

Feasibility of Newborn Screening for Guanidinoacetate Methyltransferase (GAMT) deficiency

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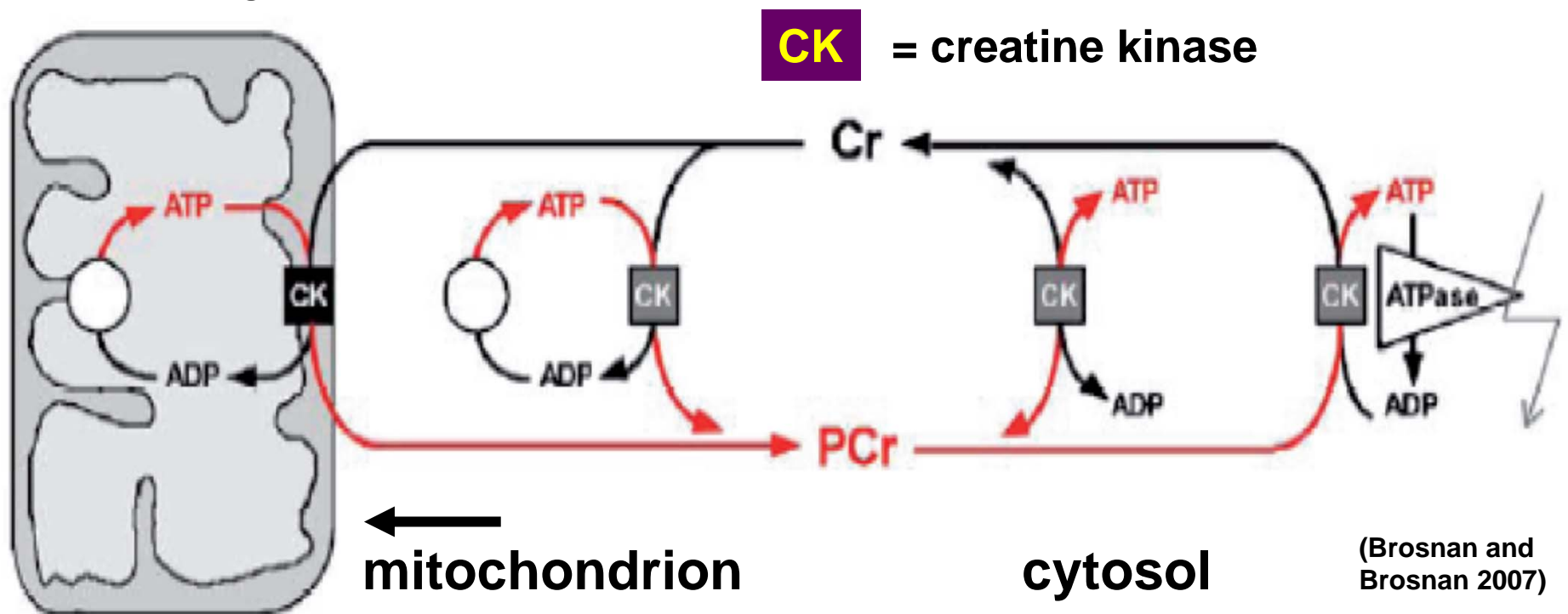


