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Review

Biochemical and clinical characteristics of creatine deficiency syndromes✪

Jolanta Sykut-Cegielska[1]✉, Wanda Gradowska[1], Saadet Mercimek-Mahmutoglu[2] and Sylvia Stöckler-Ipsiroglu[2]✉

[1]*Division of Metabolic Diseases, Department of Pedatrics, Children's Memorial Health Institute, Warsaw, Poland;* [2]*Department of Pediatrics, and National Newborn Screening Laboratory, University Hospital and General Hospital of Vienna, Austria*

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Creatine deficiency syndromes are a newly described group of inborn errors of creatine synthesis (arginine:glycine amidinotransferase (AGAT) deficiency and guanidinoacetate methyltransferase (GAMT) deficiency) and of creatine transport (creatine transporter (CRTR) deficiency). The common clinical feature of creatine deficiency syndromes is mental retardation and epilepsy suggesting main involvement of cerebral gray matter. The typical biochemical abnormality of creatine deficiency syndromes is cerebral creatine deficiency, which is demonstrated by *in vivo* proton magnetic resonance spectroscopy. Measurement of guanidinoacetate in body fluids may discriminate between the GAMT (high concentration), AGAT (low concentration) and CRTR (normal concentration) deficiencies. Further biochemical characteristics include changes in creatine and creatinine concentrations in body fluids. GAMT and AGAT deficiency are treatable by oral creatine supplementation, while patients with CRTR deficiency do not respond to this type of treatment. The creatine deficiency syndromes are underdiagnosed, so their possibility should be considered in all children affected by unexplained mental retardation, seizures and speech delay.

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✉Correspondence to: Sylvia Stöckler-Ipsiroglu, Department of Pediatrics, Vienna University, Währingergürtel 18-20, A-1090 Vienna, Austria; tel.: (43 1) 404 003 210; fax: (43 1) 406 3484; e-mail: stoeckler@metabolic-screening.at; e-mail address of Jolanta Sykut-Cegielska: cegielska@czd.waw.pl

Abbreviations: AGAT, L-arginine:glycine amidinotransferase (EC 2.1.4.1); CRTR, creatine transporter; GAMT, guanidinoacetate *N*-methyltransferase (EC 2.1.1.2); MRI, magnetic resonance imagnig.

Creatine (α-methyl-guanidinoacetic acid) and phosphocreatine play an essential role in the storage and transmission of phosphate-bound energy. Despite the importance of creatine, its metabolism and distribution in humans are not well understood (Bianchi *et al.*, 2000).

Creatine is synthesized mainly in the liver and pancreas by the action of arginine:glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase (GAMT). Creatine reaches muscle and brain *via* an active transmembrane creatine transport system (CRTR). Creatine is then utilized in the cellular pool of creatine/phosphocreatine, which together with creatine kinase and ATP/ADP provides a high energy phosphate buffering system. Intracellular creatine and creatine phosphate are non-enzymatically converted to creatinine, with a constant daily turnover of 1.5% of body creatine. Creatinine is excreted in urine and the daily urinary creatinine excretion is directly proportional to total body creatine (Fig. 1).

