Molecular Analysis to Enhance Newborn Screening
Population – Based Risk Assessment

Tests must be universally available and timely
The Declining Cost of Genome Sequencing

HiSeq X Ten Next Gen Sequencer

- Produces 16 human genomes in 3 days at 30x coverage
- Projected costs per genome
  - Reagents $797
  - Machine depreciation $137
  - Technician $55–65
- Does not include overhead, infrastructure and analysis costs
- Instrument cost $10 Million USD

http://www.nature.com/news/technology-the-1-000-genome-1.14901

S. Cordovado, Ph.D.
Does Molecular Testing Add Value??

- Increase in sensitivity of a primary test, effect on specificity
- Identification of carriers; teaching moments
- Predictions regarding phenotype
- Clinicians’ perception, diagnostic tool
- Timeliness??

OR
Where Are We Currently??

- **Second tier molecular tests**
  - Increase sensitivity or specificity of primary assay
    - Cystic Fibrosis (CF)
  - Clarify an ambiguous result
    - Hemoglobinopathies
  - Supplemental “Just in Time” assay
    - Galactosemia

- **Primary molecular test**
  - When no other assay is available – e.g. severe combined immunodeficiency; spinal muscular atrophy

*In 2015, 23 countries participated in CDC PT*
What Must We Consider??

- Cost
- Value added?
- Impact on TAT; timeliness big concern
- Staff time and qualifications
- Bioinformatics needs
- Instrumentation requirements
- Practical issues
- Are we now diagnostic laboratories?
DNA Sequencing
1975 to 2015 and Beyond

Radioactive Sanger Sequencing


http://www.uvm.edu/~cgep/Ed ucation/Sequence.html

S. Cordovado, Ph.D.
DNA Sequencing
1975 to 2015 and Beyond

The Broad Institute of MIT and Harvard large-scale Sanger DNA sequencing center

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DNA Sequencing
1975 to 2015 and Beyond

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Technology and Redundancy Considerations
Molecular Analysis in Newborn Screening
A Staged Approach

Genotyping Panel of Mutations -- Single Gene

Sequencing Single Gene

Sequencing Panel of Genes

Sequencing of NBS Genes

- Ongoing in routine NBS
- Experimental in NBS
- Offered clinically and research outside NBS

S. Cordovado, Ph.D.
Targeted mutation panels – population-specific?

Cystic Fibrosis

Galactosemia

CFTR2 panel of disease causing mutations

5-9 mutations commonly tested

First Level
Example: Increasing Specificity – DNA Sequence Analysis Without A Loss of Timeliness

KRABBE DISEASE
emergent results

- Biochemistry first
- Molecular second
- Phenotype predictions
- 41.3% reduction in referrals

Familial anxiety decreases with increased specificity
Challenges of Sequencing – 1 Gene, Several Genes, or Genome/Exome

- **Major Challenge**: Determining whether any given variant is pathogenic

- 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
Example: Increasing Specificity – DNA Sequence Analysis With A Loss of Timeliness

Issue: Most referrals for cystic fibrosis don’t have disease – high rate of false positive results

Screen positive – ↑Immunoreactive trypsin (IRT) and at least 1 CF causing mutation

Most assays detect a panel of 39-100+ mutations that cause CF
>2000 known mutations/variants in CFTR gene

… And not all CFTR mutations cause classic CF
Will identify CF related metabolic syndrome (CRMS) or unknown variants

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NYS CF Newborn Screening Algorithm (2010-2013)

IRT Assay

Elevated IRT (top 5%)

Normal IRT (bottom 95%)

39 Mutation Panel (Hologic)

2 Mut

1 Mut

VHIRT (top 0.1%)

0 Mut

IRT (bottom 99.9%)

Screen Negative

Overall (All Screen Positive)
(900 referrals, 29-65 cases)

2 Mut Screen Positive:
Most confirmed (30-40 referrals, 19-37 cases)

39 Mutation Panel (Hologic)

1 Mut Screen Positive:
Most healthy single mutation carriers (650 referrals, 9-26 cases)

0 Mut/VHIRT Screen Positive:
Most healthy (250 referrals, 1-4 cases)

Overall (All Screen Positive)
(900 referrals, 29-65 cases)

D. Kay, Ph.D.
<table>
<thead>
<tr>
<th># Infants Referred</th>
<th>Hologic 39-Mut</th>
<th>Illumina 139-Mut</th>
<th>Illumina CSA+</th>
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<tr>
<td>350</td>
<td>2 MUT N=256</td>
<td>2 MUT N=300</td>
<td>2 MUT N=378</td>
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<td>6,851</td>
<td>1 MUT N=114</td>
<td>1 MUT N=79</td>
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<td>6,341</td>
<td>VHIRT N=22</td>
<td>VHIRT N=13</td>
<td>VHIRT N=0</td>
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</table>

D. Kay, Ph.D., Hughes EE et al., Hum Mutat, 37:201-208
NYS CF Newborn Screening Algorithm

IRT Assay

Elevated IRT (top 5%)

Normal IRT (bottom 95%)

NYS Mutation Panel (TruSeq)

2 Mut

1 Mut

0 Mut

VHIRT (top 0.1%)

IRT (bottom 99.9%)

All Others

Screen Negative

TruSeq Bioinformatics

(2 Mut)

