Creatine deficiency syndrome caused by guanidinoacetate methyltransferase deficiency: Diagnostic tools for a new inborn error of metabolism

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Hepatic guanidinoacetate methyltransferase deficiency induces a deficiency of creatine/phosphocreatine in muscle and brain and an accumulation of guanidinoacetic acid (GAA), the precursor of creatine. We describe a patient with this defect, a 4-year-old girl with a dystonic-dyskinetic syndrome in addition to developmental delay and therapy-resistant epilepsy. Several methods were used in the diagnosis of the disease: (1) the creatinine excretion in 24-hour urine was significantly lowered, whereas the creatinine concentration in plasma and in randomly collected urine was not strikingly different from control values; (2) the Sakaguchi staining reaction of guanidino compounds in random urine samples indicated an enhanced GAA excretion; (3) GAA excretion measured quantitatively by guanidino compound analysis using an amino acid analyzer was markedly elevated in random urine samples; (4) in vivo 1H magnetic resonance spectroscopy (MRS) revealed a strong depletion of creatine and an accumulation of GAA in brain; (5) in vivo phosphorus 31 MRS showed a strong decrease of the phosphocreatine resonance and a resonance identified as guanidinoacetate phosphate; and (6) in vitro 1H MRS showed an absence of creatine and creatinine resonances in cerebrospinal fluid and the occurrence of GAA in urine. For early detection of this disease, we recommend the Sakaguchi staining reaction of urine from patients with dystonic-dyskinetic syndrome, seizures, and psychomotor retardation. Positive results should result in further investigations including quantitative guanidino compound analysis and both in vivo and in vitro MRS. Although epilepsy was not affected by orally administered creatine (400 to 500 mg/kg per day), this treatment resulted in clinical improvement and an increase of creatine in cerebrospinal fluid and brain tissue. (J Pediatr 1997;131:626-31)
CASE REPORT

The female patient was born after an uncomplicated pregnancy and labor at term to healthy Kurdish parents (first cousins). Five siblings are healthy. At 6 months of age, retarded psychomotor development was noticed. At 15 months, she could hardly grasp. She exhibited an extrapyramidal syndrome with dyskinetic-dystonic involuntary movements in which, except during sleep, the upper extremities were more involved than the lower ones. Moreover, she had nonepileptic myoclonic jerks, and she had pyramidal signs characterized by increased muscle tone of the lower limbs and increased deep tendon reflexes. Cranial magnetic resonance imaging revealed marked bilateral myelination delay of white matter, a large cisterna magna, and multiple dermoids. An electroencephalogram showed nonepileptiform discharges. At 3 years of age, she was able to pull up for standing and walking, but there was no speech. At 5 years of age a loss of acquired functions was reported. Within a few months she was no longer able to sit, to crawl, or to pull up for standing. She lost social contact, stopped playing, and appeared sedated and dull. At 14 months of age, she had a prolonged febrile convulsion. Since that time she has had epilepsy with rare grand mal seizures, frequent drop attacks, and absences. Antiepileptic drug therapy including phenobarbital, valproate, ethosuximide, vigabatrin, carbamazepine, lamotrigine, and corticoids showed no benefit.

We saw the girl for the first time at the age of 3 years 8 months. She had microcephaly and wasting, was unable to sit up or to pull up for standing or walking, and she could hardly grasp. She exhibited an extrapyramidal syndrome with dyskinetic-dystonic involuntary movements in which, except during sleep, the upper extremities were more involved than the lower ones. Moreover, she had nonepileptic myoclonic jerks, and she had pyramidal signs characterized by increased muscle tone of the lower limbs and increased deep tendon reflexes. Cranial magnetic resonance imaging revealed marked bilateral myelination delay of white matter, a large cisterna magna, and multiple dermoids. An electroencephalogram showed bilateral synchronous and diffuse slow spike waves, not significantly influenced by progression of disease nor by antiepileptic drug therapy. Acoustic evoked potentials and motor nerve conduction velocity were normal. Laboratory investigations revealed no striking abnormalities except mild hyperammonemia (65 to 145 μmol/L) and intermittently elevated lactate concentrations in blood and CSF (1.0 to 3.7 mmol/L and 1.2 to 2.6 mmol/L, respectively).

