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AGAT, GAMT and SLC6A8 distribution in the central nervous system, in relation to creatine deficiency syndromes: a review.

Olivier Braissant and Hugues Henry.

Inborn Errors of Metabolism, Clinical Chemistry Laboratory,

Centre Hospitalier Universitaire Vaudois and University of Lausanne,

CH-1011 Lausanne, Switzerland.

Correspondence to: **Dr. Olivier Braissant**,

Inborn Errors of Metabolism, Clinical Chemistry Laboratory,

Centre Hospitalier Universitaire Vaudois and University of Lausanne,

CI 02/33, Avenue Pierre-Decker 2, CH-1011 Lausanne, Switzerland

Tél : (+41.21) 314.41.52, Fax : (+41.21) 314.35.46

e-mail : Olivier.Braissant@chuv.ch

Summary

Creatine deficiency syndromes, either due to AGAT, GAMT or SLC6A8 deficiencies, lead to a complete absence, or a very strong decrease, of creatine within the brain, as measured by magnetic resonance spectroscopy. While the mammalian CNS express AGAT, GAMT and SLC6A8, the lack of SLC6A8 in astrocytes around blood-brain barrier limits the brain capacity to import creatine from periphery, and suggests that CNS has to rely mainly on endogenous creatine synthesis through AGAT and GAMT expression. This seems contradictory with SLC6A8 deficiency, which, despite AGAT and GAMT expression, also leads to creatine deficiency in CNS. We present novel data showing that in cortical grey matter, AGAT and GAMT are expressed in a dissociated way: e.g. only a few cells co-express both genes. This suggests that to allow synthesis of creatine within CNS, at least for a significant part of it, guanidinoacetate must be transported from AGAT- to GAMT-expressing cells, possibly through SLC6A8. This would explain the creatine deficiency observed in SLC6A8-deficient patients. By bringing together creatine deficiency syndromes, AGAT, GAMT and SLC6A8 distribution in CNS, as well as a synthetic view on creatine and guanidinoacetate levels in the brain, this review presents a comprehensive frame, including new hypotheses, on brain creatine metabolism and transport, both in normal conditions and in case of creatine deficiency.

1 sentence "take-home message": This review brings together creatine deficiency syndromes with AGAT, GAMT and SLC6A8 distribution in CNS, and presents a comprehensive frame on brain creatine metabolism and transport, both in normal conditions and in case of creatine deficiency.

Abbreviated title: CNS creatine deficiencies, AGAT, GAMT & SLC6A8

References to electronic databases: L-arginine:glycine amidinotransferase (EC 2.1.4.1; AGAT/GATM; Agat/Gatm) deficiency: OMIM 602360; Guanidinoacetate N-methyltransferase (EC 2.1.1.2; GAMT; Gamt) deficiency: OMIM 601240; Creatine transporter (SLC6A8; Slc6a8) deficiency: OMIM 300352.

List of abbreviations: AGAT: L-arginine:glycine amidinotransferase; BBB: blood-brain barrier; CAT: cationic amino acid transporter (system y^+); CK: creatine kinase; CNS: central nervous system; Cr: creatine; CSF: cerebrospinal fluid; GAA: guanidinoacetate; GAMT: guanidinoacetate methyltransferase; MCEC: microcapillary endothelial cell; MRS: magnetic resonance spectroscopy; SLC6A8: creatine transporter; tCr: total creatine (creatine + phosphocreatine).

Introduction

In mammals, creatine (Cr) is taken up from the diet, or can be synthesized endogenously by a two-step mechanism involving (**i**) L-arginine:glycine amidinotransferase (AGAT), which, from arginine and glycine as substrates, yields the intermediate guanidinoacetate (GAA), and (**ii**) guanidinoacetate methyltransferase (GAMT), which converts GAA to Cr. Cr is distributed through the blood and is taken up by cells with high energy demands through a specific Cr transporter, SLC6A8, also abbreviated CT1, CRT1, CRTR, CTR or CreaT (for a review, see Wyss and Kaddurah-Daouk, 2000). With the discovery of Cr deficiency syndromes due to either AGAT, GAMT or SLC6A8 deficiency (Item et al 2001; Salomons et al 2001; Stöckler et al 1994; for a review, see Stöckler et al 2007), the last 15 years have seen a boost in the Cr research field, particularly in the central nervous system (CNS). In this review, we aim at bringing together what is known on Cr deficiency syndromes with the latest research on AGAT, GAMT and SLC6A8 distribution within the brain, in order to delineate a comprehensive frame on Cr metabolism and transport in CNS, both in normal conditions and in case of Cr deficiency. New hypotheses will also be presented.

Functions of creatine within the brain

The Cr / phosphocreatine / creatine kinase (CK) system is essential for the buffering and transport of high-energy phosphates. In CNS, Cr has been shown essential in the growth cones migration as well as dendritic and axonal elongation, in Na^+/K^+-ATPase activity, neurotransmitter release, maintenance of membrane potential, Ca^{2+} homeostasis and the restoration of ion gradients (Wallimann et al 1992; Wyss and Kaddurah-Daouk 2000). Cr was also recently hypothesized to act as a central neuromodulator, and particularly as co-

transmitter on GABA postsynaptic receptors (Almeida et al 2006). Finally, Cr has been proposed to regulate appetite and weight by acting on specific hypothalamic nuclei (Galbraith et al 2006).

