Am. J. Hum. Genet. 56:1052–1059, 1995

Seven Novel Mutations in the Methylenetetrahydrofolate Reductase Gene and Genotype/Phenotype Correlations in Severe Methylenetetrahydrofolate Reductase Deficiency

Philippe Goyette,[1] Phyllis Frosst,[1] David S. Rosenblatt,[1,2] and Rima Rozen[1]

Departments of [1]Human Genetics, Pediatrics, Biology, and [2]Medicine, McGill University, Montreal

Summary

5-Methyltetrahydrofolate, the major form of folate in plasma, is a carbon donor for the remethylation of homocysteine to methionine. This form of folate is generated from 5,10-methylenetetrahydrofolate through the action of 5,10-methylenetetrahydrofolate reductase (MTHFR), a cytosolic flavoprotein. Patients with an autosomal recessive severe deficiency of MTHFR have homocystinuria and a wide range of neurological and vascular disturbances. We have recently described the isolation of a cDNA for MTHFR and the identification of two mutations in patients with severe MTHFR deficiency. We report here the characterization of seven novel mutations in this gene: six missense mutations and a 5′ splice-site defect that activates a cryptic splice site in the coding sequence. We also present a preliminary analysis of the relationship between genotype and phenotype for all nine mutations identified thus far in this gene. A nonsense mutation and two missense mutations (proline to leucine and threonine to methionine) in the homozygous state are associated with extremely low activity (0%–3%) and onset of symptoms within the 1st year of age. Other missense mutations (arginine to cysteine and arginine to glutamine) are associated with higher enzyme activity and later onset of symptoms.

Introduction

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a carbon donor in the methylation of homocysteine to methionine. MTHFR, a flavoprotein which transfers electrons to NADP, is allosterically inhibited by S-adenosylmethionine. Deficiency of MTHFR, which leads to homocysteinemia, is an autosomal recessive disorder. Patients with severe MTHFR deficiency (residual enzyme activity of 0%–20%) show a wide range of clinical symptoms, such as developmental delay, severe mental retardation, perinatal death, psychiatric disturbances, and later-onset neurodegenerative disorders (Rosenblatt 1995). Different amounts of residual activity, as well as variable heat inactivation profiles of the enzyme, have led to the postulation of strong genetic heterogeneity at this locus (Rosenblatt and Erbe 1977). A milder deficiency of MTHFR, with 50% residual enzyme activity and marked thermolability, has been described in patients with coronary artery disease (Kang et al. 1991).

We recently isolated a human cDNA for MTHFR and reported the first two mutations in this inborn error of folate metabolism (Goyette et al. 1994). We describe here seven novel mutations in the MTHFR gene and examine the association between genotype, enzyme activity, and clinical phenotype in severe MTHFR deficiency.

Received December 14, 1994; accepted for publication February 10, 1995.

Address for correspondence and reprints: Dr. Rima Rozen, McGill University—Montreal Children's Hospital Research Institute, 4060 St. Catherine St. West, Room 242, Montreal, Quebec H3Z 2Z3, Canada.

0002-9297/95/5605-0005$02.00

Patients, Material, and Methods

Patient Description

The clinical and laboratory findings of the patients have been reported elsewhere (Mudd et al. 1972; Freeman et al. 1975; Rosenblatt and Erbe 1977; Harpey et al. 1981; Erbe 1986; Beckman et al. 1987; Rosenblatt et al. 1992; Kishi et al., in press). Residual MTHFR activity was measured in cultured fibroblasts at confluence (Rosenblatt and Erbe 1977).

Patient 354 (Family 2, LM) (Erbe 1986), an African American girl, was diagnosed at age 13 years with mild mental retardation. Her sister, patient 355 (Family 2, BM), was diagnosed at age 15 years with anorexia, tremor, hallucinations, and progressive withdrawal (Freeman et al. 1975). In patient 354, residual MTHFR activity was 19% of control values, and, in her sister, 355, it was 14% of control values (Rosenblatt and Erbe 1977). The residual activity after heating had equivalent

thermal stability to control enzyme (Rosenblatt and Erbe 1977; Rosenblatt et al. 1992).

Patient 1807, a Japanese girl whose parents are first cousins (Kishi et al., in press), had delayed walking and speech until age 2 years, seizures at age 6 years, and a gait disturbance with peripheral neuropathy at age 16 years. Residual activity of MTHFR was 3%, and the enzyme was thermolabile (Rosenblatt et al. 1992).

Patient 735 (Family 11, SM) (Erbe 1986), an African Indian girl, was diagnosed at age 7 mo with microcephaly, progressive deterioration of mental development, apnea, and coma. Residual activity of MTHFR was 2% of control levels. Thermal properties were not determined.

