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Advances in Brief

A Methylenetetrahydrofolate Reductase Polymorphism and the Risk of Colorectal Cancer[1]

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Abstract

We examined the relationship of a common polymorphism ($^{667}C\rightarrow T$) of the *methylenetetrahydrofolate 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-methylenetetrahydrofolate 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 disproportionally 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).

MTHFR[3] 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-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary methyl donor for the remethylation of homocysteine to methionine. A common $^{677}C\rightarrow T$ (*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 $(NH_4)_2SO_4$, 10 mM KCl, 7.5 mM $MgSO_4$, 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*

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

homozygotes, but substantially higher among *val/val* homozygotes (9), we combined the *val/ala* and *ala/ala* genotypes into a single reference category.

Results

The frequencies of *val/val*, *val/ala*, and *ala/ala* genotypes among the controls were 13.4, 41.9, and 44.6%, respectively (Table 1). The frequency of *val/val* genotype among the cases (9.0%) was lower compared with the controls; the age-adjusted OR for this genotype was 0.62 (95% CI, 0.33–1.15; Table 1). After adjustment for age, family history, and intakes of folate, methionine, and alcohol, the OR was 0.57 (95% CI, 0.30–1.06; Table 1). The ORs were similar among men with and without a family history of colorectal cancer. Intakes of folate, methionine, and alcohol were not substantially correlated with one another (Pearson correlation coefficients all <0.18).

Consistent with data from the overall cohort (1), consumption of alcohol, a methyl antagonist, was positively associated with risk of colorectal cancer in this study (P, trend = 0.08). Men who consumed five drinks or more per week were at significantly elevated risk (OR, 1.61; 95% CI, 1.01–2.58) compared with men who consumed one drink or less per week. We observed a significant interaction (P, interaction = 0.02) between alcohol consumption and MTHFR genotype. The positive association between alcohol consumption and colorectal cancer was stronger among *val/val* (P, trend = 0.01) than *val/ala* or *ala/ala* (P, trend = 0.36) individuals. Men who consumed one drink or less per week and who had *val/val* genotype were at a significantly lower risk of developing colorectal cancer (OR, 0.11; 95% CI, 0.01–0.85) compared with those who had *val/ala* or *ala/ala* genotype (Table 2); consumption of five or more drinks per week abolished the reduced risk among men with the *val/val* genotype. Men consuming more methionine were at a reduced risk of developing colorectal cancer (OR, 0.27; 95% CI, 0.06–1.20). This inverse association was slightly stronger among individuals with *val/val* genotype compared with those with *val/ala* or *ala/ala* genotype (OR, 0.70; 95% CI, 0.43–1.13), although the test for interaction was not statistically significant (P, interaction = 0.23). Folate consumption was nonsignificantly inversely associated with colorectal cancer (P, trend = 0.47), and we did not observe any interaction between folate intake and MTHFR genotype (P, interaction = 0.64; Table 2).

Table 1 *Relationship of MTHFR genotype to colorectal cancer in a case-control study nested in the HPFS*

	Cases		Controls		Age-adjusted	Multivariate
Genotype	*n*	%	*n*	%	OR (95% CI)	OR[a] (95% CI)
val/val	13	(9.0)	84	(13.4)	0.62 (0.33–1.15)	0.57 (0.30–1.06)
val/ala	64	(44.4)	263	(41.9)		
ala/ala	67	(46.5)	280	(44.6)	1.00[b]	1.00[b]
Total	144		627			

[a] Adjusted for age, family history, and intakes of folate, methionine, and alcohol.
[b] Combined genotype *val/ala* and *ala/ala* is the reference category.

