CHAPTER 9

PRE-SYMPTOMATIC TREATMENT OF CREATINE BIOSYNTHESIS DEFECTS

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Abstract: Recent observations in two patients, one with AGAT deficiency (AGAT-D) and one with GAMT deficiency (GAMT-D), both diagnosed already at birth, provide first evidence for important therapeutic effects of pre-symptomatic treatment with creatine (Cr) supplementation in AGAT-D and Cr supplementation plus guanidinoacetate lowering strategies in GAMT-D. Although long-term data are lacking, the results suggest that complete prevention of neurological sequelae in early treated patients could be feasible (Battini *et al.*, 2006; Schulze *et al.*, 2006)

1. INTRODUCTION

Creatine (Cr) deficiency syndromes represent a group of recently discovered inborn errors of metabolism (Stöckler *et al.*, 1994; Schulze, 2003; Bianchi *et al.*, 2000; Salomons *et al.*, 2001). Cr deficiency syndromes are caused by defects in either the biosynthesis or the transport proteins of Cr, resulting in deficiency of Cr and phosphocreatine (PCr), mainly in the brain (Schulze, 2003). Within this group of disorders, there are two synthesis defects (arginine:glycine amidinotransferase deficiency, AGAT-D, and guanidinoacetate methyltransferase deficiency, GAMT-D) and one cellular Cr transport defect (CT1, SLC6A8); the first two defects respond to Cr supplementation.

2. CREATINE REPLACEMENT THERAPY IN AGAT AND GAMT DEFICIENCY

Cr is substituted as oral Cr monohydrate at a 15- to 20-fold dosage of the normal daily Cr requirement, corresponding to 350–400 mg/kg/d in children aged 4 to 12 years. A high blood Cr concentration is achieved by frequent administration of Cr

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monohydrate (6–8 times a day) which might be favourable with respect to transport of Cr across the blood-brain barrier (BBB) (Stöckler *et al.*, 1997). Administration of higher amounts of Cr (up to 1.5–2.0 g/kg/d), with larger time intervals in between individual servings (e.g. every 8–12 hours), or in an alternating manner (e.g. 4 days application followed by a break for 3 days) do not seem to be more effective in further enhancing transport of Cr across the BBB (Schulze, 2005).

The optimal dosage of Cr monohydrate for recovery and maintenance of the cerebral Cr pool still has to be established. Long-term observation of the three AGAT-D patients described so far has allowed confirmation of clinical and neuropsychological improvement even after a reduction of the dosage of daily Cr supplementation to 200 mg/kg after three years from the start of therapy (Bianchi *et al.*, 2007). Accordingly, when studied with proton magnetic resonance spectroscopy (^{1}H-MRS), the Cr pool did not change significantly over that time interval.

Besides only incomplete restoration of brain Cr pools by Cr replacement therapy in both AGAT-D and GAMT-D patients, the ^{1}H-MRS results indicate a faster slope and a more complete recovery of brain Cr concentrations in AGAT-D patients than in GAMT-D patients (Figure 1) (Schulze, 2005; Bianchi *et al.*, 2007). This

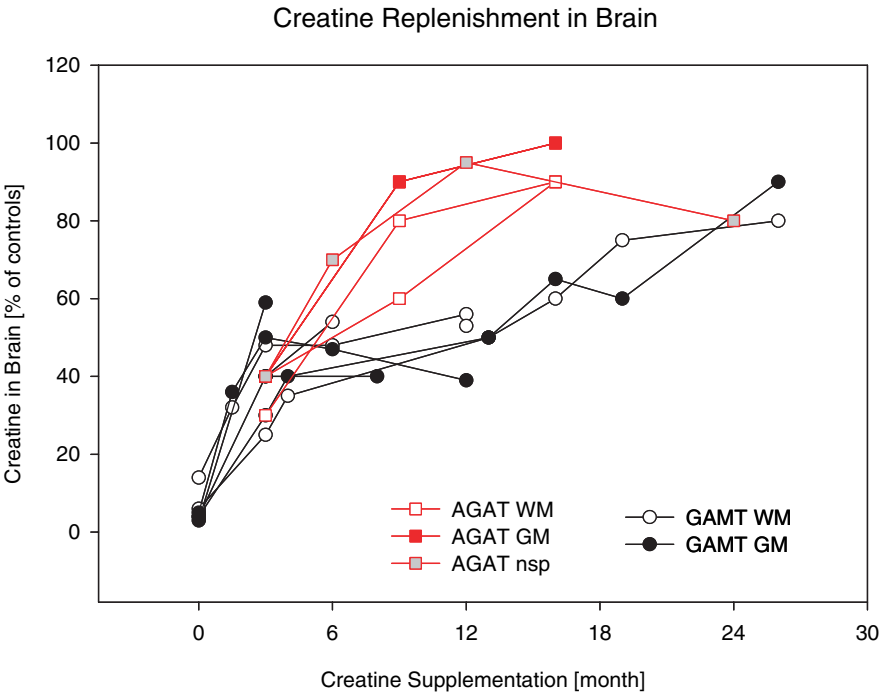


Figure 1. Creatine replenishment in the brain of patients with GAMT-D and AGAT-D in the course of creatine supplementation, estimated by *in vivo* proton magnetic resonance spectroscopy. WM, white matter; GM, grey matter; nsp, not specified. Reprinted from Schulze (2005) with kind permission from SPS Verlagsgesellschaft mbH, Heilbronn, Germany.

