Guanidinoacetate methyltransferase (GAMT) deficiency

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Abstract
Guanidinoacetate methyltransferase (GAMT, EC 2.1.1.2) deficiency is a newly recognized inborn error of creatine synthesis. The clinical phenotype is variable including a spectrum of neurological involvement from progressive extrapyramidal movement disorder and severe muscular hypotonia, to epilepsy and mental retardation. Biochemical findings include high urinary excretion of guanidinoacetate (immediate precursor of creatine and substrate to the deficient enzyme activity), low urinary excretion of creatinine, and depletion of creatine in brain and muscle. Enzymatic diagnosis is possible by the demonstration of deficient GAMT activity in liver, skin fibroblasts and virus transformed lymphoblasts. Prenatal diagnosis has not been performed so far. Symptoms are partly reversible under oral supplementation of creatine-monohydrate. GAMT deficiency is an autosomal recessive inherited disorder. In the 9 patients known so far in the literature, 5 mutant alleles have been identified which are located in exon 2 and exon and intron 6 of the GAMT gene. The most efficient way for investigation of patients at risk seems to be determination of guanidinoacetate in body fluids. Several analytical methods including gas chromatography/mass spectrometry, tandem mass spectrometry and column chromatography are available for this purpose.

Keywords
Guanidinoacetate, creatine, creatinine, mental retardation, epilepsy, extrapyramidal symptoms, creatine-monohydrate, oral supplementation, *in vivo* magnetic resonance spectroscopy.

Disease name and synonyms
Guanidinoacetate methyltransferase (GAMT, MIM 601240) is the second enzyme in creatine synthesis converting guanidinoacetate and S-adenosylmethionine to creatine and S-adenosylhomocysteine. *GAMT* is located on chromosome 19p13.3. In humans, GAMT activity is expressed mainly in liver and pancreas. For diagnostic purpose, GAMT activity is measured in virus transformed lymphoblasts, and in

cultivated fibroblasts. GAMT deficiency is an autosomal recessive inborn error of creatine synthesis (1,2), resulting in deficiency of creatine an in accumulation of guanidinoacetate in tissues and body fluids.

Excluded diseases

Creatine deficiency syndromes are a newly described group of inborn errors of creatine synthesis (arginine:glycine amidinotransferase (AGAT) deficiency and guanidinoaceteate methyltransferase (GAMT) deficiency) and creatine transport (creatine transporter (CRTR) deficiency). The common clinical denominator of creatine deficiency syndromes is mental retardation and epilepsy, suggesting the main involvement of cerebral grey matter (grey matter disease). (36)

Creatine transporter (CRTR) deficiency is differentiated from GAMT deficiency by the 3 following characteristics:

- creatine deficiency is in tissues but not in body fluids;
- normal guanidinoacetate concentrations exist in tissues and body fluids;
- the brain creatine pool is not rapidly restored upon oral supplementation of creatine-monohydrate.

Arginine glycine amidinotransferase (AGAT), which still remains to be discovered as a human inborn error, is differentiated from GAMT by the 3 following characteristics:

- creatine deficiency is in tissues and body fluids;
- normal guanidinoacetate concentrations exist in tissues and body fluids;
- the brain creatine pool is expected to be rapidly restored upon oral supplementation of creatine-monohydrate.

Diagnostic criteria / definition

Guanidinoacetate is the most specific marker of GAMT deficiency and its accumulation in tissues and body fluids is pathognomonic for the disease.

Creatine deficiency is the second diagnostic marker of GAMT deficiency. Creatine is below the normal range both in body fluids (e.g. plasma, urine, cerebrospinal fluid (CSF)) (3) and in tissues (e.g. brain, muscle) (4). Complete lack of creatine in the brain, in the presence of a normal spectral pattern of the remaining metabolites, is a striking and unique pattern which can be found by *in vivo* proton magnetic resonance spectroscopy (4).

