REVIEW

Human guanidinoacetate n-methyl transferase (GAMT) deficiency: A treatable inborn error of metabolism

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Abstract: The creatine biosynthetic pathway is essential for cellular phosphate associated energy production and storage, particularly in tissues having higher metabolic demands. Guanidinoacetate N-Methyl transferase (GAMT) is an important enzyme in creatine endogenous biosynthetic pathway, with highest expression in liver and kidney. GAMT deficiency is an inherited autosomal recessive trait that was the first among creatine deficiency syndrome to be reported in 1994 having characteristic features of no comprehensible speech development, severe mental retardation, muscular hypotonia, involuntary movements and seizures that partly cannot be treated with anti-epileptic drugs. Due to problematic endogenous creatine biosynthesis, systemic depletion of creatine/phosphocreatine and accumulation of guanidinoacetate takes place that are the diagnostic features of this disease. Dietary creatine supplementation alone or along with arginine restriction has been reported to be beneficial for all treated patients, although to various extent. However, none of the GAMT deficient patient has been reported to return to complete normal developmental level.

Keywords: CDS; GAMT deficiency; creatine/phosphocreatine system; guanidinoacetate; mental retardation.

INTRODUCTION

Creatine phospho creatine energy shuttle

Creatine (Cr) and Phospho creatine (PCr) system provides an instant energy source of high-energy phosphate and serves as ATP derived energy reservoir (Iqbal *et al., 2015*). It is also involve in energy transport from production site, in mitochondria, to the consumption sites, such as muscles and brain due via active sodium dependent creatine transporter (Guimbal and Kilimann, 1993; Sora *et al*., 1994; Iqbal *et al*., 2011). Most of creatine in body comes through diet as well as it can be synthesized endogenously in liver and pancrease by using glycine and arginine as substrates and Arginine: Glycine Amidino Transferase (AGAT) and Guanidinoacetate N-Methyl Transferase (GAMT) as the catalyst Iqbal, 2009). AGAT is responsible for the reversible transport of amidino group from arginine to glycine yielding ornithine and guanidinoacetate (GAA). Methylation of GAA, catalyzed by GAMT, results in the production of creatine (Cr) and S-adenosyl-L-methionine (SAM) converts into S-adenosyl-L-homocysteine after donating its methyl to GAA (Schulze, 2003). In Cr storing tissues, Creatine Kinase (CK) mediates Phosphorylation/ dephosphorylation of Cr and thus provides a high-energy phosphate buffer during ATP synthesis and utilization (Battini *et al*., 2006). Ultimately, Intracellular Cr and phosphocreatine (PCr) are non-enzymatically cycled to creatinine (fig. 1). This conversion has a constant daily turnover of 1.5% of total body creatine. Creatinine is mainly excreted in urine and daily urinary creatinine excretion is directly proportional to total body creatine concebtrations (Bianchi *et al*., 2000).

Creatine deficiency syndrome (CDS)

Despite its critical importance, until 1994, no primary metabolic disorders of creatine synthesis and transport were identified. Stöckler *et al*. (1994) had reported the first case of GAMT deficiency. X-linked inherited alteration of creatine metabolism was second among CDS to be reported in 2001 in two pedigrees having central nervous system with disturbed creatine transporter 1 (Cecil *et al*., 2001; Salmons *et al*., 2001). Finally in 2001, two Italian sisters, having a previously unclassified brain Cr deficit, were confirmed by Item *et al*. (2001). to be suffering from AGAT deficiency

Biochemical and molecular characterization of GAMT

GAMT is believed to be the major enzyme involved in the metabolic conversion of SAM to SAH and the endogenous biosynthesis of Cr is reported to represent 75% of the total utilization of methionine through SAM in human beings (Mudd *et al*., 1980). GAMT is responsible for the SAM dependent methylation of GAA to yield Cr and SAH. Maximum GAMT expression is reported in kidney, liver and pancreas (Braissant *et al*., 2001) and also detected in lower extents in brain (Stöckler *et al*., 1996; Braissant *et al*., 2008), lymphocytes and fibroblasts (Mudd *et al*., 1980). It has been demonstrated that most SAH dependent methyltransferases including GAMT are inhibited by SAH (Lion *et al*., 2006). The remaining methyl groups are used for DNA, protein and other methylation reactions (Wyss and Kaddurah-Daouk, 2000).

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GAMT gene consists of 6 exon and mapped to chromosome 19p13.3 (Schulze, 2003). GAMT protein has α/ß open sandwich structure and its 1-42 residues in N-terminal section covers the active site entrance, hence making it unavailable for reaction. To open the active site entrance, N-terminal section moves through Brownian motion and SAM along with GAA molecules enters and binds firmly to their respective sites by hydrogen bonds and to get excluded from the active site, unbound water molecules (Komoto *et al*., 2004).