Creatine deficiency syndromes

The CNS is the main organ affected in patients suffering from Cr deficiency syndromes caused by either AGAT, GAMT or SLC6A8 deficiency (Item et al 2001; Salomons et al 2001; Stöckler et al 1994). These patients present neurological symptoms in infancy (Battini et al 2002; DeGrauw et al 2002; Schulze et al 1997). In particular, mental retardation and delays in speech acquisition can be observed (AGAT, GAMT and SLC6A8 deficiencies), as well as epilepsy (GAMT and SLC6A8 deficiencies), autism, automutilating behavior, extrapyramidal syndrome and hypotonia (GAMT deficiency) (for a review, see Stöckler et al 2007).

AGAT, GAMT and SLC6A8 present a wide pattern of expression in the mammalian brain, which has been demonstrated in rat (AGAT, GAMT and SLC6A8), mouse (GAMT and SLC6A8) and human (GAMT) (see below; and Braissant et al 2001a; Braissant et al 2005; Galbraith et al 2006; Schmidt et al 2004; Tachikawa et al 2004). This may, at least in part, contribute to the diverse phenotypic spectrum of neurological symptoms observed in AGAT, GAMT and SLC6A8 deficient patients (Anselm et al 2006; Battini et al 2006; Mercimek-Mahmutoglu et al 2006; Schulze 2003). The recently proposed roles of Cr as co-transmitter on GABA postsynaptic receptors (Almeida et al 2006), and of regulator of appetite and weight on specific hypothalamic nuclei (Galbraith et al 2006), might also contribute to this phenotypic diversity. Specific features of GAMT deficiency are probably due to the

epileptogenic effect of the accumulated GAA (Schulze et al 2001), the activation of $GABA_A$ receptors by GAA (Neu et al 2002) and its inhibitory effect on Na^+/K^+-ATPase and CK (Zugno et al 2006).

All three deficiencies are characterized by an absence, or a severe decrease, of Cr in CNS, as measured by magnetic resonance spectroscopy (MRS) (Stromberger et al 2003; Sykut-Cegielska et al 2004). AGAT and GAMT deficient patients can be treated with oral Cr supplementation. Although very high doses of Cr are being used, the replenishment of cerebral Cr takes months and results only in the partial restoration of the cerebral Cr pool (Battini et al 2002; Ganesan et al 1997; Item et al 2001; Schulze et al 1998; Stöckler et al 1996b). The pre-symptomatic treatment of AGAT- and GAMT-deficient patients has been reported, and appears to ameliorate the outcome for these patients (Battini et al 2006; Schulze et al 2006; Schulze and Battini 2007). For GAMT deficiency, lowering GAA by arginine-restricted diet with low-dose ornithine supplementation (Schulze et al 2001) or by sole high-dose supplementation of ornithine (Schulze et al 2005) have been shown effective. Cr supplementation of SLC6A8 deficient patients is inefficient to restore cerebral Cr levels (Bizzi et al 2002; Cecil et al 2001; DeGrauw et al 2002; Póo-Argüelles et al 2006).

Expression of AGAT, GAMT and SLC6A8 within the central nervous system

It has long been thought that most, if not all, of the Cr necessary for the brain is of peripheral origin, be it taken from the diet or synthesized endogenously through AGAT and GAMT activities in kidney, pancreas and liver (Wyss and Kaddurah-Daouk 2000). It is known however since a long time that the mammalian brain is able to synthesize Cr (Pisano et al 1963; Van Pilsum et al 1972), which is also true for primary cultures of brain cells and nerve

cell lines (Braissant et al 2002; Braissant et al 2008; Cagnon and Braissant 2007; Daly 1985; Dringen et al 1998). It has now been clearly established that both AGAT and GAMT are expressed within the brain, both during development and in adulthood (Braissant et al 2001b; Braissant et al 2005; Braissant et al 2007; Lee et al 1998; Nakashima et al 2005; Schmidt et al 2004; Tachikawa et al 2004; Tachikawa et al 2007). AGAT is expressed throughout the adult rat CNS, including the retina, and can be found in all the main types of brain cells, namely neurons, astrocytes and oligodendrocytes (Braissant et al 2001b; Nakashima et al 2005). In the structures regulating exchanges between periphery and CNS, as well as between brain parenchyma and cerebrospinal fluid (CSF), AGAT is expressed in microcapillary endothelial cells (MCEC) and the astrocytes contacting them at the blood-brain barrier (BBB), as well as in the choroid plexus and ependymal epithelia (Braissant et al 2001b). GAMT is also expressed throughout the main structures of the adult mammalian brain, as shown in rat, mouse and human; furthermore, GAMT is expressed by neurons, astrocytes and oligodendrocytes, with higher levels found in both glial cell types (Braissant et al 2001b; Nakashima et al 2005; Schmidt et al 2004; Tachikawa et al 2004). GAMT is not expressed in MCEC but is present in the astrocytes contacting them (at the BBB), as well as in the choroid plexus and ependymal epithelia (Braissant et al 2001b; Tachikawa et al 2004).

