Newborn Screening & Methods for Diagnosing Inborn Errors of Metabolism

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Learning Objectives

• Justify the purpose of newborn screening (NBS)
• Outline the history of NBS
• Describe the testing used for NBS and for diagnosing inborn errors of metabolism (IEM)
• Assess several cases of IEM based on their test results

Why newborn screening?

• IEM are rare
  • "Common":
    • PKU. MDA - 1:14,000
  • Uncommon:
    • MSUD - 1:185,000
    • Galactosemia - 1:60,000
  • Collectively genetic disorders are NOT uncommon - overall rate:
  • 1:4000 live births
• In selected populations:
  • 1:275 – MSUD in Amish in Pennsylvania
  • 1:2500 – SCID in Navajo population

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Why newborn screening?

- IEM presentations are not straight-forward and often critical
  - Initial symptoms are extremely non-specific
    - Metabolic acidosis with $\uparrow$ anion gap, $\uparrow$ transaminases, $\downarrow$ glucose, $\uparrow$ ammonia

- Untreated individuals have significant morbidity and/or mortality

- Early treatment improves everything: length and quality of life and financial burden

NBS origins

- Early 1960’s - Dr. Robert Guthrie –
  - Bacterial inhibition assay for phenylalanine
  - Screen for Phenylketonuria (PKU)

- 1962 - Maine - PKU screening

- Not nationally mandated
  - Individual States fund and decide on what disorders are screened for
  - Each disorder tested for required a blood punch and a separate test – 30+ years

US NBS

<table>
<thead>
<tr>
<th>Disorder</th>
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<tr>
<td>PKU</td>
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<tr>
<td>Congenital Hypothyroidism</td>
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</tr>
<tr>
<td>Galactosemia</td>
<td>47</td>
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<td>Sickle Cell Disease</td>
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<td>GAA</td>
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<td>Branched Chain Acyl-CoA Synthetase Deficiency</td>
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<tr>
<td>MSUD</td>
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<tr>
<td>Hemocytinuria</td>
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<td>Cystic Fibrosis</td>
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<tr>
<td>MCAD</td>
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~1995 – Tandem MS

- Development of DBS MS/MS assay - combination acylcarnitine/amino acid assay
- “Expanded NBS”
- ~2002 – American College of Medical Genetics (ACMG) Newborn Screening Expert Group
- 29 core disorders should be screened for by all States

- Appropriately detectable (soon enough, reliable test) Treatable

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3111605/
US NBS

ACMG - 29 core disorders should be screened for by all States

<table>
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<th></th>
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<td>Sickle Cell Disease</td>
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<td>50</td>
<td>50</td>
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<td>CAH</td>
<td>18</td>
<td>26</td>
<td>47</td>
<td>50</td>
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<td>Biotinidase Deficiency</td>
<td>20</td>
<td>25</td>
<td>46</td>
<td>50</td>
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<td>MSUD</td>
<td>21</td>
<td>25</td>
<td>46</td>
<td>50</td>
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<td>Homocystinuria</td>
<td>14</td>
<td>16</td>
<td>46</td>
<td>50</td>
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<td>Cystic Fibrosis</td>
<td>3</td>
<td>7</td>
<td>33</td>
<td>50</td>
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<tr>
<td>SCID</td>
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<td>0</td>
<td>0</td>
<td>18</td>
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</table>

Molecular diagnostics

NBS and Diagnosing IEM

Looking for biochemical markers indicative of disease

- Normal metabolites $\rightarrow$ to abnormal (toxic) levels
  - Amino acids – phenylalanine in PKU
  - Pathway intermediates – 2-ketoisocaproic acid in MSUD (next intermediate after leucine deamination)
  - Whole molecules - glycogen in glycogen storage diseases

- Abnormal metabolites occur
  - Body attempts to bypass block:
    - Acetyl-CoA + OAA $\rightarrow$ citrate (TCA cycle)
    - Propionyl-CoA + OAA $\rightarrow$ methylcitrate (in Propionic acidemia (PA) and methylmalonic acidemia (MMA))
    - Alloisoleucine in MSUD

Specific IEM diagnostic tests

- Organic acid analysis of urine samples by GC/MS
  - Organic acid disorders (MMA, PA, IVA, GA1)
  - One Urea Cycle disorders (OTC)
  - Some Fatty acid oxidation disorders (MCAD, LCHAD)
  - Some amino acid disorders (Tyr1, MSUD)

- Amino acid analysis of serum samples by HPLC, MS/MS
  - Amino acid disorders (PKU, Tyr, MSUD)
  - Urea cycle disorders (ASA, CITN, ARG)

- Acylcarnitine analysis of serum samples by Tandem MS
  - Some organic acid disorders (MMA, PA, IVA)
  - Fatty acid oxidation disorders (MCAD, CPT2, VLCAD)
Diagnosing IEM

