2

Genetic Variation in Folate Metabolism

Impact on Development

Rima Rozen

INTRODUCTION

Folate derivatives are utilized in single-carbon transfer reactions for many critical pathways, including methionine and DNA synthesis (*see* Fig. 1). For example, 5,10-methylene tetrahydrofolate (5,10-methylene THF) is required for conversion of dUMP to dTMP. It can also be converted to 10-formyltetrahydrofolate for the synthesis of the purine ring, or reduced to 5-methyltetrahydrofolate (5-methylTHF) for the remethylation of homocysteine to methionine. Methionine is the precursor for *S*-adenosymethionine (SAM), the methyl donor in numerous methylation reactions. The enzyme 5,10-methylene tetrahydrofolate reductase (MTHFR) converts 5,10-methylene THF to 5-methylTHF, thereby regulating a balance between folate required for DNA synthesis and folate required for methionine/SAM synthesis and methylation reactions. The importance of MTHFR to SAM supply is highlighted by the fact that SAM is a MTHFR inhibitor.

A common sequence variant in MTHFR has been implicated in several different complex conditions, including cardiovascular disease, neural tube defects, pregnancy complications, and cancer. This chapter will review our current knowledge on the biology of MTHFR and on the developmental problems associated with this common variant in the MTHFR gene. Other relevant enzymes in the homocysteine remethylation pathway and their sequence variants will be mentioned briefly, but additional studies are required to assess their impact on development.

BIOCHEMICAL AND MOLECULAR GENETIC ASPECTS OF MTHFR

5-Methylene tetrahydrofolate reductase is utilized to convert 5,10-methylene THF to 5-methylTHF in many different species. The bacterial and

From: *Folate and Human Development*
Edited by: E. J. Massaro and J. M. Rogers © Humana Press Inc., Totowa, NJ

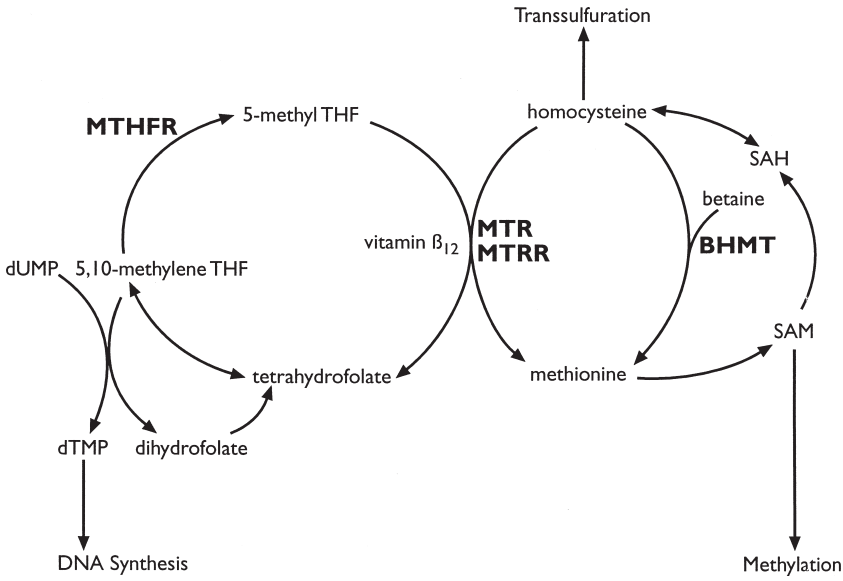


Fig. 1. Interconversion of folate derivatives by MTHFR and homocysteine remethylation pathway. MTR, gene for methionine synthase; MTRR, gene for methionine synthase reductase; BHMT, gene for betaine homocysteine methyltransferase.

porcine enzymes have been purified to homogeneity *(1,2)*. Although they both share a catalytic domain, the mammalian enzyme has an additional regulatory C-terminal domain that accounts for the larger size of the mammalian enzyme (77-kDa subunit in pig vs 33-kDa subunit in *Escherichia coli*). This C-terminal domain contains the binding site for SAM, an allosteric inhibitor of the enzyme. The bacterial enzyme is a tetramer, whereas the porcine enzyme is a dimer. The bacterial enzyme has recently been crystallized and its crystal structure determined; this work is critical for the understanding of how mutations in MTHFR impact on enzyme function *(1)*.

Using amino acid sequence information from the porcine enzyme, a partial cDNA for human MTHFR (1.3 kb) was isolated in 1994 by Goyette et al. *(3)*. This cDNA was used to isolate and express a cDNA of 2.2 kb *(4)*, which resulted in the synthesis of a 70-kDa protein that was catalytically active. Western blotting has suggested the presence of at least two protein isoforms of the human enzyme, with some degree of tissue specificity. A 77-kDa polypeptide was identified in most tissues, whereas the 70-kDa minor form was observed in human fetal liver *(4)*.

The human MTHFR gene has 11 exons encoding the 2.2-kb cDNA, with a size of approx 17 kb for this region of the gene that contains the coding sequence *(5)*. However, the presence of significant 5'UTRs and 3'UTRs, in addition to the 2.2-kb coding sequence, contributes to the large mRNAs of approx 8 kb and indicates that the final size of the gene will be greater than 17 kb *(5,6)*. The large size and complex pattern of splicing in the 5'UTR suggest that MTHFR may have some interesting regulatory properties.

