Newborn screening & Metabolic disease

Patti Jones, PhD
Professor of Pathology
UT Southwestern Medical Center
Director of Chemistry
Children’s Medical Center Dallas
Objectives

• Describe the rationale behind the practice of Newborn screening (NBS)
• Delineate the history of NBS
• Describe the tests used for the diagnosis of Inborn Errors of Metabolism (IEM)
• Assess the presentation and diagnosis of several common IEM
Why newborn screening?

• Genetic disorders are rare
  – Common:
    • Cystic Fibrosis – 1:3000
    • PKU, MCAD – 1:14,000
  – Uncommon:
    • MSUD – 1:185,000
      (1:175 – Amish in Pennsylvania)
# Why IEM screening?

<table>
<thead>
<tr>
<th>Program</th>
<th>Duration</th>
<th>Number Screened</th>
<th>Total Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>New South Wales, Australia</td>
<td>1 year</td>
<td>137,000</td>
<td>31</td>
</tr>
<tr>
<td>Australia</td>
<td></td>
<td></td>
<td>(1:4400)</td>
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<tr>
<td>New England, USA</td>
<td>2 years</td>
<td>164,000</td>
<td>42</td>
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<tr>
<td>USA</td>
<td></td>
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<tr>
<td>Neo Gen Screening</td>
<td>7 years</td>
<td>&gt;700,000</td>
<td>163</td>
</tr>
<tr>
<td>Neo Gen Screening</td>
<td></td>
<td></td>
<td>(1:4300)</td>
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<tr>
<td>Bavaria, Germany</td>
<td>7 months</td>
<td>87,000</td>
<td>22</td>
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<tr>
<td>Germany</td>
<td></td>
<td></td>
<td>(1:3950)</td>
</tr>
<tr>
<td>Ave</td>
<td></td>
<td></td>
<td>1:4100</td>
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</tbody>
</table>
IEM presentation

• Common symptoms (non-specific)
  – Failure to thrive
  – Hypoglycemia
  – Hyperammonemia
  – Metabolic acidosis (↑ AGAP)
  – Elevated transaminases
  – Unexplained death of a sibling in infancy
Screening for Inborn Errors of Metabolism

• Over-riding purpose:

  – Screen all newborn infants for diseases in which symptoms are not clinically evident until irreversible damage has occurred and for which effective treatments are available
NBS origins

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

- 1962 - Maine - PKU screening

- Not nationally mandated
  - States fund and decide on what disorders are screened for
Criteria for disorder inclusion

• have a significant incidence in the population screened
• are clinically well defined with the untreated natural history characterized
• have a well defined biochemical phenotype
• cause significant morbidity and/or mortality
• are treatable, where treatment improves outcome
• testing is safe, simple and sufficiently sensitive
• specific confirmatory testing is available
• testing, treatment and treatment outcome are cost effective with respect to non-treatment
## Inborn Errors of Metabolism

<table>
<thead>
<tr>
<th>Disorder</th>
<th>States screening</th>
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<tr>
<td>PKU</td>
<td>50</td>
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<tr>
<td>Congenital Hypothyroidism</td>
<td>50</td>
</tr>
<tr>
<td>Galactosemia</td>
<td>47</td>
</tr>
<tr>
<td>Sickle Cell Disease</td>
<td>43</td>
</tr>
<tr>
<td>CAH</td>
<td>18</td>
</tr>
<tr>
<td>Biotinidase Deficiency</td>
<td>20</td>
</tr>
<tr>
<td>MSUD</td>
<td>21</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>14</td>
</tr>
<tr>
<td>Cystic Fibrosis</td>
<td>3</td>
</tr>
<tr>
<td>MCAD</td>
<td>0</td>
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</table>

Each test performed required a blood punch and an individual test.