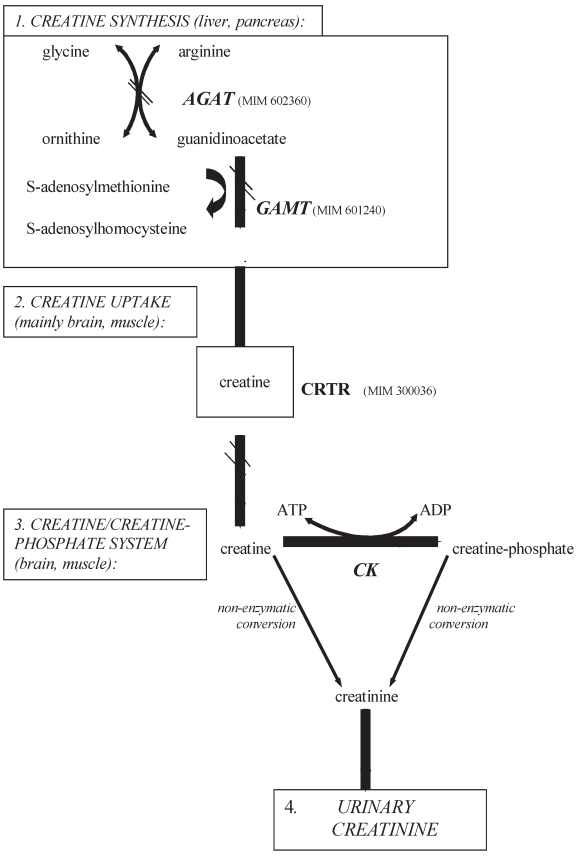

Figure 1. Metabolic pathway of creatine/phosphocreatine.

AGAT, arginine:glycine amidinotransferase; GAMT, guanidinoacetate methyltransferase; CRTR, creatine transporter; CK, creatine kinase.

According to the metabolic pathway of creatine, creatine deficiency syndromes may be due to disorders of creatine synthesis including AGAT (MIM 602360) and GAMT (MIM 601240) deficiency and disorders of creatine transport including the transmembrane creatine transporter (CRTR, MIM 300036) deficiency. GAMT deficiency was recognized as the first inborn error of creatine metabolism in 1994 (Stöckler *et al.*, 1994), and a few years later, AGAT deficiency (Item *et al.*, 2001) and CRTR (SLC6A8) deficiency (Salomons *et al.*, 2001) were described. Inheritance of GAMT and AGAT deficiency is autosomal recessive, while CRTR deficiency is X-linked. So far, about 20 patients with GAMT deficiency, 4 patients with AGAT deficiency, and more than 20 patients with CRTR deficiency have been diagnosed worldwide.

CLINICAL CHARACTERISTICS

The main clinical symptoms observed in all three creatine deficiency syndromes are: mental retardation, seizures and speech delay. Patients with GAMT deficiency exhibit a more complex clinical phenotype with severe to mild presentation. The severe phenotype includes intractable epilepsy, early global developmental delay, extrapyramidal movement disorder and abnormal signal intensities of the basal ganglia. Patients with the intermediate type exhibit moderate to severe mental retardation, speech delay, behavioural changes (autistic, hyperkinetic behaviour), and epilepsy (treatable with common anticonvulsive drugs) with minor or unspecific EEG changes. The few patients described so far with the mild phenotype presented with mental retardation, autistic behavior, and

speech delay (Mercimek-Mahmutoglu *et al.*, 2004, submitted*). Interestingly, patients with disorders of creatine synthesis and creatine transport do not have signs of cardiac myopathy nor do they have pronounced signs of skeletal myopathy, although muscle tissue might be another site of creatine depletion.

BIOCHEMICAL AND MOLECULAR DIAGNOSTICS

Extra- and intracellular creatine pool

Patients with disorders of creatine synthesis have systemic depletion of creatine and creatine phosphate due to impairment of *de novo* creatine biosynthesis. Patients with CRTR deficiency – due to impairment of cellular creatine transport – have intracellular depletion of creatine and creatine phosphate, while extracellular (urinary) creatine concentrations are normal or even elevated.

Creatine in brain

A common denominator of GAMT, AGAT and CRTR deficiency is depletion of the cerebral creatine pool. Direct measurement of total creatine levels in the brain is possible by *in vivo* proton magnetic resonance spectroscopy: a complete lack of creatine, in the presence of a normal spectral pattern of the remaining metabolites, is a striking and unique pattern. Creatine has a prominent proton magnetic spectrum in the brain, and its deficiency cannot be overlooked (Fig. 2). However, still little is known about brain creatine uptake and brain creatine distribution (Bianchi *et al.*, 2000). In the first described patient with GAMT deficiency creatine and phosphocreatine concentrations were measured by *in vivo* proton magnetic resonance spectroscopy in white and gray matter and revealed profound generalized deficiency; 0.3 mmol/L (control values: 5.1 ± 0.9) and 0.2 mmol/L (control values: 5.5 ± 0.8), respectively (Stöckler *et al.*, 1994).