Treatment with creatine monohydrate was started with a dosage of 400 mg/kg per day, which increased 3 months later to 500 mg/kg per day. During the first 2 months of treatment we noticed improvement in mental and motor functions. However, it soon became obvious that the patient's psychomotor status was determined predominantly by the course of the epilepsy. After a decrease in the frequency of absences and drop attacks, the girl became more alert, started to play, and tried to pull up for standing and supported walking. However, epilepsy proved to be drug resistant, and the child went many times and sometimes for weeks into minor motor status with loss of mental and motor function. Nevertheless, it was our impression that the dystonic-dyskinetic syndrome abated with creatine supplementation.

METHODS

Biochemical Determination of Creatinine

Creatinine measurements in blood and urine samples were performed biochemically by means of a modified Jaffé method.10

Sakaguchi Reaction

Urinary guanidino compounds were detected after their thin-layer chromatography separation using the Sakaguchi reaction.11,12 In this simple screening method, staining of monosubstituted guanidines such as arginine, argininosuccinic acid, homoarginine, or GAA shows clearly an increased excretion of GAA.

Determination of Guanidino Compounds

The concentration of the guanidino compounds was determined with a model LC 6001 amino acid analyzer (Biotronik, Mainz, Germany) adapted for guanidino compound determination. The guanidino compounds were separated over a cation exchange column as described elsewhere in detail.13

In Vivo 1H and Phosphorus 31 MRS

In vivo 1H and 31P MRS examinations of the brain were performed at $B_0 = 1.5T$ in a whole-body magnetic resonance system (Magnetom 63/84 SP 4000; Siemens, Erlangen, Germany). Spectra were acquired before and during oral therapy with L-arginine and creatine monohydrate. Localized 63 MHz 1H MRS spectra were obtained in single-voxel technique with the stimulated echo pulse sequence.14 The voxel was placed in the basal ganglia (voxel size = 8 cm3). Measurement parameters in the first and second examination were $T_R = 6000$ msec (repetition time), $T_E = 20$ msec (echo time), $T_M = 30$ msec (middle interval), and $T_R = 1500$ msec, $T_E = 50$ msec, $T_M = 30$ msec in the third examination number of experiments (NEX) = 170 excitations. Nonlocalized 31P MRS spectra were acquired with a one-pulse sequence ($T_R = 2000$ ms, NEX = 200) with a crossed Helmholz head coil.

High-resolution In Vitro 1H MRS

In vitro 1H MRS of CSF and urine was carried out as recently described.15,16 Depending on the body fluid, analyzed samples were directly lyophilized (urine) or first filtered with a 10 kD filter (CSF). Samples were dissolved in deuterium oxide with trimethylsilyl 2,2,3,3-tetradeteropropionic acid as the internal standard. Care was taken to standardize the pH of the samples ($\text{pH} = 7.26$ ± 0.10). Subsequently a 600 MHz 1H MRS spectrum was obtained at 298 K with a 60-degree radiofrequency pulse and $T_{1R} = 12$ seconds.

Determination of Enzyme Activity

The GAMT activity of liver tissue was determined by measuring the transfer of [methyl-3H] groups from S-adenosylmethionine to GAA.17

RESULTS

Creatinine in Plasma and Urine (Jaffé Method)

Plasma creatinine was found to be constantly in the lower normal range (18 μmol/L; normal range, 18 to 90). The creatinine concentration in random urine samples was not suspect and ranged from low to normal values (1300 to 5400 μmol/L; normal range, 1800 to 4400). In
Fig. 1. Localized in vivo $^1$H MRS of the brain of the patient with creatine deficiency syndrome at $B_0 = 1.5$ T. The voxel (8 cm$^3$) was placed in the basal ganglia. Spectra (63 MHz Larmor frequency) were acquired, A, before therapy, B, during oral treatment with L-arginine (300 mg/kg per day), and C, during creatine monohydrate supplementation (400 mg/kg per day). Ino, Inositols; tCho, choline-containing compounds; tCr, total creatine (mainly creatine and phosphocreatine); NAA, N-acetyls-l-aspartate.

Repetition of the Sakaguchi reaction

GAMT deficiency leads to an increase in GAA in body fluids and tissues. At the time of diagnosis we investigated, by means of the Sakaguchi staining reaction on TLC plates, the urine samples of the two patients known at that time to have creatine deficiency syndrome. There was repeatedly a positively stained spot with the chromatographic running behavior of GAA (fading orange to red) on TLC plates, with simultaneous negative control results. The detection limit for GAA by this method was estimated to be 2.5 mmol/L.