The human gene maps to chromosome 1 (*1p36*) and the mouse gene maps to distal chromosome 4, a region that is homologous to the human *1p36* region *(3,7)*. The mouse gene structure is very similar to that of the human gene, with over 85% identity in the amino acid sequence *(5)*. There is 30% identity in amino acids between the *E. coli* enzyme, encoded by the *metF* gene, and the N-terminal catalytic domain of the human enzyme *(3,5)*.

SEVERE MTHFR DEFICIENCY

Severe MTHFR deficiency, with less than 20% of control enzyme activity, is the most common inborn error of folate metabolism *(8)*. These patients have dramatic elevations of plasma homocysteine with homocystinuria, and low or low–normal levels of plasma methionine. This relatively rare condition can have devastating consequences. Some patients die in the first year of life; others have variable clinical features, including developmental delay, motor and gait disturbances, seizures, and psychiatric manifestations. Pathological findings have included demyelination and vascular changes *(8)*. Over 20 different mutations in MTHFR have been reported in this group of patients *(3,9–12)*. Some of these mutations have been expressed in vitro and confirmed to impact enzyme function *(13)*.

The early-onset clinical symptoms in these homocystinuric patients emphasize the important role of MTHFR and folate metabolism in normal development, particularly in the development of the central nervous system. Other enzyme deficiencies, the most common being a deficiency of cystathionine-β-synthase (CBS), can cause homocystinuria through a disruption of homocysteine trans-sulfuration, but, in contrast to MTHFR-deficient patients, CBS deficiency is associated with high methionine levels in plasma *(14)*. This is the result of the fact that MTHFR deficiency results in a disruption of the homocysteine remethylation pathway, whereas CBS-deficient patients have normal remethylation, with a block in the first enzyme of the trans-sulfuration pathway. Although CBS-deficient patients also have some CNS problems, such as mental retardation, MTHFR deficiency appears to be associated with additional significant neurological features. Because

MTHFR-deficient patients are likely to be compromised with respect to SAM synthesis, it is possible that some of their distinct neurological problems are related to a decrease in methylation reactions in the CNS, such as decreased phospholipid synthesis or decreased neurotransmitter synthesis.

Common to both types of homocystinuria are vascular changes, presumably reflecting the increased levels of homocysteine in the circulation. With the recognition of hyperhomocysteinemia as a risk factor for cardiovascular disease, many studies have addressed the nature of the pathogenic effects of homocysteine on the vasculature. These include toxicity to the vascular endothelium, enhanced proliferation of smooth muscle cells, thrombogenic effects and an increase in oxidative stress *(15)*. However, because most studies have been performed in vitro, the physiologic relevance of these findings requires confirmation.

A mouse model for severe MTHFR deficiency has recently been generated *(16)*. Mice that are homozygous for a knockout of the MTHFR gene have severe hyperhomocysteinemia with reduced survival or delayed development and cerebellar pathology. Additional investigations of these mice should provide important information regarding the pathogenicity of hyperhomocysteinemia and the role of folate in normal development.

MILD MTHFR DEFICIENCY: 677C→T MUTATION

Based on the presence of thrombotic episodes in patients with severe hyperhomocysteinemia and homocystinuria, several investigators suggested that milder elevations in homocysteine could also be a risk factor for cardiovascular disease *(17)*. The observation that some patients with cardiovascular disease had a mild deficiency of MTHFR, with a thermolabile enzyme *(18)*, led to molecular genetic studies that identified a C to T mutation at bp 677 (an alanine to valine substitution) *(4)*. This mutation was expressed in vitro and shown to encode a thermolabile enzyme. Individuals who were homozygous mutant also had reduced enzymatic activity at 37°C (approx 35% of control values). These individuals are at risk for mild hyperhomocysteinemia, particularly when their plasma folate is low *(19)*. Folate supplementation has been demonstrated to lower homocysteine in these individuals *(20)*. These clinical observations are supported by biochemical studies that have demonstrated that the mutant human enzyme can be stabilized by both folate and its cofactor flavin adenine dinucleotide (FAD) *(1)*. Thus, supplemental folate might prevent hyperhomocysteinemia in mutant individuals by improving enzyme function. Studies with the mutagenized bacterial enzyme have yielded similar results to those of the human enzyme and have predicted, on the basis of crystal structure informa-

tion, that the mutant valine residue may indirectly affect FAD binding and/or increase the dissociation of the active bacterial tetramer into a dimer *(1)*.

The mutation has been shown to decrease total plasma folate *(21)*, as 5-methylTHF is the primary circulatory form of folate. It has also been shown to affect the distribution of folates in red blood cells *(22)*. Individuals with the mutation have decreased amounts of methylTHF and increased amounts of the formylated derivatives. Because 5,10-methylene THF conversion to 5-methylTHF is compromised by reduced MTHFR activity, there is an increased amount of 5,10-methylene THF available for conversion to formyltetrahydrofolate. The decreased 5-methylTHF accounts for hyperhomocysteinemia because of the reduced conversion of homocysteine to methionine by methionine synthase; this reduction could also affect methylation reactions. The increased amount of other folate derivatives might improve thymidine and purine availability for DNA synthesis (*see* Fig. 1). Although total folate in red blood cells was not different in mutant individuals in this study *(22)*, others have suggested decreased *(23)* or increased *(24)* total folate in erythrocytes. The discrepancy could be the result of the variable methodologies employed in different laboratories.

