Between now and tomorrow

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Professor, Department of Pediatrics, UMASS Med

Transforming Public Health in a Changing World
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Anticipating future applications of genomic technology in NBS

• Expand the list of treatable conditions that can be screened
• Strengthen interpretations of current screening results
• Provide that single black box?

• Generate a knowledge base from which we develop other (non-genomic) screening assays
Tomorrow

• Pre-determined pathogenic profiles for
• Preventable disease
Hmmm

- Some new instrumentation, software, skilled technical staff
- Genomic info from baby
- List of preventable diseases
- List of pathogenic profiles
- Flexibility to modify profiles
- Your understanding
Anticipating future applications of genomic technology in NBS

• Is it here to stay? - likely
• Is it a transient fad? - parts may be
• What is IT?
• Can we do this? - Yes
• Should we do this? - May need to
• How do we do this? - Carefully
Anticipating future applications of genomic technology in NBS

• Is it here to stay? - likely
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• Can we do this? - Yes
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• How do we do this? - Carefully
Current It: Next-Generation Sequencing
Newborn Screening is …

a public health program that provides an opportunity for early identification and early treatment of infants with conditions that otherwise would go unrecognized prior to irreversible clinical damage.
Relative Proportions of Infants Identified by Newborn Screening
Texas Newborn Screening Laboratory

8 plates are distributed to 5 areas to test for 29 disorders.

Hemoglobinopathy Screening:
One test is used to identify:
- Sickle Cell Anemia
- Sickle Hemoglobin C Disease
- Sickle/Beta Thalassemia Disease
- Other hemoglobinopathy diseases and traits

Galactosemia & Biotinidase Screening:
Two tests are used to identify:
- Galactosemia
- Biotinidase Deficiency

Endocrine & Cystic Fibrosis Screening:
Three tests are used to identify:
- Congenital Hypothyroidism
- Congenital Adrenal Hyperplasia
- Cystic Fibrosis

SCID Screening:
One molecular test is used to identify:
- Severe Combined Immunodeficiency

Tandem Mass Spectrometry Screening:
One test is used to identify:
- 6 amino acid disorders (e.g. PKU)
- 5 fatty acid disorders (e.g. MCAD)
- 9 organic acid disorders (e.g. glutaric acidemia type 1)
## Hi Throughput – Large Menus

<table>
<thead>
<tr>
<th></th>
<th>Conditions</th>
<th>Daily Tests</th>
<th>Confirmed Cases</th>
<th>Newborns Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>New York NBS:</strong></td>
<td>30</td>
<td>29,299</td>
<td>782</td>
<td>242,208</td>
</tr>
<tr>
<td><strong>New England NBS:</strong></td>
<td>30</td>
<td>12,602</td>
<td>301</td>
<td>116,236</td>
</tr>
<tr>
<td><strong>Wisconsin NBS:</strong></td>
<td>30</td>
<td>7,099</td>
<td>143</td>
<td>67,057</td>
</tr>
<tr>
<td><strong>Minnesota NBS:</strong></td>
<td>30</td>
<td>6,780</td>
<td>155</td>
<td>67,780</td>
</tr>
<tr>
<td><strong>TXNBSP:</strong></td>
<td>29*</td>
<td>71,740</td>
<td>743</td>
<td>379,255</td>
</tr>
</tbody>
</table>

*Note: TXNBSP has 29 conditions instead of 30.*
Molecular Assays in Use in Newborn Screening 2013

Second-tier and first and second-tier assays are shown on this map. The states are color-coded to indicate the tier of screening assay in use. The map is courtesy of CDC.
NEWBORN SCREENING CALLS TO THE FRONTLINE OF DEFENSE
EVERY 3 MINUTES:

1 high risk
6 additional actionable

NEWBORN SCREENING CALLS TO THE FRONTLINE OF DEFENSE
Anticipating future applications of genomic technology in NBS

- Expand the list of treatable conditions that can be screened
- Strengthen interpretations of current screening results
- Provide that single black box?

