MTHFR Polymorphism, Methyl-Replete Diets and the Risk of Colorectal Carcinoma and Adenoma among U.S. Men and Women: An Example of Gene-Environment Interactions in Colorectal Tumorigenesis1,2,3

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ABSTRACT Our studies on interactions of a folate-metabolizing gene polymorphism and dietary intake in colorectal tumorigenesis demonstrate the potential importance of studying interactions between genotype and environmental exposure in relation to cancer risk. We observed an inverse association of a polymorphism (667C → T, ala → val) in the methylenetetrahydrofolate reductase (MTHFR) gene with colorectal cancer but not with colorectal adenomas. The inverse association of methionine and adverse association of alcohol with colorectal cancer were stronger among val/val individuals. These interactions were not present in studies of colorectal adenomas. Our studies illustrate that studying gene-environment interactions in relation to cancer can be of importance in clarifying cancer etiology as well as pointing to preventive dietary modifications. J. Nutr. 129: 560S–564S, 1999.

KEY WORDS: • MTHFR polymorphism • colorectal cancer • colorectal adenomas • methyl diet

Colorectal cancer is the second leading cause of cancer death in the U.S. (Silverberg et al. 1990); up to 90% of these malignancies can be attributed to environmental factors, including diet (Doll and Peto 1981). Genes that interact with environmental or dietary exposures may play a large role in colorectal carcinogenesis. The attributable risk for functional polymorphisms in a common nutrient- or carcinogen-metabolizing gene with a relatively modest relative risk (e.g., relative risk of 2.0) may be higher than that of rare mutations in a major proto-oncogene or tumor-suppressor gene with much higher relative risk. In addition, studying gene-environment interactions in relation to risk of cancer may be valuable because positive findings would clearly implicate the substrates with which the gene interacts as disease-causing exposures, clarify cancer etiology and point to preventive dietary and other environmental modifications.

In this paper, we present our studies on interactions of a folate-metabolizing gene polymorphism and dietary intake in colorectal tumorigenesis to illustrate the potential importance of studying interactions between genotype and environmental exposure in relation to cancer risk.

METHYL-REPLETE DIETS AND COLORECTAL CARCINOGENESIS

Direct evidence from two cohort studies, one of men, the other of women, suggests that a methyl-deplete diet (diet that is low in folate and methionine and high in alcohol) is associated with increased risk of colon carcinoma and adenoma (Giovannucci et al. 1993 and 1995). In a prospective study of 47,931 male health professionals in the U.S. (Giovannucci et al. 1995), folate and methionine intakes were inversely associated with risk of colon carcinoma and adenoma (Giovannucci et al. 1993). In a prospective study of 47,931 male health professionals in the U.S. (Giovannucci et al. 1995), folate and methionine intakes were inversely associated with risk of colon carcinoma and adenoma (Giovannucci et al. 1993). In a prospective study of 47,931 male health professionals in the U.S. (Giovannucci et al. 1995), folate and methionine intakes were inversely associated with risk of colon carcinoma and adenoma (Giovannucci et al. 1993). In a prospective study of 47,931 male health professionals in the U.S. (Giovannucci et al. 1995), folate and methionine intakes were inversely associated with risk of colon carcinoma and adenoma (Giovannucci et al. 1993). In a prospective study of 47,931 male health professionals in the U.S. (Giovannucci et al. 1995), folate and methionine intakes were inversely associated with risk of colon carcinoma and adenoma (Giovannucci et al. 1993).
intake yielded more than a threefold increase in risk of colon cancer. A methyl-replete diet was also inversely related to risk of colon adenomas in the same population of male health professionals as well as in another prospective study of 88,756 women (Giovannucci et al. 1993); those in the highest quintile of folate intake had a 35% reduction in risk of adenomas compared with those in the lowest quintile of intake. Those drinking >30 g of alcohol per day (~2 drinks) had a significant 80% elevation in risk compared with those who did not consume alcohol. Combinations of alcohol, folate and methionine were formed to examine the joint influence on risk of adenomas. High intake of alcohol and low intake of folate and methionine constituted a strong risk factor for adenomas, especially adenomas <1 cm, resulting in close to a threefold increase in risk compared with individuals with high intake of folate and methionine and low or no alcohol intake.

