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Normal Brain Myelination in a Patient Homozygous for a Mutation That Encodes a Severely Truncated Methionine Adenosyltransferase I/III

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Two isozymes of mammalian methionine adenosyltransferase, MAT I and MAT III, are expressed solely in adult liver. They are, respectively, tetramers and dimers of a single subunit encoded by the gene *MAT1A*. A third isozyme, MAT II, contains a catalytic subunit encoded by a separate gene, *MAT2A*, and is expressed in a variety of tissues, including (to a slight extent) adult liver. Based on a recent finding that 2 children with isolated hypermethioninemia and brain demyelination were homozygous for *MAT1A* mutations predicted to produce severely truncated proteins, and devoid of activity when expressed, it was concluded that complete lack of MAT I/III activity may be associated with neurological symptoms and demyelination. We now report that a 43-year-old man with persistent isolated hypermethioninemia, previously demonstrated to have deficient MAT activity in his liver, has normal brain myelination on MRI and normal neurological function, despite being homozygous for a 539 TG insertion in exon V of *MAT1A*, so that the gene is predicted to encode a protein of only 184 rather than the normal 395 amino acids. This patient's exon V mutation was demonstrated by SSCP analysis and verified by sequencing. Both parents are heterozygous for the same insertion. This suggests that *MAT1A* mutations producing severely truncated proteins do not necessarily produce brain demyelination. This finding has relevance to a previously reported 4-year-old girl who was also homozygous for the 539insTG mutation. Finally, our patient's 7% residual hepatic MAT activity, measured at 1 mM methionine, may reflect the hepatic activity of the more ubiquitous enzyme form, MAT II. Am. J. Med. Genet. 75:395–400, 1998. © 1998 Wiley-Liss, Inc.†

KEY WORDS: methionine adenosyltransferase; hypermethioninemia; adenosylmethionine; demyelination

INTRODUCTION

Methionine adenosyltransferase (MAT; E.C.2.5.1.6) catalyzes the first step in the transsulfuration pathway, i.e., the synthesis of *S*-adenosylmethionine (AdoMet) from L-methionine and ATP [Mudd et al., 1995a]. AdoMet serves as a methyl donor in a large number of reactions, and is responsible for the formation of spermine, spermidine, phosphatidylcholine, carnitine, creatine, the methylated derivatives of DNA, RNA, protein, and catecholamines, and other compounds [Mudd and Poole, 1975]. AdoMet also regulates the partitioning of homocysteine between degradation via cystathionine and remethylation to methionine [Gahl et al., 1988]. Different forms of MAT have been identified in mammalian tissues [Okada et al., 1981; Sullivan and Hoffman, 1983; Kotb and Kredich, 1985; Cabrero et al., 1987; Mitsui et al., 1988]. MAT I/III, encoded by the single copy gene *MAT1A*, is found as both tetramers (MAT I) and dimers (MAT III) formed from identical $\alpha 1$ subunits [Kotb et al., 1997]. This gene is expressed solely in adult liver [Okada et al., 1981; Sullivan and Hoffman, 1983; Cabrero et al., 1987; Alvarez et al., 1993]. MAT II, encoded by a separate gene, *MAT2A* [Horikawa and Tsukada, 1992; De La Rosa et al., 1995], is found in fetal liver and to a lesser extent in

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adult liver, as well as in the kidney, brain, testis, and lymphocytes [Kotb and Kredich, 1985; Mitsui et al., 1988; Horikawa and Tsukada, 1992; De La Rosa et al., 1995].

