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Haploinsufficiency of Cytosolic Serine Hydroxymethyltransferase in the Smith-Magenis Syndrome

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Summary

Folate-dependent one-carbon metabolism is critical for the synthesis of numerous cellular constituents required for cell growth, and serine hydroxymethyltransferase (SHMT) is central to this process. Our studies reveal that the gene for cytosolic SHMT (*cSHMT*) maps to the critical interval for Smith-Magenis syndrome (SMS) on chromosome 17p11.2. The basic organization of the *cSHMT* locus on chromosome 17 was determined and was found to span ~40 kb. The gene for *cSHMT* was found to be deleted in all 26 SMS patients examined by PCR, FISH, and/or Southern analysis. Furthermore, with respect to haploinsufficiency, cSHMT enzyme activity in patient lymphoblasts was determined to be ~50% that of unaffected parent lymphoblasts. Serine, glycine, and folate levels were also assessed in three SMS patients and were found to be within normal ranges. The possible effects of *cSHMT* hemizygosity on the SMS phenotype are discussed.

Introduction

One-carbon metabolism is central to the biosynthesis of lipids, hormones, thymidylate, and purines, as well as to the metabolism of several amino acids, including serine, glycine, methionine, and histidine. Folate coenzymes act as donors and acceptors of one-carbon units in a variety of these reactions, and the β-carbon of serine is the major source of one-carbon units for folate-dependent one-carbon metabolism (Schirch 1982). Serine hydroxymethyltransferase (SHMT) is a highly conserved pyridoxal phosphate–containing enzyme that catalyzes the reversible conversion of serine and tetrahydrofolate (THF) to glycine and 5,10-methylene THF, respectively (Schirch 1982; Usha et al. 1994). During the S-phase of the cell cycle and when cells are stimulated to proliferate, incorporation of the β-carbon of serine into DNA, as well as SHMT activity, is increased (Eichler et al. 1981; Snell et al. 1987). Isozymes of SHMT exist in the cytosol (cSHMT) and in mitochondria (mSHMT) facilitating one-carbon exchange within and between the respective compartments. cDNAs for both the cytosolic and the mitochondrial isozymes have been cloned and mapped to chromosomes 17p11.2 and 12q13, respectively (Garrow et al. 1993).

While the enzyme is ubiquitously expressed, SHMT is likely to play an important role in the CNS (Daly and Aprison 1974; Snell 1984; Waziri et al. 1990). In the brain, cleavage of serine by SHMT is the main source of glycine and one-carbon units (Shank and Aprison 1970). Reduced SHMT activity may cause an increase in glycine and serine levels (Waziri et al. 1990; Devor and Waziri 1993), which may play enhancing roles in *N*-methyl-D-aspartate (NMDA) receptor-produced neurotoxicity (Deutch et al. 1989; Waziri et al. 1990, 1992; Devor and Waziri 1993). Several studies have suggested that this process occurs in the brains of psychotics and schizophrenics, where SHMT activity has been found to be reduced (Waziri et al. 1990, 1992; Devor and Waziri 1993). In addition, decreased SHMT activity can lead to low levels of adenosine (which acts as a neuromodulator of dopamine and glutamate and also as a neuroprotective agent). Therefore, decreased SHMT activity may promote neuronal damage (Devor and Waziri 1993).

An interstitial deletion in band p11.2 of chromosome 17 results in the multiple congenital anomaly/mental retardation disorder called Smith-Magenis syndrome (SMS) (Smith et al. 1986). SMS encompasses a complex phenotype that includes developmental and growth delay, facial anomalies, and unusual behaviors (Smith et al. 1986; Greenberg et al. 1991). SMS was first described in 1986, and, while its prevalence is estimated to be 1:25,000 (Greenberg et al. 1991), it is likely underdiagnosed in the general population. The complex phenotypic abnormalities may be due to haploinsufficiency of several contiguous genes.

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The critical interval for SMS is quickly being narrowed and further defined. To date, three genes have been mapped within the common SMS deletion interval: (i) small nuclear RNA U3 (Chevillard et al. 1993), (ii) the human homologue of the *Drosophila flightless-I* gene (*FLI*) (Chen et al. 1995), and (iii) a human microfibril-associated protein, MFAP4 (Zhao et al. 1995). The possible role of each of these genes in the SMS phenotype is not yet defined. Here, we report that the gene for the cytosolic isozyme of serine hydroxymethyltransferase (cSHMT) maps within the SMS critical region on chromosome 17p11.2 and is deleted in all SMS patients examined. We have assessed levels of cSHMT activity in cell lines derived from three SMS patients. The possible effect of cSHMT deficiency on serine, glycine, and folate levels in SMS patients has also been examined. We discuss the plausible role of cSHMT in the SMS phenotype.