Patient 1084 (Family 15, JI) (Erbe 1986), a Caucasian male, was diagnosed at age 3 mo with an infantile fibrosarcoma. He was found to be hypotonic and became apneic. He died at the age of 4 mo (Beckman et al. 1987). Residual activity of MTHFR was not detectable. Thermal properties were not determined.

Patient 356, the first patient reported with MTHFR deficiency (Family 1, CP) (Erbe 1986), is an Italian American male who presented at age 16 years with muscle weakness, abnormal gait, and flinging movements of the upper extremities (Mudd et al. 1972). MTHFR residual activity was 20% of control values; activity was rapidly and exponentially inactivated at 55° (Rosenblatt and Erbe 1977).

Patient 458 (Family 3, WMa) (Erbe 1986), a Caucasian male, was diagnosed at age 12 years with ataxia and marginal school performance. Residual MTHFR activity was ~10% (Rosenblatt et al. 1977, 1992), and the activity was thermolabile.

Patient 1396, (JJ) (Rosenblatt et al. 1992), a Caucasian female, was described as clumsy and as having a global learning disorder in childhood. At age 14 years, she developed ataxia, foot drop, and inability to walk. She developed deep vein thrombosis and bilateral pulmonary emboli. Residual activity of MTHFR was 14%, and the enzyme was thermolabile.

Genomic Structure and Intronic Primers

Exon nomenclature is based on available cDNA sequence as reported by Goyette et al. (1994). Exon 1 has been arbitrarily designated as the region of cDNA from base pair (bp) 1 to the first intron. Identification of introns was performed by amplification of genomic DNA, using cDNA primer sequences. PCR products that were greater in size than expected cDNA sizes were sequenced directly.

Mutation Detection

Specific exons (see table 1 for primer sequences) were amplified by PCR from genomic DNA and were analyzed by the SSCP protocol. SSCP was performed with the Phastgel system (Pharmacia), a nonisotopic rapid SSCP protocol, as described elsewhere (Goyette et al. 1994), or with ^{35}S-labeled PCR products run on 6% acrylamide: 10% glycerol gels at room temperature (6 w, overnight). In some cases, the use of restriction endonucleases, to cleave the PCR product before SSCP analysis, enhanced the detection of band shifts. PCR fragments with altered mobility were sequenced directly (GIBCO, Cycle Sequencing kit). If the sequence change affected a restriction endonuclease site, then the PCR product was digested with the appropriate enzyme and was analyzed by PAGE. Otherwise, allele-specific oligonucleotide (ASO) hybridization was performed on a dot blot of the PCR-amplified exon (John et al. 1989).

Results

Mutation Analysis of Severe MTHFR Deficiency

5′ Splice-site mutation.—Amplification of cDNA, bp 653–939, from reverse-transcribed total fibroblast RNA, revealed two bands in sisters 354 and 355: a smaller PCR fragment (230 bp) in addition to the normal 287-bp allele (table 2; fig. 1*A*). Sequencing of the smaller fragment identified a 57-bp in-frame deletion, which would remove 19 amino acids (fig. 1*B*). Analysis of the sequence at the 5′ deletion breakpoint in the undeleted fragment revealed an almost perfect 5′ splice-site consensus sequence (AG/gcatgc) (Senapathy et al. 1990). This observation suggested the presence of a splicing mutation in the natural 5′ splice site, which might activate this cryptic site, to generate the deleted allele. The sequence following the deletion breakpoint, in the mutant allele, corresponded exactly to the sequence of the next exon. Amplification of genomic DNA, using the same amplification primers as those used for reverse-transcribed RNA, generated a 1.2-kb PCR product, indicating the presence of an intron. Direct sequencing of this PCR fragment in patient 354 identified a heterozygous G→A substitution in the conserved GT dinucleotide of the intron at the 5′ splice site (fig. 1*C*). This substitution abolished a *Hph*I restriction endonuclease site that was used to confirm the mutation in the two sisters (fig. 1*D*).

Patients with homozygous coding substitutions.—SSCP analysis of exon 4 for patient 1807 revealed an abnormally migrating fragment, which was directly sequenced to reveal a homozygous C→T substitution (bp 764) converting a proline to a leucine residue. This change creates a *Mnl*I restriction endonuclease site, which was used to confirm the homozygous state of the mutation (fig. 2*A*). Fifty independent control Caucasian chromosomes and 12 control Japanese chromosomes were tested by restriction analysis; all were negative for this mutation. Homo-

Table 1

PCR Primers for DNA Amplification and Mutation Analysis of MTHFR

Exon	Primer Type	Primer Sequence (5′→3′)	Location[a]	Fragment Size (bp)
1	Sense	AGCCTCAACCCCTGCTTGGAGG	C	271
	Antisense	TGACAGTTTGCTCCCCAGGCAC	I	
4	Sense	TGAAGGAGAAGGTGTCTGCGGGA	C	198
	Antisense	AGGACGGTGCGGTGAGAGTGG	I	
5	Sense	CACTGTGGTTGGCATGGATGATG	I	392
	Antisense	GGCTGCTCTTGGACCCTCCTC	I	
6	Sense	TGCTTCCGGCTCCCTCTAGCC	I	251
	Antisense	CCTCCCGCTCCCAAGAACAAAG	I	

[a] C = cDNA; I = intronic.

zygosity in this patient is probably due to the consanguinity of the parents.

