Newborn Screening for Guanidinoacetate Methyltransferase (GAMT) deficiency

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GAMT Deficiency

**Cause:** recessive deficiency of guanidinoacetate methyltransferase impairs brain creatine synthesis. Accumulation of guanidinoacetate is toxic for the brain.

**Incidence:** 1:120,000 (Utah)

**Presentation:** Developmental delays, hypotonia, seizures, autistic-like behavior.

**Diagnosis:** Lack of creatine peak in MR spectroscopy, plasma and urine creatine panel: increased plasma guanidinoacetate, decreased creatine.

**Therapy:** Creatine (300 to 1,000 mg/kg/day) initiated preferably early in life in AGAT and GAMT deficiency. GAA levels can be reduced by ornithine supplementation (400-800 mg/kg/day) and Benzoate (50-250 mg/kg/day) to reduce glycine levels and GAA synthesis.

**Outcome:** Good if therapy is initiated before brain damage, newborn screening in development.
Guanidinoacetate

Arginine

Glycine

L-Arginine: Glycine Amidino Transferase
AGAT

Ornithine

Guanidinoacetate

Guanidino Acetate Methyl Transferase
GAMT

S-Adenosyl-L-Methionine

S-Adenosyl-L-Homocysteine

Creatine Synthesis

Creatine

Plasma Membrane

CT1 Creatine Transporter (SLC6A8 gene)

Creatinine

GAMT deficiency treatment

- Treatment Goal:
- Restore creatine, reduce guanidinoacetate (GAA)

BRAIN MR SPECTROSCOPY

GAMT deficiency treatment

- Creatine (300 to 1000 mg/kg/day) initiated preferably early in life
- In GAMT deficiency, GAA levels can be reduced by ornithine supplementation (400-800 mg/kg/day) and Benzoate (50-250 mg/kg/day) to reduce glycine levels and GAA synthesis.

![Metabolic pathway diagram](image)
Outcome

• Patients with 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).

Feasibility of GAMT newborn screening

• 10,000 de-identified DBS were analyzed using our routine NBS method, with d₃-creatine and d₂-GAA added in the Internal Standards mixture. Creatine and GAA were measured using SRM.

• Abnormal results (elevated GAA, > 99.5%) were followed up with 2nd tier test using LC-MS/MS.

• Aims:
  – evaluate feasibility of screening for creatine deficiency syndromes (especially GAMT deficiency)
  – evaluate false positive rate
  – evaluate effectiveness of second tier testing
Newborn Screening for GAMT Deficiency

Screening

- Normal
- Abnormal

Elevated GAA

Second tier Testing by UPLC-MS/MS

- Normal
- Abnormal

Confirmatory tests
Summary of data

- 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
Creatine and Guanidinoacetate in dried blood spots

The ratio GAA/Creatine increases the specificity

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 2\textsuperscript{nd} tier tests = 60
- Positives after 2\textsuperscript{nd} tier test = 7 samples (three patients with GAMT deficiency, 1\textsuperscript{st} and 2\textsuperscript{nd} screens)

- No false positives were identified after the second tier test.
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 (2nd 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=3)
- False positive rate was 0% with second tier testing (n=10,000)
Summary

• NBS for GAMT deficiency is feasible

• False positive rate can be reduced to virtually 0% with a second tier test

• Utah will start screening for GAMT deficiency probably by the Summer.