Pre-symptomatic Screening for Duchenne Muscular Dystrophy [DMD] a model for Infant Screening

Paul Fernhoff, MD, FAAP, FACMG
Division of Medical Genetics
Department of Human Genetics
Emory University School of Medicine

Visiting Scientist
NCEH, Div. Lab. Sciences, Newborn Screening and Molecular Biology Branch

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Pre-symptomatic Screening for DMD

• Current U.S. NBS programs
• Rationale for pre-symptomatic screening of DMD and other late onset disorders
• Newborn vs. Infant/Well Child screening
• Infant screening for DMD
Newborn Screening: WHY?

• Detect an affected infant before symptoms to prevent or reduce morbidity and mortality
• Provide parents and family reproductive options for future pregnancies
~95 to 98% of the 4.3 million newborns a year in the U.S. have a heel-stick before discharge to screen for at least 28 core genetic disorders plus hearing screening.

• Significant morbidity and/or mortality
• Available and effective treatment
• Time exists before onset of symptoms so that intervention can be effective
• Clinical validity and clinical utility of screen
• Economically “reasonable” and cost-beneficial
• Natural history of the disease understood
• Known and significant incidence in population to be screened
U.S. Newborn Screening

Mandated Disorders – March 2003

(Note: Other disorders may be offered but are not mandated)
29 disorders on “Uniform Panel”
- 9 Organic Acidemias
- 5 Fatty Acid Oxidation Defects
- 6 Amino Acidopathies
- 3 Hemoglobinopathies
- 6 Others: CH, CAH, Galactosemia, Cystic Fibrosis, Hearing

25 additional “Reportable” disorders found by TMS
Newborn Screening Tests by U.S. States, 2008

- 21 or more core conditions (49)
- 10 – 20 core conditions (1)
- Fewer than 10 core conditions (1)

Hatch marks indicate screening for additional core conditions required but not yet implemented.

Screening 29 Core Conditions

Source: March of Dimes.
Data reported from NNISGRIC as of December 31, 2008.
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Addition of Disorders to NBS
Uniform Panel: ACHDGDNC
Candidate Disorders for Pre-symptomatic screening

- Disorders where early intervention prevents or reduces morbidity &/or mortality. Improves quality of life for child and family, but not yet on Uniform Panel
- Primary immunodeficiencies
- Congenital infections: CMV, Toxo, HIV
- Lysosomal storage disorders [LSDs]
- Early onset neuromuscular disorders: DMD, Spinal Muscular Atrophy...
- Fragile X syndrome, Chromosomal abnormalities [sex chromosomes, microdeletions...]
Screening for late onset disorders: “Diagnostic Odyssey”
Dystrophinopathies: DMD

- Spectrum of degenerative muscle disorders caused by mutations in DMD gene. Inadequate dystrophin
- X-linked. Affects males, but late onset heart failure in female carriers
- Affects approximately 1 in 3500 live male births or ~575 cases/yr in U.S.
- ~2/3 cases no family history
- Delayed motor milestones
- Diagnosis usually between 3 and 6 years
- Progressive: Wheelchair early teens, death late teens to early adulthood. Cardiac failure.
- Becker's: milder, but serious form. Death in 40s
Dystrophinopathies

- Isolated NBS programs
- Screening: Elevated creatine kinase (CK) on dried blood spot
- Molecular confirmation
- Supportive therapies +/- Prednisone
- Curative therapies: Exon skipping, “Ataluren” [PTC 124], aminoglycosides codons, stem cells, etc.
Ataluren [PTC 124]: Skips nonsense mutations
Concerns about NBS for later-onset disorders

- Rx not needed for years
- Rx may be partially or not effective
- Early identification could interfere with family bonding with child
- Limitations on health insurance with a pre-existing condition
- Confusion with screening for “Mandated Disorders”
Newborn vs. Infant/Well Child Screening
Infant Screening