Subjects, Material, and Methods

SMS Patients

All SMS patients examined in this study (except HOU114-476, 338-911, and 339-913) have been reported either individually or in summary previously (patients HOU52-147, 65-241, 67-246, 69-251, 70-254, 71-255, 74-263, 75-266, 95-363, 108-429, 110-468, 113-475, and 245-725 [Greenberg et al. 1991]; patient HOU142-540 [Finucane et al. 1993*b*; Juyal et al., in press]; patients HOU138535, 140-536, 161-566, 165-572, 166-575, 171-590, 213-661, and 227-689 [Finucane et al. 1993*a*, 1993*b*, 1994]; and patient HOU202-641R [Juyal et al. 1995]).

Somatic Cell Hybrids

Hybrids used for regional mapping, including MH22-6 (containing chromosome 17), 88H5, LS-1, and Hy357-2D and their rodent parents have been described elsewhere (Patel et al. 1992). The construction of somatic cell hybrids retaining a chromosome 17 with a deletion from SMS patients has been reported elsewhere (Guzzetta et al. 1992; Zori et al. 1993).

PCR Analysis of cSHMT in Somatic Cell Hybrids

166-bp sequence-tagged site (STS).—Approximately 175 ng of genomic DNA from each hybrid cell line and human and rodent controls was subjected to PCR in a 25-μl reaction containing 10 mM Tris-HCl (pH 8.3), 100 mM KCl, 1.5 mM $MgCl_2$, 400 nM of each STS primer (forward, 5′-GCCACCCTGAAAGAGTTCAAGG-3′; reverse, 3′-GGGTGGACCGCGGTGTCTCC-5′), 0.5 mM dNTP, 10% glycerol, and 1–2 U *Taq* polymerase. The PCR was run for 30 cycles in the GeneAmp Thermal Cycler (model 9600) with a predenaturing step at 95°C for 4 min, denaturing at 94°C for 45 s, annealing at 60°C for 1 min, and elongation at 72°C for 1 min, followed by a 10 min final elongation. Approximately 8 μl of each reaction was analyzed by electrophoresis in a 1% agarose (Gibco/BRL)/TAE buffer.

600-bp STS.—Approximately 175 ng of genomic DNA from each hybrid cell line and human and rodent controls was subjected to PCR in a 25-μl reaction containing 60 mM Tris-HCl (pH 8.5), 15 mM $(NH_4)SO_4$, 1.5 mM $MgCl_2$, 400 nM of each STS primer (forward, 5′-TGACGTCCCGTGGACTTTTGGA-3′; reverse, 3′-GGGTGGACCGCGGTGTCTCC-5′), 2.5 mM dNTP, and 1–2 U *Taq* polymerase. The reaction was amplified and analyzed as described above. The expected size of the fragment was ~270 bp on the basis of the cDNA sequence, but the presence of an intron within this sequence was confirmed by doing nested PCR to obtain the 166-bp fragment described above.

Full-Length cSHMT DNA.—The full-length *cSHMT* cDNA was PCR amplified as described above from 440 ng of a λ-ACT B-cell cDNA library (Durfee et al. 1993) with primers (forward, 5′-GAACCAGTGCAATGACGATGCC-3′; reverse, 3′-GGGTGGACCGCGGTGTCTCC-5′). Reactions were as for the 600-bp STS, except with 1 mM dNTP and 10% dimethylsulfoxide. Two similar-sized PCR products were obtained from this protocol, and each was cloned into the pCRII vector (pSHE1 and pSHE2) using the Original TA cloning kit (Invitrogen). Sequencing was performed using an ABI automated fluorescent DNA sequencer at the Nucleic Acid Core facility of the Department of Molecular and Human Genetics at Baylor College of Medicine. Computer analysis of sequences was performed using the Eugene/SAM software package developed by the Molecular Biology Computational Resource Information Technology Program and the Department of Cell Biology at Baylor College of Medicine. Both cloned sequences were identical to the published *cSHMT* cDNA at the 5′ and 3′ ends.

