

# 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.

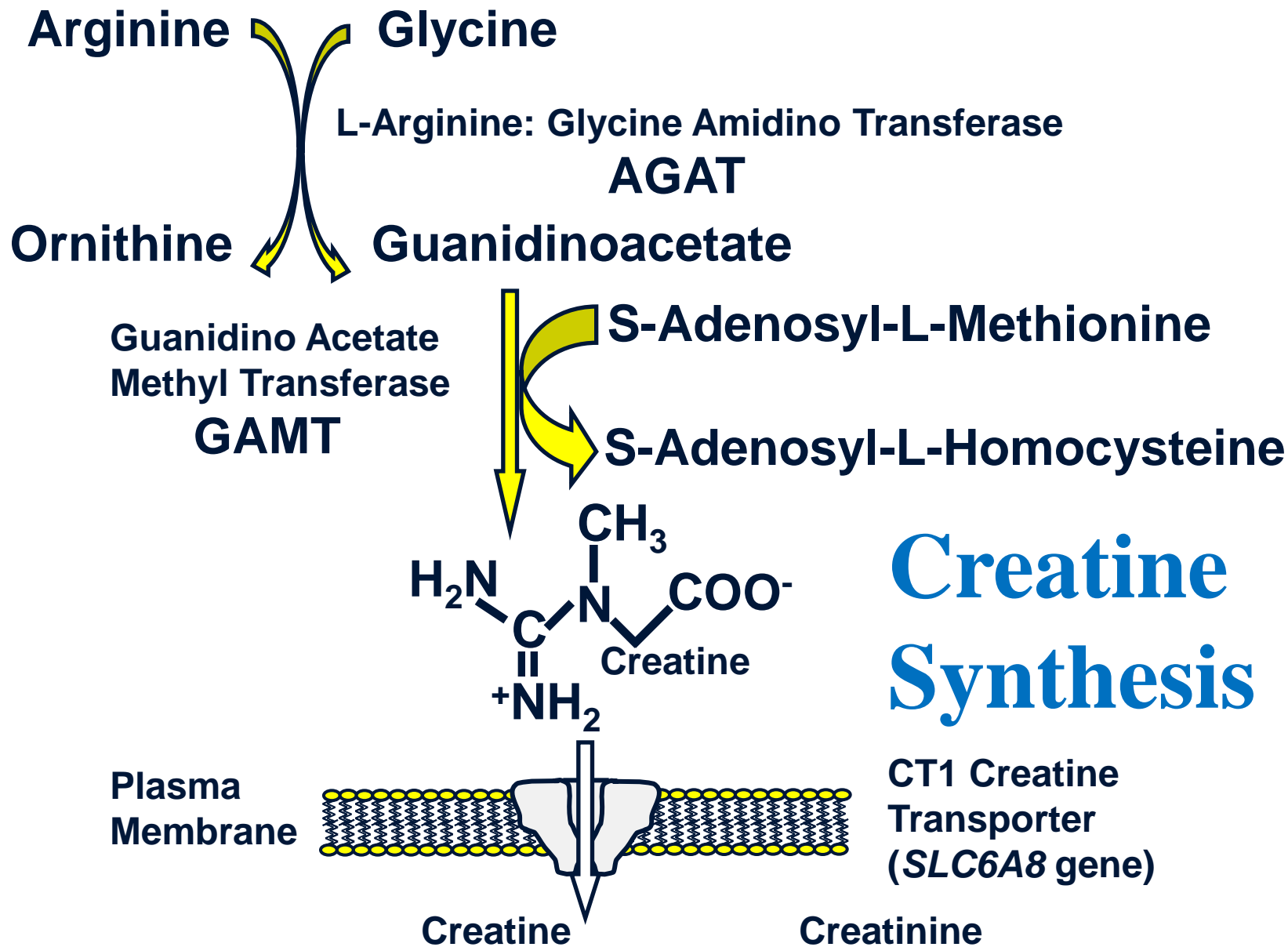
**Incidence:** 1:120,000 (Utah)

