ARTICLE

Disorders of Creatine Transport and Metabolism

NICOLA LONGO,* ORLY ARDON, RENA VANZO, ELIZABETH SCHWARTZ, AND MARZIA PASQUALI

Creatine is a nitrogen containing compound that serves as an energy shuttle between the mitochondrial sites of ATP production and the cytosol where ATP is utilized. There are two known disorders of creatine synthesis (both transmitted as autosomal recessive traits: arginine: glycine amidinotransferase (AGAT) deficiency; OMIM 602360; and guanidinoacetate methyltransferase (GAMT) deficiency (OMIM 601240)) and one disorder of creatine transport (X-linked recessive SLC6A8 creatine transporter deficiency (OMIM 300036)). All these disorders are characterized by brain creatine deficiency, detectable by magnetic resonance spectroscopy. Affected patients can have mental retardation, hypotonia, autism or behavioral problems and seizures. The diagnosis of these conditions relies on the measurement of plasma and urine creatine and guanidinoacetate. Creatine levels in plasma are reduced in both creatine synthesis defects and guanidinoacetate is increased in GAMT deficiency. The urine creatine/creatinine ratio is elevated in creatine transporter deficiency with normal plasma levels of creatine and guanidinoacetate. The diagnosis is confirmed in all cases by DNA testing or functional studies. Defects of creatine biosynthesis are treated with creatine supplements and, in GAMT deficiency, with ornithine and dietary restriction of arginine through limitation of protein intake. No causal therapy is yet available for creatine transporter deficiency and supplementation with the guanidinoacetate precursors arginine and glycine is being explored. The excellent response to therapy of early identified patients with GAMT or AGAT deficiency candidates these condition for inclusion in newborn screening programs.

KEY WORDS: creatine; X-linked mental retardation; guanidinoacetate; creatine transport; GAMT; AGAT

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INTRODUCTION

Creatine was initially identified as a constituent of meat (*kreas* in Greek) in 1832 and is required for the utilization of ATP-derived energy at sites of high energy utilization (muscle, brain, heart) [Wallimann et al., 1992; Wyss and Kaddurah-Daouk, 2000]. In adult humans, about half of the creatine needed is obtained from diet mostly from meat and dairy products and the other half is synthesized in the kidney and liver [Brosnan and Brosnan, 2007]. Creatine and its phosphorylated form, phosphocreatine, spontaneously break down to creatinine that is excreted in the urine [Brosnan and Brosnan, 2007]. Losses of creatine (as creatinine) are restored by new synthesis and dietary intake; creatine deficiency can develop when synthesis is defective.

Two enzymes are needed for the synthesis of creatine: arginine: glycine amidinotransferase (AGAT or GATM, OMIM 602360) and guanidinoacetate methyltransferase (GAMT, OMIM 601240). AGAT catalyzes the transfer of a guanidino group from arginine to glycine to form ornithine and guanidinoacetate (Fig. 1). Guanidinoacetate methyltransferase catalyzes the transfer of a methyl group from S-adenosylmethionine to guanidinoacetate to form S-adenosylhomocysteine and creatine. The resulting creatine can enter cells and tissues through specific membrane transporters, the most important of which is creatine transporter 1 (CT1, CRTR, CRT, OMIM 300036) encoded by the *SLC6A8* gene.

Brain creatine deficiency syndromes are a group of rare disorders that include two recessive conditions that impair the synthesis of creatine (AGAT deficiency, OMIM 612718; and GAMT deficiency, OMIM 612736) or its transfer to the brain (X-linked recessive

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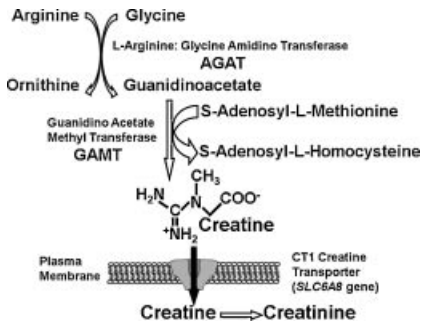

Figure 1. Creatine synthesis and transport. Creatine is synthesized from the amino acids arginine and glycine through the action of the enzymes AGAT and GAMT. AGAT synthesizes guanidinoacetate to which a methyl group is added from S-adenosylmethionine by GAMT to generate creatine. Creatine enters cells and the brain through the CT1 creatine transporter encoded by the *SLC6A8* gene. Metabolism of creatine leads to formation of creatinine that is excreted in urine.

