suppressor sequence in exon 31
BMD-associated stop codon mutation in exon 31
c.4250T>A  p.1417Leu>X
BMD versus DMD nonsense mutations

In-frame exons (39) shaded  Out-of-frame (40) unshaded

p.Trp3X

DMD 176
I/BMD 26
Phenotype and flanking reading frame

\[ p = 0.004 \]
161 unique nonsense mutations
3' (acceptor) and 5' (donor) splice sites metrics

Scoring of splice site sequence motifs:

1. Weight Matrix Model (WMM)
2. First-order Markov Model (MM)
3. Maximum Dependence Decomposition Model (MDD)
4. Maximum Entropy Model (MaxENT)

From Z. Wang & C. Burge, Splicing regulation: From a parts list of regulatory elements to an integrated splicing code. RNA (2008), 14:802–813
Association of splice site strength and intron/exon size with exon frame and BMD phenotypes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>In-Frame</th>
<th></th>
<th>Out-of-Frame</th>
<th></th>
<th>p-value</th>
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<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
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<tr>
<td>5'ss strength (MaxEnt)</td>
<td>7.47</td>
<td>1.85</td>
<td>8.74</td>
<td>1.50</td>
<td>0.002</td>
</tr>
<tr>
<td>5'ss strength (MDD)</td>
<td>11.74</td>
<td>2.06</td>
<td>12.68</td>
<td>2.05</td>
<td>0.05</td>
</tr>
<tr>
<td>5'ss strength (MM)</td>
<td>6.72</td>
<td>1.81</td>
<td>8.02</td>
<td>1.65</td>
<td>0.003</td>
</tr>
<tr>
<td>5'ss strength (WMM)</td>
<td>7.12</td>
<td>1.76</td>
<td>8.50</td>
<td>1.84</td>
<td>0.004</td>
</tr>
<tr>
<td>3'ss strength (MaxEnt)</td>
<td>7.69</td>
<td>2.40</td>
<td>8.23</td>
<td>2.76</td>
<td>0.24</td>
</tr>
<tr>
<td>3'ss strength (MM)</td>
<td>8.06</td>
<td>2.53</td>
<td>8.61</td>
<td>3.22</td>
<td>0.17</td>
</tr>
<tr>
<td>3'ss strength (WMM)</td>
<td>7.38</td>
<td>3.81</td>
<td>9.08</td>
<td>4.83</td>
<td>0.06</td>
</tr>
<tr>
<td>Upstream intron size (kb)</td>
<td>16.53</td>
<td>29.24</td>
<td>36.76</td>
<td>52.59</td>
<td>0.005</td>
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<tr>
<td>Downstream intron size (kb)</td>
<td>14.62</td>
<td>19.12</td>
<td>34.66</td>
<td>51.09</td>
<td>0.02</td>
</tr>
<tr>
<td>Exon size (nts.)</td>
<td>151</td>
<td>33</td>
<td>155</td>
<td>53</td>
<td>0.40</td>
</tr>
</tbody>
</table>

*a Rod domain in-frame exons: 25, 27, 29, 31, 37, 38, 40*
Aggregator site for multiple algorithms predicting exon splice enhancers (ESE) and exon splice suppressors (ESS)

Generated ESE/ESS density measures for all exons (motifs/nucleotides)
Search for intronic splicing regulatory sequences in flanking exons of known mammalian cassette exons (1736 exons in 1473 genes)

Suyama M et al. Nucl. Acids Res. 2010;nar.gkq705

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### Table 1. Conserved pentamers with statistical significance