difference is likely correlated to guanidinoacetate (GAA) accumulation in GAMT-D. GAA, like some other guanidino compounds, is a competitive inhibitor of Cr transport (Ohtsuki *et al.*, 2002). In accordance with these findings, the clinical response to oral Cr supplementation is more pronounced for AGAT-D patients than for GAMT-D patients, including good language development and complete disappearance of autistic-like behaviour (Battini *et al.*, 2002; Bianchi *et al.*, 2000).

3. GUANIDINOACETATE LOWERING THERAPY IN GAMT DEFICIENCY

Accumulation of GAA, known for its neurotoxic and epileptogenic effects, contributes to the pathophysiology in GAMT-D. Cr replacement therapy causes an approximately 50% reduction of GAA in body fluids. Considering the 10- to 100-fold elevated GAA concentrations in affected individuals (Mercimek-Mahmutoglu *et al.*, 2006), this reduction is far from normal GAA levels. Two different approaches have proven effective in further lowering GAA in GAMT-D patients, namely (i) dietary arginine restriction by a protein restricted diet combined with low-dose ornithine supplementation (Schulze *et al.*, 2001) and (ii) high-dose ornithine treatment (Schulze, 2005).

The strategy for dietary treatment is substrate deprivation (arginine, glycine) of the AGAT reaction, and consists of protein restriction, supplementation of arginine-free amino acids, low-dose ornithine, and sodium benzoate. Intake of arginine by nutritional protein is restricted to 15–25 mg arginine/kg/d (corresponding to 0.4–0.7 g natural protein/kg/d). An arginine-free essential amino acid mixture is substituted with 0.2–0.7 g/kg/d to meet age-dependent physiological protein requirements. Ornithine in low dosage (50–100 mg/kg/d) seems necessary to keep arginine low. Sodium benzoate (100 mg/kg/d) is given for additional substrate deprivation. It removes glycine, the other substrate of the AGAT reaction, and lowers the flux through the arginine-forming urea cycle (Schulze *et al.*, 2001; Stoeckler-Ipsiroglu *et al.*, 2006).

More recently, treatment with high-dose ornithine has shown to be at least as effective in lowering GAA levels as dietary arginine restriction. Ornithine is a competitive inhibitor of the AGAT enzyme. AGAT inhibition is achieved by administration of 800 mg ornithine/kg/d given in 5–6 doses per day (dpd) (Schulze, 2005). Whether a combination of dietary treatment with high-dose ornithine therapy is best for decreasing GAA levels still has to be elucidated. Figure 2 illustrates our current knowledge about the effectiveness of different treatments on GAA reduction in blood and urine.

Cr replenishment and GAA lowering improve the clinical symptoms in GAMT-D patients. This applies mainly to the autistic behaviour, the movement disorder, and to some cognitive capabilities, whereas language skills and general cognitive development remain poor or improve only slightly. Control of severe seizures so far refractory to antiepileptic drugs and Cr treatment is obviously achievable by GAA lowering therapy (Schulze *et al.*, 2001). Even treatment only initiated in

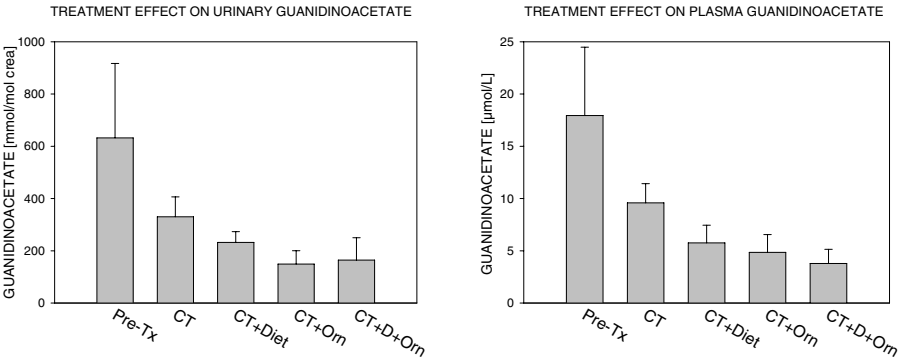


Figure 2. Effect of different treatments on guanidinoacetate levels in urine and plasma of patients with GAMT-D. Pre-Tx, pre-treatment (n = 6 patients), CT, Cr treatment (n = 3); CT+Diet, Cr treatment plus dietary arginine restriction (n = 1); CT+Orn, Cr treatment plus high-dose ornithine treatment (n = 5); CT+D+Orn, Cr treatment plus dietary arginine restriction plus high-dose ornithine treatment (n = 2).

adulthood led to an impressive improvement in epileptic seizures, mental capabilities, movement disorder and behaviour in one patient (Schulze, 2003).

