Feasibility of Newborn Screening for Guanidinoacetate Methyltransferase (GAMT) deficiency

Marzia Pasquali, PhD, FACMG
Professor of Pathology and Pediatrics
Medical Director, Biochemical Genetics and Newborn Screening
University of Utah and ARUP Laboratories

Atlanta, 9 May 2013
Creatine functions in energy homeostasis in the cell

• Creatine is a nitrogen-containing compound that serves as an energy shuttle between the mitochondrial sites of ATP production and the cytosolic sites of ATP utilization, regenerating ATP from ADP.

(Brosnan and Brosnan 2007)
Creatine is obtained 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.
Guanidinoacetate

L-Arginine: Glycine Amidino Transferase
AGAT

S-Adenosyl-L-Methionine

S-Adenosyl-L-Homocysteine

Guanidinoacetate Methyl Transferase
GAMT

Creatine

Plasma Membrane
CT1 Creatine Transporter (SLC6A8 gene)

Creatinine

CREATINE SYNTHESIS

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.
Creatine Deficiency Syndromes: Diagnosis

- MR spectroscopy: lack of the creatine peak
Creatine Deficiency Syndromes: Diagnosis

- Plasma and urine guanidinoacetate (GAA) and creatine:

<table>
<thead>
<tr>
<th></th>
<th>P Creatine</th>
<th>P GAA</th>
<th>U 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>

- Confirmation by DNA testing and/or enzyme/transporter assay
Creatine deficiency syndromes treatment

• Goal of treatment:
  – Restore creatine (AGAT) and reduce guanidinoacetate (GAMT)

BRAIN MR SPECTROSCOPY
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).
Evaluation of 10,000 dried blood spots – ARUP Lab

- 10,000 de-identified DBS were analyzed using our routine NBS method, with $d_3$-creatine and $d_2$-GAA added in the Internal Standards mixture. Creatine and GAA were measured using SRM.

- Results above an established cut-off for GAA (> 99.5%) were followed up with 2nd tier test using UPLC-MS/MS.

- Aims:
  - evaluate feasibility of screening for creatine deficiency syndromes (especially GAMT deficiency)
  - evaluate false positive rate
  - evaluate effectiveness of second tier testing
Samples Information

- 9,288 viable DBS
  - < 7 days: n=4,691
    - 5.4% collected at <1 day
    - 88.7 % collected at 1-2 days
    - 5.9 % collected at ≥ 3 days
  - > 7 days: n=4,597
    - 47.6 % collected at 8-14 days
    - 44.8 % collected at 15-21 days
    - 7.6 % collected at > 21 days

- 7 blood spots from 3 patients with GAMT deficiency
  - collected at 1 – 21 days
# GAMT screening: Summary

<table>
<thead>
<tr>
<th>GAA (first screen results)</th>
<th>Average (µmol/L)</th>
<th>Std Dev</th>
<th>99.9% (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP (NBS)</td>
<td>1.21</td>
<td>0.34</td>
<td>3.55</td>
</tr>
<tr>
<td>GAMT (NBS)</td>
<td>15.0</td>
<td>7.4</td>
<td>NA Min = 6.0</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 (2\textsuperscript{nd} tier test)</td>
<td>11.0</td>
<td>5.5</td>
<td>NA Min = 4.9</td>
</tr>
</tbody>
</table>
Creatine and Guanidinoacetate in dried blood spots

Patients with GAMT deficiency had markedly elevated guanidinoacetate even in the newborn period. Creatine levels physiologically decrease after birth even in normal controls. They were normal at birth in patients with GAMT deficiency, but decreased with time below normal.
Creatine and Guanidinoacetate in dried blood spots: Specificity

60 samples (0.64%) had elevated GAA levels with the routine screening. 53 resolved with the 2\textsuperscript{nd} tier test, 7 were confirmed with GAMT deficiency. No clear predictor for false positivity was identified.
Guanidinoacetate/Creatine ratio increases specificity

GAA/Creatine

GAMT patients
False positives

Age, days
Second tier test for GAA and creatine

- Creatine and GAA were extracted from DBS (4.7 mm punches) using methanol containing deuterated internal standards.
- The extract was dried, derivatized using 3N HCl in butanol, dried, and reconstituted with water/acetonitrile.
- The analysis was performed using a XEVO-TQ UPLC-MS/MS system with a BEH C18 column for the chromatographic separation.
Second tier testing for GAA

• Positive screen results (> 2.44 μmol/L) = 60
• Total number of 2nd tier tests = 60
• Positives after 2nd tier test = 7 samples (three patients with GAMT deficiency, 1st and 2nd screens)

• No false positives were identified after the second tier test.
Second tier testing for GAA differentiates true from false positives
Second tier testing for creatine cannot differentiate affected patients from normal controls.
Conclusions

- NBS for GAMT deficiency is feasible
- False positive rate can be reduced to virtually 0% with a second tier test
- The test is fully integrated with the routine screening
- The cost of screening for GAMT deficiency is very low
Acknowledgements

University of Utah

• ARUP Laboratories and Department of Pathology
  – Elisabeth Schwarz
  – Maren Jensen
  – Tatiana Yuzyuk PhD
  – Irene De Biase PhD

• Department of Pediatrics-Biochemical Genetics
  – Nicola Longo MD PhD

Utah Department of Health

• Newborn Screening lab
  – Norm Brown

• Follow-up program
  – Harper Randall MD
  – Kim Hart