Molecular Mechanisms of an Inborn Error of Methionine Pathway

Methionine Adenosyltransferase Deficiency

Tsuneyuki Ubagai,* Ke-Jian Lei,* Sonia Huang,* S. Harvey Mudd,‡ Harvey L. Levy,§ and Janice Yang Chou*

**Human Genetics Branch, National Institute of Child Health and Human Development; ‡Laboratory of General and Comparative Biochemistry, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20892; and §Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115*

Abstract

Methionine adenosyltransferase (MAT) is a key enzyme in transmethylation, transsulfuration, and the biosynthesis of polyamines. Genetic deficiency of α/β-MAT causes isolated persistent hypermethioninemia and, in some cases, unusual breath odor or neural demyelination. However, the molecular mechanism(s) underlying this deficiency has not been clearly defined. In this study, we characterized the human α/β-MAT transcription unit and identified several mutations in the gene of patients with enzymatically confirmed diagnosis of MAT deficiency. Site-directed mutagenesis and transient expression assays demonstrated that these mutations partially inactivate MAT activity. These results establish the molecular basis of this disorder and allow for the development of DNA-based methodologies to investigate and diagnose hypermethioninemic individuals suspected of having abnormalities at this locus. (*J. Clin. Invest.* 1995. 96:1943–1947.) Key words: methionine adenosyltransferase deficiency • genetic mutations • human methionine adenosyltransferase gene • single-stranded conformation polymorphism

Introduction

Methionine adenosyltransferase (MAT,[1] ATP:L-methionine *S*-adenosyltransferase, EC 2.5.1.6) catalyzes the biosynthesis of *S*-adenosylmethionine (AdoMet) from methionine and ATP (1) and thus plays a central role in cellular metabolism. AdoMet acts as the methyl donor for most transmethylation reactions, participates in the transsulfuration pathway and, after decarboxylation, serves as a propylamine group donor in the biosynthesis of polyamines (2, 3). Three forms of MAT (α, β, and γ) have been identified in mammalian tissues (4–8). The α and β MATs, composed of homotetramers and homodimers, respectively, are expressed primarily in adult liver (6, 9). Although the α and β forms have distinct kinetic properties, no difference is observed in their subunit structures (4–6) suggesting that both are encoded by the same gene. Southern analysis of genomic DNA using either a human (9) or a mouse (10) MAT cDNA probe further shows that the α/β-MAT is encoded by a single copy gene. The γ form is expressed in fetal liver, kidney, brain, and lymphocytes (7, 8). Sequence comparison of the cDNAs (9–11) suggests that γ-MAT is probably encoded by a separate gene(s).

Hepatic α/β MAT deficiency has been positively diagnosed in seven patients by demonstrating reduced MAT activity in liver biopsy specimens (12–15). However, clinical and biochemical characteristics have been published on only six patients (12–17). These individuals are characterized by isolated persistent hypermethioninemia. Absent are hyperhomocysteinemia, tyrosinemia, or serious liver disease, signs of conditions in which hypermethioninemia is a secondary feature. The plasma methionine concentration in α/β-MAT–deficient patients can be as high as 1,300 μM, some 35 times above the upper limit of $\sim$ 35 μM in normal adults. In some patients, an unusual breath odor occurs due to the presence of unusually large amounts of dimethylsulfide (17). Most of these patients have been free of major clinical difficulties (3). When assayed, MAT activity in their erythrocytes, lymphocytes, or fibroblasts has been normal (14, 17, 18). Routine screening of newborns for hypermethioninemia has identified additional individuals with biochemical abnormalities suggestive of α/β-MAT deficiency (3, 19, Mudd, S. H., H. L. Levy, A. Tangerman, C. Boujet, N. Buist, and A. Davidson-Mundt, unpublished observations). One such individual developed abnormal neurological symptoms and neural demyelination that responded favorably to AdoMet therapy (19). Because most of these hypermethioninemic individuals do not exhibit severe clinical manifestations, a liver biopsy sampling was not recommended and definitive diagnosis of MAT deficiency was not made. To understand the molecular basis of isolated persistent hypermethioninemia and to develop a noninvasive, DNA-based diagnosis, we characterized the cDNA and the gene for human α/β-MAT.