SLC6A8 creatine transporter deficiency, OMIM 300036)[Leuzzi, 2002; Stockler et al., 2007] (Fig. 1). All these disorders are characterized by brain creatine deficiency, detectable by magnetic resonance spectroscopy (MRS) [Leuzzi, 2002; Stockler et al., 2007]. Affected patients have mental retardation, hypotonia, autism, behavioral problems, and seizures [Leuzzi, 2002; Schulze, 2003; Stockler et al., 2007]. This review will describe the function of creatine and the different brain creatine deficiency syndromes.

Brain creatine deficiency syndromes are a group of rare disorders that include two recessive conditions that impair the synthesis of creatine (AGAT deficiency, OMIM 612718; and GAMT deficiency, OMIM 612736) or its transfer to the brain (X-linked recessive SLC6A8 creatine transporter deficiency, OMIM 300036).

FUNCTION OF CREATINE

The major function of creatine is the transfer of high energy groups from the site of production within mitochondria (or in limited amounts from glycolysis) to the sites of energy (ATP) consumption in the cytoplasm (all sorts of cellular ATPases). ATP and ADP can diffuse in very limited amounts. Phosphocreatine and creatine are much smaller than ATP and ADP, can diffuse more easily and can accumulate to higher concentrations within cells without affecting regulatory feedback loops [Wyss and Kaddurah-Daouk, 2000]. This establishes a system capable of constantly regenerating ATP from ADP in tissues with high energy requirements such as the muscle and others.

The transfer of phoshocreatine from mitochondria to the cytoplasm requires a system of kinases (creatine kinases, CK, CPK) in mitochondria at sites of ATP production and in the cytoplasm at sites of ATP utilization. Mitochondrial creatine kinases are usually located at contact sites between the inner and outer mitochondrial membrane. There are at least two human isoforms, sarcomeric sMtCK and ubiquitous uMtCK, that transfer the phosphorus from ATP to creatine to generate phosphocreatine. The phosphocreatine generated is transferred to the cytoplasm and used by another creatine kinase in the cytoplasm (muscle-type MM-CK, brain-type BB-CK, and a heterodimeric MB-CK). The latter is usually anchored to structures requiring high amounts of ATP and transfer the phosphorus back to ADP generated by energy-requiring processes [Brosnan and Brosnan, 2007]. Surprisingly, knocking out the genes for the mitochondrial (sMtK) and the cytosolic (M-CK) CK isoforms in mice produce only mild changes in muscle physiology [Steeghs et al., 1997], possibly related to the presence of alternative mechanisms for the transfer of phosphorus in these tissues including expansion of the mitochondrial pool. By contrast, mice deficient for both BB-CK and uMtCK have a severely abnormal phenotype, with permanently reduced body weight, impaired spatial learning, low nestbuilding activity, impaired hearing, vestibular dysfunction, and partially abnormal morphology of the hippocampal brain structures [Streijger et al., 2009]. Creatine and the creatine kinase system play a major role in the functioning of ATPases essential for restoring ionic gradients, neurotransmitter cycling, molecular synthesis and transport or motility of cell constituents [Wyss and Kaddurah-Daouk, 2000], possibly explaining part of the changes in brain functions seen with defective kinases. Still, the complete biological function of creatine in the brain is not completely understood.