- Done at ~1 yr. when most infants have a finger stick for anemia and lead
- Detects disorders where curative treatment is unavailable or partially effective or when Rx does not need to start during 1st year of life. Disorders not on Uniform Panel
- Early recognition and intervention is beneficial for child and family
- Examples: Duchenne Muscular Dystrophy, LSDs, Fragile X Syndrome, Sex Chromosome Abnormalities, etc.
<table>
<thead>
<tr>
<th></th>
<th>Newborn Screening</th>
<th>Infant Screening</th>
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<tbody>
<tr>
<td><strong>Pros</strong></td>
<td>• Avoids diagnostic odyssey</td>
<td>• Avoids diagnostic odyssey</td>
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<tr>
<td></td>
<td>• Provides reproductive options</td>
<td>• Provides reproductive options</td>
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<tr>
<td></td>
<td>• Provides life planning options</td>
<td>• Provides life planning options</td>
</tr>
<tr>
<td></td>
<td>• Well establish public health system</td>
<td>• More time for informed consent</td>
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<td></td>
<td></td>
<td>• Reduces potential for impaired family bonding</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Potential for an “infant” screening panel: FraX, LSDs, chromosomal abnormalities...</td>
</tr>
<tr>
<td><strong>Cons</strong></td>
<td>• Distress of diagnosis prior to symptoms</td>
<td>• Distress of diagnosis prior to symptoms</td>
</tr>
<tr>
<td></td>
<td>• Stigmatization</td>
<td>• Stigmatization</td>
</tr>
<tr>
<td></td>
<td>• Discrimination</td>
<td>• Discrimination</td>
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<tr>
<td></td>
<td>• Limited time to educate parents about risks/ potential benefits</td>
<td>• Not population based. Not all infants tested.</td>
</tr>
<tr>
<td></td>
<td>• Potential impaired bonding with affected child</td>
<td>• Role of public health labs?</td>
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<td></td>
<td></td>
<td>• Requires new PCP office procedure, consenting parents</td>
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CDC Cooperative Agreement: Early Screening and Diagnosis for DMD

• Newborn Screening
  - Columbus Children’s Research Institute in collaboration with the Cincinnati Children’s Hospital and the Ohio Dept. of Health

• Infant Screening
  - Dept. Human Genetics, Division of Medical Genetics, Emory University
Emory/CDC DMD Screening: Project Goal

• Demonstrate the feasibility of screening for DMD in a general population of healthy males, ages 6-15 months

• Voluntary, requiring informed consent

• Incorporate into routine pediatric practice
DMD Infant Screening: Overview

- IRBs: CDC, Emory and Children’s Healthcare of Atlanta
- Focus Groups with DMD affected and unaffected families: Brochure, posters, fact sheets and consent forms
- Filter Paper Assay
  - 3 tiers:
    - Creatine Kinase (CK)
    - CK Isoenzymes
    - Multiplex Ligation-dependent probe amplification (MLPA)
- Recruitment/sample collection
  - 4 metro Atlanta pediatric offices
DMD Infant Screening: Project Overview

• At 9 month well-baby visit:
  - Introduce study to parents
  - Provide parents with information packet

• At 12 month well-baby visit:
  - Introduce study to parents not approached at 9 month well-baby visit
  - Consent interested parents
  - Collect blood spots at the same time blood drawn for hematocrit/lead level testing - No additional finger stick!
DMD Infant Screening: Program Evaluation

- Laboratory Performance [CDC and Ohio]
- Program Satisfaction
  - Parent Decliner’s Survey
  - Screen Negative Survey, includes Parenting Stress Index (PSI)
  - False Positives Survey, includes PSI
  - Provider Survey
  - Positives Screens
    - Face to face interview and survey (including PSI)
    - 6 months post-diagnosis
DMD Infant Screening: Project Enrollment n=264

Average age = 12.31 months (range = 11-16 months)

Average CK=113.8 U/L (range = 12-221 U/L)