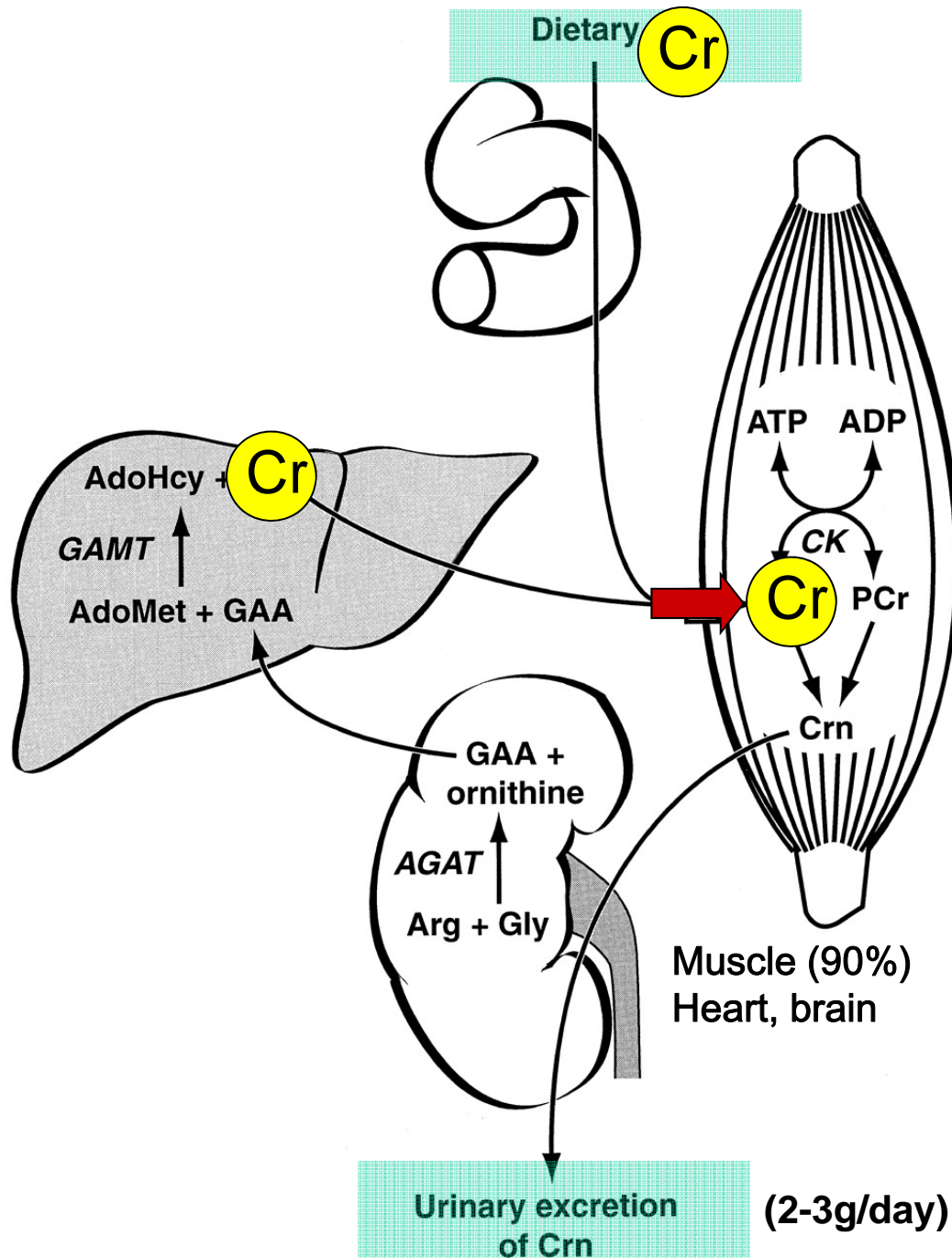
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Creatine functions in energy homeostasis in the cell

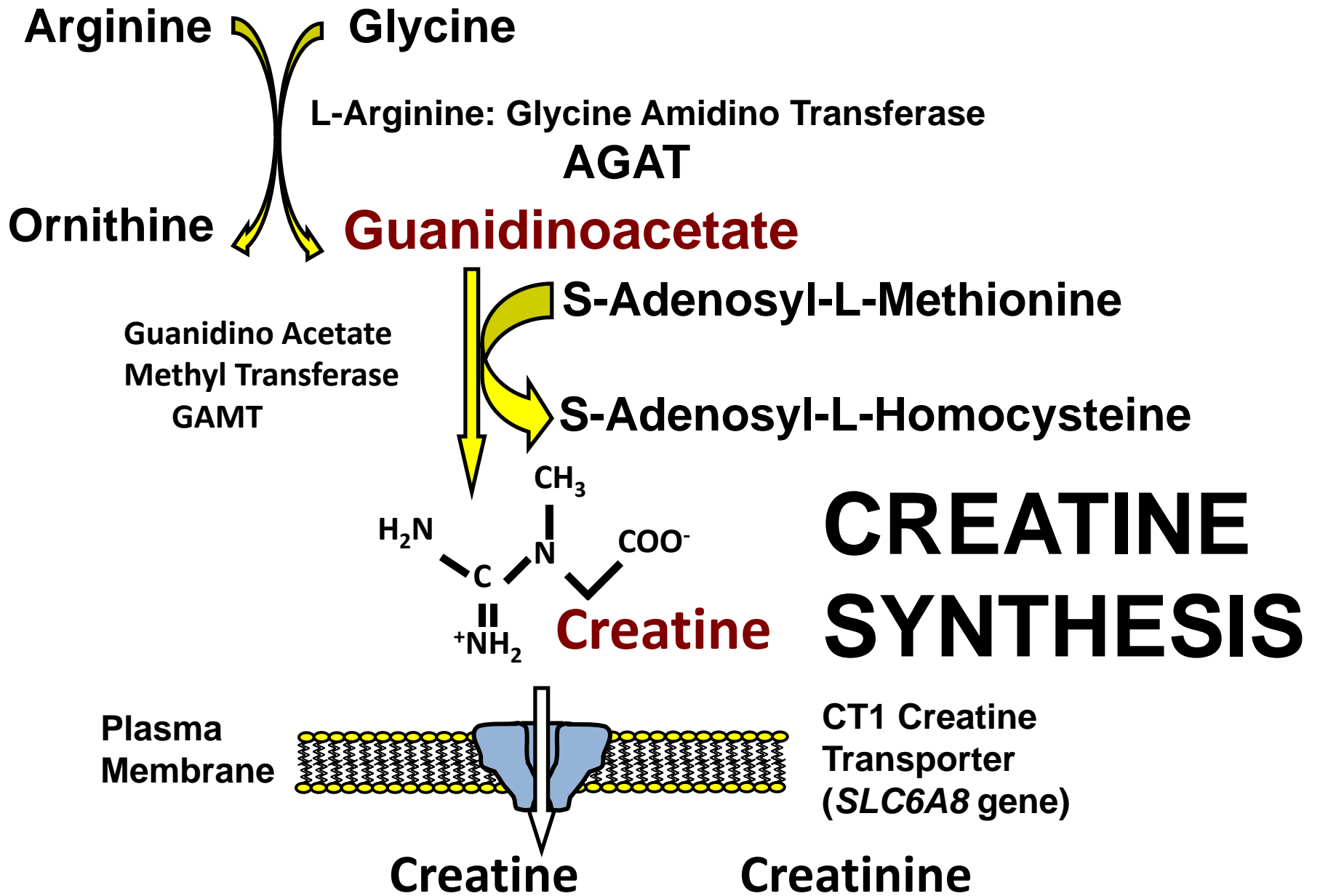
- Creatine is a nitrogen-containing compound that serves as an energy shuttle between the mitochondrial sites of ATP production and the cytosolic sites of ATP utilization, regenerating ATP from ADP.