Patients 735 and 1084 had the same mutation in exon 4, in a homozygous state: a C→T substitution (bp 692) that converted an evolutionarily conserved threonine residue to a methionine residue and abolished an *Nla*III restriction endonuclease site. Allele-specific oligonucleotide hybridization to amplified exon 4 (figs. 3*A* and 3*B*) was used to confirm the mutation in these two patients and to screen 60 independent chromosomes, all of which turned out to be negative.

Patient 356 showed a shift on SSCP analysis of exon 5. Direct sequencing revealed a homozygous C→T substitution (bp 985) that converted an evolutionarily conserved arginine residue to cysteine; the substitution abolished an *Aci*I restriction endonuclease site. This was used to confirm the homozygous state of the mutation in patient 356 (fig. 2*B*) and its presence in the heterozygous state in both parents. Fifty independent control chromosomes, tested in the same manner, were negative for this mutation.

Patients who are genetic compounds.—Patient 458 is a compound heterozygote of a mutation in exon 5 and a mutation in exon 1. The exon 5 substitution (C→T at bp 1015) resulted in the substitution of a cysteine residue for an arginine residue; this abolished a *Hha*I restriction endonuclease site, which was used to confirm the mutation in patient 458 (fig. 2*C*) and to show that 50 control

Table 2

Summary of Genotypes, Enzyme Activity, Age at Onset, and Background of Patients with MTHFR Deficiency

Patient[a]	BP Changes[b]	Amino Acid Changes	% Activity	Age at Onset	Background
1807	C764T/C764T	Pro→Leu/Pro→Leu	3	Within 1st year	Japanese
735	C692T/C692T	Thr→Met/Thr→Met	2	7 mo	African Indian
1084	C692T/C692T	Thr→Met/Thr→Met	0	3 mo	Caucasian
1554	C559T/C559T	Arg→Ter/Arg→Ter	0	1 mo	Native American (Hopi)
1627	C559T/C559T	Arg→Ter/Arg→Ter	1	1 mo	Native American (Choctaw)
356	C985T/C985T	Arg→Cys/Arg→Cys	20	16 years	Italian American
458	C1015T/G167A	Arg→Cys/Arg→Gln	10	11 years	Caucasian
1396	C1081T/G167A	Arg→Cys/Arg→Gln	14	14 years	Caucasian
1779[c]	G482A/?	Arg→Gln/?	6	15 years	French Canadian
1834[c]	G482A/?	Arg→Gln/?	7	Asymptomatic at 37 years	French Canadian
1863	G482A/?	Arg→Gln/?	14	21 years	Caucasian
354[d]	792+1G→A/?	5′ splice site/?	19	13 years	African American
355[d]	792+1G→A/?	5′ splice site/?	14	11 years	African American

[a] Patients 1554, 1627, 1779, 1834, and 1863 were previously reported by Goyette et al. (1994).

[b] ? = unidentified mutation.

[c] Patients 1779 and 1834 are sibs.

[d] Patients 354 and 355 are sibs.