Patients with MAT I/III (hepatic MAT) deficiency have persistent isolated hypermethioninemia, meaning that they do not have, as a cause of their hypermethioninemia, tyrosinemia type I, liver disease, or hyperhomocyst(e)inemia and homocystinuria due to cystathionine β-synthase deficiency [Mudd et al., 1995b]. Most individuals with isolated hypermethioninemia have been identified coincidentally through newborn screening programs designed to ascertain infants with homocystinuria due to cystathionine β-synthase deficiency, who display a secondary elevation in plasma methionine. Patients with isolated hypermethioninemia are often incompletely investigated; only 7 of the 50 or more identified to date have been subjected to liver biopsy. When performed, this procedure has produced evidence of deficient MAT activity in liver. MAT activity has been normal in cultured skin fibroblasts, red blood cells, and lymphoid cells [Gahl et al., 1987, 1988; Gaull and Tallan, 1974; Finkelstein et al., 1975; Gout et al., 1977; Gaull et al., 1981; Uetsuji, 1986]. It was long considered that these individuals with deficient MAT activity in liver exhibited a benign disorder, but this belief was based largely on scattered reports in children. The only adult with proven deficient MAT activity in liver, a male who presented with an unusual breath odor at age 31 years [Gahl et al., 1987], also appeared clinically normal, bolstering the impression that partial MAT deficiency and chronic hypermethioninemia are not deleterious. In contrast, 2 patients with isolated hypermethioninemia (but without liver biopsy evidence of MAT deficiency) displayed brain demyelination and neurological symptoms or mental deficits [Surtees et al., 1991; Mudd et al., 1995b]. Hence, there remained a question as to whether isolated hypermethioninemia, presumably due to hepatic MAT deficiency, causes neurological deterioration.

After the genomic organization of the mouse *MAT1A* gene was elucidated [Sakata et al., 1993], the molecular basis of hepatic MAT deficiency was established in 3 of the 6 humans with the diagnosis previously established by assay of liver extract [Ubagai et al., 1995]. Eight additional individuals with isolated hypermethioninemia, including the 2 patients with evidence of demyelination, were then studied [Chamberlin et al., 1996]. All 11 patients exhibited mutations in the *MAT1A* gene, and the 2 with demyelination were homozygous for mutations predicted to produce severely truncated MAT proteins. It was concluded that complete lack of MAT I/III activity can lead to neurological abnormalities. Furthermore, a 4-year-old girl with a homozygous *MAT1A* mutation (539insTG) predicted to produce a truncated MAT I/III transcript with negligible activity (patient 3 of Chamberlin et al. [1996]) was considered to be at risk for developing demyelination at a later age.

We now report the results of investigations into the current clinical condition and the *MAT1A* mutation of the oldest known patient with hepatic MAT deficiency [Gahl et al., 1987], now 43 years old. This man has the identical homozygous 539insTG mutation as the 4-year-old girl mentioned above, and provides further indications as to her prognosis, specifically with respect to brain demyelination.

MATERIALS AND METHODS

Patient

The patient and his parents were enrolled in a protocol approved by the NICHD Institutional Review Board for the study of inborn errors of metabolism and gave informed consent for these investigations.

Amino Acid Analyses

Plasma and urinary amino acids were quantitated using a 4151 Alpha Plus analyzer (LKB-Biochrom, Cambridge, UK), with five lithium buffers [Gahl et al., 1988]. Specimens were frozen at −20°C, thawed, and deproteinized by ultrafiltration with Amicon Centrifree micropartition cartridges (Amicon, Danvers, MA). The ultrafiltrates were analyzed immediately or were refrozen and analyzed within 1 week of filtering.

Breath Dimethylsulfide and Urinary Methionine Transamination (TA) Metabolites

Measured volumes of breath (100 and 250 ml) were collected in Tenax (Chrompack, Middleburg, The Netherlands) tubes, and dimethylsulfide was analyzed as previously described [Tangerman et al., 1983]. Urinary methionine TA metabolites were determined as the sum of the methanethiol released into the gas phase at pH 12.5–13.0 [Blom et al., 1989]. For urine, these TA metabolites consist mainly of a base-labile methylthio-containing mixed disulfide (X-S-S-CH_3) and, to a minor extent, 4-methylthio-2-oxobutyrate.

