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Guanidinoacetate Methyltransferase Deficiency: The First Inborn Error of Creatine Metabolism in Man

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Summary

In two children with an accumulation of guanidinoacetate in brain and a deficiency of creatine in blood, a severe deficiency of guanidinoacetate methyltransferase (GAMT) activity was detected in the liver. Two mutant GAMT alleles were identified that carried a single base substitution within a 5′ splice site or a 13-nt insertion and gave rise to four mutant transcripts. Three of the transcripts encode truncated polypeptides that lack a residue known to be critical for catalytic activity of GAMT. Deficiency of GAMT is the first inborn error of creatine metabolism. It causes a severe developmental delay and extrapyramidal symptoms in early infancy and is treatable by oral substitution with creatine.

Introduction

We have recently reported a 22-mo-old male infant with muscular hypotonia, progressive extrapyramidal movement disorder and an extremely low excretion of creatinine, deficiency of creatine and creatine phosphate, and simultaneous accumulation of guanidinoacetate in brain, as detected by in vivo proton and phosphorus magnetic resonance spectroscopy (Stöckler et al. 1994). The clinical symptoms and biochemical abnormalities improved significantly after oral substitution of creatine monohydrate. These observations were suggestive of an enzyme defect in creatine biosynthesis at the level of guanidinoacetate methyltransferase (GAMT) activity (see fig. 1). Here we report on the deficiency of GAMT in the liver of this and a second unrelated female patient with similar biochemical characteristics. The identification of the mutations in the GAMT alleles of these patients established the genetic nature of this treatable disorder of creatine metabolism.

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Patients, Material, and Methods

Patients

Patient 1 developed progressive muscular hypotonia and extrapyramidal symptoms with hemiballistic voluntary movements after the first few months of life. At the age of 22 mo, his developmental age was that of a 8-wk-old infant. The electroencephalogram (EEG) showed abnormally low background activity with multifocal spikes. Brain magnetic resonance imaging revealed bilateral abnormal signal intensities in the globus pallidus. Plasma creatinine levels and urinary creatinine excretion were consistently low. A severe deficiency of creatine and creatine phosphate and simultaneous accumulation of guanidinoactetate were detected by in vivo proton and phosphorous magnetic resonance spectroscopy of the brain (Stöckler et al. 1994).

In patient 2, severe developmental delay, muscular hypotonia, ataxia, and intractable seizures were the predominant clinical symptoms at the age of 4 years. Biochemical and spectroscopy findings were similar to those of patient 1 (A. Schulze and D. Rating, personal communication).

Liver Samples

With the informed consent from the parents, liver tissue (220–300 mg) was obtained by laparoscopic surgery by use of an electrocautery device (patient 1, Stöckler et al. 1994) or a surgical knife (patient 2). Control tissue was obtained from three adults undergoing hepatic lobectomy for liver cancer. The samples were collected from tumor-free areas, with a surgical knife or by an electrocautery device. The samples were frozen in liquid nitrogen and stored at −80°C until liver extracts were prepared. Light microscopy was performed by staining unfixed cryostat sections with hematoxylin/eosin and oil red O (Filipe and Lake 1990).

Preparation of Liver Extracts

Procedures for preparation of liver extracts were carried out at 0°C–4°C. Liver homogenates were prepared in three volumes of 10 mM potassium phosphate/2 mM EDTA, pH 7.2 (buffer A), with a Dounce homogenizer. The homogenate was adjusted to pH 5.5 by addition of 2 M sodium acetate pH 4.4, and centrifuged at 10,000

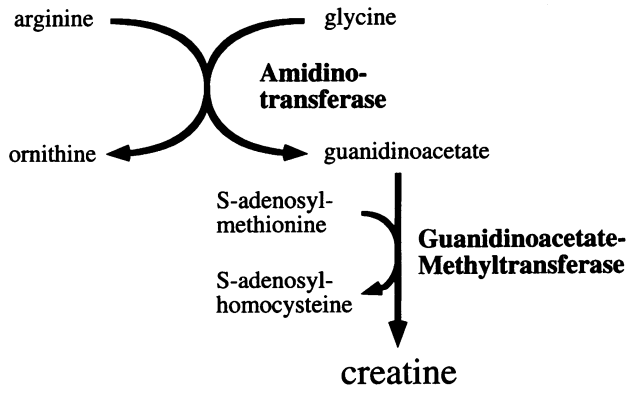

Figure 1 Biosynthesis of creatine. Biosynthesis of creatine in human liver and pancreas involves arginine:glycine amidinotransferase and guanidinoacetate methyltransferase as enzymes and arginine, glycine, and S-adenosylmethionine as substrates. Creatine is the substrate of creatinekinase in muscle and brain.

g for 15 min. The supernatant was readjusted to pH 7.4 with 2 N KOH, and aliquots were taken for determination of protein and reference enzymes. The supernatant was then subjected to consecutive ammonium sulfate fractionation with 21 g/100 ml (1.5 M) and 10 g/100 ml (2.0 M) as described by Ogawa et al. (1983). The second precipitate containing 65%–75% of the GAMT activity present in the homogenate was dissolved (1.25 ml/g wet weight), dialyzed against buffer A, and stored at −20°C until determination of protein, GAMT, and reference enzymes.