DISORDERS OF CREATINE SYNTHESIS

Arginine: Glycine Amidinotransferase (AGAT) Deficiency (OMIM 612718)

AGAT deficiency was initially described in 2000 and to date less than 10 patients have been reported worldwide [Bianchi et al., 2000; Battini et al., 2002]. The clinical presentation is nonspecific, with developmental delays, mental retardation, autistic behavior, febrile seizures, hypotonia and failure to thrive or slow somatic growth [Bianchi et al., 2000; Battini et al., 2002; Johnston et al., 2005]. The delays in development are more marked in speech and usually present before 1 year of age.

The *GATM* (glycine amidinotransferase, mitochondrial) gene that encodes AGAT spans about 17 kb on chromosome 15q15.3 and is composed of 9 exons (Fig. 2, [Item et al., 2001]). Only few mutations have been identified in

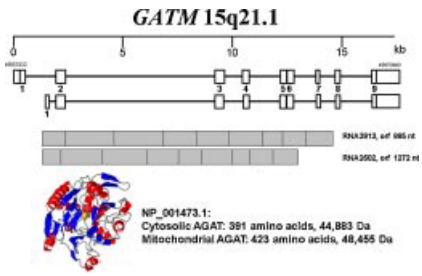

Figure 2. Arginine: glycine amidinotransferase: Schematic of the gene and the protein. Crystal structure reconstructed with coordinates from [Humm et al., 1997].

this gene, due to the rarity of the condition. The patients part of the original family are all homozygous for c.G446A/p.W149X, whereas the other patients are homozygous for IVS3+1G>T leading to abnormal splicing resulting in a frameshift [Item et al., 2001; Johnston et al., 2005]. A recently described patient is homozygous for a frameshift mutation (c.1111_1112insA/p.M371fsX5) [Edvardson et al., 2010].

The *GATM* gene in mice is imprinted and maternally expressed in placenta, but has biallelic expression in other tissues [Sandell et al., 2003]. In vertebrates, the highest levels of expression are seen in liver, kidney, pancreas, or decidua, with expression in kidneys being confined to the cortex [Wyss and Kaddurah-Daouk, 2000]. AGAT is synthesized as a 423 amino acid polypeptide of which the 37 residues in the N-terminus should function as a mitochondrial targeting sequence [Humm et al., 1994]. A second isoform with 5 different amino acids in the N-terminus has a predicted cytoplasmic localization [Humm et al., 1997]. The mature enzyme in human kidney has a Km of about 2.5 mM toward the two substrates, arginine and glycine, and functions as a dimer of two identical subunit each of mass 44,000 [Gross et al., 1986]. Based on studies in rodents, the kidney is the organ in which the activity of AGAT is physiologically most relevant, with the production of guanidinoacetate that transfers to the liver to complete creatine synthesis [Wyss and Kaddurah-Daouk, 2000; Brosnan and Brosnan, 2007]. The creatine produced is then distributed to organs than need more creatine of the amount they can synthesize. AGAT is the rate limiting step in creatine biosynthesis and creatine deficiency, growth hormone and thyroxine increase expression of *GATM* gene [Guthmiller et al., 1994]. By contrast, AGAT activity is inhibited by ornithine (the end product of the reaction [Sipila, 1980]) and its expression is repressed by creatine (the final product of the cycle [McGuire et al., 1984; Guthmiller et al., 1994]).

Guanidinoacetate Methyltransferase (GAMT) Deficiency (OMIM 601240)

GAMT deficiency was initially described in 1996 and to date about 40 patients have been reported [Stockler et al., 1996]. Patients present from a few months of age to 4–5 years of age with developmental delays, seizures (in some cases resistant to therapy), hypotonia (in some cases very severe), autistic behavior, and occasional movement disorder with involuntary movements. Seizures in these patients are many times difficult to control with standard anticonvulsivant therapy.