**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.

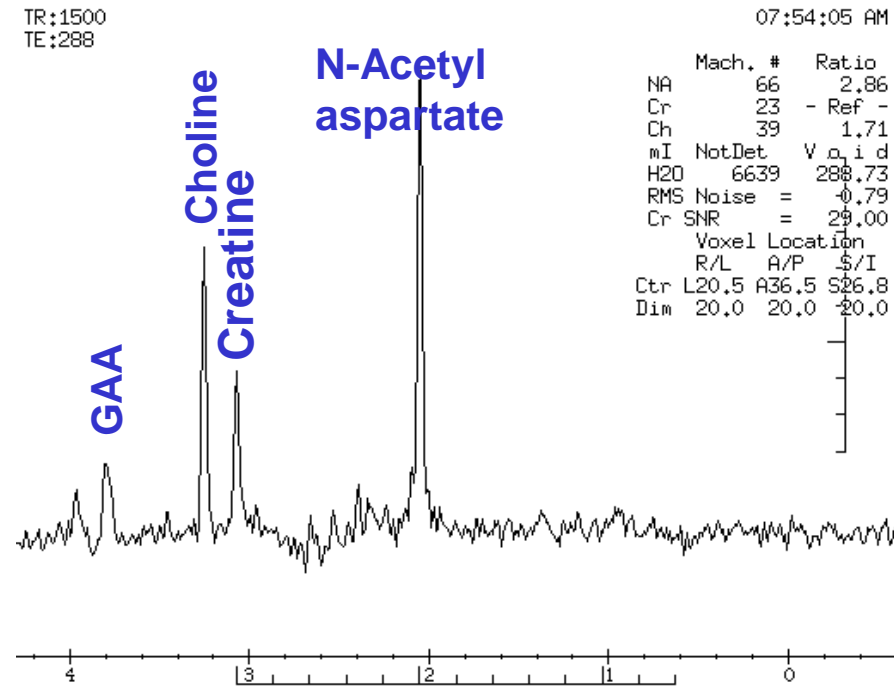
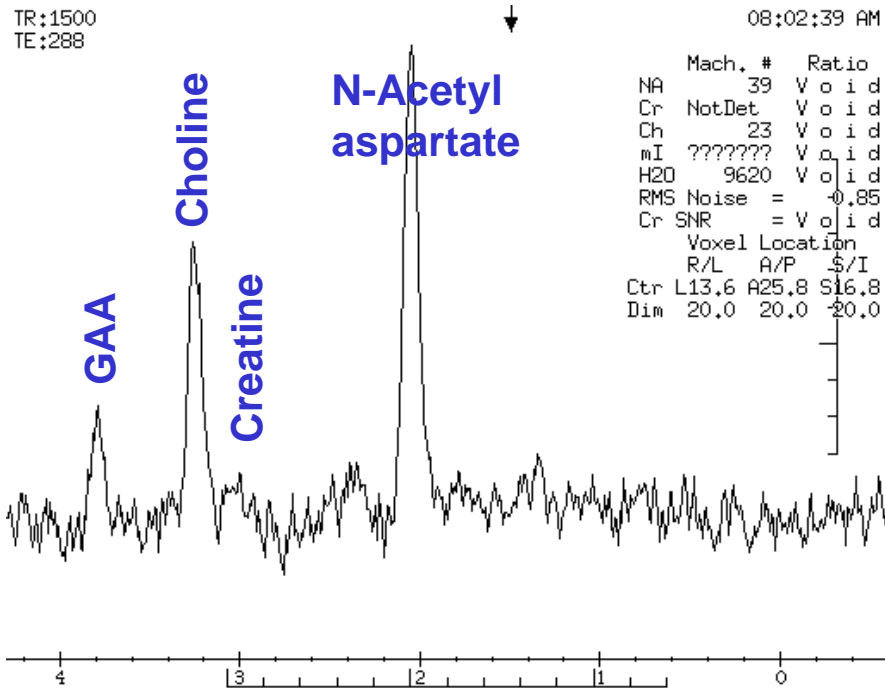
**Therapy:** Creatine (300 to 1,000 mg/kg/day) initiated preferably early in life in AGAT and GAMT deficiency. GAA levels can be reduced by ornithine supplementation (400-800 mg/kg/day) and Benzoate (50-250 mg/kg/day) to reduce glycine levels and GAA synthesis.