The 677C→T mutation is common in North American, European, and many Asian countries, with homozygosity frequencies ranging from 5% to 25% *(25)*. The highest frequencies of this variant have been reported in southern Mediterranean populations and Hispanic populations in North America. The mutation is relatively infrequent in African-Americans *(26)*.

The initial identification of this polymorphism was made on the basis of its role in the elevation of homocysteine, a risk factor for cardiovascular disease. However, because severe MTHFR deficiency is associated with a wide variety of developmental and neurologic problems, it is not too surprising that mild MTHFR deficiency might also have an impact on disorders involving the central nervous system (CNS).

DEVELOPMENTAL ABNORMALITIES ASSOCIATED WITH THE 677C→T VARIANT

Neural Tube Defects

Clinical studies have clearly demonstrated that folate supplementation reduces the occurrence and recurrence of neural tube defects (NTD) *(27,28)*. Mothers of children with NTD had been shown to have low folate levels and were suspected of having an altered folate metabolism *(29)*. The observation that mothers of NTD cases had mild hyperhomocysteinemia *(30,31)* led to the investigation of the MTHFR variant as a genetic risk factor for this

birth defect *(24)*. Several studies have reported that the 677C→T variant in the homozygous state in the child or in the mother can increase the risk for NTD. A recent review of the literature indicated a pooled odds ratio of 1.8 (95% confidence interval [CI] = 1.4 – 2.2) and 2.0 (95% CI = 1.5 – 2.8) for children and mothers, respectively, with the homozygous mutant genotype *(25)*; these values are quite similar to those reported in an earlier meta-analysis *(32)*. The combination of the mutant genotype in both the mother and child could have an even greater risk, according to one report *(33)*. As mentioned earlier for hyperhomocysteinemia, nutritional folate status is a critical determinant in the magnitude of the genetic risk conferred by this mutation. Low folate status combined with the homozygous mutant genotype may result in a higher risk than either variable alone *(33)*. The lack of an association between NTD and the MTHFR variant in some studies may reflect the nutritional status of the study group during the critical period of development of the neural tube.

The mechanism by which this mutation increases risk is not clear. Homocysteine has been shown to be teratogenic in studies of chicken embryos *(34)*. Alternatively, a decrease in methylation reactions or dysregulated DNA synthesis in critical cells could compromise neural tube closure.

Pregnancy Complications

Women with placental abruption *(35)* or recurrent pregnancy loss *(36)* have been reported to have mild hyperhomocysteinemia. These findings have culminated in several publications that have documented an increased frequency of the 677C→T variant in women with the aforementioned complications and with preeclampsia *(36–38)*. A recent review of the relevant literature has reported pooled odds ratios of 2.3 (95% CI = 1.1 – 4.9), 3.3 (95% CI = 1.2 – 9.2), and 2.6 (95% CI = 1.4 – 5.1) for placental abruption, recurrent pregnancy loss, and preeclampsia, respectively, in women who were homozygous mutant for the 677-bp variant *(38)*. The risk for these problems is also augmented in the presence of other thrombophilic risk factors, such as Factor V Leiden and the 20210-bp mutation in the prothrombin gene, either alone or in combination with the MTHFR mutation *(39–41)*. These various types of pregnancy complication could be caused by homocysteine-mediated effects on the placental vasculature.

A few reports in the literature have alluded to genetic selection, with possible intrauterine losses, based on MTHFR genotypes. One study has suggested that the frequency of the homozygous mutant genotype is increased in the younger population in Spain, compared to an older group, because folate supplementation during pregnancy in the past two decades has im-

proved the nutritional status of pregnant women and decreased the number of losses of mutants *in utero (42)*. Another study has suggested heterozygote advantage in families with NTD *(43)* and one publication has reported decreased amounts of control female newborns that are homozygous for this mutation *(44)*.

Other Emerging Developmental Problems

Oral Clefts

Maternal use of multivitamins with folic acid has been reported to reduce the risk of a cleft lip with or without cleft lip palate *(45)*. The first study of MTHFR in children with this congenital anomaly did not identify a statistically significant risk in a Hispanic California population, although there was an increased nonsignificant odds ratio for cleft lip (1.8, 95% CI = 0.3–7.9) in children of non-Hispanic white mothers who did not use vitamins *(46)*. The same group reported the absence of an effect on an isolated cleft palate *(47)*. In contrast, maternal hyperhomocysteinemia was observed in mothers of children with nonsyndromic orofacial clefts in the Netherlands *(48)*, and a recent small study in Ireland reported a significantly higher frequency of mutant MTHFR in subjects with an isolated cleft palate *(49)*.