Consistent with these findings, other epidemiologic studies have reported that individuals with high folate intake have a reduced risk of colorectal carcinoma and adenoma (Benito et al. 1991 and 1993, Ferraroni et al. 1994, Freudenheim et al. 1991). The majority of studies also indicate an association between alcohol intake and higher risk of colorectal adenoma (Landler et al. 1993) as well as large bowel cancer (Kune and Vitetta 1992), especially cancers in the distal colorectum (Klatsky et al. 1988). Moreover, indirect evidence implicates the benefit of a methyl-replete diet; fruits and vegetables, sources of methionine, are associated with decreased risk of colorectal cancer and adenomas (Giovannucci et al. 1992, Willett et al. 1992).

In light of this evidence indicating a protective effect of methyl-replete diet against colon cancer, it is reasonable to hypothesize that functional polymorphisms in genes associated with methyl metabolism, such as the methylenetetrahydrofolate reductase (MTHFR) gene, may confer differential susceptibility to colorectal cancer.

**METHYLENETETRAHYDROFOLATE REDUCTASE POLYMORPHISM**

Methylenetetrahydrofolate reductase is a critical enzyme regulating the metabolism of folate and methionine, both of which are important factors in DNA methylation and synthesis. MTHFR irreversibly converts 5,10-methylenetetrahydrofolate, the major form of intracellular folate, to 5-methyltetrahydrofolate, the major form of circulating folate in plasma. The former donates its one-carbon moiety to deoxyuridate to synthesize thymidylate, which is the rate-limiting nucleotide in DNA synthesis. The latter is the primary methyl donor for the remethylation of homocysteine to methionine, and subsequently to S-adenosylmethionine (SAM). Declines in SAM level, possibly a result of a methyl-deficient diet, enhance MTHFR activity and direct folate cofactors away from DNA synthesis (Scott and Weir 1981). Severe hyperhomocysteinemia due to MTHFR deficiency causes neurological abnormalities, mental retardation, arteriosclerosis and thrombosis (Rosenblatt 1989). Mild hyperhomocysteinemia is considered to be an independent risk factor for arterial diseases such as myocardial infarction (Stampfer et al. 1992).

A common 677C→T (ala→val) polymorphism, presumably at the folate-binding region of the gene, was found to decrease the activity and enhance the thermostability of the enzyme (Frosl et al. 1995). The variant homzygous genotype is associated with a significant elevation in plasma homocysteine levels as well as a significant decrease in plasma folate levels (Ma et al.1996, van der Put et al.1995). The polymorphism appears to be a risk factor for neural-tube defects (van der Put et al. 1995) and endometrial cancer (Esteller et al. 1997).

**MTHFR-NUTRIENT INTERACTIONS AND RISK OF COLORECTAL CARCINOMA AND ADENOMAS**

We conducted two nested case-control studies within large prospective cohort studies, the Health Professional’s Follow-up Study (HPFS) and the Nurses’ Health Study (NHS), to investigate the association of the MTHFR (ala→val) polymorphism and risk of colorectal carcinoma and adenoma; additional prospective data are available from the Physicians’ Health Study (PHS). The HPFS in men and NHS in women are population-based cohort studies on the association of nutritional and environmental factors and risk of cancer and other chronic diseases; PHS is a randomized trial of aspirin in the reduction of cardiovascular mortality and β-carotene in the reduction of cancer incidence in men. The association of the MTHFR polymorphism with colorectal cancer in HPFS (Chen et al. 1996) and PHS (Ma et al. 1997) as well as colorectal adenomas in NHS (Chen et al. 1998) was investigated.

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**TABLE 1**

**Relation of the MTHFR ala → val polymorphism to colorectal cancer and polyps**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Population</th>
<th>Cases</th>
<th>Controls</th>
<th>RR²</th>
<th>95% CI</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal cancer</td>
<td>HPFS</td>
<td>144</td>
<td>627</td>
<td>0.57</td>
<td>(0.30–1.06)</td>
<td>Chen et al. 1996</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>PHS</td>
<td>202</td>
<td>306</td>
<td>0.46</td>
<td>(0.25–0.84)</td>
<td>Ma et al. 1997</td>
</tr>
<tr>
<td>Colorectal polyps</td>
<td>NHS</td>
<td>257</td>
<td>720</td>
<td>1.37</td>
<td>(0.85–2.19)</td>
<td>Chen et al. 1998</td>
</tr>
</tbody>
</table>

1 Abbreviations used: HPFS, Health Professional’s Follow-up Study; PHS, Physicians’ Health Study; NHS, Nurses’ Health Study.