Human GAMT deficiency; Clinical manifestation

GAMT deficiency is an inherited autosomal recessive disorder. Around 50 cases of human GAMT deficiency has been reported, till now from all over the world, since its discovery in 1994 (Van der Knapp *et al*., 2000; Leuzzi, 2002; Stöckler *et al*., 1997; Schulze *et al*., 1997; Dhar *et al*., 2009). The pathophysiology of GAMT deficiency may involve neuromodulatory and/or neurotoxic action of GAA, which is a partial agonist at GABA receptors (Neu *et al*., 2002).

GAMT deficiency has heterogeneous clinical presentations. However, generally developmental delay which is observed at 6 to 12 months of age along with/or developmental arrest in the second year of life, no active or comprehensible speech development, muscular hypotonia, dyskinetic involuntary movements, severe mental retardation and seizures, which partially can not be treated by anti-epileptic drugs, are considered as characteristic features of this disease. In older patients, autism with self injurious behaviour has been reported by Item *et al*. (2004). Intensive extra pyramidal movement disorder and epilepsy are common features observed in patients with a severe phenotype of the disease. Whereas only show developmental delay and mild epilepsy has been reported in mildly affected patients (Stromberger *et al*., 2003).

Human GAMT deficiency; Diagnostic findings

Impaired de novo creatine biosynthesis results in systemic depletion of Cr and PCr and is the hallmark of GAMT deficient patients. *In vivo* proton magnetic resonance spectroscopy has shown that extremely low brain Cr concentrations is the characteristic feature of patients with GAMT deficiency. While GAA accumulates in unusually high concentrations which is the immediate precursor of Cr and substrate of GAMT. Stöckler *et al*. (1994) had reported and confirmed by Leuzzi (2002) that deficiency of PCr is due to reduced availability of Cr for the action of CK. Abundant GAA in the brain gets phosphorylated by CK, instead of Cr, and represents the major proportion of high energy phosphate in GAMT deficient patients. The daily rate of urinary creatinine excretion and creatinine concentrations in plasma and cerebrospinal fluid reduces as a consequence of systemic depletion of Cr and PCr (Ganesan *et al*., 1997; Stöckler *et al*., 1997; Schulze *et al*., 1997; Dhar *et al*., 2009; Bodamer *et al*., 2001).

Mutation analysis

Mutation analysis of GAMT gene is also a routine procedure and it is considered as the confirmatory test for the GAMT deficient patients. Fifteen different type of mutations have been reported so far by using various techniques including PCR (Almedia *et al*., 2007; Dhar *et al*., 2009), DGGE (Almedia *et al*., 2007), DHPLC (Bodamer *et al*., 2009; Iqbal *et al*., 2011) and direct sequencing. These mutations include nonsense, splice site mutations as well as small deletions and insertions. Many mutant alleles has splice site mutation, c.327G>A, indicating that it is one of the most common mutations in *GAMT* gene (Matthews *et al*., 1999; Bodamer *et al*., 2009; Dhar *et al*., 2009).

Treatment and outcome

The treatment approaches focus to restore depleted brain Cr through Cr supplementation in pharmacological doses to GAMT deficient patients. All the patients supplemented with Cr are reported to be benefited by this treatment, although to various extents; however none has been reported to return to completely normal developmental level. An improvement affecting muscular hypotonia, dyskinesia, seizures, social contact, alertness and behaviour is commonly observed in the first few months after initiating Cr treatment. Cr/PCr concentration start increasing during the first months of treatment but generally prolonged supplementation is required (Bodamer *et al*., 2009). The clinical course is timely correlated with the observed changes of Cr/PCr in the brain. However, further clinical improvement, thereafter delays, especially in patients with the severe phenotype, therapy refractory seizures reoccur and the clinical circumstances may deteriorates (Schulze *et al*., 1997;).

Oral supplementation with 0.35-2.0g/day of Cr slowly increases the Cr/PCr concentration in the brain. However, even after several months, Cr/PCr in these patient's brain remains significantly below the normal brain ranges (Bodamer *et al*., 2001). Cr replacement also causes decreased GAA formation in GAMT deficient patients as GAA concentrations are reported to remain largely elevated in cerebrospinal fluid, serum and urine of these patients (Schulze *et al*., 2001). These high GAA concentrations may explain the persistence of epilepsy, one of the common features of the disease (Bodamer *et al*., 2009).

