Tandem Mass Spectrometry in New Born Screening

Detlef Knoll
National Testing Centre
Short History of NBS  
History NBS and NZ  
Tandem Mass Spectrometry
Short History of NBS- IEM

Archibald Garrod (1857-1936)

• First to describe ‘Inborn Errors of Metabolism’
• Described Alcaptonuria in 1902
# Short History of NBS-IEM

<table>
<thead>
<tr>
<th>Authors</th>
<th>Garro et al</th>
<th>Stanbury et al</th>
<th>Scrive et al</th>
<th>Scrive et al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>1909</td>
<td>1983</td>
<td>1995</td>
<td>2001</td>
</tr>
<tr>
<td>Disorders</td>
<td>4</td>
<td>200</td>
<td>460</td>
<td>?</td>
</tr>
<tr>
<td>Chapters</td>
<td>6</td>
<td>91</td>
<td>154</td>
<td>255</td>
</tr>
<tr>
<td>Pages</td>
<td>168</td>
<td>2048</td>
<td>4605</td>
<td>6750</td>
</tr>
<tr>
<td>Weight of book</td>
<td>300 g</td>
<td>3060 g</td>
<td>11000 g</td>
<td>10 g</td>
</tr>
</tbody>
</table>
• Bob Guthrie (1916-1995) developed filter paper test for PKU (Identified newborns with PKU whose diet could be modified thus preventing mental retardation.)
Status of Newborn Screening Worldwide - 2003

- Full Population Program
- Little or No Screening (<40%)
- Developing Screening (40% or More)

Brad Therrell  National Newborn Screening and Genetics Resource Center  Austin, Texas
Phenylketonuria (PKU) 1:15 000 (1969)
Maple syrup urine disease (MSUD) > 1:100 000 (1969)
Galactosemia 1:40 000 (1970)
Congenital hypothyroidism (CH) 1:3500 (1978)
Cystic Fibrosis (CF) 1:2500 (1979)
Biotinidase deficiency > 1:100 000 (1983)
Congenital adrenal hyperplasia (CAH) 1:15 000 (1983)

1 Dec 2006... Tandem Mass Spectrometry added
EXPANDED SCREENING
Selected other Amino Acidopathies and Organic Acidaemias and Fatty acid oxidation defects
Previously - One test one result

Phe result for PKU..

TSH result for CH
TMS - One test MANY results

TANDEMMS

>25 Other disorders

TSH result for CH
Expanded Screening NZ

Amino Acid Breakdown Disorders
- Phenylketonuria (phenylalanine hydroxylase deficiency)
- Biopterin deficiencies (synthase and regeneration deficiencies)
- Maple Urine Disease
- Arginono succinic lyase deficiency
- Citrulinenia (Arginono succinic synthase deficiency, citrin deficiency)
- Glutaric Acidemia type I (glutaryl CoA Dehydrogenase deficiency)
- 3 Hydroxy-3-Methyl CoA lyase deficiency (HMGCoA def)
- Isovaleric Acidemia (Isovaleryl CoA dehydrogenase deficiency)
- Beta-ketothiolase deficiency
- 3-Methylcrotonyl CoA carboxylase deficiency
- Methylmalonic acidurias (Mutase deficiency, CblA, CblB, CblD defects)
- Propionic acidemia (propionyl CoA carboxylase deficiency)
- Tyrosinemia (fumaryl acetoase deficiency, tyrosine aminotransferase deficiency)

Fatty acid Oxidation Disorders
- CACT (carnitine acylcarnitine translocase deficiency)
- Carnitine transporter defect
- CPTI (Carnitine palmitoyltransferase -I deficiency)
- CPTII (Carnitine palmitoyltransferase -II deficiency)
- LCHAD (3-hydroxy long-chain acyl CoA dehydrogenase deficiency)
- TFP (trifunctional protein deficiency)
- MADD (multiple acyl CoA dehydrogenase deficiency)
- MCAD (medium-chain acyl CoA dehydrogenase deficiency)
- VLCAD (very-long-chain acyl CoA dehydrogenase deficiency)

http://www.moh.govt.nz
Method

- 1x 3.2 mm DBS per baby in 96 well plate
- 180 µl of working solution (MeOH + 22 Deut labelled Isotopes)
- Incubation & Extraction
- 25 µl of the extracted sample injected onto TMS
- Run time per sample 2.3 minutes
- Data extraction with Neolynx
- Calibration and Validation of controls with an Excell Interface.
- Data validated daily against In-house and CDC controls
- Data processed with In-House developed Database
How does it work