In the present study we used a combination of single-stranded conformation polymorphism (SSCP) (20) and DNA sequence analyses to characterize the α/β-MAT gene in three unrelated patients, who were previously diagnosed, by enzyme assay, as having MAT deficiency (12, 14). All three patients were found to contain mutations in the coding region of the α/β-MAT gene and each of these mutations partially inactivates MAT activity. These studies have enabled us to establish the molecular basis of this disorder.

Methods

Characterization of human α/β-MAT cDNA and genomic clones. A phMAT-1 cDNA clone containing nucleotides 1–1188 of the entire coding region of human α/β-MAT was isolated by reverse transcriptase PCR amplification of human liver poly(A)$^+$ RNA using two oligonucle-

Address correspondence to Janice Yang Chou, Building 10, Room 9S242, National Institutes of Health, Bethesda, MD 20892. Phone: 301-496-1094; FAX: 301-402-0234.

Received for publication 5 May 1995 and accepted in revised form 15 June 1995.

1. *Abbreviations used in this paper:* AdoMet, S-adenosylmethionine; MAT, methionine adenosyltransferase; MDE, mutation detection enhancement; SSCP, single-stranded conformation polymorphism.

The Journal of Clinical Investigation, Inc.
Volume 96, October 1995, 1943–1947

otide primers derived from nucleotides 1–24 (5′-ATGAATGGACCG-GTGGATGGCTTG-3′, Ms, sense) and 1188–1161 (5′-CTAAAA-TACAAGCTTCCTGGGAACCTCC-3′, Mas, antisense) of the human α/β-MAT cDNA (9, 21). The sense and antisense primers contain additional XhoI and XbaI linkers, respectively, and after digestion with XhoI and XbaI, the amplified fragment was subcloned into a pSVL vector (Pharmacia LKB Biotechnology Inc., Piscataway, NJ).

A human placenta genomic library in λEMBL-3 (Clontech, Palo Alto, CA) was screened with the phMAT-1 cDNA and one genomic clone, hMATG3, which lacks exon I, was obtained and characterized. A fragment of 2.4 kb containing exon I and part of intron 1 of the α/β-MAT gene was isolated by PCR amplification of human genomic DNA using a sense primer containing nucleotides −67 to −50 of the human α/β-MAT cDNA, pSHL (9), and an antisense primer (5′-ATA-CATGCTGGCCACCTTAGGCTT-3′, I1as) containing sequence within intron 1. Both strands of the cDNA and genomic clones were sequenced, and the genomic sequences were compared to cDNA sequences as a means to identify intron–exon junctions.

MAT-deficient individuals. We have analyzed the α/β-MAT gene of three MAT-deficient patients positively diagnosed as having reduced MAT activities in liver biopsy samples. The defects in patient G1 (Caucasian American female) (14) and G3 (African American female) (14) have been extensively characterized. Genomic DNA preparations from G1 and the mother of G1 were extracted from the blood samples. The genomic DNA preparation from patient G3 was isolated from cultured skin fibroblasts kindly sent to us by Dr. Susan Sklower of the Institute for Basic Research in Developmental Disabilities, Staten Island, NY. The genomic DNA preparation from patient MG was isolated from cultured fibroblasts (repository No. GM 00911) deposited at the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository, Camden, NJ, by Dr. R. Longhi. It was noted that patient MG was deficient in hepatic MAT activity, while fibroblast MAT activity was normal. The DNAs were isolated using a Nucleon II kit obtained from Scotlab (Strathclyde, United Kingdom). Peripheral blood samples were obtained with the informed consent of the patients.

