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The Human Guanidinoacetate Methyltransferase (GAMT) Gene Maps to a Syntenic Region on 19p13.3, Homologous to Band C of Mouse Chromosome 10, but GAMT Is Not Mutated in Jittery Mice[1]

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Guanindinoacetate methyltransferase (gene symbol, GAMT) catalyses the synthesis of creatine from guanidinoacetate and S-adensylmethionine. Pathological mutations in the coding region of GAMT were recently identified in two children with symptoms of muscular hypotonia, ataxia, seizures, and abnormal extrapyramidal movements. During contig construction in the telomeric region of human chromosome 19 we identified a cosmid clone carrying the entire GAMT gene. This clone was shown to overlap with cosmids from a contig that was previously mapped to chromosome 19p13.3. The human GAMT gene has a size of about 5 kb and consists of six exons which agree with the published cDNA sequence. Since the mouse mutations jittery/hesitant are located on band C of mouse chromosome 10 in a region of conserved synteny with 19p13.3 and jittery mice exhibit ataxia and abnormal movement behaviour, the genomic sequence of GAMT was determined in wild-type and jittery mice. The coding region of the GAMT gene, however, was not mutated in these mutant mice. Our linkage and sequence data will facilitate the identification of new GAMT mutations in patients suffering from an abnormal creatine metabolism.

The creatine/creatine-phosphate system plays an important role in phosphate bound energy metabolism in particular in those tissues with high and fluctuating energy demands. Creatine is synthesized from guanidinoacetate in a methylation reaction which is catalysed by the enzyme guanidinoacetate methyltransferase with S-adenosylmethionine as the methyl donor (1). The major site of endogenous creatine biosynthesis is the liver, but considerable amounts of creatine are also taken up with food and milk. Peripheral tissues take up creatine from the bloodstream by active transport and passive diffusion. Certain cell types including neuronal cells, Sertoli cells and epithelial cells of the epidydimis are capable of synthesizing considerable amounts of creatine and may contribute significantly to the local creatine requirement (2).

Recently, two unrelated children with an inborn error of creatine metabolism were reported (3). One patient presented with developmental delay, progressive muscular hypotonia and extrapyramidal symptoms after a few months of life. The other patient developed severe ataxia and seizures at the age of 4 years and showed a severe developmental delay. Long term treatment of the former child with oral creatine resulted in an impressive improvement of all clinical symptoms (4). Cloning and sequencing of the cDNA for human guanidinoacetate methyltransferase (gene symbol: GAMT) revealed that two mutant alleles were present in both patients (3). One patient was homozygous for a mutation in the codon for Lys-39 which was mutated from AAG to AAA. Since this codon is immediately followed by the 5′ donor of an adjacent intron (AAGgt), incorrect splicing of the intron after Lys-39 occurred and resulted in abberrant 5′ splice site selection. The other patient was a compound heterozygote. In addition to the same AAG to AAA mutation, a direct 13-bp tandem duplication was found within the second exon. These mutations caused an

[1] Sequence data from this article have been deposited in GenBank. Accession numbers are AF010246, AF010247, AF010248; AF015887, AF010498, and AF010499.

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TABLE 1

GAMT Primer Sequences

Primer	Exon	Strand	5′ to 3′ sequence
		(1) Murine and human GAMT	
DJ542	1	+	GTGATGGAGCGCTGGGAGACC
DJ557	2	−	TGGCCATACCGAAGCCCACTT
DJ561	3	+	AGGCCTGTGGGAGGATGTGG
DJ558	4	−	TGGCCATACCGAAGCCCACTT
DJ562	5	+	GGGGGAGCTGATGAAGTCCAA
DJ543	5	−	CCCAGGAGGTGAGGTTGCAGTA
DJ544	6	−	GGAAGGCGTAGTAGCGGCAGTC
		(2) Human GAMT only	
DJ580	2	−	AGTCCCGGAGCCGCTGGAAG
		(3) Murine GAMT only	
DJ555	1	+	GCCTGGTTTGCACAGCCTCAC
DJ579	1	−	GGAGCCGGGACCTCTTTCCT
DJ572	2 + 3	+	GCCAGGCCTCACATCTAAGTCC
DJ565	2 + 3	−	GCAGGCACAGGCTGCCAAGT
DJ563	4 + 5	+	TGAGGTTGGGCAAGGCTGTG
DJ564	4 + 5	−	GGCCTTCCTGACTTCAGAATGG
DJ556	6	−	CCCACAATCCAGCCACAAAGG

almost complete loss of GAMT activity in the liver of both patients (3).

