EXPANDED NEWBORN SCREENING: EARLY RESULTS, SURPRISES, AND THE FUTURE

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Stanford University

Newborn Screening Goal

The early detection of conditions for which early and timely interventions can lead to the elimination or reduction of associated mortality, morbidity and disabilities


Wilson-Junger Criteria for Screening (1968)

1. Condition an important health problem
2. There is an accepted treatment
3. Diagnostic and treatment centers available
4. Latent or early symptomatic stage
5. Suitable screening test
6. Test acceptable to public
7. Natural history of disorder understood
8. Agreed policy on whom to treat
9. Cost/benefit ratio
10. Case finding a continuing process

Current California Newborn Screening

560,000 Newborns per year
(~1/8 of neonates born annually in USA)
>99% screened

THE “HEELSTICK TEST”
Newborn Screening Components

- Screening
- Short-term follow up
- Diagnosis
- Treatment / Management
- Evaluation
- Education

Newborn Screening Program Area Service Centers

- Lucile Packard Children’s Hospital, Stanford
- Valley Children’s Hospital, Madera
- UCLA Medical Center, Los Angeles
- Harbor/UCLA Medical Center, Torrance
- SCICDS, Inc., San Diego

“Find the babies
Treat the babies
Save the babies”

-LPCH ASC Motto

Communication in Newborn Screening

Private Sector Laboratories → Central Laboratory

Specialists: Biochemical Geneticist, Endocrinologist, Hematologist

Area Service Center

Family → Primary Care Provider

History of Newborn Screening in California

- 1966 - PKU
- 1980 - Hypothyroidism, Galactosemia
- 1990 - Sickle Cell Disease, Other Hemoglobinopathies

- 2002 - 2003 - MS/MS Pilot Project

Mandatory since 7/11/05
California Newborn Screening - Fatty acid oxidation defects

<table>
<thead>
<tr>
<th>DISORDER</th>
<th>N</th>
<th>PREVALENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTD</td>
<td>16</td>
<td>1/55,000</td>
</tr>
<tr>
<td>CPT2 deficiency</td>
<td>3</td>
<td>1/294,000</td>
</tr>
<tr>
<td>CAT deficiency</td>
<td>1</td>
<td>1/877,000</td>
</tr>
<tr>
<td>SCAD</td>
<td>18</td>
<td>1/49,000</td>
</tr>
<tr>
<td>SCHADD</td>
<td>8</td>
<td>1/110,000</td>
</tr>
<tr>
<td>MCADD</td>
<td>42</td>
<td>1/21,000</td>
</tr>
<tr>
<td>LCHADD</td>
<td>1</td>
<td>1/877,000</td>
</tr>
<tr>
<td>VLCADD</td>
<td>12</td>
<td>1/74,000</td>
</tr>
</tbody>
</table>

California Newborn Screening - Organic acidemias

<table>
<thead>
<tr>
<th>DISORDER</th>
<th>N</th>
<th>PREVALENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMA - mutase</td>
<td>11</td>
<td>1/80,000</td>
</tr>
<tr>
<td>MMA - cobalamin</td>
<td>17</td>
<td>1/52,000</td>
</tr>
<tr>
<td>Propionic acidemia</td>
<td>1</td>
<td>1/877,000</td>
</tr>
<tr>
<td>Isovaleric acidemia</td>
<td>8</td>
<td>1/110,000</td>
</tr>
<tr>
<td>Glutaric acidemia</td>
<td>6</td>
<td>1/147,000</td>
</tr>
<tr>
<td>MADD</td>
<td>5</td>
<td>1/179,000</td>
</tr>
<tr>
<td>MCD</td>
<td>2</td>
<td>1/455,000</td>
</tr>
<tr>
<td>3-MCC deficiency</td>
<td>22</td>
<td>1/40,000</td>
</tr>
<tr>
<td>IBDHD</td>
<td>1</td>
<td>1/877,000</td>
</tr>
</tbody>
</table>

California Newborn Screening - Aminoacidemias

<table>
<thead>
<tr>
<th>DISORDER</th>
<th>N</th>
<th>PREVALENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKU</td>
<td>50</td>
<td>1/18,000</td>
</tr>
<tr>
<td>Citrullinemia</td>
<td>5</td>
<td>1/179,000</td>
</tr>
<tr>
<td>ASAL deficiency</td>
<td>2</td>
<td>1/455,000</td>
</tr>
<tr>
<td>Arginase deficiency</td>
<td>1</td>
<td>1/877,000</td>
</tr>
<tr>
<td>Hypermethioninemia</td>
<td>3</td>
<td>1/294,000</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>1</td>
<td>1/877,000</td>
</tr>
<tr>
<td>MSUD</td>
<td>8</td>
<td>1/110,000</td>
</tr>
<tr>
<td>Prolinemia</td>
<td>2</td>
<td>1/222,000</td>
</tr>
<tr>
<td>Citrin deficiency</td>
<td>1</td>
<td>1/877,000</td>
</tr>
</tbody>
</table>