SMS Patient Deletion Analyses

Patients were analyzed for hemizygosity of the *cSHMT* locus by either Southern hybridization, when hybrids were available (patients, HOU52-147, 65-241, 67-246, 69-251, 70-254, 71-255, 74-263, 75-266, 95-363, 108-429, 110-468, and 113-475), or FISH (patients HOU114-476, 138-535, 140-536, 142-540, 161-566, 165-572, 166-575, 171-590, 202-641R, 213-661, 227-689, 245-725, 338-911, and 339-913). Southern transfer and hybridizations were performed essentially as described elsewhere (Patel et al. 1990). Electrophoretic transfer was used for all blots (BioRad Transblot System), and probes were labeled using the RediPrime random labeling kit (Amersham). FISH was performed

as described elsewhere (IJdo et al. 1992; Juyal et al. 1995). Approximately 30 metaphases were scored for each patient.

Restriction Mapping of cSHMT Cosmids

Approximately 1.5 μg of cosmid DNA was digested for 1 h at 37°C with 10 U of *Eco*RI (Pharmacia). Digests were run on a 1% agarose/TAE gel and stained with .5 mg/ml ethidium bromide. Duplicate filters were prepared by "sandwich blotting" for 4 h. Approximately 1 μg of full-length *cSHMT* cDNA was digested simultaneously with 10 U each of *Bam*HI and *Stu*I (Boehringer). The products were separated at 5 V/cm on a 1.5% agarose/TAE gel, excised from the gel, and purified again on a 1% low-melt agarose gel at 5 V/cm. Labeled products were hybridized individually to the *Eco*RI-digested cosmids and *Eco*RI digests of human genomic DNA. The restriction map was assembled by grouping together cosmids having restriction products of similar size. These shared fragments were ordered according to the hybridization pattern seen when hybridized to the various cDNA probes.

Measurement of cSHMT Enzyme Activity

cSHMT enzyme activity was determined in fractionated patient lymphoblasts by measuring the SHMT-catalyzed exchange of the pro2-S proton of glycine as described elsewhere (P. Stover, L. Chen, D. M. Stover, K. Keyomarsi, and B. Shane, unpublished information). The assay is based on the observation that THF accelerates the SHMT-catalyzed exchange of the pro-2S proton of glycine, while 5-formyl THF inhibits this reaction (Stover and Schirch 1992). Cytoplasmic and mitochondrial fractions were prepared and the isolated mitochondria were purified as described elsewhere (Lin et al. 1993). Lactate dehydrogenase (LDH) and glutamate dehydrogenase activities were determined in each fraction to correct for mitochondrial breakage and cytosolic contamination (Lin et al. 1993). Control reactions containing 5-formyl THF showed <10% proton exchange compared to the THF-catalyzed exchange reaction. All assays were performed in duplicate, and all experiments were repeated at least twice. Total tritium exchanged never exceeded 5% of the total [^{3}H]glycine. By use of marker enzyme activities, cSHMT activity was corrected for mSHMT contamination, and the contribution of mSHMT activity to the cytosolic fraction never exceeded 10% of cSHMT activity. Purified mitochondria were determined to be free of lactate dehydrogenase activity; therefore, the mitochondrial fraction was not corrected for cSHMT contamination.

Plasma and Urine Metabolic Assays

All plasma and urine samples were analyzed at the Pediatric Amino Acid Laboratory at the University of Iowa by standard procedures.

Results

Mapping of Cytosolic SHMT to the SMS Critical Region of Chromosome 17p11.2

A recent report mapped the gene for cytosolic serine hydroxymethyltransferase (cSHMT) to chromosome 17p11.2 (Garrow et al. 1993). In order to determine whether this gene was located within the SMS region of 17p11.2, primers specific for a 166-bp STS at the 3′ end of the gene were used in PCR analysis. This STS was found by PCR to be present in the hybrid MH22-6, which retains a single normal human chromosome 17. The *cSHMT*-specific STS was deleted in somatic cell hybrids retaining a del(17)(p11.2) chromosome representative of SMS patients (Hy147-20D and Hy485-3D) but was present in hybrids retaining the proximal 17p11.2 region (88H5, Hy357-2D) (fig. 1). These results, shown in figure 1, indicate that *cSHMT* maps within the proximal 17p11.2 region, typically deleted in SMS patients (R. C. Juyal, L. Figuera, and P. I. Patel, unpublished information).