Analysis of the α/β-MAT gene by SSCP and DNA sequencing. The MAT gene of the patients and of the mother of patient G1 was characterized by amplifying the coding regions of each of exons I to IX and the corresponding intron–exon junctions by PCR using nine pairs of oligonucleotide primers containing 5′-untranslated, 3′-untranslated and intron sequences of the MAT gene in the presence of [^{32}P]dCTP (6,000 Ci/mmol). The sense and antisense primers for amplifying exon I are 5′-AAAGAACGCTGAGTGGAGAA-3′ (1s) and 5′-TCAGTATAG-GCTTGGAATGA-3′(1as), exon II, 5′-GTGATTCTGACCCATAT-TTG-3′ (2s) and 5′-TTTCTCAGTCTAGCCCACTT-3′(2as), exon III, 5′-TGCTGGCACTTGGCTGCTAA-3′ (3s) and 5′-TTGACTCA-GCACTGGGGTCT-3′(3as), exon IV, 5′-ATCCATTGGGAAGT-TTGTGG-3′ (4s) and 5′-CATGAACTTTCCCAGCCAGC-3′(4as), exon V, 5′-TTCAGGCTTGGAGAGAGCTG-3′ (5s) and 5′-TAT-TAAAGCTTCTGTCTAGG-3′ (5as), exon VI, 5′-GCCTCAGCC-TCACAGTATCT-3′ (6s) and 5′-CTGCTCTGAGACATAAGCAA-3′ (6as), exon VII, 5′-CTGCGTCTGCTTCTTATCTT-3′ (7s) and 5′-CCTCACTCAGGGCAGAACTG-3′ (7as), exon VIII, 5′-ACAGAG-GCTTCAATCCCTGA-3′ (8s) and 5′-GGTGAGCATCTGGGC-AAGGA-3′ (8as), and exon IX, 5′-ACCCTGCTCCTGTTCCATCT-3′ (9s) and 5′-ACCAGGTGCCTCCAGGGTGAGA-3′ (9as).

The PCR-amplified fragments, I (175 bp), II (164 bp), III (239 bp), IV (238 bp), V (263 bp), VI (330 bp), VII (292 bp, VIII (236 bp), and IX (201 bp) were analyzed for single-strand conformation polymorphisms (20) by electrophoresing wild-type and mutant target DNAs side by side through mutation detection enhancement (MDE) nondenaturing gels (AT Biochem, Malvern, PA) following the protocols provided by the manufacturer. To increase sensitivity of SSCP analysis, we also analyzed the amplified fragments on MDE gels containing 5% glycerol. Mutations in the target DNA were visualized by the differential migration of one or both of the mutant strands.

Construction of α/β-MAT mutants. The pSVLhMAT-ApaI-5′ and pSVLhMAT-ApaI-3′ fragments were obtained by digestion of pSVLhMAT-1 cDNA with XbaI/ApaI and XhoI/ApaI, respectively, and the respective fragments were purified on a low melting agarose gel. The pSVLhMAT-ApaI-5′ fragment (containing nucleotides 1–663 of hMAT) was used as template for constructing mutants containing mutations in exons I to VI by site-directed mutagenesis (22) and the pSVLhMAT-ApaI-3′ fragment (containing nucleotides 664–1188 of hMAT) for construction mutants containing mutations in exons VI through IX. Each construct was shown to be correct by DNA sequencing.

Expression in COS-1 Cells. Wild-type or mutant α/β-MAT cDNAs were transfected into COS-1 cells by the DEAE-dextran/chloroquine method (23). Mock transfections of COS-1 cultures with the pSVL vector were used as controls. Two independent isolates of each construct were used in quadruplet cultures. After incubation at 37°C for 3 d, the transfected cultures were homogenized in 0.3 ml of a buffer containing 0.25 M Tris-HCl, pH 7.4, and 4 mM β-mercaptoethanol and used for measuring enzyme activity.

MAT was assayed essentially as described (8). The enzyme was incubated for 30 min at 37°C in a reaction mixture (100 μl) containing 0.1 M Tris-HCl, pH 8.2, 20 mM $MgCl_2$, 150 mM KCl, 10 mM ATP, 5 mM β-mercaptoethanol, and 500 μM L-[methyl-^{3}H]methionine (0.5 μCi, sp act, 84 Ci/mmol). The reaction was stopped with 10 μl of 2 M $HClO_4$ containing 5 mM methionine. After centrifugation, 50 μl of the supernatant solution was spotted onto a phosphocellulose circle, washed in water, dried, and radioactivity measured in a scintillation counter.

Results

Characterization of the human α/β-MAT transcription unit. The hMATG-3 genomic clone, which lacks exon I, was isolated from a human placenta genomic library using a phMAT-1 cDNA probe containing nucleotides 1–1188 of the entire coding region of human α/β-MAT. Exon I and the associated intron 1 containing genomic fragment was obtained by PCR amplification of human genomic DNA. The structural organization of the human α/β-MAT gene was defined by restriction endonuclease mapping, Southern-blot hybridization using the phMAT-1 cDNA probe, and DNA sequencing. The exon–intron junctions were assigned by sequence alignment of cDNA and genomic sequences.

