LOW CITRULLINE AS A MARKER FOR THE PROXIMAL UREA CYCLE DEFECTS
EXPERIENCE OF THE NEW ENGLAND NEWBORN SCREENING PROGRAM

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Urea Cycle Disorders

Nitrogen Pool

Glutamate → N-acetyl glutamate (NAGS) → N-acetyl glutamate

Ammonia → Carbamoyl phosphate synthetase (CPS) → Carbamoyl phosphate

Carbamoyl phosphate transporter (Citrin) → Aspartate-glutamate carrier (Citrin) → Aspartate

Aspartate → Argininosuccinic synthetase (ASS) → Argininosuccinate

Argininosuccinate lyase (ASL) → Arginine

Arginine → Arginase (ARG) → Urea

Fumarate
Proximal Urea Cycle Disorders

- CPS (*Carbamoylphosphate synthetase*)
- OTC (*Ornithine transcarbamylase*)
- NAGS (*N-acetylglutamate synthase*)

- Acute neonatal presentation in 2/3
- Milder phenotypes (Late Onset/Intermittent forms): Progressive neurological disorder. Episodic headaches, ataxia, scotomas, sleep disturbances, behavioral abnormalities, ADD, psychosis.

- OTC is X-linked (~15% females affected)
- CPS and NAGS autosomal recessive
NENSP screening for amino acids & acylcarnitines using MS/MS since Feb 1999.

Targeted analysis with MRM.

Amino acids targeted:
Arginine, Argininosuccinic acid, Citrulline, Leucine, Methionine, Ornithine, Phenylalanine, Tyrosine, Valine

Low Citrulline cut-off introduced in 2004
Based on analysis of approximately 250,000 randomly selected newborn screening technically satisfactory specimens collected within 24 hrs to 7 days of birth during Aug 2004 to Aug 2013. Although distribution shown is log transformed, values on x-axis reconverted to actual concentrations and ratios. Mean values shown in blue; others shown are 2, 3 and 4 SD from the mean.
Positive Screens for Proximal UCDs

- Citrulline $\leq$ 3uM

OR

- Citrulline > 3uM to $\leq$ 3.8 &

  $\frac{\text{Citrulline}}{[\text{Tyrosine} \times \text{Methionine}]} \leq 0.002.$
Positive Screen: Categories

- **High Risk**
  - Cit ≤ 3.8 uM &
  - Citrulline/[Tyrosine x Methionine] < 0.002 &
  - Ornithine / Citrulline > 15

- **Moderate Risk**
  - Cit ≤ 3.8 uM & Citrulline/[Tyrosine x Methionine] ≤ 0.002
  - OR
  - Cit ≤ 3 uM & Ornithine / Citrulline > 15

- **Low Risk**
  - Cit ≤ 3uM & both ratios in range
1.2 Million
Screened Aug 2004 - Aug 2013
Technically satisfactory
Collected 24 hrs-7 DOL

Positive Screens (Infants) : 25

20
Initial Specimen

5
Repeat Specimen
(Initial Normal)
<table>
<thead>
<tr>
<th>Disorder</th>
<th>Birth Weight</th>
<th>NICU Status</th>
<th>Initial Screen</th>
<th>Neonatal Clinical Status</th>
<th>Follow-up</th>
<th>Repeat Screen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Age (in days)</td>
<td>Citrulline (in uM)</td>
<td>Risk Category</td>
<td>Age</td>
</tr>
<tr>
<td>OTC 1</td>
<td>3236</td>
<td>NO</td>
<td>2</td>
<td>2.82</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>OTC 2</td>
<td>3033</td>
<td>NO</td>
<td>1</td>
<td>3.32</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>OTC 3</td>
<td>2440</td>
<td>YES</td>
<td>4</td>
<td>1.79</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>OTC 4</td>
<td>4220</td>
<td>NO</td>
<td>2</td>
<td>2.8</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>OTC 5</td>
<td>4875</td>
<td>YES</td>
<td>7</td>
<td>1.13</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>CPS 1</td>
<td>4005</td>
<td>YES</td>
<td>2</td>
<td>2.45</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>CPS 2</td>
<td>3657</td>
<td>NO</td>
<td>1</td>
<td>2.09</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>NAGS1</td>
<td>3930</td>
<td>NO</td>
<td>2</td>
<td>2.68</td>
<td>High</td>
<td></td>
</tr>
</tbody>
</table>
## Initial Specimens: False Positives