- NBS follow-up testing
  - Important to know what was abnormal about the newborn screen
- Disorder may be picked up by only one of these tests
- Many disorders are picked up by more than one of these assays
- Combination of tests may confirm diagnosis
  - MSUD, Ty type 1 – amino acid, confirmed by organic
  - PKU – amino acid – can be picked up on organic
  - MMA, PA – picked up elevated C3 carnitine on acylcarnitine analysis, differentiated by organic

GC/MS – Organic acid analysis

GC separates compounds by retention

MS blasts compounds into a pattern of fragments - mass spectrum

Organic acid analysis

Identify compounds; look for a pattern of compounds
Amino acid analysis

HPLC ion-exchange chromatography

Amino acids all have same basic structure

\[ \text{R} \quad \text{CH} - \text{COO}^- \quad \text{NH}_3^+ \]

Amino acid charge is dependent on the pH of the solution

Separate them based on charge using a pH gradient

Post-column addition of ninhydrin makes them absorb light at 570 nm (440 nm)

Acylcarnitine analysis (& amino acid analysis)

Precursor ion/fragment

Collision gas

Product ion/fragment

“Neutral loss”

“MRM”

Tandem Mass Spectrometry

IEM Diagnosis

Example Case Studies
Case 1

- History and Physical:
  - 4 day old, Hispanic female
  - Presented with respiratory distress, vomiting and refusal to feed
  - Family history was unremarkable
  - Lethargic and hypotonic and appeared dehydrated.

Case 1

- Principle Laboratory Findings:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Interval</th>
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</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.10</td>
<td>7.35 - 7.45</td>
</tr>
<tr>
<td>pCO2</td>
<td>21 (2.79)</td>
<td>34 - 50 mm/Hg (4.52 - 6.65 kPa)</td>
</tr>
<tr>
<td>HCO3</td>
<td>6</td>
<td>16 - 24 mmol/L</td>
</tr>
<tr>
<td>Sodium</td>
<td>151</td>
<td>139 - 146 mmol/L</td>
</tr>
<tr>
<td>Chloride</td>
<td>116</td>
<td>96 - 106 mmol/L</td>
</tr>
<tr>
<td>AGAP</td>
<td>28</td>
<td>5 - 14</td>
</tr>
<tr>
<td>Glucose</td>
<td>24 (1.3)</td>
<td>74 - 127 mg/dL (4.1 - 7.0 mmol/L)</td>
</tr>
</tbody>
</table>

Case 1 - Other tests

- Expected possible IEM
- Look for other negative ions causing Anion Gap
- Ordered:
  - Urine organic acid analysis
  - Serum amino acid analysis
Case 1

- Diagnostic laboratory findings:
  - Metabolic workup:
    - Test                     Result               Reference range
    - Amino acid analysis:
      - Leucine 4375 μmol/L  47 – 160
      - Isoleucine 588 μmol/L  26 – 91
      - Valine 1155 μmol/L  64 – 336
    - Alloisoleucine (abnormal metabolite)
    - Organic acid analysis:
      - Presence of: 2-hydroxy-isovaleric acid, 2-hydroxy-isocaproic acid and 2-hydroxy-3-methylvaleric acid
Maple Syrup Urine Disease (MSUD)

<table>
<thead>
<tr>
<th>Leucine</th>
<th>Isoleucine</th>
<th>Valine</th>
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<tbody>
<tr>
<td>Branched chain amino acid transaminase</td>
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<tr>
<td>α-ketoisocapric acid</td>
<td>α-keto-β-methylvaleric acid</td>
<td>α-ketoisovaleric acid</td>
</tr>
<tr>
<td>Branched-chain α-keto acid dehydrogenase (BCKD) Complex</td>
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</tr>
</tbody>
</table>

MSUD

- Treatment:
  - Restricted diet: sufficient for growth, but prevent toxic effects of excess branch-chain amino acids
- Prognosis:
  - Dependent on specific defect
  - Enzyme activity: <2% - 30% of normal
  - Age at diagnosis!