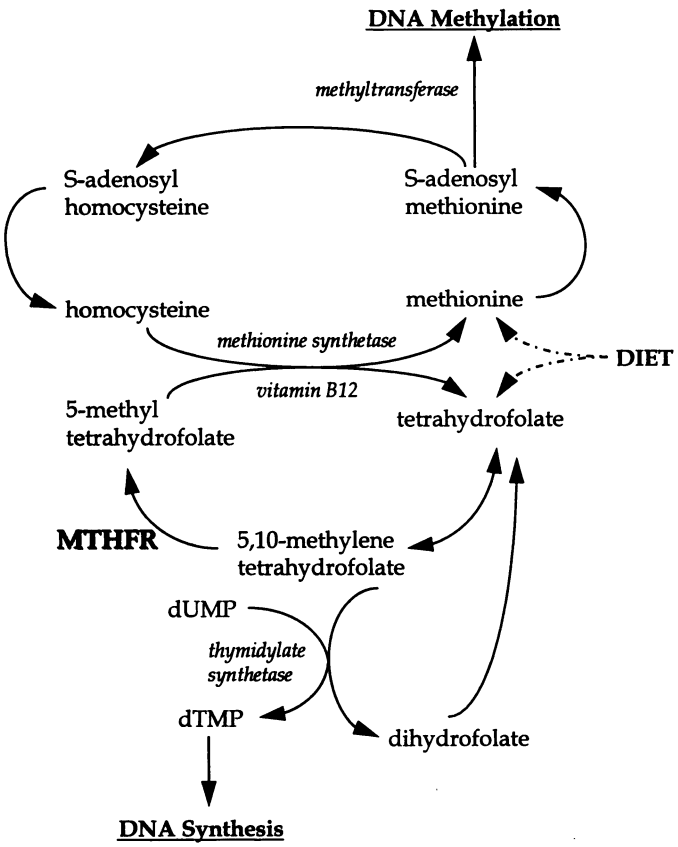

Fig. 1. The metabolic role of MTHFR in folate metabolism involving DNA methylation and DNA synthesis.

Discussion

Among *val/val* individuals, the MTHFR enzyme is less efficient in converting 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, thus potentially preventing depletion of 5,10-methylenetetrahydrofolate, a cofactor for *de novo* synthesis of nucleotides necessary for DNA synthesis, especially dTMP (Fig. 1). As a result, cells may be less prone to "dTMP stress," which has been shown to promote cancer-associated genetic alterations (5) due to alterations in the pool of nucleotide precursors available for DNA synthesis. Alteration in these precursor pools induced by methyl (folate) deficiency significantly increases the uracil content and the frequency of chromosome breaks in human leukocyte DNA (6).

MTHFR *val/val* individuals have lower levels of plasma 5-methyltetrahydrofolate (8). Thus, alcohol, which depletes 5-methyltetrahydrofolate (10), would be predicted to be particularly disadvantageous among *val/val* men. This is consistent with the abolition of the protective association with the *val/val* genotype by increasing alcohol intake. A slightly stronger, statistically significant inverse association of the *val/val* genotype with colorectal cancer and a similar abolition of this effect among alcohol consumers have been observed in a nested case-control study in the Physicians' Health Study.[4] As low levels of 5-methyltetrahydrofolate probably result in lower cellular methionine and *S*-adenosylmethionine levels, potentially leading to aberrant DNA methylation, high methionine intake may be particularly beneficial among *val/val* men, consistent with the nonsignificant, stronger inverse trend with methionine intake we observed among *val/val* men. The lack of association between folate intake and colorectal cancer in this study may reflect the fact that the HPFS cohort is a relatively health-conscious population; very few men are folate deficient, and we had reduced power in this smaller case-control study to detect an association observed between colorectal cancer and folate intake in the larger cohort study.

This study suggests that the relationship of the MTHFR genotype with colorectal cancer may depend on the methyl supply of the diet. When the methyl supply is replete, MTHFR *val/val* individuals may be at reduced risk due to a reduction in nucleotide pool imbalance. When there is a shortage of methyl groups (*i.e.*, low methionine or high alcohol), abnormal methylation may become the primary mech-

[4] J. Ma, personal communication.

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