<table>
<thead>
<tr>
<th>False Positives</th>
<th>Birth Weight</th>
<th>NICU Status</th>
<th>Initial Screen</th>
<th>Neonatal Clinical Status</th>
<th>Follow-up</th>
<th>Repeat Screen</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP 1</td>
<td>2680</td>
<td>YES</td>
<td>3 2.89 High</td>
<td>Pneumothorax, Respiratory distress, Sepsis on DOL 1 Amino acids, NH3 Normal. CPS &amp; OTC molecular analysis negative</td>
<td></td>
<td>8 7 Normal</td>
</tr>
<tr>
<td>FP 2</td>
<td>2521</td>
<td>NO</td>
<td>2 2.77 Moderate</td>
<td>Asymptomatic.</td>
<td>Plasma citrulline persistently low.</td>
<td>8 4.67 Normal</td>
</tr>
<tr>
<td>FP 3</td>
<td>3028</td>
<td>NO</td>
<td>2 2.52 Moderate</td>
<td>Asymptomatic</td>
<td>Amino acids, NH3 Normal.</td>
<td>8 3.11 Low</td>
</tr>
<tr>
<td>FP 4</td>
<td>3235</td>
<td>NO</td>
<td>3 2.69 Moderate</td>
<td>Asymptomatic</td>
<td>Plasma citrulline low. NH3 normal Developmental delays, hypotonia, hypospadias, pulmonary stenosis.</td>
<td>... ... ...</td>
</tr>
<tr>
<td>FP 5</td>
<td>1838</td>
<td>YES</td>
<td>5 2.31 Moderate</td>
<td>Ex 32 weeker. Poor feeding</td>
<td>…</td>
<td>8 7.94 Normal</td>
</tr>
<tr>
<td>FP 6</td>
<td>2072</td>
<td>YES</td>
<td>6 1.95 Moderate</td>
<td>Ex 33 weeker. Poor feeding Bowel resection.</td>
<td>Amino acids, NH3 Normal. Immunodeficiency</td>
<td>10 6.7 Normal</td>
</tr>
<tr>
<td>FP 7</td>
<td>2510</td>
<td>YES</td>
<td>1 1.86 Moderate</td>
<td>Respiratory Distress. Hyperbilirubinemia on DOL 1 Amino acids, NH3 Normal.</td>
<td>…</td>
<td>5 7 Normal</td>
</tr>
<tr>
<td>FP 8</td>
<td>2704</td>
<td>YES</td>
<td>3 2.92 Moderate</td>
<td>Ex 35 weeker. Poor feeding Infant of diabetic mother.</td>
<td>Transient hyperammonemia. Persistent orotic aciduria. OTC molecular analysis negative</td>
<td>... ... ...</td>
</tr>
<tr>
<td>FP 9</td>
<td>3365</td>
<td>YES</td>
<td>2 2.24 Moderate</td>
<td>Ex 35 weeker. Poor feeding.</td>
<td>Aysmptomatic at 6 months of age. Moved out of state.</td>
<td>7 4.44 Normal</td>
</tr>
<tr>
<td>FP 10</td>
<td>1870</td>
<td>YES</td>
<td>3 3.13 Low</td>
<td>Ex 32 weeker.</td>
<td>…</td>
<td>8 4.36 Normal</td>
</tr>
<tr>
<td>FP 11</td>
<td>2530</td>
<td>YES</td>
<td>1 3.32 Low</td>
<td>Ex 35 weeker. Poor feeding. Hyperbilirubinemia &amp; bloody stools.</td>
<td>…</td>
<td>5 6.23 Normal</td>
</tr>
<tr>
<td>FP 12</td>
<td>2596</td>
<td>YES</td>
<td>3 3.22 Low</td>
<td>Ex 36 weeker. Poor feeding ...</td>
<td>…</td>
<td>5 7.46 Normal</td>
</tr>
</tbody>
</table>
Repeat Specimens

