Polymorphisms of Methylenetetrahydrofolate Reductase and Other Enzymes: Metabolic Significance, Risks and Impact on Folate Requirement[1]

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ABSTRACT A common genetic polymorphism results from a C→T substitution in the gene encoding methylenetetrahydrofolate reductase (MTHFR), the enzyme that produces 5-methyltetrahydrofolate (5-methyl-THF) required for the conversion of homocysteine to methionine. In individuals with the T/T genotype (T/T), functional metabolic effects include changes in one-carbon folate derivatives, elevations in plasma homocysteine and differences in response to folic acid supplementation compared with normal (C/C) or heterozygous (C/T) genotypes. The metabolic changes associated with the T/T genotype are postulated to modify risk for chronic disease (e.g., vascular disease and cancer) and neural tube defects (NTD) when accompanied by folate deficiency. The modulation of these metabolic abnormalities by increasing folate intake suggests that folate requirements may be different in affected individuals (T/T) relative to normal (C/C) or heterozygous (C/T) individuals. The complex interaction between this common genetic polymorphism of MTHFR and folate intake is the focus of intense investigation. J. Nutr. 129: 919–922, 1999.

KEY WORDS: • folate • MTHFR polymorphism • homocysteine • vascular disease • cancer • neural tube defects

The interrelationship among genetics, metabolic needs and dietary adequacy is a topic of intense interest in the area of folate nutrition. A major impetus is the clear public health connection, i.e., a rapidly expanding linkage between health and the adequacy of vitamin-dependent one-carbon metabolism. The existence of several common genetic polymorphisms of key enzymes of one-carbon metabolism has been documented in recent years. These common polymorphisms differ from generally more profound inborn errors of metabolism because the mutation may exhibit more subtle effects on metabolism and health. We have recently summarized folate-dependent reactions in one-carbon metabolism and the evidence supporting changes in recommendations for folate intake (Bailey and Gregory 1999). In the current article, we focus on issues of common genetic polymorphisms involving one of the key enzymes in one-carbon metabolism, methylenetetrahydrofolate reductase (MTHFR),[3] and summarize other relevant genetic polymorphisms. The focus of this review is the metabolic impact of the genetic polymorphisms, their possible effects on health or risk of disease and whether nutritional requirements are affected.

Methylenetetrahydrofolate Reductase. MTHFR catalyzes the biologically irreversible reduction of 5,10-methylenetetrahydrofolate (5,10-methylene-THF) to 5-methyltetrahydrofolate (5-methyl-THF), the methyl donor for methionine synthesis from homocysteine (**Fig. 1**). The most common mutation of MTHFR is a C → T substitution at bp 677 that causes a substitution of valine for alanine in the functional enzyme (Frosst et al. 1995). This single substitution can be detected functionally because it reduces the stability of the enzyme during in vitro incubation of cell (i.e., lymphocyte) extracts at 46°C for 5 min (Kang and Wong 1996). This is an autosomal recessive mutation. Individuals who are homozygous for the C677T mutation (i.e., T/T) exhibit lower specific activity of MTHFR in lymphocytes (per mg of total protein) and reduced stability of the enzyme in this in vitro screening procedure; hence it is termed "thermolabile MTHFR." Typical data (from Frosst et al. 1995) are shown in **Figure 2**. Although the full kinetic properties of the human thermolabile MTHFR variant have not been reported, total cellular MTHFR activity is reduced in T/T individuals. Whether this reflects reduced in vivo stability (i.e., lower steady-state level of active MTHFR protein) or another process affecting activity is unclear. Studies with an analogous MTHFR mutant from a bacterial expression system suggest that the thermolabile form of MTHFR may lose activity by facile loss of its flavin adenine dinucleotide (FAD) coenzyme (Matthews et al. 1998).