1997 – Mississippi did not require NBS
NBS changes driven by technology

• Tandem mass spectrometry
  • Development of MS/MS assays using blood spots
  • Combination acylcarnitine – amino acid assay - 1999
  • MS/MS can detect >50 different disorders
  • “Expanded NBS”

• 1999 AAP Newborn Screening Taskforce recommended Health Resources and Services Administration (HRSA) of US Dept of Health and Human Services do something about the fact that there is no nationally standardized NBS program
  – Maternal and Child Health bureau of HRSA commissioned ACMG in 2002
NBS changes driven by technology

• Expanded NBS
  – American College of Medical Genetics (ACMG) Newborn Screening Expert Group – “Newborn Screening: Toward a Uniform Screening Panel and System” (ACMG website – executive summary; full report published 2006 Genetics IN Medicine)
  
  – 20 disorders recommended for inclusion in NBS as primary targets by the expert group

  – 22 more secondary targets – conditions part of differential diagnosis for core disorders
20 Core conditions - included

**AMINO ACIDS**
- Homocystinuria
- Argininosuccinic Acidemia
- Citrullinemia
- Maple Syrup Urine Disease
- Phenylketonuria
- Tyrosinemia, type 1

**FATTY ACIDS**
- Long-Chain 3-Hydroxy-acyl CoA Dehydrogenase (LCHAD)
- Trifunctional Protein (TFP) deficiency
- Medium-chain Acyl CoA Dehydrogenase (MCAD) deficiency
- VLCAD deficiency
- Carnitine uptake defect

**ORGANIC ACIDS**
- Glutaric Acidemia, type 1
- 3-Hydroxy-3-Methyl-Glutaryl CoA Lyase deficiency
- Isovaleric acidemia
- 3-Methyl-Crotonyl CoA Carboxylase deficiency
- Methylmalonic Acidemia, mut
- Methylmalonic Acidemia Cbl A,B
- Propionic Acidemia
- 3-Ketothiolase deficiency
- Multiple Carboxylase Deficiency

6 amino acid, 5 FAO, 9 organic acid disorders – 1 blood punch
Secondary targets

**AMINO ACIDS**
- Hyperphenylalaninemia
- Hypermethioninemia
- Biopterin cofactor synthetic defects
- Biopterin cofactor regulatory defects
- Tyrosinemia, type 2
- Tyrosinemia, type 3
- Argininemia
- Citrullinemia, type 2

**FATTY ACIDS**
- Carnitine Palmitoyltransferase 1 (CPT1) deficiency
- CPT 2 deficiency
- Carnitine/Acylcarnitine Translocase defect
- SCAD deficiency
- M/SCHAD deficiency
- MCKAT deficiency
- Glutaric Acidemia, type 2
- Dienoyl CoA reductase deficiency

**ORGANIC ACIDS**
- Malonic acidemia
- Isobutyryl CoA dehydrogenase deficiency
- 3-Methylglutaconic aciduria
- Methylmalonic Acidemia, Cbl C,D
- 2-Methyl-3-hydroxybutyric aciduria
- 2-Methylbutyryl CoA dehydrogenase deficiency

8 amino acid, 8 FAO, 6 organic acid disorders
Disorders not recommended for NBS inclusion

• Refined criteria
  – the disorder can be identified at a phase (24-48 hrs after birth) at which it would not ordinarily be clinically detectable
  – a test is available for it that has appropriate sensitivity and specificity
  – there are demonstrated benefits of early detection, timely intervention and efficacious treatment of the disorder

• Not recommended
  – Not identified soon enough
  – Not reproducibly detected
  – No treatment available
# Inborn Errors of Metabolism

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<td>50</td>
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<td>7</td>
<td>33</td>
<td>50</td>
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<tr>
<td>MCAD</td>
<td>0</td>
<td>8</td>
<td>47</td>
<td>50</td>
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</table>

*1997 – Mississippi did not require NBS
From NBS to Diagnosis

• NBS – DBS samples for MS/MS, etc
  – All newborns in US now get this screening

• Follow up of positive screening results:
  – Other testing required
  – Only performed on infants with positive screen or presenting with symptoms