4. PRE-SYMPTOMATIC TREATMENT IN AN AGAT-D PATIENT

AGAT-D (MIM 602360) is an autosomal recessive disease characterized by mental retardation, severe language impairment and behavioural disturbances. Supplementation of Cr has been shown to improve clinical symptoms in previously reported symptomatic cases (Battini *et al.*, 2002; Bianchi *et al.*, 2000). Pre-symptomatic treatment has been reported in only one subject (Battini *et al.*, 2006).

The boy is the third child of healthy non-consanguineous Italian parents. Both parents are carrying the c.446G>A mutation that results in the replacement of tryptophan by a stop codon at residue 149 (p.Trp149X) in the *AGAT* gene. His two older sisters were previously diagnosed with AGAT-D and are being treated for 6 years. Both sisters are homozygous for the above described pathogenic nonsense mutation (Bianchi *et al.*, 2000). The boy was born after unremarkable pregnancy and delivery (birth weight: 3 kg; length: 49 cm; head circumference: 35 cm). Neurological examination at the age of 3 days was normal. He was breast-fed. Serum panel chemistries were normal. Analysis of Cr deficiency metabolites by GC/MS showed the following: serum GAA (0.13 µM vs. normal range 0.22–3.14 µM) and Cr (16.2 µM vs. 18–141 µM); urine GAA (0.54 µM vs. 55–698 µM) and Cr (24.6 µM vs. 200–5500 µM). Sequencing of the proband's *AGAT* gene confirmed the presence of the same homozygous mutation as previously detected in DNA of the sisters. The diagnosis was also confirmed by means of a new AGAT activity assay in lymphocytes or lymphoblasts, using GC coupled with a quadrupole detector, without using labelled substrates. The lymphocytes or lymphoblasts were incubated in a reaction mixture containing glycine and arginine at pH 7.5. The reaction was

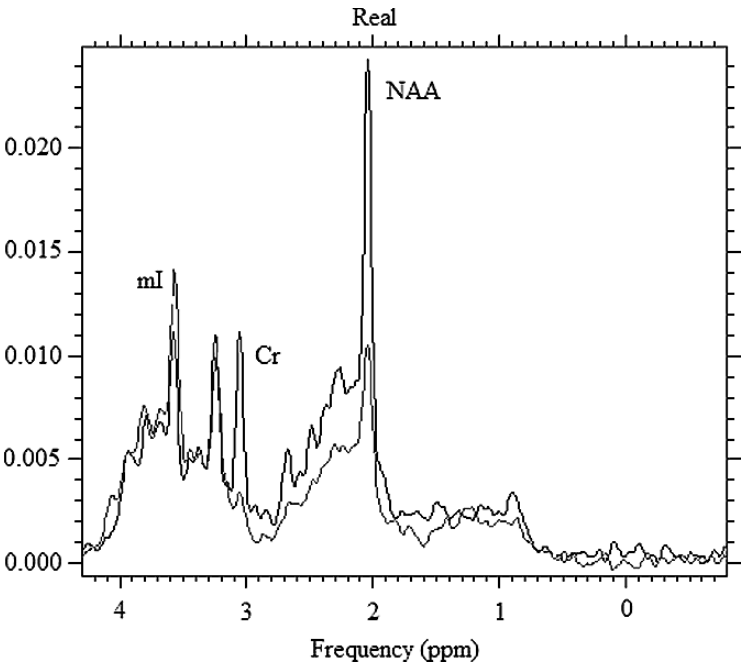


Figure 3. [1]H-MRS of the patient with AGAT deficiency at birth (grey line) and at 24 months, after 20 months of oral creatine supplementation (black line). Note the absence of a creatine (Cr) peak at 3.05 ppm at birth and its partial restoration after early treatment. Other differences in the spectrum represent the physiological changes with age and mainly reflect myelination during the first years of life. *Myo*-inositol (mI) is higher and N-acetylaspartate (NAA) is lower at birth.

stopped by addition of 1 N PCA after 4 hours of incubation at 37 °C. The end-product of the reaction (GAA) was separated and quantified with an Agilent GC/MS, set in Electron Ionization – SIM mode (Alessandri *et al.*, 2005). In control samples, AGAT activity was 0.243–0.425 and 0.950–1.470 nmol/mg/h for lymphocytes and lymphoblasts, respectively. Enzyme activity in AGAT-D patients was below the detection limit in both cell types.