The frequency of the C677T polymorphism of MTHFR varies among racial and ethnic groups. Analysis of Caucasian and Asian populations typically shows rates of ~12% for homozygous (T/T) and up to 50% for those who are heterozygous (C/T) (Brattstrom et al. 1998, Frosst et al. 1995, Gudnason et al. 1998). African-Americans exhibit very low incidence of T/T genotype (Austin et al. 1997, Franco et al. 1998, McAndrew et al. 1996, Stevenson et al. 1997), whereas European Caucasians exhibit substantial variation (Abbate et al. 1998, Gudnason et al. 1998). The C677T genotype can be determined by a polymerase chain reaction–based procedure (Frosst et al. 1995, Ulvik et al. 1997). The consequences of this polymorphism are discussed below.

Alterations in Folate and One-carbon Metabolism. The actual metabolic consequences of genetic polymorphisms such as the MTHFR C677T mutation can be inferred by analysis of cellular metabolites. Bagley and Selhub (1998) recently reported such evidence, suggesting that the MTHFR (T/T) genotype has significantly reduced the production of 5-methyl-THF, which causes changes in cellular composition of one-carbon folate derivatives. These investigators examined erythrocyte folates from groups of T/T and C/C individuals with the use of an improved HPLC method. As expected, they found that erythrocyte folates of C/C subjects were entirely 5-methyl-THF polyglutamates. However, individuals homozygous for the C677T MTHFR mutation (i.e., T/T) had a mean of 22% formyl-THF polyglutamates in addition to the typical

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[3] Abbreviations used: FAD, flavin adenine dinucleotide; 5,10-methylene-THF, 5,10-methylenetetrahydrofolate; 5-methyl-THF, 5-methyltetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; NTD, neural tube defects; THF, tetrahydrofolate.

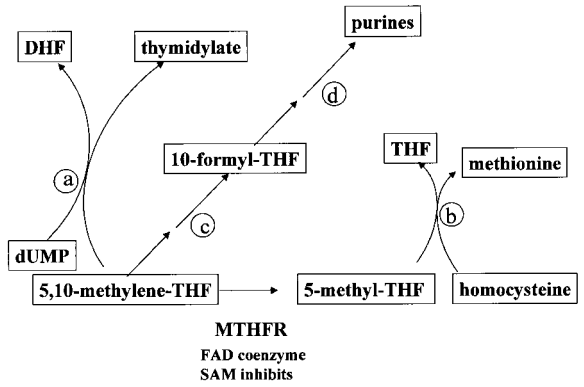

FIGURE 1 Role of methylenetetrahydrofolate reductase (MTHFR) as a key branch point in folate-dependent one-carbon metabolism. MTHFR reduces 5,10-methylene-THF to 5-methyl-THF, a process that commits the one-carbon unit to methylation of homocysteine to form methionine. 5,10-Methylene-THF also functions as a carbon donor in nucleotide synthesis in the thymidylate synthase–catalyzed reaction and indirectly via oxidation to 10-formyl-THF, a carbon donor in two transformylase-catalyzed steps of de novo purine synthesis. Abbreviations: THF, tetrahydrofolate; DHF, dihydrofolate; dUMP, deoxyuridine monophosphate; FAD, flavin adenine dinucleotide; SAM, S-adenosyl methionine. Reactions circled: a, thymidylate synthase; b, methionine synthase; c, sequential reactions of methylenetetrahydrofolate dehydrogenase and methenyltetrahydrofolate cyclohydrolase; d, de novo purine synthesis pathway.

5-methyl-THF derivatives. Existence of formyl-THF species in erythrocytes only from individuals with the thermolabile MTHFR is consistent with the hypothesis that there is in vivo impairment in the MTHFR activity, resulting in an altered distribution of cellular one-carbon units. In view of this altered ratio of erythrocyte formyl/methyl folates caused by the T/T genotype, it is quite likely that other quantitative metabolic changes also will become apparent. Isotopic studies are underway in the authors' laboratories to provide an in vivo assessment of the effect of the C677T mutation.