• Diagnosing IEM rather than screening for them
Diagnosing IEM

• Normal metabolites build up to 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 → citrate (TCA cycle)
    • Propionyl-CoA + OAA → methyl-citrate (in Propionic acidemia and methylmalonic acidemia)
    • Alloisoleucine in MSUD
IEM Diagnosis

• Tests that look for abnormal amounts of normal metabolites or abnormal metabolites or both
  
  • **Organic acid analysis** of urine samples by GC/MS
  
  • **Acylcarnitine analysis** of serum samples by MS/MS
  
  • **Amino acid analysis** of serum samples by HPLC or LC/MS/MS
Organic acid analysis

GC/MS
Organic Acid Analysis

- Essentially all done by GC/MS
- Reporting is different between institutions
  - Report specific list of metabolites with reference intervals
  - Report above plus interpretive comment
  - Report presence of abnormal metabolites and interpretive comment
Acylcarnitine analysis

MS1  CC (MS2)  MS3

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

“Neutral loss”

“MRM”

Tandem Mass Spectrometry

Butyl-formate - 102

Ala - 90
Phe - 160
Ala - 44
Phe - 120
Ala - 40
Phe - 120

Butylated

Butylated
Acylcarnitine Analysis

• Most people use neutral loss ($m/z$ 85)

• Report many quantitative results

<table>
<thead>
<tr>
<th>species (acyl group)</th>
<th>LOW</th>
<th>HIGH</th>
<th>RESULT</th>
<th>status</th>
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<tbody>
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<td>C2 (ACETYL)</td>
<td>0.21</td>
<td>20.5</td>
<td>11.5</td>
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<td>C3:1 (PROPYL)</td>
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<td>0.3</td>
<td>0.05</td>
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<td>C3 (PROPYL)</td>
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<td>C4:0C (OMA, ULCIN)</td>
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<td>C4:OH (HYDROXYBUTYRYL)</td>
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<td>C5:1 (TERTYLAMETOXYL)</td>
<td>0</td>
<td>0.2</td>
<td>0.05</td>
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<td>C5 (ISOVALERYL)</td>
<td>0</td>
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<td>C5:OH (ISOISOVALERYL)</td>
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<td>0.11</td>
<td>0.07</td>
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<td>C5:DC (GLUTARYL)</td>
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<td>C6 (HEXANOL)</td>
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<td>0.22</td>
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<td>C6:DC (METHYLGLUTARIC)</td>
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<td>0.1</td>
<td>0.12</td>
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<td>C8:1 (OCTENYL)</td>
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<td>0.7</td>
<td>0.36</td>
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<td>C8 (OCTANOL)</td>
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<td>0.8</td>
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<tr>
<td>C8:DC (MALONYL)</td>
<td>0</td>
<td>0.13</td>
<td>0.06</td>
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<tr>
<td>C10:2 (DECADENYL)</td>
<td>0</td>
<td>0.11</td>
<td>0.14</td>
<td>ELEVATED</td>
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<tr>
<td>C10:4 (DECADENOYL)</td>
<td>0.01</td>
<td>0.49</td>
<td>1.02</td>
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<td>C12 (ODODENYL)</td>
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<td>0.9</td>
<td>0.93</td>
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<td>C12:1 (OEODENYL)</td>
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<td>C12:OH (ODODENYL)</td>
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<td>0.08</td>
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<td>C14:1 (TETRADECENOYL)</td>
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<td>C14:OH (3-OH-14)</td>
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<td>C16 (PENTAMITOL)</td>
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<td>C18:OH (3-OH-PALMITOL)</td>
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<td>0.1</td>
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<tr>
<td>C18:2 (LINOLENOYL)</td>
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<tr>
<td>C18:1 (OLEOYL)</td>
<td>0</td>
<td>0.12</td>
<td>0.05</td>
<td>NORMAL</td>
</tr>
<tr>
<td>C18:2:OH (3-OH-LINOLEOYL)</td>
<td>0</td>
<td>0.05</td>
<td>BQL</td>
<td>NORMAL</td>
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<tr>
<td>C18:1:OH (3-OH-OLEOYL)</td>
<td>0</td>
<td>0.03</td>
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<td>NORMAL</td>
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<td>C19 (OCTEADENOYL)</td>
<td>0.01</td>
<td>0.11</td>
<td>BQL</td>
<td>NORMAL</td>
</tr>
</tbody>
</table>