Molecular Studies

DNA was prepared from peripheral blood lymphocytes [Sambrook et al., 1989]. For single-strand conformational polymorphism analysis (SSCP), *MAT1A* gene exons were first amplified using the primers listed in Ubagai et al. [1995]. PCR was performed using 50 mM KCl, 1.5 mM $MgCl_2$, 5 mM Tris, pH 8.3, 200 μM dNTPs, 0.01% gelatin, 2.5 U Taq polymerase, 0.4 μM of each primer, and 0.1 μg of genomic DNA in a final volume of 50 μl. Products were radiolabeled by inclusion of 1 μCi of α-^{32}P[dCTP]. After an initial denaturation step at 94°C for 3 min, PCR was conducted for 30 cycles with a denaturation step at 94°C for 30 sec, annealing at 53°C (exons I–V, VII, and IX) or 56°C (exons VI and VIII) for 30 sec, extension at 72°C for 1 min, and a final elongation step at 72°C for 10 min. PCR products were denatured with 10 μl of formamide and heating at 94°C for 10 min, and were fractionated at room temperature or at 4°C by electrophoresis for 12 hr on a 0.5 × MDE (mutation detection enhancer) gel (AT Biochemical, Malvern, PA). The gel was prepared in 0.6 × TBE (Tris-borate-EDTA) with or without 5% glycerol and subjected to autoradiography. Mutations in the

MATA1 gene were visualized by the differential migration of one or both of the mutant strands.

Direct, automated fluorescent sequencing was performed by Biotech Research Laboratories (Rockville, MD) on the exon V fragment of the patient's *MAT1A* gene. In addition, after subcloning exon V into the PCR 2.1 vector (InVitrogen, Carlsbad, CA), several clones were selected and both strands of DNA were sequenced.

RESULTS

Case Report

The patient, ascertained because of a breath odor due to dimethylsulfide [Gahl et al., 1988], was extensively investigated at age 31 [Gahl et al., 1987]. Except for mild aortic insufficiency attributable to rheumatic fever, he was developmentally, physically, and mentally normal. A 25–30-fold elevation of plasma methionine, along with a transiently elevated bilirubin level, led to a liver biopsy that demonstrated only 7% of the normal activity of hepatic methionine adenosyltransferase when measured at 1.0 mM methionine; hepatic activities of cystathionine β-synthase, γ-cystathionase, and betaine homocysteine methyltransferase were normal. Liver histology was normal, and a diagnosis of Gilbert's disease was made.

The patient had been a long-distance runner, but at age 37 he developed atrial fibrillation which spontaneously converted to normal sinus rhythm. His ejection fraction was measured at 63%, and he was placed on prophylactic penicillin. By age 38, the isolated aortic insufficiency had progressed in severity and heavy exercise was restricted. The patient continued to lead a normal, active life as husband and father of 4 children.

At age 43, the patient was in good health except for his aortic insufficiency and recent carpal tunnel syndrome. Vital signs were normal. Physical findings were normal except for a III/VI diastolic blowing murmur at the left sternal border. In particular, his neurologic status was normal. A family history showed that the patient's father's maternal grandfather and his mother's paternal grandmother shared the same name and had lived their entire lives in an isolated West Virginia community.

Laboratory studies showed hemoglobin of 14.0 g/dl, white blood cell count of 6,400/mm^3 with a normal differential, and a platelet count of 143,000/mm^3. The sedimentation rate was 35 mm/hr (normal, 0–25 mm/hr). Electrolytes and blood chemistries were normal, with a cholesterol of 132 mg/dl. Serum creatinine was 0.9 mg/dl (normal, 0.7–1.3 mg/dl), and the creatinine clearance was 105 ml/min (normal, 90–125 ml/min). Total bilirubin was 1.5 mg/dl (normal, 0.1–1.0 mg/dl), with a direct bilirubin of 0.2 mg/dl, reflecting this man's presumed Gilbert's disease. Aspartate aminotransferase (SGOT) was 27 units/l (normal, 9–34 units/l), alanine aminotransferase (SGPT) was 30 units/l (normal, 6–41 units/l), lactate dehydrogenase was 217 units/l (normal, 113–226 units/l), alkaline phosphatase was 62 units/l (normal, 37–116 units/l), and creatine phosphokinase was 56 units/l (normal, 52–386 units/l). Serum albumin was 3.9 g/dl (normal, 3.7–4.7 g/dl), calcium 2.13 mmol/l (normal, 2.05–2.50 mmol/l, and phosphorus 2.7 mg/dl (normal, 2.3–4.3 mg/dl). Plasma testosterone was 576 ng/dl (normal, 300–1,200 ng/dl).