Organotypic rat cortical cultures, primary brain cell cultures – either neuronal, glial or mixed – and neuroblastoma cell cultures have Cr uptake activity (Almeida et al 2006; Braissant et al 2002; Braissant et al 2008; Daly 1985; Möller and Hamprecht 1989). *In vivo*, mouse and rat CNS are able to take up Cr from the blood against its concentration gradient, but this uptake of Cr through BBB seems relatively inefficient (Ohtsuki et al 2002; Perasso et al 2003). SLC6A8 is expressed throughout the adult mammalian brain (Braissant et al 2001b; Galbraith et al 2006; Guimbal and Kilimann 1993; Happe and Murrin 1995; Saltarelli et al 1996; Schloss et al 1994). In rat and mouse, SLC6A8 is found in neurons and oligodendrocytes, but,

in contrast to AGAT and GAMT, cannot be detected in astrocytes (Braissant et al 2001b; Ohtsuki et al 2002; Tachikawa et al 2004). This holds true also for the retina, where SLC6A8 is expressed in retinal neurons, but not in astrocytes (Acosta et al 2005; Nakashima et al 2004). In contrast to the absence of SLC6A8 in astrocytes lining microcapillaries, MCEC which form the BBB and the blood-retina barrier do express SLC6A8 (Acosta et al 2005; Braissant et al 2001b; Nakashima et al 2004; Ohtsuki et al 2002; Tachikawa et al 2004), and are able to take up Cr (Ohtsuki et al 2002). SLC6A8 is also expressed by choroid plexus and the ependymal epithelia (Braissant et al 2001b).

Creatine and guanidinoacetate within the normal *versus* creatine deficient CNS

In normal conditions, Cr within human CSF is maintained in the 17-90µM range (**Table 1** and references therein). By MRS, tCr is measured between 5.5mM and 6.4mM in the cortical gray matter, and between 4.8mM and 5.1mM in the cortical white matter (**Table 1**). GAA is maintained in human CSF at a 1000x lower level than Cr, with a 0.015-0.114µM range, while its levels in gray and white matters were estimated to 1.6mM and 0.9mM respectively (**Table 1**).

With the exception of SLC6A8-deficient heterozygous females, where brain Cr deficiency appears partial (Cecil et al 2003), all three Cr deficiencies present the virtual absence (or a very stong decrease) of the Cr peak measured by MRS in the cortical gray and white matters or in basal ganglia (Stöckler et al 2007). However, despite the lack of detection or decrease under MRS measure, Cr remains present within the brain of Cr deficient patients (**Table 1**).

In SLC6A8 deficiency, Cr levels in CSF do not seem different from age-matched controls (Cecil et al 2001; DeGrauw et al 2002; Salomons et al 2001) (**Table 1**). In AGAT deficiency, tCr levels in cortical gray and white matters are decreased to 12% and 10% respectively of age-matched controls (Battini et al 2002) (**Table 1**), which suggests that tCr levels in these regions are in the 500µM range. In GAMT deficiency, CSF levels of Cr are strongly decreased (<2µM) as compared to controls (Ensenauer et al 2004; Schulze et al 1997; Schulze et al 2003), while in cortical gray and white matters, tCr were found to be in the 0.2-1.5mM and 0.3-1.9mM ranges respectively (Mancini et al 2005; Stöckler et al 1994) (**Table 1**).

GAA accumulation in body fluids is characteristic of GAMT deficiency, and the CSF of GAMT-deficient patients presents levels of GAA 60-1000x higher than age-matched controls (**Table 1**), while it was estimated to be 3.6mM and 3.4mM within cortical gray and white matters respectively. No precise data are available on GAA levels within the AGAT- and SLC6A8-deficient CNS, but it was shown recently by MRS that GAA can also accumulate in the brain of SLC6A8-deficient patients (Sijens et al 2005) (see also below).

In the rodent brain, Cr concentrations were 8.5mM (rats) and 8.2mM (mice) (Renema et al 2003), or 10-11µmol/g of tissue (mice) (Schmidt et al 2004; Torremans et al 2005) (**Table 2**). In mice, GAA is maintained at a 1000x lower level than Cr within CNS (0.012µmol/g of tissue). As expected, $GAMT^{-/-}$ KO mice show decreased levels of Cr within their brain, which however still reach 1.4mM or 0.4-0.5µmol/g of tissue, and a very significant increase in GAA (1.9µmol/g tissue; **Table 2**). As for GAMT-deficient patients, $GAMT^{-/-}$ KO mice slowly replenish their brain Cr upon Cr treatment (Kan et al 2007).

Synthesis or uptake of creatine by the brain ?

The *in vivo* expression of AGAT and GAMT within the mammalian brain, as well as the *in vitro* endogenous synthesis of Cr by various types of cultured brain cells, suggest that the CNS synthesizes Cr (for a review, see Braissant et al 2007). However, it was thought for a long time that most, if not all, of the Cr needed by the brain comes from the periphery through BBB (for a review, see Wyss and Kaddurah-Daouk 2000).

The discovery that SLC6A8 cannot be detected in astrocytes, particularly in their feet sheathing microcapillaries at BBB suggested however that in the mature brain, the BBB has a limited permeability for Cr, despite the expression of SLC6A8 by MCEC and their capacity to import Cr (Acosta et al 2005; Braissant et al 2001b; Nakashima et al 2004; Ohtsuki et al 2002; Tachikawa et al 2004). This is further confirmed *in vivo*, both in rodents and humans. The blood to brain transport of Cr through BBB has been demonstrated in rats and mice, but is relatively inefficient (Ohtsuki et al 2002; Perasso et al 2003). Moreover, the long-term treatment of AGAT- and GAMT-deficient patients with high doses of Cr allows the replenishement of their brain Cr pools, but is very slow and only partial (Stromberger et al 2003; Sykut-Cegielska et al 2004). Similarly, $GAMT^{-/-}$ KO mice treated with high doses of Cr replenish their brain Cr, but only slowly (Kan et al 2007). One possibility for the limited entry of Cr into the brain parenchyma, without going through astrocytes, could be the use of the limited surface of CNS capillary endothelium that is free of astrocytic endings (Ohtsuki 2004; Virgintino et al 1997). This would explain that the AGAT- or GAMT-deficient CNS, despite its very significant decrease in Cr, still presents measurable levels of Cr (**Tables 1 and 2**).

