A Polymorphism of the Methionine Synthase Gene: Association with Plasma Folate, Vitamin B_{12}, Homocyst(e)ine, and Colorectal Cancer Risk[1]

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Abstract

We previously reported (J. Chen *et al.*, Cancer Res., *56:* 4862–4864, 1996; J. Ma *et al.*, Cancer Res., *57:* 1098–1102, 1997) that a 5,10-methylenetetrahydrofolate reductase (MTHFR) polymorphism (677C→T, *ala→val*) was associated with lower risk of colorectal cancer. In this study, we examined the relationship of a polymorphism (2756A→G, *asp→gly*) in the gene (*MTR*) for methionine synthase, another important enzyme in the same folate/methionine/homocyst(e)ine metabolic pathway, with risk of colorectal cancer among 356 cases and 476 cancer-free controls. The frequency of the homozygous variant genotype (*gly/gly*) was slightly lower among cases (3%) than controls (5%). The odds ratio for the *gly/gly* genotype was 0.59 [95% confidence interval (CI), 0.27–1.27] compared with those with the homozygous wild type (*asp/asp*). There were no significant differences in plasma levels of folate, vitamin B12, and homocyst(e)ine (tHcy) among the *MTR* genotypes, in contrast to the MTHFR polymorphism. However, similar to the interaction observed for the MTHFR polymorphism among men who consumed less than 1 alcoholic drink/day, those with the *gly/gly* genotype had a lower risk of colorectal cancer with an odds ratio of 0.27 (95% CI, 0.09–0.81) compared with those with the *asp/asp* genotype. The possible association of the *MTR* polymorphism with lower risk of colorectal cancer especially among those with low alcohol consumption, in the same direction as for the MTHFR polymorphism, is intriguing. However, our study had limited statistical power because of the low frequency of the *MTR* variant genotype, which is reflected in the wide CIs. Hence, these findings need to be confirmed in larger populations.

Introduction

Methionine synthase, a vitamin B_{12}-dependent enzyme, plays an important role in folate metabolism (1). It catalyzes the transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine, producing methionine and tetrahydrofolate. Methionine synthase is critical for maintaining adequate intracellular methionine, an essential amino acid and the precursor of SAM.[4] SAM is a crucial methyl group donor involved in over 100 methylation reactions including DNA methylation. Hypomethylation of the promoter regions of proto-oncogenes (2) or hypermethylation of these regions of tumor suppressor genes (3) may cause selective growth and transformation of cells. *De novo* methylation of CpG islands within the promoter regions of growth-regulatory genes, which may inactivate their transcription, is frequently observed in colonic tumors (4). Methionine synthase is also essential for maintaining adequate intracellular folate pools and ensuring that homocysteine concentrations do not reach toxic levels. Severe deficiency of vitamin B_{12} or of methionine synthase causes hypomethioninemia, hyperhomocysteinemia, and homocystinuria (5). It may also result in an accumulation of 5-methyltetrahydrofolate and depletion of intracellular folate derivatives including 5,10-methylenetetrahydrofolate required for thymidylate biosynthesis, the basis of the well-documented "methyl folate trap" leading to deoxynucleotide pool imbalances and megaloblastic anemia (1, 6, 7). This leads to the accumulation of deoxyuridylate in DNA, and removal of this abnormal base may damage DNA, perhaps leading to strand breaks commonly seen in colorectal cancers (7, 8). Pernicious anemia, caused by vitamin B_{12} malabsorption, has been associated with an elevated risk of cancer of the esophagus, stomach, and colon (9, 10). Recently, a polymorphism in the methionine synthase gene (*MTR*; 2756A→G), resulting in the substitution of aspartic acid (D919) by glycine (G), was identified in patients with methionine synthase deficiency and was found to be polymorphic among healthy controls (11).