Human α/β-MAT is encoded by a gene containing 9 exons and 8 introns spanning ~ 20 kb (Fig. 1 *A*). The sizes of exons I (162 bp, including 91 bp coding and 71 bp 5′-untranslated sequences), II (78 bp), III (123 bp), IV (113 bp), V (144 bp), VI (219 bp), VII (183 bp), and VIII (134 bp) were determined by DNA sequencing. The size of exon IX (2,061 bp, including 103 bp coding and 1,958 bp 3′-untranslated sequences) contained in hMATG-3 was initially estimated from nucleotide sequence of the human α/β-MAT cDNA (pSHL) reported by Alvarez et al. (9). This was confirmed by PCR amplification of the hMATG-3 genomic clone using oligonucleotide primers, 9s (containing intron 8 sequence adjacent to exon IX) and 3UT1as (nucleotides 3117 to 3098 in pSHL); 9s and 3UT2as (nucleotides 2222–2203 in pSHL); and 3UT2s and 3UT1as. Three fragments of 2,083, 1,188, and 895 bp were obtained as predicted. The 5′ donor and 3′ acceptor splice sites in each intron conform to the GT-AG rule (24) and agree with the consensus sequence compiled for exon–intron boundaries.

Southern-blot analysis (Fig. 1 *B*) of human genomic DNA hybridizing with the phMAT-1 cDNA probe showed that the exon sequences were contained within two BamHI (> 20- and 4.5-kb) or two EcoRI (14- and 3.8-kb) fragments, consistent with the map of the human MAT gene. The simple pattern

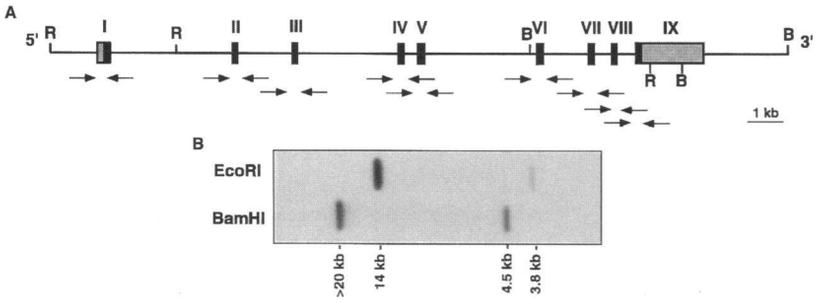

Figure 1. (*A*) Structural organization of the human α/β-MAT gene. The exons (I–IX) are indicated by boxes with the untranslated regions stippled. Arrows illustrate oligonucleotide primers used for amplification of exon containing fragments; B, BamHI; R, EcoRI. (*B*) Southern-blot hybridization of human genomic DNA digested with BamHI or EcoRI with the phMAT-1 cDNA probe.

suggests that the human α/β-MAT is encoded by a single copy gene.

Identification of mutations in the α/β-MAT gene of MAT-deficient patients. To characterize mutations in the α/β-MAT gene, we used nine pairs of oligonucleotide primers containing 5′-, 3′-untranslated and intron sequences (shown diagrammatically in Fig. 1) to amplify individually the coding regions contained in exons I through IX and the associated intron–exon junctions. The amplified fragments were analyzed by SSCP (20) to detect mutation-containing fragments.

SSCP analysis on MDE gels in the absence (Fig. 2 *A*) or presence (Fig. 2 *B*) of glycerol revealed the presence of mutations in the α/β-MAT gene of all three patients, G1, G3, and MG. The mutation in patient G1 was not clearly detected by SSCP on regular MDE gels (data not shown). However, this mutation could be identified using MDE gels containing 5% glycerol (Fig. 2 *B*). The mutation-containing fragments were subcloned, and five or more subclones of each fragment were sequenced. Sequence data of the defective genes were compared with the normal gene to identify mutations.

SSCP analysis (Fig. 2 *B*) of G1 suggests that this patient may be homozygous for a mutation in exon VIII. This analysis also suggests that the mother of G1 is heterozygous for the same mutation. Sequence analysis of five exon VIII subclones of patient G1 revealed that all subclones contained a T to G mutation at nucleotide 966 that converts Ile-322 to Met (I322M) (Fig. 3). Four of the seven exon VIII subclones of the mother of G1 contained this mutation.

