Spinal Muscular Atrophy: Overview of Available Screening Methods

Thursday, June 28, 2018

Dial in: 866.740.1260
(passcode 4852701#)
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Agenda

Moderator: Patricia Hunt, Texas Department of State Health Services

1:00 - 1:05 Welcome and Introduction
1:05 - 1:20 Overview of Available Screening Methods
   Francis Lee, PhD, Centers for Disease Control and Prevention

1:20 – 2:00 State Implementation Experiences: NY, MA, UT, MN
   Michele Caggana, ScD, FACMG New York State Department of Health, Wadsworth Center
   Anne Comeau, PhD, New England Newborn Screening Program
   Andy Rohrwasser, PhD, MBA, Utah Department of Health
   Carrie Wolf, MBS, Minnesota Department of Health

2:00 – 2:15 Overview of Second Tier Screening Methods
   Mei Baker, MD, FACMG, Wisconsin State Laboratory of Hygiene

2:15 - 2:30 Q&A and Closing
SMA is the leading genetic cause of death among infants

- A neuromuscular disease caused by progressive degeneration of motor neurons
- Major signs and symptoms include loss of normal motor function and respiratory difficulty/failure; can result in death in severe cases
- 3 clinical types based on age of onset and severity
  - Type I: Birth – 6 mos.
  - Type II: 6 mos. – 2 years
  - Type III: 18 mos. – 3+ years
- Birth prevalence ~ 1 : 10,000
Newborn screening for SMA can lead to early diagnosis and treatment

- In SMA type 1, motor neuronal death begins perinatally; >90% loss within 6 months

- FDA approved drug available since December 2016
Advisory Committee on Heritable Disorder in Newborns and Children

- Submitted recommendation to the Secretary of Health and Human Services to “Expand the Recommend Uniform Screening Panel (RUSP) to include the addition of SMA due to homozygous deletion of exon 7 in SMN1”  Mar 8, 2018

- Deputy HHS Secretary interim response – April 19, 2018 will provide “detailed response regarding actions on the recommendation within 120 days”
Different molecular assays have been used to detect SMA

- Restriction Fragment Length Polymorphism (RFLP) analysis
- High Resolution Melting (HRM) analysis
- Multiplex Ligation-Dependent Probe Amplification (MLPA)
- Luminex Genotyping
- DNA sequencing
- Quantitative Real-time PCR (qPCR)
Real-time PCR emerges as the preferred method in newborn screening for SMA

- Real-time PCR allows for high throughput screening
- Most state newborn screening labs are already using this method to detect Severe Combined Immunodeficiency
  - Labs are equipped with the necessary instrumentation
  - Staff is familiar with procedure
- Reactions can be multiplexed into current SCID assay
  - Reduced the cost of adding SMA
  - Does not require added labor cost to run
SMA Real time PCR Taqman assays used in state newborn screening labs

- CDC ver. 1*: target *SMN1* Exon 7 – Intron 7 (LNA probe and LNA rev primer)
- CDC ver. 2**: target *SMN1* Exon 7 (LNA probe)
- Perkin Elmer: target *SMN1* Exon 7 (LNA probe)

* adopted by New England NBS lab in stand-alone assay
** adopted by UT and MN NBS labs in multiplex assay with TREC
What are the challenges in designing a real-time PCR assay to screen for SMA?
Challenge #1:

**SMN1** has a paralog, the **SMN2** gene, which has nearly identical genomic sequence

- Only 5 nucleotide differences between the two genes in this region
- It is critical to avoid cross signal from **SMN2** when trying to identify the loss of **SMN1**

Need to be able to discriminate single nucleotide polymorphism
Locked Nucleic Acid (LNA) Nucleotide

LNA : A modified RNA nucleotide with extra bridge connecting the 2' oxygen and 4' carbon

"locks" the ribose in the 3'-endo conformation

- PCR primers and probes with some nucleotides substituted by LNAs can differentiate single nucleotide mismatch
- LNA primers and probes can be ordered from multiple commercial sources
The LNA modified probe (in green) was designed to selectively bind *SMN1* by discriminating between the mismatch nucleotides of *SMN1* and *SMN2*

- *SMN1* nucleotide A and *SMN2* nucleotide (G)

Forward and reverse primers (in grey) will amplify both *SMN1* and *SMN2* sequences

**Initial SMA assay developed at CDC targeted intron 7 sequence**

CTTGTGAA_ACAAAAATGCTTTTTAACATCCATATAAAGCTATCTATATATA
GCTATCTATG/ATCTATATAGCTATTTTTTTACTTTCTTTATTTTCT
TACAGGGTTTC(T)AGACAAAAATCAAAAAAGAAGGAAGGTGCTCACAATTTCTCT
TAATAAAGGA_GTAAGTCTGCCCAGCATTATGAAAGTGAATCTTTACTTTTG
AAAAACTTTATGTTTTGCTGGAAAAACAAATGTTTTTGAACATTTAAAAAGT
TCAGATGTTA(A)AAAGTGGAAAGGTAAATGTAAATCAATATTAAA
GAATTTTGAATCCAAACTATTAGATAAAAAGGTTAATCTACATCCTACT

Characters in red = SMN 1(2) exon 7

- The LNA modified probe (in green) was designed to selectively bind *SMN1* by discriminating between the mismatch nucleotides of *SMN1* and *SMN2*
Challenge #2: Chimeric gene