2 Multivariate-adjusted relative risk comparing val/val to ala/ala and val/val combined.
We did not observe any material association between the MTHFR polymorphism and risk of colorectal adenomas, nor did we observe an interaction between this polymorphism and the consumption of folate, methionine and alcohol. Because of the relatively low val/val genotype frequency and the consumption of folate, methionine and alcohol, the carcinogenic effect of alcohol may be due to an adverse impact on methyl-group metabolism (Barak et al. 1987). On the other hand, folate/methyl deficiency may also interrupt DNA repair, leading to enhanced mutagenesis and apoptosis in hamster cells (James et al. 1994) as well as DNA strand breaks and hypomethylation of p53 in rats (Kim et al. 1997). In a recent study by Blount et al. (1997), folate deficiency in humans resulted in deficient methylation of DNA and subsequent chromosomal breaks.

The mechanism by which the methyl-replete diet exerts an anticarcinogenic effect is not well understood. It has been hypothesized that low methyl diets reduce levels of SAM, which is required for DNA methylation; DNA hypomethylation has been linked with the formation of colon adenomas and cancer (Goelz et al. 1985). The cancer-enhancing effect of alcohol may be due to an adverse impact on methyl-group metabolism (Barak et al. 1987). On the other hand, folate/methyl deficiency may also interrupt DNA repair, leading to enhanced mutagenesis and apoptosis in hamster cells (James et al. 1994) as well as DNA strand breaks and hypomethylation of p53 in rats (Kim et al. 1997). In a recent study by Blount et al. (1997), folate deficiency in humans resulted in deficient methylation of DNA and subsequent chromosomal breaks.

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limited sample size, it is possible that a very modest association could exist but was not detected because of the limited power of the study.

However, the absence of these associations, if confirmed in other studies, seems to suggest that MTHFR may play a role only in a late stage (adenoma → carcinoma) of colorectal tumorigenesis. Even though the observation of a protective association between a methyl-replete diet and risk of colorectal adenomas supports the notion that methylation is an early event in the colorectal tumorigenesis, our data suggest that the MTHFR polymorphism may protect against potential defects in DNA synthesis, which might be more crucial toward a later stage. The protective effect of this polymorphism in DNA synthesis may be abolished once the optimal methylation is challenged. We speculate that as adenomas progress to carcinoma, colorectal epithelial cells divide faster and are more likely to be prone to nucleotide pool imbalances. In particular, dUMP may replace dTMP, the limiting nucleotide for DNA synthesis, and its misincorporation into DNA may result in strand break–induced genomic instability (Blount et al. 1997). This mechanism may be less crucial in the early stage of tumorigenesis when cells divide less often. Another possibility is that the MTHFR val/val polymorphism is protective only in a subset of colorectal adenomas that have potential to progress to malignant tumors. It has been shown that the cumulative incidence of colorectal cancer among patients with an adenoma ≥10 mm was only 10% over 15 y of follow-up (Morson 1984), indicating that only a fraction of adenomas undergo metastatic progression to cancer. If the benefit of the MTHFR val/val genotype were limited to a small subset of premalignant adenomas, then an overall association with adenomas would be difficult to detect.

ADVANTAGES AND LIMITATIONS

The case-control study nested within a prospective cohort offers some advantages in studying gene-environment interaction. First, the control group is derived from the same population in which cases occurred. This minimizes the potential for confounding by race/ethnicity or other colon cancer risk factors. It is especially important in our studies because the frequency of the val/val genotype varies greatly among ethnic and geographic populations (range 0–16%; Stevenson et al. 1984), indicating that only a fraction of adenomas undergo metastatic progression to cancer. If the benefit of the MTHFR val/val genotype were limited to a small subset of premalignant adenomas, then an overall association with adenomas would be difficult to detect.

LITERATURE CITED