During the first 15 days after birth, serum and urine Cr and GAA levels decreased even further (serum Cr 3.2 μM; serum GAA 0.04 μM; urine Cr 20.7 μM; urine GAA 0.34 μM). Brain MRI was normal but [1]H-MRS showed an almost complete absence of the cerebral Cr peak at 3.05 ppm, confirming the diagnosis of AGAT-D (Figure 3) (Battini *et al.*, 2006). In order to replenish the Cr depletion and considering that the child was breast-fed, we initially tried to supplement the maternal diet with Cr monohydrate (3 to 9 g/d). After one month of maternal supplementation, an increase in Cr concentration in the maternal milk (190 μM; normal range 82.3–128.9 μM) was detected; the serum and urine Cr concentrations were 4.04 μM and 6.3 μM, respectively, while GAA was undetectable in both serum and urine. Unfortunately, the Cr increase in blood, urine and brain of the child was unremarkable. At the

age of 4 months, following weaning, dietary supplementation of the child with Cr monohydrate was initiated. We started with a low dosage of oral Cr (100 mg/kg/d) divided in five dpd. This dosage was selected based on (i) the lack of toxicological data in neonates and (ii) results obtained in his affected relatives for which optimization of Cr therapy led to the conclusion that 100 mg/kg/d might be the minimal optimal dose of Cr to be administered chronically (Bianchi *et al.*, 2007). Subsequent assessment of serum and urine Cr levels revealed a progressive replenishment of body Cr pools by the end of the first week of treatment: after 1 month of supplementation of the child, serum and urine Cr concentrations were 222 μM and 1.75 mM respectively; after 3 months 164 μM and 4.32 mM after 6 months 172 μM and 5.35 mM, and after 20 months 67.7 μM and 12.7 mM respectively. GAA levels in body fluids remained undetectable (Battini *et al.*, 2006). Serum concentration of Cr overlapped with those obtained in the affected relatives which were treated with a higher dosage of Cr (Battini *et al.*, 2002; Bianchi *et al.*, 2000). At the age of 12 months, after 8 months of treatment, we performed a control examination with brain ^{1}H-MRS and demonstrated the restoration of about 60% of normal brain Cr levels. Growth and psychomotor development of the child remained completely normal. At the age of 12 months, he walked unaided and uttered single words. At the age of 16 months he was able to ask by gestures and from the age of 18 months he produced some two-word combinations and understood simple verbal requests. His general developmental quotient was 105 (Griffiths Developmental Scales), and his growth was normal (Battini *et al.*, 2006). It is of interest that in the affected relatives, the first clinical symptoms appeared at an age of around 8–10 months, while at around 18 months, an important delay in somatic growth and psychomotor development, associated with hypotonia or autistic-like behaviour, was already apparent (Battini *et al.*, 2002; Bianchi *et al.*, 2000). At the age of 24 months, after 20 months of therapy at the same oral Cr dosage as from the start (100 mg/kg/d), a new ^{1}H-MRS examination showed similar Cr replenishment as at 12 months (Figure 3). The baby was healthy; his psychomotor development and social interaction were according to age. Cr supplementation was always well tolerated. No side effects were observed except for some episodes of diarrhoea at the beginning of treatment and when the dosage was increased according to weight gain.

This patient is the first subject with AGAT-D diagnosed in the neonatal period and treated when still asymptomatic. The observation in this patient has proven that blood GAA and Cr levels, which can be measured both in plasma and dried blood spots, are significantly low from the first days of life, supporting their use as early diagnostic markers for AGAT-D (Carducci *et al.*, 2002, 2006). Severe brain Cr depletion, as detected by ^{1}H-MRS, was already present since the first days of life and, even with Cr being a component of maternal milk, we found that this nutrient has a limited efficacy in the maintenance of Cr pools when a defect in endogenous Cr synthesis is present. The latency in clinical manifestation in Cr disorders may be related to a relatively low need for the Cr/PCr system during the early phases of brain development.

Despite the expression of the *GAMT*, *AGAT* and *CT1* genes in different stages of the developing rat embryo (Braissant *et al.*, 2005) and the disturbed Cr and

GAA levels in brain of neonates affected with AGAT-D or GAMT-D, no clinical abnormalities are found in newborns. Probably, brain Cr depletion causes a slow and cumulative effect on nervous development, in particular on higher cortical function, as shown by the presence of mental retardation and severe language deficits as specific hallmarks of all Cr disorders (Battini *et al.*, 2002; Schulze, 2003; Mancini *et al.*, 2005; Mercimek-Mahmutoglu *et al.*, 2006). Although congenital depletion of brain Cr would support very early treatment, it is of interest that a 4-month delay in starting Cr administration does not seem to have affected the development of the child so far. The Cr dosage we used was about a quarter of that administered initially in previously reported AGAT-D patients (Battini *et al.*, 2002; Bianchi *et al.*, 2000) and proved to be safe and effective in replenishing both the peripheral Cr pools and partially also the brain Cr pool.

5. PRE-SYMPTOMATIC TREATMENT IN A GAMT-D PATIENT

GAMT-D (MIM 601240) is an autosomal-recessive inherited Cr synthesis defect and has the most severe phenotype among the Cr deficiency syndromes. Patients are clinically affected by mental retardation, lack of speech, autistic behaviour, extrapyramidal movement disorder, and epilepsy (sometimes refractory to therapeutic intervention) (Schulze, 2003). Cr deficiency and accumulation of GAA, the latter known for its neurotoxic and epileptogenic effects (da Silva *et al.*, 1999; D'Hooge *et al.*, 1992; Neu *et al.*, 2002), contribute to the pathophysiology of GAMT-D (Schulze *et al.*, 2001). Treatment in GAMT-D is directed towards Cr replenishment and decreasing of GAA levels (Schulze, 2005). Despite treatment, the clinical outcome of patients diagnosed in childhood or adulthood is still unfavourable (Mercimek-Mahmutoglu *et al.*, 2006). Pre-symptomatic disease detection and early initiation of treatment, which are potentially essential for a good outcome, have only been reported in one patient (Schulze *et al.*, 2006).

