Molecular Analysis to Enhance Newborn Screening........

Michele Caggana, Sc.D., FACMG
February 29, 2016
....Are You Ready to Jump In??
Population – Based Risk Assessment
Characteristics of Newborn Disorders Include

- Significant disease
- Treatment possible
- Not evident until harm is done
- Mass testing methods available
- Benefits justify costs
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??
It Is Here – 23 Countries Participate in CDC’s Molecular PT Programs in 2015

- Countries Participating only in CF PT (14)
- Countries Participating only in SCID PT (3)
- Countries Participating in CF & SCID PT (6)

Note that not all NSQAP PT participating countries offer universal screening

S. Cordovado, Ph.D.
Current Molecular Testing in Newborn Screening Laboratories

- **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
Will Molecular Testing Take NBS by Storm?

Or Will We Ride the Wave?

Either way, we are going to get wet...

S. Cordovado, Ph.D.
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?
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

Genome Exome

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
Entire coding sequence of an entire gene

**KRABBE DISEASE**

*emergent results*

- VOUS
- Phenotype predictions
- Timeliness
- 41.3% reduction in referrals

Other LSDs? -- pseudodeficiency
Next Gen Sequencing and Cystic Fibrosis Newborn Screening

94% of referred CF screens are false positives in NYS

Screen positive – ↑IRT and at least 1 CF causing mutation
Most assays detect a panel of mutations that cause CF
>2000 known mutations/variants in CFTR gene

Not all CFTR mutations cause classic CF
Will identify CF related metabolic syndrome or unknown variants
Can limit sequence detection to known mutations but will miss cases?
How many missed cases can we live with?
Can’t we do better?

Hughes EE et al., Hum Mutat, 37:201-208
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)

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)

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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Hughes EE et al., Hum Mutat, 37:201-208
# Variants Not Detected on Clinical Sequencing Assay

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<tr>
<th>CF ID</th>
<th>Mut 1</th>
<th>Mut 2</th>
<th>Final Dx</th>
<th>Sweat Test (mmol/L)</th>
<th>Race/Ethnicity</th>
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E Hughes, MLS
NYS CF Newborn Screening Algorithm

IRT Assay

- Elevated IRT (top 5%)
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NYS Mutation Panel (TruSeq)

- 2 Mut
- 1 Mut
- VHIRT (top 0.1%)
- 0 Mut
- IRT (bottom 99.9%)

TruSeq Bioinformatics

- All Others

Screen Negative

(2 Mut)
Referral to Specialty Care Center for Dx

D. Kay, Ph.D.
Next Gen Sequencing and SCID Newborn Screening

Issue: 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
- Screening labs can provide timely mutation analysis
- When public health provides mutational analysis, ensures health equality
Current NBS for severe combined immunodeficiency:

- Measure T-cell receptor excision circles
- <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

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<tr>
<th>ADA</th>
<th>AK2</th>
<th>ATM</th>
<th>BLNK</th>
<th>BTK</th>
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<td>TBX1</td>
<td>WAS</td>
<td>ZAP70</td>
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</table>

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*Department of Health*
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
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
Acknowledgements

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Thank You !!