

A Methylene tetrahydrofolate Reductase Polymorphism and the Risk of Colorectal Cancer¹

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Abstract

We examined the relationship of a common polymorphism (⁶⁶⁷C→T) of the *methylene tetrahydrofolate reductase (MTHFR)* gene with the risk of colorectal cancer in a case-control study conducted in the Health Professionals Follow-up Study. MTHFR genotypes were ascertained from blood samples among 144 men previously diagnosed with colorectal cancer and 627 controls. The adjusted odds ratio (OR) for the MTHFR variant homozygous (*val/val*) genotype was 0.57 [95% confidence interval (CI), 0.30-1.06]. High dietary intake of methionine (OR, 0.27; 95% CI, 0.06-1.20) and low consumption of alcohol (OR, 0.11; 95% CI, 0.01-0.85) were associated with reduced incidence of colorectal cancer. Alcohol intake was a stronger risk factor among men with the *val/val* genotype (*P*, trend = 0.01), and consumption of five or more alcoholic drinks per week abolished the reduced risk of colorectal cancer among *val/val* individuals (*P*, interaction = 0.02). The inverse association of methionine with colorectal cancer risk was slightly stronger among individuals with the MTHFR *val/val* genotype. These data suggest that dietary methyl supply is particularly critical among MTHFR *val/val* individuals. When dietary methyl supply is high, MTHFR *val/val* individuals may be at reduced risk of colorectal cancer probably because higher levels of 5,10-methylene tetrahydrofolate may prevent imbalances of nucleotide pools during DNA synthesis. In contrast, when 5-methyltetrahydrofolate is depleted by alcohol consumption, *val/val* individuals may be less able to compensate, leading to potentially oncogenic alterations in DNA methylation.

Introduction

Risk of developing colorectal cancer has been linked to diets that are low in the methyl donors folate and methionine and high in alcohol, a methyl group antagonist (1). Dietary methyl group availability may influence cancer risk by altering DNA methylation or by influencing the rate of DNA mutation. Selective growth and transformation of cells can result from DNA hypomethylation of proto-oncogenes (2) or hypermethylation of tumor suppressor genes (3) in their promoter regions. In contrast to these mechanisms, in which aberrant DNA methylation influences gene expression, the mutation-mediated hypothesis proposes that the oncogenic process is influenced by a disproportionately high rate of CpG→TpG transitions, such as those frequently observed in the *p53* gene in colorectal tumors (4), potentially due to deamination of 5-methylcytosine. Finally, methyl-deficient diets may cause imbalances in the pools of nucleotide precursors leading to DNA strand breaks and mutations (5, 6).

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MTHFR³ is a critical enzyme regulating the metabolism of folate and methionine (Fig. 1), both of which are important factors in DNA methylation and synthesis. MTHFR irreversibly converts 5,10-methylene tetrahydrofolate to 5-methyltetrahydrofolate, the primary methyl donor for the remethylation of homocysteine to methionine. A common ⁶⁷⁷C→T (*Ala*→*Val*) mutation of the gene was found to enhance the thermolability of the enzyme (7), and the variant homozygous genotype is associated with elevation in plasma homocysteine levels (7), decrease in plasma 5-methyltetrahydrofolate levels (8), as well as risk of spina bifida (9).

We hypothesized that men who inherited the MTHFR homozygous variant genotype may respond differently to the methyl content of their diets. We designed a case-control study within a prospective cohort to study the association between colorectal cancer and the MTHFR polymorphism.

Materials and Methods

Subjects. HPFS is a prospective study of 51,529 predominantly Caucasian-American male health professionals ages 40-75 enrolled in 1986. Participants completed a validated semiquantitative food frequency questionnaire at baseline and were subsequently monitored for incident cancer through self-report on mailed questionnaires; cases of cancer were confirmed by obtaining medical records.

Among 18,025 men who gave a blood sample between 1993 and 1994, the 144 men who had been diagnosed with colorectal cancer between 1986 and 1994 were the cases in this analysis; 627 participants who had not been diagnosed with colorectal cancer were selected as controls. We calculated ORs and 95% CIs for the association of the MTHFR genotype with colorectal cancer using unconditional logistic regression. We also used unconditional logistic regression to estimate the associations of folate, methionine, and alcohol consumption (categorized into three groups based on the distribution in controls) with colorectal cancer, as well as to assess whether the associations of alcohol and these nutrients with colorectal cancer differ according to MTHFR genotype.

MTHFR Genotype. There are three MTHFR genotypes: variant homozygotes (*val/val*), variant heterozygotes (*val/ala*), and wild-type homozygotes (*ala/ala*). Genotyping for MTHFR was carried out using a modification of the PCR-RFLP method of Frosst *et al.* (7). In brief, two primers were designed from the cDNA sequence to generate a 198-bp fragment. The primer sequences are: 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' and 5'-AGGACGGTGCG-GTCAGAGTG-3'.

Amplification was performed using initial denaturation at 95°C for 2 min followed by 29 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s with a final extension at 72°C for 10 min. The buffer for PCR reaction contained 20 mM Tris (pH 8.8), 10 mM (NH₄)₂SO₄, 10 mM KCl, 7.5 mM MgSO₄, and 0.1% Triton X. Laboratory personnel were blind to case-control status, and blinded quality control samples were included. Because of the overlapping activity between *val/ala* and *ala/ala* genotype, and the fact that plasma homocysteine levels are only slightly higher among *val/ala* heterozygotes than *ala/ala*

³ The abbreviations used are: MTHFR, methylene tetrahydrofolate reductase; HPFS, Health Professionals Follow-up Study; OR, odds ratio; CI, confidence interval.