Enzyme Assays

Protein (Lowry et al. 1951) and activities of lactate dehydrogenase (Richterich 1971) and β-hexosaminidase (Carrol 1978) as reference enzymes were determined as described elsewhere. The activity of GAMT was determined by measuring the transfer of [methyl-^{3}H] groups from S-adenosylmethionine to guanidinoacetate (Im et al. 1979). The assay mixture contained 100 mM Tris-HCl pH 8, 10 mM mercaptoethanol, 100 μM L-[methyl-^{3}H] S-adenosylmethionine (New England Nuclear, diluted to a specific activity of 6 Ci/mol), and 5 μl liver extract in a final volume of 50 μl. After preincubation for 10 min at 37°C, the reaction was started by the addition of 20 mM guanidinoacetate to a final concentration of 2 mM. Blanks were incubated in the absence of guanidinoacetate. After incubation (normally 10 h) 50 μl water were added, and samples were stored at −20°C until determination of [methyl-^{3}H] creatine. The formation of creatine was proportional to incubation time and amount of liver extract, provided that incubation time and substrate turnover did not exceed 12 h and 60%, respectively.

Separation of [methyl-^{3}H] Adenosylmethione from [methyl-^{3}H] Creatine

The samples were mixed with 10 μl 5 mM creatine and subjected to high-performance liquid chromatography (Waters Millipore) with a strong cation-exchange column (Mono S HR 5/5, Pharmacia Biotech) equilibrated in 40 mM acetic acid (pH 3.0). Following a 5-min isocratic run, creatine was eluted at 2 ml/min with a 10-min linear gradient from 0 to 0.1 M NaCl at 40 mM NaCl and detected at 210 nm. Radioactivity was determined in the fractions collected according to the UV signal. In blanks, ~3% of the starting radioactivity were recovered in the fractions containing creatine. For calculation of GAMT activity, all values were corrected for the radioactivity in the blanks. One unit of GAMT activity was defined as the amount of enzyme catalyzing the formation of 1 nmol product/h.

RNA Isolation, Northern Blot Hybridization, DNA Isolation

Total RNA was isolated from liver, leukocytes, and cultured fibroblasts from controls and patients (Chirgwin et al. 1979), electrophoresed under denaturing conditions, transferred to Hybond-N membranes (Amersham), and hybrized with a [^{32}P]-labeled probe by using standard protocols (Sambrook et al. 1979). The probe was prepared with the Megaprime labeling system (Amersham) by using human GAMT cDNA (Isbrandt and von Figura 1995) as a template. Genomic DNA from blood was isolated as described by Sambrook et al. (1979).

Reverse Transcription, PCR, and Sequencing

First-strand cDNA synthesis was done with a kit (Pharmacia Biotech) according to the supplier's instructions by using primer 2 (see fig. 2) and total RNA from liver, fibroblasts, or leukocytes. PCR amplifications were performed in a PE9600 thermocycler (Perkin Elmer Cetus) by using *Taq* polymerase (Pharmacia Biotech) and primers 1 and 2 (see fig. 3). Cycling conditions were 1 s denaturation at 98°C, 30 s annealing at 60°C, and 90 s extension at 72°C for 30 cycles. After purification of the 869-bp product, GAMT fragments A and B (see fig. 3) were amplified by seminested PCR with primers 1–4. PCR amplification of genomic DNA was done with primers 5 and 7. After removal of nucleotides and primers by filtration, the products were subjected to sequencing directly or after subcloning using Prism-Dye Terminator cycle sequencing kit (Applied Biosystems), and primers 1–7, and sequence analysis was performed on an automated 373A DNA sequencer (Applied Biosystems). The primer sequences are as follows: primer 1, CGGAATTCCGCGCGATCGAGGTCGGGTC; primer 2, CGGGATCCCGAAGCCGGGAAAGCTTCTG-

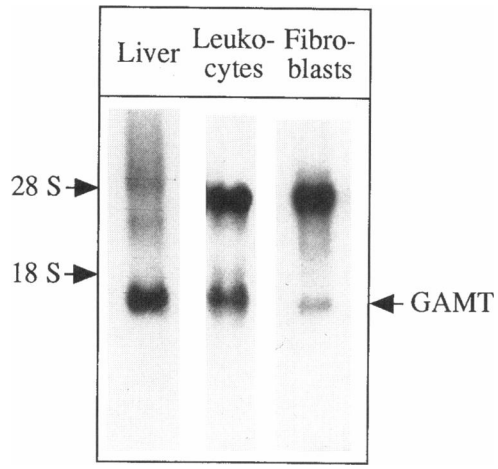

Figure 2 Northern blot analysis of total RNA. RNA (10 μg) isolated from liver, leukocytes, or cultured fibroblasts of controls was hybridized with a GAMT cDNA probe. The positions of 18 S (1.87 kb) and 28 S (5.77 kb) rRNA and of the 1.1 kb GAMT RNA are indicated. The 28 S RNA gave a hybridization signal of variable intensity. Ethidium bromide staining confirmed that equal amounts of RNA had been loaded (not shown).