SSCP analysis of patient G3 revealed that the fragments representing exons II, VII, and VIII contained mutations. Sequence analysis revealed that two of the five exon II subclones had a C to A transversion at nucleotide 164 that converts Ala-55 to an Asp (A55D) (Fig. 4). 3 of the 10 exon VIII subclones had a C to T transition at nucleotide 1070 that converts Pro-357 to a Leu (P357L) (Fig. 4). Three of the five exon VII subclones had a C to T mutation in the associated intron 7 which may represent sequence polymorphism. Our results suggest that patient G3 is a compound heterozygote with different mutations in each of the two α/β-MAT alleles.

SSCP analysis of patient MG revealed that the fragments representing exons V (data not shown), VII (Fig. 2 *A*), VIII (Fig. 2 *A*), and IX (data not shown) contained mutations. Sequence analysis of the subclones indicated that the exon V mutation (a C to T at nucleotide 426) did not change the code for Ala-142, while the exon IX mutation (a T to C at nucleotide 1131) did not change the code for Tyr-377. Two mutations were contained in the exon VII fragment, a G to A transition at nucleotide 870 that did not change the code for Val-290 (data not shown) and a T to C transition at nucleotide 914 (two of the eight subclones) that converts Leu-305 to a Pro (L305P) (Fig. 5). Two of the five exon VIII subclones contained a T to G transversion at nucleotide 966 that converts Ile-322 to a Met (I322M) (Fig. 5). Our findings suggest that patient MG is also a compound heterozygote.

The liver biopsy samples of patients G1 and G3 had low levels of MAT activity (14). Although the details of patient MG have not been published, the available data indicate that MAT activity in the liver biopsy sample of this patient was also very low. This suggests that MAT harboring the A55D, L305P,

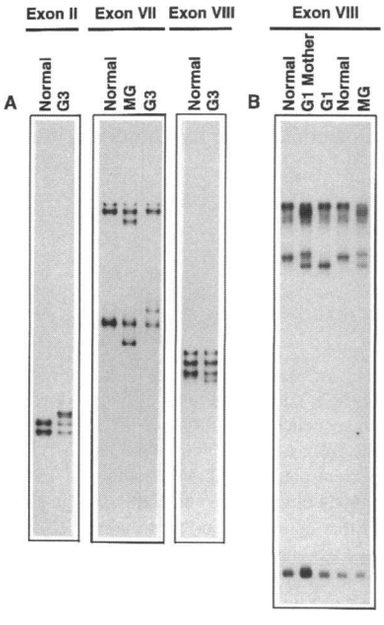

Figure 2. SSCP analysis of mutations in the α/β-MAT genes of hepatic MAT-deficient patients. The exon-containing fragments were amplified by PCR and analyzed by electrophoresis on MDE gels (*A*) or MDE gels containing 5% glycerol (*B*). Normal indicates the pattern of an individual with the wild-type gene.

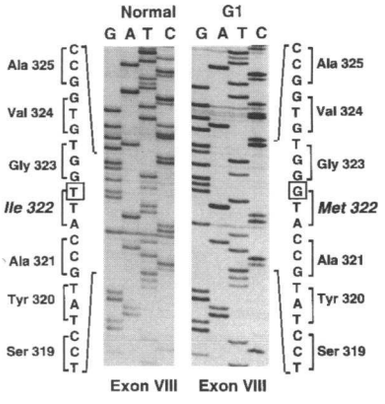

Figure 3. Autoradiograms of Sanger nucleotide sequencing reactions of the α/β-MAT gene from a normal individual and MAT-deficient patient G1. G1 contains a T to G (boxed) transversion at nucleotide 966 in exon VIII that converts Ile-322 to a Met (I322M) and is homozygous for this mutation.