California Expanded Newborn Screening by MS/MS: 07/05-01/07

- 877,489
- 250 disorders identified
- Prevalence 1/3,500
California Expanded Newborn Screening by MS/MS: 07/05-01/07

- Sensitivity 99.21%
- Specificity 99.82%
- PPV 14%
- NPV 100%
- False +ve 0.18%

California Expanded Newborn Screening Congenital Adrenal Hyperplasia

<table>
<thead>
<tr>
<th>TYPE</th>
<th>N</th>
<th>PREVALENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt Wasting</td>
<td>36</td>
<td>1/24,000</td>
</tr>
<tr>
<td>Simple Virilizing</td>
<td>9</td>
<td>1/98,000</td>
</tr>
<tr>
<td>Non-Classical</td>
<td>3</td>
<td>1/294,000</td>
</tr>
<tr>
<td>11βOH deficiency</td>
<td>4</td>
<td>1/222,000</td>
</tr>
</tbody>
</table>

The Stanford Metabolic Center Experience

- ~2 years
- 195 cases referred = 2 cases/week
  - 128 (66%) = no disorder identified
  - 41 (21%) = confirmed diagnosis
  - 12 (6%) = still pending
  - 14 (7%) = 6 deceased w/o metabolic diagnosis; 8 transferred out of area or lost to follow-up

The Stanford Metabolic Center Experience

- Confirmed Diagnoses
  - PKU/Hyperphenylalaninemia 9
  - Cobalamin C disease 7
  - 3-MCC deficiency 5
  - D/G galactosuria 3
  - MCAD deficiency 3
  - VLCAD deficiency 3
  - SCAD deficiency 2
  - Inositol acidemia 2
  - Galactosemia 1
  - Carnitine transporter deficiency 1
  - Argininosuccinic acid lyase deficiency 1
  - Hypermethioninemia 1
  - Hyperprolinemia 1

Cobalamin

- Cofactor
  - Methymlmalonyl-CoA mutase
  - Methionine synthase

The Cobalamin Pathway
**Cobalamin C (cblC) Disease**
- Most common inborn error of vitamin B12
- Decreased enzyme activity
- Autosomal recessive inheritance
- Estimated incidence of 1:200,000 births
- ~300 known cases

**cblC Disease**
- Biochemical characteristics
  - ↑ methylmalonic acid (MMA) and homocysteine (HCY)
  - cblC disease identified by elevations of C3 and increased C3/C2 ratio on MS/MS NBS
- Clinical findings
  - Mental retardation, nystagmus, pigmentary retinopathy, seizures, neurodegeneration

**Incidence**
- 830,727 newborns screened
  - Half of all births are of Latin American descent
- 13 with cblC disease
  - 10 of Latin American descent
- Incidence in California
  - General population 1 in 64,000
  - Latin American population 1 in 42,000

**Demographics**
- Sex
  - 7 male, 6 female
- Ethnicity
  - Latin American
  - Asian
  - Middle Eastern
  - Caucasian
- Birth weight
  - 1555 to 4215 grams
- Diet

**Clinical Findings at Presentation**
- Asymptomatic (8)
- Mild hypotonia (1)
- Moderate hypotonia and seizures (1)
- Megaloblastic anemia, hypotonia, strabismus, inability to fix and follow (1)

**Confirmatory Testing**
- **MMLA4HC mutation analysis (n=7)**
  - One identified mutation (1)
  - Homozygous (2)
  - Compound heterozygote (4)

**Allele 1** | **Allele 2**
--- | ---
G3760A | G3760A
G9155G | G9155G
T2750G | T2750G
G2347G | G2347G
G9138G | G9138G
G2842G | G2842G
T628G | T628G
T658G | T658G
G2841A | G2841A
G2842A | G2842A
G3759A | G3759A
G9155G | G9155G
T2750G | T2750G
G2347G | G2347G
G9138G | G9138G
G2842G | G2842G
T628G | T628G
T658G | T658G
G2841A | G2841A
G2842A | G2842A
G3759A | G3759A
G9155G | G9155G
T2750G | T2750G
G2347G | G2347G
G9138G | G9138G
G2842G | G2842G
T628G | T628G
T658G | T658G
G2841A | G2841A
G2842A | G2842A
G3759A | G3759A
G9155G | G9155G
T2750G | T2750G
G2347G | G2347G
G9138G | G9138G
G2842G | G2842G
T628G | T628G
T658G | T658G
G2841A | G2841A
G2842A | G2842A
G3759A | G3759A
G9155G | G9155G
T2750G | T2750G
G2347G | G2347G
G9138G | G9138G
G2842G | G2842G
T628G | T628G
T658G | T658G
G2841A | G2841A
G2842A | G2842A
G3759A | G3759A
G9155G | G9155G
Treatment