GTG; primer 3, AGGATCCCATCAAAGTGAC; primer 4, CCTGTGGGAGGATGTGGCAC; primer 5, AGCGTCAAAGGTGCAGGAGG; primer 6, ACTCGATGATCCAATGCTCATC; and primer 7, CGGGATCCCGCTCCCACAGGCCTTTCAAGG.

Results

Deficiency of GAMT in Liver

Activity of GAMT was assessed by following the transfer of [^{3}H]-methyl groups from [^{3}H] S-adenosylmethionine to guanidinoacetate, which results in the formation of [^{3}H]-creatine. Activity was detectable only after ammonium sulfate fractionation of the homogenate and dialysis. The fraction precipitating between 1.5 and 2.0 M ammonium sulfate contained 65%–75% of the GAMT activity present in the fractions and was used for further determinations. The GAMT activity in three control livers varied between 34.1 to 38.2 units/g liver tissue (see table 1).

The liver specimen from the two patients with an elevation of guanidinoacetate and a deficiency of creatine showed histologically a normal cytoarchitecture but fat droplets as a sign of a mild steatosis. The residual GAMT activity varied between levels below the limit of detection and 1.9 units/g liver. It should be noted that

Table 1

Activity of GAMT in Extracts from Liver

Liver Extract	GAMT (nmol/h · g)	LDH (U/g)	β-Hex (U/g)	Protein (mg/g)
Patient 1	1.35 (.5–1.9) $n = 4$	69 (61–77) $n = 2$	2.9 (2.7–3.0) $n = 2$	.6 $n = 1$
Patient 2	.5 $n = 2$	89.0 $n = 1$	5.3 $n = 1$	.8 $n = 1$
Controls	36.4 (33.8–38.8) $n = 5$	87.7 (54.7–120) $n = 4$	7 (6.2–9.0) $n = 4$	1.1 (.6–1.9) $n = 4$

NOTE.—Activities of GAMT, lactate dehydrogenase (LDH), and β-hexosaminidase (β-Hex), and protein content were determined in liver extracts from two patients and from three controls. The values refer to the mean and range of the number of determinations indicated.

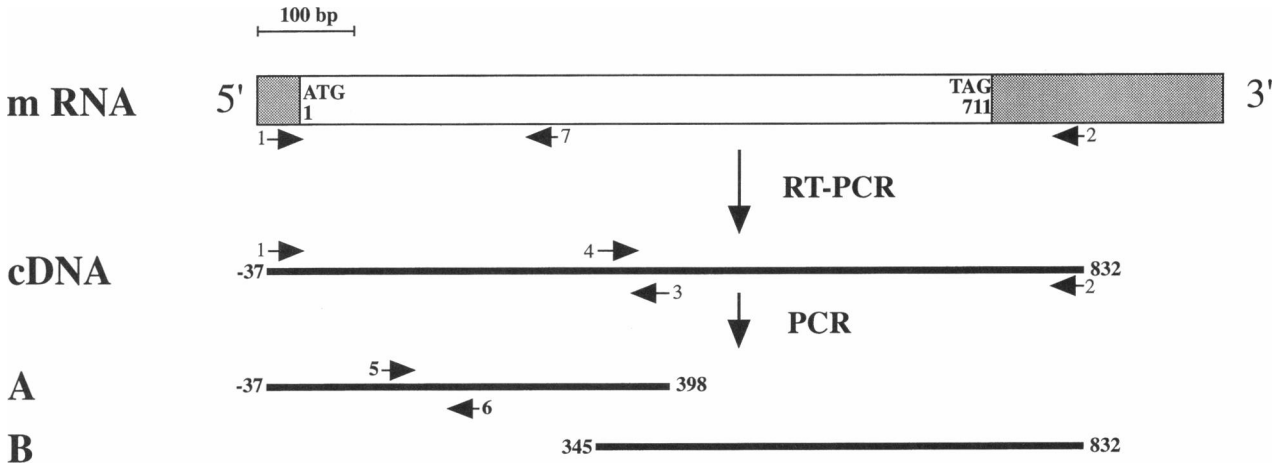

Figure 3 Amplification of GAMT RNA by RT-PCR. The scheme depicts the position of the primers used for RT-PCR of GAMT cDNA (upper row), for amplification of fragments A and B by seminested PCR (middle row), and for sequencing (bottom row). In the upper bar, the 5′- and 3′-untranslated sequences of GAMT mRNA are shaded. The numbering of nucleotides follows Isbrandt and von Figura (1995).

the liver biopsy of one patient had been taken by an electrocauter. Control experiments with normal liver showed that this procedure can lower the activity of GAMT by 40%.

When 1:1 mixtures of liver extracts from the two patients and control livers were incubated, the GAMT activity exceeded the calculated activity by 40%–60%. This excludes the possibility that the deficiency of GAMT in the patients' livers is due to an inhibitor. It, rather, points to the presence of an activator of GAMT in the patients' livers.

The activities of two unrelated enzymes (cytosolic lactate dehydrogenase and lysosomal β-hexosaminidase) and the protein content, which served as reference parameters, were within the same range of controls (table 1). Taken together, these results confirm the suspected deficiency of GAMT activity in the liver of the two patients.