RATIOS

| D3/C2 | 0.08 |
| D3/C4 | 1.69 |
| D4/C2 | 0.05 |
| D4/C3 | 2.73 |
| D5/C3 | 1.26 |
| D5/D3/C3 | 0.28 |
| D5/D3/G3 | 0.25 |
Amino acid analysis

Amino acids carry a charge, which is dependent on the pH of the solution.

Separate them based on charge by using a pH gradient.

HPLC ion-exchange chromatography

Post-column addition of ninhydrin makes them absorb light at 570 nm (440 m).
Amino Acid Analysis

• Other methods:
  – LC/MS/MS – requires separation
  – Pre-column treatment with chemical for UV detection and C18 reverse phase separation

• Report results as:
  – 40+ quantitative individual amino acids
  – Screen: no abnormalities or abnormal with results
Methods

• All NBS and IEM diagnostic methods are LDTs!

• Must be CLIA validated completely

• Subject to FDA regulation

• Proficiency testing
  – BGL survey
  – Internal PT
  – Trade samples with other labs performing this testing
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>
</tr>
</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>
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<td>6</td>
<td>16 – 24 mmol/L</td>
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<td>Sodium</td>
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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

Normal patient

Valine, isoleucine, leucine

Patient

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

- Diagnostic laboratory findings:
  - Metabolic workup:
    | Test               | Result     | Reference Interval |
    |--------------------|------------|--------------------|
    | 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 | | |
Case 1 - Diagnosis

Maple Syrup Urine Disease
MSUD
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, low bicarb, elevated AGAP
  – Intubated to ICU
Case 2

- Suspected IEM
- Ordered:
  - Acylcarnitine analysis
  - Organic acid analysis
Case 2 - acylcarnitine

<table>
<thead>
<tr>
<th>species</th>
<th>(ACYL GROUP)</th>
<th>LOW (nmol/mL)</th>
<th>HIGH (nmol/mL)</th>
<th>RESULT (nmol/mL)</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>
<td>NORMAL</td>
</tr>
<tr>
<td>C3:1</td>
<td>(PROPENOYL)</td>
<td>0</td>
<td>0.3</td>
<td>0.03</td>
<td>NORMAL</td>
</tr>
<tr>
<td>C3</td>
<td>(PROPIONYL)</td>
<td>0</td>
<td>1.6</td>
<td>12.5</td>
<td>ELEVATED</td>
</tr>
<tr>
<td>C4</td>
<td>(BUTYRYL/ISOBUTYRYL)</td>
<td>0</td>
<td>1</td>
<td>0.69</td>
<td>NORMAL</td>
</tr>
<tr>
<td>C4-DC</td>
<td>(MMA, SUCCINIC)</td>
<td>0</td>
<td>0.2</td>
<td>0.08</td>
<td>NORMAL</td>
</tr>
<tr>
<td>C4-OH</td>
<td>(HYDROXYBUTYRYL)</td>
<td>0</td>
<td>0.4</td>
<td>0.04</td>
<td>NORMAL</td>
</tr>
<tr>
<td>C5:1</td>
<td>(THIGLYLME-CROTOXYL)</td>
<td>0</td>
<td>0.2</td>
<td>0.13</td>
<td>NORMAL</td>
</tr>
<tr>
<td>C5</td>
<td>(ISOVALEYL/2ME-BUTYRYL)</td>
<td>0</td>
<td>0.7</td>
<td>0.63</td>
<td>NORMAL</td>
</tr>
<tr>
<td>C5-OH</td>
<td>(3OH-ISOVALEYL)</td>
<td>0</td>
<td>0.11</td>
<td>0.17</td>
<td>ELEVATED</td>
</tr>
</tbody>
</table>