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.

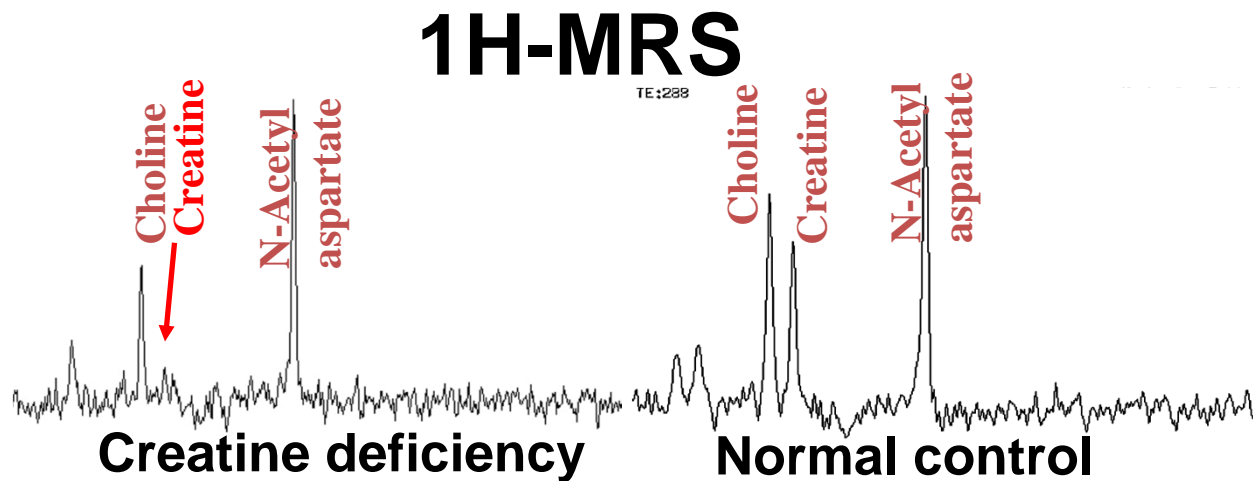


Brain Creatine Deficiency Syndromes

- **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

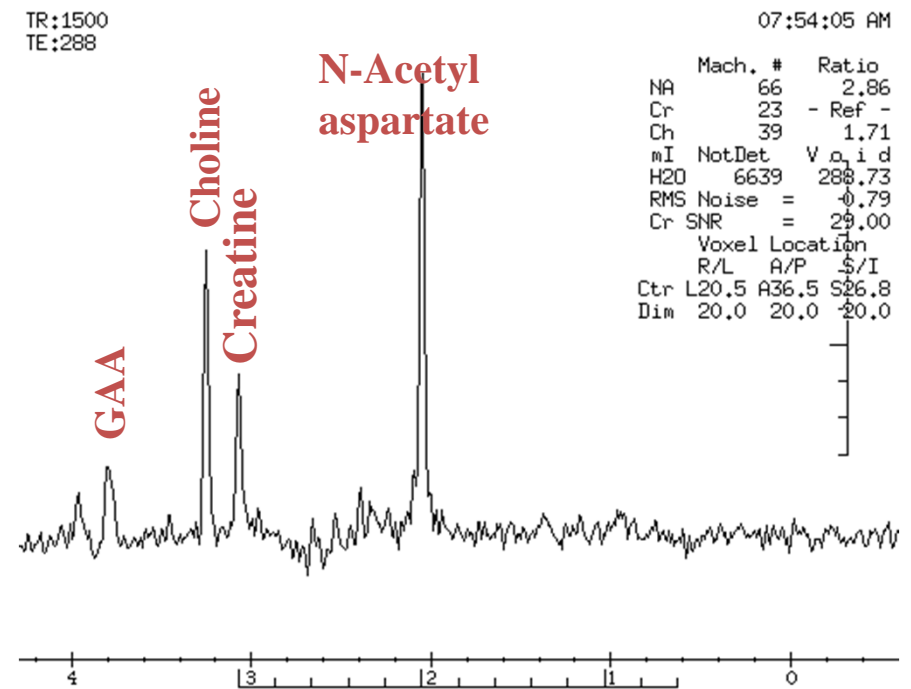
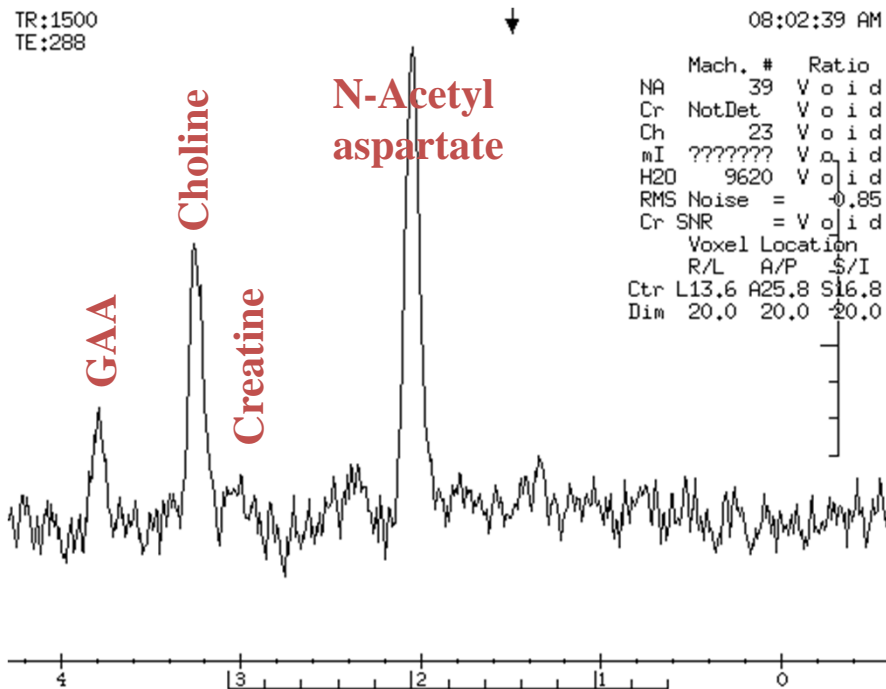
- **Plasma and urine guanidinoacetate (GAA) and creatine:**

	P Creatine	P GAA	U Creat/Creatinine
AGAT	Low	Low	NA
GAMT	Low	High	NA
Transporter	Normal	Normal	High

