CREATINE DEFICIENCY SYNDROMES: BIOCHEMICAL AND MOLECULAR ASPECTS

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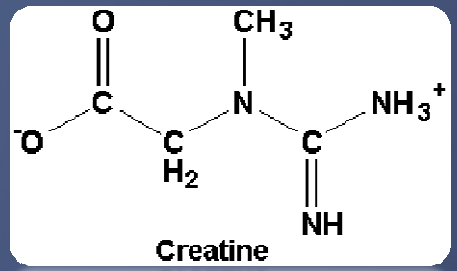
Creatine (Cr)



- Cr and Phospho-Cr play essential roles in the storage and transmission of phosphate-bond energy in several tissues.
  - Muscle and brain – tissues with high and flutuating energy demands.
  - Maintaining the high energy levels necessary for CNS development and functions through regeneration and buffering of ATP levels.

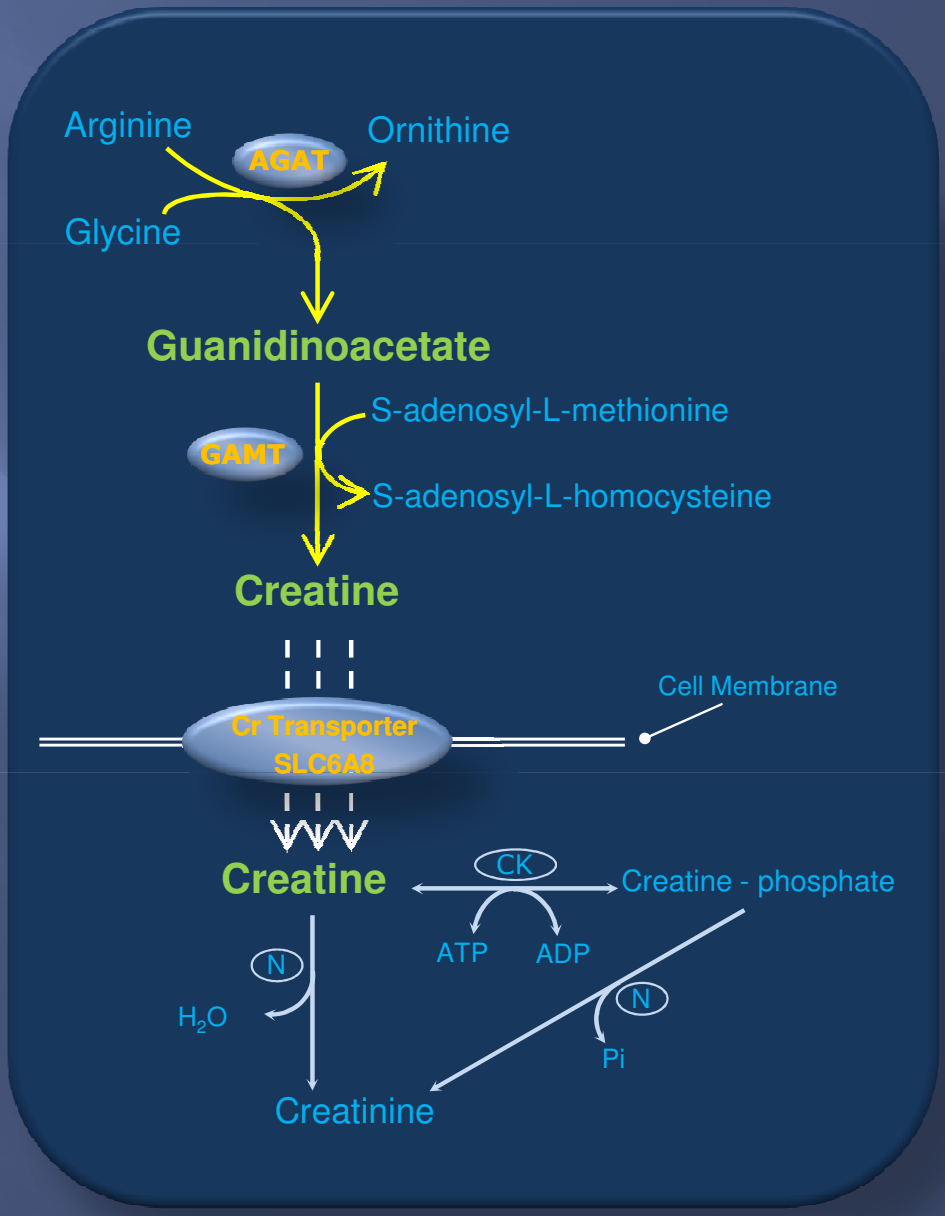
- Recent studies showed that Cr may act as a neuromodulator in CNS.