Quantitative Guanidino Compound Analysis

The urinary excretion levels of GAA determined by ion exchange chromatography were markedly increased (361 to 509 μmol/mmol creatinine; normal range, 28 to 96), whereas the creatine excretion was decreased (17.6 to 29.3 μmol/mmol creatinine; normal range, 45 to 362). Furthermore, a small increase in urinary levels of guanidinosuccinic acid, γ-guanidinobutyric acid, and guanidine was found (17.0 to 19.1, 4.9 to 7.1, and 5.3 to 5.6 μmol/mmol creatinine, respectively; normal ranges, 2.8 to 11.3, 0.6 to 3.4, and 1.1 to 2.8, respectively). Creatine treatment caused an excessive increase in creatine excretion without normalization of GAA in urine (10,753 and 617 μmol/mmol creatinine, respectively).

In Vivo $^1$H and $^{31}$P MRS of the Brain

The patient’s localized $^1$H MRS spectrum (Fig. 1, A), obtained before treatment, shows a strongly reduced signal of total creatine (mainly creatine and phosphocreatine) in comparison with spectra obtained from the brain tissue of healthy volunteers. Besides the resonances of $N$-acetyl-l-aspartate, choline-containing compounds, and inositol, peaks of lower intensity are found at 2.1 to 2.6 ppm. The resonance at $\delta = 3.8$ ppm is assigned to GAA. No lactate signal was seen.

The $^{31}$P MRS spectra (Fig. 2) show strong signals of phosphodiesters and phosphomonoesters. Evaluation of the chemical shift difference of phosphocreatine ($\delta = 0.0$ ppm) and inorganic phosphate ($\delta = 5.1$ ppm) yields an apparent intracellular pH of 7.24. For comparison, mean pH is 7.07 ± 0.02 in healthy human brain tissue. A well-resolved peak was found at $\delta = -0.4$ ppm, close to and stronger than the resonance of phosphocreatine at $\delta = 0.0$ ppm (Fig. 2, A). Concomitant with the findings in the $^1$H contrast, the creatinine excretion in 24-hour urine samples was diminished (38 to 46 μmol/kg per day; normal range, 88 to 132). During treatment with creatine, the plasma creatinine concentration increased (27 to 35 μmol/L) and the daily creatinine excretion became normal (131 μmol/kg per day).
MRS spectra, this intense signal was assigned to guanidinoacetate phosphate on the basis of its chemical shift.\textsuperscript{18} The unusual low intensity of the phosphocreatine signal before treatment agrees with the reduced total creatine resonance in the $^1$H MRS spectra.

After oral substitution of L-arginine (300 mg/kg per day for 1 month), no significant changes were seen in the $^1$H MRS spectra (Fig. 1, B). $^{31}$P MRS could not be performed at this stage because the patient was too agitated. In contrast, oral substitution of creatine produced a significant increase of creatine and phosphocreatine signal intensities in $^1$H (Fig. 1, C) and $^{31}$P (Fig. 2, B) spectra. In the $^1$H MRS spectrum a decrease in GAA and an increase in choline-containing compound signal intensities were seen. At this stage the $^{31}$P MRS resonance of guanidinoacetate phosphate could no longer be resolved. A relative decrease in phosphodiesters and, particularly, in phosphomonoesters signals was found. Intensity, line width, and chemical shift of the inorganic phosphate resonance were reduced ($\delta = 4.8$ ppm); the calculated pH was approximately 7.07 ($\Delta$ pH $\equiv -0.17$) (i.e., in the normal range) after administration of creatine. The signal-to-noise ratio of the three nucleoside $5'\$ triphosphate resonances was lower than that of the spectrum in Fig. 2, A.

**In Vitro $^1$H MRS of CSF, Plasma, and Urine (Table)**

In CSF the creatine resonance was lacking completely, and the creatinine resonance was strongly reduced. Resonances of these metabolites were always present in CSF samples of 60 patients with other neurologic diseases.\textsuperscript{15} The concentrations of creatine and creatinine in CSF clearly distinguish control values and our patient. GAA could not be quantified reliably in CSF with in vitro $^1$H MRS. Fig. 3 shows the spectrum of the patient’s CSF (Fig. 3, A) compared with a normal CSF spectrum (Fig. 3, B).

In random urine samples, creatine was not detectable, but the reference range for urinary creatine excretion is broad, ranging from very low to high values. In contrast, the creatinine values were normal in comparison with values determined by conventional techniques.