<table>
<thead>
<tr>
<th>Rank</th>
<th>Conserved pentamer</th>
<th>Clustered motif</th>
<th>$P$-value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GCATG</td>
<td>TGCATG</td>
<td>$1.0 \times 10^{-29}$</td>
</tr>
<tr>
<td>2</td>
<td>TGCTG</td>
<td>TGCATG</td>
<td>$1.4 \times 10^{-20}$</td>
</tr>
<tr>
<td>3</td>
<td>ACTAA</td>
<td>ACTAAC</td>
<td>$7.5 \times 10^{-12}$</td>
</tr>
<tr>
<td>4</td>
<td>CTAAC</td>
<td>ACTAAC</td>
<td>$9.8 \times 10^{-10}$</td>
</tr>
<tr>
<td>5</td>
<td>TGCTG</td>
<td>CTGCTGC</td>
<td>$8.7 \times 10^{-08}$</td>
</tr>
<tr>
<td>6</td>
<td>GCTGC</td>
<td>CTGCTGC</td>
<td>$3.3 \times 10^{-07}$</td>
</tr>
<tr>
<td>7</td>
<td>TGCTT</td>
<td>CTGCTTT</td>
<td>$7.2 \times 10^{-06}$</td>
</tr>
<tr>
<td>8</td>
<td>CTTGC</td>
<td>CTGCTTT</td>
<td>$8.2 \times 10^{-06}$</td>
</tr>
<tr>
<td>9</td>
<td>GTGGG</td>
<td>GTGGTGGG</td>
<td>$1.1 \times 10^{-05}$</td>
</tr>
<tr>
<td>10</td>
<td>TTTCT</td>
<td>TTTCT</td>
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<td>11</td>
<td>AAGAT</td>
<td>AAGAT</td>
<td>$3.6 \times 10^{-05}$</td>
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<td>12</td>
<td>TGGAA</td>
<td>TGGAA</td>
<td>$4.2 \times 10^{-05}$</td>
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<td>13</td>
<td>GCTAA</td>
<td>GCTAA</td>
<td>$5.6 \times 10^{-05}$</td>
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<tr>
<td>14</td>
<td>CTGCT</td>
<td>CTGCTGC</td>
<td>$5.6 \times 10^{-05}$</td>
</tr>
<tr>
<td>15</td>
<td>AAAGG</td>
<td>AAAGG</td>
<td>$6.8 \times 10^{-05}$</td>
</tr>
<tr>
<td>16</td>
<td>GTGGT</td>
<td>GTGGTGGG</td>
<td>$9.8 \times 10^{-05}$</td>
</tr>
<tr>
<td>17</td>
<td>TCTTG</td>
<td>TCTTG</td>
<td>$1.2 \times 10^{-04}$</td>
</tr>
<tr>
<td>18</td>
<td>GTGGG</td>
<td>GTGGTGGG</td>
<td>$1.3 \times 10^{-04}$</td>
</tr>
</tbody>
</table>

Pentamers are sorted by $P$-values. The pentamers that cover only a part of known motifs are excluded from the list (see Supplementary Table S4 for a complete list of the pentamers sorted by $P$-value).

$^a$ $P$-values are calculated by Fisher’s exact test.

---

1.) Binding site for RNA-binding proteins of Quaking-like (Qk and STAR) family

Association of ESE/ESS sites with exon frame and BMD phenotypes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>In-Frame</th>
<th></th>
<th>Out-of-Frame</th>
<th></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>p-value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESE density (hexESE hexamers)</td>
<td>0.26 (0.06)</td>
<td>0.22 (0.08)</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESE density (RESCUE-ESE hexamers)</td>
<td>0.20 (0.06)</td>
<td>0.16 (0.07)</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESE density (PESE octamers)</td>
<td>0.10 (0.03)</td>
<td>0.08 (0.03)</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESE density (ESEfinder SR motifs)</td>
<td>0.13 (0.04)</td>
<td>0.14 (0.04)</td>
<td>0.08</td>
<td></td>
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</tr>
<tr>
<td>ESE density (EIE)</td>
<td>0.42 (0.07)</td>
<td>0.42 (0.08)</td>
<td>0.77</td>
<td></td>
<td></td>
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<tr>
<td>ESE density (Tra2b 9G8 motifs)</td>
<td>0.15 (0.03)</td>
<td>0.14 (0.04)</td>
<td>0.39</td>
<td></td>
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<tr>
<td>ESS density (hexESS hexamers)</td>
<td>0.04 (0.03)</td>
<td>0.04 (0.03)</td>
<td>0.69</td>
<td></td>
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<tr>
<td>ESS density (Sironi Silencer motifs)</td>
<td>0.12 (0.03)</td>
<td>0.12 (0.02)</td>
<td>0.74</td>
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<tr>
<td>ESS density (hnRNP A1 motifs)</td>
<td>0.06 (0.02)</td>
<td>0.05 (0.02)</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISR density (Suyama et al.)</td>
<td>0.024 (0.007)</td>
<td>0.022 (0.007)</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Rod domain in-frame exons: 25, 27, 29, 31, 37, 38, 40

b Rod domain in-frame exons: 23, 24, 26, 28, 30, 32, 33, 34, 35, 36, 39, 41, 42
In-frame exons with BMD nonsense mutations have:

- The weakest aggregate splice site signals of all *DMD* exons
- Weaker competing 3’-ss strengths than those exons that only have DMD mutations
- Lower ESE densities than those exons that only have DMD mutations
- Higher flanking ISR density than those that have only DMD mutations
- May be particularly sensitive to ESE or ESS sequence alterations
Exon splice regulatory elements alterations occur within an exon definition context

described in A. Disset et al., Human Molecular Genetics 2006
Quantitative RT-PCR of exon 31 (c.4240C>T)