- Taiwan pilot newborn screening for SMA
  Feasibility trial for pre-symptomatic diagnosis
  Nov 2014 – Sept 2016

Total Screened: 120,267
- Tier-One Positive: 15 (by absence of SMN1 intron 7)
- Tier-Two Positive and Confirmed: 7 (by ddPCR & MLPA)

False positive due to recombination between \textit{SMN1} and \textit{SMN2} resulting in a hybrid genotype

We replaced the reverse primer with an *SMN1*-specific LNA primer (in yellow) to eliminate *SMN2* amplification

The LNA probe targets the exon 7 region with the mismatch between SMN1 *C* and SMN2 *(T)*

Assay has two layers of specificity to eliminate any X-reaction to *SMN2*
No background signal from SMN2 (maximum sensitivity in detecting SMN1 absence)
However, no signal if either SMN1 exon or intron is absent
Requires confirmation with second tier assay specific for exon 7 or intron 7
Limitations associated with LNA primer

While highly specific, LNA primers are technically more demanding

- Sensitive to quality of DNA extract
- Sensitive to type of Taqman master mix
- Sensitive to temperature accuracy
- PCR efficiency around 90%
Revised SMA Assay ver. 2 – Targeting exon 7

- Reverse primer moved to exon 7 region: the unmodified forward and reverse primer will amplify exon 7 of both SMN1 and 2.
- The LNA probe (in green) for exon 7 was further optimized for maximum specificity.

**Characters in red = SMN 1(2) exon 7**

CTTGTGAAACAAAAATGCTTTTTAACATCCATATAAAAGCTATCTATATATATA  
GCTATCTATAG(A)TCTATATAGCTATTTTTTTAAACCTTTCTTTTTTCT  
TACAGGGTTTC(T)AGACAAATCAAAAAAGAAGGAAGGTGCTCACATTCTCT  
**TAAATTAAGGAGA**TAAGTCTGCCAGCATTATGAAAGGTGAATCTTACTTTTG  
TAAAACTTTATGTTTTGTGGAAAAAACAAATGTTTTTGAAACATTTAAAAAGTT  
TCAGATGTAA(A(G))AAAGTTGAAAGGTTAATGTAAAAACAATCAATATTAAA  
GAATTCTGATGACCAAAACTATTAGATAAAAAGGTAAAATCTACACATCCCTACT
LNA probe was redesigned for maximum specificity

- Factors important in the design of LNA probe for mismatch discrimination:
  - short length (10-12 nucleotides)
  - Location of mismatch nucleotide in the center of probe
  - LNA substitution in triplet at site of mismatch

Probe with LNA modification of pyrimidine (C or T) at mismatch site within probe

The Assay ver. 2 utilizes an SMN1-specific LNA probe with forward strand sequence.

- We do not observe any non-specific signal in SMN1 null samples even when challenged with an excess of SMN2 sequence.
**SMN1** can also be multiplexed into the current TREC assay (SMN1-TREC-RPP30)
SMA patients are correctly identified from dried blood spots when using the multiplex assay

<table>
<thead>
<tr>
<th>Donor Number</th>
<th>Cq - SMN1 Exon 7</th>
<th>SMN1 Result</th>
<th>Clinical Category</th>
<th>SMA Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No Cq</td>
<td>Absent</td>
<td></td>
<td>Affected</td>
</tr>
<tr>
<td>2</td>
<td>No Cq</td>
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<td></td>
<td>Affected</td>
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<tr>
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<tr>
<td>12</td>
<td>22.6</td>
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<td>Unaffected/Carrier</td>
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<td>13</td>
<td>23.2</td>
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<td>14</td>
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<td>Present</td>
<td></td>
<td>Unaffected/Carrier</td>
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<td>15</td>
<td>24.4</td>
<td>Present</td>
<td></td>
<td>Unaffected/Carrier</td>
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<tr>
<td>16</td>
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<td>Present</td>
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<td>Unaffected/Carrier</td>
</tr>
<tr>
<td>17</td>
<td>24.6</td>
<td>Present</td>
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<td>Unaffected/Carrier</td>
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<tr>
<td>18</td>
<td>24.7</td>
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<td>Unaffected/Carrier</td>
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<tr>
<td>19</td>
<td>24.9</td>
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<tr>
<td>25</td>
<td>26.7</td>
<td>Present</td>
<td></td>
<td>Unaffected/Carrier</td>
</tr>
<tr>
<td>26</td>
<td>28.4</td>
<td>Present</td>
<td></td>
<td>Unaffected/Carrier</td>
</tr>
</tbody>
</table>
Technology Transfer to state newborn screening laboratories

Both versions of CDC SMA assay have been validated in state NBS labs, and is being used in state-wide screening

- Massachusetts (January 29, 2018)
- Utah (January 29, 2018)
- Minnesota (March 5, 2018)

- As of June, > 40,000 newborns have been screened
- Three SMA infants have been identified, confirmed and treated
Discussion

- *SMN1* assay is the first newborn screening 1st tier test based on genotype alone
- High specificity required to discriminate *SMN2* sequence to avoid false negative results
- Possible unknown non-pathogenic SNP, if present in the probe region can potentially lead to false positive
- Clinical diagnostic lab confirmation of screen positive cases, and determination of *SMN2* copy numbers are important for medical management
CDC SMA NBS resources available to state labs

- If a state lab decides to try CDC assays, we provide reagents (enough for assay development), primers and probe sequences, QC materials and technical support.