Table 2 Relationship of methionine, alcohol, and folate to colorectal cancer risk stratified by MTHFR genotype among 144 cases and 627 controls in the HPFS

Genotype		Alcohol		
		Low ^a	Medium	High
<i>val/ala</i> and <i>ala/ala</i>	Cases	48	49	34
	Controls	196	247	100
	<i>P</i> , trend = 0.36			
	OR ^b (95% CI)	1.00 (ref.)	0.82 (0.53–1.28)	1.35 (0.82–2.24)
<i>val/val</i>	Cases	1	4	8
	Controls	35	29	20
	<i>P</i> , trend = 0.01			
	OR (95% CI)	0.11 (0.01–0.85)	0.55 (0.18–1.64)	1.56 (0.65–3.81)
			<i>P</i> , interaction = 0.02	
		Methionine		
		Low	Medium	High
<i>val/ala</i> and <i>ala/ala</i>	Cases	49	45	37
	Controls	182	174	187
	<i>P</i> , trend = 0.16			
	OR (95% CI)	1.00 (ref.)	0.94 (0.59–1.49)	0.70 (0.43–1.13)
<i>val/val</i>	Cases	8	2	3
	Controls	29	29	26
	<i>P</i> , trend = 0.09			
	OR (95% CI)	0.95 (0.41–2.15)	0.37 (0.11–1.27)	0.27 (0.06–1.20)
			<i>P</i> , interaction = 0.23	
		Folate		
		Low	Medium	High
<i>val/ala</i> and <i>ala/ala</i>	Cases	44	46	41
	Controls	178	179	186
	<i>P</i> , trend = 0.56			
	OR (95% CI)	1.00 (ref.)	0.98 (0.62–1.57)	0.86 (0.54–1.39)
<i>val/val</i>	Cases	6	4	3
	Controls	32	28	24
	<i>P</i> , trend = 0.23			
	OR (95% CI)	0.73 (0.29–1.89)	0.56 (0.19–1.68)	0.44 (0.13–1.55)
			<i>P</i> , interaction = 0.64	

^a The cutoff points for low and high categories are: alcohol: ≤ 1 drink/week, ≥ 5 drinks/week; methionine: ≤ 1.89 g/day, ≥ 2.22 g/day; folate: ≤ 317 mg/day, ≥ 461 mg/day.

^b Age-adjusted OR.

anism of colorectal tumorigenesis (1), and the benefit of MTHFR *val/val* genotype is offset by a methyl-deficient diet. Our findings suggest that multiple pathways, affecting methylation as well as DNA synthesis, are involved in the relation of methyl group metabolism and colorectal cancer, and individuals differ in their response to their dietary methyl supply, in part, due to inherent variability in MTHFR activity.

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References

- Giovannucci, E., Rimm, E. B., Ascherio, A., Stampfer, M. J., Colditz, G. A., and Willett, W. C. Alcohol, low-methionine-low-folate diets, and risk of colon cancer in men. *J. Natl. Cancer Inst.*, 87: 265–273, 1995.
- Fearon, E. R., and Vogelstein, B. A. A genetic model for colorectal tumorigenesis. *Cell*, 61: 759–767, 1990.
- Issa, J.-P., Ottaviano, Y. L., Celano, P., Hamilton, S. R., Davidson, N. E., and Baylin, S. B. Methylation of the oestrogen receptor CpG island links aging and neoplasia in human colon. *Nat. Genet.*, 7: 536–540, 1994.
- Greenblatt, M. S., Bennett, W. P., Hollstein, M., and Harris, C. C. Mutations in the *p53* tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res.*, 54: 4855–4878, 1994.
- James, S. J., Basnakian, A. G., and Miller, B. J. *In vitro* folate deficiency induces deoxynucleotide pool imbalance, apoptosis, and mutagenesis in Chinese hamster ovary cells. *Cancer Res.*, 54: 5075–5080, 1994.
- Blount, B. C., and Ames, B. N. DNA damage in folate deficiency. *Baillieres Clin. Haematol.*, 8: 461–478, 1995.
- Frosst, P., Blom, H. J., Milos, P., Goyette, P., Sheppard, C. A., Matthews, R. G., Boers, G. J. H., den Heijer, M., Kluijtmans, L. A. J., van der Heuvel, L. P., and Rozen, R. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat. Genet.*, 10: 111–113, 1995.
- Ma, J., Stampfer, M. J., Selhub, J., Malinow, M. R., Willett, W. C., Hennekens, C. H., and Rozen, R. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. *Circulation*, in press.
- 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., Mariman, E. C. M., den Heijer, M., Rozen, R., and Blom, H. J. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. *Lancet*, 346: 1070–1071, 1995.
- Shaw, S., Jayatilake, E., and Herbert, V. Cleavage of folates during ethanol metabolism: role of acetaldehyde/xanthine oxidase-generated superoxide. *Biochem. J.*, 257: 277–280, 1989.

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