- **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 d₃-creatine and d₂-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

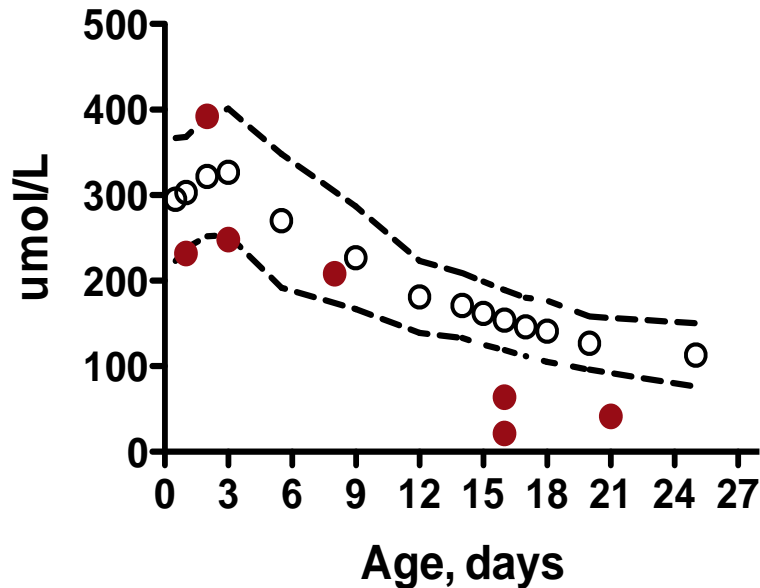
- **9,288 viable DBS**
 - **< 7 days: n=4,691**
 - 5.4% collected at <1 day
 - 88.7 % collected at 1-2 days
 - 5.9 % collected at \geq 3 days
 - **> 7 days: n=4,597**
 - 47.6 % collected at 8-14 days
 - 44.8 % collected at 15-21 days
 - 7.6 % collected at > 21 days
- **7 blood spots from 3 patients with GAMT deficiency**
 - collected at 1 – 21 days

