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Intermediate Hyperhomocysteinemia Resulting from Compound Heterozygosity of Methylenetetrahydrofolate Reductase Mutations

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Summary

Four subjects with thermolabile methylenetetrahydrofolate reductase (MTHFR) were discovered among 16 "obligate" heterozygotes for severe MTHFR deficiency and their family members. All four subjects had less than 25% of normal mean MTHFR specific activity in lymphocyte extracts. Three of them with normal serum folate and cyanocobalamin had intermediate hyperhomocysteinemia, and one with high serum folate and cyanocobalamin had no excessive accumulation of serum homocysteine. The biochemical features in these four subjects are distinguishable from subjects homozygous for the thermolabile MTHFR, whose specific activity is approximately 50% of the normal mean, and from heterozygotes for severe MTHFR deficiency, in whom the enzyme is thermostable and has a specific activity of about 50% of the normal mean. We propose that these four subjects are genetic compounds of the allele for the severe mutation and the allele for thermolabile mutation of the MTHFR gene. It is postulated that subjects with this genetic compound are more susceptible to the development of intermediate hyperhomocysteinemia despite normal folate and B_{12} levels. Nonetheless, hyperhomocysteinemia due to this compound heterozygosity is correctable by oral folic acid therapy.

Introduction

Severe classical methylenetetrahydrofolate reductase (MTHFR) deficiency results in hyperhomocystinemia, homocystinuria, mental retardation, neurological abnormalities, and early demise (Mudd et al. 1972; Wong et al. 1977*b*). Parents of these patients who are obligate heterozygotes have about 50% of normal mean MTHFR specific activity in their lymphocytes and the MTHFR is usually thermostable (Wong et al. 1977*a;* Kang et al. 1991).

Recently, a "new" variant of MTHFR deficiency was discovered among subjects without neurologic abnormalities (Kang et al. 1988*a*). Specific activity of lymphocyte MTHFR was about 50% of the normal mean. In contrast to the obligate heterozygotes of severe MTHFR deficiency, this variant was thermolabile (Kang et al. 1988*a*, 1988*b*). Most subjects with this variant had a mild elevation of serum total homocysteine and cardiovascular disease in adulthood (Kang et al. 1988*a*). However, some subjects had more than a threefold increase of total serum homocysteine or intermediate hyperhomocysteinemia (Kang et al. 1988*b*).

We propose that a significant number of subjects with intermediate hyperhomocysteinemia are compound heterozygotes of an allele for severe MTHFR deficiency and an allele for the thermolabile variant. This hypothesis is based on the observation that subjects homozygous for thermolabile MTHFR have about 50% enzyme activity and homozygotes for classical MTHFR deficiency have virtually no enzyme activity in lymphocyte extracts. Hence, the subjects with compound heterozygosity are expected to have ap-

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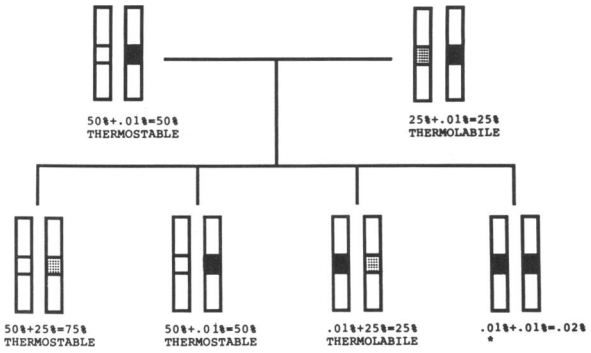

Figure 1 Hypothetical pedigree describing the mating between a classical heterozygote with thermostable MTHFR and a compound heterozygote of the classical and thermolabile mutants with thermolabile MTHFR. The offspring of this mating may have (1) thermostable MTHFR with specific activity of 75% (heterozygote for thermolabile MTHFR) (2) thermostable MTHFR with specific activity of 50% (heterozygotes for severe MTHFR deficiency) (3) thermolabile MTHFR with specific activity of 25% (compound heterozygosity), and (4) severe MTHFR deficiency with undeterminable thermostability (homozygote for severe MTHFR deficiency). □ = Normal; ■ = severe mutant; ▨ = thermolabile mutant; * = thermosensitivity not determinable.

proximately 25% of specific activity, thermolabile MTHFR (fig. 1), and a serum homocysteine level between that observed in the classical patients with severe MTHFR deficiency and that observed in the subjects homozygous for thermolabile MTHFR.