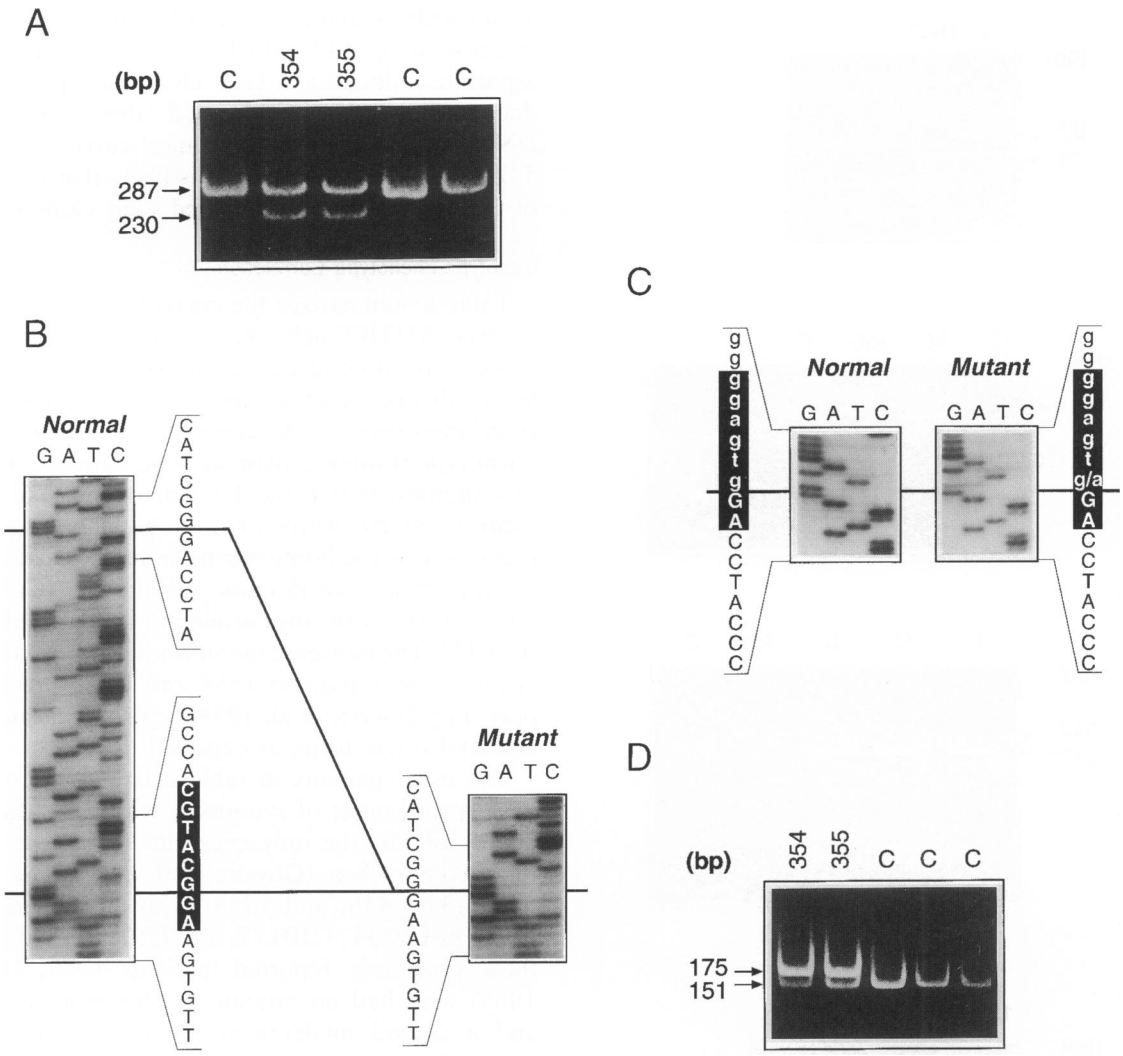

Figure 1 Identification of a 5′ splice-site mutation leading to a 57-bp in-frame deletion of the RNA. *A*, PAGE analysis of amplification products of cDNA bp 653–939, from reverse-transcribed RNA. Controls have the expected 287-bp fragment, while patients 354 and 355 have an additional 230-bp fragment. *B*, Direct sequencing of the PCR products from patient 354. The 57-bp deletion spans bp 736–792 of the cDNA. An almost perfect 5′ splice site (boxed) is seen at the 5′ deletion breakpoint. *C*, Sequencing of the 5′ splice site in control and patient 354. The patient carries a heterozygous G→A substitution in the 5′ splice site (boxed). Intronic sequences are in lower case. *D*, *Hph*I restriction endonuclease analysis on PCR products of DNA for exon 4 of patients 354 and 355 and of three controls (C). The 198-bp PCR product has two *Hph*I sites. The products of digestion for the control allele are 151, 24, and 23 bp. The products of digestion for the mutant allele are 175 and 23 bp, due to the loss of a *Hph*I site. The fragments of 24 and 23 bp have been run off the gel.

chromosomes were negative. The second mutation was a heterozygous G→A substitution (bp 167) converting an arginine to a glutamine residue. Allele-specific oligonucleotide hybridization to amplified exon 1 confirmed the heterozygous state of this mutation in patient 458 and identified a second patient (1396) carrying this mutation, also in the heterozygous state (figs. 3*C* and 3*D*); none of the 62 control chromosomes carried this mutation.

The second mutation in patient 1396 was identified in exon 6: a heterozygous C→T substitution (bp 1081) that converted an arginine residue to a cysteine residue and abolished an *Hha*I restriction endonuclease site. Restriction analysis confirmed the heterozygous substitution in 1396 (fig. 2*D*) and showed that 50 control chromosomes were negative.