It has been reported that a significant and permanent decrease of GAA in body fluids of some GAMT deficient patients has been observed following dietary arginine restriction (15mg/Kg/day) in combination with ornithine supplementation (100mg/Kg/day) (Schulze, 2003). These subjects showed marked clinical improvement including

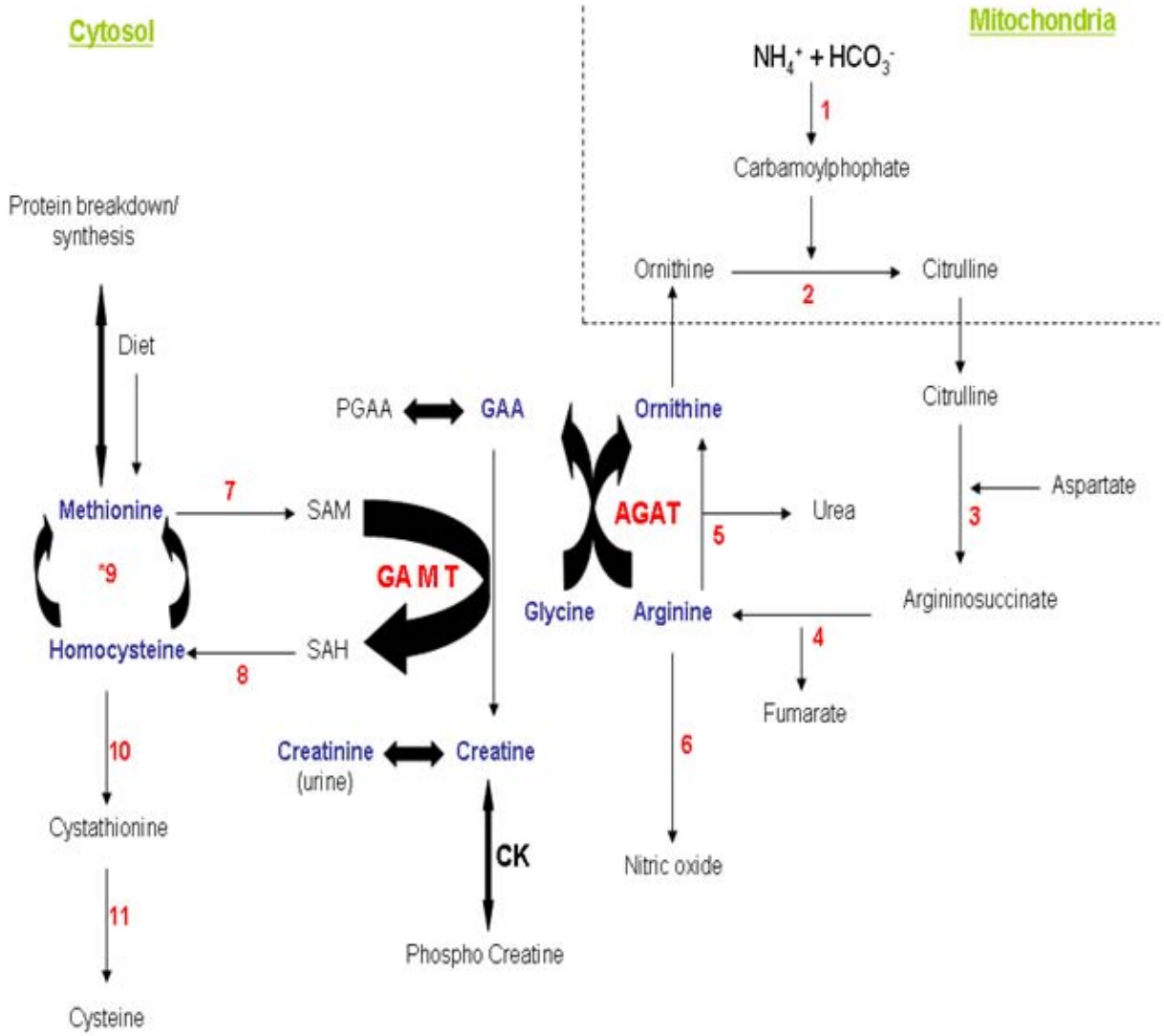

Fig. 1: The creatine/phosphocreatine, methionine-homocysteine and urea cycle pathways in human and animals. Metabolites of interest are in blue while enzymes under study are in red colour. Other enzymes involved are numbered: 1. Carbamoylphosphate synthase 2. Ornithinecarbamoyl transferase 3. Argininosuccinate synthase 4. Argininosuccinate lyase 5. Arginase 6. Nitric oxide synthase 7. Methionine adenosyl transferase 8. S-Adenosyl homocysteine hydrolase 9. 5-methyl THF: Homocysteine methyltransferase 10. Cystationine ß-synthase 11.γ-Cystathionase * Methionine synthase and betaine-homocysteine methyltransferase are also involved in this reaction.

distinctly reduced epileptogenic activities accompanied by almost complete disappearance of seizures and demonstrates the positive effect of GAA reduction in these patients (Schulze *et al*., 2001).

CONCLUSION

GAMT deficiency is a rare recessive metabolic disorder in which endogenous creatine synthesis is compromised. Dietary creatine supplementation alone or along with arginine restriction has been reported to be beneficial for all treated patients, although to various extent. However, none of the GAMT deficient patient has been reported to return to complete normal developmental level.

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