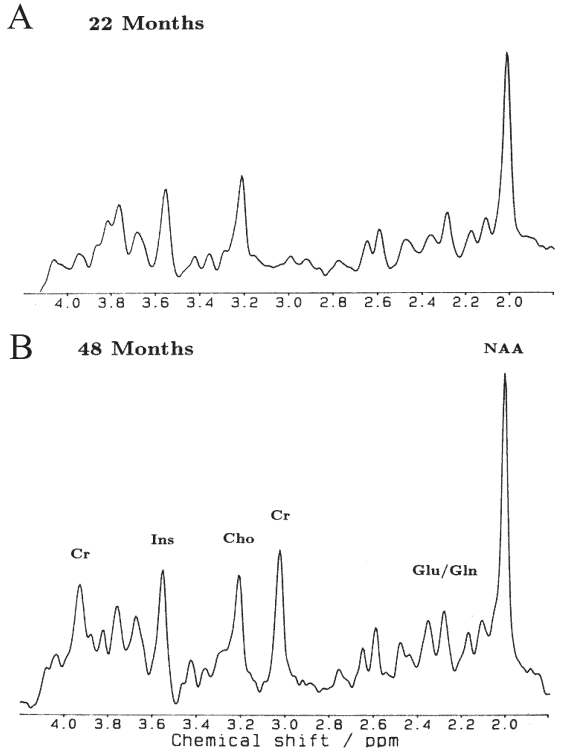

Figure 2. *In vivo* proton magnetic resonance spectroscopy (1H MRS) of the brain of a patient with cerebral creatine deficiency due to GAMT deficiency.

A. Complete lack of creatine resonance. B. Normalisation of creatine spectrum after 6 months of treatment with oral creatine monohydrate.

Creatine in muscle

Muscle contains more than 90% of the body creatine pool. As in the brain, creatine is taken up from blood against a concentration gradient by an active transporter (CRTR). Creatine concentration was low in muscle biopsy of the first reported patient with GAMT

*Mercimek- Mahmutoglu S, Stöckler-Ipsiroglu S, Item CB *et al.*, (2004) Clinical, biochemical and molecular characteristics of guanidinoacetate methyltransferase (GAMT) deficiency, a newly recognized inborn error of creatine biosynthesis. *Ann Neurol.*; submitted.

deficiency (2.4 μmol/g wet weight; normal range: 25.3 ± 5.8) and in another patient with GAMT deficiency muscle creatine, measured by proton magnetic resonance spectroscopy, was detectable, but lower than in normal controls (Ensenauer *et al.*, 2000).

Creatine in body fluids

In patients with GAMT deficiency, plasma and urinary creatine concentrations are low; mean value – 18 μmol/L (normal range: 18–90 μmol/L) and 38–46 μmol/kg per 24 h (normal range: 88–132 μmol/kg per 24 h), respectively (Schulze *et al.*, 1997). In the proton magnetic resonance spectroscopy of random urine samples, of plasma and cerebrospinal fluid the values were found to be below the detection limit: <5 μmol/mmol creatinine, undetectable and <2 μmol/L (control values: 30–1140 μmol/mmol creatinine, 100–264 μmol/L and 25–70 μ/L), respectively (Schulze *et al.*, 1997).

In contrast, in patients with AGAT deficiency, plasma creatine was found to be within the normal range in two patients: 122 and 95 μmol/L, and urinary creatine concentration was only moderately reduced (Bianchi *et al.*, 2000). Therefore, determination of creatine in body fluids seems to be a specific marker of GAMT deficiency, but not for AGAT deficiency. In the patients with CRTR deficiency, the urinary creatine excretion relative to the creatinine excretion is elevated, and the ratio creatine/creatinine can be used as a first biochemical diagnostic marker for this disease.

Guanidinoacetate

The accumulation of guanidinoacetate in tissues and body fluids is pathognomonic for GAMT deficiency, while levels below normal are characteristic for AGAT deficiency. Guanidinoacetate is not altered in CRTR deficiency.

Creatinine

Urinary creatinine excretion is directly related to the intracellular creatine pool. As the cellular creatine pool is diminished both in disorders of creatine synthesis and in disorders of creatine transport, assessment of the daily creatinine excretion in 24-h urine samples may be helpful in the diagnosis of GAMT, AGAT, and CRTR deficiency. However, in various conditions with reduced muscle mass (e.g. in newborns and very young infants and in patients with muscle disease) this test may not be reliable as it merely reflects an unspecific reduction of the body creatine pool.