Fetal Anticonvulsant Syndrome

One study has reported an increased frequency of this variant in women on anticonvulsant medication who had children with this syndrome *(50)*. Three common anticonvulsants (carbamazepine, phenytoin, and sodium valproate) have been shown to interfere with folate metabolism; consequently, these women may have a higher requirement for folate during pregnancy. In a related study, epileptic women on anticonvulsants were shown to have hyperhomocysteinemia and low folate, particularly when they were homozygous for this variant *(51)*.

Down Syndrome

Two studies *(52,53)* have reported an increase in the frequency of the MTHFR variant in mothers of children with Down syndrome. The postulated mechanism is an increase in DNA hypomethylation, which could promote nondisjunctional events by altering centromere methylation patterns or chromosome stability.

Schizophrenia

Abnormalities in methyl group metabolism have been observed in schizophrenics *(54)*, and patients with severe MTHFR deficiency can have

psychiatric disturbances *(8)*. Consequently, a few studies have examined the common MTHFR variant as a risk factor for this condition. Positive associations *(55,56)* have been observed in some but not all studies. This discrepancy could be the result of the fact that the association may only be present in subgroups of patients (e.g., those who are good responders to neuroleptic medication) as reported in a recent study *(56)*.

Congenital Heart Defects

Multivitamin supplementation has been shown to decrease the risk of congenital heart defects. Mothers of children with congenital heart defects have been reported to have higher homocysteine levels than control subjects *(57)*. Although the MTHFR variant was not examined in this study, the association between vitamin responsiveness and hyperhomocysteinemia suggests that the MTHFR variant could be a candidate risk factor, particularly because neural-crest cells contribute to the formation of the septum.

Cancer

The high frequency of a polymorphism that can affect genetic fitness in several different conditions raises the question of a possible selective advantage. One hypothesis relates to the fact that a mutation in MTHFR should increase DNA synthesis or repair through an elevation of 5,10-methylene THF levels for the synthesis of thymidine or purines. Several publications have reported a protective effect of the MTHFR variant in colorectal cancer *(58,59)*, possibly through the aforementioned mechanism; a recent study has demonstrated a similar protective effect in adult acute lymphocytic leukemia *(60)*. Although the age of onset of these disorders is in the adult period and therefore may have little impact on selection or early development, it is possible that enhanced DNA synthesis may be advantageous during early development or in the protection against early-onset childhood cancers.

OTHER COMMON VARIANTS IN FOLATE METABOLISM

MTHFR 1298A→C

A second common variant in MTHFR, at bp 1298, converts a glutamate to an alanine codon. This variant is present at a similar frequency (approx 9–10% homozygosity) to that of the 677-bp variant *(61,62)*. However, by itself, it may not sufficiently disrupt enzymatic function to alter homocysteine remethylation. The mutant enzyme has recently been expressed in vitro; the activity associated with this mutation (approx 68% of control activity) is intermediate between that of the wild-type enzyme and the enzyme

Table 1
Comparison of MTHFR Activities (at 37°C) Obtained by In Vitro Expression with Activities Reported in Lymphocyte Extracts

	Recombinant enzyme expression[a]	NTD patients and their parents and controls[b]	NTD mothers and controls	NTD children and controls[c]
AE	100%	100%	100%	100%
AA	68% (± 5.0)	61%	66%	57%
VE	45% (± 10.8)	25%	32%	31%
Residual activity (%)				
AE	31.1%	66%		
AA	48.9%	61%		
VE	11.9%	17.6%		

Note: The designations for the wild-type (AE) and mutant (AA, VE) enzymes are based on the single-letter amino acid code. AE (wild type) indicates the alanine codon at bp 677 and the glutamate codon at bp 1298. AA indicates the wild-type alanine codon at bp 677 and the mutant alanine codon at bp 1298. VE indicates the mutant valine codon at bp 677 and the wild-type glutamate codon at bp 1298. Residual activity refers to the activity after heating at 46°C for 5 min, an indicator of thermolability.

[a]The activities for the mutagenized enzyme in vitro were obtained from ref. *63*.

[b]The activities in lymphocyte extracts were obtained from ref. *61*.

[c]The activities in lymphocyte extracts were obtained from ref. *62*.

carrying a mutation at bp 677 (45% of control activity) *(63)*. These results are consistent with the enzyme activities determined in lymphocyte extracts in vivo (*see* Table 1). The mutation at bp 1298 does not affect thermostability of the enzyme, unlike the mutation at bp 677.

Hyperhomocysteinemia has not been observed in individuals who are mutant only for the 1298-bp mutation, reflecting the higher levels of enzyme activity associated with this mutation, as compared to the 677-bp change *(61,62)*. On the other hand, individuals who are heterozygotes for both the 1298 and 677 variants may be at risk for hyperhomocysteinemia *(61,63)* and, consequently, for the conditions associated with disruption of this pathway. One report has observed an increased odds ratio for neural tube defects in children who were double heterozygotes, but this risk was not statistically significant *(61)*.

Methionine Synthase (MTR) 2756A→G

This vitamin B_{12}-dependent enzyme utilizes 5-methylTHF for conversion of homocysteine to methionine. A polymorphism at bp 2756 has been

described, which converts an aspartate to a glycine residue *(64)*. This variant, however, is much less common than the MTHFR variants, with homozygosity frequencies of approx 4%. An increased risk for neural tube defects has not been observed *(33,65)*, but one report has suggested a protective effect in colon cancer *(66)*, as previously observed for the MTHFR variant.