<table>
<thead>
<tr>
<th>Ethnic Categories</th>
<th>Totals</th>
</tr>
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<tbody>
<tr>
<td>Hispanic/Latino</td>
<td>9 (3.4%)</td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>255 (96.6%)</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Racial Categories</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black/African American</td>
<td>50 (18.9 %)</td>
</tr>
<tr>
<td>White</td>
<td>189 (71.6 %)</td>
</tr>
<tr>
<td>Asian</td>
<td>10 (3.8 %)</td>
</tr>
<tr>
<td>American Indian/Native Alaskan</td>
<td>2 (0.8 %)</td>
</tr>
<tr>
<td>Native Hawaiian/Other Pacific Islander</td>
<td>0</td>
</tr>
<tr>
<td>Multi-racial</td>
<td>12 (4.5%)</td>
</tr>
<tr>
<td>Don’t know</td>
<td>1 (0.4%)</td>
</tr>
</tbody>
</table>
### DMD Infant Screening: Survey Demographics: n=160

<table>
<thead>
<tr>
<th></th>
<th>Negative Screen Result Survey (n=138)</th>
<th>Decliner Survey (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race/Ethnicity</td>
<td>• 79.4% White</td>
<td>• 72.7% White</td>
</tr>
<tr>
<td></td>
<td>• 96.3% Non-Hispanic/Latino</td>
<td>• 100% Non-Hispanic/Latino</td>
</tr>
<tr>
<td>Relationship to Child</td>
<td>• 88.2% Mothers</td>
<td>• 90.9% Mothers</td>
</tr>
<tr>
<td>Marital Status</td>
<td>• 91.9% Married</td>
<td>• 94.7% Married</td>
</tr>
<tr>
<td>Education</td>
<td>• 90.3% 4-year college or higher education</td>
<td>• 100% 4-year college or higher education</td>
</tr>
</tbody>
</table>
## DMD Infant Screening: Reasons to Accept or Decline

<table>
<thead>
<tr>
<th>Participants’ Reasons</th>
<th>%</th>
<th>Decliners’ Reasons</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>I just wanted to know</td>
<td>54.3</td>
<td>My child is healthy</td>
<td>63.6</td>
</tr>
<tr>
<td>If he had DMD, I wanted to get treatment for my son earlier</td>
<td>42.0</td>
<td>No other family member with DMD</td>
<td>45.5</td>
</tr>
<tr>
<td>I wanted to rule it out to have one less thing to worry about</td>
<td>31.2</td>
<td>There is no real benefit of knowing right now</td>
<td>22.7</td>
</tr>
<tr>
<td>If he had DMD, I wanted to be able to make plans for the future</td>
<td>23.2</td>
<td>I would not want the stress of knowing before symptoms start</td>
<td>18.2</td>
</tr>
<tr>
<td>If he had DMD, I wanted to mentally prepare myself</td>
<td>19.6</td>
<td>Right now there is no cure or proven treatment</td>
<td>13.6</td>
</tr>
</tbody>
</table>

*Participants and decliners were asked to check up to three reasons*
Parents’ Attitudes Toward Screening

• **Support for screening:**
  - 65.2% supported boys being screened for DMD
  - 34.8% were unsure
  - No parents indicated that boys should not be screened for DMD

• **Preferred timing of screening:**
  - 49.4%: After birth when the routine heel stick for newborn screening takes place
  - 46.1%: When blood is taken for other tests between 6 and 15 months of age

• **Optional or mandatory screening?**
  - 78.3% indicated DMD screening should be optional
  - 12.3% indicated DMD screening should be required
  - 9.4% had no opinion
DMD Infant Screening: Stressfulness of Screening

- 95.6% of the participants felt the experience was only a little or not at all stressful
- 4.3% reported the experience was somewhat stressful
- None of the participants indicated that they felt the experience was very or extremely stressful
- Parent scores on the Parenting Stress Index [PSI] indicated a low level of stress
DMD Infant Screening: Parents Evaluation of Informed Consent

- 96.8% understood the written material “well enough” or better
- 68.8% said the material was at least “somewhat helpful” in making a decision
  - 7.0% felt the material was not helpful in making a decision
- 78.5% felt they had enough time to read the material
- 94.3% said the person who explained the study was at least “somewhat helpful”
- 92.7% said they were given enough time to ask questions about the study
Knowledge of DMD Screening

Five questions were asked to determine whether participants understood basic concepts about DMD and screening.