Additional sequence changes.—*Hha*I analysis of exon 6, mentioned above, revealed a DNA polymorphism. This change is a T→C substitution at bp 1068, which does not alter the amino acid (serine) but creates a *Hha*I recognition site. T at bp 1068 was found in 9% of tested chromosomes. Sequence analysis identified two discrep-

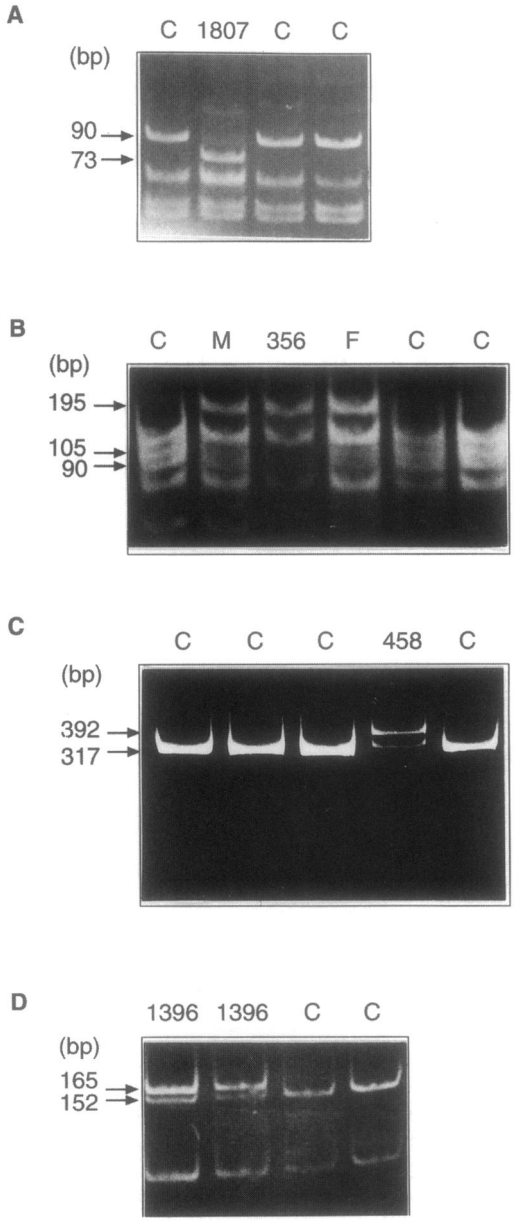

Figure 2 Diagnostic restriction endonuclease analysis of four mutations (see table 1 for PCR primers). *A*, *MnlI* restriction analysis of exon 4 PCR products for patient 1807 and three controls (C). Expected fragments: control allele—90, 46, 44, and 18 bp; mutant allele—73, 46, 44, 18, and 17 bp. An additional band at the bottom of the gel is the primer. *B*, *AciI* restriction analysis of exon 5 PCR products for patient 356, his father (F), his mother (M), and three controls (C). Expected fragments: control allele—129, 105, 90, and 68 bp; mutant allele—195, 129, and 68 bp. *C*, *HhaI* restriction analysis of exon 5 PCR products for patient 458 and four controls (C). Expected fragments: control allele—317 and 75 bp; mutant allele—392 bp. The 75-bp fragment is not shown in the figure. *D*, *HhaI* restriction analysis of exon 6 PCR products for patient 1396 and two controls (C). Expected fragments: control allele—152, 86, and 13 bp; mutant allele—165 and 86 bp. The 13-bp fragment has been run off the gel.

ancies with the published cDNA sequence: a G→A substitution at bp 542, which converts the glycine to an aspartate codon, and a C→T change at bp 1032, which does not alter the amino acid (threonine). Since all DNAs tested (>50 chromosomes) carried the A at bp 542 and the T at bp 1032, it is likely that the sequence of the original cDNA contained some cloning artifacts.

Genotype/Phenotype Correlation

Table 2 summarizes the current status of mutations in severe MTHFR deficiency. In eight patients, both mutations have been identified; in five patients (three families), only one mutation has been identified. Overall the correlation between the genotype, enzyme activity, and phenotype is quite consistent. Five patients, with onset of symptoms within the 1st year of life, had ≤3% of control activity. Three of these patients had missense mutations in the homozygous state: two patients with the threonine-to-methionine substitution (C692T) and one patient with the proline-to-leucine substitution (C764T). The nonsense mutation (C559T) in the homozygous state in patients 1554 and 1627 (previously reported by Goyette et al. 1994) is also associated with a neonatal severe form, as expected.