Methionine Synthase Reductase (MTRR) 66A→G

This enzyme is required for the reductive activation of methionine synthase. A very common mutation has been identified that converts an isoleucine to a methionine codon *(67)*. Homozygosity frequencies of approx 25% have been observed in North Americans. This variant has been reported to increase the risk of neural tube defects when vitamin B_{12} levels are low or when the MTHFR 677-bp variant is present *(67)*. Of additional interest is the finding that this variant may also increase the risk of Down syndrome *(53)* or vascular disease *(68)*. However, all of the aforementioned disease associations have been the subject of single reports, which clearly require confirmation.

CONCLUSION

The important role of adequate dietary folate for normal development has been clearly established. The association of genetic variants in folate metabolism with increased risk for disease suggests that some individuals may have higher requirements for this nutrient.

The 677-bp variant in MTHFR has been investigated more thoroughly than other polymorphisms and appears to influence several developmental processes. The interaction between the genetic mutation and nutrient status is interesting and offers a reasonable approach (i.e. folate supplementation) to overcome the consequences of the mutation. In addition to nutritional influences, however, there may be other variants in the aforementioned enzymes or in other enzymes involved in remethylation, such as betaine homocysteine methyltransferase (BHMT), which can modify risk. The challenge will be to study interactions of multiple genetic and environmental factors in disorders that are clearly multifactorial in nature. Large numbers of individuals will have to be assessed, with various combinations of these risk factors, before a comprehensive understanding is achieved. The availability of mouse models with defects in folate metabolism *(16,69)* should complement clinical studies in elucidating the mechanisms of folate-dependent disease states.

REFERENCES

1. Guenther, B. D., Sheppard, C. A., Tran, P., Rozen, R., Matthews, R. G., and Ludwig, M. L. (1999) The structure and properties of methylenetetrahydrofolate reductase from *Escherichia coli*: a model for the role of folate in ameliorating hyperhomocysteinemia in humans. *Nature Struct. Biol.* **6**, 359–365.
2. Daubner, S. C. and Matthews, R. G. (1982) Purification and properties of methylenetetrahydrofolate reductase from pig liver. *J. Biol. Chem*. **257**, 140–145.
3. Goyette, P., Sumner, J. S., Milos, R., Duncan, A. M. V., Rosenblatt, D. S., Matthews, R. G., et al. (1994) Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. *Nature Genet.* **7**, 195–200.
4. Frosst, P., Blom, H. J., Milos, R., Goyette, P., Sheppard, C. A., Matthews, R. G., et al. (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nature Genet.* **10**, 111–113.
5. Goyette, P., Pai, A., Milos, R., Frosst, P., Tran, P., Chen, Z., et al. (1998) Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). *Mammal. Genome* **9**, 652–656.
6. Chan, M., Tran, P., Goyette, P., Chen, Z., Milos, R., Artigas, C., et al. (1999) Analysis of the 5' region of the methylenetetrahydrofolate reductase (MTHFR) gene reveals multiple exons with alternative splicing, and an overlapping gene. *FASEB J.* **13**, A1375.
7. Frosst, P., Zhang, Z-X., Pai, A., and Rozen, R. (1996) The methylenetetrahydrofolate reductase (MTHFR) gene maps to distal mouse chromosome 4. *Mammal. Genome* **7**, 864–865.
8. Rosenblatt, D. S. (1995) Inherited disorders of folate transport and metabolism. In *The Metabolic and Molecular Bases of Inherited Disease*. 7th ed. Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds. McGraw-Hill: New York, pp. 3111–3128.
9. Goyette, P., Frosst, P., Rosenblatt, D. S., and Rozen, R. (1995) Seven novel mutations in the methylenetetrahydrofolate reductase gene and genotype/phenotype correlations in severe MTHFR deficiency. *Am. J. Hum. Genet*. **56**, 1052–1059.
10. Goyette, P., Christensen, B., Rosenblatt, D. S., and Rozen, R. (1996) Severe and mild mutations in cis for the methylenetetrahydrofolate reductase (MTHFR) gene, and description of 5 novel mutations in MTHFR. *Am. J. Hum. Genet*. **59**, 1268–1275.
11. Kluijtmans, L. A. J., Wendel, U., Stevens, E. M. B., van den Heuvel, L. P. W. J., Trijbels, F. J. M., and Blom, H. J. (1998) Identification of four novel mutations in severe methylenetetrahydrofolate reductase deficiency. *Eur. J. Hum. Genet.* **6**, 257–265.
12. Sibani, S., Christensen, B., O'Ferrell, E., Saadi, I., Hiou-Tim, F., Rosenblatt, D. S., et al. (2000) Characterization of six novel mutations in the methylenetetrahydrofolate reductase (MTHFR) gene in patients with homocystinuria. *Hum. Mutat.* **15**, 280–287.