- Hands-on technical training at CDC, if requested.

- SMA positive QC dried blood spot material: prepared from patient cell lines spiked into leukocyte-depleted blood.

- CDC started SMA pilot proficiency testing program in June 6, 2018 (10 labs participating).
Screening for SMA
- Completing assay validation
- At planning stage
Acknowledgments

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  - Shu-Chuan Chiang
  - Wuh-Liang Hwu

- **State NBS lab collaborators:**
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  - **New Jersey**
    - Alyssa MacMillan
  - **Wisconsin**
    - Mei Baker, Sean Mochal
  - **Massachusetts**
    - Anne Comeau, Lan Ji
  - **Utah**
    - Andreas Rohrwasser
    - Katelyn Logerquist
Thank you for your attention!

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For more information please contact Centers for Disease Control and Prevention

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.
Spinal Muscular Atrophy Screening in New York State
APHL Webinar – June 28, 2018

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Director, Newborn Screening Program
Wadsworth Center, NYS Department of Health
Disclosures

- Biogen, Idec funded this study (screening, recruitment).

- Biogen had no role in data analysis, interpretation, or decisions regarding patient counseling or care.
Spinal Muscular Atrophy (SMA)

- Progressive degeneration & loss of spinal cord & brainstem motor neurons
- Muscle weakness, atrophy
- Difficulty breathing, poor weight gain, pneumonia, scoliosis, joint contractures

Age at onset, symptoms, severity and survival vary
Most common genetic cause of infant & toddler death

- Incidence: 1 in 6,000 to 1 in 10,000
- Carriers: 1 in 50 to 1 in 60

95%–98% homozygous deletion of Survival of Motor Neuron 1 (SMN1) exon 7

**SMN1** (5q13)

\[\begin{align*}
\text{exon 1} & \quad 2a & \quad 2b & \quad 3 & \quad 4 & \quad 5 & \quad 6 & \quad 7 & \quad 8 \\
\end{align*}\]

**SMN2** = **SMN1** homologue (differ by few nucleotides | both code for SMN)

\[\begin{align*}
\text{full-length SMN} (100\%) \\
\text{truncated, non-functional SMN} (\sim 85\%-95\%) \\
\text{full-length SMN} (\sim 5\%-15\%)
\end{align*}\]

# genomic copies of **SMN2** varies (0–5)

\[\begin{align*}
\uparrow \text{SMN2} & \approx \text{less severe, later onset}
\end{align*}\]
Spinal Muscular Atrophy (SMA)

Age at onset, symptoms, severity and survival vary

<table>
<thead>
<tr>
<th>SMA Type</th>
<th>Age</th>
<th>Life-span</th>
<th>Motor Function Achieved</th>
<th>Major Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>&lt;6 mo</td>
<td>≤2 yr</td>
<td>Never sit without support</td>
<td>Profound hypotonia and flaccidity, no head control, paradoxical breathing, bell-shaped upper torso, poor suck &amp; swallow. Respiratory and nutritional problems.</td>
</tr>
<tr>
<td>Type II</td>
<td>6–12 mo</td>
<td>70% alive at 25 yr</td>
<td>Maintain sitting; Never walk independently</td>
<td>Muscle weakness, kyphoscoliosis, fine tremors, weak swallow. Respiratory and nutritional problems.</td>
</tr>
<tr>
<td>Type III</td>
<td>&gt;1 yr</td>
<td>Adult/</td>
<td>Reach all major milestones; walk independently (≥25m)</td>
<td>Variable weakness, legs &gt;arms, frequent falls, lose ability to walk (childhood-adults), some use wheelchairs; most w/ scoliosis. No respiratory, nutritional issues.</td>
</tr>
<tr>
<td>Type IV</td>
<td>20's–30's</td>
<td>Adult/</td>
<td>Reach all major milestones; walk independently</td>
<td>Variable weakness; walk as adults; motor impairment mild. No respiratory, nutritional issues.</td>
</tr>
</tbody>
</table>

SMN2 copy #1,2

<table>
<thead>
<tr>
<th>Copy</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 (7-13%)</td>
<td>1 (---)</td>
<td>1 (---)</td>
<td>1 (---)</td>
</tr>
<tr>
<td>2</td>
<td>2 (73-83%)</td>
<td>2 (11%)</td>
<td>2 (0-4%)</td>
<td>2 (0-4%)</td>
</tr>
<tr>
<td>3</td>
<td>3 (4-20%)</td>
<td>3 (82%)</td>
<td>3 (51-78%)</td>
<td>3 (51-78%)</td>
</tr>
<tr>
<td>4</td>
<td>4 (---)</td>
<td>4 (7%)</td>
<td>4 (7%)</td>
<td>4 (22-46%)</td>
</tr>
</tbody>
</table>

Mailman, 2002, Genet Med 2
Feldkotter, 2002, Am J Hum Genet
Path to SMA
Newborn Screening

Should SMA be screened?