The girl is the second child of healthy, non-related Turkish parents. Her 5-year-old brother has GAMT-D which was diagnosed and treated at age 2 ¾ years. Because of the 25% probability of being affected, the girl was followed clinically and biochemically since birth. She was born at term by spontaneous delivery after an unremarkable pregnancy. Clinical examination up to age 3 weeks was normal. She was breast-fed with addition of preterm formula. Red blood cell count and serum chemistries (electrolytes, liver function tests, urea, glucose, protein) as well as clotting were normal. EEG and brain MRI revealed no abnormalities.

Blood was taken from the umbilical cord and subsequently every 12 hours until day 5 by heel prick, and was spotted onto filter paper. In the dried blood spot specimens, measurement of GAA, Cr, and creatinine was performed by means of electrospray-ionization tandem mass spectrometry (modified from Bodamer *et al.*, 2001). The results were informative for the diagnosis of GAMT-D in all specimens from birth to day 5 (Figure 4). GAA was already elevated in cord blood. A subsequent increase during the first 24 hours of life was followed by a decline thereafter. However, GAA remained permanently elevated until day 5 by exceeding the 99.5th percentile for healthy newborns. The levels of Cr and creatinine, both

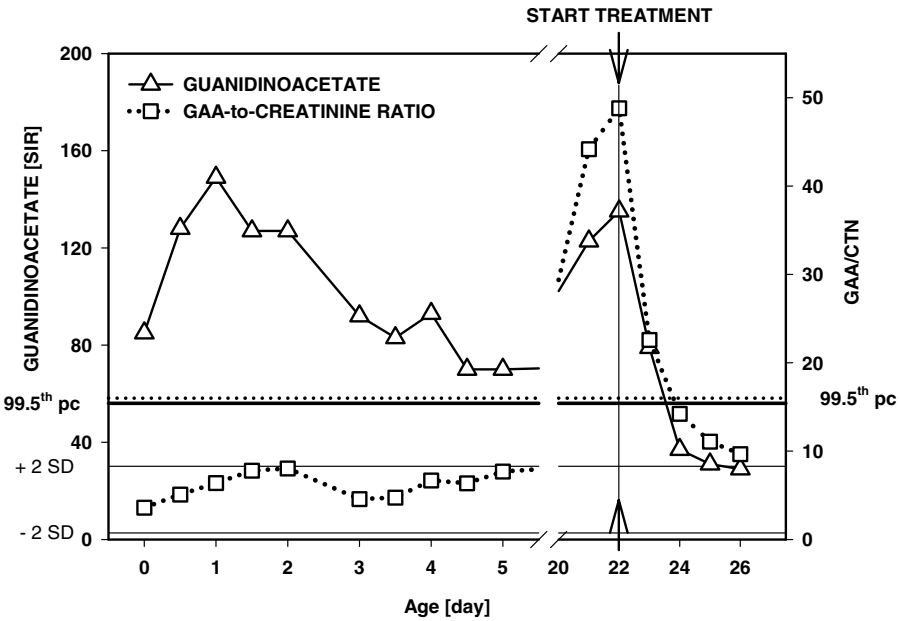


Figure 4. Course of guanidinoacetate (GAA) level and GAA-to-creatinine ratio (GAA/CTN) in dried blood spots of a patient with GAMT-D during the first 5 days of life and at the start of treatment. Guanidino compounds were analyzed by tandem-mass spectrometry. Normal range of GAA (±2 standard deviations, SD) and cut-off limit for GAA and GAA/CTN in neonates (99.5th percentile, pc) were calculated from results in healthy neonates (n = 3, 407). SIR, signal intensity ratio (Permitted reprint from: Schulze A., Hoffmann G.F., Bachert P., Kirsch S., Salomons G.S., Verhoeven N.M., and Mayatepek E., 2006, Presymptomatic treatment of neonatal guanidinoacetate methyltransferase deficiency. *Neurology* **67:** 719–721).

usually decreased in GAMT-D, were of no diagnostic relevance during the neonatal period. Creatinine was within the normal range at birth and decreased only gradually until day 5. The resulting GAA/creatinine ratio was unrevealing. Only in the third week of life did the GAA/creatinine ratio reach higher diagnostic sensitivity owing to a decrease in creatinine levels after the first week of life (Figure 4) (Schulze *et al.*, 2006).

Guanidino compounds in urine and plasma were measured by cation-exchange liquid chromatography (Schulze *et al.*, 1997). In urine, GAA was already slightly increased from the second day of life, whereas Cr was below the normal range. In the third week of life, the GAA increase in urine was even more pronounced, and in plasma, the GAA concentration was elevated, Cr decreased, and creatinine close to the lower limit of controls (Table 1). We investigated the total Cr content in brain by single-voxel ^{1}H-MRS (Schulze, 2003). Intensity ratios calculated from ^{1}H-MR spectra showed a largely decreased but still detectable Cr level in the brain at the age of three weeks ($I_{Cr}/I_{H2O} \sim 8 \times 10^{-5}$) (Figure 5) (Schulze *et al.*, 2006).