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



# GAMT deficiency treatment

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

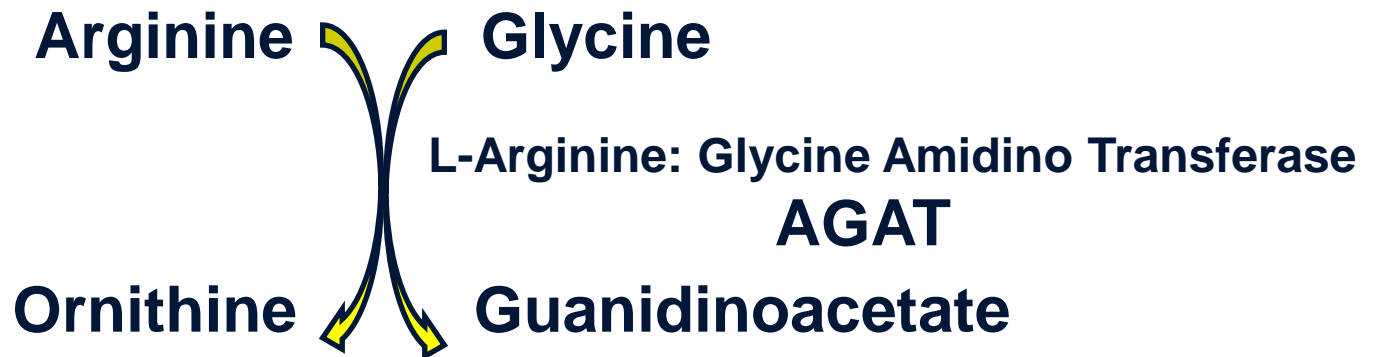


## BRAIN MR SPECTROSCOPY

Viau KS, Ernst SL, Pasquali M, Botto LD, Hedlund G, Longo N. Evidence-based treatment of guanidinoacetate methyltransferase (GAMT) deficiency. Mol Genet Metab. 2013 Nov;110(3):255-62. doi: 0.1016/j.ymgme.2013.08.020. Epub 2013 Sep 8. PMID: 24071436

