Newborn Screening and Diagnosis of Fatty Acid Oxidation Disorders

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Newborn Screening

A Public Health Program

- Aimed at identification of conditions for which early intervention can prevent mortality, morbidity, and disabilities
- Performed by analysis of diagnostic markers in blood spots collected on filter paper at birth
- Largest population-based genetic screening effort in the US: ~4 Million babies screened annually
The Newborn Screening System

• Screening
  – Laboratory analysis of newborn bloodspot

• Follow-up of an abnormal result
  – Rapid location, follow-up and referral of the screen-positive infant

• Diagnosis
  – Clinical and biochemical evaluation to diagnose or rule out the disorder

• Management
  – Rapid planning and implementation of long-term therapy

• Evaluation
  – Assessment of the NBS program: efficiency of follow-up & intervention, benefit to society
Newborn Screening: Short Term Follow-up

Abnormal result obtained from the State Lab

CMS nurse from follow-up team notifies Genetics on-call Physician about the Abnormal Newborn Screen result

Genetics on-call Physician locates patient, makes clinical assessment of patient status – recommends clinic visit

Infant sample collected (Plasma, Urine, Whole Blood) Diagnostic evaluation performed

Positive cases: long-term patient management & ongoing care
Follow-up Diagnostic Testing

• Routine Chemistries
  – Electrolytes, blood gases, anion GAP
  – Blood sugar, ketones, lactate, ammonia

• Biochemical Genetics Testing
  – Plasma acylcarnitine (Green top tube – 0.5 ml min whole blood)
  – Carnitine Status (Red top tube – 0.5 ml min whole blood)
  – Urine organic acids (No preservatives – 3 ml min vol)
  – Plasma amino acids (Green top tube – 0.5 ml min whole blood)

• Other specialized testing
  – Enzyme analysis (eg Fatty acid oxidation Probe Assay)
  – Mutation testing
Use of Tandem Mass Spectrometry for Multianalyte Screening of Dried Blood Specimens from Newborns

Donald H. Chace, Theodore A. Kalas, and Edwin W. Naylor
Tandem Mass Spectrometry

- Identification of unknown compounds
- Quantitation of known analytes using stable isotopes
- Provides structural and chemical information of molecules
- High sensitivity and specificity
- Fast analytical time
- Potential for automation for high throughput tests

(modified from ASMS, 1989)
An Acylcarnitine molecule has the following structure:

\[
\begin{align*}
\text{CH}_3 & \quad \text{R} \quad \text{COO} \\
\text{CH}_3 & \quad \text{+N - CH}_2 - \text{CH - CH}_2 - \text{COO - C}_4\text{H}_9 \\
\text{CH}_3 & 
\end{align*}
\]

Members of this family of compounds have varying lengths of their R-Group:

- C3 acylcarnitine ... 3 carbon units
- C8 acylcarnitine ... 8 carbon units
- C16 acylcarnitine ... 16 carbon units

- Specific Acylcarnitines accumulate in fatty acid oxidation disorders (and certain organic acidemias) and form a distinct “pattern”
- Acylcarnitine analysis is very important in: prenatal diagnosis, newborn screening, evaluation of symptomatic patients, and postmortem screening
Collective incidence
1:2-4,000 newborns

**Newborn Screening by MS/MS**

**Disorders of fatty acid oxidation**
- 2,4-Dienoyl-CoA reductase deficiency
- Carnitine acylcarnitine translocase deficiency
- Carnitine palmitoyltransferase I deficiency
- Carnitine palmitoyltransferase II deficiency
- Carnitine transport defect
- Electron transfer flavoprotein deficiency
- ETF ubiquinone oxidoreductase deficiency
- Long-chain L-3-OH acyl-CoA dehydrogenase deficiency
- Medium-chain acyl-CoA dehydrogenase deficiency
- Medium-chain L-3-OH acyl-CoA dehydrogenase deficiency
- Medium chain ketoacyl-CoA thiolase deficiency
- Short-chain acyl-CoA dehydrogenase deficiency
- Trifunctional protein deficiency
- Very long-chain acyl-CoA dehydrogenase deficiency

**Disorders of amino acid metabolism**
- Arginase deficiency
- Argininosuccinate lyase deficiency
- Argininosuccinate synthase deficiency
- Maple syrup urine disease (MSUD)
- Citrin deficiency
- Cystathionine β-synthase deficiency
- Methionine adenosyltransferase deficiency
- Mitochondrial ornithine transport defect (HHH)
- Phenylalanine hydroxylase deficiency (PKU)
- Defects of biotinioin metabolism
- Fumaracetoacetase deficiency
- Tyrosine aminotransferase deficiency