The patient's isolated hypermethioninemia persisted, with continued elevation of urinary transamination metabolites and breath dimethylsulfide (Table I).

Myelination

Magnetic resonance imaging of the brain was performed, with 5-mm slices including T2 axonals and coronals, and T1 and proton density axonals. Fluid was evident in the right sphenoidal sinus, but the white and gray matter structures were normal in signal and size, with a normal-sized ventricular system. The cerebral cortex and posterior fossa structures were also normal, with no evidence of demyelination.

Mutation Analysis

In order to identify mutations within the patient's DNA, all nine exons and the associated intron-exon junctions of the *MAT1A* gene were subjected to SSCP analysis using an MDE gel. For 8 of the 9 exons, no aberrant band migration was observed. However, the SSCP pattern of exon V suggested a homozygous mutation (Fig. 1). Exon V was subcloned, and five different clones were sequenced. All these clones exhibited a TG insertion at nucleotide 539 (data not shown). This 2 base-pair insertion alters the reading frame of the transcript and generates a premature stop at codon 185.

On SSCP analysis, each parent appeared heterozygous for the patient's mutation in exon V (Fig. 1). To confirm that the patient was homozygous and the parents heterozygous for the TG insertion, direct sequencing of exon V was performed. Sequencing gel chromatography verified the patient's homozygosity for the TG insertion (Fig. 2B). Both his father (Fig. 2C) and his

TABLE I. Plasma, Urine, and Breath Amino Acids and Methionine Metabolites

	Patient			
	Age 31	Age 38	Age 43	Controls[a]
Plasma (μM)				
Methionine	716	941	793	26 (15–40)
Tyrosine	33	73	61	47 (31–90)
Homocystine (free)	<0.5	<0.5	<0.5	<0.5
½ cystine	9	44	10	26 (0–70)
Urine (μmol/day)				
Methionine	552	352	578	99 (39–225)
Tyrosine	137	94	177	97 (46–170)
Homocystine (free)	Trace	Trace	Trace	0–trace
½ cystine	26	109	50	145 (105–203)
Urine (mmol/mol C)[b]				
TA metabolites	174	ND	254	~1.8
Breath (nmol/l)				
Dimethylsulfide	5.86	ND	2.52	0.34 (0.13–0.65)

[a]Mean (range).

[b]Urinary transamination (TA) metabolites, expressed in mmol/mol of creatinine, include a base-labile methylthio-containing mixed disulfide and 4-methylthio-2-oxobutyrate [Gahl et al., 1988; Blom et al., 1989].