- Important health problem
- Natural hx known
- Recognizable latent stage
- Biomarker & test (2000’s)
- Acceptable Tx (2016)
- Demonstrated benefit of early detection, intervention & Tx (2016)

Screening criteria adapted from Wilson and Jungner (1968) Principles and practice of screening for disease
Nomination of SMA for addition to RUSP (2017)

- Evidence review by ACHDNC
  - NBS assay validated and implemented in traditional public health lab
  - Spinraza FDA-approved in 2016
  - Clinical trials (Spinraza, AVXS-101) published in 2017

- Recommendation to Secretary: Newborn Screening for SMA due to homozygous deletion of exon 7 in SMN1 should be added to the RUSP as a core condition.
  (February 8, 2018; 8-5 vote → June 9, 2018 was due)
Pilot Newborn Screening for SMA

Columbia University Medical Center, Columbia Presbyterian Hospitals, and NYS Newborn Screening Program

**Major Goals**

- Develop *SMN1* assay
- Demonstrate feasibility of high-throughput newborn SMA screening
- Offer screening, assess uptake and outcomes; including carrier status

Morgan Stanley Children’s Manhattan 4,400 births/yr

Allen Hospital Upper Manhattan/Bronx 2,000 births/yr

Weill-Cornell Medical Center Manhattan 5,800 births/yr
Recruitment – Opt-in model

Hospitals - 3 NYC hospitals, 12,000 births/yr

Materials - video & brochure

Coordinators - describe study, answer questions, obtain consent on tablet (REDCap), mark Guthrie card
Screening – SMN1 exon 7 deletion assay

- No biomarker; DNA-first test
- DNA extracted from dried blood spot
- TaqMan real-time qPCR assay
  - SMN1 exon 7
  - RPPH1 (internal control gene)
- ABI 7900HT / QuantStudio 12K Flex
- ΔΔCt to calculate SMN1 copy number

SMA Assay Validation

45 Positive Controls

≥1 copy SMN1 (1-2 copies SMN2)

0 copies SMN1 (2≥4 copies SMN2)

RPPH1 amplification

SMN1 amplification

Probe
5’-FAM (SMN1)
5’-VIC (RPPH1)
3’ quencher – MGB-NFQ
ROX standard

4,028 DBS

*each point=mean RQ, 3 replicates

screen neg (3,929)

borderline (14)

fail (34)

het del (51)
Biogen Samples

Both detectors

RPPH1

SMN1
SMA Assay Controls

*All in triplicate*

*RQ = relative quantity = $2^{\Delta\Delta Ct}$*

- Known 2 SMN1 copies
- As calibrators
- 0 copy SMN1 control
- 1 copy SMN1 control
- 2 copies SMN1 control

**ΔCt sample – cal median ΔCt**

FAM = SMN1  VIC = RPPH1
SMA Assay

All in triplicate

Equivocal (0.001-0.299 or 0.600-0.799)

2 or more SMN1 copies

1 copy of SMN1
## Exon 7 DNA sequence

- High CV X2
- Equivocal X2
- Equivocal on repeat
- 1 copy SMN1
- 0 copies SMN1
Results

January 15, 2016 – June 15, 2018
Infants screened: 14,089 (200 carriers)
Opt in rate: 91-93%

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Recruitment period</th>
<th>Infants Screened</th>
<th>Carriers (Freq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morgan Stanley Children’s Hospital</td>
<td>1/14/2016 – 5/9/2018*</td>
<td>5,840</td>
<td>74 (1 in 79)</td>
</tr>
<tr>
<td>Weill-Cornell Medical Center</td>
<td>7/13/2016 – 5/9/2018</td>
<td>4,851</td>
<td>95 (1 in 51)</td>
</tr>
<tr>
<td>Allen Hospital</td>
<td>1/26/2016 – 5/9/2018</td>
<td>2,523</td>
<td>20 (1 in 126)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>13,214</strong></td>
<td><strong>189 (1 in 70)</strong></td>
</tr>
</tbody>
</table>

False positives: 0% (0/13,214)
False negatives: 0% (0/13,214)

Retest rate ~1%; mostly around carrier calls; live = no CV fails
Follow-up – Carriers

14.1% (16/113) agreed to genetics referral
- 73.3% (11/15) made appt
- 72.7% (8/11) maintained appt

Most parents expressed concern; after speaking with counselor, expressed understanding of "carrier" status versus "affected"

42.9% (81/189) knew they were carriers
- less concerned, better understanding
Results

Affected infant identified by NBS

Genotype:
- $SMN1$: homozygous Δ exon 7
- $SMN2$: 2 copies

Predicts SMA type 1

SMA Type 1 Natural History
- Onset: <6 months
- Survival: ≤2 years
- Major motor milestones reached: None; never sit unassisted.
- Sx: Profound hypotonia and flaccidity, no head control, poor suck & swallow; respiratory and nutritional problems

@ 29 months – tolerates medication, meeting milestones on time, walking, running, talking
Results

SMA Assay
Detected homozygous deletion
Conclusions

- SMA newborn screening is feasible
  - Sensitive, specific, robust, high-throughput
  - No false positives/negatives
- NYS families want testing (93%)
- Carrier rate = 1 in 70
- 1 infant predicted to have type 1 infantile SMA (1 in 13,214)
  - treated with nusinersen (Spinraza)
  - asymptomatic at 29 months
Population-wide Screening in NYS