The other patients in table 2 had ≥6% of control activity and onset of symptoms within or after the 2d decade of life; the only exception is patient 1834, as reported elsewhere (Goyette et al. 1994). The three patients (356, 458, and 1396) with missense mutations (G167A, C985T, C1015T, and C1081T) are similar to those previously reported (patients 1779, 1834, and 1863) who had an arginine-to-glutamine substitution and a second unidentified mutation (Goyette et al. 1994). The sisters with the 5′ splice mutation and an unidentified second mutation also had levels of activity in the same range and onset of symptoms in the 2d decade, but the activity is likely due to the second unidentified allele.

The patients come from diverse ethnic backgrounds. Although patients 1554 and 1627 are both Native Americans, the mutations occur on different haplotypes, suggesting recurrent mutation rather than identity by descent (data not shown). Since the substitution occurs in a CpG dinucleotide, a "hot spot" for mutation, recurrent mutation is a reasonable hypothesis. It is difficult to assess whether some mutations are population specific, since the numbers are too small at the present time.

Discussion

MTHFR deficiency is the most common inborn error of folate metabolism and is a major cause of hereditary homocysteinemia. The recent isolation of a cDNA for MTHFR has permitted mutational analysis in this gene,

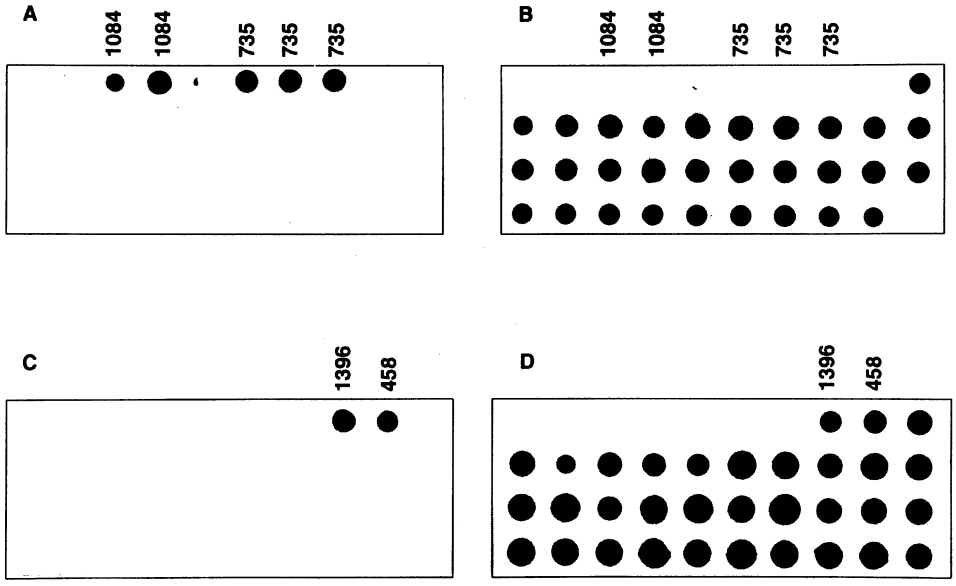

Figure 3 ASO hybridization analysis of two mutations (see table 1 for PCR primers). *A*, Hybridization of mutant oligonucleotide (692T) to exon 4 PCR products from patients 735 and 1084 and 30 controls. Only DNA from patients 735 and 1084 hybridized to this probe. *B*, Hybridization of normal oligonucleotide (692C) to stripped-dot blot from *A*. All control DNAs hybridized to this probe. *C*, Hybridization of mutant oligonucleotide (167A) to exon 1 PCR products from patients 458 and 1396 and 31 controls. Only DNA from patients 458 and 1376 hybridized to this probe. *D*, Hybridization of normal oligonucleotide (167G) to stripped-dot blot from *C*. DNA from patients 1396 and 458, as well as DNA from controls, hybridized to this probe.

with the aims of defining important domains for the enzyme and of correlating genotype with phenotype in MTHFR-deficient patients.

Our definition of a disease-causing substitution, as distinct from a benign polymorphism, is based on three factors: (1) absence of the change in ≥ 50 independent control chromosomes; (2) presence of the amino acid in the bacterial enzyme, attesting to its evolutionary significance; and (3) whether the change in amino acid is conservative. Although expression of the substitutions is required to prove formally that they are not benign, the criteria above allow us to postulate that the changes described in this report are likely to affect activity.

The seven mutations described here (six single amino acid substitutions and a 5′ splice-site mutation) bring the total to nine mutations identified thus far in this gene and complete the mutation analysis for eight patients. The identification of each mutation in only one or two families points to the striking degree of genetic heterogeneity in this gene. Seven of the nine mutations are located in CpG dinucleotides, which are prone to mutational events.