Expression of GAMT in Human Tissues

The cDNA of human GAMT has been isolated from a liver cDNA library with the aid of a partial cDNA of rat GAMT. It contains an open reading frame of 711 nt (Isbrandt and von Figura 1995). The cDNA was used as a probe for Northern blot analysis of RNA from liver, leukocytes, and fibroblasts of controls. A single GAMT-RNA species of 1.1 kb was detected in all three tissues (fig. 2). In leukocytes and fibroblasts, the frequencies of GAMT-RNA were 5- and 17-fold lower, respectively, than in liver.

GAMT-Transcripts in Patients with GAMT Deficiency

By use of total RNA from liver, leukocytes, or fibroblasts as a template, GAMT cDNA was amplified by RT-PCR with two primers derived from the 5′ and 3′ noncoding region of GAMT-cDNA, followed by a seminested PCR amplification of the two overlapping fragments A and B covering nt −37–398 and nt 345–832, respectively (fig. 3).

From patient 1, total RNA of leukocytes and fibroblasts was available for amplification. Direct sequencing of both strands of fragment A revealed on variable backgrounds an insertion of 13 nt following nt 309 and a G→A change of nt 327 followed by an insertion of 44 nt. The sequence of fragment B was identical to that of wild-type GAMT. Subcloning and sequencing of fragment A identified the presence of three different transcripts, which gave rise to the complex background in direct sequencing. One transcript carried the 327 G→A substitution followed by an insertion of 44 nt (327 G→A, ins 44 transcript in fig. 4*D*), another the insertion of 13 nt (309 ins 13 transcript in fig. 4*B*), and a third transcript, 309 ins 13, 327 ins 44 (fig. 4*C*), carried in addition to the 13-nt insertion an insertion of 44 nt following nt 327. The 44-nt insertion is identical to that of the 327 G→A, ins 44 transcript, but is preceded at position 327 by G, as in wild-type GAMT. This transcript was present in fibroblasts and leukocytes at about the same frequency as the 309 ins 13 transcript.

When translated into an amino acid sequence, the 327 G→A, ins 44 mutation causes a frameshift after the codon for His 108 and premature translational stop at codon 128. The 309 ins 13 mutation causes a frameshift with a change of sequence after Pro 104 and a premature translational stop at codon 127. In the longer transcript of this allele, the 44-nt insertion after nt 327 corrects the frameshift introduced by the 13-nt insertion at nt 309. This transcript encodes a polypeptide in which the wild-type pentapeptide RQTHK (105–110) is replaced by a novel sequence consisting of 24 residues.

From patient 2, total RNA from liver and leukocytes was available for amplification of fragments A and B. Direct sequencing of fragment A with primer 1 revealed a deletion of 146 nt following nt 181 on the background of the wild-type sequence. Direct sequencing with primer 5 yielded a single sequence with a 327 G→A substitution followed by an insertion of 44 nt that was already identified in patient 1. The sequence of fragment B was identical to that of wild-type GAMT. Subcloning of fragment A revealed that one type of transcripts carried the 327 G→A, ins 44 mutation (D), and the other the deletion of 146 nt (fig. 4*E*). The 181 del 146 transcript (see fig. 4), which was present in liver and leukocytes at about the same level, encodes a truncated polypeptide of 76 residues with a change of sequence after Gly 61, because of a frameshift.

Mutations in GAMT-Deficiency Alleles

To identify the mutations that give rise to the four mutant transcripts, the fragment between nt 222 and 354 of GAMT cDNA was amplified using primers 5 and 7 and genomic DNA as a template (see Methods). Sequencing of the fragment amplified from control DNA showed that the GAMT gene contains an intron of 205-bp length following G 327. Furthermore, the 44-nt insertion in the 327 G→A, ins 44 and 309 ins 13, 327 ins 44 transcripts is identical to the 5′ sequence of the intron.

Analysis of the DNA from patient 1 and his parents showed that he had inherited from his father the allele with the 327 G→A mutation and from his mother the allele with the 309 ins 13 mutation, while his brother was heterozygous for the 327 G→A mutation (fig. 5*A*). Patient 2 was homozygous for the 327 G→A mutation, and her parents heterozygous (fig. 5*B*). This indicated that the 327 G→A allele gives rise to the 181 del 146 transcript as well as to the 327 G→A, ins 44 transcript.