Primers

- ex30
- ex31
- ex32
- ex33

![Graph showing percent DMD transcript comparison between different conditions: ctrl 30/31, ctrl 30/32, c.4240C>T 30/31, and c.4240C>T 30/32. The graph illustrates the relative expression levels and variability.]
Nonsense mutations and BMD

1. Exon splice regulatory elements alterations occur within a specific exon definition context

2. Only a subset of in-frame exons – those with a weaker exon definition metrics – host nonsense-associated BMD mutations

3. We cannot yet give definitive prognostic information based upon which exon contains a nonsense mutation

4. Genotype alone cannot predict phenotype, and phenotypic predictions should be made with caution in an in-frame flanking context
Potential Therapies in Trials

- Stop Codon Readthrough
- Exon Skipping
- Gene Transfer
Potential Therapies in Trials

- Stop Codon Readthrough
- Exon Skipping
- Gene Transfer
Aminoglycoside antibiotics restore dystrophin function to skeletal muscles of mdx mice.
Barton-Davis ER, Cordier L, Shoturma DI, Leland SE, Sweeney HL.
Gentamicin-Induced Readthrough of Stop Codons in Duchenne

PTC124 targets genetic disorders caused by nonsense mutations

Ellen M. Welch1*, Elisabeth R. Barton2*, Jin Zhuo1, Yuki Tomizawa1, Westley J. Friesen1, Panayiota Trifillis1, Sergey Paushkin1, Meenal Patel1, Christopher R. Trotta1, Seongwoo Hwang1, Richard G. Wilde1, Gary Karp1, James Takasugi1, Guangming Chen1, Stephen Jones1, Hongyu Ren1, Young-Choon Moon1, Donald Corson1, Anthony A. Turpoff1, Jeffrey A. Campbell1, M. Morgan Conn1, Atiyya Khan1, Neil G. Almstead1, Jean Hedrick1, Anna Mollin1, Nicole Risher1, Marla Weetall1, Shirley Yeh1, Arthur A. Branstom1, Joseph M. Colacino1, John Babiak1, William D. Ju1, Samit Hirawat1, Valerie J. Northcutt1, Langdon L. Miller1, Phyllis Spatrick3, Feng He3, Masataka Kawana2, Huisheng Feng2, Allan Jacobson3, Stuart W. Peltz1 & H. Lee Sweeney2

Welch et al, Nature 2007; 447(7140):87-91
Welch et al, Nature 2007; 447(7140):87-91
Nonaminoglycoside compounds induce readthrough of nonsense mutations

Liutao Du,1 Robert Damoiseaux,5 Shareef Nahas,1 Kun Gao,2 Hailiang Hu,1 Julianne M. Pollard,1 Jimena Goldstine,3 Michael E. Jung,6 Susanne M. Henning,2 Carmen Bertoni,4 and Richard A. Gatti1,3

1Department of Pathology and Laboratory Medicine, 2Center for Human Nutrition, 3Department of Human Genetics, and 4Department of Neurology, David Geffen School of Medicine, 5Molecular Shared Screening Resources, California NanoSystems Institute, 6Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, CA 90095

Figure 8. RTCs restored full-length dystrophin protein in mouse mdx myotubes (TAA). Cells were prepared and treated with RTCs for 3 d. Dystrophin proteins were detected by Western blot analysis using immunoprecipitation. Wild-type cells were used in the experiment to localize dystrophin protein. Gentamicin was used as a positive readthrough control. Both RTC#13 and #14 induced a significant amount of dystrophin protein. Results were consistent in three independent experiments.
Quantitative RT-PCR of exon 31 (c.4240C>T)
Potential Therapies in Current Trials

- Stop Codon Readthrough
- Exon Skipping
- Gene Transfer
A

DMD deletion at exon 50

Exon 49 ▶ Intron 49/50 ▶ Exon 51 ▶ Intron 51 ▶ Exon 52 ▶ Pre-mRNA

Splicing

Exon 49 ▶ Exon 51 ▶ Out-of-frame mRNA

No dystrophin

B

PRO051

Exon 49 ▶ Intron 49/50 ▶ Exon 51 ▶ Intron 51 ▶ Exon 52 ▶ Pre-mRNA

Splicing

Exon 49 ▶ Exon 52 ▶ In-frame mRNA

BMD-like dystrophin

van Deutekom et al, N Engl J Med (2007) 357;2678-2786
Local Dystrophin Restoration with Antisense Oligonucleotide PRO051

Local restoration of dystrophin expression with the **morpholino** oligomer AVI-4658 in Duchenne muscular dystrophy: a single-blind, placebo-controlled, dose-escalation, proof-of-concept study