Creatine deficiency syndromes-CDS

Half of the Cr may be obtained from diet, the other half is synthesized endogenously by a two-step mechanism involving:

- L-arginine-glycine amidinotransferase – **AGAT**
- guanidinoacetate methyltransferase – **GAMT**

Cr is distributed to tissues and taken up by cells through a specific Cr transporter - **SLC6A8**.



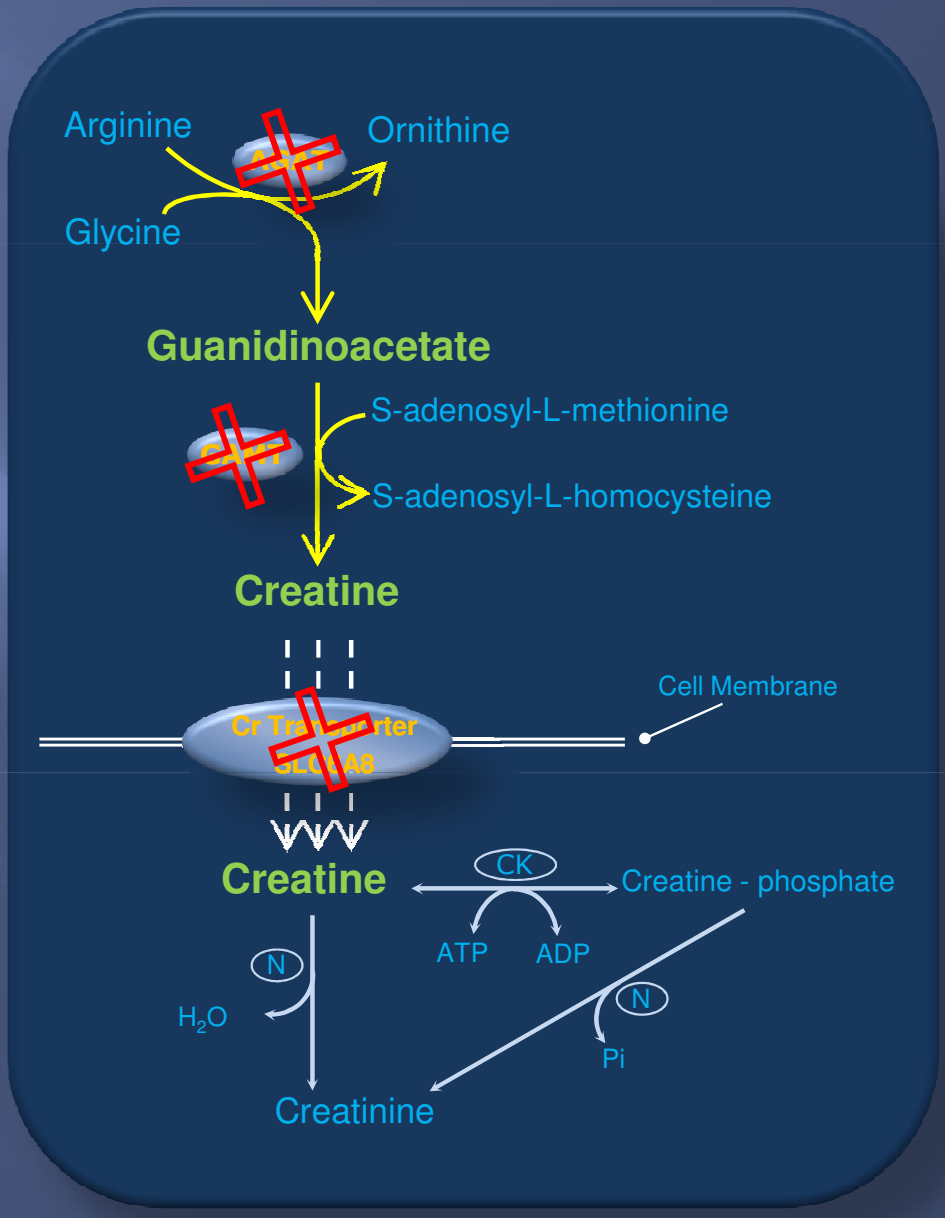
Creatine deficiency syndromes-CDS

Inborn errors of Cr biosynthesis

- **AGAT deficiency**
  - GAA and Cr - ↓
- **GAMT deficiency**
  - GAA - ↑↑ and Cr - N-↓

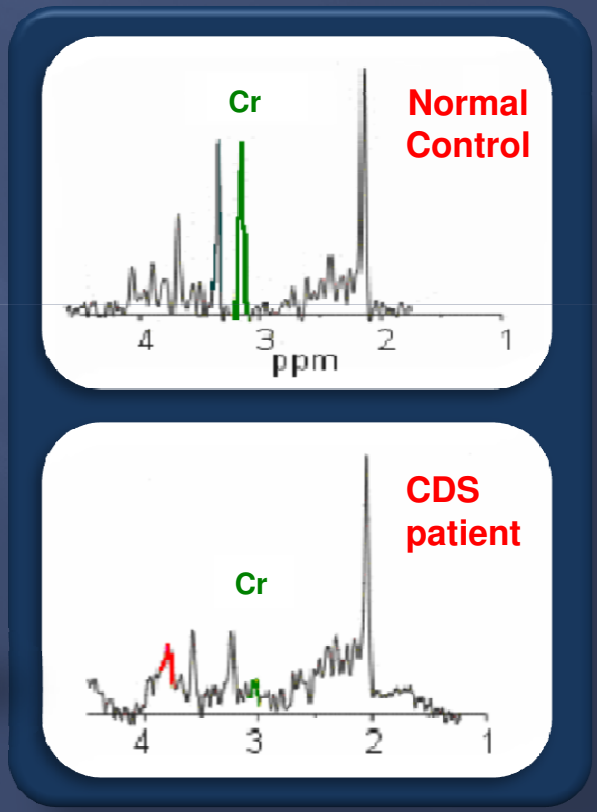
Cr transporter deficiency

- GAA - N and Cr - ↑↑



Creatine deficiency syndromes-CDS

- AGAT and GAMT deficiencies are autossomal recessive disorders (gene locus 15q15.3 and 19p13.3 respectively).
- SLC6A8 deficiency is an X-linked disorder (gene locus Xq28).
- The common denominator of these disorders is the depletion of the brain creatine pool, that can be detected by ^{1}H-MRS.



Creatine deficiency syndromes-CDS

- Patients with CDS may present with **mental retardation** (MR), expressive speech and language delay, and epilepsy.
- Patients with GAMT or SLC6A8 deficiency may also exhibit autistic-like behavior.
- The prevalence of SLC6A8 deficiency is estimated at 2% of all X-linked MR and at 1% of males with MR of unknown etiology.