GAMT screening: Summary

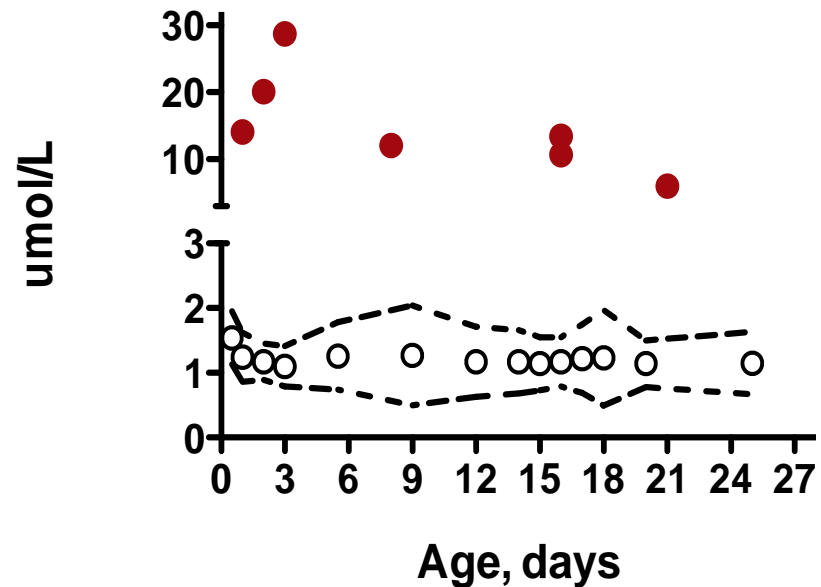
GAA (first screen results)	Average ($\mu\text{mol/L}$)	Std Dev	99.9% ($\mu\text{mol/L}$)
NP (NBS)	1.21	0.34	3.55
GAMT (NBS)	15.0	7.4	NA Min = 6.0
NP (2nd tier test)	1.42	0.54	3.08
GAMT (2nd tier test)	11.0	5.5	NA Min = 4.9

Creatine and Guanidinoacetate in dried blood spots

Creatine



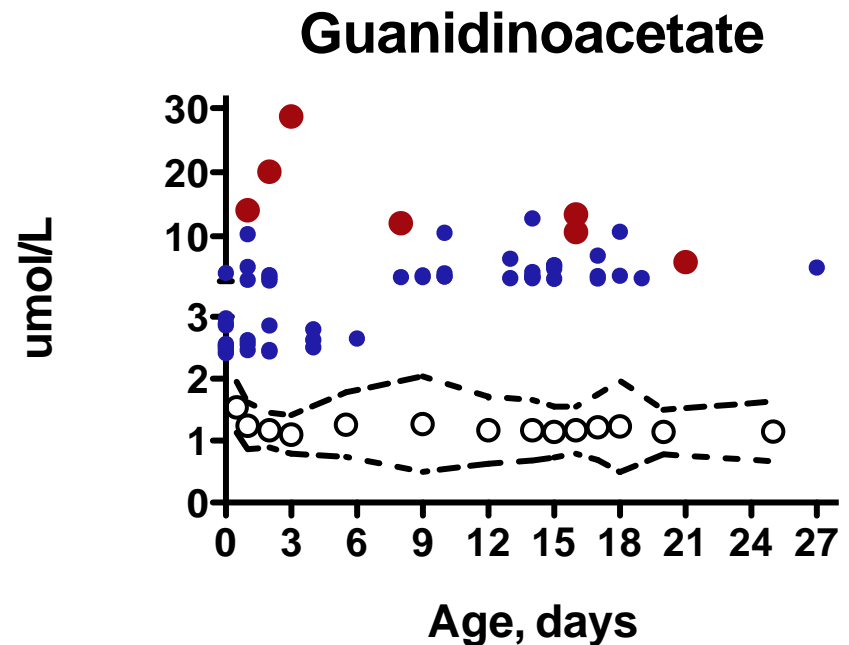
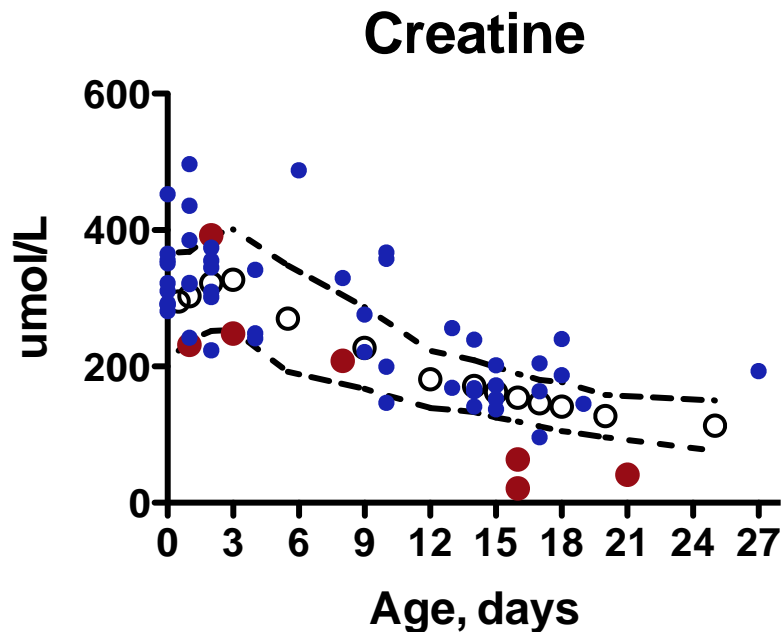
Guanidinoacetate