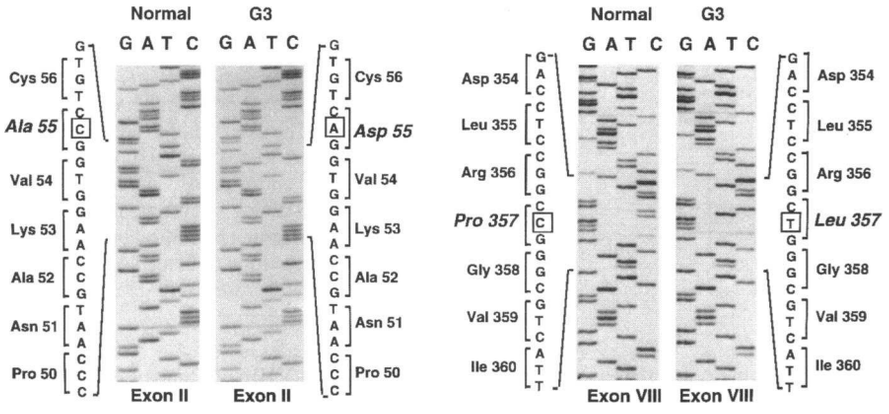

Figure 4. Autoradiograms of Sanger nucleotide sequencing reactions of the α/β-MAT gene from a normal individual and MAT-deficient patient G3. G3 contains a C to A (boxed) transversion at nucleotide 164 in exon II that converts Ala-55 to a Asp (A55D) and C to T (boxed) transition at nucleotide 1070 in exon VIII that converts Pro-357 to a Leu (P357L).

I322M, or P357L mutation will have reduced enzymatic activity. To further investigate these points, four α/β-MAT mutants were constructed, each containing one of the four mutations identified in the patients. All four mutations yielded mutant MAT with low but detectable enzyme activity (Table I). Northern hybridization analysis of MAT transcripts from transfected cells showed that wild-type and mutant MAT mRNAs were expressed at similar levels (data not shown).

Discussion

We describe for the first time the organization of the human α/β-MAT gene and show it to contain 9 exons and 8 introns. The exons account for the full coding region in the cDNA. Based on gene structure, we characterized several mutations in the gene that account for the decreases in MAT activity in liver biopsy samples of three patients with confirmed MAT deficiency (14). Two of the patients are compound heterozygotes and one is a homozygote. The I322M mutation found in the homozygous patient G1 was detected also in the mother of G1 who is heterozygous for this mutation. This mother is clinically well (3). Her plasma methionine concentration is 28 μM, and her hepatic MAT activity was well within the normal range (14). Our data suggest that the I322M mutation behaves in a Mendelian recessive manner in the presence of a normal allele. The amino acid sequences of all known forms of MAT including bacteria, yeast, plants, and mammals have been highly conserved (10). It is noteworthy Ala-55, Ile-322, and Pro-357, altered in the α/β-MAT deficient patients, have been virtually invariant during evolution. In the case of Leu-305 only conservative changes to Ile (bacteria) or Val (plants) occurred. Our data suggest that these residues play important roles in MAT catalysis.

Each of the six patients with confirmed α/β-MAT deficiency for whom data are available had some detectable activity of MAT in his or her liver (13–15). When tested, these patients had normal γ-MAT activities in erythrocytes, cultured skin fibroblasts, and lymphocytes (14, 17, 18). Since adult liver also expresses low levels of the γ form of MAT (25), it has been unclear whether the residual MAT activity detected in the livers of these hypermethioninemic patients was due solely to this γ-MAT, to residual activity contributed by mutant α/β-MAT, or to some combination thereof. In our transient expression assays of wild-type and mutant α/β-MAT cDNAs in COS-1 cells, we found that the mutations A55D, L305P, I322M, and P357L only partially inactivated α/β-MAT activity. Thus, the residual hepatic activities detected in these patients are most likely contributed, at least in part, by the mutant α/β-MAT isozyme and the phenotype of a complete loss of α/β-MAT activity remains unknown.

One clinically normal patient retaining hepatic MAT activity equivalent to 7% of normal and having greatly elevated plasma methionine concentrations synthesized AdoMet at a rate equal to that of a normal person on a normal methionine intake (17). However, this patient lacked the normal capacity to catabolize an increased methionine intake (17). These results suggest that mutations that lead to lower residual α/β-MAT activities might have more serious clinical outcomes than that observed in this