Many investigators have reported that plasma folate concentrations are significantly lower in individuals with the T/T genotype (Brattstrom et al. 1998, Jacques et al. 1996, Nelen et al. 1998, van der Put et al. 1995). Molloy and co-workers (1997) reported that both plasma and erythrocyte folate concentrations were significantly lower in individuals homozygous for the T/T MTHFR genotype. In contrast, other investigations indicated that erythrocyte folate concentrations were elevated in T/T individuals (Nelen et al. 1998, van der Put et al. 1995). An explanation of these conflicting findings regarding erythrocyte folate can be found in the inherent differences in response of the competitive binding radioassay and microbiological assays used in these studies (Molloy et al. 1998). Comparative studies using more specific analytical methods are needed to resolve this issue.

Additional evidence of functional metabolic effects of the C677T mutation has been reported regarding the response to supplemental folic acid. Differences in plasma homocysteine and folate concentrations in response to low-dose (0.5 mg/d) folic acid supplementation have been detected between the T/T and C/C genotypes (Malinow et al. 1997, Nelen et al. 1998). In the study by Nelen et al. (1998), before supplementation, women with the T/T genotype had the highest fasting plasma homocysteine concentration and the lowest plasma folate concentration compared with the T/C or C/C genotypes. After 2 mo of supplementation, women with the T/T genotype showed the greatest decline in median fasting homocysteine concentrations, resulting in a plasma homocysteine concentration that was not different from that for the other genotypes. The women with the T/T genotype were less responsive to the folic acid supplementation on the basis of the absolute increase in serum folate concentration relative to the other genotypes. The persistence of lower plasma folate concentration in the T/T women apparently reflects the impaired production of 5-methyl-THF, the primary form of plasma folate, by the thermolabile MTHFR variant.

Elevated Plasma Homocysteine and Vascular Disease Risk. A large body of data indicates that individuals with the T/T C677T MTHFR genotype are prone to mildly elevated plasma total homocysteine levels compared with individuals with either the heterozygous C/T or homozygous wild-type C/C genotype (Brattstrom et al. 1998, Gudnason et al. 1998). The association of the T/T MTHFR genotype with elevated plasma homocysteine exists primarily in individuals with poor folate status (Jacques et al. 1996). Several investigators have observed a steeper negative slope in plots of serum folate vs. serum homocysteine concentration in T/T individuals. One hypothesis is that a higher folate status may increase the in vivo stability of the MTHFR enzyme, thus reducing the difference in enzyme activity between T/T and control subjects. Therefore, it is expected that the adverse effects of this polymorphism related to elevated plasma homocysteine concentration would affect primarily people with poorer folate status (Jacques et al. 1996). When folate status is adequate, plasma homocysteine levels are normal and independent of genotype. This observation led to the hypothesis that the intake of folate needed to normalize homocysteine metabolism may be greater in the T/T genotype, which may actually indicate a higher folate requirement for T/T individuals (Rosenberg and Rosenberg 1998).

A small elevation in plasma homocysteine concentration ($>\sim 15$ μmol/L) is considered to be an independent risk factor for a vascular disease, including significantly increased risk of myocardial infarction, stroke, peripheral arterial disease and venous thrombosis (Brattstrom et al. 1998, Refsum et al. 1998). Initial observations by Kang and associates suggested that the thermolabile MTHFR was a risk factor in cardiovascular disease (Kang and Wong 1996). However, in the past several years, many studies have indicated little or no evidence that the T/T C677T MTHFR genotype exhibits greater rates of vascular disease. However, a number of reports linking the T/T genotype with incidence of certain forms of vascular disease in selected populations (beyond the scope of this review) cannot be disregarded.

The widely documented elevations in plasma homocysteine

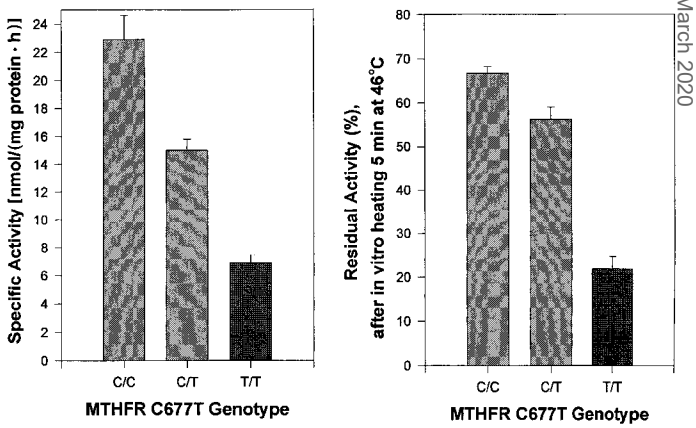

FIGURE 2 Typical effects of the C677T (thermolabile) polymorphism of methylenetetrahydrofolate reductase (MTHFR) on lymphocyte MTHFR specific activity (*left panel*) and in vitro retention of MTHFR activity after 5 min incubation at 46°C. (data adapted from Frosst et al. 1995).