# 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.**



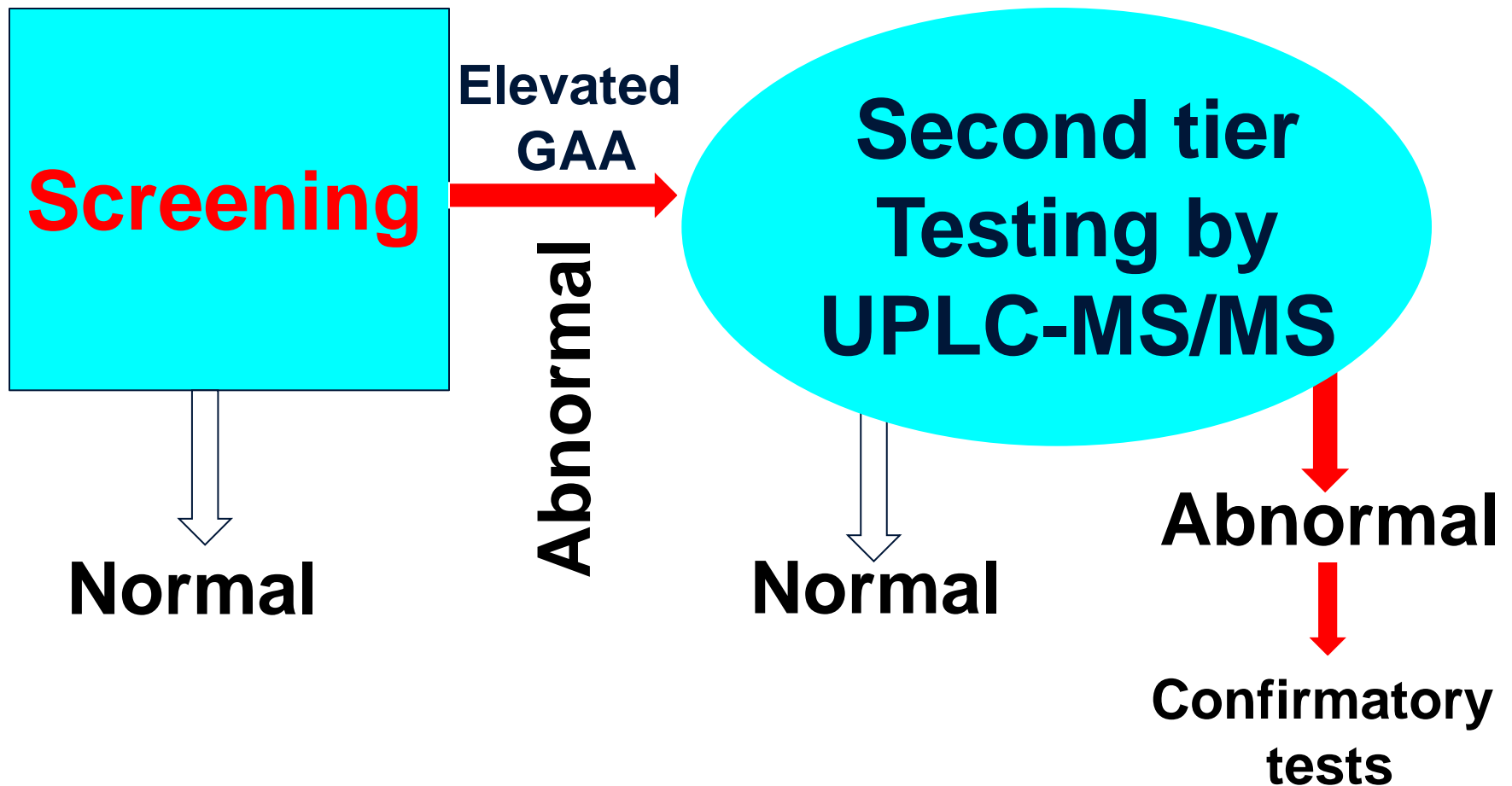
# Outcome

- **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 d<sub>3</sub>-creatine and d<sub>2</sub>-GAA added in the Internal Standards mixture. Creatine and GAA were measured using SRM.**
- **Abnormal results (elevated GAA, > 99.5%) were followed up with 2<sup>nd</sup> 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