The cDNA of human GAMT was isolated from a liver cDNA library with the aid of a partial cDNA of rat GAMT [Isbrandt and von Figura, 1995]. It has an open reading frame of 711 nucleotides (Fig. 3). Northern blot analysis of RNA from liver, leukocytes, and fibroblasts detects a single GAMT mRNA species of 1.1–1.2 kb in all tissues and cells [Stockler et al., 1996]. The gene is widely expressed including in liver, brain, skin, lung, pectoral muscle, melanotic melanoma and hepatocellular carcinoma cell lines and many other tissues. High amounts of GAMT mRNA are in skeletal muscle, liver, heart, kidney, and in smaller amounts in brain [Schmidt et al., 2004]. Within the brain, high levels of expression are in cerebellum, cerebral cortex, medulla, caudate nucleus, and thalamus, but significant expression seems present in almost all areas [Schmidt et al., 2004]. The *GAMT* gene that encodes GAMT spans about 4.5 kb on chromosome 19p13.3 and is composed of 6 exons (Fig. 3, [Stockler et al., 1996]). Many different mutations have been identified in this gene, with nonsense mutations representing about two thirds of all mutations [Dhar et al., 2009]. Most mutations are private and have been reported in single or few patients. A splice mutation (c.327G>A) and a missense mutation (c.59G>C, p.W20S) have been reported in multiple families of different genetic background [Item et al., 2001, 2004; Mercimek-Mahmutoglu et al., 2006; Dhar et al., 2009].

In some species, creatine synthesis and transport are not very active before

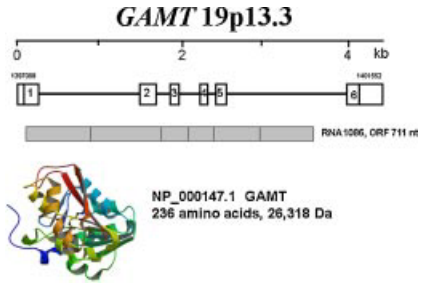

Figure 3. Guanidinoacetate methyltransferase: Schematic of the gene and the protein.

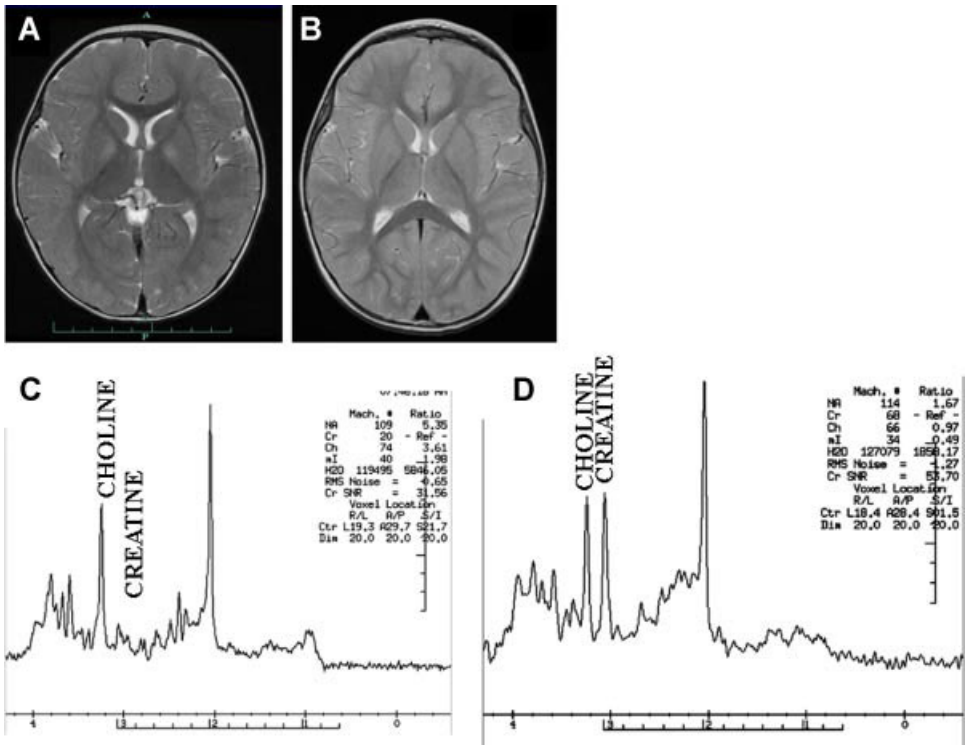

Figure 4. Neuroimaging and MR spectroscopy from a patient with GAMT deficiency. (**A**) Brain MRI at diagnosis demonstrated basal ganglia hyperintensities; (**B**) Brain MRI 15 months after initiation of therapy shows lesion improvement; (**C**) MRS at diagnosis demonstrating reduced creatine peak; (**D**) MRS 15 months after initiation of therapy.