concentration associated with the T/T genotype led to a meta-analysis conducted to explore the risk of cardiovascular disease in the T/T vs. that in C/C genotypes (Brattstrom et al. 1998). The conclusions of this analysis were that although the T/T MTHFR mutation is a major cause of mild hyperhomocysteinemia (~25% higher mean total plasma homocysteine concentration than C/C genotype), the mutation does not increase cardiovascular risk. An example of the discrepancy between cardiovascular risk attributable to homocysteine and the T/T mutation is the U.S. Physicians' Health Study (Stampfer et al. 1992). In this study, the relative risk for myocardial infarction for the highest 5% of the homocysteine distribution (>15.8 μmol/L) vs. the bottom 90% was significant: 3.4. The T/T genotype was present in 21% of hyperhomocysteinemic subjects (>15.8 μmol/L) and in 12% of normohomocysteinemic subjects, and the mean plasma homocysteine was 2.0 μmol/L higher in those with T/T vs. C/C genotypes. In spite of the associated higher homocysteine concentrations, the T/T genotype was found less frequently in patients than in controls (Ma et al. 1997). Further, the T/T genotype was not associated with risk of myocardial infarction. In the Health Professionals Follow-up Study, the T/T genotype was present in 12.2% of men with coronary artery disease or myocardial infarction and 14.2% of male control subjects (Verhoef et al. 1997). These and other findings (e.g.Wilcken et. al 1996) indicate the lack of a direct association between the T/T genotype, which is frequently accompanied by a mild elevation in plasma homocysteine concentration, and risk for cardiovascular disease. Finally, one must ask whether these data regarding homocysteine concentration and risk, irrespective of MTHFR genotype, would eliminate elevated plasma homocysteine as a risk factor. Data from a large European population-based study indicate that plasma homocysteine is positively and strongly associated with major well-recognized risk factors for cardiovascular disease (Nygård et al. 1995), thus providing a possible explanation for the common finding of mild hyperhomocysteinemia in patients who have or will develop vascular disease. Aside from nutritional and genetic factors metabolically governing homocysteine concentration, it is now known that impaired renal function can also be a major determinant of plasma homocysteine level (Refsum et al. 1998). A compelling interpretation of these data has been presented by Refsum and Ueland (1998) who suggested that elevated plasma homocysteine may not necessarily be deleterious, but it could promote vascular blockage under conditions predisposing to vascular disease. Finally, low levels of plasma 5-methyl-THF and whole-blood S-adenosylmethionine, irrespective of homocysteine concentration, are common in coronary heart disease (Loehrer et al. 1996), which suggests that these parameters should be more closely assessed in evaluating genetic and nutritional influences on vascular disease.

Neural Tube Defect Risk. Incidence of neural tube defects (NTD) has been shown to be dramatically responsive to supplemental folic acid taken during the periconceptional period (Scott et al. 1995). Lower blood folate concentration (Daly et al. 1995) was a significant risk factor in NTD-affected pregnancies in a large case-controlled study. The potential role of elevated plasma homocysteine concentrations in the etiology of NTD has been proposed by many investigators, as reviewed previously (Molloy et al. 1998). Several studies have shown that the T/T C677T genotype is a significant risk factor for neural tube defects (Ou et al. 1996, van der Put et al. 1995 and 1997a, Whitehead et al. 1995) and the association would account for ~15% of NTD cases (van der Put et al. 1997). The complexity of this field is illustrated by the opposite conclusions from investigations of NTD and fetal genotype. Mornet et al. (1997) concluded that the C677T MTHFR mutation cannot be regarded as a genetic risk factor for NTD, whereas Ou et al. (1996) reported that genotype was highly associated with risk.