We previously reported, from the same group of participants (12, 13), that a polymorphism (677C→T) that encodes a

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[4] The abbreviations used are: SAM, *S*-adenosylmethionine; MTHFR, 5,10-methylenetetrahydrofolate reductase; tHcy, homocyst(e)ine; OR, odds ratio; CI, confidence interval; PHS, Physician's Health Study; HPFS, Health Professional Follow-up Study.

Table 1 Frequency of *MTR* genotypes and age-adjusted risk of colorectal cancer by *MTR* genotype among participants of the Physicians' Health Study (PHS) and the Health Professional Follow-up Study (HPFS)

MTR genotype	Cases		Controls		OR	95% CI
	N	(%)	*N*	(%)		
PHS						
asp/asp	145	(68)	235	(68)	1.00	ref.[a]
asp/gly	61	(29)	95	(27)	1.04	0.71–1.53
gly/gly	6	(3)	16	(5)	0.63	0.24–1.64
Total	212		346			
HPFS						
asp/asp	103	(72)	82	(63)	1.00	ref.
asp/gly	37	(26)	42	(32)	0.71	0.42–1.20
gly/gly	4	(3)	6	(5)	0.53	0.14–1.93
Total	144		130			
PHS and HPFS						
asp/asp	248	(70)	317	(66)	1.00	ref.
asp/gly	98	(28)	137	(29)	0.92	0.67–1.25
gly/gly	10	(3)	22	(5)	0.59	0.27–1.27
Total	356		476			

[a] ref., reference.

Table 2 Age-adjusted mean of folate (geometric mean), B_{12}, and homocyst(e)ine by case/control status and genotypes of *MTR* and *MTHFR* among the PHS participants

	Case		Controls	
	N	Mean	*N*	Mean
MTR genotype				
Folate				
asp/asp	126	4.13	218	4.29
gly/asp	53	4.17	87	3.80
gly/gly	6	4.60	14	3.88
B_{12}				
asp/asp	126	452	218	477
gly/asp	53	494	87	476
gly/gly	6	542	14	506
Homocyst(e)ine				
asp/asp	115	12.9	200	12.3
gly/asp	49	11.6	79	11.9
gly/gly	6	12.8	11	11.4
MTHFR genotype				
Folate				
ala/ala	81	4.54	138	4.39
val/ala	81	3.87	116	4.07
val/val	14	3.12[a]	46	3.50[a]
B_{12}				
ala/ala	81	471	138	503
val/ala	81	462	116	468
val/val	14	431	46	440
Homocyst(e)ine				
ala/ala	79	12.0	135	12.2
val/ala	78	12.3	111	11.8
val/val	13	17.6[b]	43	13.1

[a] $P \leq 0.05$.
[b] $P \leq 0.01$ for *val/val* genotype versus *ala/ala* genotype.

thermolabile MTHFR and causes a decrease in plasma 5-methyltetrahydrofolate was associated with a lower risk of colorectal cancer. This protective effect could result from increased availability of intracellular 5,10-methylenetetrahydrofolate and consequent reductions of uracil incorporation into DNA or from decreased SAM levels leading to DNA hypomethylation. Low folate intake or high alcohol consumption (which interferes with folate metabolism) seemed to negate some of the protective effect (12, 13). In the present study nested in two large cohorts, we examined the association of the MTR polymorphism with the risk of colorectal cancer and whether the association differs by plasma levels of folate, vitamin B_{12}, tHcy, or alcohol intake. We hypothesize that, if the variant genotype (*gly/gly*) of this MTR polymorphism is associated with a decreased activity of methionine synthase, men with the *gly/gly* genotype would have lower cellular methionine and folate derivatives, elevated tHcy levels, and an increased risk of colorectal cancer. Alternatively, lower methionine and SAM may lead to DNA hypomethylation, which would modify the cancer risk. For comparison, we also present some results for the MTHFR polymorphism.