Elevated C3-carnitine (propionyl-carnitine)
Metabolism of odd-chain fatty acids

\[
\text{CH}_3-\text{CH}_2-C-S-\text{CoA}
\]

propionyl CoA

\[
\text{CO}_2
\]

propionyl-CoA carboxylase requires biotin

\[
\overset{\text{O}}{\text{C}}-S-\text{CoA}
\]

H-\overset{\text{O}}{\text{C}}-CH_3

\overset{\text{COO}^-}{\text{COO}^-}

d-methylmalonyl CoA

\[
\overset{\text{O}}{\text{C}}-S-\text{CoA}
\]

H_2\overset{\text{O}}{\text{C}}-CH

\overset{\text{COO}^-}{\text{COO}^-}

l-methylmalonyl-CoA

\[
\overset{\text{O}}{\text{C}}-C-H_2-C-S-\text{CoA}
\]

succinyl-CoA

methylmalonyl-CoA racemase

methylmalonyl-CoA mutase requires vitamin B_{12}, cobalamin
Case 2

Abundance

Methylmalonic acid

TIC: MAR1405-13.D

hippuric/citric

methyl-citric

3-OH-propionic

IS
Case 2: Methylmalonic aciduria
Case 2:

- Known defects in adenosyl- and Methyl- Cbl
  - $\uparrow$ MMA AND $\uparrow$ Homocysteine
  - Cbl C, Cbl D, and Cbl F

- 2 known defects in adenosyl Cbl
  - $\uparrow$ MMA
  - Cbl A - Cbl reductase
  - Cbl B - Cbl adenotransferase

- 2 known defects in mutase
  - $\uparrow$ MMA
Methylmalonic aciduria

- Organic acids:
  - Methylmalonic, methylcitric, 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 methylmalonic precursors – branched chain, methionine, threonine)

• Prognosis:
  – Good for some cbl mutations
  – Not so good for mutase mutations
## Methylmalonic aciduria

<table>
<thead>
<tr>
<th>Analyte (ref. interval)</th>
<th>original</th>
<th>After cobalamin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylmalonic Acid (0 – 0.4 μmol/L)</td>
<td>77.8</td>
<td>12.3</td>
</tr>
<tr>
<td>Homocysteine (4-12 μmol/L)</td>
<td>160</td>
<td>90</td>
</tr>
<tr>
<td>Methionine (6-60 μmol/L)</td>
<td>2</td>
<td>13</td>
</tr>
</tbody>
</table>
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
• Diagnostic strategies look for abnormal metabolites and normal metabolites in high concentrations
• IEM present with very non-specific clinical and laboratory findings
Self-assessment Questions

1. All of the following have been used as criteria for including disorders in NBS programs, **EXCEPT**:
   a) Disorder has no effective treatment
   b) A test can detect the disorder within hours of birth
   c) Cost of testing and treatment is less than the cost of the untreated disorder
   d) Disorder has an appropriately sensitive and specific screening test
Self-assessment Questions

2. The first newborn screening test:
   a) Was instituted in Maine and was for tyrosinemia
   b) Was a bacterial growth inhibition assay
   c) Was developed on the tandem mass spectrometer
   d) Screened for 5 disorders
Self-assessment Questions

3. Common laboratory findings in IEM presentations do NOT include:
   a) Hyperammonemia
   b) Low concentrations of transaminases
   c) Metabolic acidosis with elevated anion gap
   d) Hypoglycemia
Self-assessment Questions

4. Methods used for confirming diagnosis of positive newborn screening results do NOT include:
   a) Organic acids by GC/MS
   b) Acylcarnitines by tandem mass spectrometry
   c) Amino acids by GC/MS
   d) PCR for specific genetic defects
Answers

1. A
2. B
3. B
4. C