Creatinine excretion is directly related to the intracellular creatine pool and the assessment of the daily creatinine excretion in 24-hour urine

samples may be helpful in the diagnosis of creatine deficient states (3). In creatine deficient states with persistently low urinary creatinine excretion, urinary creatinine concentration is not - as usual - a general marker of urinary concentration (6). Therefore various metabolites excreted in the urine (e.g. organic acids and amino acids) may be unspecifically high when given in relation to the urinary creatinine concentration. The unspecific elevation of urinary organic acids was an important diagnostic hint in some patients with GAMT deficiency (4,7,8). Plasma creatinine concentrations have been found both below and within (the lower) normal range in patients with GAMT deficiency.

GAMT deficiency is confirmed enzymatically by determination of GAMT activity in virus transformed lymphoblasts and in fibroblasts (9). Finally mutation analysis will disclose the underlying defect at the DNA level.

Prevalence

The first patient with GAMT deficiency has been described in 1994 (4). Since then, 9 patients have been reported with the disease including: 7 children from families of German (4), Kurdish (7), Welsh (10), Italian (11), Dutch (8), Turkish (8,12) origin, and 2 adult patients (2 sisters) from the Kosovo region (13). As GAMT deficiency is a newly described disorder and diagnostic methods are not generally established, prevalence data are not yet available.

Clinical description

Common denominators of GAMT deficiency are epilepsy, slow background activity in EEG, global developmental delay, failure of active speech, altered signal intensities in the basal ganglia and extrapyramidal movement disorder. The clinical phenotype varies widely from predominance of extrapyramidal encephalopathy (4,7,11) and intractable epilepsy (7,10,12) to moderate mental retardation only (8). Patients with GAMT deficiency do not have signs of cardiac myopathy nor do they have pronounced signs of skeletal myopathy, although muscle tissue seems to be another site of creatine depletion (4).

Management including treatment

Systemic creatine deficiency can be corrected by oral supplementation of creatine-monohydrate. Dosages from 350 mg to 2 g / kg BW (body weight) / day have been used (7,10,13). The dose level of 350 mg / kg BW / day is about 20 times the daily creatine requirement and has been reported not to induce side effects in healthy volunteers (14). Marked increase of

plasma creatine concentrations with peak values after 1 hour of creatine-monohydrate ingestion and normalization of urinary creatinine excretion reflect intestinal creatine absorption and replenishment of intracellular, mainly muscular and brain creatine pool (3,13). Restoration of brain creatine and creatine-phosphate can be monitored by *in vivo* proton and phosphorus magnetic resonance spectroscopy. During a 25-month period of treatment, almost complete recovery of brain creatine was demonstrated in the first described patient (13). Clinical response to oral creatine supplementation include resolution of extrapyramidal sings and symptoms, developmental progress, improvement of epilepsy and of general condition (7,10,13). Although creatine supplementation leads to substantial clinical benefit, none of the patients has achieved normal development.

In contrast to creatine deficiency, accumulation of guanidinoacetate cannot be sufficiently corrected by therapeutic means. Synthesis of guanidinoacetate is catalyzed by the activity of AGAT, the regulating enzyme of creatine biosynthesis. The gene expression of this enzyme is mainly controlled by a creatine-dependent negative-feedback mechanism. Investigations in one patient with GAMT deficiency have shown, that repression of (highly expressed) AGAT activity by exogenous creatine leads to decrease but by far not to normalization of guanidinoacetate in body fluids (3). Further reduction of guanidinoacetate concentrations via competitive inhibition of AGAT activity by additional substitution with high dose ornithine failed (3). Restriction of dietary arginine, which is the immediate precursor of guanidinoacetate and substrate to AGAT activity, has failed to lower guanidinoacetate levels either (15). Combined arginine restriction and ornithine supplementation is able to decrease 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 (16).

Suppression of the neurotoxic effect of guanidinoacetate by selective GABAA receptor antagonists as shown in a knockout mouse model for GAMT deficiency (17) may provide a specific pharmacological approach to the treatment of GAMT deficiency.