Patients and Methods

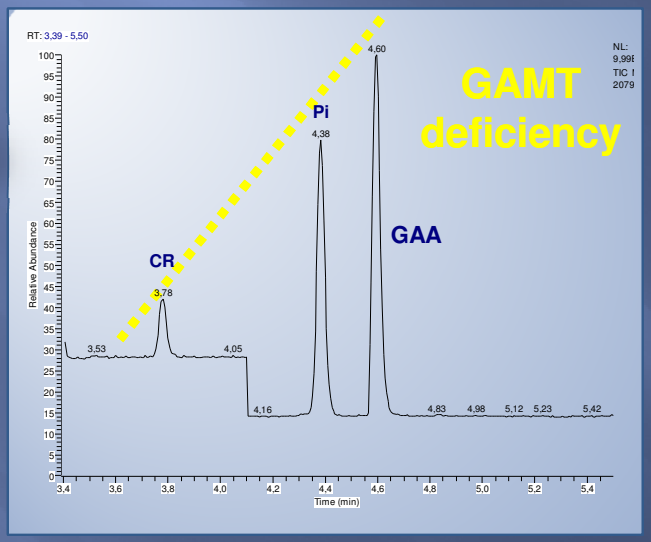
- We studied **6,600 urine samples** from Portuguese children and young adults with MR, speech delay and autistic features, for CDS.
- We started with the determination of guanidinoacetate and creatine in urine by GC-MS-SIM.
- DNA mutation analysis was performed in all biochemically suspected cases, in order to confirm the diagnosis.
  - DNA extraction
  - PCR
  - DNA sequence analysis

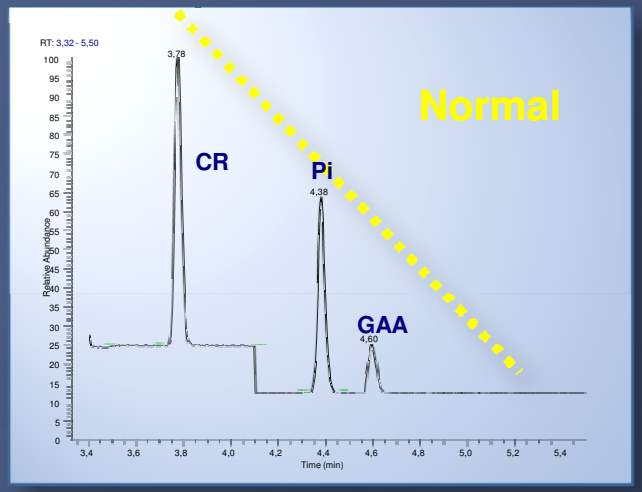
Molecular analysis was performed in colaboration with the Department of Clinical Chemistry, Metabolic Unit, VU Medical Center, Netherlands

Results: Biochemical analysis

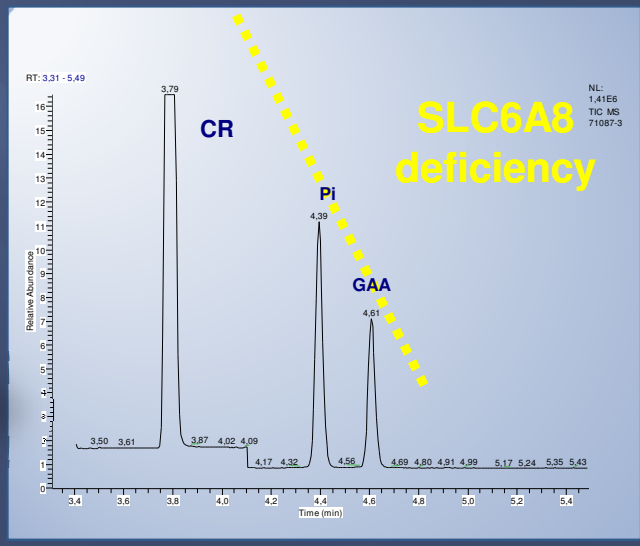
6,600 urine samples

7 cases presented increased excretion of guanidinoacetate





15 cases presented increased levels of creatine/creatinine ratio



Up to now no AGAT deficient patient was diagnosed in our lab

Results - GAMT deficiency

- All GAMT deficient patients show the same mutation **(c.59G>C, exon1; p.Trp20Ser)** which suggests a founder effect in our population.