Treatment with creatine led to an excessive increase in creatine concentrations in urine and plasma but only to a rise into the normal range in CSF. In contrast to the creatine excess, creatinine increased only within the normal range in all investigated body fluids. The GAA excretion was found to be elevated during both arginine and creatine treatment. Unfortunately, quantitative values before treatment could not be obtained with this method because of an interfering metabolite.

**Investigation of Liver Tissue**

A needle biopsy of liver revealed a slight centrilobular fatty liver and cirrhosis. The activity of GAMT was almost completely deficient (0.5 nmol/hr per gram liver; control range, 33.8 to 38.8; $n = 5$), confirming the diagnosis of hepatic GAMT deficiency.\textsuperscript{8}

**Discussion**

The most predominant clinical feature of the creatine deficiency syndrome seems to be extrapyramidal symptoms mainly characterized by dyskinetic-dystonic involuntary movements. The progressive loss of motor and mental functions points to the neurodegenerative nature of the disease. The course of the disease in our patient was complicated by therapy-resistant epilepsy with myoclonic and astatic seizures, as well as grand mal convulsions.

Therapy for the creatine deficiency syndrome consists of the oral supplementa-
tion of creatine. We noticed a striking clinical improvement within the first 2 months of treatment but no further progress during the following 18 months. The epilepsy, which seemed to be responsible for the psychomotor and mental status, could not be influenced. This might be explained by an incomplete restoration of intracellular creatine homeostasis or by the neurotoxic effects of GAA, which could not be completely removed by creatine substitution. Moreover, the worse clinical course may be a result of intractable epilepsy and perhaps may be independent of cerebral creatine/GAA.

The deficient GAMT activity in our patient is based on a homozygous 327 G → A mutation. The GAMT deficiency is biochemically characterized by lack of creatine and its derivatives and by the accumulation of GAA. Thus the finding of lowered creatine and its derivatives and the accumulation of GAA both facilitate the diagnosis of this disease. Diagnosis of the disease in the presently known two patients was primarily based on in vivo MRS of the brain, which demonstrated the diagnostic utility of MRS. In vitro $^1$H MRS of CSF is still under investigation as a general screening method in neurometabolic disorders. As clearly demonstrated in our patient, this method proved to be useful for the diagnosis of creatine deficiency syndrome. In our patient's CSF samples, creatine and creatinine concentrations as

**Table.** Metabolite profile in $^1$H MRS of random urine samples and of plasma and cerebrospinal fluid

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Body fluid</th>
<th>Without</th>
<th>Arginine</th>
<th>Creatine</th>
<th>Control values* $(\pi = 15)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine</td>
<td>Urine</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>12000</td>
<td>30-1140</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td></td>
<td></td>
<td>496</td>
<td>100-264</td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>46</td>
<td>25-70</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Urine</td>
<td>4500</td>
<td>3200</td>
<td>1800</td>
<td>750-14,960</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>92</td>
<td>20-100</td>
</tr>
<tr>
<td>GAA</td>
<td>Urine</td>
<td>IFM</td>
<td>1000</td>
<td>520</td>
<td>&lt;96*</td>
</tr>
</tbody>
</table>

Measurements: urine, micromoles per millimole of creatinine; plasma and CSF, micromoles per liter. IFM, Interfering metabolite.

Reference data for creatine and creatinine were derived from a group of 15 children (ages 1 to 5 years) and were measured with MRS.

Reference data for GAA were derived from a group of 12 children and were measured by ion exchange chromatography.

![Fig. 5. A](image.png) In vitro 600 MHz $^1$H MRS of cerebrospinal fluid of the patient with creatine deficiency syndrome, in comparison with B, a normal CSF spectrum.
measured by in vitro $^1$H MRS were found to be below the detection limit. This finding seems to be pathognomonic, suggesting that analysis of creatine and creatinine in CSF may be a reliable diagnostic method for the disease. In the diagnostic study, we recommend the Sakaguchi reaction in randomly collected urine after TLC separation as a simple screening method and, in addition, the measurement of creatinine in 24-hour urine samples. Subsequently, further diagnostic study should include quantitative determination of guanidino compounds in 24-hour urine samples. As clearly demonstrated in our patient, in vitro $^1$H MRS and in vivo $^1$H/$^3$P MRS, which can be added to routine magnetic resonance imaging, are useful diagnostic tools and should, whenever possible, at least be performed in all patients with neurologic symptoms such as extrapyramidal signs, seizures, and psychomotor retardation.

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**REFERENCES**