This paper described four subjects with thermolabile MTHFR among heterozygotes for severe MTHFR deficiency. Three of them have intermediate hyperhomocysteinemia.

Material and Methods

Eight patients with severe MTHFR deficiency who had less than 2% of normal mean MTHFR activity in lymphocyte extracts and their family members were the subjects of this study (Kang et al. 1991). No investigation was performed to verify paternity.

For the determination of MTHFR activity, blood was drawn in EDTA tubes and lymphocytes were immediately separated using Ficoll-Hypaque (Jondal et al. 1971) at the referring laboratories. The isolated lymphocytes were washed twice with Hank's buffer and were mailed in dry ice for overnight delivery to Presbyterian-St. Luke's Medical Center. Each batch included control samples from "normal" subjects. The samples were frozen at −72°C until enzyme assay. The methods for the determination of MTHFR specific activity and thermostability were described elsewhere (Kang et al. 1988*a*). Thermostability was determined by heat inactivation at 46°C for 5 min, and the residual activity was expressed as a percentage of the unheated enzyme activity. The enzyme was defined as thermolabile when residual activity was less than 20% (Kang et al. 1991). For the determination of serum total homocysteine, folate, and cyanocobalamin, serum was separated from venous blood, mailed in dry ice, and stored at −21°C until analysis. Serum homocysteine was determined by the measurement of protein-bound homocysteine in the serum after storage at −21°C for more than 4 wk (Kang et al. 1979, 1986). The methods of Dunn and Foster (1973) and Herbert et al. (1966) were used for the measurement of folic acid and cyanocobalamin, respectively.

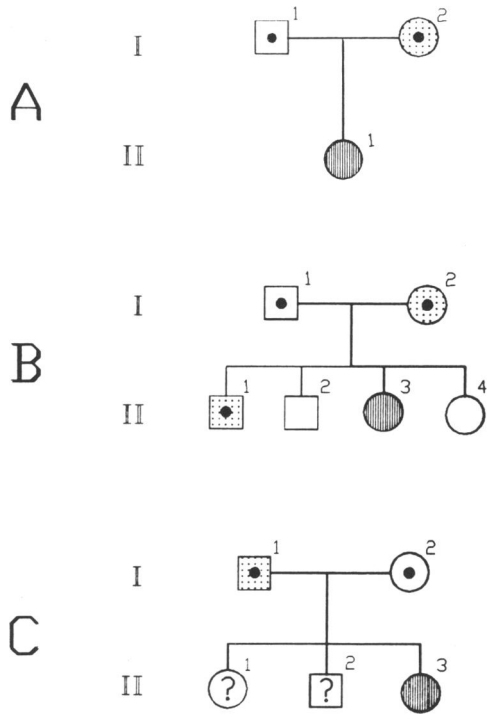

Figure 2 Pedigrees of three families with offspring suffering from severe MTHFR deficiency and one parent with thermolabile MTHFR. ⊡ = Obligate heterozygote for severe MTHFR deficiency; ◍ = thermolabile MTHFR; ◍ = severe MTHFR deficiency.

Results

Among 16 "obligate" heterozygotes for severe MTHFR deficiency, three had thermolabile MTHFR with specific activity of less than 25% of the normal mean.

Kindred A was a Native American family (fig. 2*A*).

Proband II-1 was an only child who presented at 4 wk of age with coma and failure to thrive. Subsequently she had neurological deterioration. A computed tomogram showed focal intracranial hemorrhage, hydrocephalus, and thrombosis of the superior sagittal sinus. She was found to have 11 nmol plasma methionine/ml, 32 nmol free homocystine/ml, which is equivalent to 64 nmol homocysteine/ml, 21 nmol cysteine-homocysteine disulfide/ml, and 9 nmol cystine/ml. Serum total homocysteine was 120 nmol/ml. Lymphocyte MTHFR specific activity in the proband, II-1, was 0.02 nmol formaldehyde (HCHO) formed/mg protein/h (table 1). Both parents were neurologically normal and had no history or clinical evidence of vascular disease. Lymphocyte MTHFR specific activity, thermolability, and plasma total homocysteine in the 17-year-old mother, I-2, were different from those of the controls. MTHFR specific activity was 1.76 nmol HCHO formed/mg protein/h, and residual activity after heat inactivation was 11.5%. Serum homocysteine was 3.5-fold greater than normal (table 1). In contrast, both serum folate and cyanocobalamin values were within the normal range (table 1). On the other hand, MTHFR specific activity, thermostability, and serum homocysteine in the father, I-1, were within the normal range.