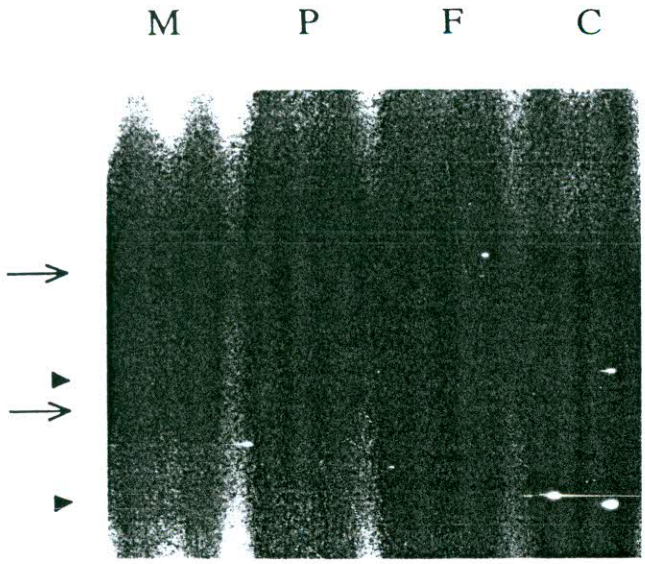

Fig. 1. SSCP analysis of exon V of the patient and his parents. The control **(lane C)** displays two bands representing the forward and reverse strands of DNA (arrowheads). The patient **(lane P)** exhibits two differently migrating fragments (arrows), reflecting forward and reverse strands of DNA with charges different from those of the two normal strands. Heterozygous mother **(lane M)** and father **(lane F)** each exhibit all four bands, two for the normal and two for the mutant allele. The mother's normal allele is fainter than her mutant allele.

mother (data not shown) were heterozygous for this mutation.

DISCUSSION

The cloning, characterization, and expression of the human *MAT1A* gene have proven useful for establishing a molecular basis for isolated hypermethioninemia. Thus far, 11 mutations have been documented that abolish or reduce MAT I/III activity; since only 6 of the 34 patients with defined mutations, including 21 with a dominant negative R264H mutation [Blom et al., 1989; Chamberlin et al., 1997; Nagao and Oyanagi, 1997], had undergone a liver biopsy to confirm their disorder, the molecular data have contributed greatly to our understanding of the MAT isozymes.

However, in spite of recent molecular insights, uncertainty continues regarding whether MAT I/III deficiency represents a benign disorder or, in some cases, may cause neurological damage. Patients C and 8 of Chamberlin et al. [1996] were reported to have homozygous mutations within the *MAT1A* gene resulting in truncated MAT proteins with zero activity when expressed in bacterial cells; both these individuals experienced a demyelinating disorder beginning prior to age 11. Based upon these data, it was suggested that patient 3 of Chamberlin et al. [1996], who was homozygous for the 539insTG mutation leading to early termination at codon 185, might suffer from a demyelinating disease later in life.

We have now determined that the oldest and most thoroughly studied MAT I/III-deficient patient is homozygous for the identical 539insTG mutation present in patient 3 of Chamberlin et al. [1996]. However, our patient at age 43 years exhibits no signs of neurological abnormality, and his MRI showed normal brain myelination. This was true despite the patient's persistent elevation of plasma methionine, breath dimethylsulfide, and urinary methionine transamination metabolites (Table I). The finding of the identical mutation in our patient and in patient 3 [Chamberlin et al., 1996] suggests that patient 3 could possibly live a completely normal life without ever developing neurological problems.

Why, then, do patients C and 8 [Chamberlin et al., 1996] have early demyelinating disease, while our patient thrives with normal myelination at age 43? It is theoretically possible that the demyelination manifested by patients C and 8 is attributable to insults other than their homozygous *MAT1A* mutations. Patient C was born with a cleft lip and palate, often associated with more general defects, including neurological involvement. Patient 8 had a younger brother with dystonia and verbal dysarticulation [Mudd et al., 1995b], but with normal plasma amino acids.

Nevertheless, there remains considerable evidence linking presumably low AdoMet concentrations, which can result from hepatic MAT deficiency, with demyelination. Rats fed cycloleucine, an inhibitor of MAT activity, develop abnormalities of myelin which are preventable, at least in part, by the administration of AdoMet [Bianchi et al., 1997]. In humans, defective remethylation due to disorders such as cobalamin deficiency and methylene tetrahydrofolate reductase deficiency has been associated with a variety of neurological disorders [Scott et al., 1981; Weir et al., 1988; Bottiglieri et al., 1994; Rosenblatt, 1995], some of which have been reversed by treatment with betaine, a source of methyl units [Wendel and Bremer, 1984; Roback et al., 1989]. Moreover, the demyelination of pa-