- Regulatory amendment *(bill pending currently)*
- Specialty Care Centers *(certifying)*
  - Genetics, neuromuscular specialists (n = 11)
- No carrier reporting
- Multiplex with severe combined immunodeficiency (SCID) qPCR assay; singlicate
  - $0.10/baby for SMA FOR TEST
- SMN2 dosage (digital droplet PCR), about $25 per baby
Screening – *SMN1* exon 7 deletion assay

**SMN1 Exon 7 Deletion Assay** (Multiplexed SCID and SMA)

≥1 copy *SMN1*  
0 copies *SMN1*

**SCREEN NEGATIVE**  
No Further Action Required

**SCREEN POSITIVE**  
Referral for Evaluation & Diagnostic Testing

Model for universal screening

Will use Ct cut-off rather than ΔΔCt to calculate *SMN1* copy number

Probes  
5’-VIC (SMN1)  
5’-ABY (RPPH1)  
5’-FAM (TREC)  
3’ quencher – MGB-NFQ (SMN1 + TREC)  
3’ quencher – QSY (RPPH1)

Purple haze standard
Universal SMA Screening – New York Plan

Multiplex with SCID TREC assay

Carriers
• Not reported

Late onset SMA
• SMN2 copy number
• When to treat
• How will detection impact the incidence of SMA?

Non-deletion mutations
• Will not be detected; report language important
• 2 – 5%

Treatment
• Long-term effects? Renal toxicity?
• Availability, cost and compliance?
• Insurance Coverage
Acknowledgement

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• Bianca Haser, BS
• Veronica Ortiz, MHS
• Anthony Albertorio, BA
• Emilia Naranjo
• Talia Weitz
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• Jacqueline Gomez, RN
• Angela Pena
• Columbia Presbyterian Hospitals

Clinical
• Wendy Chung, MD, PhD
• Carrie Koval, MS, CGC
• Julia Wynn, MS
• Lilian Cohen, MD
• Sarah Andrew, BA
• Sally Dunaway Young, PT, DPT
• Nicole LaMarca, DNP, MSN, CPNP
• Darryl De Vivo, MD
• Columbia University Medical Center

Funding
• Biogen, Idec

Controls
• Pediatric Neuromuscular Research Clinic (PNRC)
• Biogen, Idec
Screening for Spinal Muscular Atrophy
Early Data from Massachusetts Newborn Screening

APHL SMA Webinar Series Part Two:
Overview of Available Screening Methods

Anne Marie Comeau, Ph.D
Deputy Director, New England Newborn Screening Program
Professor of Pediatrics, UMass Medical School
The University of Massachusetts holds intellectual property that is used in 1 of 17 pipeline therapies that are listed by Cure SMA.
Spinal Muscular Atrophy (SMA)

- Most common lethal autosomal recessive disorder in infants.
- Progressive muscle weakness resulting from degeneration of an anterior horn neurons
- FDA-approved therapy
- Recommended for RUSP by SACHDNC
- Estimated Incidence: 1 in 6,000 to 20,000
- 1 in 40 people are heterozygote carriers
Assay Development for SMA NBS

Francis K Lee and Kristina Mercer

Newborn Screening and Molecular Biology Branch, Centers for Disease Control and Prevention

Lan Ji and Jennifer Navas

New England Newborn Screening Program
UMMS
Assay Development for SMA NBS

Two factors key to development:

- SMA is related to the absence of a fully functional gene that produces a Survival of Motor Neuron (SMN) protein, $SMN1$
- 95% SMA patients show homozygous loss of $SMN1$ exon 7
Validation

Pre-characterized samples from Corielle  n=7

Pre-characterized samples from CDC n= 2

Pre-characterized samples from Biogen
n= 22 SMA patients
n= 44 obligate carriers (parents)

100% pass
The Massachusetts SMA NBS Workgroup

Representatives from Newborn Screening, Neurology, Genetics

Mary Alice Abbott, MD
Basil Darras, MD
Beverly N. Hay, MD
Kathryn J. Swoboda, MD
Anne Marie Comeau, PhD
Jaime E. Hale, MS
Inderneel Sahai, MD
Roger B. Eaton, PhD
Number of Babies Screened for SMA

21,341

As of 6/26/2018

New England Newborn Screening Program
Number of infants with a specimen prompting Tier 2

n = 29 (0.14%)

n = 21,312

Prompted Tier 2

Normal NBS by Tier 1
Infants with a specimen prompting Tier 2
n = 29
Infants with a specimen prompting Tier 2
n = 29

72% prompting Tier 2 have been NICU specimens

- WNL NBS - SMN1 Hybrid
- WNL NBS
- Positive NBS
Infants with a specimen prompting Tier 2
n = 29

72% prompting Tier 2 have been NICU specimens

False positive; specimen apparently contained an inhibitor

New England Newborn Screening Program
Implementation of SMA/TREC LDT Assay

Katelyn Logerquist, MLS(ASCP)\textsuperscript{CM}
David E. Jones, PhD
Andy Rohrwasser, PhD

SMA Webinar
June 28, 2018
SMA/TREC Assay Method

• PCR-Based Triplex Assay (described by Dr. Lee)
  – \textit{SMN1} – Deletion of exon 7 of \textit{SMN1} gene (SMA)
  – TREC – T-cell receptor excision circles (SCID)
  – \textit{RPP30} – Internal control

