Newborn Screening for Brain Creatine Deficiency Syndromes

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Creatine is a nitrogen-containing organic chemical that helps to supply energy to muscle and nerve cells. It was identified in 1832 as a component of skeletal muscle and named creatine after the Greek word for flesh, \textit{Kreas}. 

\textbf{CREATINE}  
\[ \text{H}_2\text{N} \rightleftarrows \text{N} \rightleftarrows \text{N} \rightleftarrows \text{N} \rightleftarrows \text{CH}_3 \text{O} \rightleftarrows \text{O} \rightleftarrows \text{NH}_2^+ \]  
\text{2-(carbamimidoyl-methyl- amino) acetic acid}
Creatine functions in energy homeostasis in the cell

Creatine serves as an energy shuttle between the mitochondrial sites of ATP production and the cytosolic sites of ATP utilization, regenerating ATP from ADP.

CK = creatine kinase

(Brosnan and Brosnan 2007)
Creatine can be taken from the diet (about 50%) or synthesized by the body using two enzymes: AGAT (L-arginine:glycine amidinotransferase) and GAMT (Guanidinoacetate methyltransferase).

Specific transporters allow creatine to reach all organs including muscle and brain.
L-Arginine: Glycine Amidino Transferase (AGAT)

- Arginine
- Glycine
- Ornithine
- Guanidinoacetate

Guanidino Acetate Methyl Transferase (GAMT)

- S-Adenosyl-L-Methionine
- S-Adenosyl-L-Homocysteine

Creatine Synthesis

Plasma Membrane

CT1 Creatine Transporter (SLC6A8 gene)

Creatine → Creatinine

BRAIN CREATINE DEFICIENCY SYNDROMES

- Defects in creatine synthesis (AGAT or GAMT deficiency) or transport (CT1 deficiency) result in brain creatine deficiency and neurological symptoms.
- Characterized by mental retardation, hypotonia, seizures, autistic features and disturbance of cognitive and expressive speech. Can also present as moderate mental retardation, attention deficit, hyperactivity and semantic-pragmatic language disorder.

Disorders were initially diagnosed for the lack of the creatine peak in MR spectroscopy.
MR Spectroscopy can measure chemicals *in vivo* based on the resonance of paramagnetic nuclei.

Safe, non-invasive technique to study metabolites. Atomic nuclei spin and if they have an uneven number of charges will generate a magnetic moment, with the ability to resonate. For biomedical purposes the most important nuclei are \( ^1\text{H} \), \(^{31}\text{P} \), and \(^{13}\text{C} \).

Metabolites have different peaks in the spectrum which appear at known frequency.
2. Plasma and urine guanidinoacetate and creatine

<table>
<thead>
<tr>
<th></th>
<th>CREATINE</th>
<th>GUANIDINOACETATE</th>
<th>CREAT/CREATININE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGAT</td>
<td>Low</td>
<td>Low</td>
<td>NA</td>
</tr>
<tr>
<td>GAMT</td>
<td>Low</td>
<td>High</td>
<td>NA</td>
</tr>
<tr>
<td>Transporter</td>
<td>Normal</td>
<td>Normal</td>
<td>High</td>
</tr>
</tbody>
</table>

3. Confirmation by enzyme/transporter assay and/or DNA testing.

**DIAGNOSIS: CREATINE DEFICIENCY SYNDROMES**
Biochemical predictors of long-term outcome in patients with Guanidinoacetate Methyltransferase (GAMT) Deficiency

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GAMT DEFICIENCY

• Patients present from a few months of age to 4–5 years of age with developmental delays, seizures (in many cases resistant to therapy), hypotonia (in some cases very severe), autistic behavior, and occasional movement disorder with involuntary movements.