- Generate a knowledge base from which we develop other (non-genomic) screening assays
Current Purposes of DNA in NBS

(data generated prior to full diagnostic evaluation)

• Enhance capacity of screening for conditions not otherwise included...
  TREC assay for SCID: molecular in **FIRST TIER**

• Enhance specificity of 1st tier test....
  CFTR mutation assay after IRT: molecular in **SECOND TIER**

• Supplemental just-in-time
  Increase available information to aid diagnostic evaluation…
  GALT mutation assay: molecular in **SECOND TIER**
Current DNA testing:

Regardless of purpose, the DNA target might be:

A specific mutation
  A specific structure
A foreign element
Qualitative or Quantitative

Like other targets, these can be multiplexed.
DNA Testing in the 2nd Tier
(data generated prior to full diagnostic evaluation)

• Enhance capacity of screening for conditions not otherwise included...
  TREC assay for SCID: molecular in FIRST TIER

(Conventional genetic)

• Enhance specificity of 1st tier test....
  CFTR mutation assay after IRT: molecular in SECOND TIER

• Supplemental just-in-time
  Increase available information to aid diagnostic evaluation...
  GALT mutation assay: molecular in SECOND TIER
Current DNA testing:

Multiple mutations are already tested on a subset of babies

• IRT – top 5% infants tested for 39 mutations in CFTR
Genotype Distribution Among 450 New England CF infants relative to common allele

- No DF508
- DF508/DF508
- DF508/Other
Proportion of New England CF Infants shown to carry one or two mutations by newborn screening
Carriers confirmed after diagnostic testing - identified in order to find CF

~5% will have two carrier parents
Relative Proportions of Infants Identified by Newborn Screening
<table>
<thead>
<tr>
<th>Nucleic Acid Testing</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applications</td>
<td>Genetic mutations, viral load, infectious disease, cancer detection, etc.</td>
</tr>
<tr>
<td>Verification</td>
<td>Accuracy, precision, reproducibility, sensitivity, specificity, robustness</td>
</tr>
<tr>
<td>Validation</td>
<td>Identify analytic and clinical performance characteristics and test limitations</td>
</tr>
</tbody>
</table>

**Nucleic Acid Testing Examples**

- **Luminex-based assays**
  - Traditional PCR followed by post-amplification analysis. Detection of the final product is after the amplification is complete.
  - CFTR, galactosemia, MCADD

- **Quantitative real-time PCR**
  - Simultaneous amplification and quantification in a closed system measuring the increase in fluorescence produced by a reporter molecule with each reaction cycle.
  - SCID

**Advantages of Real-Time Q PCR**

<table>
<thead>
<tr>
<th>Advantages of Real-Time Q PCR</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantitative results in real-time</td>
<td>Limited multiplexing capability</td>
</tr>
<tr>
<td>Closed tube, reduced risk of contamination</td>
<td>Can be complex to set up, particularly for multiplexed reactions</td>
</tr>
<tr>
<td>Rapid cycling time (30 minutes to 2 hours)</td>
<td></td>
</tr>
<tr>
<td>Highly sequence specific</td>
<td>Intra- and inter-assay variation, hence the need for an internal monitoring control</td>
</tr>
</tbody>
</table>
To be determined:

Analytic validity
- Promising –
- known issues with large deletions, rearrangements, copy number variants

Analytic validity in high throughput
- Promising –
- Scan or target…

Clinical validity
- Ongoing learning…complex traits…
Next Gen interrogates

- A whole genome
- A whole exome
- Targeted genes
- A targeted gene
- ....there is the capacity to target regions for sequencing
<table>
<thead>
<tr>
<th>GENE</th>
<th>REFERENCE</th>
<th>POSSIBLE SEQUENCE VARIANTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFTR</td>
<td>COLOR</td>
<td>CAWPR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COLAR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COLOUR</td>
</tr>
<tr>
<td>PKU</td>
<td>RED</td>
<td>REE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DEAD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>READ</td>
</tr>
<tr>
<td>MCAD</td>
<td>BLUE</td>
<td>BECN</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BLOO</td>
</tr>
<tr>
<td></td>
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<td>BLEU</td>
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<tr>
<td>SCID</td>
<td>SOUND</td>
<td>SWIMD</td>
</tr>
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<td></td>
<td></td>
<td>SOUDD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SOWND</td>
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<tr>
<td>GALT</td>
<td>QUACK</td>
<td>HONK</td>
</tr>
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<td></td>
<td></td>
<td>QUICK</td>
</tr>
<tr>
<td></td>
<td></td>
<td>QUAKK</td>
</tr>
<tr>
<td>HGB</td>
<td>BELL</td>
<td>BLOB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BALL</td>
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<td>CAWPR, COLAR, COLOUR</td>
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<tr>
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<td>HGB</td>
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<td>BLOB, BALL, BELLE</td>
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</table>

