A 3-year pilot study for Guanidinoacetate Methyltransferase (GAMT) deficiency in British Columbia

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Conflicts of interest

• None
Cerebral Creatine Deficiency

- Creatine from systemic biosynthesis and diet
- Creatine-P is a key energy source
  - Local immediate energy reserve for ADP/ATP recycling
- Little creatine synthesis in the brain
- Cerebral creatine deficiency
  - Developmental delay
  - Autistic features
  - Seizures
  - Movement disorder

## Creatine Disorders

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Blood</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine:Glycine Amidinotransferase (AGAT)</td>
<td>↓ GAA ↓ Creatine</td>
<td>↓ GAA ↓ Creatine</td>
</tr>
<tr>
<td>Guanidinoacetate methyltransferase (GAMT)</td>
<td>↑↑ GAA ↓ Creatine</td>
<td>↑↑ GAA ↓ Creatine</td>
</tr>
<tr>
<td>Creatine Transporter (X-CRTR, SLC6A8)</td>
<td></td>
<td>↑ Creatine</td>
</tr>
</tbody>
</table>

### Creatine Biosynthesis

- **Arginine** → **Glycine** via **AGAT**
- **Ornithine** → **Guanidinoacetate** via **GAMT**
- **Guanidinoacetate** → **Creatine**
- **Creatine** exported to **Cerebral** and **Blood** via **X-CRTR**
GAMT Treatment

- Supplement
  - Creatine
  - Ornithine
- Restrict
  - Arginine
- Increased cerebral creatine
- Decreased GAA

Early Intervention improves outcomes
(only 4 infants treated from birth)
GAMT Newborn Screening

Previous Trials:
- Austria, Portugal
- Terminated due to false positive rates

Multi-tiered Approaches:
- Texas, British Columbia, Utah, Italy
- Second-tier testing reduces false positives

Ongoing Screening:
- Australia (Victoria) since 2002
- No cases in 770,000 infants
BC GAMT Pilot

- Clinical detection: 2 cases in 5 years
- Population-wide pilot (deidentified)
  - 3 years (~120,000 infants)
  - 3-tiers
    - GAA by FI-MS/MS (integrated into AA/AC assay)
    - GAA by LC-MS/MS (integrated into MSUD assay)
    - GAMT sequencing in bloodspot (6 exons)
  - 1 or 2 GAMT mutations = abnormal screen
  - Re-identification of abnormal screens
Initial Newborn Screening Card (Punched, Extracted and Derivitized)

All samples deidentified (coded)

1st-Tier GAA by Flow injection (Integrated with routine AA/AC analysis)

Amino Acids

Major Acylcarnitines

Minor Acylcarnitines and GAA

2nd-Tier GAA by LC-MS/MS (Integrated with existing MSUD assay)

Internal Standards

Patient

2nd-Tier GAA by LC-MS/MS (Integrated with existing MSUD assay)

3rd-Tier GAMT Sequencing (Directly from initial bloodspot card)

If GAA > 6uM (99 ppm)

If GAA > 6uM

NEGATIVE SCREEN = No further action

POSITIVE SCREEN = Reidentify and refer for treatment

No mutations

1 or more mutations

NEGATIVE SCREEN

POSITIVE SCREEN
First Tier Performance

- As of Oct 2014: 91,000 infants screened

First-Tier

- Mean GAA = 1.65 uM (±0.55)
  - Consistent with the literature

- 0.14% over 1st-tier cutoff (125 newborns)

LOW QC

CV=9.6%

HIGH QC

CV=9.7%
Second-Tier Performance

• Integrated with MSUD
• 5 minute run-time
• Most normal on 2\textsuperscript{nd}-Tier
  – Mean = 1.52 uM (Range 0.3-6.8)
• Only one positive on 2\textsuperscript{nd} tier (GAA=6.8uM)
  – No GAMT mutations found
• FPR= 0% but TPR =0%
• Retrospective Clinical Cases
  – GAA = 9.1 and 10.7 uM (5yr RT storage)
First-Tier Positive Screens

• Enriched for premature infants
  – 63% <2500g (Population = 5.6%)
• Enriched for repeat specimens
  – 42% Repeat collections
• Interference is likely a product of therapy
  – Exogenous compound?

LC-MS/MS Removes Interference
Is GAMT a good NBS candidate?

✓ Natural History
  – Universally poor outcomes without treatment

✓ Treatment
  – Excellent outcomes with early intervention
  – Inexpensive treatment (Creatine and diet)

✓ Test performance
  – Two-tier approach required
  – Integration into existing assays (very low cost)

? Incidence
  – A very rare disorder (0/770,000 in Australia)
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