The full-length cDNA was PCR-amplified with primers representing the 5′ and 3′ termini of the published *cSHMT* sequence using, as template, plasmid DNA derived from a human B-cell λ-ACT cDNA library (Durfee et al. 1993). Each of the two resulting PCR products was cloned: one with an insert size of ~1.5 kb (pSHE1), the expected cDNA size, and a shorter fragment with an insert size of ~1.4 kb (pSHE2), which likely represents an alternatively spliced mRNA. The cloned PCR products (pSHE1 and pSHE2) were sequenced and their identities confirmed by comparison with the GenBank sequence (accession L11931). The longer *cSHMT* cDNA (pSHE1) was subsequently hybridized to a chromosome 17p11.2 mapping panel containing *Eco*RI-digested hybrid and control DNAs to confirm the results obtained with PCR (data not shown). A positive signal was observed only in controls and those hybrids retaining the proximal 17p11.2 region (MH22-6, 88H5, and Hy357-2D) and was absent in Hy147-20D, Hy485-3D, and LS-1 (an iso17q-retaining hybrid). Thus, *cSHMT* maps in the interval of p11.2 common to both hybrids 88H5 and Hy357-2D. This interval is known to also contain the *FLI* locus (Chen et al. 1995) and the anonymous marker FG2 (D17S447). Two other candidate genes, *U3* (Chevillard et al. 1993) and MFAP4 (Zhao et al. 1995), map proximal to this interval. In addition, the *cSHMT* cDNA hybridized to an ~390-kb YAC (CEPH 52B10) previously obtained through a screen with an STS for the *FLI* gene (data not shown) indicating that the *cSHMT* locus maps close to the *FLI* locus.

Genomic Organization of the cSHMT Locus on Chromosome 17

In order to identify genomic clones for the *cSHMT* locus, a cosmid library constructed from flow-sorted

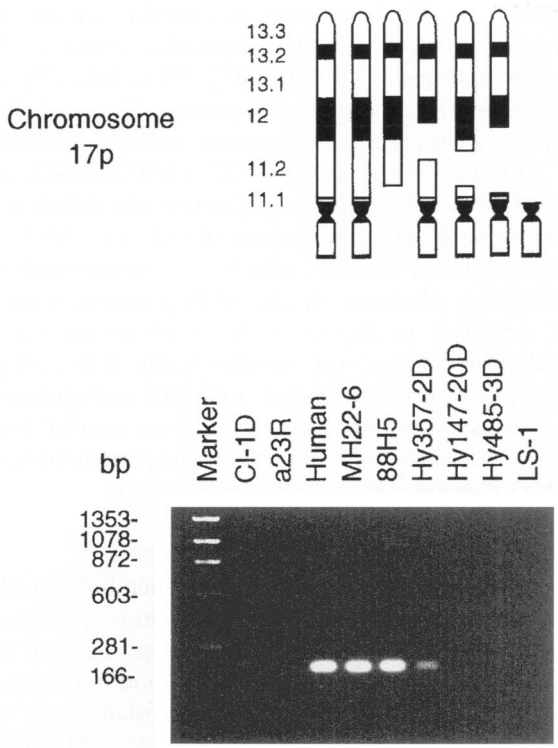

Figure 1 Mapping of *cSHMT* to the SMS region of 17p11.2. The 166-bp STS described in Subjects, Material, and Methods was PCR amplified from each of the indicated control and hybrid DNAs and electrophoresed on a 1% agarose gel. The absence of the *cSHMT* gene is indicated by the absence of the STS in hybrids Hy147-20D and Hy485-3D. No signals are observed in the mouse and hamster controls either. Above each lane, a chromosome 17p ideogram is shown, indicating the approximate breakpoints for each of the chromosomes 17 retained in these cell lines. All hybrids, except for 88H5, retain a complete long arm of chromosome 17. LS-1 is an iso17q-retaining hybrid.

chromosome 17 DNA (Kallioniemi et al. 1994) was screened using an ~600-bp STS that was PCR-amplified from human genomic DNA, as described in Subjects, Material, and Methods. Eight cosmids were identified: c98F9, c108D4, c110D8, c113D9, c123G9, c155D9, c157A9, and c159D8. Subsequent screening with the full-length *cSHMT* cDNA identified an additional cosmid, c92D4. The full-length *cSHMT* cDNA (pSHE1) was then hybridized to each of these cosmids, and common fragments were observed in *Eco*RI-digested human and cosmid DNAs (data not shown).