Materials and Methods

Study Population. The PHS is a randomized, double-blind, placebo-controlled 2×2 factorial trial of low-dose aspirin and β-carotene among 22,071 predominantly Caucasian-American male physicians, ages 40–84 years. Blood samples were collected at baseline, in 1982, from 14,916 (68%) of the randomized physicians. Alcohol consumption (drinks of beer, wine, or liquor) was ascertained from the baseline questionnaire. The men were subsequently followed for incident cancer through biannual mailed questionnaires. After 13 years of follow-up, 212 cases of colorectal cancer were identified and confirmed by medical records. Three hundred forty-six men who were free from diagnosed cancer at the time of case ascertainment were selected as controls and were matched on age (1 year) and smoking status (never, past, current).

The HPFS is a prospective study of 51,529 predominantly Caucasian-American male health professionals, ages 40–75, enrolled in 1986. Alcohol consumption (drinks of beer, wine, or liquor) was ascertained from a semiquantitative food frequency questionnaire at baseline. Blood samples were collected between 1993 and 1994 from 18,025 participants, among whom 144 had been diagnosed with colorectal cancer between 1986 and 1994; 130 cancer-free men were selected as controls. These cancer cases were also confirmed by medical records. The participants were predominantly Caucasians (over 95% in both the PHS and the HPFS).

***MTR* Genotype and Other Laboratory Assays.** DNA from cases and controls from both studies was extracted and *MTR* genotype was analyzed in Dr. Rozen's laboratory; investigators and laboratory personnel were blinded to case-control status. The presence of the mutation was determined by PCR of genomic DNA, followed by *Hae*III restriction digestion, as previously described (11). Because plasma levels of folate, vitamin B_{12}, and tHcy may be altered by change of diet or treatment after the diagnosis of cancer, these biomarkers were measured only for the PHS participants. Samples from cases and their matched controls were assayed in the same batch to minimize interassay variability, and aliquots from a pool of quality control plasma were inserted randomly. Laboratory personel were unable to distinguish among case, control, and quality control samples. They were also unaware of the genotype status. Plasma levels of folate and vitamin B_{12} were determined using a radioassay kit (Ciba-Corning, Walpole, MA) in the laboratory of the Jean Mayer United States Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA. Total tHcy levels were determined in the same laboratory as described previously(14). The average intra-assay coefficient of variation for folate, vitamin B_{12}, and tHcy were 6.8%, 3.0%, and 2.9%, respectively.

Table 3 Age-adjusted risk of colorectal cancer according to *MTR* and *MTHFR* genotypes and levels of folate and homocyst(e)ine among the PHS participants

	Folate (ng/ml)		Homocyst(e)ine (mg/dl)	
	Tertile 1	Tertile 2 and 3	Tertile 1 and 2	Tertile 3
MTR asp/asp or *gly/asp*				
Number	51/102	128/203	100/186	64/93
OR (95% CI)	0.84 (0.56–1.27)	1.00 (ref)	1.00 (ref)	1.21 (0.81–1.82)
MTR gly/gly				
Number	3/4	3/10	4/7	2/4
OR (95% CI)	1.25 (0.27–5.69)	0.51 (0.14–1.90)	1.10 (0.31–3.90)	1.05 (0.19–5.85)
	$P_{interaction} = 0.22$		$P_{interaction} = 0.37$	
MTHFR ala/ala or *val/ala*				
Number	45/83	117/171	100/167	57/79
OR (95% CI)	0.88 (0.56–1.36)	1.00 (ref)	1.00 (ref)	1.16 (0.76–1.78)
MTHFR val/val				
Number	8/18	6/28	4/25	9/18
OR (95% CI)	0.57 (0.24–1.38)	0.29 (0.12–0.73)	0.25 (0.08–0.74)	0.69 (0.29–1.62)
	$P_{interaction} = 0.16$		$P_{interaction} = 0.24$	

Table 4 Age-adjusted risk of colorectal cancer according to *MTR* genotype and alcohol intake among United States physicians and health professionals in the PHS and HPFS