Plasma creatinine concentrations have been found both below and within (the lower) normal range in patients with creatine deficiency syndromes. Therefore, determination of plasma creatinine concentrations alone is not a suitable diagnostic tool for the recognition of these disorders.

Enzymatic diagnosis

GAMT and AGAT deficiency are confirmed enzymatically by determination of the respective enzyme activities. The highest activities are measured in liver biopsy samples. GAMT is a monomeric, cytosolic protein (relative molecular mass 31000) catalyzing the final step in the biosynthesis of creatine by the transfer of a methyl group from *S*-adenosylmethionine to guanidinoacetate (Fig. 1). The GAMT activity in three control livers varied between 34.1 to 38.2 units/g liver tissue and the residual GAMT activity – below the limit of detection, i.e. 1.9 units/g liver tissue (Stöckler *et al.*, 1996). For a less invasive diagnosis, sensitive assays for the measurement of GAMT and AGAT activities have been developed in fibroblasts and virus (EBV) transformed lymphoblasts. The GAMT activity determined in control human cultivated fibroblasts, virus transformed lymphoblasts and amniotic cells were as follows: 0.38–0.56,

0.61–0.84 and 0.38–0.56 nmol/h per mg protein, respectively. The four described patients with GAMT deficiency had the enzyme activity in fibroblasts and lymphoblasts below the detection limit, i.e. <0.1 nmol/h per mg protein (Ilas *et al.*, 2000).

A radiochemical assay for the determination of AGAT activity in fibroblasts and lymphoblasts, based on the separation of radioactive labelled substrate from reaction product by HPLC, revealed no detectable activity in cell lines from patients with AGAT deficiency (in normal control cell lines the enzyme activity was clearly measurable) (Item *et al.*, 2001). Moreover, for GAMT and AGAT assays the method of stable isotope dilution has been introduced (Verhoeven *et al.*, 2001).

The creatine transporter (CRTR) belongs to the sodium-dependent plasma membrane transporter family. CRTR deficiency may be diagnosed by creatine uptake studies in cultured fibroblasts. Salomons *et al.* (2001) reported recently a non-radioactive creatine uptake method that allows the identification of creatine uptake defect in cultured cells. In fibroblasts of two unrelated patients affected with creatine transporter deficiency the uptake defect was negligible, when the cells were cultured at physiological creatine levels. Only incubations at very high (500 μmol) creatine levels resulted in some uptake (approximately 25% of the values found in control cells).

Mutation analysis

Molecular analysis of the GAMT, AGAT and CRTR genes is available. Thirteen different mutations located in various exons of the GAMT gene have been found in patients with GAMT deficiency (Item *et al.*, 2002; 2004). The four patients with AGAT deficiency (three of them from the same pedigree) were homozygous for the T149X nonsense mutation (Battini *et al.*, 2002; Item *et al.*, 2001; and unpublished results). Different mutations have also been identified in the CRTR deficient families (Bizzi *et al.*, 2002; Hahn *et al.*, 2002; Salomons *et al.*, 2001).

For a review of diagnostic procedures see (Stöckler *et al.*, 2003).

For an overview of clinical and biochemical characteristics see Table 1 and (Stromberger *et al.*, 2003).

TREATMENT AND OUTCOME

The systemic creatine deficiency caused by disorders of creatine synthesis (GAMT and AGAT deficiency) can be corrected by oral supplementation of creatinemonohydrate. Dosages from 350 mg to 2 g/kg body weight per day have been used in patients with GAMT and AGAT deficiency. The dose of 350 mg/kg body weight per day is about 20 times the daily creatine requirement and has been reported not to induce side effects in healthy volunteers (Greenhaff *et al.*, 1993).