- Hydroxocobalamin 1mg daily intramuscular injections
- Oral supplementation of
  - Folic acid 1mg/day
  - L-carnitine 100mg/kg/day
  - Betaine-HCl 300mg/kg/day
  - One also required methionine supplementation
- Propimex formula started in four

Initial and Treated Values

<table>
<thead>
<tr>
<th>MMA (&lt;0.5 µmol/L)</th>
<th>HCY (&lt;1.5 µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>Treated</td>
</tr>
<tr>
<td>254</td>
<td>0.6</td>
</tr>
<tr>
<td>67</td>
<td>2.5</td>
</tr>
<tr>
<td>208</td>
<td>0.5</td>
</tr>
<tr>
<td>113</td>
<td>1.2</td>
</tr>
<tr>
<td>40</td>
<td>0.7</td>
</tr>
<tr>
<td>106</td>
<td>8.6</td>
</tr>
<tr>
<td>25</td>
<td>0.3</td>
</tr>
<tr>
<td>—</td>
<td>0.2</td>
</tr>
<tr>
<td>—</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Treated MMA 1.7 ± 2.7  Treated HCY 25 ± 21

Clinical Outcome

- Normal growth and development (7)
- Nystagmus and delayed development (2)
- Developmental delay (1)
- Lost to follow up (1)

Cobalamin C Disease in California

- Incidence is unexpectedly high
- The discrepancy between those ascertained by NBS and those presenting clinically is unclear at this time
- cblC disease comprises a spectrum
- DNA studies reveal multiple causative mutations
- Genotype-phenotype correlation is needed
- Long term follow up is needed

Very long chain acyl-CoA dehydrogenase (VLCAD) deficiency

- 1/74,000
- Mildly elevated C14:1-carnitine
- Tendency for repeat samples to normalize
- Further diagnostic evaluations are needed
  - DNA analysis
  - Skin biopsy for enzymology or intracellular acylcarnitine profile

3-Methylcrotonyl-CoA carboxylase (3-MCC) deficiency

- 1/40,000
- Elevated C5OH-carnitine
- 3-Methylcrotonylglycinuria
- Decreased total & free carnitine
- Leukocyte enzymology
- Asymptomatic mothers identified
Carnitine Transporter Deficiency (CTD)

- 1/55,000
- Decreased free carnitine (C0)
- Elevated urine carnitine
- Skin biopsy for carnitine uptake studies
- DNA analysis
- Asymptomatic mothers identified

Non-MS/MS-Based Testing (disease-specific platforms)

- Galactosemia
- Congenital Hypothyroidism
- Hemoglobinopathies
- Congenital adrenal hyperplasia
- Biotinidase deficiency
- Cystic fibrosis
- G6PD deficiency

MS/MS Newborn Screening: Some Final Truths

- Abnormal screening results require follow up diagnostic testing
  - Urine organic acids
  - Plasma acylcarnitine profile
  - Plasma amino acids
  - Plasma carnitine (free and total)

MS/MS Newborn Screening: Some Final Truths

- MS/MS detects analytes not diseases
  - e.g. phenylalanine elevation –
    - Classic PKU
    - Mild non-PKU hyperphenylalaninemia
    - Biopterin-responsive PAH deficiency
    - Biopterin biosynthetic defects
    - TPN
    - Liver disease

MS/MS Newborn Screening: Some Final Truths

- MS/MS newborn screening identifies conditions with unclear natural history and treatment benefit
  - Short chain acyl-CoA dehydrogenase deficiency (SCAD)
  - 3-Methylcrotonyl-CoA carboxylase deficiency
  - Mild variants of classic diseases

Neonatal Hyperglycinemia (n=9)
**MS/MS Newborn Screening: Some Final Truths**

- False Positives
  - Preterm infants
  - TPN

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**Newborn Screening for Biotinidase Deficiency – August 1, 2007**