Both parents in kindred B were British descendants (fig. 2*B*). Proband II-3 was a 22-year-old female who presented with neurological abnormalities, mental deterioration, and seizures at 12 years of age. A computed tomographic study demonstrated diffuse cortical atrophy. One of us (A.H.) found plasma free homocystine concentrations of 40–60 nmol/ml, which are equivalent to 80–120 nmol homocysteine/ml. Serum total homocysteine was 91.6 nmol/ml and lymphocyte MTHFR specific activity was 0.10 nmol HCHO formed/mg protein/h (table 1). She was treated with oral folic acid. Neither parent had any history or clinical evidence of neurologic or vascular disease. The proband's 46-year-old mother, I-2, and 26-year-old brother, II-1, had thermolabile MTHFR, and their enzyme specific activities were 2.34 and 2.52 nmol HCHO/mg protein/h, respectively (table 1). Serum total homocysteine in I-2 and II-1 was 3.3- and 4.0-fold greater than the normal mean, respectively. In contrast, neither the specific activity nor the thermostability of MTHFR in her father, I-1, and her siblings, II-2 and II-4, was distinguishable from those in the controls.

Kindred C was an East Indian family (fig. 2*C*). Proband II-3, the youngest of three children, had severe MTHFR deficiency. At 12 mo of age, she presented

Table 1

Initial Specific Activity of Lymphocyte MTHFR and Residual Activity after Heat Treatment, and Serum Total Homocysteine, Folate, and Cyanocobalamin Concentration

Family and Individual	Specific Activity of MTHFR (nmol HCHO/mg protein/h)	Residual Activity of MTHFR (%)	Serum Total Homocysteine (nmol/ml)	Serum Folate (ng/ml)	Serum Cyanocobalamin (pg/ml)
A:					
I-1	10.03	43.2	7.60	4.8	541
I-2	1.76	11.5	29.80	4.0	319
II-1	.02	...	120.00		
B:					
I-1	8.98	28.3	8.88	2.9	266
I-2	2.34	9.1	27.70	2.2	270
II-1	2.52	11.2	34.12	3.8	419
II-2	12.50	31.5	8.00	3.4	486
II-3	.10	...	91.64	25.3	288
II-4	9.55	36.0	5.68	2.9	328
C:					
I-1	1.91	13.8	10.28	26.0	1,113
I-2	4.28	43.9	6.86	7.4	450
II-3	ND	...	199.60	28.7	>2,000
Obligate heterozygotes	5.50 ± 1.96	36.0 ± 7.1	...	...	...
Controls ($n = 34$)	10.33 ± 2.89	33.3 ± 4.5	8.50 ± 2.80	2-18	190–1,000

NOTE. — Families A, B, and C correspond to VI, VII, and IV of our previous studies (Kang et al. 1991), respectively. ND = not determined.

with neurological abnormalities and mental deterioration. A computed tomogram showed hydrocephalus and a suggestion of cerebrovascular abnormalities. Her total serum homocysteine was 199.6 nmol/ml, and lymphocyte MTHFR activity was less than 0.01 nmol HCHO formed/mg protein/h. Neither parent had any history or clinical evidence of neurological or vascular disease. Her 42-year-old father, I-1, had thermolabile MTHFR with specific activity of 1.91 nmol/mg protein/h and residual activity of 13.8%. His serum total homocysteine was 10.28 nmol/ml, serum folate 26 ng/ml, and serum cyanocobalamin 1,113 pg/ml (table 1). The serum folate and cyanocobalamin values were distinctly above the normal limits, indicating that he was taking vitamin B_{12} and folic acid. His serum homocysteine level remained within the normal limits, possibly because of oral vitamin therapy. Siblings II-1 and II-2 were unavailable for study.

Discussion

Genetic compounds are frequently found among patients with inborn errors of metabolism. These patients often have clinical presentations different from the classical cases and may be identified by biochemical characteristics of the mutant enzymes or by molecular studies.

In severe MTHFR deficiency, the homozygotes have virtually no detectable enzyme activity in lymphocyte extracts (Wong et al. 1977*a*). The obligate heterozygotes, who have a single dose of the normal gene, are expected to have approximately 50% of the normal mean MTHFR specific activity and thermostability as found in the normal subjects. They do not have any neurological abnormalities.