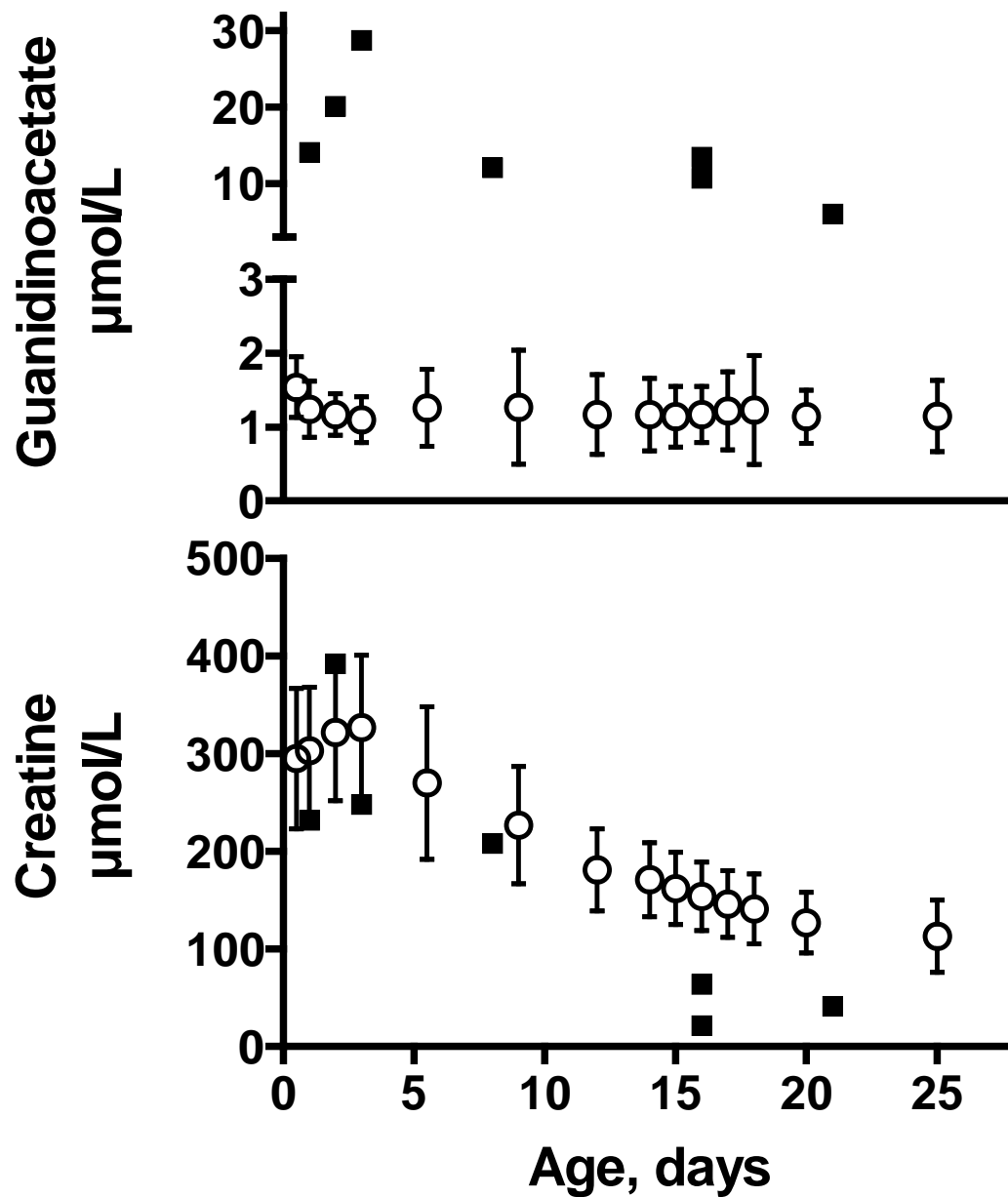




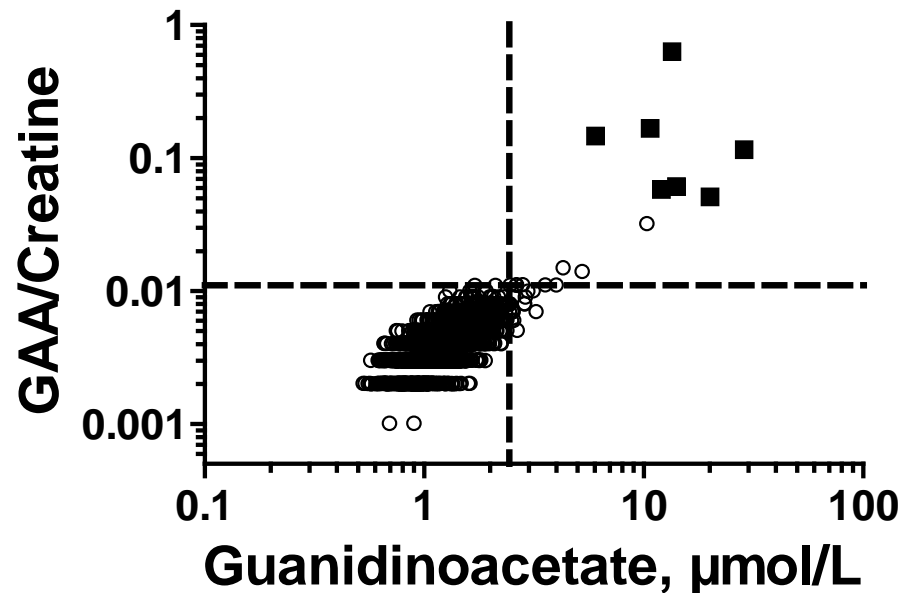
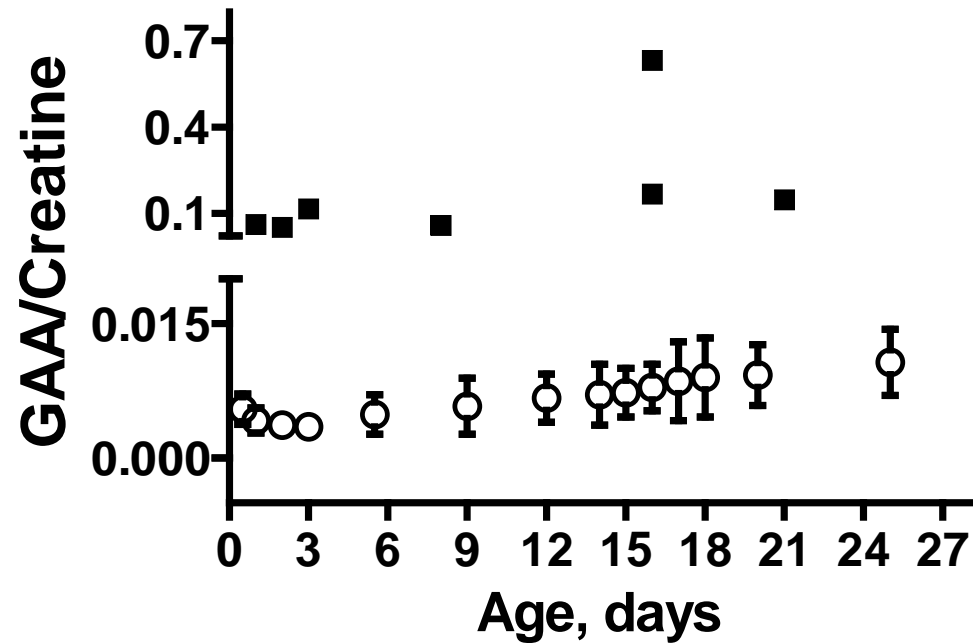
# Summary of data