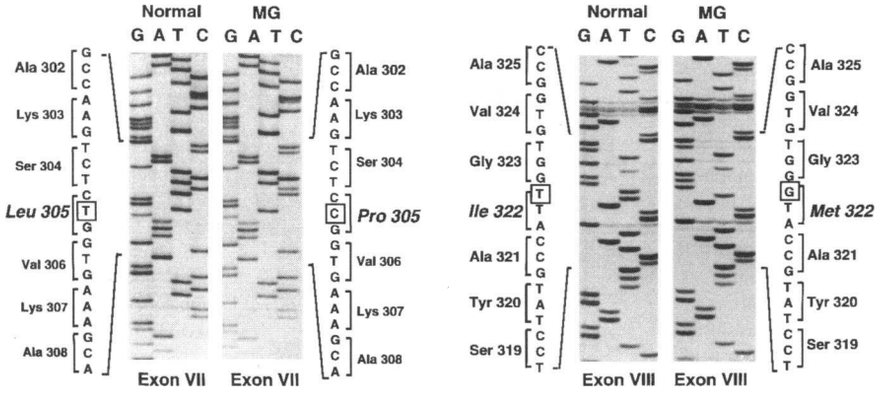

Figure 5. Autoradiograms of Sanger nucleotide sequencing reactions of the α/β-MAT gene from a normal individual and MAT-deficient patient MG. MG contains a T to C (boxed) mutation at nucleotide 914 in exon VII that converts Leu-305 to a Pro (L305P) and T to G (boxed) transversion at nucleotide 966 in exon VIII that converts Ile-322 to a Met (I322M).

Table I. MAT Activity Encoded by the Wild-Type and Mutant α/β-MAT cDNAs

Constructs	Specific enzyme activity	Constructs	Specific enzyme activity
	nmol/min per mg protein		*nmol/min per mg protein*
Mock	0.27±0.01	L305P	0.61±0.01
Wild-type	2.38±0.23	I322M	0.51±0.04
A55D	0.41±0.06	P357L	0.74±0.08

MAT activity in transfected COS-1 cells was assayed as described in Methods. Data are presented as the mean±SEM.

patient. Indeed it is possible that a total lack of α/β-MAT activity would be lethal. Routine screening of newborns has led to the identification of a number of individuals with persistent isolated hypermethioninemia compatible with hepatic MAT deficiency (3, 19, Mudd et al., unpublished observations). However, none of these individuals has been subjected to liver biopsy to confirm α/β-MAT deficiency. Although most of these individuals are clinically well, one girl at age 11 yr developed neurological problems and had neural demyelination as evidenced by magnetic resonance imaging (19). Both manifestations responded favorably to treatment with AdoMet. Other patients with isolated persistent hypermethioninemia have evidence of dystonia, learning disability, and magnetic resonance imaging evidence of myelination arrest, while others have subnormal intelligence quotients (Mudd et al., unpublished observations). Thus, isolated persistent hypermethioninemia may not always be a clinically benign condition. Furthermore, a few patients have been described with isolated persistent hypermethioninemia yet normal activities of MAT in biopsied liver material (3). An accurate molecular diagnosis of the underlying genetic lesion in patients with isolated hypermethioninemia coupled with determination of the levels of mutant α/β-MAT activity should be useful in arriving at a prognosis and a rational plan of therapy for α/β-MAT deficient patients. The cloning, characterization, and expression of the human α/β-MAT gene, reported here, should provide accurate and noninvasive gene-based methods for achieving both these objectives and for defining further the clinical spectrum of α/β-MAT deficiency.

Acknowledgments

We thank Drs. Brian Mansfield, Margaret Chamberlin, Anil Mukherjee, and Ida Owens for critical reading of the manuscript.

References

1. Cantoni, G. L. 1953. S-Adenosylmethionine: a new intermediate formed enzymatically from L-methionine and adenosinetriphosphate. *J. Biol. Chem.* 204:403–416.

2. Kotb, M., and A. M. Geller. 1993. Methionine adenosyltransferase: structure and function. *Pharmacol. & Ther.* 59:125–143.

3. Mudd, S. H., H. L. Levy, and F. Skovby. 1994. Disorders of transsulfuration. *In* The Metabolic Basis of Inherited Diseases. C. R. Scriver, A. L. Beaudet, R. Charles, W. S. Sly, and D. Valle, editors. McGraw-Hill Inc., New York, 1279–1327.

4. Okada, G., H. Teraoka, and K. Tsukada. 1981. Multiple species of mammalian S-adenosylmethionine synthetase. Partial purification and characterization. *Biochemistry.* 20:934–940.

5. Sullivan, D. M., and J. L. Hoffman. 1983. Fractionation and kinetic properties of rat liver and kidney methionine adenosyltransferase isozymes. *Biochemistry.* 22:1636–1641.

6. Cabrero, C., J. Puerta, and S. Alemany. 1987. Purification and comparison of two forms of S-Adenosyl-L-methionine synthetase from rat liver. *Eur. J. Biochem.* 170:299–304.