• Extraction
  – Automated – TECAN Freedom EVO
  – PBS/Tween 20 wash/Qiagen Solution 2 wash and elution
  – 96 well format to 384 well format

• Real-Time PCR
  – Roche LightCycler 480 II
  – 384 well block
Extraction

1. 3.2 mm punch
2. Wash 1: 80ul PBS/Tween 20, 8 mins, shaking 700rpm (RT, Inheco)
3. Wash 2: 80ul Qiagen Solution 2, 8 mins, RT, shaking 700rpm
4. Elution: 140ul Qiagen solution 2, 30 mins, 70C, shaking 700rpm
5. Transfer 3.5 ul into 384 well, PCR volume 12 ul
SMA/TREC Assay Results

• Normal Control
  – Pooled known normal specimens

• Abnormal Control
  – Negative control
    • SMN1
    • TREC
SMN1

Amplification Curves

Fluorescence (618-660)

Cycles
Validation of SMA/TREC Assay

- Reproducibility Study
- Limited Case Control Study (BLINDED!)
- Population Analysis (5000 (SMA), 3000 (SCID))
### SMA Abnormals

<table>
<thead>
<tr>
<th>Patient</th>
<th>Origin</th>
<th>$SMN1$ Cp</th>
<th>$RPP30$ Cp</th>
<th>LDT Determination</th>
<th>Dx</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Biogen</td>
<td>No Amp</td>
<td>27.64</td>
<td>Abnormal</td>
<td>SMA</td>
</tr>
<tr>
<td>2</td>
<td>Biogen</td>
<td>No Amp</td>
<td>26.41</td>
<td>Abnormal</td>
<td>SMA</td>
</tr>
<tr>
<td>3</td>
<td>Biogen</td>
<td>No Amp</td>
<td>27.61</td>
<td>Abnormal</td>
<td>SMA</td>
</tr>
<tr>
<td>4</td>
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<td>Abnormal</td>
<td>SMA</td>
</tr>
<tr>
<td>5</td>
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<td>28.45</td>
<td>Abnormal</td>
<td>SMA</td>
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<tr>
<td>6</td>
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<td>No Amp</td>
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<td>SMA</td>
</tr>
<tr>
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<td>Biogen</td>
<td>No Amp</td>
<td>29.82</td>
<td>Abnormal</td>
<td>SMA</td>
</tr>
<tr>
<td>8</td>
<td>Biogen</td>
<td>No Amp</td>
<td>29.67</td>
<td>Abnormal</td>
<td>SMA</td>
</tr>
<tr>
<td>9</td>
<td>Biogen</td>
<td>No Amp</td>
<td>27.91</td>
<td>Abnormal</td>
<td>SMA</td>
</tr>
<tr>
<td>10</td>
<td>Biogen</td>
<td>No Amp</td>
<td>28.85</td>
<td>Abnormal</td>
<td>SMA</td>
</tr>
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<td>No Amp</td>
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<td>Abnormal</td>
<td>SMA</td>
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<tr>
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<td>28.12</td>
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<td>SMA</td>
</tr>
<tr>
<td>13</td>
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<td>No Amp</td>
<td>29.92</td>
<td>Abnormal</td>
<td>SMA</td>
</tr>
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<td>No Amp</td>
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<td>SMA</td>
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<td>Abnormal</td>
<td>SMA</td>
</tr>
<tr>
<td>16</td>
<td>CDC</td>
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<td>Abnormal</td>
<td>SMA</td>
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<td>Abnormal</td>
<td>SMA</td>
</tr>
<tr>
<td>18</td>
<td>Utah</td>
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<td>28.59</td>
<td>Abnormal</td>
<td>SMA</td>
</tr>
<tr>
<td>19</td>
<td>Utah</td>
<td>No Amp</td>
<td>29.08</td>
<td>Abnormal</td>
<td>SMA</td>
</tr>
<tr>
<td>20</td>
<td>Utah</td>
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<td>Abnormal</td>
<td>SMA</td>
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<tr>
<td>21</td>
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<td>Abnormal</td>
<td>SMA</td>
</tr>
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<td>22</td>
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<td>Abnormal</td>
<td>SMA</td>
</tr>
<tr>
<td>23</td>
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<td>No Amp</td>
<td>29.82</td>
<td>Abnormal</td>
<td>SMA</td>
</tr>
<tr>
<td>24</td>
<td>Utah</td>
<td>25.58</td>
<td>26.21</td>
<td>Normal</td>
<td>Normal</td>
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</table>
## SCID Abnormals