Patient	Age (years)	Gender	GAA (umol/mmol crn)	Cr (umol/L)	Mutation analysis	Protein
GAMT-1	16	Male	827 ↑	456	c.59G>C	p.Trp20Ser
GAMT-2	20	Male	406 ↑	366	c.59G>C	p. Trp20Ser
GAMT-3	21	Male	423 ↑	337	c.59G>C	p. Trp20Ser
GAMT-4	19	Male	546 ↑	462	c.59G>C	p. Trp20Ser
GAMT-5	15	Male	1230 ↑	78	c.59G>C	p. Trp20Ser
GAMT-6	9	Female	1064 ↑	110	c.59G>C	p. Trp20Ser
GAMT-7	12	Female	911 ↑	129	c.59G>C/c.521G>A	p. Trp20Ser.Trp174X

Controls: 5-11y: GAA: 18-130; Cr: 146-8560; >12y: GAA: 18-130; Cr: 142-5952

Patient	Age (years)	Cr (umol/L)	Cr/Crn	Mutation analysis	Protein
SLC6A8-1	4	12 337	5.87	c.IVS11+1G>A, intron 11	(*)
SLC6A8-2	8	19 684	3.45	c.1261G>C, exon 9	p.Gly421Arg (*)
SLC6A8-3	3	4680	4.25	c.1A>G, exon 1	p.Met1? (*)
SLC6A8-4	12	11 889	2.90	c.1169C>T, exon 8	p.Pro390Leu (*)
SLC6A8-5	4	5922	4.93	c.1222_1224delTTC, exon 8	p.Phe408del (N)
SLC6A8-6	2	5371	3.58	c.1432dupG, exão 10	p.Ala478GlyfsX24 (*)
SLC6A8-7	2	10 092	2.80	c.884_885delCT, exon 5	p.Pro295ArgfsX169 (*)
SLC6A8-8	15	4932	2.24	c.1456C>T, exon 10	p.Gln476X (*)
SLC6A8-9	15	5891	1.84	c.1456C>T, exon 10	p.Gln476X (*)
SLC6A8-10	6	6098	2.26	c.1661C>T, exon 12	p.Pro554Leu (?)
SLC6A8-11	2	20 475	9.31	c.986G>T, exon 6	p.Ser329Ile (N)
SLC6A8-12	5	27 807	7.72	c.321_323delCCT, exon 2	p.Phe107del
SLC6A8-13	4	30 996	6.20	c.1299_1309del, exon 9	p.Pro434LeufsX27 (*)
SLC6A8-14	6	4009	2.00	c.355G>T, exon 2	p.Gly119Cys (?)
SLC6A8-15	4	10 590	2.71	No mutation found	(?)

(*) - carrier mother; (N)- Non carrier mother (?) – DNA analysis of the mother not available

Controls: **<4y:** Cr: 140-7910; Cr/Crn: 0.04-1.51; **5-11y:** Cr: 146-8560; Cr/Crn:0.04-1.07; **>12y:** Cr: 142-5952; Cr/Crn: 0.04-0.56

Conclusion

- So far, **22 patients with CDS** were identified in our laboratory (1:300).
- We believe these defects are still under diagnosed, so this group of disorders should be considered in all subjects affected by **unexplained MR, seizures, and speech delay.**
- SLC6A8 defect should also be considered in males with X-linked MR and negative fragile-X testing.

Conclusion

- GAMT deficiency treatment
  - Oral creatine monohydrate and ornithine supplementation with arginine dietary restriction.
  - GAA is a neurotoxic and epileptic substrate, treatment in a pre-simptomatic phase leads to the restoration of cerebral Cr levels, favorable clinical response and prevents neurological sequelae.

- SLC6A8 deficiency treatment
  - No successful treatment as been reported.
  - Attempts with Cr supplementation in males showed no marked improvement.
  - It is important however to female siblings with intellectual disability.

Conclusion

- Molecular analysis of GAMT and SLC6A8 genes are now available at our lab.

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Thank you for your attention