- 1/61,000 (autosomal recessive)
- Enzyme-based (colorimetric) assay
  - Partial deficiency
- Clinical features - seizures, hypotonia, ataxia, developmental delay, hearing loss, alopecia, skin rash, lactic acidosis, and ketosis
- Therapy – Biotin

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**Major Clinical Features of Children with Biotinidase Deficiency**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seizures</td>
<td>10/15</td>
</tr>
<tr>
<td>Ataxia</td>
<td>9/15</td>
</tr>
<tr>
<td>Skin rash</td>
<td>10/15</td>
</tr>
<tr>
<td>Alopecia</td>
<td>12/15</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>7/14</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>5/15</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>9/15</td>
</tr>
<tr>
<td>Fungal infections</td>
<td>3/15</td>
</tr>
<tr>
<td>Developmental delay</td>
<td>5/15</td>
</tr>
<tr>
<td>Metabolic acidosis</td>
<td>9/14</td>
</tr>
<tr>
<td>Lactic acidosis</td>
<td>9/14</td>
</tr>
<tr>
<td>Hypotanninuria</td>
<td>5/12</td>
</tr>
<tr>
<td>Organic acidaemia</td>
<td>9/15</td>
</tr>
</tbody>
</table>

**IMPROVED WITH BIOTIN** 12/12

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**Newborn Screening for Cystic fibrosis – August 1, 2007**

- Initial screen
  - Immunoreactive trypsin (IRT)
- Follow-up screen
  - 38 DNA mutation panel
- 2 mutations identified → automatic referral to CF center
- 1 mutation identified → carrier (phone counseling offered)
**Expected number and percent of births and CF cases in CA per year** (Based on Year 2003)

- Annual Births – 540,827
- Birth Prevalence – 1/5876

**Proposed 4-Step Screening Model**

1. **IRT**
   - IRT Analysis
     - All Births
     - Low IRT
     - Screen Negative
   - Low IRT
     - Screen Negative

2. **DNA Panel**
   - DNA Analysis
     - All Mutations
     - 1 Mutation
     - No Mutations

3. **DNA Sequencing**
   - Sequencing
     - All Mutations
     - 1 Mutation
     - No Mutations

4. **Sweat Test**
   - Sweat Test
     - 2 Mutations
   - Questionable Case
   - CF Center
   - Genetic Counseling
   - Genetic Counseling
   - Genetic Counseling
   - No Sweat Test
     - Carrier
     - Telephone
     - Genetic Counseling
     - Genetic Counseling
     - Genetic Counseling

**Newborn Screening for LSD**

- LSD are considered candidates for NBS
  - Patients are usually normal at birth
  - LSD are progressive, debilitating, and often life-threatening
  - Treatment is available for some LSD
  - Early intervention may make a significant difference
    - Magnitude remains to be determined
    - Can only be determined through NBS

**Newborn Screening for Lysosomal Storage Disorders**

- LSD are considered candidates for NBS
  - Patients are usually normal at birth
  - LSD are progressive, debilitating, and often life-threatening
  - Treatment is available for some LSD
  - Early intervention may make a significant difference
    - Magnitude remains to be determined
    - Can only be determined through NBS

**Pompe Disease**

- Infantile onset < 12 months
  - Head lag
  - Enlarged tongue
  - Respiratory insufficiency
  - Delayed motor development
  - Muscle weakness
- Late onset > 12 months
  - Morning headache
  - Respiratory insufficiency
  - Shortness of breath
  - Sleep apnea
  - Snoring
  - Snuffling
  - Low back pain
  - Muscle weakness

**LSD Enzyme Assay Targets for MS/MS**

- Developed
  - Pompe disease
  - Krabbe disease
  - Fabry disease
  - Gaucher disease
  - Niemann-Pick A/B
  - MPS I
  - MPS II
- In queue
  - MPS VI
  - MLD
  - CLN2
  - Tay Sachs disease
- In search of method
  - Cystinosis

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  - MPS VI
  - MLD
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  - MPS I
  - MPS II
- In queue
  - MPS VI
  - MLD
  - CLN2
  - Tay Sachs disease
- In search of method
  - Cystinosis
For Further Information…

- www.dhs.ca.gov/pcfh/gdb/html/NBS/Mainmenu.htm
- http://www.genes-r-us.uthsc.edu/
- www.questdiagnostics.com

For Further Information…

- www.stanfordlab.com/index.html
- LPCH Biochemical Genetics Lab:
  - (650) 724-3118
  - (650) 724-7858 [Dr. Tina Cowan]
- Stanford Area Service Center:
  - (650) 812-0353
- Newborn Screening Fact Sheets:
  - Pediatrics 2006;118(3):e934-e963