Table 1. Guanidino compounds in plasma and urine prior and during treatment of a pre-symptomatically diagnosed and treated patient with GAMT-D (Permitted reprint from: Schulze A., Hoffmann G.F., Bachert P., Kirsch S., Salomons G.S., Verhoeven N.M., and Mayatepek E., 2006, Presymptomatic treatment of neonatal guanidinoacetate methyltransferase deficiency. *Neurology* **67:** 719–721) .

Age, d	Plasma [μmol/L]			Urine [mmol/mol creatinine]	
	Guanidinoacetate	Creatine	Creatinine	Guanidinoacetate	Creatine
2				232	13
19	8.27	6	13	340	11
20				326	20
21	9.09	5	7	349	35
22					
	Start of Treatment				
23	3.60	238	54	456	6,795
26	6.75	258	39	454	8,026
28	2.88	512	14		
62	3.21	795	39	133	10,352
113	4.07	278	109		
216	4.15	388	16	174	8,297
419	4.10	498	25	179	13,195
421	3.23	251	28	173	8,665
Controls	0.20–1.46*	50–124*	5.2–35.2*	28–180†	28–1,700†

* Normal range, derived from healthy subjects at age 1 week to 2 years (n = 17)
† Normal range, derived from healthy subjects at age 1 month to 2 years (n = 16)

Mutation analysis of the *GAMT* gene of the girl (and her parents) using direct sequencing of genomic DNA (Caldeira Araujo *et al.*, 2005) confirmed compound heterozygosity for the two mutations known from her brother (Item *et al.*, 2004). c.152A>C in exon 1 results in replacement of a highly conserved histidine by proline at position 51 (p.His51Pro) and was not detected in 210 control chromosomes, suggesting that the mutation is pathogenic. The frameshift mutation c.526dupG in exon 5 (p.Glu176GlyfsX15) predicts a truncated protein. This indicates that the mutation is pathogenic. GAMT activity in lymphoblasts was analyzed by an enzyme assay using substrates labelled with stable isotopes (Verhoeven *et al.*, 2004). Activity was < 1 pmol/h/mg protein (control range: 63–443 pmol/h/mg protein) confirming the diagnosis of GAMT-D.

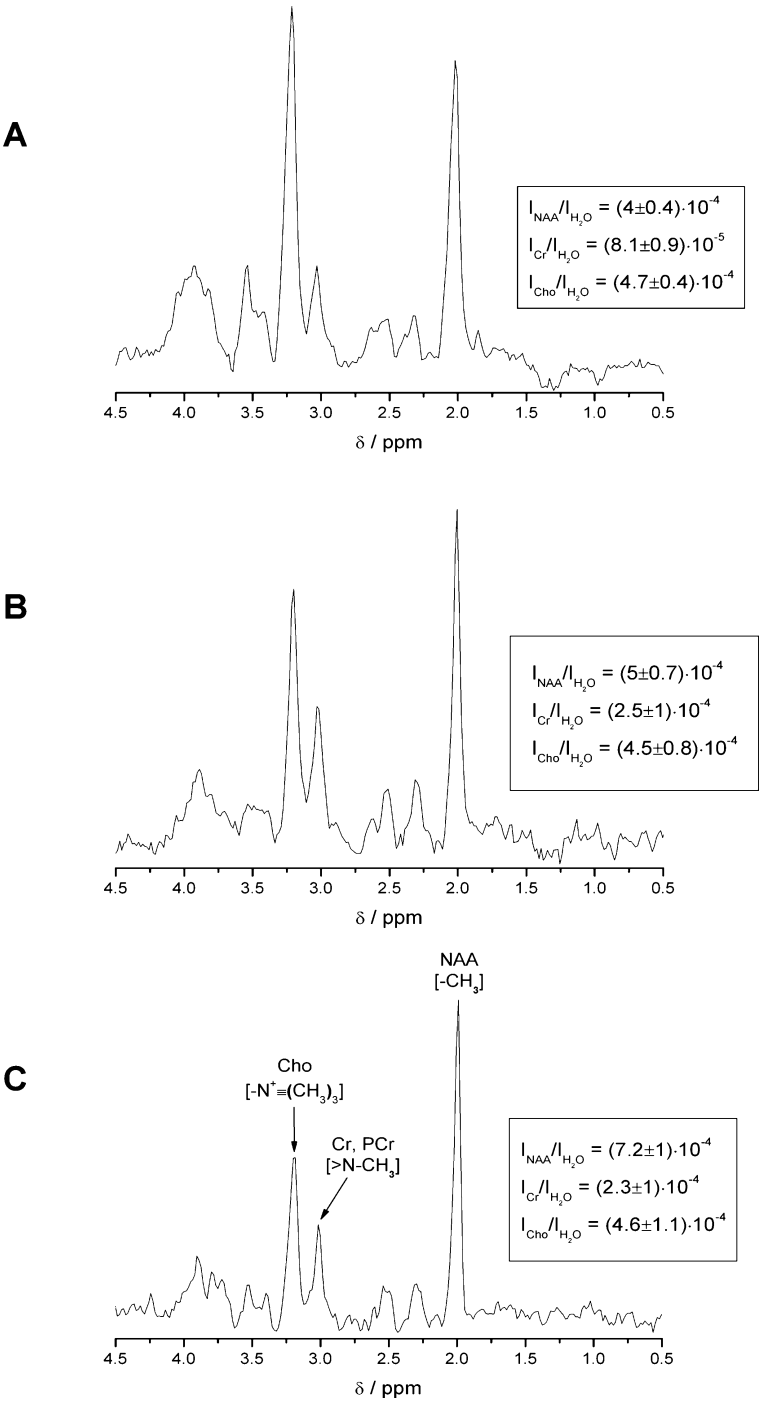
At the age of 22 days, treatment was initiated. Oral Cr monohydrate was given (400 mg/kg/d in 6 dpd). Sodium benzoate was given at 100 mg/kg/d in 3 dpd. We started oral ornithine hydrochloride supplementation with a dosage of 400 mg/kg/d in 6 dpd, increased the dosage to 600 mg/kg/d after two days, and to the final dosage of 800 mg/kg/d after another two days. The parents opted for an additional dietary treatment for their baby. Dietary arginine restriction was started with (per day) 50 ml breast milk, 8 g preterm formula, 35 g basic-p® (SHS, Germany), 20 g maltodextrin® (SHS, Germany), and 6 g E-AM1® (SHS, Germany). Thus, the daily intake of natural protein, of protein from arginine-free amino acid mixture, and of calories was 0.4 g/kg, 1.0 g/kg, and 102 kcal/kg, respectively. As a result of