Molloy et al. (1998) found that the proportion of T/T homozygotes was marginally higher ($P = 0.054$) among NTD mothers compared with controls. However, it was concluded that there may be negligible NTD risk attributable to the genotype per se when controlling for plasma homocysteine and low folate status. This conclusion was based on their observation that the T/T genotype in both NTD-affected cases and controls had significantly lower blood folate concentrations and elevated plasma homocysteine concentrations than either the C/C or C/T genotype, which suggests an indirect association of the mutation. These results point to the need to evaluate such possible indirect effects of other genetic polymorphisms.

Cancer. The topic of environmental-genetic linkages with respect to colorectal cancer, with emphasis on the role of C677T MTHFR polymorphism, has been recently reviewed (Chen et al. 1999). Strong interactive effects have been noted between MTHFR genotype, folate status and methionine intake. Chen et al. (1996 and 1998), found a significant inverse relationship between the T/T genotype for MTHFR with the risk for colorectal cancer (i.e., a protective effect) in a case-control study conducted in the Health Professionals Follow-up Study. Similar results were found in participants in the Physicians' Health Study with the T/T genotype in which the risk of colorectal cancer was reduced by 50% relative to that of normal genotypes (Ma et al. 1997). Among men with the T/T genotype for MTHFR and normal plasma folate concentrations, there was a threefold decrease in risk compared with either the C/C or C/T genotype. In contrast, men with the T/T genotype who were folate deficient were at comparable risk for colorectal cancer as the other genotypes. The low folate status appeared to negate the protective effect of the MTHFR mutation. A plausible explanation for this phenomenon is that reduced activity of the thermolabile MTHFR variant could have a positive effect on nucleotide synthesis by increasing the availability of 5,10-methylene-THF required for normal DNA synthesis and cell division. Chen et al. (1996) hypothesized that cells from folate-replete individuals with the T/T genotype may be less prone to insufficiencies in the pools of nucleotide precursors available for DNA synthesis. Folate deficiency has been shown to depress thymidylate synthesis, which increases the misincorporation of uracil and increases the frequency of chromosome breaks in human leukocyte DNA (Blount et al. 1997).

Incidence and Effects of Other Genetic Polymorphisms. Although many mutations have been detected in enzymes of one-carbon metabolism, most are rare relative to the MTHFR polymorphism discussed here. However, several additional genetic polymorphisms merit attention. A second common polymorphism of MTHFR has been shown to involve an A → C substitution at bp 1298, which causes a Glu → Ala substitution in the MTHFR protein (van der Put et al. 1998) with an allele frequency similar to that of the C677T mutation. Initial observations suggest that the A1298C polymorphism is not associated with elevation in plasma homocysteine concentration and exhibits no interaction with plasma folate as is observed with the C677T MTHFR polymorphism. The significance of this polymorphism, if any, requires further investigation.

Evidence of polymorphism also has been reported for methionine synthase (Chen et al. 1997, van der Put et al. 1997b). At least two reasonably prevalent polymorphisms exist. Initial evidence suggests that the D919G mutation, which yields an Asp → Gly substitution in the methionine synthase protein, is probably a benign polymorphism with little or no discernible effect on risk of NTD or vascular disease.

Summary. The complexity of interactions between genetics and nutrition is clearly evident in the case of folate and one-carbon metabolism. The thermolabile variant of MTHFR is associated with reduced activity, impairment of carbon flux to methylate homocysteine and altered partitioning of one-carbon units in favor of nucleotide synthesis. The extent to which this and other polymorphisms alter risk of disease and folate requirements has not yet been fully evaluated. Further analysis of genes encoding the various enzymes involved, coupled with continued assessment of interactions of polymorphisms, nutrition and disease prevalence, is needed. In addition, in vivo measurement of rates of key metabolic processes, as can be accomplished with existing isotopic techniques, will provide a clearer understanding of the actual metabolic effect of genetic and nutritional variables.

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