The cosmids identified by the *cSHMT* cDNA probe were then used to determine the overall organization of the gene (fig. 2). The full-length cDNA was subdivided into fragments I, II, and III by restriction digestion with *Bam*HI and *Stu*I. Each of these fragments was then independently hybridized to *Eco*RI digests of the *cSHMT* cosmids. Fragment I hybridized to >12 kb and 8.3 kb; fragment II hybridized to 8.3 kb and 5.5 kb; and fragment III hybridized to 10.5-kb, 5.5-kb, and 0.3-kb *Eco*RI bands. On the basis of these results and the restriction pattern of the cosmids seen by ethidium bro-

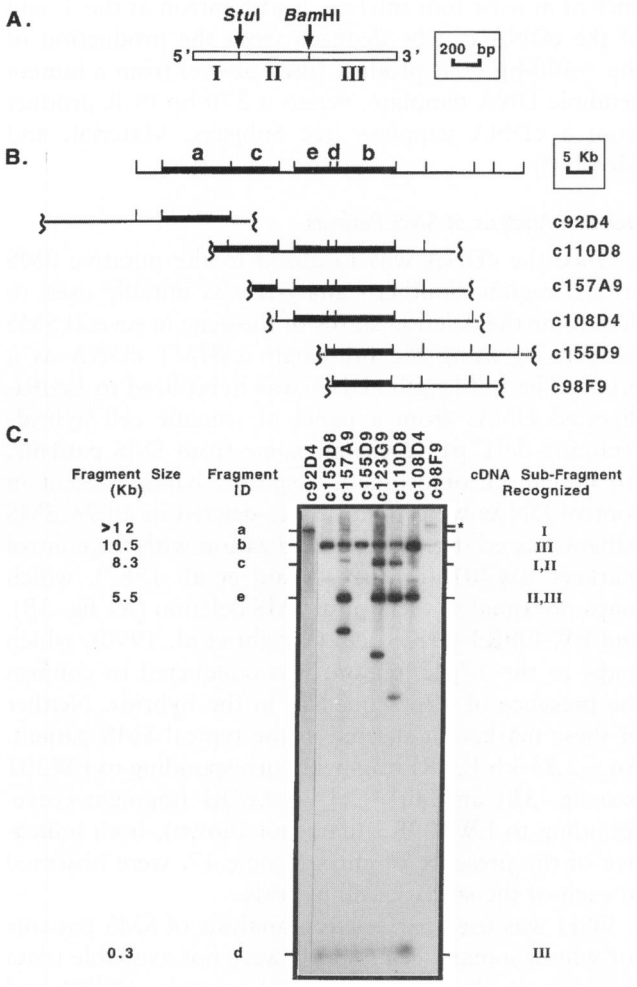

Figure 2 Genomic organization of the *cSHMT* locus on chromosome 17. *A*, Schematic representation of the *cSHMT* cDNA (pSHE1) restriction products (I, II, and III) produced by digestion with *Stu*I and *Bam*HI as described in Subjects, Material, and Methods and used as probes for hybridization to cosmid and genomic DNA. *B, above,* Deduced layout of *Eco*RI restriction fragments at the *cSHMT* locus on chromosome 17; *below, Eco*RI restriction maps of overlapping cosmids, identified to the right of each map. Heavy lines indicate fragments hybridizing to *cSHMT* cDNA. Dotted lines represent fragments having positions that are interchangeable. Cosmid 155D9 is very similar to c113D9 and c159D8, which are not shown. Cosmid 110D8 is similar to c123G9 (not shown), except for a 3.8-kb fragment of unknown location. *C*, Hybridization of the full-length cSHMT cDNA to *Eco*RI digests of cosmids in the region. Sizes for chromosome 17–specific *cSHMT* fragments identified in genomic DNA (see fig. 3) are shown on the left. The same blot was hybridized to cDNA subfragments indicated in panel A (data not shown), and the resulting data are summarized to the right. The asterisk (*) identifies a fragment resulting from partial digestion.

mide staining of the gel, the cDNA spans an ~40-kb area in the genome. A restriction map of the cosmids is shown in figure 2*B*, along with a deduced map for the genomic region. Since the cDNA does not have any *Eco*RI restriction sites, the identification of five genomic fragments containing cDNA sequence indicates the presence of at least four introns. A fifth intron at the 3′ end of the cDNA can be deduced from the production of the ~600-bp PCR product (used above) from a human genomic DNA template, versus a 270-bp PCR product from a cDNA template (see Subjects, Material, and Methods).

