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, MCAD – 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:
  - 1:175 – MSUD in Amish in Pennsylvania
  - 1:2500 – SCID in Navajo population
Why newborn screening?

- IEM presentations are not straight-forward and often critical
  - Initial symptoms are extremely non-specific
    - Metabolic acidosis with ↑ anion gap, ↑ transaminases, ↓ glucose, ↑ 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

- Development of DBS MS/MS assay - combination acylcarnitine/amino acid assay
- “Expanded NBS”
- ~2002 – American College of Medical Genetics (ACMG) Newborn Screening Expert Group

<table>
<thead>
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<tr>
<td>Sickle Cell Disease</td>
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<td>CAH</td>
<td>18</td>
</tr>
<tr>
<td>Biotinidase Deficiency</td>
<td>20</td>
</tr>
<tr>
<td>MSUD</td>
<td>21</td>
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<tr>
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<td>14</td>
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<tr>
<td>Cystic Fibrosis</td>
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</tr>
<tr>
<td>MCAD</td>
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</table>

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

<table>
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<td>46</td>
<td>50</td>
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<tr>
<td>MCAD</td>
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<td>8</td>
<td>47</td>
<td>50</td>
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<tr>
<td>SCID</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18</td>
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Molecular diagnostics
NBS and Diagnosing IEM

Looking for biochemical markers indicative of disease

- **Normal metabolites \( \uparrow \) to abnormal (toxic) levels**
  - Amino acids – phenylalanine in PKU
  - Pathway intermediates – 2-keto-isocaproic 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 \( \rightarrow \) methyl-citrate (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, Tyr 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
\[ R \quad CH \quad COO^- \quad NH_3^+ \]

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)

MS1  CC (MS2)  MS3

- Precursor ion/fragment
- Collision gas
- Product ion/fragment

“Neutral loss”

Tandem Mass Spectrometry

“MRM”

Butyl-formate - 102

Ala - 146
Phe - 222
Ala - 90
Phe - 160

Ala - 44
Phe - 120
Ala - 40
Phe - 120
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>
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</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
Case 1 amino acids

Valine, isoleucine, leucine

Peak # 16 = alloisoleucine; co-elutes with methionine
Case 1

- Diagnostic laboratory findings:
  - Metabolic workup:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference range</th>
</tr>
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<tr>
<td><strong>Amino acid analysis:</strong></td>
<td></td>
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<tr>
<td>- Leucine</td>
<td>4375 μmol/L</td>
<td>47 – 160</td>
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<tr>
<td>- Isoleucine</td>
<td>588 μmol/L</td>
<td>26 – 91</td>
</tr>
<tr>
<td>- Valine</td>
<td>1155 μmol/L</td>
<td>64 – 336</td>
</tr>
<tr>
<td>- Alloisoleucine (abnormal metabolite)</td>
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</tbody>
</table>

**Organic acid analysis:**
- Presence of: 2-hydroxy-isovaleric acid, 2-hydroxy-isocaproic acid and 2-hydroxy-3-methylvaleric acid
Maple Syrup Urine Disease (MSUD)

Leucine → Branched chain amino acid transaminase → α-ketoisocaproic acid

Isoleucine → Branched chain amino acid transaminase → α-keto-β-methylvaleric acid

Valine → Branched chain amino acid transaminase → α-ketoisovaleric acid

Branched-chain α-keto acid dehydrogenase (BCKD) Complex
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>RESULT</th>
<th>status</th>
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<tbody>
<tr>
<td>C2</td>
<td>(ACETYL)</td>
<td>4.21</td>
<td>20.6</td>
<td>8.29</td>
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<tr>
<td>C3:1</td>
<td>(PROPENOYL)</td>
<td>0</td>
<td>0.3</td>
<td>0.03</td>
<td>NORMAL</td>
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<td>C3</td>
<td>(PROPIONYL)</td>
<td>0</td>
<td>1.6</td>
<td>12.5</td>
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<tr>
<td>C4</td>
<td>(BUTYRYL/ISOBUTYRYL)</td>
<td>0</td>
<td>1</td>
<td>0.69</td>
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<tr>
<td>C4-DC</td>
<td>(MMA,SUCCINIC)</td>
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<td>0.2</td>
<td>0.08</td>
<td>NORMAL</td>
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<tr>
<td>C4-OH</td>
<td>(HYDROXYBUTYRYL)</td>
<td>0</td>
<td>0.4</td>
<td>0.04</td>
<td>NORMAL</td>
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<tr>
<td>C5:1</td>
<td>(TIGLYL/ME-CROTONYL)</td>
<td>0</td>
<td>0.2</td>
<td>0.13</td>
<td>NORMAL</td>
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<tr>
<td>C5</td>
<td>(ISOVALERYL/2ME-BUTYRYL)</td>
<td>0</td>
<td>0.7</td>
<td>0.63</td>
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<tr>
<td>C5-OH</td>
<td>(3OH-ISovaleryl)</td>
<td>0</td>
<td>0.11</td>
<td>0.17</td>
<td>ELEVATED</td>
</tr>
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</table>
Case 2

BCAA
Threonine
Methionine
Odd chain FA
Cholesterol side chain
Case 2 – organic acid

Methylmalonic aciduria
Case 2:

2 known defects in mutase
- ↑ MMA

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

2 known defects in adenosyl Cbl
- ↑ MMA
- Cbl A - Cbl reductase
- Cbl B - Cbl adenotransferase
Case 2 - Methylmalonic aciduria

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

- **Amino acids:**
  - Homocystine – 160 µmol/L (ref range < 12 µmol/L)
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>Lipid</th>
<th>Description</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
<th>Status</th>
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<tbody>
<tr>
<td>C4-DC</td>
<td>(MMA, SUCINIC)</td>
<td>0</td>
<td>0.2</td>
<td>0.2</td>
<td>NORMAL</td>
</tr>
<tr>
<td>C4-OH</td>
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<tr>
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<tr>
<td>C5-DC</td>
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<td>0.08</td>
<td>BQL</td>
<td>NORMAL</td>
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</table>
Case 3

- **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
Ornithine Transcarbamylase deficiency
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

Monitor glutamine concentrations

Monitor ammonia – treat aggressively!

Complex to excrete in urine

- Na phenylacetate
- Na benzoate

Supplement