SLC6A8 deficient patients have normal levels of Cr in CSF (**Table 1**), but are unable to import Cr from the blood (Bizzi et al 2002; Cecil et al 2001; DeGrauw et al 2002; Póo-

Argüelles et al 2006). In contrast, GAMT-deficient patients have strongly decreased levels of Cr in CSF (**Table 1**), but are able to import Cr from the blood (Schulze et al 1997; Stöckler et al 1994). These observations are in favour of endogenous synthesis of Cr within CNS, which would still be operational, at least in part of brain cells, under SLC6A8 deficiency, while completely blocked in AGAT and GAMT deficiencies (**Figure 1**).

Thus, under normal physiological conditions, the adult mammalian brain might depend more on its own Cr synthesis, through the expression of AGAT and GAMT, than on Cr supply from the blood (Braissant et al 2001b; Braissant et al 2007). The brain capacity for Cr synthesis would thus depend on the efficient supply of arginine, the limiting substrate for Cr synthesis, from blood to CNS, and then also on the local trafficking of arginine between brain cells. We and others have shown that the cationic amino acid transporters (CATs) might fulfill these roles in the adult rat brain, as CAT1 is expressed at the BBB as well as ubiquitously in neuronal and glial cells, as CAT2(B) is present in neurons and oligodendrocytes, and as CAT3 is restricted to neurons (Braissant et al 2001a; Braissant et al 1999; Hosokawa et al 1999).

However, the hypothesis of endogenous Cr synthesis in the brain might seem contradictory with the *in vivo* characteristics of SLC6A8 deficiency, which, despite expression of AGAT and GAMT within CNS, shows an absence or a very low level of brain Cr by MRS, as in AGAT and GAMT deficiencies (Salomons et al 2003). This apparent contradiction might be explained by the AGAT, GAMT and SLC6A8 expression pattern in CNS: AGAT and GAMT can be found in every cell type of the brain (Braissant et al 2001b), while they rarely seem co-expressed within the same cell.

Dissociated expression of AGAT, GAMT and SLC6A8 within the brain

To elucidate this, we hypothesized that within the different cell types of the brain, AGAT, GAMT and SLC6A8 might be expressed in a dissociated way, and that GAA, which is known to compete for Cr uptake through SLC6A8 (Ohtsuki et al 2002; Saltarelli et al 1996), had to be transported from AGAT- to GAMT-expressing cells, possibly through SLC6A8, for Cr to be synthesized within CNS (Braissant et al 2007). This could explain the absence of Cr synthesis in the brain of SLC6A8 deficient patients. Our aim was thus first to dissect the cell-to-cell (co-)expression of AGAT, GAMT and SLC6A8 within the adult rat brain.

To achieve this, *in situ* hybridization coupled to immunohistochemistry was applied to cryosections of the rat brain (Braissant 2004), where the expression pattern of AGAT, GAMT and SLC6A8 was analyzed within the grey matter of cortex. Specific RNA probes and polyclonal antibodies were used (Braissant et al 2001b; Braissant et al 2005) to unravel, on adjacent sections, the 3 different "2 by 2" combinations of the 3 genes (AGAT+GAMT; AGAT+SLC6A8; GAMT+SLC6A8). For each combination, *in situ* hybridization for gene n°1 was coupled to immunohistochemistry for gene n°2, followed on adjacent section by *in situ* hybridization for gene n°2 coupled to immunohistochemistry for gene n°1. All combinations were repeated twice, allowing a total of 4 labelling "2 by 2" of each combinations of the 3 genes. With each combination, the proportion of cells with (**i**) no expression of either genes 1 or 2, (**ii**) expression of gene 1 only, (**iii**) expression of gene 2 only, or (**iv**) co-expression of genes 1 and 2, was obtained, which finally allowed the calculation of the expression pattern of AGAT, GAMT and SLC6A8 taken "3 by 3" (**Table 3**).

These experiments revealed that within grey matter of the rat cortex, significant proportions of cells do no express either AGAT, GAMT or SLC6A8 (30.9%), or express AGAT only (14.8%), GAMT only (13.4%) or SLC6A8 only (13.9%). Cortical cells co-expressing AGAT+GAMT but not SLC6A8 were 7.9%, AGAT+SLC6A8 but not GAMT were 6.7%, and GAMT+SLC6A8 but not AGAT were 7.9%. Finally, cells co-expressing AGAT+GAMT+SLC6A8 were 4.1%.

Altogether, we show that in the rat cortex, a low proportion of cells (12%) appears able of its own Cr synthesis (i.e. co-express AGAT+GAMT), in agreement with the Cr deficiency observed by MRS in SLC6A8-deficient patients. Cells co-expressing GAMT+SLC6A8, thus equipped for Cr synthesis if GAA is taken up by SLC6A8, were also 12%.

Future work will aim at deciphering whether the proportions in the cortical expression pattern of AGAT, GAMT and SLC6A8 are respected within the other regions of the brain, or if differential expression patterns for AGAT, GAMT and SLC6A8 occurs between these structures.

