Newborn Screening: It’s Complicated!
DNA testing in Newborn Screening

Denise M. Kay, Ph.D.
NYS Newborn Screening Program
Wadsworth Center, NYS DOH
Why test DNA?

Most conditions screened are genetic...

... is DNA testing necessary?
Tiered approach

Initial screen for abnormal biomarker: Immunoreactive trypsinogen (IRT) ↓ Reflex to DNA testing
Benefits

- Triage follow-up
- Family planning
- Reduce disparities
- Molecular therapy

Aim for high sensitivity
- Reduce false positive referrals
- Rapid molecular dx at birth
- Complement slow, painful, invasive dx testing
- Phenotype prediction (some)
DNA, tests and conditions

No mutation analysis

Target common mutation(s)

Mutation panels

Gene sequencing

Gene panels

Exome sequencing

Genome sequencing

Medium-chain acyl-coenzyme A dehydrogenase (MCAD) deficiency

- Fatty acid oxidation disorder
- 1 in 17,000
- Mendelian, autosomal recessive
- Can’t convert certain fats to energy
- Hypoglycemia, lethargy, vomiting, rapid deterioration, seizures, brain damage, coma, sudden death
- Target p.K304E – common mutation
- Dollars/sample, couple hours

C8 → p.K304E

MS/MS target mutation
DNA, tests and conditions

No mutation analysis

Target common mutation(s)

Mutation panels

Gene sequencing

Gene panels

Exome sequencing

Genome sequencing

Cystic Fibrosis (CF)

- p.F508del – most common, varies by race/ethnicity
- >2,000 CFTR gene variants
- Commercial panels – 40–80%
- ~$35/sample, >1 day

IRT → Mutation panel

Abbreviation: CF, cystic fibrosis; DNA, deoxyribonucleic acid; IRT, immunoreactive trypsinogen. From Cystic Fibrosis Foundation.14

Contemporary Pediatrics, 2014
DNA, tests and conditions

No mutation analysis

Target common mutation(s)

Mutation panels

Gene sequencing

Gene panels

Exome sequencing

Genome sequencing

Pompe disease

- Lysosomal storage disorder
- 1st tier assay high FPR
- No commercial assays
- No major mutations – diverse
- Pseudodeficiency alleles
- Sequencing (Sanger)
- $ hundreds/sample, >1 day

α-glucosidase activity

↓

Sanger sequence (GAA)
DNA, tests and conditions

Severe Combined Immunodeficiency (SCID)

- Low/absent T-cells (B, NK)
- Lack of adaptive immunity
- Untreated, fatal by 2 yrs
- Spectrum, multiple genes
- Tx varies by genetic defect
- Diagnostics include genetics – iterative, slow, expensive

Gene panel (NGS)
- Gene panel (NGS)
- $ hundreds/sample, >1 day

2nd tier gene panel (NGS)
(research, pilot)
DNA, tests and conditions

No mutation analysis

Target common mutation(s)

Mutation panels

Gene sequencing

Gene panels

Exome sequencing

Genome sequencing

DNA not needed if # false positives low, positive predictive value high, molecular dx not required
**DNA, tests and conditions**

**Severe Combined Immunodeficiency (SCID)**

- Low/absent T-cells
- Lack of adaptive immunity
- No biochemical biomarker

**DNA-first molecular assay (T-cell receptor excision circles; TRECS)**

- No mutation analysis
- Target common mutation(s)
- Mutation panels
- Gene sequencing
- Gene panels
- Exome sequencing
- Genome sequencing

TRECs
↓
Gene panel (NGS) (research, pilot)
<table>
<thead>
<tr>
<th></th>
<th>NY</th>
<th>TX</th>
<th>WA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Galactosemia</strong></td>
<td>5 common</td>
<td>4 common</td>
<td>4 common</td>
</tr>
<tr>
<td><strong>Cystic Fibrosis</strong></td>
<td>Panel (39), NGS</td>
<td>Panel (60)</td>
<td>Panel (planned 2018)</td>
</tr>
<tr>
<td><strong>MCADD</strong></td>
<td>1 common</td>
<td>4 common</td>
<td>[No DNA]</td>
</tr>
<tr>
<td><strong>VLCADD</strong></td>
<td>[No DNA]</td>
<td>Seq</td>
<td>[No DNA]</td>
</tr>
<tr>
<td><strong>Krabbe Disease</strong></td>
<td>Seq, 2 del</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>X-ALD</strong></td>
<td>Seq</td>
<td>---</td>
<td>[No DNA]</td>
</tr>
<tr>
<td><strong>Pompe Disease</strong></td>
<td>Seq, 1 del</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Hemoglobinopathies</strong></td>
<td>---</td>
<td>PCR, Seq</td>
<td>4 common</td>
</tr>
<tr>
<td><strong>SCID</strong></td>
<td>TREC (custom)</td>
<td>TREC (custom)</td>
<td>TREC</td>
</tr>
<tr>
<td><strong>SMA</strong></td>
<td>1 common (pilot)</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

--- Condition not screened
Spinal Muscular Atrophy (SMA)

- Neuromuscular disorder
- 1 in 10,000 (most common genetic cause of infant and toddler death)
- Not currently screened, likely soon
  - No biomarker, but primarily caused by single mutation
  - Universal SCID screening (47 states)

Multiplex real-time qPCR:
TRECs + SMA mutation
Pilot studies are essential

Pilot studies ≠ universal, population screening

Retrospective
- Feasibility – infrastructure, logistics
- Evaluate platform, technology, DBS
- Determine reference ranges, cutoffs

Prospective
- Blinded/unblinded
- Data for RUSP assessment
- Human subjects – informed consent (opt in/out)
- Assess population prevalence

Resources – infrastructure, reagents, staff, time
Logistic Challenges

• Test availability
• Cost
• Infrastructure
• Expertise
• Turnaround time
Other challenges

• Interpretation difficult
  – Mild, late-onset alleles
  – Variable penetrance or expressivity
  – Variants of uncertain significance (VOUS)
  – Novel variants
  – ‘Patients in waiting’

• Access to genetic counseling
• Provider education
• Screening vs diagnostic testing
• Next generation sequencing
It’s complicated!!!