● GAMT patients

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

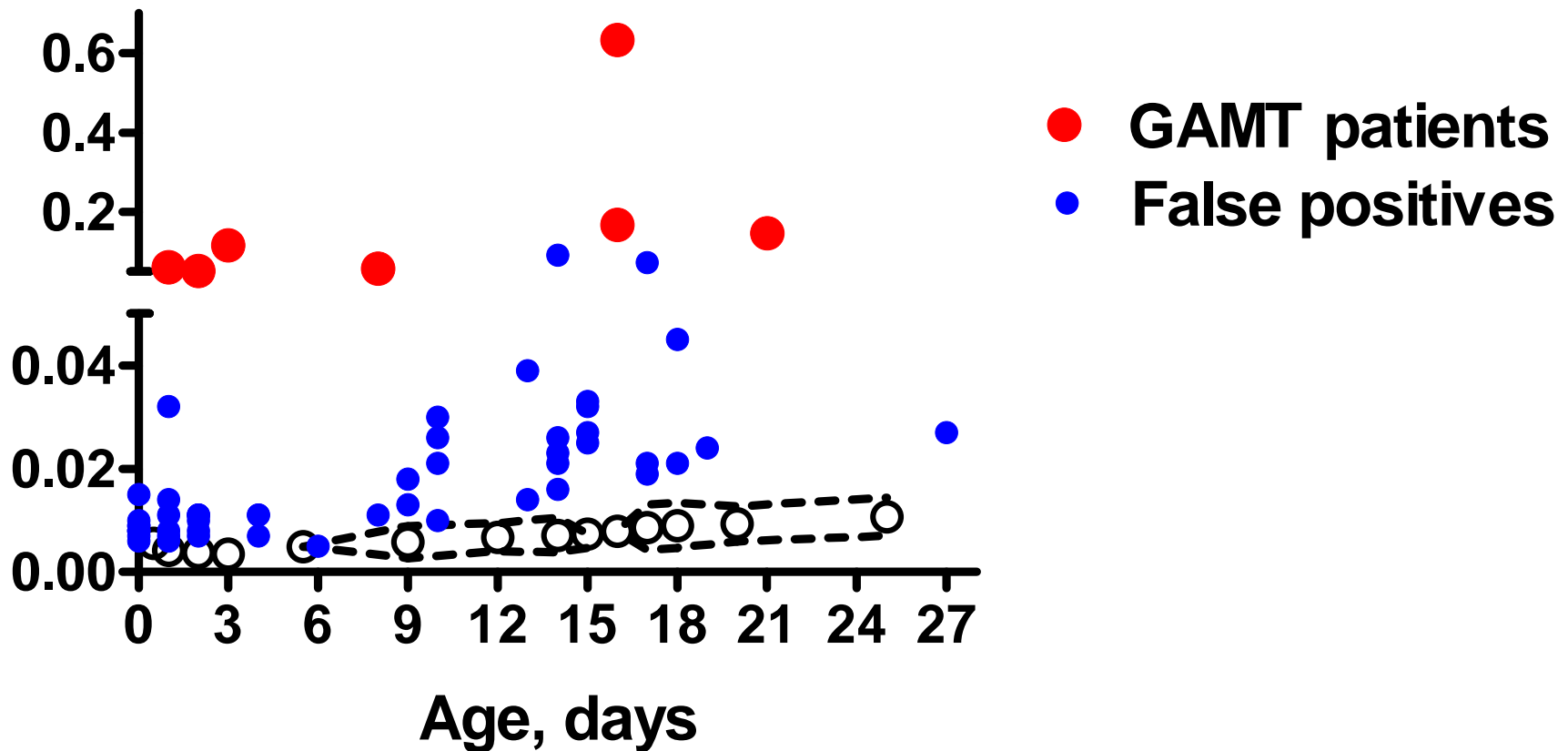


- GAMT patients
- False positives

60 samples (0.64%) had elevated GAA levels with the routine screening. 53 resolved with the 2nd tier test, 7 were confirmed with GAMT deficiency. No clear predictor for false positivity was identified.

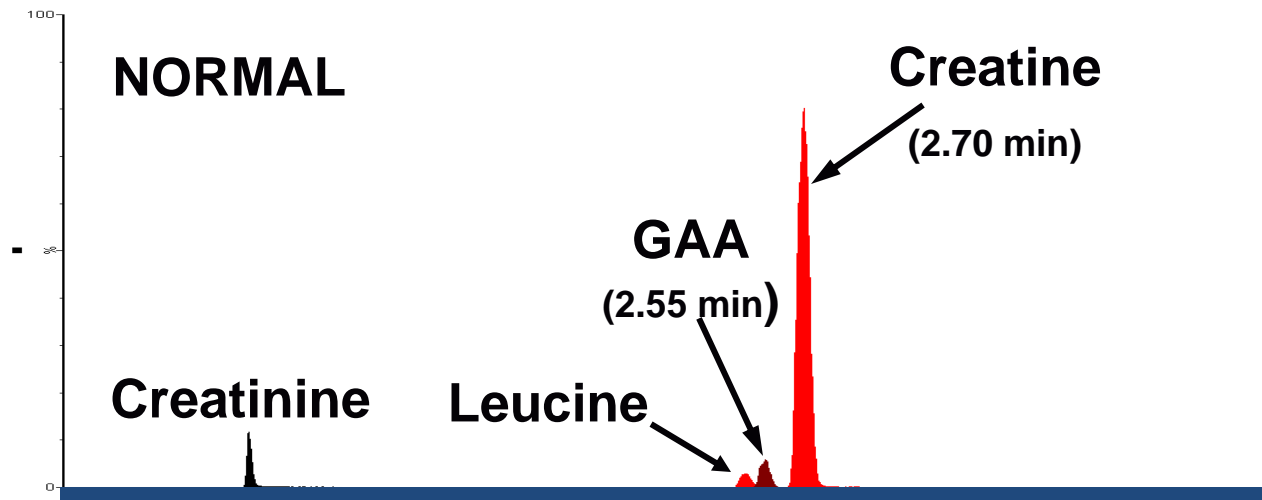
Guanidinoacetate/Creatine ratio increases specificity

GAA/Creatine