Here we report the exon-intron organization of the human GAMT gene and its physical mapping to human 19p13.3 which is homologous to a region on mouse chromosome 10, containing the jittery locus. Since the neurological symptoms and developmental delay of jittery mice resemble the clinical picture of GAMT deficiency in humans, we have also determined the sequence of the murine GAMT gene in wild-type and jittery mice. Our data indicate that murine GAMT is not mutated in jittery mice.

MATERIAL AND METHODS

Cloning and sequencing of the human GAMT gene. Cosmid clone 32929 containing the GAMT gene was isolated from a chromosome 19 specific cosmid library and was identified as a member of the cosmid contig containing the FISH mapped cosmid 19401 (5,6) by partial restriction site mapping and fingerprinting of Eco RI sites. The insert of cosmid 32929 was partially sequenced at the ends using the vector specific (Lawrist 16) primers DJ180 (5′-CGACTCACTATAGGGAGACCCA-3′) and DJ181 (5′-CCTCGAGAATTACCCTCACTAA-3′). Sequencing of cosmid and plasmid templates, gel-purified PCR products and cDNA fragments was performed by the dideoxy chain-termination method using an ABI PRISM 377 DNA sequencer (Perkin Elmer).

PCR analysis. Elongase (GIBCO BRL) or TAQ polymerase-catalysed (AmpliTAQ, Perkin Elmer) amplification of genomic or cDNA fragments was performed as described by the manufacturers. The murine cDNA of GAMT was amplified from cDNA which had been reverse-transcribed from murine bone marrow poly(A)+ RNA (Marathon cDNA amplification kit, Clontech). Primers for the sequencing of the human and murine GAMT gene were designed on the basis of the published human cDNA sequence (7) and the murine cDNA sequence. These primer sequences are listed in Table 1. The murine cDNA sequence was initially assembled from expressed sequence TAGs (ESTs) that were found in GenBank using the program BLAST and the human cDNA as query sequence. Ambiguities of the murine GAMT cDNA sequence were eliminated by sequencing RT-PCR products. Genomic DNA of homozygous jittery (*ji*) mice was a generous gift of M. Burmeister.

RESULTS AND DISCUSSION

During the course of contig construction and cosmid walking in the telomeric region of chromosome