MTR genotype	Alcohol intake	
	<1 drink/day	≥1 drink/day
asp/asp		
Number	162/210	86/104
OR (95% CI)	1.00 (ref.)	1.04 (0.73–1.49)
gly/asp		
Number	57/86	41/50
OR (95% CI)	0.88 (0.59–1.30)	1.03 (0.64–1.63)
gly/gly		
Number	4/19	6/3
OR (95% CI)	0.27 (0.09–0.81)	2.64 (0.65–10.82)
	$P_{trend} = 0.04$	$P_{trend} = 0.52$
	$P_{interaction} = 0.04$	

Statistical Analyses. We examined the age-adjusted OR and 95% CI for the association of the *MTR* genotype with the risk of developing colorectal cancer in the PHS and HPFS separately and combined. We conducted unconditional logistic regression analysis because of the combined study population. Using conditional logistic analysis yielded similar results when the matched case-control subgroup was examined. Prospective data on blood nutrients levels were availible only for the PHS colorectal cancer cases and controls. The age-adjusted geometric mean of plasma folate (because of the skewed distribution), mean vitamin B_{12}, and tHcy concentration within strata of the *MTR* and *MTHFR* genotypes by case-control status were calculated by analysis of covariance. In the PHS, we assessed the age-adjusted ORs for the joint effect of the *MTR* and *MTHFR* genotypes and status of plasma folate, vitamin B_{12}, and tHcy (categorized into two groups based on control tertile distribution, lower one-third *versus* upper two-thirds for folate and vitamin B_{12}, lower two-thirds *versus* upper one-third for tHcy) using an indicator variable for each category in logistic regression models. Because alcohol consumption was measured prospectively in both the PHS and the HPFS, we assessed the joint effect of the *MTR* genotype and alcohol consumption (<1 drink/day, and ≥1 drink/day) within the PHS as well as the PHS and the HPFS combined. We compared the log likelihood statistics of the main effect model with the joint effect model to assess interaction. Because of small numbers in some of the stratified analyses, we used an exact statistic method (LogXact; Ref. 15) and obtained virtually identical results; we, therefore, present all of the results from unconditional logistic regression analyses. All of the *P*s are two-sided, and all of the analyses were done using SAS (16).

Results

The overall frequency of the homozygous variant *gly/gly* genotype was 5% among controls and 3% among cases (Table 1). The *gly* allele frequencies were 0.19 among controls and 0.17 among cases, not materially different from the 0.15 reported by Leclerc *et al.* (11) and the 0.16 reported by van der Put *et al.* (17). The genotype and allele frequencies were similar in the two cohorts. Overall, there was a nonsignificant inverse association of the *gly/gly* genotype with risk of colorectal cancer (OR, 0.59; 95% CI, 0.27–1.27) compared with the *asp/asp* genotype (Table 1).

Plasma folate, tHcy, and B_{12} were measured only among the PHS participants. There were no apparent associations between the MTR homozygous variant *gly/gly* genotype and plasma levels of folate and tHcy (Table 2). Similar to our previous observation using the microbiological method for folate measurement (13), the MTHFR *val/val* genotype was significantly associated with lower plasma folate levels determined by the radioassay method, among both cases and controls (Table 2). The observation that the MTHFR homozygous variant genotype *val/val* was significantly associated with lower plasma folate levels is also consistent with findings from a separate case-control study of myocardial infarction nested in the PHS (18). In the present study, we also measured plasma vitamin B_{12} and tHcy levels and observed significantly higher tHcy levels among cases with the MTHFR *val/val* genotype (Table 2). Although men with the MTR homozygous variant *gly/gly* genotype had higher levels of vitamin B_{12} and men with the MTHFR *val/val* genotype had lower levels, none of these differences were statistically significant.