Second tier test for GAA and creatine

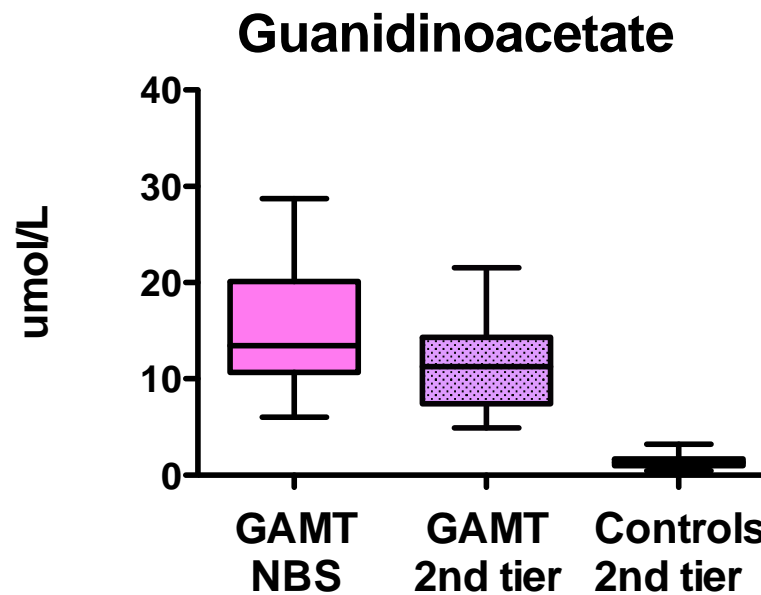
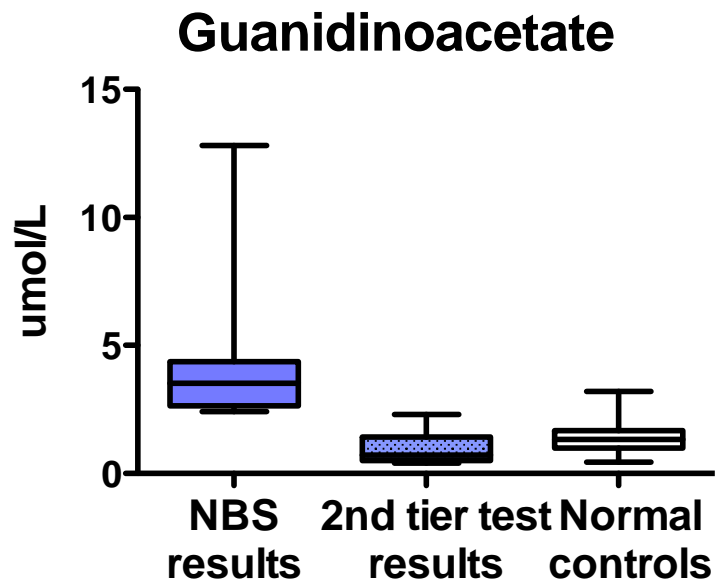
- Creatine and GAA were extracted from DBS (4.7 mm punches) using methanol containing deuterated internal standards.
- The extract was dried, derivatized using 3N HCl in butanol, dried, and reconstituted with water/acetonitrile.
- The analysis was performed using a XEVO-TQ UPLC-MS/MS system with a BEH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