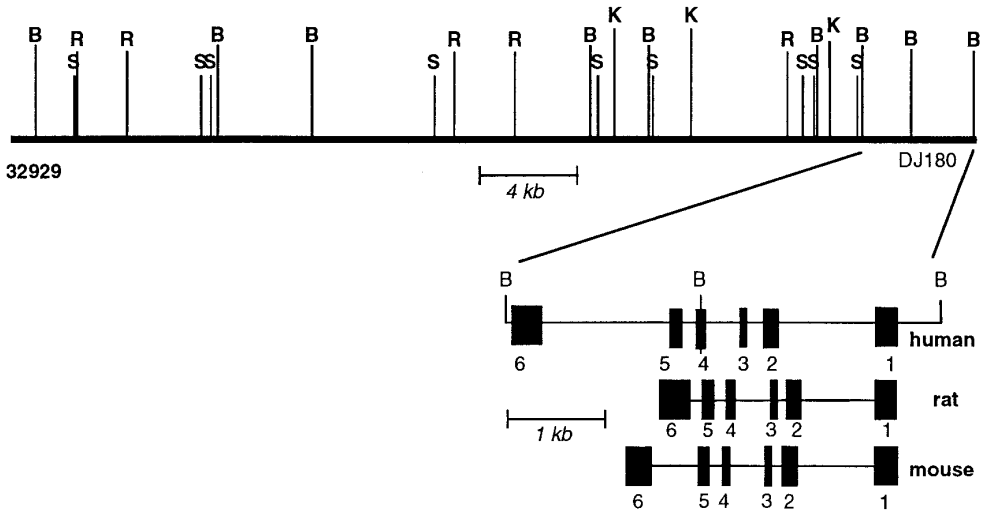

FIG. 1. Physical map and organization of the guanidinoacetate methyltransferase (GAMT) gene in human, rat and mouse. Upper part: restriction site map of cosmid 32929 which covers the human GAMT gene. Restriction enzymes: B, *Bam* HI; R, *Eco* RI, S; *Sac* I; K, *Kpn* I. Lower part: exon-intron structure of the human, murine and rat genes. Exons are depicted by black boxes and introns by thin connecting lines. Exon-intron distribution of the rat gene is taken from a previous report (9).

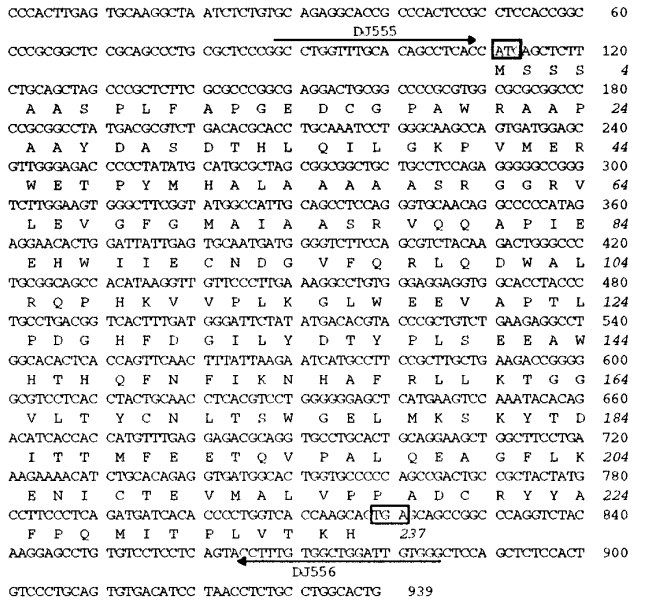

FIG. 2. Nucleotide sequence of mouse guanidinoacetate aminotransferase (GAMT) and cDNA derived amino acid sequence of mouse GAMT. The nucleotide numbering starts with the first base of the assembled cDNA sequence and is shown on the left. The predicted amino acid residues are shown by standard one-letter symbols below the first base of the respective codon. Amino acid residues numbers (italicized) are given at the end of each row. The methionine codons of the translational start site and the stop codon are boxed, while the position of the primers DJ555 and DJ556 are marked by an arrow above (forward primer) and below (backward primer) the cDNA sequence, respectively. The cDNA sequence has been completely sequenced between the primers DJ555 and DJ556 and has been deposited into Genbank under accession number AF015887.

19p13.3, we characterized cosmid 32929 by restriction site mapping and partial sequence analysis in more detail (Fig. 1). Using the polylinker primer DJ180 for the cosmid vector lawrist 16, we directly sequenced the end of the cosmid insert. Sequence comparison with the Genbank database revealed a perfect match with the 5′ end of the human GAMT cDNA sequence (7). Based on the human and rat cDNA sequences (7,8) and the genomic structure for the rat homologue of GAMT (9), we designed several sequencing and PCR primers (Table 1). Long distance PCRs were performed to identify the location and size of the five introns. Fig. 1 illustrates the restriction site map and genomic structure of the human GAMT gene. The first and the last intron is considerably larger than the introns between exons 2 and 5. Sequence analysis of the exon-intron boundaries confirmed complete identity between the human and rat genomic structure. A continuous sequence covering the exons 2 to 5 and part of the flanking introns as well as sequences determined for the exons 1 and 6 have been deposited in the Genbank database (accession numbers AF010246, AF010247, AF010248). These genomic sequences will facilitate the analyses of GAMT exons in other patients with a suspected defect in creatine biosynthesis.