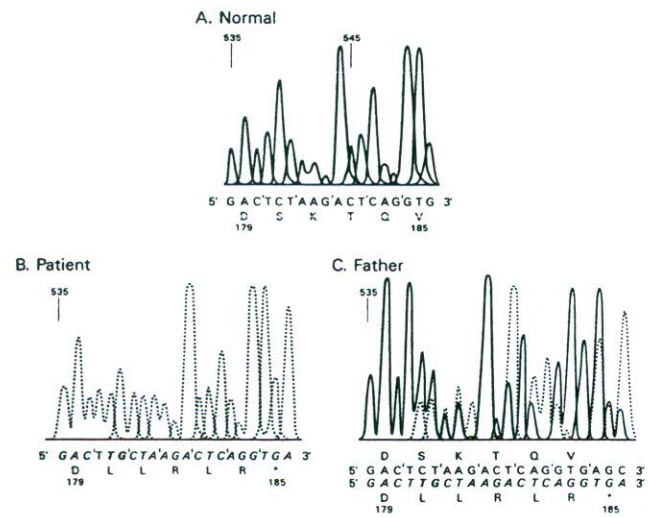

Fig. 2. DNA sequencing of normal control **(A)**, patient **(B)**, and heterozygous father **(C)** in the area of the 539insTG mutation. The patient is homozygous for a TG insertion causing a frameshift with altered amino acids incorporated in positions 180–184 and a termination codon at amino acid 185. C: Bases of both alleles are shown after base 539, and the predicted peptide sequences are given. Sequencing of the mother's DNA gave a pattern identical with that of the father.

tient C herself was reversed by administration of AdoMet [Surtees et al., 1991].

These lines of evidence make it worthwhile tentatively to retain the working hypothesis of a causative connection between truncating mutations in *MAT1A* and delayed demyelination, and to search for factors that may explain the differences between our patient and patients C and 8 [Chamberlin et al., 1996]. For example, dietary or other environmental differences may have occurred, including the intake of methionine and/or choline at critical periods of development. There may also be differences in the consequences of the various truncating mutations. The truncated polypeptide encoded by the mutant *MAT1A* in our patient is only 184 amino acids long, and lacks many structural features of the wild-type catalytic α1 subunit encoded by *MAT1A*, including the arginine residue at position 264. That residue appears critical in the salt bridge formation involved in the normal dimerization of these subunits [Takusagawa et al., 1996; Chamberlin et al., 1997]. The polypeptides encoded by the mutant *MAT1A* of patients C and 8 contain 350 and 349 amino acids (of the normal 395), including several abnormal residues but a normal R264. These polypeptides may be more capable of interacting with the catalytic α2 subunits encoded by *MAT2A*, and may adversely affect their activity and/or intracellular stability. In human liver, catalytic α1 and α2 subunits are 84% homologous in amino acid sequence [Horikawa and Tsukada, 1992], including extremely highly conserved regions both 5′ and 3′ to R264. Both subunits are formed within hepatocytes [Horikawa et al., 1993]. Thus, the possibility of deleterious interaction(s) in patients C and 8, but not in our patient, must be considered. The result might be less hepatic activity of MAT II, the presence of which may be crucial in preventing demyelination. Patients with point mutations in *MAT1A* have not developed demyelination, but such patients have residual activities of MAT I/III [Chamberlin et al., 1996] that may contribute to preventing demyelination.

Together, the results of the present and previous investigations suggest that our patient may provide the best estimate of how much hepatic MAT activity is derived from the *MAT2A* gene. His highly truncated *MAT1A*-derived protein presumably has no MAT activity and, as noted above, is unlikely to interact with α2 subunits. Hence, his 7% residual hepatic MAT activity at 1 mM methionine [Gahl et al., 1987] could reflect activity attributable to hepatic *MAT2A*, and this may define the contribution of *MAT2A* toward total adult hepatic MAT activity.

Finally, these investigations indicate that patient 3 of Chamberlin et al. [1996] may not necessarily develop demyelination, even if untreated. Predictions concerning the clinical consequences associated with mutant *MAT1A* genes will be difficult until further information has been obtained. Patients with isolated hypermethioninemia should be clinically investigated thoroughly; as more patients appear, a combination of clinical, biochemical, and molecular studies may deepen our understanding of the relationship of MAT I/III deficiency and demyelination.

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