Investigation of skeletal muscle by proton and phosphorus magnetic resonance spectroscopy before therapy demonstrated the presence of considerable amounts of creatine and phosphocreatine, and accumulation of phosphorylated guanidinoacetate in a 7-year-old boy diagnosed with GAMT deficiency, suggesting separate mechanisms for creatine uptake and synthesis in brain and skeletal muscle. The combination of creatine supplementation and a guanidinoacetate-lowering therapeutic approach resulted in improvement of clinical symptoms and metabolite concentrations in brain, muscle, and body fluids.

Etiology

Creatine Metabolism
The creatine/creatine-phosphate system plays an important role in the storage and transmission of phosphate bound energy. In humans, creatine is synthesized in liver and pancreas involving arginine and glycine as substrates. (AGAT) and (GAMT) as enzymes (18). Creatine accumulates mainly in muscle and brain via an active transmembrane creatine transport system (19,20,21) and is utilized in the cellular pool of creatine/creatine-phosphate which together with creatine kinase and ATP/ADP, provides a high energy phosphate buffering system (22). Intracellular creatine and creatine-phosphate are non-enzymatically cycled to creatinine, with a constant daily turnover of 1.5% of body creatine. Creatinine is mainly excreted in urine and its daily excretion is directly proportional to total body creatine (23).

According to the creatine pathways in the body, two main categories of disorders in creatine metabolism can be expected:
- Disorders in creatine synthesis,
- Disorders of cellular creatine transport.

GAMT deficiency is the first inborn error of creatine synthesis (1). (AGAT), the potential second disorder of creatine synthesis has not been described so far. Recently the first patient with creatine transporter (CRTR) deficiency has been described (24).

Genetics
Inheritance of GAMT deficiency is autosomal recessive. In the 12 alleles investigated from 6 patients (2,25,26), 3 different mutations have been identified on exon 2, all resulting from truncated enzyme proteins. GAMT activity was below detection limit in the patients bearing these mutations either in homozygous or in compound heterozygous form, while it was within the expected heterozygote range in their parents and heterozygous siblings (9). A G insertion following nucleotide 491 of the cDNA (c.491-492insG) in exon 5 and a transversion at nt -3 in intron 5 (IVS5-3C>G) mutation was found on the remaining 2 alleles of the GAMT gene (25) which have been identified in an

Italian patient described by Leuzzi et al (11). One mutation encodes for a truncated enzyme protein, the other mutation prevents splicing of the last exon of the gene. Although values of GAMT activity have not been reported, it is most probable that these mutations cause a non-functional enzyme protein in this compound heterozygous patient.

In a recent study, Item et al. investigated seven new patients by screening the promoter, 3'UTR, and six exons and exon/intron boundaries using direct sequencing and denaturing gradient gel electrophoresis. The clinical and biochemical phenotype was characterized by scoring the degree of main clinical manifestations and by determination of urinary guanidinoacetate concentrations and of GAMT activity in fibroblasts / lymphoblasts, respectively. They identified 7 novel mutations, including c.64dupG (exon 1; 4/14 alleles); c.59G>C (exon 1; 3/14 alleles); c.491delG (exon 5; 2/14 alleles); c.160G>C (exon 1; 2/14 alleles); and c.152A>C (exon 1; 1/14 alleles); c.526dupG (exon 5; 1/14 alleles); c.521G>A (exon 5; 1/14 alleles), and two polymorphisms c.626C>T (exon 6) and c.459+71G>A (intron 4). Frameshift and missense mutations in exon 1 were prevalent in the 4 patients with the severe phenotype, however a clear genotype-phenotype correlation has not been established in the limited number of patients characterized so far. (37)

Diagnostic methods
Guanidinoacetate can be detected by several methods: semiquantitative detection is possible by thin layer chromatography or high voltage electrophoresis of urinary aminoacids and by staining with the Sakaguchi reagent (27). Quantitative methods for the determination of guanidinoacetate include cation exchange chromatography in aminoacid analyzers adapted for guanidinocompound determination (3,7,28). Determination of urinary guanidinoacetate by gas chromatography / mass spectrometry requires separate derivatisation of samples designated for organic acid screening (29). Stable isotope dilution methods (30) allow exact quantification and might also be valid for determination of guanidinoacetate in amniotic fluid. Guanidinoacetate is also determined by tandem mass spectrometry (10,31,32).