5′ Splice-Site Mutation

The G→A substitution at the GT dinucleotide of the 5′ splice site in patients 354 and 355 results in a 57-bp in-frame deletion of the coding sequence, which should delete 19 amino acids of the protein. This deletion occurs as a result of the activation of a cryptic 5′ splice site (AG/gc), even though this cryptic site does not have a perfect 5′ splice-site consensus sequence (AG/gt). However, GC (instead of GT) as the first two nucleotides of an intron have been reported in several naturally occurring splice sites, such as in the genes for human prothrombin (Degen and Davie 1987) and human adenine phosphoribosyltransferase (Broderick et al. 1987) and twice within the gene for the largest subunit of mouse RNA polymerase II (Ahearn et al. 1987). The remaining nucleotides of the cryptic site conform to a normal splice-site consensus sequence with its expected variations (A_{60} $G_{79}/g_{100}t_{100}a_{59}a_{71}g_{82}t_{50}$) (Senapathy et al. 1990). It is unlikely that the deleted enzyme resulting from this aberrantly spliced mRNA would have any activity; 8 of the 19 deleted amino acids are conserved in the *Escherichia coli* enzyme. Although the two patients show residual enzyme activity in the range of 20% of controls, the activity is probably due to the unidentified second allele in these patients.

Six Missense Mutations

The Arg→Cys substitution (C1081T) in patient 1396 is within a hydrophilic sequence previously postulated to be the linker region between the catalytic and regulatory domains of MTHFR (Goyette et al. 1994). These two

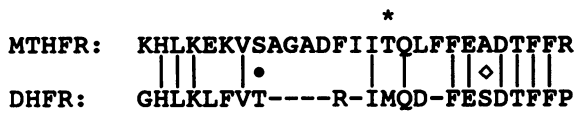

Figure 4 Region of homology between human methylenetetrahydrofolate reductase (MTHFR) and human dihydrofolate reductase (DHFR). | = identity; ● = homology; and ◇ = identity to bovine DHFR enzyme (Volz et al. 1982). An asterisk (*) indicates location of Thr→Met substitution in patients 735 and 1084. MTHFR sequence is GenBank accession number U09806. Human DHFR sequence is GenBank accession number J00140.

domains are readily separable by mild trypsinization of the porcine enzyme. The linker domain, a highly charged region, is likely to be located on the outside surface of the protein and is therefore more accessible to proteolysis. Because the Arg→Cys substitution converts a charged hydrophilic residue to an uncharged polar residue, it cannot be considered a conservative change, and it could affect the stability of the enzyme.

The two Arg→Cys substitutions identified in patients 356 and 458 (C985T and C1015T, respectively) may be involved in binding the flavin adenine dinucleotide (FAD) cofactor. Previous work (Rosenblatt and Erbe 1977) showed that heating fibroblast extracts at 55°, in the absence of the FAD cofactor, inactivated MTHFR completely. The addition of FAD to the reaction mixture before heat inactivation restored some enzyme activity to control extracts and to extracts from some patients, while the extracts of patients 356 and 458 were unaffected. Based on these observations, it was suggested that these two patients had mutations affecting a region of the protein involved in binding FAD (Rosenblatt and Erbe 1977). The two mutations are found in close proximity to each other, within 11 amino acids. In patient 356, the Arg residue is evolutionarily conserved in *Escherichia coli* and is found in a stretch of 9 conserved amino acids, suggesting a critical role for this residue; the altered Arg residue in patient 458 is not evolutionarily conserved. Crystal structure analysis of medium-chain acyl-CoA dehydrogenase (MCAD), a flavoprotein, has defined critical residues involved in the binding of FAD (Kim et al. 1993). Two consecutive residues of the MCAD protein, Met165 and Trp166, involved in interactions with FAD, can also be identified in MTHFR, 3 and 4 amino acids downstream, respectively, from the Arg residue altered in patient 458.

The Thr→Met substitution (C692T), is found in a region of high conservation with the *Escherichia coli* enzyme and in a region of good homology with human dihydrofolate reductase (DHFR) (fig. 4). Considering the early onset phenotype of the patients, one can assume that the residue is critical for activity or that it contributes to an important domain of the protein. This region of homology in DHFR contains a residue, Thr136, that has been reported to be involved in folate binding (Davies et al. 1990). This Thr residue in DHFR aligns with a Ser residue in MTHFR, an amino acid with similar biochemical properties. The Thr→Met substitution is located 8 amino acids downstream from this Ser codon, in the center of the region of homology between the two enzymes. We therefore hypothesize that the Thr→Met substitution may alter the binding of the folate substrate.

The G167A (Arg→Gln) and C764T (Pro→Leu) substitutions both affect nonconserved amino acids. Their importance in the development of MTHFR deficiency cannot be determined at the present time. All the mutations identified thus far are located in the 5′ end of the coding sequence, the region thought to encode the catalytic domain of MTHFR. Mutation analysis has been useful in beginning to address the structural and functional properties of the enzyme as well as to understand the diverse phenotypes in severe MTHFR deficiency.