Cosmid 32929 belongs to the same contig as cosmid 19401 which was previously mapped to 19p13.3 between proteinase 3 (gene symbol, PRTN3) and transcription factor 3 (immunglobulin enhancer binding factor E2A; gene symbol, TCF3) using fluorescence in situ hybridization (5,6). Thus GAMT is located in a region which is homologous to mouse chromosome 10 (10). The recessive jittery (*ji*) mutation was mapped to this region on mouse chromosome 10. Like patients suffering from GAMT deficiency jittery mice develop their first symptoms after birth and deteriorate progressively during the first 40 days. Jittery mice show seizures and are unable to walk without falling. Finally they die from starvation and dehydration. Hesitant is another mutation of the same gene leading to an uncoordinated abnormal movement behaviour and reduced fertility in males. In contrast to jittery mice, hesitant homozygotes have a milder phenotype and a normal lifespan (10).

Because of some similarities between the GAMT deficiency syndrome in humans and the *ji*/*hes* phenotype, GAMT appeared to be a good candidate for being mutated in *ji* mice. A search of GenBank revealed the presence of several mouse expressed sequence tags (ESTs) with high homology to the rat and human GAMT cDNA sequence (Genbank accession numbers: W13274, AA238539, AA057971, AA255018, AA033104, AA049899, AA030269, AA212732, W85027, W83179, AA222935, AA261309, W98830, W53067, AA048544, W71697, AA238076, AA008953, AA271145, W89424, AA064314, AA153502, AA22240). The murine GAMT cDNA sequence was tentatively assembled from these EST sequences and aligned with the known nucleotide and amino acid sequences for the human and rat enzyme. Sequence ambiguities were eliminated by sequencing a PCR generated cDNA fragment which covers the entire coding region of murine GAMT using the primers DJ555 and DJ556. The nucleotide sequence of this PCR product is available from GenBank under the accession number AF015887 (Fig. 2).

Several additional primers were designed to amplify the six exons and flanking regions of the murine GAMT gene (see Fig. 3 for designations and annealing positions) from total mouse DNA. The following PCR products were partially sequenced from both ends: DJ555<>DJ556 (covering exons 1 to 6), DJ542<>DJ544 (exons 1 to 6), DJ542<>DJ543 (exons 1 to 5), DJ572<>DJ565 (exons 2 and 3), DJ563<>DJ564 (exons 4 and 5), DJ562<>DJ556 (exons 5 and 6) using total wild-type and homozygous *ji* DNA as a template. In this way most of the murine genomic sequence has been established except for a 50 bp segment of the first intron (Genbank accession numbers AF010498, AF010499). Comparisons of the