ES or APcl Probe

Sample Cone and Source Block at Cone Voltage

Ions

RF Lens

Extraction Cone and RF Lens at Lower Voltages and Lower Pressure

To Rough Pump

Analyzer Section (at Lowest Pressure (Best Vacuum))

Quads, Collision Cell and Detector
Analyzing by Parent Ions

MS1
Scanning

Collision Cell (w/Argon)
5-40 eV

MS2
Fixed

Find ions that will produce via CID, daughter ions with a particular m/z
Fragmentation of acylcarnitines in MS-MS as free acids

$$\text{CID}$$

- $$\text{N(CH}_3\text{)}_3$$
- $$\text{RCO}_2\text{H}$$

$$\text{m/z 85}$$
Consider a class of compounds that are similar in structure:

Different Compounds
That Are Somewhat
Similar In Structure

Different Neutral Fragments

Same Charged Fragment

Parent Ion Scans can be used to detect those compounds whose molecular ions produce the same charge fragment.
Analyzing by Neutral Loss

**MS1**
Scanning

**Collision Cell (w/Argon)**
-5V
5-40 eV
1V

**MS2**
Scanning

\[ m_1 - \text{offset} \]
\[ m_2 - \text{offset} \]

Q1 and Q2 scan together. m/z of Q2 is m/z of Q1 minus an offset.
Fragmentation of $\alpha$-amino acids in MS-MS (a) as free acids

$$\text{CID} \rightarrow -\text{HCOOH}$$

(neutral loss = 46)
Analyzing by Multiple Reaction Monitoring (MRM)

MS1
Fixed

Collision Cell (w/Argon)

MS2
Fixed

Precursor ion set

Fragmentation (CID)

Product ion set

Scans/ Time
Analyzing New Born Sample

Ave 14 Scans

Signal Intensity

20 µL/min
Allows for slow introduction of sample and collection of data over a long time

500 µL/min
Expels final sample

MRM for 46 analyte comb

2.2 minutes

200 µL/min

Ave 14 Scans

200 µL/min
Acyl Carnitine Profile

NORMAL

Internal standards Deuterium labelled
Isotopes marked by *

[Graph showing various peaks labeled C2, C3, C4, C4DC, C16, C18, and C18:1]
Note: C3 most reliable; C4DC is variable
<table>
<thead>
<tr>
<th>Primary Metabolite Monitored</th>
<th>Second Metabolite Monitored</th>
<th>POSSIBLE PRIMARY DISORDER</th>
<th>Seen also in</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine (↑)</td>
<td></td>
<td>1. Nonketotic hyperglycinemia</td>
<td>1. Organic Acidemias</td>
</tr>
<tr>
<td>Alanine (↑)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoleucine, Leucine &amp; allo-isoleucine</td>
<td>Valine (↑)</td>
<td>1. MSUD (Maple Syrup Urine disease)</td>
<td>1. Marker same MW as for Hydroxyprolinuria</td>
</tr>
<tr>
<td>Methionine (↑)</td>
<td></td>
<td>1. CBS (Cystathionine synthase deficiency) (homocystinuria)</td>
<td>1. Tyrosinemia type I 2. Methionine adenosyltransferase deficiency (Methionine (↑)) 3. Methylmalonic academia (cobalamin disorders) (Methionine N- (↓)) 4. GAMT</td>
</tr>
<tr>
<td>Phenylalanine (↑)</td>
<td>Tyrosine (↑)</td>
<td>1. PKU (Phenylketonuria) 2. Biopterin disorders</td>
<td></td>
</tr>
<tr>
<td>Tyrosine (↑)</td>
<td></td>
<td>1. Tyrosinemia type I 2. Tyrosinemia types II-III</td>
<td>1. Most liver disorders</td>
</tr>
<tr>
<td>Ornithine (↑)</td>
<td>Arginine (↓)</td>
<td>1. HHH syndrome</td>
<td>1. Creatine deficiency (Arginine (↓))</td>
</tr>
<tr>
<td>Citrulline (↑)</td>
<td></td>
<td>1. Citrullinemia (type I and II) 2. Argininosuccinic aciduria</td>
<td>1. Pyruvate carboxylase deficiency (type B)</td>
</tr>
<tr>
<td>Citrulline (↓)</td>
<td></td>
<td>1. CPS (Carbamyl phosphate synthase) 2. OTC (Ornithine transcarbamylase)</td>
<td>1. Lysinuric protein intolerance</td>
</tr>
<tr>
<td>Arginine (↑)</td>
<td></td>
<td>1. Arginase deficiency</td>
<td></td>
</tr>
</tbody>
</table>
# Fatty acid oxidation disorders and Organic Acidemias