In total RNA from fibroblasts of patient 1, who is

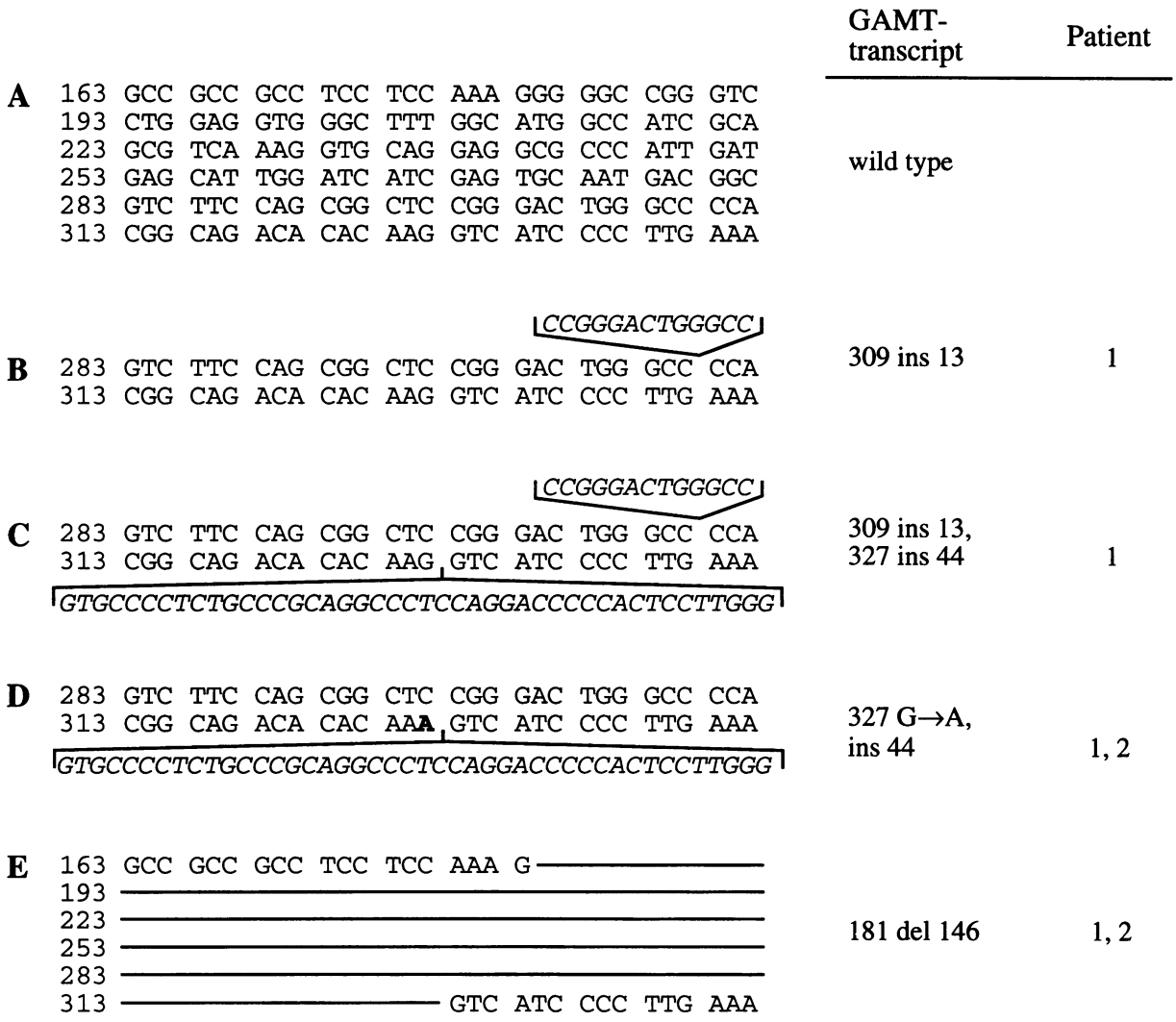

Figure 4 Mutations in GAMT-deficiency transcripts. *A*, Nucleotide sequence of GAMT cDNA between nt 163 and nt 342. *B–E*, Mutations in the four GAMT-deficiency transcripts 309 ins 13, 309 ins 13 327 ins 44, 327 G→A ins 44, and 181 del 146. The numbering of nucleotides follows Isbrandt and von Figura (1995). Substitutions are indicated in bold, insertions in italic, and deletions by a bar. The designation of the GAMT transcripts A–E and their occurrence in patients 1 and 2 are indicated on the right.

heterozygous for the 327 G→A allele, only the 327 G→A, ins 44 transcript was detected. Following the identification of two products of the 327 G→A allele in patient 2, RNA of patient 1 was reanalyzed. On reinvestigation, the 181 del 146 transcript was also detected among the RT-PCR products when total RNA from fibroblasts and leukocytes of patient 1 was used as a template.

Discussion

In two unrelated patients of German and Turkish origin, a profound deficiency of GAMT activity was observed in liver. GAMT deficiency had been suspected in both patients because of a deficiency of creatine and creatine phosphate and a simultaneous elevation of guanidinoacetate in brain as measured by in vivo magnetic resonance spectroscopy. A secondary deficiency of GAMT due to the presence of an excess of an inhibitor of GAMT could be excluded by mixing experiments.

The hereditary nature of the GAMT deficiency was established by the identification of the mutations in the GAMT alleles of the two patients. A 327 G→A single-base-substitution allele (fig. 6*A*) was found in both patients. G 327 occupies the position −1 of the 5′ splice site of an intron. This intron is likely to represent intron 2, since in rat GAMT, which in its coding part is 86% identical with human GAMT (Isbrandt and von Figura 1995), G 327 occupies the position −1 of the 5′ splice site of intron 2 (Ogawa and Fujioka 1988). The G in