birth, suggesting that transplacental creatine transfer is the major source of creatine to the fetus [Ireland et al., 2009]. GAMT was recently identified as a p53 target gene, potentially involving it in coordinating stress response with changes in cellular metabolism [Ide et al., 2009].

Creatine Transporter Deficiency (OMIM #300352)

Conservation and inter-organ transfer of creatine require specific membrane transporters. The creatine transporter gene SLC6A8 encoding creatine transporter 1 (CT1 or CRTR) maps to Xq28 and is expressed in most human tissues, with highest levels found in skeletal muscle and kidney [Gregor et al., 1995]. A defect in the CT1 creatine transporter results in brain creatine deficiency, an X-linked disorder that in affected hemizygous males is characterized by mental retardation, delays in language and speech, autistic-like behavior, seizures in about 50% of cases, and in some cases mid-facial hypoplasia and short stature [Salomons et al., 2001; Clark et al., 2006]. Affected females can have mild cognitive impairment with behavior and learning problems [Stockler et al., 2007]. This transporter has a high affinity toward creatine (Km is about 30 micromolar in fibroblasts, [Ardon et al., 2010]) and many of the mutations identified to date completely abolish this activity. To date, more than 21 mutations in the *SLC6A8* gene have been reported in patients with varying degrees of mental retardation. The prevalence of *SLC6A8* mutations in X-linked mental retardation varies between 1% and 5.4% [Rosenberg et al., 2004; Newmeyer et al., 2005; Clark et al., 2006; Lion-Francois et al., 2006; Arias et al., 2007; Betsalel et al., 2008; Puusepp et al., 2009; Ardon et al., 2010].

Diagnosis

The clinical presentation of developmental delays or seizures might prompt brain imaging. This can be normal in all disorders (especially early in life) or show relatively nonspecific findings such as delayed myelination or hyperintensity of globi pallidi (Fig. 4). Magnetic resonance (MR) spectroscopy is characteristic and shows a decrease in the creatine peak in all disorders (Fig. 4C). Creatine is one of the major peaks in proton MR spectroscopy and is almost absent in all disorders of creatine synthesis and transport [Verhoeven et al., 2005].

Measurement of plasma and urine levels of creatine and guanidinoacetate provide additional clues to the diagnosis [Stockler et al., 2007]. Table I summarizes the expected biochemical findings in the three conditions. Plasma creatine levels are low in AGAT and GAMT deficiency, while they are usually normal in the creatine transporter deficiency. Plasma (and urine) guanidinoacetate levels are markedly increased in GAMT deficiency and normal or low normal in the other two conditions. Urinary creatine levels are usually increased in

TABLE I. Creatine and Guanidinoaceatate Levels in Blood and Urine of Patients with Defects of Creatine Synthesis and Transport

	Plasma creatine	Plasma guanidinoacetate	Urine guanidinoacetate	Urine creatine/creatinine
AGAT deficiency	Low/normal	Low	Low	Low/normal
GAMT deficiency	Low	High	High	Normal
Creatine transporter deficiency	Normal	Normal	Normal	Low

patients with creatine transporter deficiency when normalized for creatinine levels. They can also be elevated in patients receiving creatine supplements or on special diets. The confirmation of the diagnosis requires functional or molecular studies.

Enzyme assay is available only in Europe for AGAT and GAMT deficiency, while DNA testing is widely available. The diagnosis of creatine transporter deficiency can be confirmed by measurement of long-term creatine accumulation in fibroblasts [Salomons et al., 2001, 2003], measurement of creatine transport [Ardon et al., 2010] or by DNA sequencing [Salomons et al., 2003]. It is still unclear whether there are mutations leaving residual transporter activity with milder phenotype.