Referral to Specialty Care Center for Dx

Overall reduction 900 to 100 referrals
Cystic Fibrosis Newborn Screening Summary

NY Annual birth rate: ~250,000

1st tier
Babies in upper 5% IRT: ~12,500

2nd tier
Babies with 1 or 2 CFTR muts or VHIRT: ~900

3rd tier
Babies with 2 CFTR muts: ~100

Only these babies are sent for diagnostic evaluation and testing

S. Cordovado, Ph.D.
Increased Turnaround Time
89% Decrease in Referrals

- Accessioning (1)
- IRT test (1)
- Abnormal (2)
- Repeat IRT test (2)
- Extract DNA (2)
- 39-mutation screen (3)
- Extract fresh punch (3)
- 39-mutation screen (3)
- Enter results (4)
- Mailer (5)

- Accessioning (1)
- IRT test (1)
- Abnormal (2)
- Repeat IRT test (2)
- Extract DNA (2)
- 39-mutation screen (3)
- Extract fresh punch (3)
- Next Gen (3-5)
- Sanger / Suppl (5-6)
- Enter results (6)
- Mailer (7)*

*These times don’t account for any batching
Next Gen Sequencing and SCID Newborn Screening – Post-analytic to Analytic?

Severe Combined Immunodeficiency (SCID) is a spectrum of disorders that can only be differentiated by identifying causative mutations

- Many genes involved in SCID
- Immunologists can provide better care when SCID causative mutations are known quickly; now done post-analytically
- Screening labs can provide timely mutation analysis
- When public health provides mutation analysis, health care quality ensured

S. Cordovado, Ph.D.
Current NBS for severe combined immunodeficiency:

- Measure T-cell receptor excision circles (TRECs)
- <125 TRECs constitutes a referral
- Immunologists order CBC, flow, mitogen studies
- Molecular tests order by candidacy, multi-gene panel(s), insurance issues, available labs
- Becomes iterative, slow, stressful process
Specific Aims

- Validate 2 platforms for 39-gene NGS immunodeficiency panel

- Evaluate Next Gen Sequencing Utility and TAT
  Shortened time to diagnosis?
  Fewer visits to Specialist?
  Earlier, targeted treatment?
  Long-term follow-up

- Create and disseminate educational materials for parents and providers to state programs
# Severe Combined Immunodeficiency

## 39 – Gene Panel

<table>
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<tr>
<th>ADA</th>
<th>AK2</th>
<th>ATM</th>
<th>BLNK</th>
<th>BTK</th>
<th>CD3D</th>
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<tr>
<td>CD3G</td>
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<td>IL2RG</td>
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<td>IL7R</td>
<td>JAK3</td>
<td>LIG4</td>
<td>MTHFD1</td>
<td>MTR</td>
<td>NHEJ1</td>
<td>NBN</td>
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<td>PNP</td>
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<td>RAG2</td>
<td>RMRP</td>
<td>SLC46A1</td>
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<td>TBX1</td>
<td>WAS</td>
<td>ZAP70</td>
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</table>
Entire coding sequence of all known genes in a given biochemical pathway

- Modifiers
- Phenotype predictions
- Infantile, juvenile, late
Entire coding sequence of all known NBS genes

- Complete
- Only looking at NBS
- Can turn off analysis
- Easily modifiable
- Similar information
- Economy of scale
- Still ‘manageable’

- Under consideration in NY
- Establishment of NBS core

Fourth Level
Whole exome or whole genome analyses

- Complete
- All disease / onset
- VOUS
- Screening v. diagnostic
- No phenotype yet
- Consent
- No longer ‘manageable’ currently
Points to Consider

• Will we make it easier for families?
• Will we alleviate or increase burden?
• Variants of unknown significance
• Misclassified variants
• Screening programs become diagnostic
• Molecular diagnosis may not result in phenotype – patients in waiting
• Providers need education to relay information
• Availability of genetic counseling
We Can Do This Right

- Molecular subcommittee
- Expertise exists in NBS
- Community of collaboration
- Be smart about implementation
- Tools can help families
  - reduce # of referred
  - provide data for future
- Health care equality
- Information at time of referral
NBS Molecular Subcommittee

MISSION

➢ The mission of the subcommittee is to ensure continuity and responsible growth of emerging molecular technologies within the newborn screening public health environment.

➢ WA, MI, CA, NY, MN, IA, WI, TX, MA, PR, CA(2)
NBS Molecular Subcommittee

OBJECTIVES:

- To facilitate a collaborative environment for transfer of knowledge about emerging technology among newborn screening laboratories.
- To provide input to CDC’s Molecular Quality Improvement Program (MQIP) on procedures, policies and activities for molecular testing.
- To provide input to state newborn screening public health laboratories on procedures, policies and activities for molecular screening.
- To serve as a communications conduit between MQIP and newborn screening systems.
- To assist laboratories in improving newborn disorder detection sensitivity and specificity with molecular testing.
- To collaborate with newborn screening laboratories to anticipate future molecular assays and needs
- To serve as a liaison to organizations, programs and activities in order to address issues concerning molecular testing in newborn screening.
NBS Molecular Subcommittee

- Molecular Quality Improvement Program
- NBS Molecular Workshops
- Molecular Assessment Program
- Molecular Resources Website
- Paradigm for NBS Molecular Pilots
- Presentations to the Community
Molecular Subcommittee Meeting

Meeting Objective:

• Discuss current status of gene sequencing in NBS
• Discuss laboratory and follow-up needs, barriers and solutions
• Provide state experience in implementation and practice

Target Audience – NBS Lab and Follow-up Managers

Planned for first quarter 2017

laura.russell@aphl.org or snc4@cdc.gov for more information
Molecular Survey

Goals:

• To assess the status of molecular testing in US NBS laboratories currently and in the near future
• To identify states actively or planning to use sequencing for certain disorders and to identify the platforms used or under consideration

Contact laura.russell@aphl.org or snc4@cdc.gov for more information
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- CF Specialty Care Center Directors
- Applied Genomic Technologies Core [WC]
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Thank You !!