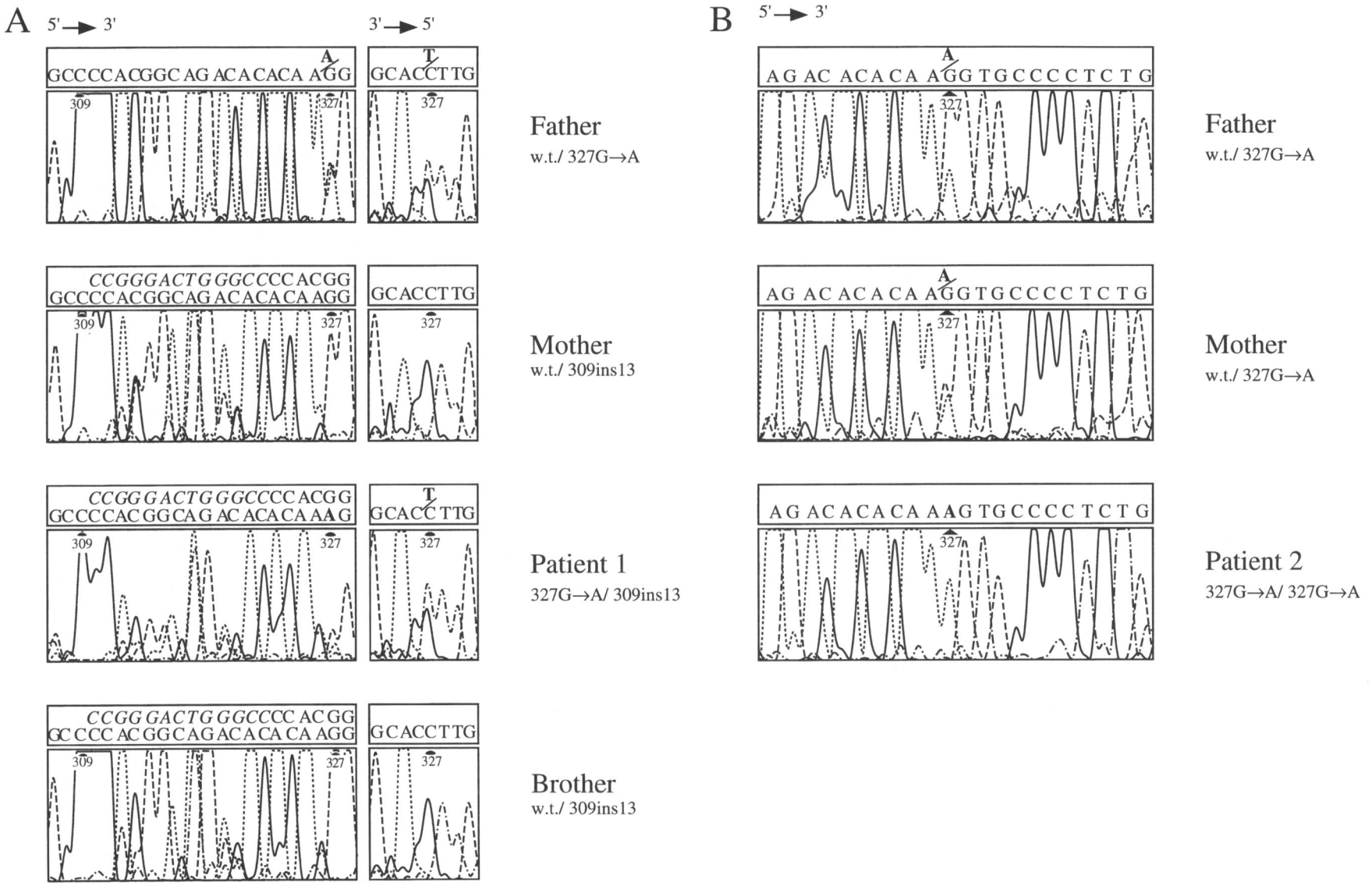

Figure 5 Sequence of GAMT alleles of patient 1 and 2 and their family. Shown are relevant parts of the coding (nt 307–328) and noncoding (nt 331–324) strand identifying the 309 ins 13 and 327 G→A alleles. The inserted sequence is shown in italics, the substitution in bold. *A*, Analysis of patient 1 and his parents and brother. *B*, Analysis of patient 2 and her parents.