<table>
<thead>
<tr>
<th>Primary Metabolite</th>
<th>Secondary Metabolite</th>
<th>POSSIBLE DISORDER</th>
</tr>
</thead>
</table>
| C0 (↓)             |                      | Fatty acid catabolism defects  
1. Carnitine plasma membrane transporter deficiency (uptake deficiency) |
| C0 (↑) (only with marked reduction of C16 and C18:1), otherwise could be normal | See C16 and C18:1 for CPT-I |
| C3 (↑)             | C4DC (↑ ?)           | Organic acids defects  
1. Propionic acidemia  
2. Methylmalonic acidemia/Cobalamine disorders  
3. Multiple Carboxylase deficiency (MCD) - this includes holocarboxylase deficiency |
| C3DC (↑)           |                      | Organic acids defects  
1. Malonic acidemia |
| C4 (↑)             | C5 (↑)               | Organic acids defects  
1. Isobuteryl CoA dehydrogenase deficiency  
Fatty acid catabolism defects  
1. Short chain acyl CoA Dehydrogenase deficiency (SCAD) |
| C4-OH (↑ ?) (Not yet tested for, to be considered) |                      | Organic acids defects  
1. SCHAD (marker more commonly seen in ketosis) |
| C4,C5 (↑)          |                      | Organic acids defects  
1. Multiple Acyl CoA Dehydrogenase deficiency (MADD)  
(Also called Glutaric acidemia Type II)  
2. Ethylmalonic encephalopathy |
| C5 (↑)             |                      | Organic acids defects  
1. Isovaleric acidemia  
2. 2-methylbutyryl CoA dehydrogenase deficiency (2MBCD) |
<table>
<thead>
<tr>
<th>Primary Metabolite</th>
<th>Secondary Metabolite</th>
<th>POSSIBLE DISORDER</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3 (↑)</td>
<td>C4DC (↑ ↑)</td>
<td>Organic acids defects</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1. Propionic acidemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Methylmalonic acidemia/Cobalamine disorders</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Multiple Carboxylase deficiency (MCD) - this includes holocarboxylase deficiency</td>
</tr>
</tbody>
</table>
Propionic acidemia – affects both isoleucine and valine

Isoleucine Metabolism

Valine Metabolism

Elevated C3 Marker on Tandem MS could.....
New Born Screening in NZ

Isoleucine Metabolism

L-Isoleucine

(s) 2-Oxo-3-methylvaleric acid

(s) -2-methylbutyryl-CoA

2-Methylbutyryl-CoA dehydrogenase

Tiglyl-CoA

(s)-2-Methyl-3-hydroxybutyryl-CoA

2-methylacetoacetate-CoA

Mitochondrial acetoacetyl-CoA thiolase

Acetyl-CoA

Propionyl-CoA

Methylmalonyl-CoA

2-Methylacetoacetic acid

Succinyl-CoA

Elevated Metabolites

2-Methylbutyric acid
2-Methylbutyrlglycine
2-Methylbutrylcarnitine

Tiglic acid
Tiglylglycine
2-Methylglutaconic acid
Tiglylcarnitine

2-Methyl-3-hydroxybutyric acid
2-Methyl-3-hydroxybutrylcarnitine

2-Methylacetoacetic acid
2-Butanone
Elevated C3 Marker on Tandem MS could.....