Recently, a new variant of MTHFR deficiency was discovered (Kang et al. 1988*a*, 1988*b*). Homozygotes of this variant had a specific activity of approximately 50% of the normal mean in lymphocyte extracts and were characterized by thermolability of MTHFR with residual activity of less than 20% after heat inactivation. Subjects with this variant were neurologically normal. The incidence of this variant was found to be 17.0% among patients with proven coronary artery disease and 5% among randomly selected subjects without history or clinical evidence of coronary artery disease (Kang et al. 1991). Hence, the frequency of compound heterozygosity consisting of the allele for the severe mutation and the allele for the thermolabile mutation in parents of offspring with severe MTHFR deficiency should be 1:4.46, or 22.4%. In contrast, the frequency of compound heterozygosity in the general population is estimated to be 1:8,177, or 0.012%. This estimate is based on the suggestion that the incidence of homozygotes for severe MTHFR deficiency seems to be one-tenth that of homozygotes for cystathionine synthase deficiency. The latter has been determined by neonatal screening to have a frequency of approximately 1:335,000 (Erbe 1986; Mudd et al. 1989).

Among the 16 obligate heterozygotes for severe MTHFR deficiency in this study, three were found to have thermolabile MTHFR, giving a frequency of 18.8%. In family A, the mother, I-2, who was classified as a compound heterozygote, had intermediate serum total homocysteine concentration despite normal serum folate and cyanocobalamin levels. This reduction of MTHFR specific activity to 25% or less of normal mean value rendered the subject susceptible to the development of hyperhomocysteinemia. In family B, the mother, I-2, who was classified as a compound heterozygote, had intermediate hyperhomocysteinemia and normal serum folate and cyanocobalamin levels. II-1, a brother of the proband II-3, also had MTHFR specific activity and thermolability compatible with a compound heterozygosity. He too had intermediate hyperhomocysteinemia despite normal serum folate and cyanocobalamin levels. In family C, the father, I-1, was classified to be a compound heterozygote because of MTHFR specific activity and thermolability but had normal total serum homocysteine concentration. Unlike other subjects with thermolabile MTHFR, he had serum folate and cyanocobalamin levels above the normal range (table 1), and this might explain the normal homocysteine level.

Specific activities of MTHFR in the lymphocyte extracts from AI-1 and BI-1 were 2 SD above the mean of thermostable obligate heterozygotes for severe MTHFR deficiency (Kang et al. 1991). However, this does not exclude heterozygosity. It is important to point out that the biochemical characteristics of AI-2 and BI-2, an increased thermolability and decreased specific enzyme activity, were clearly distinguishable from that of other heterozygotes (Kang et al. 1991).

Marked increase of plasma homocystine is observed in homozygotes of severe cystathionine synthase deficiency (Mudd et al. 1989), severe MTHFR deficiency (Mudd et al. 1972), and defects of B_{12} metabolism (Rosenblatt 1989). On the other hand, hyperhomocysteinemia due to heterozygosity for these metabolic defects or due to homozygosity for thermolabile

MTHFR is variable (Wilcken et al. 1983; Murphy-Chutorian et al. 1985; Kang et al. 1988*a*, 1991; McGill et al. 1990). Since the plasma concentration of homocysteine is also influenced by various nongenetic factors, such as folate, B_{12}, and pyridoxine intake, or hormonal status in normal subjects (Kang et al. 1987; Mudd et al. 1989; Ueland and Refsum 1989), it may be expected that subjects with moderate deficiency in the enzymes involved in homocysteine metabolism are also influenced by such nongenetic factors. Oral folic acid supplement has been demonstrated to be able to normalize the hyperhomocysteinemia in these subjects (Kang et al. 1988*b*).

In severe MTHFR deficiency, the clinical severity was found to be correlated with the severity of enzyme deficiency (Rosenblatt 1989). In subjects with thermolabile MTHFR and a specific activity of approximately 50% of the normal mean, there was a mild elevation of serum total homocysteine (Kang et al. 1991). Subjects with compound heterozygosity of MTHFR mutations and a specific activity about 25% of the normal mean are more susceptible to the development of hyperhomocysteinemia despite normal serum folic acid concentrations. The hyperhomocysteinemia may constitute an undue risk for vascular disease, particularly when folate and B_{12} intake is suboptimal.

This paper describes compound heterozygosity of allelic MTHFR mutations causing intermediate hyperhomocysteinemia. In addition, we postulate that similar intermediate hyperhomocysteinemia may be also caused by a compound of nonallelic heterozygosities. Since hyperhomocysteinemia is caused by a number of different genetic defects in methionine and/or folate metabolism, the combination of thermolabile MTHFR with heterozygosity of any of these defects, such as cystathionine synthase deficiency, may produce intermediate hyperhomocysteinemia.

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