13. Goyette P. and Rozen R. (2000) The thermolabile variant can further reduce activity when expressed in cis with severe mutations for human methylenetetrahydrofolate reductase. *Hum. Mutat*. **16**, 132–138.
14. Mudd, S. H., Levy, H. L., and Skovby, F. (1995) Disorders of transsulfuration. In *The Metabolic and Molecular Bases of Inherited Disease*. 7th ed. Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds. McGraw-Hill: New York, pp. 1279–1327.
15. Welch, G. N. and Loscalzo, J. (1998) Homocysteine and atherothrombosis. *N. Engl. J. Med.* **15**, 1042–1050.
16. Chen, Z., Karaplis, A., Ackerman, S. L., Pogribny, I. P., Melnyk, S., Lussier-Cacan, S., et al. (2001) Mice deficient in methylenetetrahydrofolate reductase exhibit hyperhomocysteinemia and decreased methylation capacity with neuropathology and aortic lipid deposition. *Hum. Molec. Genet.* **10**, 433–443.
17. Wilcken, D. E. L. (2000) Historical aspects of the relationship between homocysteine and vascular disease. In *Homocysteine and Vascular Disease*. Robinson, K., ed. Kluwer Academic: Dordrecht, pp. 5–14.
18. Kang, S-S., Wong, P. W. K., Susmano, A., Sora, J., Norusis, M., and Ruggie, N. (1991) Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary disease. *Am. J. Hum. Genet.* **48**, 536–545.
19. Jacques, P. F., Bostom, A. G., Williams, R. R., Ellison R. C., Eckfeldt, J. H., Rosenberg, I. H., et al. (1996) Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* **93**, 7–9.
20. Malinow, M. R., Nieto, F. J., Kruger, W. D., Duell, P. B., Hess, D. L., Gluckman, R. A., et al. (1997) The effects of folic acid supplementation on plasma total homocysteine are modulated by multivitamin use and methylenetetrahydrofolate reductase genotypes. *Arteriosclerosis Thromb. Vasc. Biol*. **17**, 1157–1162.
21. Harmon, D. L., Woodside, J. V., Yarnell, J. W. G., McMaster, D., Young, I. S., McCrum, E. E., et al. (1996) The common "thermolabile" variant of methylenetetrahydrofolate reductase is a major determinant of mild hyperhomocysteinaemia. *Q. J. Med.* **89**, 571–577.
22. Bagley, P. J. and Selhub, J. A. (1998) Common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of formylated tetrahydrofolates in red blood cells. *Proc. Natl. Acad. Sci. USA* **95**,13,217–13,220.
23. Molloy, A. M., Daly, S., Mills, J. L., Kirke, P. N., Whitehead, A. S., Ramsbottom, D., et al. (1997) Thermolabile variant of 5,10-methylenetetrahydrofolate reductase associated with low red-cell folates: implications for folate intake recommendations. *Lancet* **349**, 1591–1593.
24. van der Put, N. M. J., Steegers-Theunissen, R. P. M., Frosst, P., Trijbels, F. J. M., Eskes, T. K. A. B., van den Heuvel, L. P., et al. (1995) Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. *Lancet* **346**, 1070–1071.
25. Botto, L. D. and Yang, Q. (2000) 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: A HuGE review. *Am. J. Epidemiol*. **9**, 862–877.

26. Stevenson, R. E., Schwartz, C. E., Du, Y. Z., and Adams, M. J. (1997) Differences in methylenetetrahydrofolate reductase genotype frequencies, between whites and blacks. *Am. J. Hum. Genet.* **60**, 230–233.
27. Czeizel, A. E. and Dudas, I. (1992) Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N. Engl. J. Med.* **327**, 1832–1835.
28. MRC Vitamin Study Research Group (1991) Prevention of neural-tube defects: results of the Medical Research Council vitamin study. *Lancet* **338**, 131–137.
29. Scott, J. M., Kirke, P. N., and Weir, D. G. (1990) The role of nutrition in neural tube defects. *Annu. Rev. Nutr.* **10**, 277–295.
30. Steegers-Theunissen, R. P. M., Boers, G. H. J., Trijbels, F. J. M., Finkelstein, J. D., Blom, H. J., Thomas, C. M. G., Borm, et al. (1994) Maternal hyperhomocysteinemia: a risk factor for neural-tube defects? *Metabolism* **43**,1475–1480.
31. Mills, J. L., McPartlin, J. M., Kirke, P. N., Lee, Y. J., Conley, M. R., Weir, D. G., and Scott, J. H. (1995) Homocysteine metabolism in pregnancies complicated by neural-tube defects. *Lancet* **345**, 149–151.
32. van der Put, N. M. J., Eskes, T. K. A. B., and Blom, H. J. (1997) Is the common 677C→T mutation in the methylenetetrahydrofolate reductase gene a risk factor for neural tube defects? A meta-analysis. *Q. J. Med.* **90**, 111–115.
33. Christensen, B., Arbour, L., Tran, P., Leclerc, D., Sabbaghian, N., Platt, R., et al. (1999) Genetic polymorphisms in methylenetetrahydrofolate reductase and methionine synthase, folate levels in red blood cells, and risk of neural tube defects. *Am. J. Med. Genet*. **84**, 151–157.
34. Rosenquist, T. H., Ratashak, S. A., and Selhub, J. (1996) Homocysteine induces congenital defects of the heart and neural tube: Effect of folic acid. *Proc. Natl. Acad. Sci. USA* **93**, 15,227–15,232.
35. Goddijn-Wessel, T. A. W., Wouters, M. G. A. J., Molen, E. F. v. d., Spuijbroek, M. D. E. H., Steegers-Theunissen, R. P. M., Blom, H. J., et al. (1996) Hyperhomocysteinemia: a risk factor for placental abruption or infarction. *Eur. J. Obstet. Gynec. Reprod. Biol.* **66**, 23–29.
36. Nelen, W. L. D. M., Steegers, E. A. P., Eskes, T. K. A. B., and Blom, H. J. (1997) Genetic risk factor for unexplained recurrent early pregnancy loss. *Lancet* **350**, 861.
37. Sohda, S., Arinami, T., Hamada, H., Yamada, N., Hamaguchi, H., and Kubo, T. (1997) Methylenetetrahyrofolate reductase polymorphism and pre-eclampsia. *J. Med. Genet.* **34**, 525–526.
38. Ray, J. G. and Laskin, C. A. (1999) Folic acid and homocyst(e)ine metabolic defects and the risk of placental abruption, pre-eclampsia and spontaneous pregnancy loss: a systemic review. *Placenta* **20**, 519–529.
39. Grandone, E., Margaglione, M., Colaizzo, D., Cappucci, G., Paladini, D., Martinelli, P., et al. (1997) Factor V Leiden, C>T MTHFR polymorphism and genetic susceptibility to preeclampsia. *Thromb. Haemost.* **77**, 1052–1054.
40. Kupferminc, M. J., Eldor, A., Steinman, N., Many, A., Bar-Am, A., Jaffa, A., et al. (1999) Increased frequency of genetic thrombophilia in women with complications of pregnancy. *N. Eng. J. Med.* **340**, 9–13.