Deletion Analysis of SMS Patients

Once the cDNA was localized to the putative SMS critical region, Southern analysis was initially used to determine the deletion status of the gene in several SMS patients by using the full-length *cSHMT* cDNA as a probe. The full-length cDNA was hybridized to *Eco*RI-digested DNAs from a panel of somatic cell hybrids retaining del17p11.2 chromosome from SMS patients. All human chromosome 17–specific bands present in control DNAs were found to be deleted in all 26 SMS patients tested (fig. 3*A*). Hybridization with the control markers EW301 (D17S58) (Fain et al. 1987), which maps proximal to the typical SMS deletion (see fig. 3*B*), and EW409R3 (D17S122) (Wright et al. 1990), which maps to the 17p12 region, was conducted to confirm the presence of 17p sequences in the hybrids. Neither of these markers is deleted in the typical SMS patient. An ~2.85-kb *Eco*RI fragment corresponding to EW301 (see fig. 3*B*) and an ~2.1-kb *Eco*RI fragment corresponding to EW409R3 (data not shown), both indicative of the presence of chromosome 17, were observed in each of the somatic cell hybrids.

FISH was used for deletion analysis of SMS patients for whom somatic cell hybrids were not available (data not shown). Two representative cosmids, 108D4 and 155D9, were chosen for FISH analysis of the *cSHMT* locus. All SMS patients analyzed by FISH revealed a signal on only one chromosome 17 homologue, while a signal was evident on both homologues with a control probe for 17q, c123F8. A total of 26 patients have been studied to date, and the *cSHMT* locus is deleted in all of them.

cSHMT Activity in SMS Patients

In order to assess the effect of hemizygosity for the *cSHMT* locus on SMS patients, cSHMT and mSHMT enzyme activities were determined in lymphoblastoid cell lines from three SMS patients and their parents in a blinded fashion. Measurements of LDH and glutamate dehydrogenase activities were also determined as controls for cytosolic and mitochondrial preparations, respectively. As shown in table 1, cSHMT activity was decreased ~50% in the SMS lymphoblast cultures. SMS patient lymphoblasts HOU165-572, HOU166-575, and HOU202-641R all displayed between 46% and 59% cSHMT activity, relative to parental cultures HOU165-573, HOU166-576, HOU166-577, HOU202-642, and HOU202-644. These data suggest that the deletion on chromosome 17p11.2 is associated with an ~50% decrease in cSHMT activity, which is consistent with the hemizygosity observed in the SMS patients. Control LDH activities in the cytosolic fractions of patient lymphoblast cultures were similar (table 1) for all patients and parents. In addition, mSHMT and glutamate dehydrogenase activities determined from purified mitochondria were also invariant among the patient and control lymphoblast cultures (data not shown).

Serine, Glycine, and Folate Levels in SMS Patients

The availability of an easily assayed metabolic marker for SMS would be of great value in facilitating diagnosis and may also be of therapeutic interest. Toward this end, an initial metabolic screen for blood and urinary amino acid levels in addition to blood folate levels was conducted on SMS patients HOU202-641R, HOU338-911, and HOU338-913. We have shown elsewhere that patient HOU202-641 carries a smaller-than-average deletion (Juyal et al. 1995), while these other patients carry a typical SMS deletion of 17p11.2. Results indicate that serine and glycine levels in plasma and urine were within the normal ranges and that folate levels were normal except for patient HOU202-641, whose folate levels were clearly at the lower end of the range seen in normal individuals (table 2). Additional patients are currently being examined for these metabolites to determine whether there are any significant consequences of *cSHMT* haploinsufficiency.

Discussion

SHMT is central to folate-dependent one-carbon metabolism (Schirch 1982) and helps to provide the major source of glycine in the brain, by catalyzing the reversible cleavage of serine and THF into glycine and 5-10-methylene THF, respectively (Schirch 1982; Usha et al. 1994). The results of our studies demonstrate that the gene for cytosolic SHMT was deleted in all SMS patients examined and that cSHMT activity was decreased by ~50% in all three patients tested. In addition, enzyme activities were consistent and nonvariant in these three families for all of the enzymes tested, with the exception of the cSHMT activity in patient samples. This decrease in enzyme activity can lead to altered levels of glycine, serine, adenosine, and folate. The blood and urine amino acid levels of the patients tested were found to be within