**DEAD DUCK DISEASE?**
Bioinformatics will need to be able to

- Target specific mutations,
- Detect a pathogenic profile
- Be flexible to the user

- Be Unidirectional?
Between now and tomorrow

• Some new instrumentation, software, skilled technical staff
• Genomic info from baby
• List of preventable diseases
• List of pathogenic profiles
• Flexibility to modify profiles
• Your understanding
Examples of pediatric onset actionable conditions for consideration in expansion

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Age of Onset</th>
<th>Genes</th>
<th>Inheritance</th>
<th>Clinical Features</th>
<th>Management</th>
<th>Prevention of Manifestations</th>
<th>Comments</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC (autosomal dominant Blackfan-Diamond anemia)</td>
<td>Infant</td>
<td>EKLF, EPDR</td>
<td>AD</td>
<td>Anemia, growth retardation, development delay</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>1,000 - 6,000</td>
</tr>
<tr>
<td>ACD (autosomal dominant ichthyosis)</td>
<td>Infant</td>
<td>TGM2</td>
<td>AD</td>
<td>Enhanced skin cells, ichthyosis</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>1,000 - 6,000</td>
</tr>
<tr>
<td>Adenosine deaminase deficiency</td>
<td>Infant</td>
<td>ADA</td>
<td>AD</td>
<td>Adenosine deaminase deficiency</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>1,000 - 6,000</td>
</tr>
<tr>
<td>Adrenoleukodystrophy</td>
<td>Infant</td>
<td>ABCD2</td>
<td>AD</td>
<td>Adrenoleukodystrophy</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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</tr>
<tr>
<td>Adrenomyeloneuropathy</td>
<td>Infant</td>
<td>ABCD2</td>
<td>AD</td>
<td>Adrenoleukodystrophy, neuropathy</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>1,000 - 6,000</td>
</tr>
<tr>
<td>Alport syndrome</td>
<td>Infant</td>
<td>COL4A5</td>
<td>AD</td>
<td>Renal failure, sensorineural deafness, high myopia</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>1,000 - 6,000</td>
</tr>
<tr>
<td>Aromatic L-arginine deficiency</td>
<td>Infant</td>
<td>ARG1</td>
<td>AD</td>
<td>Aromatic L-arginine deficiency</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>1,000 - 6,000</td>
</tr>
<tr>
<td>Autosomal dominant polycystic kidney disease</td>
<td>Infant</td>
<td>PKD1, PKD2</td>
<td>AD</td>
<td>Polycystic kidney disease</td>
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<td>N/A</td>
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<td>1,000 - 6,000</td>
</tr>
<tr>
<td>Autosomal recessive polycystic kidney disease</td>
<td>Infant</td>
<td>PKD1, PKD2</td>
<td>AD</td>
<td>Polycystic kidney disease</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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</table>

University of Massachusetts Medical School
Challenges: the Report

Technical Report
- CLSI demographics
- Reason for testing
- Disease locus tested
- Result is In Range or Out of Range

Out of Range:
Number of DNA sequence variants detected by the screen
Report Content

- **Names** of DNA sequence variants **detected** by the screen (colloquial and (?) HGVS)
- **Names** of DNA sequence variants **TESTED**.

nomenclature
- colloquial: Delta F508
- HGVS: c.1521_1523delCTT

Human Genome Variation Society
http://www.hgvs.org/
INTERPRETATION

- Interpretation of the overall NBS result for the condition
- State interpretation of the DNA result, e.g.,
  - *infant is (at least) a carrier*
  - *Infant with 2 variants is at high risk*

RECOMMENDED ACTION
Reporting:
Some adjustments needed
Risk Assessment Process

- **4,000,000**
  - Screen Negative
  - Results mailed to HOB
  - No follow-up needed
  - NBS follow-up

- **166,451**
  - Unsuitable for Testing or DOB (request repeat)
  - Letter sent to HOB

- **299,953**
  - Presumptive Positive (request repeat)
  - Letter sent to HOB and physician of record
  - NBS follow-up

- **51,529**
  - Referral (very abnormal)
  - Phone call to treatment center and physician of record
  - NBS follow-up

**13,711 confirmed cases or 1/290 newborns have a NBS condition**

Courtesy, NY
The public health challenges are

Justifying the transition instrumentation, labor

Defining pathogenic profiles interface with research

Technology public private partnerships, validations

Flexibility to modify assay as data grows

data mining – propose expiration dates
Public Trust

www.50yearssavingbabies.org