- **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  $\geq 3$  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 Guanidino acetate in dried blood spots

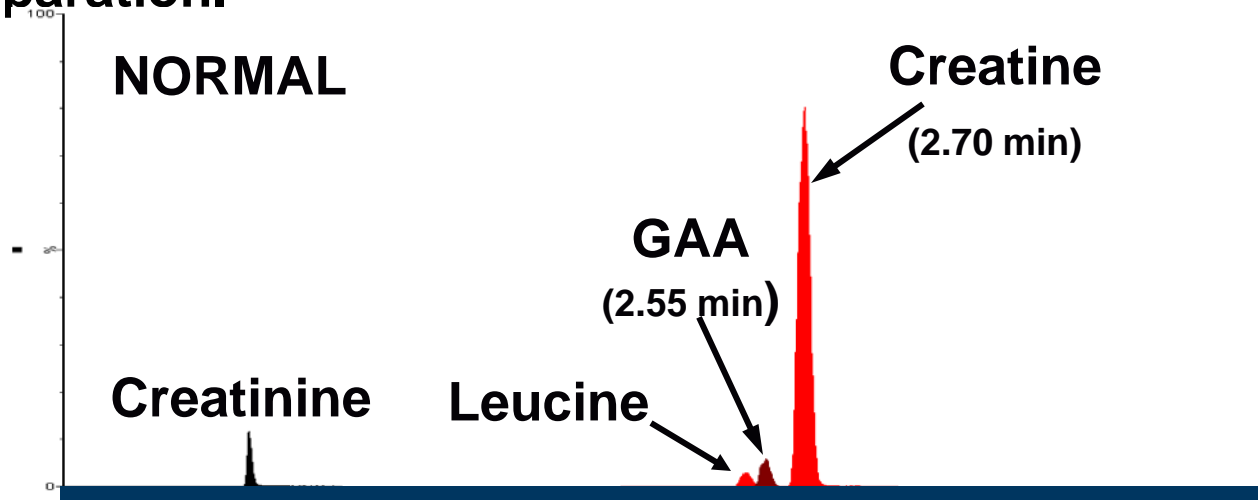


The ratio  
GAA/Creatine  
increases the  
specificity



# 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 ( $> 2.44 \mu\text{mol/L}$ ) = 60
- Total number of 2<sup>nd</sup> tier tests = 60
- Positives after 2<sup>nd</sup> tier test = 7 samples (three patients with GAMT deficiency, 1<sup>st</sup> and 2<sup>nd</sup> screens)
- ***No false positives were identified after the second tier test.***

## Feasibility of newborn screening for guanidinoacetate methyltransferase (GAMT) deficiency

Marzia Pasquali · Elisabeth Schwarz · Maren Jensen ·  
Tatiana Yuzyuk · Irene DeBiase · Harper Randall ·  
Nicola Longo

# Guanidinoacetate (GAA) levels

GAA (first screen results)	Average ( $\mu\text{mol/L}$ )	Std Dev	99% ( $\mu\text{mol/L}$ )
NP (NBS)	<b>1.25</b>	<b>0.41</b>	<b>2.20</b>
NP (2 <sup>nd</sup> tier test)	<b>1.42</b>	<b>0.54</b>	<b>3.08</b>
GAMT Deficiency	<b>32.8</b>	<b>0.54</b>	<b>N/A</b>

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.**



Department of Pathology

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