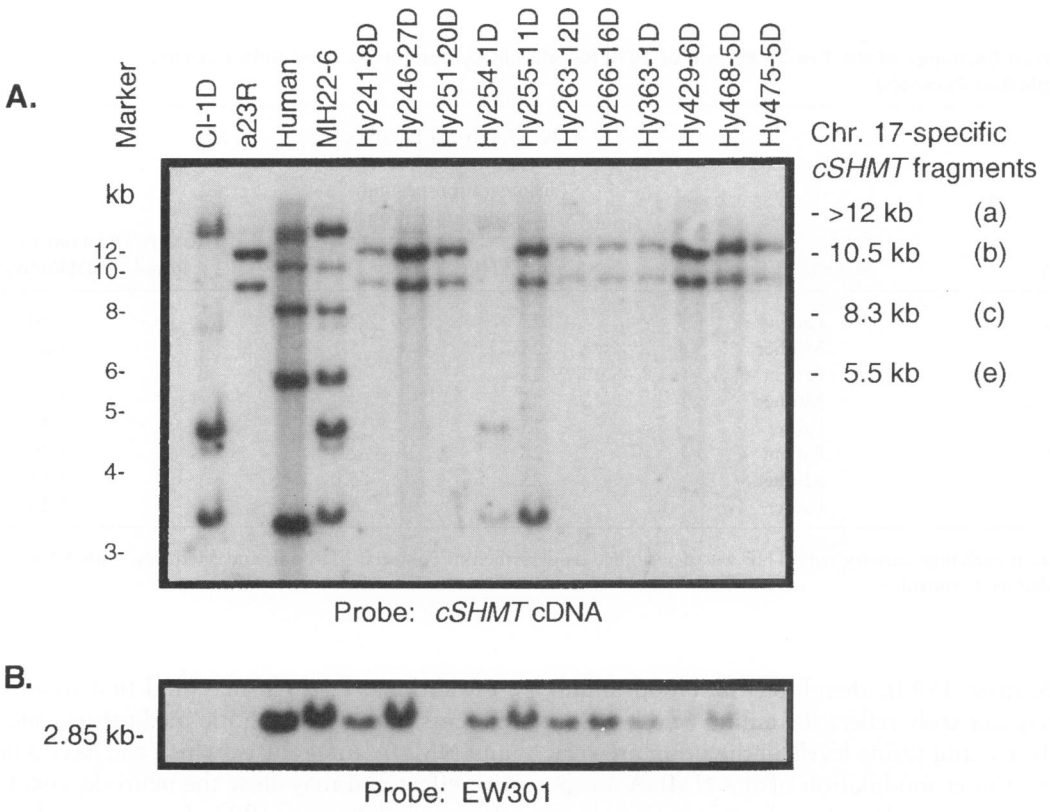

Figure 3 Southern analysis of somatic cell hybrids retaining the deleted chromosome 17 of SMS patients with the *cSHMT* cDNA probe. *A,* Five micrograms of human DNA, 10 μg of DNA from the rodent controls (Cl-1D, a23R), and 15 μg of DNA from each of the hybrids was digested with *Eco*RI and electrophoresed in a 1% agarose gel. The Southern blot was hybridized to the ~1.5 kb cDNA for *cSHMT*. The human chromosome 17–specific bands are indicated on the right and correspond to the *Eco*RI fragments from fig. 2. *B,* Probe EW301, which maps proximal to the SMS region, hybridized to the same Southern blot to indicate the presence of chromosome 17 in each of the hybrids. Hy251-20D and Hy475-5D do yield positive signals, albeit at a lower intensity. Hy255-11D also carries chromosome 12, as is indicated by the presence of the ~3.3-kb band (which comigrates with the mouse [Cl-1D] band).

normal ranges, so it does not appear that haploinsufficiency of cSHMT affects the overall glycine and serine levels in the body. However, folate levels were reduced in one patient studied and examination of additional patients is required to determine the significance, if any, of reduced folate levels in SMS patients.

It is difficult to assess the contribution of cSHMT deficiency to the SMS phenotype, as its particular role in folate-mediated one-carbon metabolism is not well understood. Mitochondria appear to be the primary source of glycine and one-carbon units required for cytoplasmic one-carbon metabolism in mammalian cells (Appling 1991). Studies of mutant Chinese hamster ovary (CHO) cells that display a range of mSHMT activities suggest a correlation between mSHMT activity, intracellular glycine concentrations, and protein synthesis rates (Pfendner and Pizer 1980). In addition, CHO cells that lack mSHMT activity are auxotrophic for glycine (Pfendner and Pizer 1980). These observations suggest that the cSHMT enzyme cannot make sufficient glycine for cell proliferation.