Differential Diagnosis

Several conditions can present with developmental delays, seizures, and autistic behavior. Many patients have been diagnosed with cerebral creatine deficiency only after an extensive evaluation has excluded other metabolic causes (among which chromosomal imbalances, mitochondrial disorders, organic acidemias, and aminoacidopathies). In some cases, muscle evaluation provided some evidence for mitochondrial dysfunction [Edvardson et al., 2010] that might possibly be caused by the abnormal creatine cycle.

Creatine synthesis requires arginine and urea cycle defects characterized by low arginine levels, may result in creatine deficiency. Hyperonithinemias (such as that observed in hyperornithinemia–hyperammonemia–homocitrullinuria syndrome or gyrate athrophy of the choroid and retina) can also impair synthesis of creatine by reducing AGAT activity. We have also found reduced creatine levels associated with hypotonia in one patient with multiple acyl CoA dehydrogenase deficiency. The reason for this is still unknown. However both hypotonia and creatine deficiency markedly improved with creatine supplements. In general, the clinical presentation and the results of "routine" metabolic tests (plasma amino acids and plasma acylcarnitine profile) should identify these conditions causing secondary creatine deficiency.

Therapy

Defects of creatine biosynthesis are treated with creatine supplements and, in GAMT deficiency, with ornithine and dietary restriction of arginine through limitation of protein intake

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[Leuzzi, 2002; Schulze, 2003; Stockler et al., 2007]. The dose of creatine in both disorders is 300–1,000 mg/kg per day divided into at least two daily administrations. Ornithine (200–800 mg/kg per day) supplements are used to inhibit the AGAT enzyme, since the toxicity in GAMT deficiency may be due in part to excess guanidinoacetate. When associated to protein restriction (to decrease supplies of arginine, one of the substrates of AGAT), ornithine can decrease guanidinoacetate levels in some patients (up to 70% of the diagnostic values, [Ensenauer et al., 2004; Dhar et al., 2009]). Benzoate (100 mg/kg per day) therapy can further reduce guanidinoacetate synthesis by binding glycine, the other substrate of AGAT. Creatine transporter deficiency responds only partially to therapy with arginine and glycine, the precursors of creatine [Fons et al., 2008]. Treatment initiated at time of symptomatic diagnosis can improve hypotonia, seizures, and overall development, but cannot reverse mental retardation [Leuzzi, 2002; Schulze, 2003]. By contrast, in the few patients with AGAT and GAMT deficiency diagnosed early because of an affected sibling, treatment immediately after birth has prevented mental retardation and all other problems [Battini et al., 2006; Schulze et al., 2006; Schulze and Battini, 2007].

The incidence of these conditions is not well established and probably underestimated. In a study conducted in Europe, 2.7% of children with mild to severe mental retardation had a disorder of creatine metabolism [Lion-Francois et al., 2006]. Because early detection and treatment greatly improve the outcome of patients with creatine biosynthesis defects (GAMT and AGAT deficiency), these conditions are good candidates for inclusion in newborn screening programs. The American College of Medical Genetics felt that disorders of creatine metabolism were adequate candidates for newborn screening, but were not included in the recommended panel because

of lack of a validated screening test [Rinaldo and Howell, 2006].

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Several methods have been developed to screen patients for disorders of creatine synthesis or transport. Methods for screening high risk patients for creatine transporter disorders using urinary creatine [Mercimek-Mahmutoglu et al., 2009] have been reported. However, at present a validated method to screen for creatine synthetic disorders is not yet well established for newborn screening. Initial studies performed in our laboratory indicate that both creatine and guanidinoacetate can be easily detected by MS/MS in newborn screening bloodspots (Schwartz E, Pasquali M, unpublished results). Evaluation of multiple bloodspots from normal controls and affected patients is still required to fully validate this method.

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