We examined the association of the *MTR* genotype and the risk of colorectal cancer according to plasma levels of folate, vitamin B_{12}, and tHcy in comparison with the *MTHFR* genotype. Because of the low prevalence of the *gly/gly* genotype, we categorized plasma folate, vitamin B_{12}, and tHcy into two groups based on the distribution among controls. For the *MTHFR* genotype, as we previously reported (13), there was a significant 70% decrease (OR, 0.29; 95% CI, 0.12–0.73) in risk

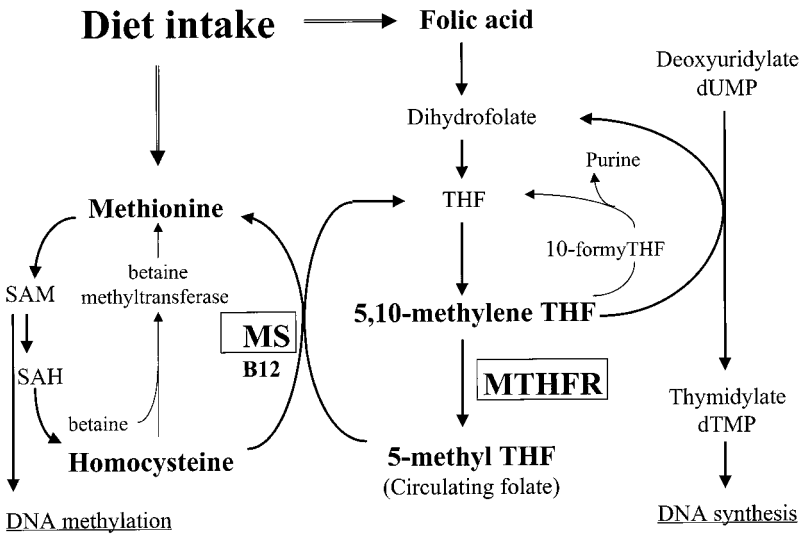

Fig. 1. Role of methionine synthase (*MS*) and *MTHFR* in folate/homocyst(e)ine/methionine metabolism.

among men with the *val/val* genotype and with plasma folate levels in the upper two tertiles of the control distribution compared with those with the *ala/ala* or *val/ala* genotype in the same folate category (Table 3). In the present analysis, we found that men with the homozygous *val/val* genotype and plasma tHcy levels in the lower two tertiles also had a significant 75% decrease (OR, 0.25; 95% CI, 0.08–0.74) in risk (Table 3). For the *MTR* genotype, we observed a nonsignificant 50% decrease (OR, 0.51; 95% CI, 0.14–1.90) in risk among men with the *gly/gly* genotype and with plasma folate levels in the upper two tertiles compared with those with the *gly/asp* or *asp/asp* genotype in the same folate category (Table 3). However, there was no apparent interaction between the *MTR* genotype and plasma tHcy (Table 3) on the risk of colorectal cancer. No apparent interactions were observed between vitamin B_{12} and both genotypes (data not shown). Adjustment for body mass index, alcohol intake, multivitamin intake, and aspirin assignment did not change the results.

Alcohol consumption was assessed at baseline in both the PHS (1982) and the HPFS (1986). We examined the alcohol-genotype interaction within the PHS cohort as well as combined with the HPFS participants. Similar to our previous observation of the interaction between the *MTHFR* genotype and alcohol intake on risk (12, 13), we found a significant interaction between the *MTR* genotype and alcohol intake ($P_{interaction} < 0.01$) in the PHS. Among men with alcohol intake less than 1 drink/day, those with the *gly/gly* genotype had an OR of 0.10 (95% CI, 0.01–0.81), those with the *gly/asp* genotype had an OR of 0.70 (95% CI, 0.44–1.12) compared with those with the *asp/asp* genotype. Compared with the same reference group, among men with alcohol intake one drink/day or more, those with the *gly/gly* genotype had an OR of 3.79 (95% CI, 0.71–20.22), those with the *gly/asp* genotype had an OR of 1.14 (95% CI, 0.61–2.13). The combined PHS and HPFS data showed a similar association ($P_{interaction} = 0.04$; Table 4). Excluding men with MTHFR *val/val* genotype ($n = 50$) or further adjusting for body mass index, multivitamin intake, and aspirin assignment yielded similar results.