<table>
<thead>
<tr>
<th>Patient</th>
<th>TREC Cp</th>
<th>Z-Score</th>
<th>RPP30 Cp</th>
<th>LDT Determination</th>
<th>Dx</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No Amp</td>
<td>No Amp</td>
<td>28.97</td>
<td>Abnormal</td>
<td>Classic SCID</td>
</tr>
<tr>
<td>2</td>
<td>No Amp</td>
<td>No Amp</td>
<td>26.98</td>
<td>Abnormal</td>
<td>Classic SCID</td>
</tr>
<tr>
<td>3</td>
<td>No Amp</td>
<td>No Amp</td>
<td>30.34</td>
<td>Abnormal</td>
<td>SCID ADA</td>
</tr>
<tr>
<td>4</td>
<td>No Amp</td>
<td>No Amp</td>
<td>29.94</td>
<td>Abnormal</td>
<td>SCID ADA</td>
</tr>
<tr>
<td>5</td>
<td>No Amp</td>
<td>No Amp</td>
<td>29.94</td>
<td>Abnormal</td>
<td>DiGeorge Syndrome</td>
</tr>
<tr>
<td>6</td>
<td>No Amp</td>
<td>No Amp</td>
<td>30.21</td>
<td>Abnormal</td>
<td>DiGeorge Syndrome</td>
</tr>
<tr>
<td>7</td>
<td>No Amp</td>
<td>No Amp</td>
<td>33.13</td>
<td>Abnormal</td>
<td>Secondary T-cell Lymphopenia</td>
</tr>
<tr>
<td>8</td>
<td>No Amp</td>
<td>No Amp</td>
<td>31.37</td>
<td>Abnormal</td>
<td>Secondary T-cell Lymphopenia</td>
</tr>
<tr>
<td>9</td>
<td>No Amp</td>
<td>No Amp</td>
<td>28.86</td>
<td>Abnormal</td>
<td>Secondary T-cell Lymphopenia</td>
</tr>
<tr>
<td>10</td>
<td>No Amp</td>
<td>No Amp</td>
<td>26.54</td>
<td>Abnormal</td>
<td>Idiopathic T-cell lymphopenia asymptomatic</td>
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<tr>
<td>11</td>
<td>No Amp</td>
<td>No Amp</td>
<td>30.58</td>
<td>Abnormal</td>
<td>Variant T-cell lymphopenia</td>
</tr>
<tr>
<td>12</td>
<td>No Amp</td>
<td>No Amp</td>
<td>27.16</td>
<td>Abnormal</td>
<td>Microdeletion syndrome</td>
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<tr>
<td>13</td>
<td>40.8</td>
<td>2.30</td>
<td>29.35</td>
<td>Normal</td>
<td>Secondary T-cell Lymphopenia</td>
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<tr>
<td>14</td>
<td>41.39</td>
<td>2.66</td>
<td>31.61</td>
<td>Normal</td>
<td>Secondary T-cell Lymphopenia</td>
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<tr>
<td>15</td>
<td>39.23</td>
<td>1.36</td>
<td>30.71</td>
<td>Normal</td>
<td>Normal</td>
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</tbody>
</table>
SMN1 Population Analysis
TREC Population Analysis
Z-Score

Population Z-score

\[ z = \frac{x - \mu}{\sigma} \]

Individual measurement:
How many standard deviations below or above the population mean?

Requires sufficiently large population study (knowledge of population mean and population standard deviation).
TREC Population Analysis

Z-Score

0 500 1000 1500 2000 2500 3000 3500

Z-Score
# SMA/TREC Assay Cut-Offs

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mean ± SD</th>
<th>2 SD</th>
<th>99&lt;sup&gt;th&lt;/sup&gt; Percentile</th>
<th>3SD</th>
<th>99.5&lt;sup&gt;th&lt;/sup&gt; Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>SMN1</em></td>
<td>29.15 ± 1.35</td>
<td>31.85</td>
<td>32.91</td>
<td></td>
<td>33.20</td>
</tr>
<tr>
<td>TREC</td>
<td>36.98 ± 1.66</td>
<td>40.31</td>
<td>41.54</td>
<td>41.97</td>
<td>42.18</td>
</tr>
<tr>
<td><em>RPP30</em></td>
<td>29.71 ± 1.39</td>
<td>32.49</td>
<td>32.99</td>
<td>33.88</td>
<td>34.14</td>
</tr>
</tbody>
</table>

*The cut-off for TREC is a Z-score of 2.8 (corresponds with a Cp ≈ 41.65).*
Term SCID Workflow

First Card

SCID

Normal

Retest

SCIDC

Normal

Indeterminate

Abnormal

Second Card

SCIDR

Normal

Retest

SCIDRC

Normal

Abnormal
SMA Production Data

<table>
<thead>
<tr>
<th>Category</th>
<th>Old Method Count (n)</th>
<th>New Method Count (n)</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Screened</td>
<td>10,989</td>
<td>5,548</td>
<td>16,537</td>
</tr>
<tr>
<td>Repeat First Screen</td>
<td>204</td>
<td>43</td>
<td>247</td>
</tr>
<tr>
<td>Second Specimens Screened</td>
<td>12</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>Total Abnormal</td>
<td>1 + 1</td>
<td>0</td>
<td>1 + 1</td>
</tr>
<tr>
<td>True SMA Case</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*Summary of patients screened from January 29, 2018 – May 31, 2018
*About 5% repeat requirement for first NBS
Abnormal Case 1

- Positive screen reported
- Assessed in clinic no symptoms present
- Confirmatory testing confirmed diagnosis of SMA (0 SMN1 and 3 SMN2)
- Patient with family history and predicted SMA Type 2 phenotype
Abnormal Case 2

• **Internal decision to send for diagnostic testing (early testing stage) instead of resorting to repeat screen/recall specimen**

• Assessed in clinic with no symptoms present

• Confirmatory testing showed 2 copies *SMN1* and 1 copy *SMN2* (confirmed in 2 independent laboratories)

• *SMN1* repeated on second NBS and was normal
Summary

• True cases show no amplification of \textit{SMN1}
• In production assay works for \textit{SMN1} and TREC
• Concordant performance with EnLite
• 384 well format allows economies of scale
• Passed initial PT
96 to 384 conversion