Models and hypotheses to understand creatine synthesis and transport within the brain

Taken together, (**i**) the expression pattern of AGAT, GAMT and SLC6A8 within CNS, (**ii**) the absence (or strong decrease) of Cr within CNS of Cr deficient patients, (**iii**) the low permeability of BBB for Cr, and (**iv**) the Cr and GAA concentrations within the brain, both in normal and Cr deficient conditions, lead us to propose the following model to understand Cr synthesis and trafficking within CNS (**Figure 1**):

In normal conditions (**Figure 1A**), SLC6A8 is expressed by CEMC, but not by the feet of surrounding astrocytes, implying that very limited amounts of Cr can enter the brain through BBB, possibly through the limited surface of CNS capillary endothelium that is free of astrocytic endings (Ohtsuki 2004; Virgintino et al 1997). Within the cortical grey matter, the high proportion of cells without expression of AGAT, GAMT and SLC6A8, and the low proportion of cells expressing SLC6A8 alone, suggest that brain cells express AGAT, GAMT and SLC6A8 on demand to timely adapt their Cr needs. Cells equipped with the full Cr synthesis pathway (i.e. co-expressing AGAT and GAMT), are only 12%. Finally, the dissociated expression of AGAT and GAMT amongst different cells suggests that to allow synthesis of Cr within CNS, at least for a significant part of it, GAA must be transported from AGAT- to GAMT-expressing cells, possibly through SLC6A8 as 12% of cortical cells co-express SLC6A8 and GAMT.

Cr supplementation of SLC6A8 deficient patients (**Figure 1B**) does not restore Cr levels in their brain, as MCEC of these patients lack functional SLC6A8. Moreover, if SLC6A8 also allows GAA uptake, SLC6A8 deficient patients should lack the Cr synthesis pathway from AGAT-expressing to GAMT+SLC6A8 co-expressing cells. This would explain why SLC6A8-deficient patients lack (or present a significant decrease in) Cr in CNS as measured by MRS, having only a small proportion of their brain cells equipped to self-synthesize Cr.

In AGAT deficiency (**Figure 1C**), no Cr can be synthesized within the brain, but the expression of SLC6A8 in MCEC allows the very limited entry of Cr within CNS. Because of the SLC6A8 expression in MCEC, the brain of AGAT-deficient patients can be replenished in Cr by oral Cr treatment.

Finally in GAMT deficiency (**Figure 1D**), no Cr can be synthesized within the brain, and GAA accumulates. As for AGAT deficiency, the expression of SLC6A8 in MCEC allows the very limited entry of Cr within CNS, as well as the replenishement of the GAMT-deficient CNS through oral Cr treatment.

To clarify these models and hypotheses, important questions remain to be solved. Future research in the brain Cr field should aim at analyzing the capacity of brain cells to take up GAA, and if yes to demonstrate whether this uptake occurs through SLC6A8 or not. Another important point is to identify how Cr (and GAA) can leave the cells, and whether SLC6A8 or another mechanism is involved. Finally, does the brain of SLC6A8 deficient patients accumulate GAA as suggested in our model? So far, data are poor on the GAA level in the brain of SLC6A8-deficient patients. However, a recent work indeed demonstrates that GAA does not accumulate in CNS only in the case of GAMT deficiency, but can also be augmented in the brain of SLC6A8 deficient patients (Sijens et al 2005). The fact that SLC6A8-deficient patients can also develop epilepsy (Hahn et al 2002; Mancardi et al 2007; Póo-Argüelles et al 2006) is also suggestive of GAA accumulation in the SLC6A8-deficient CNS.

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Figure legends :

Figure 1 : A proposed model for creatine synthesis and transport within central nervous system. A: normal conditions. A high proportion of cells do not express AGAT, GAMT and SLC6A8 (**1**). Endogenous synthesis of Cr within CNS can be achieved between AGAT- and GAMT-expressing cells and the concomitant trafficking of GAA between them (**2**), or in cells co-expressing AGAT+GAMT (**3**). A low proportion of brain cells only express SLC6A8 (**4**; i.e. Cr users-only). **B**: creatine transporter (SLC6A8) deficiency; **C**: L-arginine:glycine amidinotransferase (AGAT) deficiency; **D**: guanidinoacetate methyltransferase (GAMT) deficiency. Other abbreviations: Arg: arginine; Astr.: astrocytes ; BBB: blood brain barrier; Cr: creatine; GAA: guanidinoacetate; Gly: glycine; MCEC: microcapillary endothelial cells; Neur.: neurons; Olig.: oligodendrocytes.

Table 1 : Creatine and guanidinoacetate in the human brain of controls and SLC6A8, AGAT or GAMT deficient patients.

