Sequencing in Newborn Screening

Introduction and Background

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Division of Laboratory Sciences
Centers for Disease Control and Prevention

2017 Gene Sequencing in Public Health Newborn Screening Meeting
February 16, 2017
Newborn Screening worldwide: A quiet endeavour for 33 years, then multiplex testing...

One test / one disorder

Multiplex testing

V. Wiley, PhD
Technologies Used in Newborn Screening

- Traditional Screening Technology
  - Visible and fluorescence enzymatic assays
  - Tandem mass spectrometry
  - Electrophoreses and high-performance liquid chromatography
  - Immunochemical assays

- Molecular Screening Technology
  - Detection of mutation(s) in a gene to improve specificity for existing disorders
  - Detection of a DNA marker to screen for new disorders
Errors can Occur when DNA is Replicated for Cell Division

These errors can result in disease
Variants in the Cystic Fibrosis Gene \((CFTR)\)
Case I – unrecognizable
“Disease causing variant”
Variants in the Cystic Fibrosis Gene (CFTR)

Case II – simple error
“Variant of variable consequences”
Variants in the Cystic Fibrosis Gene (*CFTR*)

Case III – American vs. British
“Non-disease causing variant”
ACMG determined 5 categories to classify variants:

- Known pathogenic
- Likely to be pathogenic
- Unknown significance
- Likely to be benign
- Benign

Knowledge accruing daily, however the medical impact of most variants is unknown.
Current Molecular Testing in Newborn Screening Laboratories

- **Second and third tier molecular tests**
  - Increase specificity of primary assay
    - Cystic Fibrosis (CF), Krabbe, Pompe, X-ALD
  - Clarify an ambiguous result
    - Hemoglobinopathies
  - Supplemental “Just in Time” assay
    - Galactosemia, MCAD

- **Primary molecular test**
  - When no other assay is available
  - Severe Combined Immunodeficiency (SCID)

**Most states and territories are using at least one molecular test in routine screening**
<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
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</thead>
<tbody>
<tr>
<td>1952</td>
<td>Described DNA as a double helix (using x-ray diffraction)</td>
</tr>
<tr>
<td>1975</td>
<td>Devised techniques for DNA sequencing</td>
</tr>
<tr>
<td>1985</td>
<td>Conducted first polymerase chain reaction (PCR) experiments to amplify specific gene regions</td>
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<tr>
<td>2003</td>
<td>Human Genome Sequencing Project is completed</td>
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<tr>
<td>2008</td>
<td>Advent of Next Generation Sequencing</td>
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Advent of Next Generation Sequencing Technology

Cost per Genome

Sanger Sequencing Technology

Moore's Law

NIH National Human Genome Research Institute
genome.gov/sequencingcosts
“...it may soon be easier and cheaper to sequence an entire genome than to test for a number of known mutations.”

- Foundation for Genomics and Population Health

http://www.nature.com/news/technology-the-1-000-genome-1.14901
Characteristics of Newborn Disorders Include

- Significant disease
- Treatment possible
- Not evident until harm is done
- Mass testing methods available
- Benefits justify costs

M. Glass, M.S.
Gene Sequencing Timeline in Routine Newborn Screening

- Hemoglobinopathies: Texas begins \textit{HBB} sequencing 3\textsuperscript{rd} tier test
- Cystic Fibrosis: CA begins \textit{CFTR} sequencing 3\textsuperscript{rd} tier
- APHL Creates Molecular Subcommittee for NBS
- Krabbe, NY begins \textit{GALC} sequencing 2\textsuperscript{nd} tier
- Cystic Fibrosis: WI begins \textit{CFTR} Next Gen sequencing/genotyping 2\textsuperscript{nd} tier
- X-ALD, NY begins \textit{ABCD1} sequencing 3\textsuperscript{rd} tier
- X-ALD, CA begins \textit{ABCD1} sequencing 3\textsuperscript{rd} tier
- MPS 1 (\textit{IDUA}): NY: 2\textsuperscript{nd} tier
- Krabbe, Pompe & MPS 1 (\textit{GALC, GAA & IDUA}): NJ: 2\textsuperscript{nd} tier
- Pompe, MPS 1, X-ALD (\textit{GAA, IDUA, & ABCD1}): New Eng: 2\textsuperscript{nd} tier
- Pompe & MPS 1 (\textit{GAA, IDUA}): MN: 2\textsuperscript{nd} tier
- Pompe (\textit{GAA}): WI: 2\textsuperscript{nd} tier
- VLCAD (\textit{ACADVL}): TX: 2\textsuperscript{nd} tier
- Cystic Fibrosis Next Gen Seq: NY: 3\textsuperscript{rd} tier
Hemoglobinopathy Newborn Screening: Utility of *HBB* Sequencing