41. Brenner, B., Sarig, G., Weiner, Z., Younis, J., Blumenfeld, Z., and Lanir, N. (1999) Thrombophilic polymorphisms are common in women with fetal loss without apparent cause. *Thromb. Haemost.* **82**, 6–9.
42. Muñoz-Moran, E., Dieguez –Lucena, J. L., Fernandez-Arcas, N., Peran-Mesa, S., and Reyes-Engel, A. (1998) Genetic selection and folate intake during pregnancy. *Lancet* **352**, 1120–1121.
43. Weitkamp, L. R., Tackels, D. C., Hunter, A. G. W., Holmes, L. B., and Schwartz, C. E. (1998) Heterozygote advantage of the MTHFR gene in patients with neural-tube defect and their relatives. *Lancet* **351**, 1554–1555.
44. Rozen, R., Fraser, F. C., and Shaw, G. M. (1999) Decreased proportion of female newborn infants homozygous for the 677C→T mutation in methylenetetrahydrofolate reductase. *Amer. J. Med. Genet.* **83**,142–143.
45. Shaw, G. M., Lammer, E. J., Wasserman, C. R., O'Malley, C. D., and Tolarova, M. M. (1995) Risks of orofacial clefts in children born to women using multivitamins containing folic acid periconceptionally. *Lancet* **345**, 393–396.
46. Shaw, G. M., Rozen, R., Finnell, R. H., Todoroff, K., and Lammer, E. J. (1998) Infant C677T mutation in MTHFR, maternal periconceptional vitamin use, and cleft lip. *Am. J. Med. Genet.* **80**, 196–198.
47. Shaw, G. M., Finnell, R. H., Todoroff, K., Rozen, R., and Lammer, E. J. (1999) Maternal vitamin use, infant C677T mutation in MTHFR and isolated cleft palate risk. *Am. J. Med. Genet.* **85**, 84–85.
48. Wong, W., Eskes, T. K. A. B., Kuijpers-Jagtman, A., Spauwen, P. H. M., Steegers, E. A. P., Thomas, C. M. G., et al. (1999) Nonsyndromic orofacial clefts: association with maternal hyperhomocysteinemia. *Teratology* **60**, 253–257.
49. Mills, J. L., Kirke, P. N., Molloy, A. M., Burke, H., Conley, M. R., Lee, Y. J., et al. (1999) Methylenetetrahydrofolate reductase thermolabile variant and oral clefts. *Am. J. Med. Genet.* **86**, 71–74.
50. Dean, J. C., Moore, S. J., Osborne, A., Howe, J., and Turnpenny, P. D. (1999) Fetal anticonvulsant syndrome and mutation in the maternal MTHFR gene. *Clin. Genet.* **56**, 216–220.
51. Yoo, J. H. and Hong, S. B. (1999) A common mutation in the methylenetetrahydrofolate reductase gene is a determinant of hyperhomocysteinemia in epileptic patients receiving anticonvulsants. *Metabolism* **48**, 1047–1051.
52. James, S. J., Pogribna, M., Pogribny, I. I. , Melnyk, S., Hine, R. J., Gibson, J. B., et al. (1999) Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down Syndrome. *Am. J. Clin. Nutr.* **70**, 495–501.
53. Hobbs, C. A, Sherman, S. L., Yi, P., Hopkins, S. E., Torfs, C. P., Hine, R. J., et al. (2000) Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome. *Am. J. Hum. Genet.* **67**, 623–630.
54. Ismail, L., Sargent, T., Dobson, T. E. L., and Pollycove, M. (1978) Altered metabolism of the methionine methyl group in the leukocytes of patients with schizophrenia. *Biol. Psychiatry* **12**, 649–660.