Case 2

- History and Physical
  - 3 month old Caucasian female
  - Presented to ED with failure to thrive and respiratory distress
  - Hypotonic
  - Blood pH 7.28
  - Intubated to ICU
- Suspected IEM
- Ordered:
  - Acylcarnitine analysis
  - Organic acid analysis
Case 2 - acylcarnitine

<table>
<thead>
<tr>
<th>Species</th>
<th>ACYL (GROUP)</th>
<th>LOW</th>
<th>HIGH</th>
<th>RUBA</th>
<th>EBRA</th>
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<tr>
<td>CI</td>
<td>4.83</td>
<td>25.0</td>
<td>3.39</td>
<td>NORMAL</td>
<td></td>
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<tr>
<td>CI-1</td>
<td>0.3</td>
<td>0.33</td>
<td>0.63</td>
<td>NORMAL</td>
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<tr>
<td>CI-2</td>
<td>1.1</td>
<td>18.6</td>
<td>ELEVATED</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td>0.1</td>
<td>4.08</td>
<td>NORMAL</td>
<td></td>
<td></td>
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<tr>
<td>CI-3</td>
<td>0.3</td>
<td>0.68</td>
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<td>CI-4</td>
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<td>CI-5</td>
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<td>0.12</td>
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<td>CI-6</td>
<td>0.7</td>
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<td>CI-7</td>
<td>0.11</td>
<td>0.17</td>
<td>ELEVATED</td>
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</tr>
</tbody>
</table>

Case 2 - organic acid

Methylmalonic aciduria
Case 2:

- Known defects in adenosyl- and methyl-Cbl:
  - MMA and Homocysteine
  - Cbl A - Cbl reductase
  - Cbl B - Cbl adenotransferase

- Known defects in adenosyl Cbl:
  - MMA AND Homocysteine
- Cbl C, Cbl D, and Cbl F

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Case 2 - Methylmalonic aciduria

- **Organic acids:**
  - Methylmalonic, methylcitrate, 3-OH-propionic

- **Amino acids:**
  - Homocystine – 160 µmol/L
    (ref range < 12 µmol/L)

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Methylmalonic aciduria

- **Treatment:**
  - OH-Cobalamin supplementation
  - Restricted protein diet (avoid amino acid propionyl-CoA precursors – branched chain, methionine, threonine)

- **Prognosis:**
  - Good for some Cbl mutations
  - Not so good for mutase mutations
Case 3

- History and Physical
  - Apparently healthy 2 week old presenting to ED because of abnormal newborn screen

- Ordered:
  * organic acid and acylcarnitine analysis

Case 3 - acylcarnitine

<table>
<thead>
<tr>
<th>Acylicarnitines</th>
<th>03/11/2</th>
<th>02/11/2</th>
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<tr>
<td>Acetylecarnitine</td>
<td>0.2</td>
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<td>C3:0 Acylcarnitine</td>
<td>0.4</td>
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<tr>
<td>C4:0 Acylcarnitine</td>
<td>0.2</td>
<td>0.02</td>
<td>NORMAL</td>
</tr>
<tr>
<td>C6:0 Acylcarnitine</td>
<td>0.1</td>
<td>0.08</td>
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<td>C8:0 Acylcarnitine</td>
<td>0.7</td>
<td>0.08</td>
<td>NORMAL</td>
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<tr>
<td>C10:0 Acylcarnitine</td>
<td>0.1</td>
<td>0.08</td>
<td>NORMAL</td>
</tr>
<tr>
<td>C10:1 Acylcarnitine</td>
<td>0.15</td>
<td>0.15</td>
<td>ELEVATED</td>
</tr>
</tbody>
</table>

Organic acids:
- Hexanoylglycine, suberylglycine, C6-C10 dicarboxylic acids

Acylcarnitines:
- C6, C8, C10, C10:1

Medium-chain acyl CoA dehydrogenase (MCAD)
MCAD

- Poster-child disorder for NBS
- Treatment:
  - Prevent fasting!
- Prognosis:
  - Excellent

Future of IEM testing

- Multiplex assays
  - Computers allowing us to handle massive amounts of information
  - DNA micro-arrays
    - What genes are turned on or not turned on
  - Proteomic/metabolomic assays
    - Patterns of protein and metabolite expression in well vs ill children
- Continue detecting disorders that don’t meet the criteria
  - Increasing MS/MS sensitivity
  - Very mild forms, non-diseases
  - Currently untreatable

Summary

- NBS purpose is to detect treatable disorders before irreparable damage can occur
- Tandem mass spectrometry revolutionized NBS
- Availability of MS/MS testing led to more standardized NBS program
- IEM present with very non-specific clinical and laboratory findings
- Diagnostic strategies look for abnormal metabolites and normal metabolites in high concentrations
- Organic acid, amino acid and acylcarnitine analysis are predominately used to diagnose IEM
Case 4

- History and Physical
  - 2 day old male with lethargy, vomiting and arm and leg tremors
  - Non-responsive in ED – intubated
  - Ammonia = 1975 μmol/L (<100 ULN)

Case 4: Urea Cycle disorders

Case 4 – organic acid
Case 4 - OTC deficiency

- Most common deficiency of urea cycle enzymes
- X-linked defect
  - Males have more severe course
  - Females may be asymptomatic

Lower ammonia levels!

Urea cycle treatment

Complex to excrete in urine

Monitor glutamine concentrations

Monitor ammonia – treat aggressively!