- Group of inherited blood disorders caused by variants in the Globin genes

- Step 1: Identify the presence of a protein variant

- Step 2: Test for a panel of variants in the *HBB* gene known to cause a sickling disease or thalassemia

- Step 3: If β-thalassemia is suspected and negative for known variant panel or unknown variant is present, perform *HBB* sequencing
Impact of 3rd Tier Sequencing on Hemoglobinopathy Screening in Texas

TX Annual birth rate: ~400,000

1st tier
HB Variants identified by IEF & Confirmed by HPLC
~348 babies

2nd tier
DNA Variant Panel to Confirm Protein Results
~322 babies

3rd tier
Thalassemia or HB variant identified by HBB sequencing
~19 babies

Both sets of babies are sent for clinical diagnostic evaluation

R. Lee, Ph.D.
Krabbe Newborn Screening
Utility of GALC Sequencing

- Recessive genetic disorder that affects the central and peripheral nervous systems
- Onset can vary from first few weeks of life into adulthood
  - Intent of NBS – detection of Early Infantile Krabbe
- Step 1: Identify decreased GALC enzyme activity
- Step 2: Test for variants in the GALC gene using DNA sequencing and deletion analysis
**Impact of 2nd Tier Sequencing on Krabbe Screening in New York**

<table>
<thead>
<tr>
<th>Tier</th>
<th>Detection Description</th>
<th>Number of Babies</th>
</tr>
</thead>
<tbody>
<tr>
<td>NY Annual birth rate:</td>
<td>~250,000</td>
<td></td>
</tr>
<tr>
<td>1st tier</td>
<td>Detection of ↓GALC Activity (&lt;20% daily mean):</td>
<td>~1225 babies</td>
</tr>
<tr>
<td>1st tier reflex</td>
<td>Detection of ↓GALC Activity (&lt;12% daily mean):</td>
<td>~75 babies</td>
</tr>
<tr>
<td>2nd tier</td>
<td>Presence of at least 1 GALC Variant:</td>
<td>~42 babies</td>
</tr>
</tbody>
</table>

Only these babies are sent for clinical diagnostic evaluation.

M. Caggana, Sc.D

Judson Levasheff 2004 – 2007
Inherited chronic disease caused by mutations in the \textit{CFTR} gene - affects the lungs and digestive system

- Step 1: Identify elevated IRT
- Step 2: Test for CF causing variants in \textit{CFTR} gene
  - Screen positive = 1 or 2 CF causing variants
- Step 3: Sequence samples with only 1 CF causing variant
Impact of 3rd Tier Sequencing on Cystic Fibrosis Screening in California

CA Annual birth rate: ~500,000

1st tier
Babies ≥ 62 ng/mL IRT: ~8,300

2nd tier
Babies with 1 or 2 CFTR variants: ~600

3rd tier
Babies with 2 CFTR variants: ~170

Only these babies are sent for clinical diagnostic evaluation.

R. Olney, MD, MPH
Newborn Disorders Detected Using a Molecular Test in the United States

- **Primary Molecular Test**
  - Severe Combined Immunodeficiency (SCID)

- **Second- or Third-tier Molecular Tests**
  - Cystic Fibrosis (*CFTR genotyping and sequencing*)
  - Galactosemia (*GALT genotyping*)
  - MCAD (*ACADM genotyping*)
  - Hemoglobinopathies (*HBB & HBA genotyping and HBB sequencing*)
  - Krabbe (*GALC genotyping and sequencing*)
  - Pompe (*GAA sequencing*)
  - X-ALD (*ABCD1 sequencing*)
  - Maple Syrup Urine Disease (*BCKDHA genotyping*)
New Newborn Disorders to be Detected Using a Molecular Test in the United States

- **New Primary Molecular Test Coming**
  - Spinal Muscular Atrophy (*real time PCR of SMN1*)

- **New Second-tier Molecular Tests Coming**
  - Congenital Adrenal Hyperplasia (*CYP21A2 genotyping*)
  - VLCAD (*ACADVL sequencing*)
  - MPS 1 (*IDUA sequencing*)
  - SCID (*25+ genes Next Generation sequencing*)
Technical Assistance and Training
Collaboration between CDC and APHL

- Molecular laboratory training workshops at CDC
- Molecular Assessment Program (MAP) onsite visits
  - Molecular assay implementation and continuous quality improvement
- NBS Molecular Resources Online
  - Molecular tools and robotics support
- Quality assurance resources
- Molecular method transfer and technical support
- Laboratory development and design
Thank you!

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Visit: www.cdc.gov | Contact CDC at: 1-800-CDC-INFO or www.cdc.gov/info

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