7. Kotb, M., and N. M. Kredich. 1985. S-Adenosylmethionine synthetase from human lymphocytes. Purification and characterization. *J. Biol. Chem.* 260:3923–3930.

8. Mitsui, K., H. Teraoka, and K. Tsukada. 1988. Complete purification and immunochemical analysis of S-adenosylmethionine synthetase from bovine brain. *J. Biol. Chem.* 263:11211–11216.

9. Alvarez, L., F. Corrales, A. Martin-Duce, and J. M. Mato. 1993. Characterization of a full-length cDNA encoding human liver S-adenosylmethionine synthetase: tissue-specific gene expression and mRNA levels in hepatopathies. *Biochem. J.* 293:481–486.

10. Sakata, S. F., L. L. Shelly, S. Ruppert, G. Schutz, and J. Y. Chou. 1993. Cloning and expression of murine S-adenosylmethionine synthetase. *J. Biol. Chem.* 268:13978–13986.

11. Horikawa, S., and K. Tsukada. 1992. Molecular cloning and development expression of a human kidney S-adenosylmethionine synthetase. *FEBS Lett.* 312:37–41.

12. Gaull, G. E., and H. H. Tallan. 1974. Methionine adenosyltransferase deficiency: new enzymatic defect associated with hypermethioninemia. *Science (Wash. DC).* 186:59–60.

13. Finkelstein, J. D., W. E. Kyle, and J. J. Martin. 1975. Abnormal methionine adenosyltransferase in hypermethioninemia. *Biochem. Biophys. Res. Commun.* 66:1491–1497.

14. Gaull, G. E., H. H. Tallan, D. Lonsdale, H. Przyrembel, F. Schaffner, and D. B. Von Bassewitz. 1981. Hypermethioninemia associated with methionine adenosyltransferase deficiency: clinical, morphological and biochemical observations on four patients. *J. Pediatr.* 98:734–741.

15. Gahl, W. A., J. D. Finkelstein, K. D., Mullen, I. Bernardini, J. J. Martin, P. Backlund, K. G. Ishak, J. H. Hoofnagle, and S. H. Mudd. 1987. Hepatic methionine adenosyltransferase deficiency in a 31-year-old man. *Am. J. Hum. Genet.* 40:39–49.

16. Gout, J.-P., J.-C. Serre, M. Dieterlen, I. Antener, P. Frappat, M. Bost, and A. Beaudoing. 1977. Une nouvelle cause d'hypermethioninemie de l'enfant: le deficit en S-adenosyl-methionine-synthetase. *Arch. Fr. Pediatr.* 34:416–423.

17. Gahl, W. A., I. Bernardini, J. D. Finkelstein, A. Tangerman, J. J. Martin, H. J. Blom, K. D. Mullen, and S. H. Mudd. 1988. Transsulfuration in an adult with hepatic methionine adenosyltransferase deficiency. *J. Clin. Invest.* 81:390–397.

18. Tallan, H. H., and P. A. Cohen. 1976. Methionine adenosyltransferase: kinetic properties of human and rat liver enzymes. *Biochem. Med.* 16:234–250.

19. Surtees, R., J. Leonard, and S. Austin, S. 1991. Association of demyelination with deficiency of cerebrospinal-fluid S-adenosylmethionine in inborn errors of methyl-transfer pathway. *Lancet.* 338:1550–1554.

20. Orita, M., H. Iwahana, H. Kanazawa, K. Hayashi, and T. Sekiya. 1989. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc. Natl. Acad. Sci. USA.* 86:2766–2770.

21. Horikawa, S., and K. Tsukada. 1991. Molecular cloning and nucleotide sequence of cDNA encoding the human liver S-adenosylmethionine synthetase. *Biochem. Int.* 25:81–90.

22. Higuchi, R. 1990. Recombinant PCR. *In* PCR Protocols: A Guide to Methods and Applications. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, editors. Academic Press, Inc., San Diego, CA. 177–183.

23. Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. 1992. Current Protocols in Molecular Biology, Greene Publishing and Wiley-Interscience, New York. 9.2.1–9.2.6.

24. Mount, S. M. 1982. A catalogue of splice junction sequences. *Nucleic Acids Res.* 10:459–472.

25. Horikawa, S., H. Ozasa, K. Ota, and K. Tsukada. 1993. Immunohistochemical analysis of rat S-adenosylmethionine synthetase isozymes in developmental liver. *FEBS Lett.* 3:307–311.