Plate 1

Plate 2

Plate 3

Plate 4
SMN1 Reproducibility

Within Run Mean SMN1

Cp

1
2
3
TREC Reproducibility

Within Run Mean TREC

Cp

1  2  3
RPP30 Reproducibility

PCR precision Within Run Mean
RNaseP

Cp

1 2 3
Reproducibility

Between Run

RNaseP  SMN1  TREC
SMN2 Copy number Assessment in NBS for SMA

Mei Baker, MD, FACMG

Co-Director, Newborn Screening Laboratory at WSLH
Wynne Mateffy Professor, Department of Pediatrics
University of Wisconsin School of Medicine and Public Health

APHL webinar series on spinal muscular atrophy (SMA)

June 28, 2018
# SMA Types and Clinical Classification

<table>
<thead>
<tr>
<th>SMA Type</th>
<th>Age of Onset</th>
<th>Motor Ability</th>
<th>Life Expectancy</th>
<th>SMN2 Copy Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA Type I</td>
<td>&lt; 6 months</td>
<td>Cannot sit</td>
<td>&lt; 2 years</td>
<td>2 copies</td>
</tr>
<tr>
<td>SMA Type II</td>
<td>&lt; 18 months</td>
<td>Sit independently, cannot stand</td>
<td>2nd - 3rd decade</td>
<td>3-4 copies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breathing difficulty</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMA Type III</td>
<td>&gt; 18 months</td>
<td>Stand and walk independently</td>
<td>Normal life expectancy</td>
<td>3-4 copies</td>
</tr>
<tr>
<td>SMA Type IV</td>
<td>Adolescent or adult onset</td>
<td>Retain walking, muscle pain</td>
<td>Normal life expectancy</td>
<td>4-8 copies</td>
</tr>
</tbody>
</table>
SMA Type and SMN2 Copies

M. Calucho et al, Neuromuscular Disorders (2018)
SMN1 and SMN2 in SMA

M. Butchbach et al, Frontiers in Molecular Biosciences (2016)
Real-time PCR Assay

Targeting Single Base Variant in Exon 7

LNA probe specific for SMN1 target

LNA probe specific for SMN2 target
SMN2 Copy Number Assessment by Droplet Digital PCR
# SMN2 Copy Numbers in SMN1 Zero Samples

<table>
<thead>
<tr>
<th>ID</th>
<th>Clinical Diagnosis</th>
<th>SMN2 Copy Numbers</th>
<th>Real-time PCR Assay</th>
<th>Droplet Digital PCR Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>WI SMA 1</td>
<td>SMA Type II</td>
<td>3</td>
<td>4</td>
<td>3</td>
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<tr>
<td>WI SMA 2</td>
<td>SMA Type I</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
<td>WI SMA 3</td>
<td>SMA Type II</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>WI SMA 4</td>
<td>SMA Type I</td>
<td>Not Provided</td>
<td>2</td>
<td>2</td>
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<tr>
<td>WI SMA 5</td>
<td>SMA Type I</td>
<td>Not Provided</td>
<td>2</td>
<td>2</td>
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<tr>
<td>WI SMA 6</td>
<td>SMA Type I</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>WI SMA 7</td>
<td>SMA Type II</td>
<td>Not Provided</td>
<td>&gt;4</td>
<td>3</td>
</tr>
</tbody>
</table>
Wisconsin SMA Screening Protocol

NBS Specimens → RT-PCR → SMN1 Zero → ddPCR → SMN2 Copy Numbers
Wisconsin
SMA Follow-up Protocol

Confirmed
SMN1 zero & SMN2 copy#

SMN2
1-3 copies

SMN2
4 or more copies

Discuss treatment options (nusinersen, clinical trial)

Yes

Symptoms?

No

Follow clinically every 6-12 months

Yes

Symptoms?

Discuss treatment options (nusinersen, clinical trial)
SMA Screening Assay Summary

- It is technically feasible to incorporate SMA screening test into the current ongoing SCID screening test.

MULTIPLEX

- It is feasible to avoid SMA carrier identification by only detecting “SMN1 ZERO”

- Screening sensitivity of the proposed method is about 95%

- It is beneficial to include SMN2 copy number assessment in NBS for SMA protocol
Acknowledgments

- **Meredith Schultz, MD**
  Dept. of Neurology, UWSMPH
- **Matthew Harmelink, MD**
  Dept. of Neurology, CHW
- **Audrey Tluczek, PhD, RN**
  School of Nursing, UWSMPH
- **Anita Laxova**
  Dept. of Pediatrics, UWSMPH
- **Sean Mochal, BS**
- **Mandie Loehe, BS**
- **Bethany Zeitler, BS**
  Newborn Screening Laboratory at WSLH
Questions?

- Please press *7 to unmute, or type your question in the chat box.
Archived Webinar Series

The SMA webinar series has been archived and recorded. It will be posted on APHL.org within the next week.
P.A.C.E. Continuing Education Credits

- To receive 1.5 P.A.C.E. continuing education credits for attending this webinar, you must complete the post webinar evaluation, which will appear in the post webinar pop-up window and follow-up email. If you have any questions, please contact Funke Akinsola, oluwfafunke.akinsola@aphl.org, 240.485.2714