treatment, an immediate and distinct decrease of GAA levels in dried blood and plasma and, with some delay, in urine was observed (Figure 4 and Table 1) (Schulze *et al.*, 2006). At the age of 4 weeks after weaning, the diet was adapted to (per day) 27 g infant formula, 40 g basic-p®, 20 g maltodextrin®, and 7 g E-AM 1®. The resulting daily intake of natural protein, of protein from arginine-free amino acid mixture, and of calories was 0.6 g/kg, 1.0 g/kg, and 96 kcal/kg, respectively. At the age of 4 months, the parents felt comfortable with the treatment and the development of their child. Somatic growth, clinical status, and routine laboratory chemistries were normal, except for a slight metabolic acidosis (base excess [BE] ~ -5 mM). The diet and medication were adjusted further according to the child's weight. The Cr/PCr signal in ^{1}H-MRS increased significantly after 4 months of treatment ($I_{Cr}/I_{H2O} \sim 2.5 \times 10^{-4}$) and remained constant after 11 months of treatment. While I_{Cho}/I_{H2O} did not change, a continuous increase in the N-acetylaspartate level was seen during the examination period, indicating growth and formation of neuronal tissue (Figure 5) (Schulze *et al.*, 2006).

At the age of 7 months, the child's behaviour was reported as agile and friendly. She fixed and followed objects, and was babbling busily. She was able to freely sit without support already from the age of 6 months. Special neurodevelopmental investigation was normal except for slight dissociated motor development because she had not yet attained rotating skills. Medications and diet were tolerated well. The metabolic acidosis (BE -5.7 mM) persisted. The treatment was continued with adjustment for weight gain.

At 14 months of age, the girl is healthy and is developing normally. In the special neurodevelopmental investigation, her psychomotor and psychosocial development is according to age. She uses at least five meaning words correctly, understands simple verbal demands, and knows and shows several social gestures. Her gait is normal. Neurological status including muscle tone, tendon reflexes, and brain nerves are unaffected. She acquired the skills of crawling and free standing at 8 months, walking without support at 11 months, and stair climbing at 12 months. During treatment, the GAA concentration in plasma remained permanently reduced to about 50–60%, although still elevated compared to controls. GAA in urine was permanently close to normal. Creatinine in plasma and urine was normal. Cr levels in urine

Figure 5. Single-voxel 64-MHz ^{1}H-MR spectra of the brain of the patient with GAMT-D obtained at age 3 weeks (A), after 4 months (B) and after 11 months (C) of treatment. Measurement technique: double-spin echo sequence (PRESS), repetition time TR = 1500 ms, echo time TE = 135 ms, number of excitations nex = 256, water-signal suppression; B0 = 1.5 T, imaging head coil. The voxel [$(1.5\ \text{cm})^3$] was placed in the left occipital region. Peak assignments: N-acetyl-L-aspartate (NAA, chemical shift $\delta = 2.0$ ppm), creatines (Cr, $\delta = 3.0$ ppm), cholines (Cho, $\delta = 3.2$ ppm). These signals were normalized to the unsuppressed water signal obtained from the same voxel with the same measurement parameters except nex = 10. The intensity ratios I_{meta}/I_{H2O} are given in the boxes (Permitted reprint from: Schulze A., Hoffmann G.F., Bachert P., Kirsch S., Salomons G.S., Verhoeven N.M., and Mayatepek E., 2006, Presymptomatic treatment of neonatal guanidinoacetate methyltransferase deficiency. *Neurology* **67:** 719–721).