	Cr CSF µM	tCr GM[a] mM VOI	tCr WM[a] mM VOI	GAA CSF µM	GAA GM[a] mM VOI	GAA WM[a] mM VOI	References
Controls							
	n.d.	5.5 ± 0.8	5.1 ± 0.9	n.d.	1.6 ± 1.0	0.9 ± 0.9	Stöckler et al 1994
	n.d.	6.3 ± 0.7	5.1 ± 0.5	n.d.	n.d.	n.d.	Stöckler et al 1996a
	25-70	n.e	n.d.	n.d.	n.d.	n.d.	Schulze et al 1997
	n.d.	n.d.	n.d.	0.114 ± 0.068	n.d.	n.d.	Struys et al 1998
	n.d.	6.4 ± 0.3	4.8 ± 0.6	n.d.	n.d.	n.d.	Dechent et al 1999
	n.d.	n.d.	n.d.	0.062 ± 0.028	n.d.	n.d.	Leuzzi et al 2000
	35-90	n.d.	n.d.	0.015-0.100	n.d.	n.d.	Schulze et al 2001
	24-66	n.d.	n.d.	0.036-0.224	n.d.	n.d.	DeGrauw et al 2002
	24-53	n.d.	n.d.	n.d.	n.d.	n.d.	Salomons et al 2003
	17-87	n.d.	n.d.	0.020-0.560	n.d.	n.d.	Almeida et al 2004
	n.d.	6.2 ± 0.5	4.9 ± 0.4	n.d.	n.d.	n.d.	Mancini et al 2005
	n.d.	n.d.	n.d.	0.068-0.114	n.d.	n.d.	Caldeira Araujo et al 2005
SLC6A8 deficiency	62[b]	n.d.	n.d.	n.d.	n.d.	n.d.	Cecil et al 2001
	56	n.d.	n.d.	n.d.	n.d.	n.d.	DeGrauw et al 2002
	n.d.	37%[cd]	n.d.	n.d.	n.d.	n.d.	Cecil et al 2003
AGAT deficiency	n.d.	12%[c]	10%[c]	n.d.	n.d.	n.d.	Battini et al 2002
GAMT deficiency	n.d.	0.2	0.3	n.d.	3.6	3.4	Stöckler et al 1994
	< 2.0	n.d.	n.d.	n.d.	n.d.	n.d.	Schulze et al 1997
	n.d.	n.d.	n.d.	13.7	n.d.	n.d.	Struys et al 1998
	n.d.	n.d.	n.d.	11.0	n.d.	n.d.	Leuzzi et al 2000
	1.4	n.d.	n.d.	6.6	n.d.	n.d.	Schulze et al 2003
	1.8	n.d.	n.d.	15.3	n.d.	n.d.	Ensenauer et al 2004
	n.d.	n.d.	n.d.	14.0,15.0	n.d.	n.d.	Almeida et al 2004
	n.d.	1.4, 1.5	1.9, 1.6	n.d.	n.d.	n.d.	Mancini et al 2005
	n.d.	n.d.	n.d.	11.0-12.4	n.d.	n.d.	Caldeira Araujo et al 2005

a: cortical gray (GM) and white (WM) matters.
b: while on Cr treatment.
c: % as compared to age-matched controls.
d: basal ganglia, heterozygous female.

Table 2 : Creatine and guanidinoacetate in the rodent brain, including in GAMT$^{-/-}$ KO mice.

	tCr brain mM VOI	Cr brain µmol/g tissue	GAA brain µmol/g tissue	References
Control rats				
	8.5			Renema et al 2003
Control mice				
	8.2 ± 1.2			Renema et al 2003
		10.2 ± 1.1	0.012 ± 0.002	Schmidt et al 2004
		11.3 ± 0.4	0.012 ± 0.001	Torremans et al 2005
GAMT$^{-/-}$ KO mice				
	1.4 ± 0.4			Renema et al 2003
		0.43 ± 0.09	1.87 ± 0.07	Schmidt et al 2004
		0.47 ± 0.09	1.85 ± 0.06	Torremans et al 2005

Table 3 : Dissociated expression of AGAT, GAMT and SLC6A8 in the telencephalic cortex or the rat (gray matter). The proportions (%) of cells with the respective (co-)expression patterns for AGAT, GAMT and SLC6A8 are indicated. Mean ± SD (n=4).

	(Co)-expression pattern for AGAT, GAMT and SLC6A8	% of cells within gray matter (cortex)
1	- (no expression)	30.9 ± 6.5
2	AGAT alone	14.8 ± 2.3
3	GAMT alone	13.4 ± 3.6
4	SLC6A8 alone	13.9 ± 4.1
5	AGAT + GAMT	7.9 ± 2.1
6	AGAT + SLC6A8	6.7 ± 1.4
7	GAMT + SLC6A8	7.9 ± 3.3
8	AGAT + GAMT + SLC6A8	4.1 ± 1.6
No AGAT, no GAMT, no SLC6A8 (1)		**30.9 ± 6.5**
Total AGAT + GAMT (5+8)		**12.0 ± 3.7**
Total GAMT + SLC6A8 (7+8)		**12.0 ± 4.9**