Maria Kinali*, Virginia Arechavala-Gomez*, Lucy Feng, Sebahattin Cirak, David Hunt, Carl Adkin, Michela Guglieri, Emma Ashton, Stephen Abbs, Petros Nihoyannopoulos, Maria Elena Garalda, Mary Rutherford, Caroline McCulley, Linda Popplewell, Ian R Graham, George Dickson, Matthew JA Wood, Dominic J Wells, Steve D Wilton, Ryszard Kole, Volker Straub, Kate Bushby, Caroline Sewry, Jennifer E Morgan, Francesco Muntoni
Systemic Administration of PRO051 in Duchenne’s Muscular Dystrophy

Nathalie M. Goemans, M.D., Mar Tulinus, M.D., Ph.D.,
Johanna T. van den Akker, Ph.D., Brigitte E. Burm, Ph.D., Peter F. Ekhart, M.Sc.,
Niki Heuvelmans, Tjadine Holling, Ph.D., Anneke A. Janson,
Gerard J. Platenburg, M.Sc., Jessica A. Sipkens, M.Sc., J.M. Ad Sitzen, M.D., Ph.D.,
Annemieke Aartsma-Rus, Ph.D., Gert-Jan B. van Ommen, Ph.D.,
Gunnar Buyse, M.D., Ph.D., Niklas Darin, M.D., Ph.D.,
Jan J. Verschuuren, M.D., Ph.D., Giles V. Campion, M.D.,
Sjef J. de Kimpe, Ph.D., and Judith C. van Deutekom, Ph.D.

Theoretic Applicability of Antisense-Mediated Exon Skipping for Duchenne Muscular Dystrophy Mutations

Annemieke Aartsma-Rus, Ivo Fokkema, Jan Verschuuren, Ieke Ginjaar, Judith van Deutekom, Gert-Jan van Ommen, and Johan T. den Dunnen

Table 1. Overview of the Applicability of Exon Skipping for DMD Mutations

<table>
<thead>
<tr>
<th>Ranking</th>
<th>Exon(s)</th>
<th>All mutations</th>
<th>Deletions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51</td>
<td>13.0%</td>
<td>19.1%</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>8.1%</td>
<td>11.8%</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>7.7%</td>
<td>11.4%</td>
</tr>
<tr>
<td>4</td>
<td>44</td>
<td>6.2%</td>
<td>8.8%</td>
</tr>
<tr>
<td>5</td>
<td>46</td>
<td>4.3%</td>
<td>6.2%</td>
</tr>
<tr>
<td>6</td>
<td>52</td>
<td>4.1%</td>
<td>5.7%</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>4.0%</td>
<td>5.6%</td>
</tr>
<tr>
<td>8</td>
<td>43</td>
<td>3.8%</td>
<td>5.3%</td>
</tr>
<tr>
<td>9</td>
<td>6 and 7</td>
<td>3.0%</td>
<td>3.6%</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>2.3%</td>
<td>2.3%</td>
</tr>
</tbody>
</table>

Hum Mutat. 2009 Mar;30(3):293-9
Potential Therapies in Trials

- Stop Codon Readthrough
- Exon Skipping
- Gene Transfer
Potential for target cell destruction by T cells directed against non-self epitopes encoded by the transgene

**Dystrophin Immunity in Duchenne’s Muscular Dystrophy**

Jerry R. Mendell, M.D., Katherine Campbell, B.S., Louise Rodino-Klapac, Ph.D., Zarife Sahenk, M.D., Ph.D., Chris Shilling, M.S., Sarah Lewis, Dawn Bowles, Ph.D., Steven Gray, Ph.D., Chengwen Li, Ph.D., Gloria Galloway, M.D., Vinod Malik, Ph.D., Brian Coley, M.D., K. Reed Clark, Ph.D., Juan Li, M.D., Xiao Xiao, Ph.D., Jade Samulski, M.P.M., Scott W. McPhee, Ph.D., R. Jude Samulski, Ph.D., and Christopher M. Walker, Ph.D.
Summary

- Improved molecular diagnostic methods allow for lymphocyte derived DNA diagnosis of dystrophin mutations in >93-96% of patients, but:

- Prognosticating from phenotype alone should be undertaken with caution

- Novel therapies for muscular dystrophies are now approaching (or already entering) clinical trials

- Accurate *DMD* genotyping is necessary for enrollment in trials, and potentially for future therapies
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  - Julaine Florence, PhD
  - Anne Connolly, MD
  - Richard Finkel, MD
  - Carsten Bonneman, MD
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  - John Day, MD
  - Craig McDonald, MD