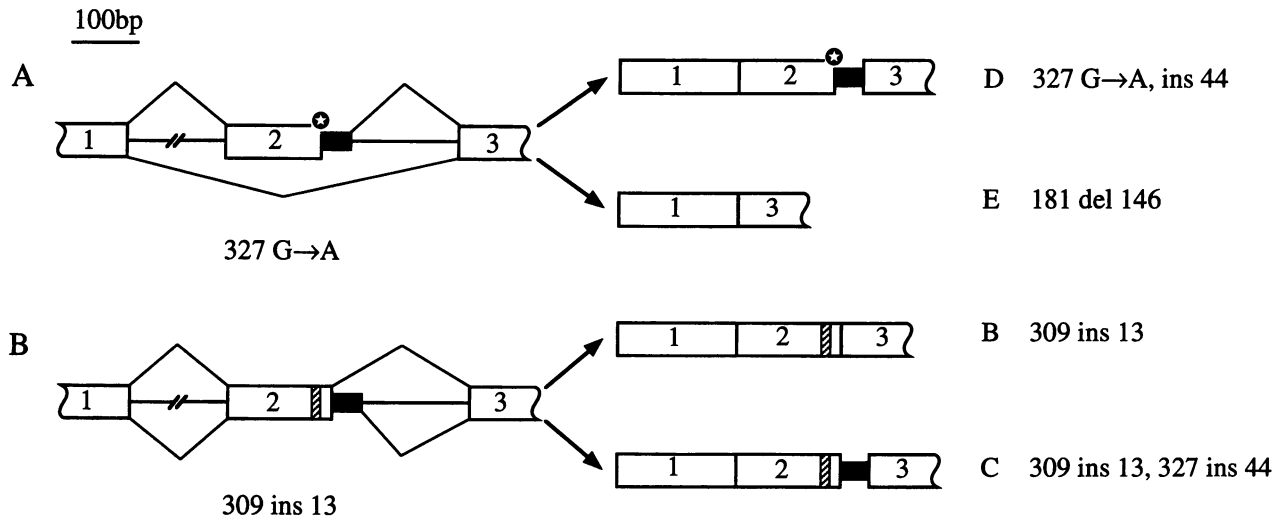

Figure 6 Generation of alternative transcripts from GAMT-deficiency alleles. Exons 1, 2, and 3 of GAMT are indicated by open bars. The filled bar represents the first 44 nt of intron 2, the hatched bar the 13 nt insertion in exon 2, and the star symbol the 327 G→A substitution. On the right, the resulting GAMT deficiency transcripts B–D (see fig. 4) are shown.

position −1 of 5′ splice sites is known to be critical for the stability of base pairing between the splice site and the complementary region of U1snRNA and a frequent target of mutations of 5′ splice sites (Zhuang and Weiner 1986; Weber et al. 1988; Cooper et al. 1995). The mutation of the 5′ splice site of intron 2 can explain the formation of the two transcripts found in each of the two patients. The 327 G→A, ins 44 transcript (fig. 6, transcript D) arises from the use of a cryptic splice site at nt 44 of intron 2. The sequence of the cryptic splice site (Ggtgag) agrees with the consensus sequence of 5′ splice sites (Cooper et al. 1995). The 181 del 146 transcript (fig. 6, transcript E) arises from skipping of exon 2. The sequence of the 146 nt deleted in this transcript corresponds in the rat GAMT gene to that of exon 2 (Ogawa and Fujioka 1988). Exon skipping can be caused by mutations of the 3′ splice site of the preceding intron or of the 5′ splice site of the following intron or a nonsense mutation within the exon (Cooper et al. 1995). The mutation of the 5′ splice site of intron 2 can therefore explain the skipping of exon 2 that characterizes the 181 del 146 transcript.

The second GAMT deficiency allele carried an insertion of 13 bp within exon 2 of GAMT (fig. *5B*). The insertion is a direct repeat, which suggests that it may have arisen from slipped mispairing during replication. Also, the 309 ins 13 allele produced two alternative transcripts (fig. 6, transcripts B and C). One transcript carried an insertion of 44 nt after G 327. It should be noted that in none of the transcripts of 14 GAMT-alleles derived from seven unrelated donors had the cryptic splice site in intron 2 been used. Therefore, it is apparent that this transcript arose from the use of the cryptic 5′ splice site within intron 2. It is tempting to speculate that the 13-nt insertion after nt 309 favors the use of the cryptic splice site within intron 2.

Three of the four mutant GAMT transcripts (327 G→A, ins 44; 181 del 146; and 309 ins 13) contain frameshift mutations altering the reading frame within the aminoterminal 127 residues of GAMT and leading to truncated polypeptides of 77–128 residues length. These polypeptides lack Asp 134. Asp 134, which is conserved between human and rat GAMT, has been shown to be critical for binding of S-adenosylmethionine. Substitution of Arg 134 in rat GAMT by alanine leads to inactivity (Takata et al. 1994). It is therefore unlikely that any of the truncated GAMT polypeptides is functional.

In one of the transcripts (309 ins 13, 327 ins 44) the frameshift introduced by the first insertion at nt 309 is corrected by the following insertion of 44 nt at nt 327. The transcript encodes a polypeptide in which a novel sequence of 24 residues replaces residues 105–110. This polypeptide may have GAMT activity and sustain a residual GAMT activity in some tissues. It should be noted that in the liver extract of the patient carrying the 309 ins 13 allele a residual activity of GAMT of $\leq 5\%$ was observed.

In humans, creatine is synthesized in liver and pancreas (Walker 1979) and involves arginine:glycine amidinotransferase and GAMT as enzymes. GAMT catalyzes the transfer of a methyl group from S-adenosylmethionine to guanidinoacetate (Cantoni et al. 1954), accounting for $>75\%$ of total body methyl group transfer

(Mudd and Poole 1975). The deficiency of GAMT readily explains the accumulation of guanidinoacetate and the depletion of the total creatine pool observed in the two patients.

Creatine is utilized in muscle and nerve tissue where the pool of creatine/creatine phosphate together with creatine kinase and ATP/ADP provides a high-energy phosphate-buffering system. Creatine and creatine phosphate are converted by nonenzymatic cyclization to creatinine, with a daily turnover accounting for 1.5% of the body creatine pool (Hoberman et al. 1948). To maintain the body creatine pool, an amount of creatine equivalent to the daily urinary creatinine excretion (1–2 g/d in adults) must be provided either from dietary sources or from endogenous synthesis.