Creatine in body fluids is quantitatively measured by the same methods as applied for guanidinoacetate (3,28,31,32). In vitro proton magnetic resonance spectroscopy is an alternative method which, however, is available only in a few centers: By this method, extremely low creatine concentrations have been demonstrated in urine and CSF in a patient with GAMT deficiency prior to treatment with creatine-monohydrate (7).

Creatinine is determined by enzymatic or color reaction methods in routine clinical chemistry laboratories. Due to several unspecific reactions (e.g. with creatine), chromatographic (High Pression Liquid Chromatography) methods or in vitro magnetic resonance spectroscopy should be applied for reliable and exact determination of creatinine concentrations in body fluids (7,33).

Direct measurement of total creatine levels in the brain is possible by in vivo proton magnetic resonance spectroscopy (34). Complete lack of creatine, in the presence of a normal spectral pattern of the remaining metabolites, is a striking and unique pattern. Guanidinoacetate has a single resonance at 3.78 ppm and can be recognized in same spectrum (11), which allows distinguishing GAMT deficiency from CRTR deficiency.

Absence of GAMT activity was first demonstrated in liver biopsy samples (2), where normally the highest GAMT activity is observed (18). We have developed a sensitive assay allowing reliable measurement of the enzyme activity in fibroblasts and virus (Epstein-Barr Virus) transformed lymphoblasts (9).

Full length cDNA sequencing of rtPCR products and subsequent sequencing of PCR products from particular regions of the genomic DNA has been applied for mutation analysis in the first two patients (2) and in a recently reported Italian patient with GAMT deficiency (25). We have developed a combination of a direct sequencing / denaturing gradient gel electrophoresis (DGGE) based method for mutation screening of the GAMT gene, including the promotor region and the 6 exons of the gene (26). Applying this method in five patients with enzymatically proven GAMT deficiency (9), we have found the c.327 G-A (splice site) mutation on 7 alleles, the c.309ins13 mutation on one allele, and a novel 151 bp deletion (g.1637-1787 del), resulting in a frameshift and premature stop codon, on two alleles.

Genetic counseling / Prenatal diagnosis
GAMT activity is clearly detectable in cultured amniotic cells and activity is comparable to values obtained in virus-transformed lymphoblasts (9). However due to the fact that a high amount of cell protein (corresponding to a minimum of four confluent 75 cm² cell monolayers) is needed for the extraction of the enzyme, determination of enzyme activity at the present state of experience might not be the primary strategy for prenatal diagnosis. Other potential tools for prenatal diagnosis include determination of guanidinoacetate in amniotic

fluid by a recently described isotope dilution method (30), and in case of known mutation in the index patient also molecular genetic analysis in cultivated amniotic cells of chorionic villi. So far none of these methods has been applied for prenatal diagnosis of GAMT deficiency.

Unresolved Problems

GAMT deficiency may present with unspecific clinical symptoms such as mental retardation, retarded speech development and epilepsy. As routine metabolic screening usually does include neither the determination of guanidinoacetate nor the determination of creatine, many patients may be unrecognized.

GAMT deficiency is treatable for creatine deficiency, but not for accumulation of guanidinoacetate. Methods which aim to suppress the production of guanidinoacetate or to treat its neurotoxicity remain to be developed.

Two female siblings with creatine deficiency, normal guanidinoacetate concentrations in body fluids, and rapid restoration of the brain creatine pool upon oral supplementation of creatine-monohydrate have been described (35). These patients might have AGAT deficiency, but biochemical and molecular investigations are pending so far.

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