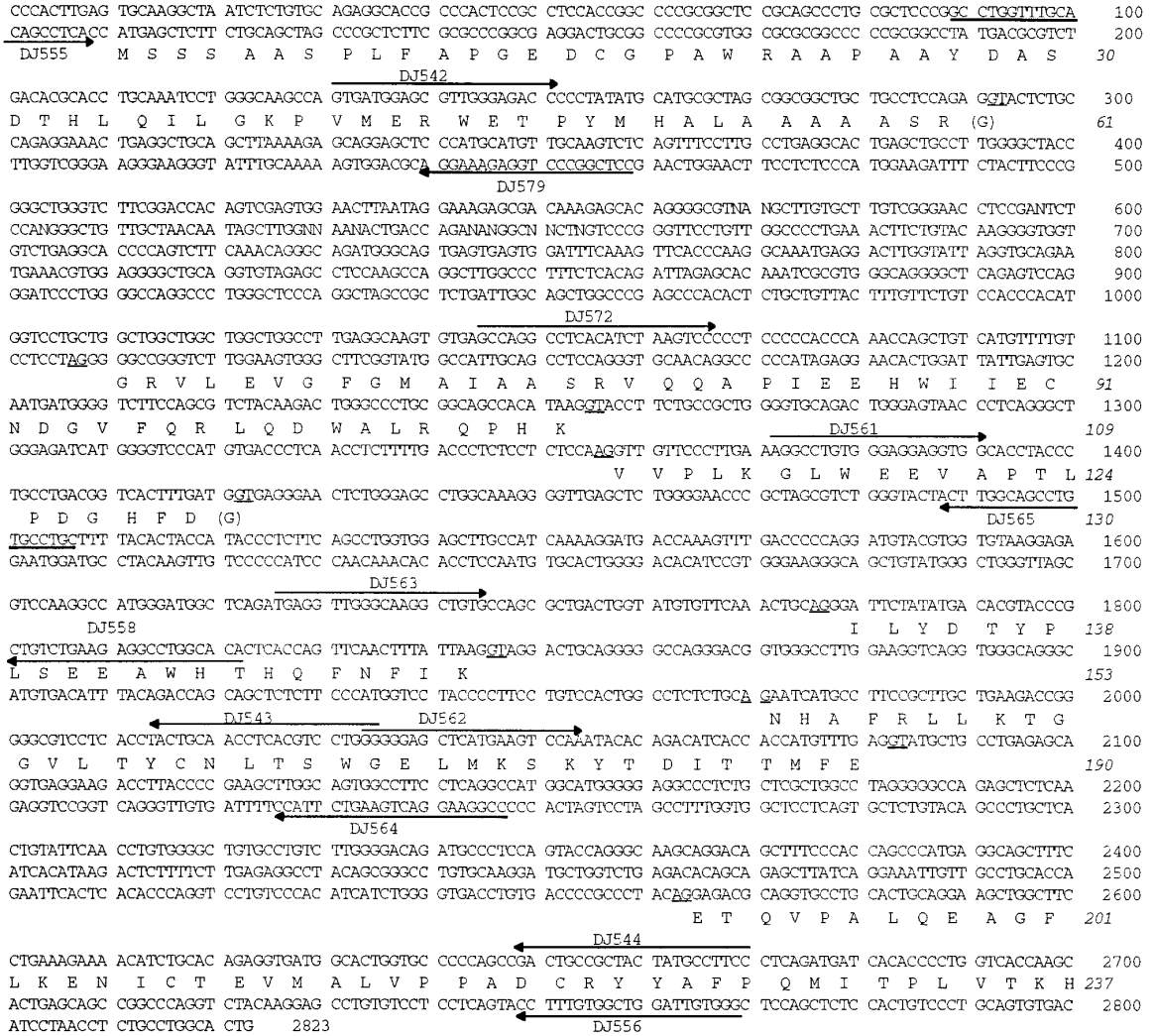

FIG. 3. Nucleotide sequence and coding regions of the murine GAMT gene. The five exons and four introns of the GAMT gene are shown together with the translation of exons in single-letter code under the first nucleotide of each codon. Amino acid residues interrupted by introns are given in parenthesis, residue numbers (italicized) are given on the right according to Fig. 2. The 5′ and 3′ splice junctions (GT and AG) are underlined, and ambiguous sequence positions within the first intron are indicated by Ns. Exon and intron sequences between the primers DJ555 and DJ556 have been submitted to GenBank under accession numbers AF010498 and AF010499 except for a short stretch within the first intron. Nucleotide numbering starts with the first base of the most 5′ murine EST sequence AA222404 (GenBank accession code) and ends with the last base of the murine EST sequence AA238539. Arrows in the forward and the backward direction above or below the GAMT sequence indicate the position and length of forward and backward primers, respectively, that were used in PCRs and sequence analyses.

coding sequences between C57Bl/6 and homozygous *ji* mice revealed no sequence difference within the GAMT coding region. Since we have not studied transcript levels for GAMT in different areas of the brain in C57Bl/6 and *ji*/*hes* mice, we cannot formally exclude the possibility that a selective local deficiency of GAMT activity in the brain of *ji*/*hes* mice is associated with a clinical picture of predominantly neurological symptoms. Functional characterization of the GAMT promoter in different cell types will be required to rule out subtle mutations in regulatory elements of the GAMT locus in *ji*/*hes* mice.

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