- All 5 false positives
- All infants with a h/o bowel resection.
- On TPN
<table>
<thead>
<tr>
<th>OTC Male</th>
<th>Birth Weight</th>
<th>NICU Status</th>
<th>Age (in days)</th>
<th>Cit (in uM)</th>
<th>Ratios</th>
<th>Neonatal Clinical Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>2125</td>
<td>YES</td>
<td>2</td>
<td>5.02</td>
<td>C/TM</td>
<td>Presented in ER after a fall at 11 months of age. H/O mild developmental delays reported. Evaluated, observed and discharged. Returned with change in mental status a week later, with rapid decline into status epilepticus and encephalopathy from which infant could not be revived. Laboratory work-up revealed hyperammonemia, low plasma citrulline and high concentrations of urinary orotic acid. Molecular analysis revealed a novel mutation.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>OTC Female</th>
<th>Birth Weight</th>
<th>NICU Status</th>
<th>Age (in days)</th>
<th>Cit (in uM)</th>
<th>Ratios</th>
<th>Neonatal Clinical Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>3380</td>
<td>NO</td>
<td>2</td>
<td>13.96</td>
<td>Normal</td>
<td>Infant born before Aug 2004. Presented with a h/o developmental delay and hemiperesis at 9 months. Diganostic work-up revealed low plasma citrulline and high concentrations of urinary orotic acid. Retrospective review of newborn screening results performed. A 2nd screen collected at 6 months of age with citrulline value of 15.33 μM.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Carrier OTC Female</th>
<th>Birth Weight</th>
<th>NICU Status</th>
<th>Age (in days)</th>
<th>Cit (in uM)</th>
<th>Ratios</th>
<th>Neonatal Clinical Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrier OTC Female</td>
<td>2805</td>
<td>YES</td>
<td>1</td>
<td>4.86</td>
<td>Normal</td>
<td>Female sibling of OTC Case 2. Citrulline concentration in repeat (DOL 5 days) 6.67 μM. NH3 &amp; Orotic acid normal. Plasma citrulline values in low normal range. Asymptomatic at 2 yrs.</td>
</tr>
</tbody>
</table>
Urea Cycle and Interrelated Pathways

- N acetyl glutamate
  - NAGS
- Acetyl-CoA
- Glutamine
- NH3
- α-keto glutarate
- HCO3-
- Carbamoyl phosphate
  - CPS
- Glutamate
  - NAGS
  - NH3
- Ornithine
  - OTC
- Citrulline
  - P5CD
- Glu-γ-semialdehyde
- Pyrroline-5-carboxylate
  - P5CS
- Proline

Mitochondria

Cytoplasm
## Additional Disorders

<table>
<thead>
<tr>
<th>Disorder</th>
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<th>Initial Screen</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Pyrroline-5-Carboxylate Synthase Deficiency</td>
<td>2268</td>
<td>NO</td>
<td>2, 4.2 (-3.2 SD) Normal</td>
<td>Hypotonia and &quot;progeroid&quot; features reported at birth. Failure to thrive and developmental delays at 3 months. Plasma amino acids revealed low citrulline and proline. Diagnosis confirmed by sequencing of P5C Synthase deficiency.</td>
</tr>
<tr>
<td>Hyperammonemia-Hyperornithinemia-Homocitrullinuria</td>
<td>3150</td>
<td>NO</td>
<td>3, 7.7 (-1.1 SD) O/C</td>
<td>Presented with gross motor delays and spasticity in lower extremeties at 18 months of age. Chronic mild hyperammonemia identified.</td>
</tr>
</tbody>
</table>
Conclusions

- Low Citrulline can identify infants with severe proximal urea cycle defects.
- However these infants presented early in the neonatal period for NBS to prevent mortality in majority.
- Late onset forms were missed.
- Adjustments in the cut-off values of citrulline may allow detection of some late onset cases of the proximal UCD and other metabolic disorders such as pyrroline-5-carboxylate synthase.