GAMT deficiency

The clinical response to oral creatine supplementation demonstrated in the first described patient with GAMT deficiency (Stöckler *et al.*, 1996) includes resolution of extrapyramidal sings and symptoms, substantial developmental progress, improvement of epilepsy and of general condition (Ganesan *et al.*, 1997; Schulze *et al.*, 1997; Stöckler *et al.*, 1996). During the 25-month period of treatment almost complete recovery of brain creatine was achieved. Although creatine supplementation leads to a substantial clinical benefit, none of the patients has achieved normal development.

The accumulation of guanidinoacetate cannot be sufficiently corrected by creatine monohydrate supplementation alone. Therefore dietary restriction of arginine, which is the rate limiting substrate for the synthesis of guanidinoacetate, and substitution of ornithine, which competetively inhibits the synthesis of guanidinoacetate, is an additional

therapeutic approach. Reduction of guanidinoacetate concentrations *via* competitive inhibition of AGAT activity by additional substitution with high doses of ornithine failed (Stöckler *et al.*, 1997). Restriction of dietary arginine, which is the immediate precursor of guanidinoacetate and AGAT substrate, has also failed to lower guanidinoacetate levels (Schulze *et al.*, 1998). Combined arginine restriction and ornithine supplementation can decrease the elevated guanidinoacetate concentrations permanently. As shown in one patient, the correction of the metabolite pattern is also associated with a significant improvement of the clinical outcome (Schulze *et al.*, 2003).

Table 1. Clinical and biochemical characteristics of GAMT, AGAT and CRTR deficiency and diagnostic tests

Disorder	Clinical characteristics	Biochemical characteristics	Diagnostic test	Confirmation
GAMT	Mental retardation	Deficiency of brain creatine	Brain MRS	GAMT activity (f,l)
	Speech delay	Accumulation of guac	Guac u, p, csf, dbs	
	Epilepsy (intractable)	Low creatinine and creatine excretion	Creatinine 24 h urine Creatine & creatinine in CSF*	GAMT mutations (b,f,l,dbs)
	(Extra) pyramidal symptoms & signs	High urinary uric acid/ creatinine ratio	Creatine, creatinine, uric acid in urine	
AGAT	Mental retardation	Deficiency of brain creatine Low guac excretion	Brain MRS Guac u, p, csf, dbs	AGAT activity (f,l)
	Speech delay	Low creatine excretion ?	Creatine urine	AGAT mutations (b,f,l,dbs)
	(Epilepsy)	Low creatinine excretion ?	Creatinine 24 h urine	
CRTR	Mental retardation	Deficiency of brain creatine	Brain MRS	CRTR activity (f,l)
	Speech delay	Low creatinine excretion ?	Creatinine 24 h urine	CRTR mutation (b,f,l)
	Epilepsy	High urinary creatine/ creatinine ratio	Creatine and creatinine urine	

Abbrevations: Guac, guanidinoacetate; u, urine; p, plasma; csf, cerebrospinal fluid; dbs, dry blood spot sample; f, fibroblasts; l, lymphoblasts; b, blood; ?, expected but not measured so far in respective patients. *Stöckler *et al.*, 1997; Schulze *et al.*, 1997.

AGAT deficiency

In three patients with AGAT deficiency, upon oral creatine supplementation (300 mg/kg) an almost complete restoration of the extremely low pretreatment cerebral creatine levels was obtained. The correction of cerebral creatine was accompanied by a favorable clinical response as shown by significant improvement of highly abnormal developmental scores (Battini *et al.*, 2002; Bianchi *et al.*, 2000). As guanidinoacetate concentration is low in AGAT deficiency, creatine substitution alone might effectively prevent neurological sequelae in early treated patients.

CRTR deficiency

Unlike in the patients with GAMT and AGAT deficiency, in CRTR deficiency oral creatine substitution does not result in an increase of brain creatine levels.

For a review of treatment see (Stöckler *et al.*, 2004).

CONCLUSION

Up to now creatine deficiency syndromes have been underdiagnosed. An alertness of clinicians is necessary to identify new patients. Therefore it is important to establish laboratories offering selective screening for specific analytes (quantitative methods for guanidinoacetate and creatine), as well as combined MRI/MRS investigations in patients at clinical risk. For interpretation of MRS results it is important to know that in creatine deficiency syndromes the almost complete lack of cerebral creatine is a striking feature.

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