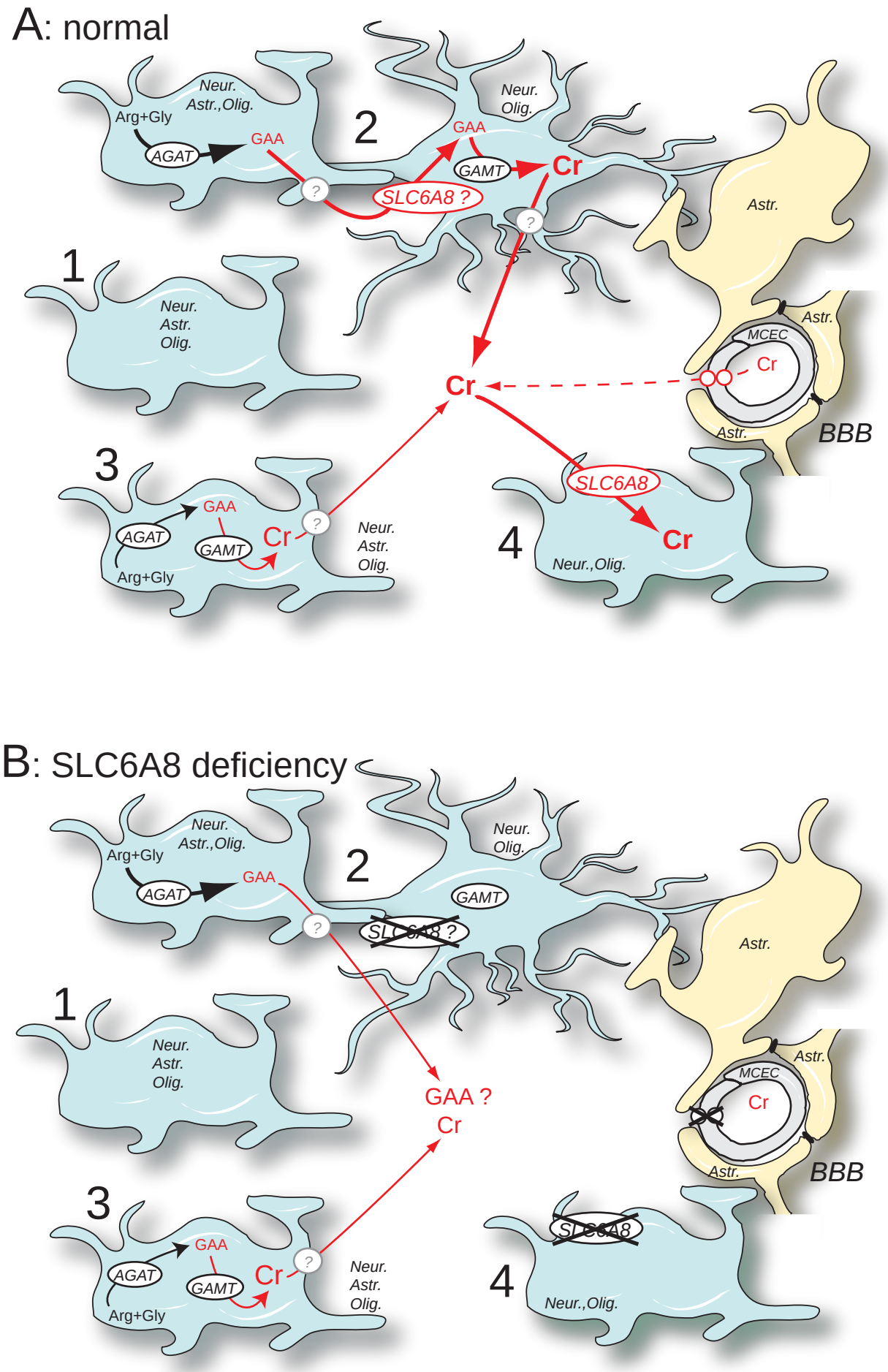
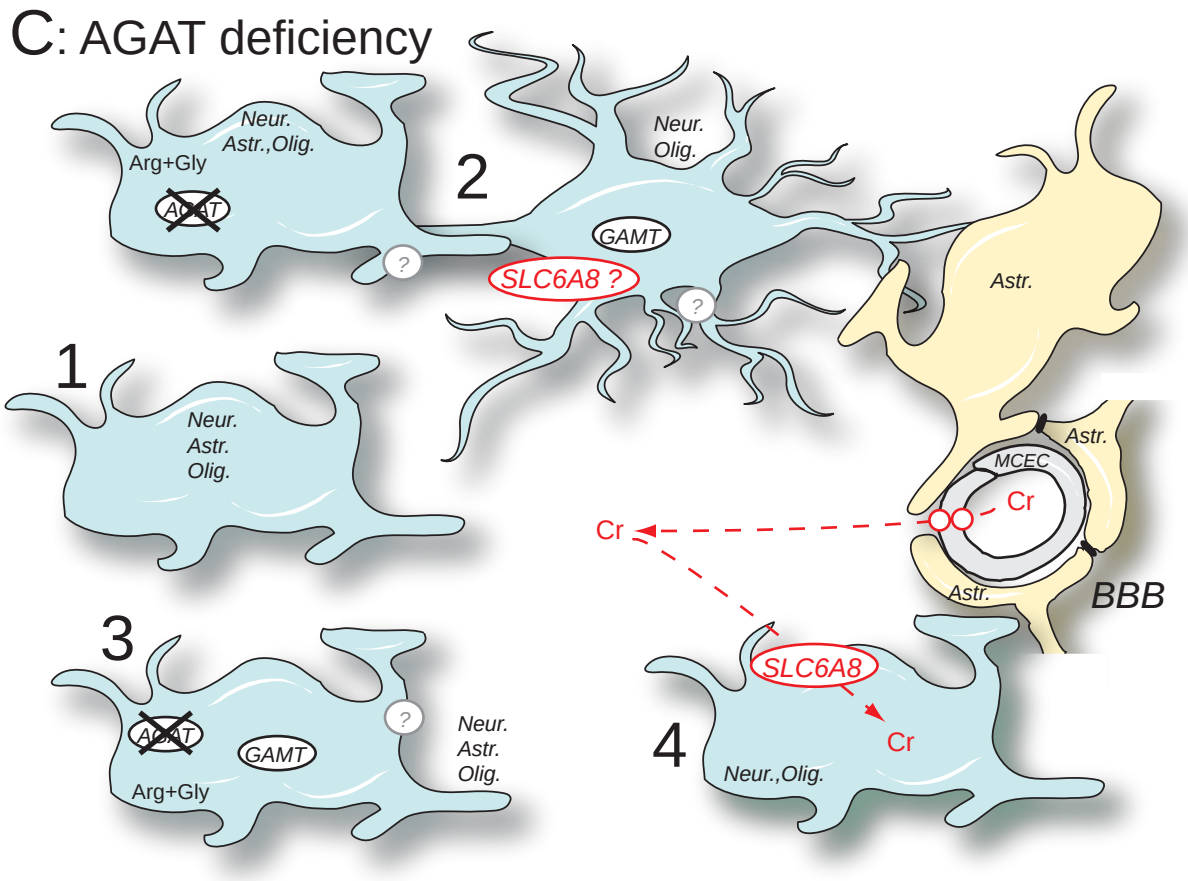