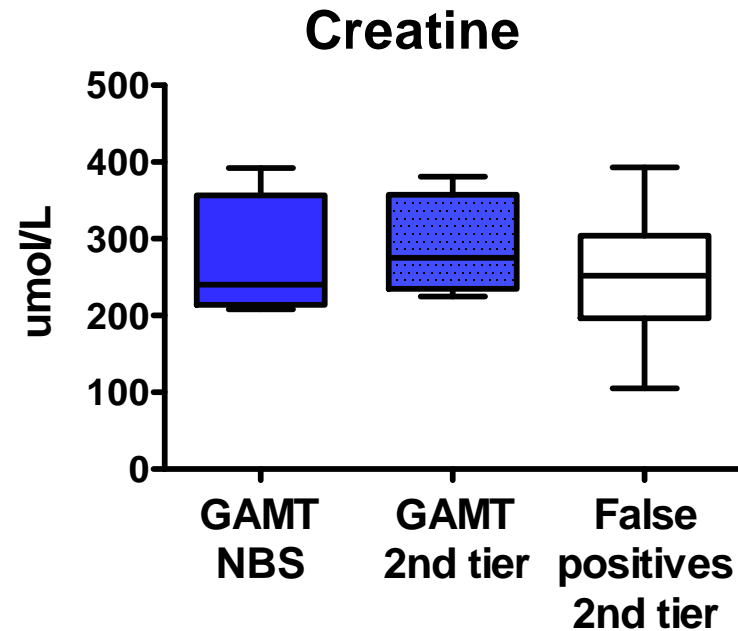
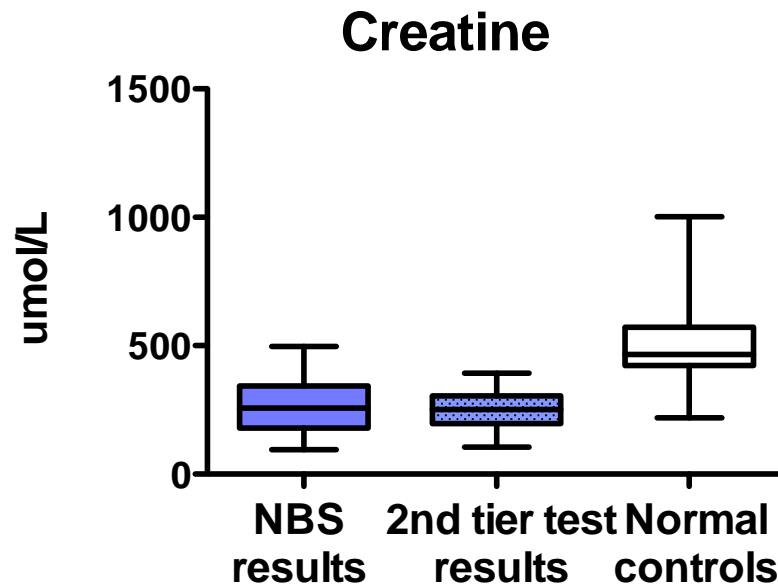
Second tier testing for GAA

- Positive screen results ($> 2.44 \mu\text{mol/L}$) = 60
- Total number of 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

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