Discussion

Although the 2756A→G polymorphism of MTR was first identified among patients with a deficiency of methionine synthase and among normal controls, the biological impact of this polymorphism is unknown (11). This A→G substitution at bp 2756 causes a substitution of glycine for aspartic acid (D919G). D919 corresponds to Q893 in the cobalamin-dependent *Escherichia coli* methionine synthase. In this highly homologous bacterial enzyme, this residue is at the penultimate position in a long helix that leads out of the cobalamin domain to the SAM-binding domain (19). It has been postulated that the glycine residue, a strong helix breaker compared with aspartic acid, could affect the secondary structure of the protein and, therefore, have functional consequences, perhaps leading to altered levels of vitamin B_{12}, folate, or tHcy (17). However, our observation that the MTR polymorphism was not associated with plasma levels of folate, vitamin B_{12}, or tHcy suggests that this aspartic acid-to-glycine change may not significantly deteriorate methionine synthase activity. In a recent Dutch study of patients and mothers of children with neural tube defects, patients with arterial disease, and population-based controls, the MTR polymorphism was also not associated with plasma tHcy levels (17). One alternative explanation is that homocysteine may be remethylated to methionine through an alternative pathway by betaine-homocysteine methyltransferase (Fig. 1; 20). Although the main physiological role of that enzyme is to catabolize excess betaine (21), it also participates in regulating tissue levels of methionine and in removing excess homocysteine during stress (22). Under normal conditions, the activity of this enzyme increases substantially as a result of (*a*) inadequate methionine intake (23); (*b*) inactivation of methionine synthase by nitrous oxide (24); (*c*) impaired methionine synthase due to ethanol administration (25); or possibly (*d*) being in the presence of the variant *MTR* genotype.

Overall, we found that the MTR *gly/gly* genotype was associated with a nonsignificant 40% decrease in risk of colorectal cancer, in the same direction as we previous reported from the same participants for the MTHFR polymorphism (12, 13). The decreased risk was consistent among the PHS and the HPFS participants analyzed separately, but the CIs were wide, reflecting the small number of participants carrying the *gly/gly* genotype. Decreased activity of MTR or MTHFR would be expected to lower SAM levels leading to decreased DNA methylation. DNA hypomethylation has been suggested to suppress tumor growth (26, 27). Alternatively, because the activ-

ities of MTHFR and methionine synthase were significantly increased in tumor-bearing animals because of increased methylation reactions for tumor growth (28–32), these polymorphisms may inhibit the excessive use of methionine by tumor cells. This inhibition probably acts on a late stage of tumorigenesis because neither the MTHFR nor the MTR polymorphisms are associated with risk of colorectal adenoma, the immediate precursor of colorectal cancer (33).

Because plasma folate levels reflect both genetic and dietary variation, and ethanol can interfere with folate and methyl group metabolism as well as methionine synthase activity, moderate enzymatic changes due to genetic polymorphisms may behave differently according to the intake of alcohol (12, 13). The interactions between the *MTR* genotype and alcohol intake are consistent with what we observed for the *MTHFR* genotype. The increased risk for colorectal cancer conferred by high alcohol intake may overcome the protective effect of these polymorphisms (12, 13). Also, the influence of alcohol on cancer risk may differ among individuals with different genetic susceptibility. Among 10 cases and 21 controls who had the MTR variant genotype *gly/gly* in our analysis, men reporting one or more drinks/day had about a 10-fold higher risk than those who drank less. Although we observed similar results within the two cohorts as well as in the combined analysis, our study has limited statistical power for both main effect and stratified analyses because of the low prevalence of the variant *MTR* genotype, which is reflected in the wide CIs. The possible association of this *MTR* genotype with the risk of colorectal cancer, especially among individuals with low alcohol intake, merits further study in larger populations.

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