A: normal
Neur.
Astr.,Olig.
Arg+Gly
AGAT
GAA
2
Neur.
Olig.
GAA
GAMT
Cr
SLC6A8 ?
?
?
Astr.
1
Neur.
Astr.
Olig.
Astr.
MCEC
Cr
Cr
Astr.
BBB
3
GAA
AGAT
GAMT
Cr
?
Arg+Gly
Neur.
Astr.
Olig.
SLC6A8
Cr
4
Neur.,Olig.
B: SLC6A8 deficiency
Neur.
Astr.,Olig.
Arg+Gly
AGAT
GAA
2
Neur.
Olig.
GAMT
SLC6A8 ?
?
Astr.
1
Neur.
Astr.
Olig.
GAA ?
Cr
Astr.
MCEC
Cr
Astr.
BBB
3
GAA
AGAT
GAMT
Cr
?
Arg+Gly
Neur.
Astr.
Olig.
SLC6A8
4
Neur.,Olig.

Figure 1, AB


C: AGAT deficiency
Neur.
Astr.,Olig.
Arg+Gly
AGAT
2
Neur.
Olig.
GAMT
SLC6A8 ?
Astr.
1
Neur.
Astr.
Olig.
Astr.
MCEC
Cr
Cr
Astr.
BBB
3
AGAT
GAMT
Arg+Gly
Neur.
Astr.
Olig.
SLC6A8
Cr
4
Neur.,Olig.

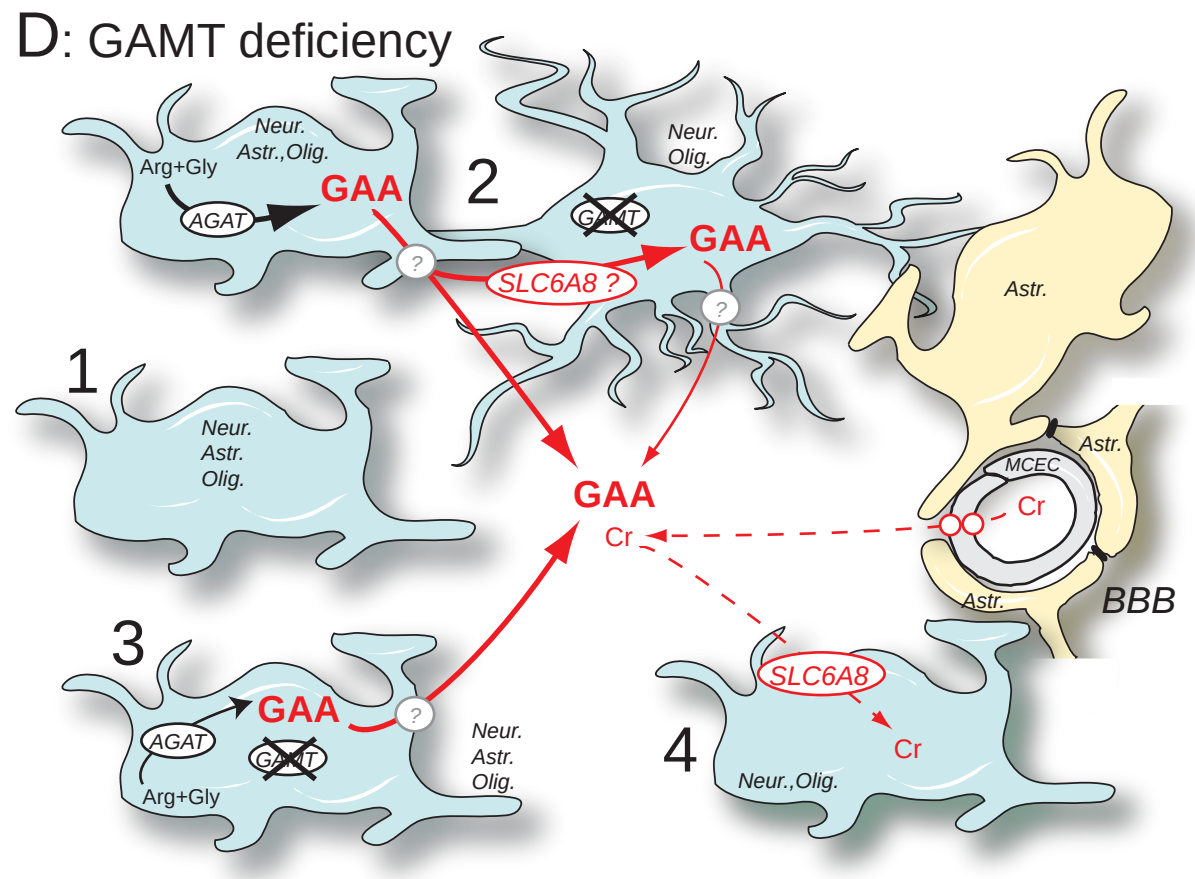

D: GAMT deficiency
Neur.
Astr.,Olig.
Arg+Gly
AGAT
GAA
2
Neur.
Olig.
GAMT
GAA
SLC6A8 ?
Astr.
1
Neur.
Astr.
Olig.
GAA
Cr
Astr.
MCEC
Cr
Astr.
BBB
3
GAA
AGAT
GAMT
Arg+Gly
Neur.
Astr.
Olig.
SLC6A8
Cr
4
Neur.,Olig.

Figure 1, CD