• Frequency: unknown, in Utah about 1:120,000 births
<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Age at Dx (mo)</th>
<th>Genotype</th>
<th>Diagnostic creatine, plasma</th>
<th>Diagnostic GAA, plasma</th>
<th>Diagnostic creatine, urine</th>
<th>Diagnostic GAA, urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.3 y</td>
<td>F</td>
<td>13</td>
<td>c.327G&gt;A/p.K109K; c.403G&gt;A/p.D135N</td>
<td>2.0</td>
<td>13.8</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9.3 y</td>
<td>F</td>
<td>65</td>
<td>*c.233T&gt;A/p.V78E; c.299_c.311dup13/p.R105Gfs X26</td>
<td>4.7</td>
<td>9.6</td>
<td>39</td>
<td>944</td>
</tr>
<tr>
<td>7.3 y</td>
<td>M</td>
<td>15</td>
<td>c.327G&gt;A/p.K109K; c.522G&gt;A/p.W174X</td>
<td>2.1</td>
<td>21.3</td>
<td>5.2</td>
<td>589</td>
</tr>
<tr>
<td>3.2 y</td>
<td>M</td>
<td>10</td>
<td>ND</td>
<td>0.4</td>
<td>15.1</td>
<td>14</td>
<td>4868</td>
</tr>
<tr>
<td>1 y</td>
<td>M</td>
<td>0</td>
<td>*c.233T&gt;A/p.V78E; c.299_c.311dup13/p.R105Gfs X26</td>
<td>19.1</td>
<td>14.2</td>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>NORMAL VALUES</strong></td>
<td><strong>37-117 mM</strong></td>
<td><strong>0.5-1.8 mM</strong></td>
<td><strong>23-1500 mmol/mol creatinin</strong></td>
<td><strong>45-250 mmol/mol creatinin</strong></td>
</tr>
</tbody>
</table>

**Table 1. Patients with guanidinoacetate methyltransferase deficiency**

GAA: guanidinoacetate; ND: not done

*Novel mutation
GAMT deficiency treatment

- Treatment: Goal:
- Restore creatine, reduce guanidinoacetate (GAA)
AGAT/GAMT deficiency treatment

• Creatine (300 to 1000 mg/kg/day) initiated preferably early in life in AGAT and GAMT deficiency.

• In GAMT deficiency, GAA levels can be reduced by ornithine supplementation (400-800 mg/kg/day) and Benzoate (50-135 mg/kg/day) to reduce glycine levels and GAA synthesis.

L-Arginine: Glycine Amidino Transferase

AGAT

Arginine
Glycine
Ornithine
Guanidinoacetate
Plasma glycine and ornithine significantly correlate with plasma GAA levels.
OUTCOME

• Patients with AGAT or GAMT deficiency respond to treatment with improvement of delays and seizures. Mental retardation is NOT reversed.

• Treatment at birth prevents mental retardation in children identified early because of family history (or newborn screening).
Marzia Pasquali PhD
University of Utah/ARUP Laboratories

Feasibility of Newborn Screening for Guanidinoacetate Methyltransferase (GAMT) Deficiency

Thursday, May 9
4:00 pm – 5:30 pm
Session 12 – Candidate Conditions

2013 Joint Meeting of the Newborn Screening and Genetic Testing Symposium and International Society for Neonatal Screening
May 5–10, 2013 | Atlanta Marriott Marquis | Atlanta, GA
Evaluation of 10,000 dry blood spots – ARUP Lab

- 10,000 DBS, half collected < 7 days of age and half collected > 7 days of age, were de-identified and sent to ARUP.

- NBS was performed according to the routine method, the only difference was the addition of GAA and creatine to the analytes recovered and to the Internal Standards.
NEWBORN SCREENING FOR GAMT DEFICIENCY

Screening

Second tier Testing: UPLC

Normal

Abnormal

Normal

Abnormal
Guanidinoacetate (GAA) levels

<table>
<thead>
<tr>
<th>GAA (first screen results)</th>
<th>Average (µmol/L)</th>
<th>Std Dev</th>
<th>99% (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP (NBS)</td>
<td>1.25</td>
<td>0.41</td>
<td>2.20</td>
</tr>
<tr>
<td>NP (2\textsuperscript{nd} tier test)</td>
<td>1.42</td>
<td>0.54</td>
<td>3.08</td>
</tr>
<tr>
<td>GAMT Deficiency</td>
<td>32.8</td>
<td>0.54</td>
<td>N/A</td>
</tr>
</tbody>
</table>

NP= Normal Population

• True positives identified on first and second screening (n=2)

• False positive rate was 0\% with second tier testing (n=10,000)
Conclusions

- Defects in creatine metabolism and transport result in mental retardation, hypotonia, developmental delays, and autism.
- Early therapy (creatine supplements, ornithine, benzoate) can prevent seizures and mental retardation.
- There is an adequate screening test and second tier testing to avoid false-positive results that can be added to current MS/MS.