Acknowledgments

The technical assistance of Renate Milos and the clerical assistance of Carolyn Mandel is gratefully acknowledged. This work was supported by the Medical Research Council of Canada. D.S.R. and R.R. are principal investigators of the MRC Group in Medical Genetics. P.G. is the recipient of a Montreal Children's Hospital Research Institute Studentship Award.

References

Ahearn JM Jr, Bartolomei MS, West ML, Cisek LJ, Corden JL (1987) Cloning and sequence analysis of the mouse genomic locus encoding the largest subunit of RNA polymerase II. J Biol Chem 262:10695–10705

Beckman DR, Hoganson G, Berlow S, Gilbert EF (1987) Pathological findings in 5,10-methylenetetrahydrofolate reductase deficiency. Birth Defects 23:47–64.

Broderick TP, Schaff DA, Bertino AM, Dush MK, Tischfield JA, Stambrook PJ (1987) Comparative anatomy of the human APRT gene and enzyme: nucleotide sequence divergence and conservation of a nonrandom CpG dinucleotide arrangement. Proc Natl Acad Sci USA 84:3349–3353

Davies JF, Delcamp TJ, Prendergast NJ, Ashford VA, Freisheim JH, Kraut J (1990) Crystal structures of recombinant human dihydrofolate reductase complexed with folate and 5-deazafolate. Biochemistry 29:9467–9479

Degen SJF, Davie EW (1987) Nucleotide sequence of the gene for human prothrombin. Biochemistry 26:6165–6177

Erbe RW (1986) Inborn errors of folate metabolism. In: Blakley RL, Whitehead VM (eds) Nutritional, pharmacological and physiological aspects, Vol 3. Wiley, New York, pp 413–465

Freeman JM, Finkelstein JD, Mudd SH (1975) Folate-responsive homocystinuria and "schizophrenia." A defect in meth-

ylation due to deficient 5,10-methylenetetrahydrofolate reductase activity. N Engl J Med 292:491–496

Goyette P, Sumner JS, Milos R, Duncan AMV, Rosenblatt DS, Matthews RG, Rozen R (1994) Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. Nat Genet 7:195–200

Harpey JP, Rosenblatt DS, Cooper BA, Le Moel G, Roy C, Lafourcade J (1981) Homocystinuria caused by 5,10-methylenetetrahydrofolate reductase deficiency: a case in an infant responding to methionine, folinic acid, pyridoxine, and vitamin B12 therapy. J Pediatr 98:275–278

John SWM, Rozen R, Laframboise R, Laberge C, Scriver CR (1989) Novel PKU mutation on haplotype 2 in French-Canadians. Am J Hum Genet 45:905–909

Kang S-S, Wong PWK, Susmano A, Sora J, Norusis M, Ruggie N (1991) Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. Am J Hum Genet 48:536–545

Kim J-J P, Wang M, Paschke R (1993) Crystal structures of medium-chain acyl-CoA dehydrogenase from pig liver mitochondria with and without substrate. Proc Natl Acad Sci USA 90:7523–7527

Kishi T, Kawamura I, Harada Y, Eguchi T, Sakura N, Ueda K, Narisawa K, et al. Effect of betaine on S-adenosylmethionine levels in cerebrospinal fluid in a patient with methylenetetrahydrofolate reductase deficiency and peripheral neuropathy. J Inherit Metab Dis (in press)

Mudd SH, Uhlendorf BW, Freeman JM, Finkelstein JD, Shih VE (1972) Homocystinuria associated with decreased methylenetetrahydrofolate reductase activity. Biochem Biophys Res Commun 46:905–912

Rosenblatt DS (1995) Inherited disorders of folate transport and metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 7th ed. McGraw-Hill, New York, pp 3111–3128

Rosenblatt DS, Erbe RW (1977) Methylenetetrahydrofolate reductase in cultured human cells. II. Studies of methylenetetrahydrofolate reductase deficiency. Pediatr Res 11:1141–1143

Rosenblatt DS, Lue-Shing H, Arzoumanian A, Low-Nang L, Matiaszuk N (1992) Methylenetetrahydrofolate reductase (MR) deficiency: thermolability of residual MR activity, methionine synthase activity and methylcobalamin levels in cultured fibroblasts. Biochem Med Met Biol 47:221–225

Senapathy P, Shapiro MB, Harris NL (1990) Splice junctions, branch point sites, and exons: sequence statistics, identification, and applications to Genome Project. Methods Enzymol 183:252–278

Volz KW, Matthews DA, Alden RA, Freer ST, Hansch C, Kaufman BT, Kraut J (1982) Crystal structure of avian dihydrofolate reductase containing phenyltriazine and NADPH. J Biol Chem 257:2528–2536