A
$I_{NAA}/I_{H_2O} = (4\pm0.4)\cdot10^{-4}$
$I_{Cr}/I_{H_2O} = (8.1\pm0.9)\cdot10^{-5}$
$I_{Cho}/I_{H_2O} = (4.7\pm0.4)\cdot10^{-4}$
4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
δ / ppm
B
$I_{NAA}/I_{H_2O} = (5\pm0.7)\cdot10^{-4}$
$I_{Cr}/I_{H_2O} = (2.5\pm1)\cdot10^{-4}$
$I_{Cho}/I_{H_2O} = (4.5\pm0.8)\cdot10^{-4}$
4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
δ / ppm
C
NAA
[-CH3]
Cho
[-N+≡(CH3)3]
Cr, PCr
[>N-CH3]
$I_{NAA}/I_{H_2O} = (7.2\pm1)\cdot10^{-4}$
$I_{Cr}/I_{H_2O} = (2.3\pm1)\cdot10^{-4}$
$I_{Cho}/I_{H_2O} = (4.6\pm1.1)\cdot10^{-4}$
4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
δ / ppm

and plasma, due to supplementation, were elevated approximately 5-fold (Table 1). The mean plasma ornithine concentration was 160 μM, close to the treatment target concentration of ~ 200 μM. The persistent metabolic acidosis (BE −4.1 mM), also observed in other GAMT-D patients receiving high-dose ornithine hydrochloride, was assigned to the administration of ornithine as hydrochloride. We therefore changed to ornithine aspartate (800 mg ornithine/kg/d), which resolved the slight acidosis immediately and permanently. This approach resulted in a similar decrease of GAA levels, without having other adverse effects.

The first prospective observation and treatment in a neonate with GAMT-D thus provides evidence that diagnosis is possible at birth and that early treatment might be beneficial in the prevention of clinical symptoms. Biochemical findings at the age of 3 weeks were similar to those found in older patients, except for Cr in the brain which – in contrast to the findings in the AGAT patient above – did not seem to be lacking entirely. This suggests (near) normal prenatal Cr supply. This hypothesis is further supported by our finding of normal Cr and creatinine levels in dried blood during the early neonatal period. Slow postnatal release of Cr/PCr pools might explain the pre-symptomatic period of 3–6 months in all GAMT-D patients.

Treatment in GAMT-D is directed towards replenishment of Cr and reduction of GAA. GAA reduction is achieved by dietary arginine restriction combined with low-dose ornithine (100 mg/kg/d) (Schulze *et al.*, 2001). In addition, benzoate is given for substrate deprivation of the AGAT reaction. In our patient we have chosen a higher ornithine dosage because high-dose ornithine supplementation (800 mg/kg/d) is supposed to inhibit AGAT and to lower GAA formation more efficiently (Schulze, 2005). The treatment was well tolerated without adverse effects, except for slight metabolic acidosis which was caused by the ornithine hydrochloride formulation. Acidosis resolved after changing to ornithine aspartate. The biochemical effect of treatment comprised, besides partial Cr replenishment, the distinct and permanent reduction of GAA levels in plasma and urine.

Diagnostic work-up in patients is generally started only when deficits in cognitive function and absence of speech become clinically evident. If these symptoms are caused by irreversible brain impairment, it is self-evident why treatment usually fails. On the other hand, when treatment can be initiated before irreversible damage occurs, clinical symptoms may possibly be prevented entirely and permanently. These considerations may explain why the clinical course for the girl diagnosed and treated early is distinct from that of her brother who was treated late. His first clinical symptoms became obvious at age 6–9 months with few spontaneous movements, little interest in surrounding and playing, and delayed general development. At age 2 years he was able to use only few words correctly with no further speech development. At age 2 ½ years he developed epileptic seizures. The normal psychomotor and psychosocial development in the pre-symptomatically treated girl also differs from the common clinical course of all other GAMT-D patients reported so far.

6. CONCLUSION

Unspecific symptoms and normal results in standard metabolic testing may mean that a substantial number of AGAT-D and GAMT-D patients remain undiscovered and others will be diagnosed late. Independent of the age at diagnosis, all patients benefit from treatment. Different treatment approaches have shown to be efficient, even if neither Cr normalisation nor GAA normalisation is completely achieved. Perhaps, complete recovery is not required for normal functioning, and early treatment prior to irreversible brain impairment is much more crucial. Besides clinical and animal studies to further improve treatment, attempts at early diagnosis are mandatory. Both cases described above have proven not only the benefit of pre-symptomatic treatment but also that diagnosis is feasible already at birth. Classical criteria for the inclusion of inherited disorders in neonatal screening programmes are a pre-symptomatic phase of the disease, the availability of treatment options, the presence of simple diagnostic tools, and a relatively high frequency of the disease. Therefore, pilot studies for neonatal screening of AGAT-D and GAMT-D are warranted.

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