<table>
<thead>
<tr>
<th>Question topic (not verbatim from survey)</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>How common is DMD and who is affected?</td>
<td>85 (63.0%)</td>
</tr>
<tr>
<td>Which muscles are weakened in DMD?</td>
<td>55 (40.7%)</td>
</tr>
<tr>
<td>Who is at risk for having a child with DMD?</td>
<td>82 (60.7%)</td>
</tr>
<tr>
<td>Screening finds affected infants before symptoms begin. (True/False)</td>
<td>128 (92.8%)</td>
</tr>
<tr>
<td>What is the availability of treatment for DMD?</td>
<td>95 (69.9%)</td>
</tr>
</tbody>
</table>

97 (74.6%) of respondents answered at least 3 of the 5 questions correctly.
DMD Infant Screening: Overall Parent Experience

- 73.7% of parents reported that they were either somewhat or very satisfied with their experience
- 9.5% indicated that they were very dissatisfied with their experience
DMD Infant Screening: Preliminary Conclusions

- Most parents wanted screening for their sons
- Screening later in infancy provides time for parents to read and understand information about DMD and screening
- Providers were in favor of screening, but do not have time to explain the benefits and limitations of DMD screening
- DMD infant screening is possible but difficult to implement in a broad clinical setting unless consent procedures are modified
DMD Infant Screening: Preliminary Conclusions

• After “informed” parental consent, screening infants for DMD, a late-onset disorder, where treatment is primarily supportive was accepted by patients and providers in these four private practice primary care settings
Infant Screening: Future Directions

• Need to determine whether acceptance of infant/well child screening would be similar in a public health setting or private practice with parents from other socioeconomic groups

• Determine feasibility and acceptability for multiplex screening of healthy infants for other late onset disorders
DMD: Acknowledgments

- Emory University, Dept. of Pediatrics
  - Julie Kable, PhD

- NCBDDD/CDC
  - Natalie Street, MS
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  - Barbara Adams, MS
  - Ajay Vatave, MD, MPH
  - Katherine Kolor, MS, PhD

- Research Assistants
  - Alissa Cyrus, MPH
  - Aneidra Leysath
  - Hollie Lawyer

- Practice Recruiting Sites
  - Children’s Medical Group
  - Dekalb Pediatric Center
  - Decatur Pediatric Group
  - Northlake Pediatrics

- MDA Clinic at CHOA - Scottish Rite
  - Ed Goldstein, MD
  - Kelley Maurielo, RN

- Emory Genetics
  - Paul Fernhoff, MD: PI
  - Chunli Yu, PhD: co PI
  - Brad Coffee, PhD: co PI
  - Sharon Quary MS: coordinator
  - Cathy Tesla, MS
  - Leonard Arthur

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**Multiplex Ligation-dependent Probe Amplification (MLPA)**

**Probe design**
- Primer X binding site
- Target specific sequences
- Stuffer sequence
- Primer Y binding site

**Multiplex hybridisation and ligation**
- No ligation of mismatched probes

**PCR with universal primers X and Y**
- No exponential amplification of non ligated probes

**Fragment Analysis**
- Sample
- Control

Adapted from www.mlpa.com
## DMD Infant Screening: Parents’ Attitudes Toward Screening

<table>
<thead>
<tr>
<th>Question</th>
<th>n</th>
<th>Mean (SD)</th>
<th>% Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMD screening was worthwhile</td>
<td>138</td>
<td>4.25 (.67)</td>
<td>87.0</td>
</tr>
<tr>
<td>It is important for baby boys to be screened for DMD.</td>
<td>135</td>
<td>3.96 (.79)</td>
<td>66.7</td>
</tr>
<tr>
<td>Babies should be screened for as many disorders as possible even if it means that more healthy babies will have false alarms.</td>
<td>137</td>
<td>3.32 (1.0)</td>
<td>44.6</td>
</tr>
<tr>
<td>If I had another baby boy, I would want him to have DMD screening.</td>
<td>137</td>
<td>4.00 (.75)</td>
<td>76.6</td>
</tr>
<tr>
<td>I would recommend DMD screening to other parents of baby boys.</td>
<td>137</td>
<td>3.82 (.82)</td>
<td>61.4</td>
</tr>
</tbody>
</table>