**Methylmalonic acidemia**

**Propionyl-CoA**

- Propionyl-CoA
- Propionyl-CoA metabolites
- Propionylcarnitine (C3)

**Succinyl-CoA**

**D-Methylmalonyl-CoA**

**L-Methylmalonyl-CoA**

**Cobalamin (B12)**

**Methylmalonyl-CoA Mutase**

**Adenosylcobalamin**

**Background**: Clinical Presentation and Major Features: Metabolic crisis, lethargy, failure to thrive, dehydration, respiratory distress, hypotonia

**Treatment**: Low protein diet, L-Carnitine, cobalamin (Vit B12), avoidance of fasting
<table>
<thead>
<tr>
<th>Compound</th>
<th>Description</th>
<th>Abnormalities/Defects</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5-OH (↑)</td>
<td>C5:1 (seen in SKAT and 3-MCC) C6-DC (Not yet tested for, seen in HMGL) No diagnostic significance to isolated mild elevations to C5:1 and C6-DC</td>
<td>Organic acids defects 1. 3-methylcrotonyl CoA carboxylase deficiency (3-MCC) 2. β-ketothiolase deficiency (SKAT) 3. 3-methyl-3-OH-glutaryl CoA lyase deficiency (HMGL) 4. 3-methyl-glutaconyl-CoA hydratase deficiency (Glutaconic aciduria ?) 5. 2-Methyl-3-Hydroxybutyryl-CoA Dehydrogenase 6. Biotidase deficiency (Duran) and holo-carboxylase deficiency</td>
</tr>
<tr>
<td>C5:1 (↑)</td>
<td>C5:0H (↑) No diagnostic significance to isolated mild elevations to C5:1</td>
<td>Organic acids defects 1. β-ketothiolase deficiency (SKAT) 2. 3-methylcrotonyl CoA carboxylase deficiency (3-MCC)</td>
</tr>
<tr>
<td>C5DC (↑)</td>
<td></td>
<td>Organic acids defects 1. Glutaric acidemia type I</td>
</tr>
<tr>
<td>C8 (↑)</td>
<td>C10 (↑) (MCAD) C10:1 (↑) (MCAD) C8:10 ratio (↑) (&gt;3:1)</td>
<td>Fatty acid catabolism defects 1. Medium chain acyl CoA dehydrogenase deficiency (MCAD)</td>
</tr>
<tr>
<td>C14:1 (↑)</td>
<td>C14:1/C12:1 ratio (↑) (&gt;3:1) (C12:1 marker not yet added)</td>
<td>Fatty acid catabolism defects 1. (Very) long chain acyl CoA dehydrogenase deficiency (VLCAD or LCAD)</td>
</tr>
<tr>
<td>C16-OH (↑)</td>
<td>C18:OH (↑) (mild) (marker not added yet, mostly seen only with marked ↑ C16-OH)</td>
<td>Fatty acid catabolism defects 1. Long chain 3-OH acyl CoA dehydrogenase deficiency (LCHAD) 2. Trifunctional protein deficiency (TFP)</td>
</tr>
<tr>
<td>C16 &amp; C18:1 (↑) (C18:1 not added yet)</td>
<td></td>
<td>Fatty acid catabolism defects 1. Carnitine palmitoyl transferase –II (CPT-II) 2. Carnitine acylcarnitine translocase deficiency (CAT or also called CACT)</td>
</tr>
<tr>
<td>C16 &amp; C18:1 (↓)</td>
<td>C0 (↑) (only with marked reduction of C16 and C18:1), otherwise could be normal</td>
<td>Fatty acid catabolism defects 1. Carnitine palmitoyl transferase –I (CPT-I)</td>
</tr>
</tbody>
</table>
To complicate matters……..