The oral substitution of high doses of creatine-monohydrate for almost 2 years in the reference patient 1 (S. Stöckler, unpublished data) has resulted in a significant increase of brain creatine and creatine phosphate and to normalization of urinary creatinine excretion, which suggests a normalization of the total body creatine pool, including muscle creatine. Even guanidinoacetate decreased, although to levels still above normal. This decrease may be ascribed to a repression of arginine:glycine amidinotransferase, the enzyme synthesizing guanidinoacetate, by creatine (McGuire et al. 1984). Clinically, the extrapyramidal movement disorder, the abnormal signal intensities in the globus pallidus, and the pathological EEG changes reversed to normal under the oral substitution of creatine-monohydrate, while the muscle tone improved but did not reach normal strength. The incomplete reversal of neurological symptoms may be due both to residual brain damage caused by chronic creatine deficiency before treatment and to the incomplete reversal of guanidinoacetate accumulation. An effective therapeutic management of GAMT deficiency may therefore require substitution of creatine as early as in the neonatal period, as well as inhibition of guanidinoacetate formation. The latter may be achieved by high doses of ornithine, which inhibits arginine:glycine amidinotransferase (Sipilä 1980).

In conclusion, GAMT deficiency is a new inborn error of metabolism that provides new insights into the physiology and pathobiochemistry of creatine metabolism and of the high-energy phosphate system in muscle and brain. The treatable nature of the disease necessitates the development of simple screening methods and noninvasive tests for the diagnosis of GAMT deficiency.

Acknowledgments

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References

Cantoni GL, Vignos PJ (1954) Enzymatic mechanism of creatine biosynthesis. J Biol Chem 209:647–659

Carrol M (1978) Characterization of proteins structurally related to human β-N-acetylglucosaminidase. Biochem J 173: 191–196

Chirgwin JM, Prybyla AE, MacDonald RJ, Rutter WJ (1979) Isolation of biologically active ribonucleic acid from sources enriched ribonuclease. Biochemistry 18:5294–5299

Cooper DN, Krawczak M, Antonarakis SE (1995) The nature and mechanisms of human gene mutations. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease. McGraw Hill, New York, pp 259–291

Filipe MI, Lake BD (1990) Histochemistry in pathology. Churchill Livingstone, Edinburgh

Hoberman HD, Sims EAH, Peters JH (1948) Creatine and creatinine metabolism in the normal male adult studied with the aid of isotopic nitrogen. J Biol Chem 172:45–48

Im YS, Cantoni GL, Chinag PK (1979) A radioactive assay for guanidinoacetate methyltransferase. Anal Biochem 95: 87–88

Isbrandt D, von Figura K (1995) Cloning and sequence analysis of human guanidinoacetate N-methyltransferase cDNA. Biochim Biophys Acta 1264:265–267

Lowry OL, Rosebrough LJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

McGuire DM, Gross MD, van Pilsum JF, Towle HC (1984) Repression of rat kidney L-arginine:glycine amidinotransferase synthesis by creatine at a pretranslational level. J Biol Chem 259:12034–12038

Mudd HS, Poole JR (1975) Labile methyl balances for normal humans on various dietary regimens. Metabolism 24:721–735

Ogawa H, Fujioka M (1988) Nucleotide sequence of the rat guanidinoacetate methyltransferase gene. Nucleic Acids Res 16:8715–8716

Ogawa H, Ishiguro Y, Fujioka M (1983) Guanidinoacetate methyltransferase from rat liver: Purification, properties, and evidence for the involvement of sulfhydryl groups for activity. Arch Biochem Biophys 226:265–275

Richterich R (1971) Klinische Chemie. Theorie und Praxis, 3d ed. S. Karger, Basel

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning, 2d ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Sipilä Z (1980) Inhibition of arginine-glycine amidinotranser-

ase by ornithine: a possible mechanism for the muscular and chorioretinal atrophies in gyrate atrophy of the choroid and retina with hyperornithinemia. Biochim Biophys Acta 613: 79–84

Stöckler S, Holzbach U, Hanefeld F, Marquardt I, Helms G, Requart M, Hänicke W, et al (1994) Creatine deficiency in the brain: a new, treatable inborn error of metabolism. Pediatr Res 36:409–413

Takata Y, Konishi K, Gomi T, Fujioka M (1994) Rat guanidinoacetate methyltransferase: effect of site directed alternation of an aspartic acid residue that is conserved across most mammalian S-adenosylmethionine-dependent methyltransferases. J Biol Chem 269:5537–5542

Walker JB (1979) Creatine: biosynthesis, regulation, and function. Adv Enzymol 50:177–242

Weber S, Aebi M (1988) In vitro splicing of mRNA precursors: 5′ cleavage site can be predicted from the interaction between the 5′ splice region and the 5′ terminus of U1suRNA. Nucleic Acids Res 16:471–486

Zhuang Y, Weiner AM (1986) A compensatory base change in U1suRNA suppresses a 5′ splice site mutation. Cell 46: 827–835