The phenotype associated with disruption in cSHMT activity in mammalian cells is unknown. However, cSHMT activity appears to be compromised by the relative distribution of folate coenzymes in the cytoplasm. Both 5-CH_3-H_4PteGlu and 5-CHO-H_4PteGlu polyglutamates are powerful inhibitors of cSHMT activity and represent >80% of total folate in the cytoplasm (Stover and Schirch 1991). It is not known whether these inhibitory folates are bound to cSHMT in vivo, but it is likely that at least some of the cSHMT enzyme is inhibited by 5-CHO-H_4PteGlu, as cSHMT has been demonstrated to catalyze its formation in vivo. Therefore, it is not surprising that the intracellular concentrations of serine and glycine were not greatly affected in SMS patients. However, complicating matters is the fact that glycine is synthesized de novo from glucose metabolism in the glial cells of the brain (Shank and Aprison 1970; Reyn-

Table 1

cSHMT-Catalyzed Exchange of the Pro-2S Proton of 2-[^{3}H]Glycine in Lymphoblasts from SMS Patients and Their Unaffected Parent(s)

Individual	Affection Status	cSHMT Pro-2-S Proton Exchange Activity[a] (disintegration per min exchanged/min/μg protein)		Lactate Dehydrogenase Activity (μmol NADH/min/μg protein)
		5-formyl THF	THF	
HOU165-572	Patient	.22	2.4	1.30
HOU165-573	Mother	.25	5.1	1.25
HOU166-575	Patient	.23	2.7	1.25
HOU166-576	Mother	.25	4.6	1.20
HOU166-577	Father	.25	5.2	1.25
HOU202-641R	Patient	.26	2.4	1.20
HOU202-642	Mother	.23	5.0	1.25
HOU202-644	Father	.22	4.8	1.20

[a] cSHMT proton exchange activity with THF was determined as described in Subjects, Material, and Methods, with 5-formyl THF proton exchange included as a control.

olds 1990; Scatton 1993); therefore, the blood amino acid levels may not truly reflect the amino acid levels in the brain. Glycine and serine levels in the brain are very important for proper modulation of the NMDA receptor, with glycine acting directly as a co-agonist along with glutamate (Reynolds 1990; Scatton 1993).

Additional research may find that treatment of SMS patients with antipsychotic medications such as glycine and NMDA antagonists, which can have a neuroprotective effect and may slow the neurodegenerative process (Kemp and Leeson 1993; Leeson and Iverson 1994), may be beneficial and effective toward controlling some

Table 2

Plasma and Urine Metabolic Screens of SMS Patients

	Patient		
	HOU202-641R	HOU338-911	HOU339-913
Plasma:			
Folic acid (ng/ml):			
Patient	2.5	8.3	8.0
Normal range	2–19.7	2–19.7	2–19.7
Glycine (μM):			
Patient	281.74	153.02	227.33
Normal range[a]	65–414	110–419	114–409
Serine (μM):			
Patient	143.62	125.32	80.93
Normal range[a]	44–199	53–183	54–214
Urine (μM/g creatinine):			
Folic acid	NT	NT	NT
Glycine (μM):			
Patient	794	1,045	968
Normal range[a]	<3,687	<3,687	<4,230
Serine (μM):			
Patient	336	461	404
Normal range[a]	<612	<612	92–1,132

Note.—NT = not tested.

[a] Ranges are normalized for the age of the patient.

of the negative behaviors (i.e., head banging, hand biting, nail pulling, etc.) observed in these patients. In addition, folic acid supplements, which have in recent years been shown to prevent neural tube defects, may also prove to be beneficial in the treatment of SMS. One can speculate on the possible effects of decreased cSHMT activity in the brain with respect to the SMS phenotype, but definite conclusions cannot be made in this regard until more studies are undertaken involving amino acid levels and cSHMT activity in the brain. Use of antisense or "knock-out" strategies to produce a mouse model with reduced cSHMT activity may also be useful in determining whether pharmacotherapeutic strategies compensating for reduced cSHMT activity are effective for at least partially alleviating the problematic symptoms of SMS.

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