Note: There is no diagnostic significance to isolated mild elevations of C6, C10, C12:1, C12, C14, C18:1 or C18:2
German Program (Interlab QA for NBS)
• Bi monthly Proficiency Program for Amino Acids

CDC Newborn Screening Quality Assurance Program
• Bi annual QC for 6 Amino Acids and 12 Acyl Carnitines
• Quarterly Proficiency Program for AA and AC
QA Programs continued

Region 4 Genetics Collaborative MSMS Project

Projects
• Implement universal screening and confirmatory testing of newborns for inborn errors of amino acid, organic and fatty acids oxidation metabolism
• Reduce inequities in access to genetic services

US participants = 41
International participants = 46

http://www.region4genetics.org
Region 4 Genetics Collaborative MSMS Project

Objectives
• Achieve uniformity of testing panel by MS/MS to maximize the detection of affected newborns
• Improve analytical performance
• Set and sustain lowest achievable rates of false results

US participants = 41
International participants = 46

http://www.region4genetics.org
QA Programs continued

Region 4 Genetics Collaborative MSMS Project

http://www.region4genetics.org
Analytical Performance

Sensitivity - Good

Linearity - Good

Assay Imprecision
(for Phe, 160, 334, 600 µmol/L)

<table>
<thead>
<tr>
<th>%CV</th>
<th>L</th>
<th>M</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within run</td>
<td>8.3</td>
<td>7.9</td>
<td>9.9</td>
</tr>
<tr>
<td>Between day</td>
<td>5.8</td>
<td>4.5</td>
<td>6.6</td>
</tr>
<tr>
<td>Between run</td>
<td>5.6</td>
<td>6.5</td>
<td>7.7</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>11.9</td>
<td>12.5</td>
</tr>
</tbody>
</table>
Expanded Screening NZ

2 ½ years MS/MS – Approx 150 000 births

Amino Acid Breakdown Disorders
- 7 PKU (+4 Hyperphe)
- 1 Biopterin Biosynthesis Def
- 1 Tyrosinemia type II
- 2 Glutaric Acidemia type I
- 2 3-Methyl Crotonyl CoA Carboxylase Def
- 1 Isovaleric Acidemia
- 3 Citrulinemia

Fatty acid Oxidation Disorders
- 10 Medium Chain Acyl CoA Dehydrogenase Def
- 1 Very Long Chain Acyl CoA Dehydrogenase Def
- 2 Multiple Acyl CoA dehydrogenase Def
- 1 Carnitine Acyl carnitine Translocase Def

Approximate Incidence by MS/MS alone: 1:4300

False Positives 0.35 %
New Tests available on Tandem MS

Secondary Trier Testing
Succinyl Acetone
Methyl Malonic acid

New Tests available on Tandem MS… - further markers can improve specificity of screening
Sugars eg for galactosemia
Steroids eg for congenital adrenal hyperplasia
Bile acids eg for biliary atresia
“Our metabolism is a chemical individuality”
“Our chemical individualities are due to our chemical merits as well as our chemical shortcomings”
“are inherited in our very chemical structure”
“our individualities went to the making of the chromosome from which we sprang”
END
Case: Born 1982 – Analysed Nov 08

<table>
<thead>
<tr>
<th></th>
<th>Free Carnitine (C0)</th>
<th>C16</th>
<th>C18</th>
<th>C0/(C16 +C18)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PASIENT</strong> (<strong>DOS 1982)</strong></td>
<td>209</td>
<td>0.06</td>
<td>0.08</td>
<td>1267</td>
</tr>
<tr>
<td><strong>CONTROLS</strong> (<strong>N=20</strong> (range))</td>
<td>92 (48-170)</td>
<td>0.34 (0.17-1.09)</td>
<td>0.18 (0.06-0.54)</td>
<td>236 (64-315)</td>
</tr>
<tr>
<td>Median (<strong>Fresh DBS 2-10 d old</strong>)</td>
<td>31</td>
<td>3.6</td>
<td>0.86</td>
<td></td>
</tr>
</tbody>
</table>
EXTRA SLIDES
Acyl Carnitine Profile C5

NORMAL

IVA or 2MBCD

1: MRM of 32 Channels ES+
403.30 49070
4.91e4

00:17:3518-Jun-2009
1: MRM of 32 Channels ES+
403.30 46743
4.67e4
How does it work

- Ionisation of sample by electron spray
- Select single mass and charge
- Fragmentation all compounds with this m/z
- Measure resulting fragments mass and charge
Analyzing by Daughter Ions

Determines Collision Induced Dissociation (CID) produced daughter ions of a particular parent ion
QA Programs continued