**Disorders of organic acid metabolism**
- 2-CH3 butyryl-CoA dehydrogenase deficiency
- 2-CH3 3-OH butyryl-CoA dehydrogenase deficiency
- 3-OH 3-CH3 glutaryl-CoA lyase deficiency
- 3-CH3 crotonyl-CoA carboxylase deficiency
- 3-CH3 glutaryl-CoA hydratase deficiency
- Isobutyryl-CoA dehydrogenase deficiency
- Isovaleryl-CoA dehydrogenase deficiency
- Glutaryl-CoA dehydrogenase deficiency
- Malonyl-CoA carboxylase deficiency
- Methylmalonyl-CoA mutase deficiency
- Disorders of cobalamin metabolism
- β-ketothiolase deficiency
- Multiple carboxylase deficiency
- Propionyl-CoA carboxylase deficiency
Normal Acylcarnitine Profile

Free Carnitine

C₂

C₃

C₄

C₈

C₁₂

C₁₆

Internal Standard

VLCAD Acylcarnitine Profile

Free Carnitine

C₁₂

C₁₄:1

C₁₆

C₁₈:1

*
Normal Profile in known VLCAD patient on diet

Patient (on MCT diet)

Free Carnitine
Postmortem Bile (LCHAD)

Postmortem DBS (LCHAD)

NBS DBS (LCHAD)

Precursor (85): 0.85 min (17 scans) from AC 070202P-06
Precursor (85): Expt. 3, 0.67 min (31 scans) from 070902-12 copy

Postmortem Bile (LCHAD)

Postmortem DBS (LCHAD)

NBS DBS (LCHAD)
Fatty Acid Oxidation Probe Assay

- Monolayer of skin fibroblasts / amniocytes
- Growth media removed and replaced with “in vitro probe” media containing palmitic acid and L-carnitine
- Incubated for 72 hours, media is collected and subjected to acylcarnitine analysis
- Adherent cells are harvested - total protein analysis
### Enzyme Assay for VLCAD - Results

#### Control Amniocytes vs VLCAD Patient Amniocytes

<table>
<thead>
<tr>
<th>Activity</th>
<th>Control Amniocytes</th>
<th>VLCAD Patient Amniocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCAD Activity (mU/mg Protein)</td>
<td>1.01</td>
<td>1.21</td>
</tr>
<tr>
<td>VLCAD Activity (mU/mg Protein)</td>
<td>1.52</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

#### Control Fibroblasts vs VLCAD Patient Fibroblasts

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>MCAD Activity (mU/mg Protein)</td>
<td>1.31</td>
<td>1.07</td>
</tr>
<tr>
<td>VLCAD Activity (mU/mg Protein)</td>
<td>3.61</td>
<td>Not detected</td>
</tr>
</tbody>
</table>
Urine Organic Acids

• Organic Acids – What are they?
  – Water-soluble compounds containing one or more carboxylic group as well as other functional groups (-keto, -hydroxy)
  – Intermediate metabolites of all major groups of organic cellular components: amino acids, lipids, carbohydrates, nucleic acids and steroids

• Accumulation of metabolites which are not present under physiological conditions
Urine Organic Acids

• Urine Sample Collection
  – Urine should be collected without preservatives
  – Collection should avoid fecal contamination
  – Frozen immediately

• Sample Preparation and Analysis
  – Liquid-liquid extraction of acidified urine into organic solvent
  – Evaporation of solvent and derivatization of residue
  – Gas chromatography – Mass spectrometry analysis

• Specific diseases have characteristic organic acid elevations
Organic Acid Analysis

Propionic Acidemia

3-OH propionic acid
3-OH isovaleric acid
propionylglycine
methylcitric acid
IS

Time (min)
Plasma Amino Acids

- **Plasma Sample Collection**
  - Timely centrifugation and separation of plasma specimens is critical to prevent artifacts
  - Must be refrigerated for the short term (< 4 hrs.) or frozen (-20°C) to arrest amino acid deterioration
  - Hemolyzed samples are not viable

- **Sample Preparation and Analysis**
  - Protein precipitation and centrifugation
  - Ion exchange chromatography to separate
  - Post-column derivatization & detection at 440 to 570 nm

- **Specific diseases have characteristic amino acid elevations**
Plasma Amino Acids Analysis

Tyrosinemia

* Internal Standards

Time (min)