55. Regland, B., Germgard, T., Gottfries, C. G., Grenfeldt, B., and Koch-Schmidt, A. C. (1997) Homozygous thermolabile methylenetetrahydrofolate reductase in schizophrenia-like psychosis. *J. Neural. Transm.* **104**, 931–941.
56. Joober, R., Benkelfat, C., Lal, S., Bloom, D., Labelle, A., Lalonde, P., et al. (2000) Association between the methylenetetrahydrofolate reductase thermolabile variant and neuroleptic-responsive schizophrenia. *Mol. Psychiatry*. **5**, 323–326.
57. Kapusta, L., Haagmans, M. L. M., Steegers, E. A. P., Cuypers, M. H. M., Blom, H. J., and Eskes, T. K. A. B. Congenital heart defects and maternal derangement of homocysteine metabolism. (1999) *J. Pediatr.* **135**, 773–774.
58. Chen, J., Giovannucci, E., Kelsey, K., Rimm, E. B., Stampfer, M. J., Colditz, G. A., et al. (1996) A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res.* **56**, 4862–4864.
59. Ma, J., Stampfer, M. J., Giovannucci, E., Artigas, C., Hunter, D., Fuchs, C., Willett, W., Selhub, J., Hennekens, C. H., and Rozen, R. (1997) Methylenetetrahydrofolate reductase polymorphism, reduced risk of colorectal cancer and dietary interactions. *Cancer Res.* **57**, 1098–1102.
60. Skibola, C. F., Smith, M. T., Kane, E., Roman, E. Rollinson, S., Cartwright, R. A., and Morgan, G. (1999) Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. *Proc. Natl. Acad. Sci. USA* **96**, 12,810–12,815.
61. van der Put, N. M. Y., Gabreels, F., Stevens, E. M. B., Smeitink, J. A. M., Trijbels, F. J. M., Eskes, T. K. A. B., et al. (1998) A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *Am. J. Hum. Genet.* **62**, 1044–1051.
62. Weisberg, I., Tran, P., Christensen, B., Sibani, S., and Rozen, R. (1998) A second genetic polymorphism in methylenetetrahydofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol. Genet. Metab*. **64**, 169–172.
63. Weisberg, I. S., Jacques, P. F., Selhub, J., Bostom, A. G., Chen, Z., Ellison, R. C., et al. (2001) The 1298A→C polymorphism in methylenetetrahydrofolate reductase (MTHFR): in vitro expression and association with homocysteine, *Atherosclerosis* **56**, 409–415.
64. Leclerc, D., Campeau, E., Goyette, P., Adjalla, C. E., Christensen, B., Ross, M., et al. (1996) Human methionine synthase: cDNA cloning, chromosomal localization, and identification of mutations in patients of the *cbl*G complementation group of folate/cobalamin disorders. *Hum. Mol. Genet.* **5**, 1867–1874.
65. van der Put, N. M. J., van der Molen, E. F., Kluijtmans, L. A. J., Heil, S. G., Trijbels, J. M. F., Eskes, T. K. A. B., et al. (1997) Sequence analysis of the coding region of human methionine synthase: relevance to hyperhomocysteinaemia in neural-tube defects and vascular disease. *Q. J. Med.* **90**, 511–517.
66. Ma, J., Stampfer, M. J., Christensen, B., Giovannucci, E., Hunter, D. J., Chen, J., et al. (1999) A polymorphism of the methionine synthase gene: association with plasma folate, vitamin B12, homocysteine, and colorectal cancer risk. *Cancer Epidemiol. Biomarkers Prevent.* **8**, 85–89.

67. Wilson, A., Platt, R., Wu, Q., Leclerc, D., Christensen, B., Yang, H., et al. (1999) A common variant in methionine synthase reductase combined with low cobalamin (vitamin B_{12}) increases risk for spina bifida. *Mol. Genet. Metab.* **67**, 317–323.
68. Brown, C. A., McKinney, K. Q., Kaufman, J. S., Gravel, R. A., and Rozen, R. (2000) A common polymorphism in methionine synthase reductase increases risk for premature coronary artery disease. *J. Cardiovasc. Risk* **7**, 197–200.
69. Piedrahita, J. A., Oetama, B., Bennett, G. D., van Waes, J., Kamen, B. A., Richardson, J., et al. (1999) Mice lacking the folic acid-binding protein Folbp1 are defective in early embryonic development. *Nature Genet.* **23**, 228–232.

http://www.springer.com/978-0-89603-796-0

Handbook of Neurotoxicology
Volume II
Massaro, E.J. (Ed.)
2002, XVI, 594 p. 32 illus., 11 illus. in color., Hardcover
ISBN: 978-0-89603-796-0
A product of Humana Press