Trans-HHS Workshop:
Diet, DNA Methylation Processes and Health

Epidemiologic Studies of Folate and Colorectal Neoplasia: a Review[1]

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ABSTRACT Dietary folate influences DNA methylation, synthesis and repair. Aberrations in these DNA processes may enhance carcinogenesis, particularly in rapidly proliferative tissues such as the colorectal mucosa. DNA methylation abnormalities may influence the expression of cancer-related genes, and inadequate levels of folate may lead to uracil misincorporation into DNA and to chromosomal breaks. Folate deficiency enhances intestinal carcinogenesis in several animal models. An increasing number of epidemiologic studies indicate that higher intakes of folate either from dietary sources or from supplements may lower the risk of colorectal adenoma and cancer. More limited data also suggest that dietary methionine, which might also influence methylation, may have a similar protective role. High alcohol consumption, which has a strong antifolate effect, also has been related to higher risk of colorectal neoplasia. The deleterious effects of alcohol are accentuated when folate or methionine intake is low. Some evidence also suggests that the risk of colorectal neoplasia may vary according to genetic polymorphisms in methylenetetrahydrofolate reductase, an enzyme that is involved in folate metabolism. The cumulative data indicate that maintaining adequate folate levels may be important in lowering risk of colorectal cancer. J. Nutr. 132: 2350S–2355S, 2002.

KEY WORDS: • *epidemiology* • *folate* • *colorectal neoplasia* • *diet*

Potentially, folate may reduce carcinogenesis through various mechanisms, including the maintenance of normal DNA synthesis and DNA methylation. As shown in **Figure 1**, 5-methyltetrahydrofolate is essential for DNA methylation, and folate is required in the form of 5,10-methylenetetrahydrofolate to convert deoxyuridylate into thymidylate. The latter reaction is required for DNA synthesis and repair. When levels of 5,10-methylenetetrahydrofolate are inadequate, misincorporation of uracil for thymidine during DNA synthesis occurs at a markedly elevated rate (1). Folate deficiency is related to massive incorporation of uracil into human DNA and to increased frequency of chromosomal breaks (2). These genomic aberrations are normalized following supplementation with folic acid.

Dietary methionine is also important. When cellular concentration of methionine is sufficiently low, *S*-adenosylmethionine decreases and *S*-adenosylhomocysteine increases, stimulating methylenetetrahydrofolate reductase (MTHFR[3]; EC 1.5.1.20) to convert 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (Fig. 1). 5-Methyltetrahydrofolate provides the methyl group to methylate homocysteine to form methionine. If compensatory production of methionine is hindered by insufficient 5-methyltetrahydrofolate, cellular methylation reactions may be affected. DNA methylation of specific cytosine guanine dinucleotide residue sites may be one mechanism that reduces gene expression, probably by modulating the interaction between the promoter sites of genes and the transcription machinery (3). In principle both hypo- and hypermethylation of DNA, which can cause either over- or underexpression of genes, respectively, may contribute to carcinogenesis. DNA methylation also is an important determinant of the conformational configuration and structural stability of DNA (4).

These DNA abnormalities provide plausible carcinogenic mechanisms for folate deficiency. DNA methylation aberra-

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[3] Abbreviations used: C677T, cytosine-to-thymine transition at position 677; CI, confidence interval; CT, cytosine/thymine genotype; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; RR, relative risk; TT, thymine thymine genotype.

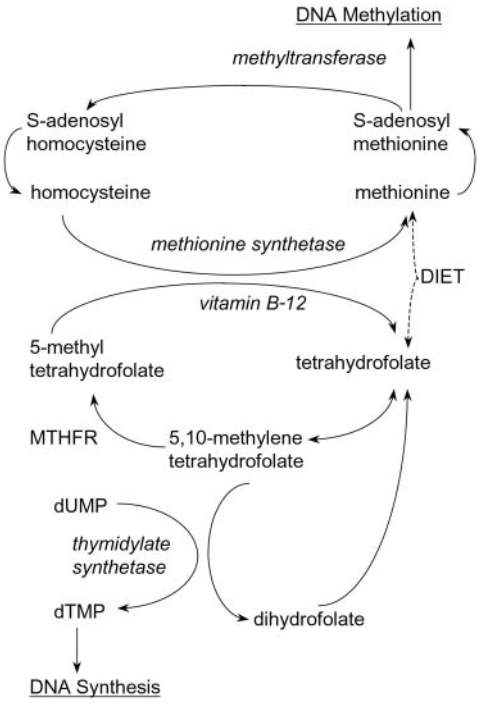

FIGURE 1 Competing pathways in folate metabolism. MTHFR, 5,10-methylenetetrahydrofolate reductase.

tions, including global hypomethylation, elevated DNA methyltransferase activity and regional hypermethylation (4), observed in human colon carcinogenesis, are strikingly similar to those observed in rats fed a diet deficient in methyl donor or transfer factors (folate, vitamin B-12, methionine and choline) (5). These animals eventually develop tumors, supporting direct carcinogenic effects of diets deficient in methyl donor or transfer factors.

In the dimethylhydrazine model in rats, a moderate dietary deficiency of folate increased incidence of colonic dysplasia and carcinoma (6). Increasing dietary folate up to four times the basal requirement led to a progressive reduction in the evolution of macroscopic neoplasms from microscopic foci, although this benefit appeared to be attenuated at higher intakes (4). In another murine carcinogenesis model based on a heterozygous mutation in the adenomatous polyposis coli (*APC*) gene and a null mutation in the DNA mismatch repair (*MSH2*) gene ($APC^{+/-}$ $MSH2^{-/-}$), dietary folate protected against neoplastic foci in the small intestine and colon if it was provided before the establishment of neoplastic foci but appeared to have opposite effects if provided at later carcinogenic stages (7). Thus, animal models indicate that folate deficiency may be especially relevant during early carcinogenic stages.

Folate and colorectal adenoma risk

Most human colorectal cancers arise from adenomas, which generally precede the malignancies by one or several decades. Adenomas allow us the opportunity to examine risk factors that act early in the carcinogenic cascade. Low dietary folate has been related consistently to an increased occurrence of colorectal adenoma (**Table 1**) (8–12). In one of these reports (10), risk of colonic adenomas was examined in two prospective cohorts, the Health Professionals Follow-Up Study and the Nurses' Health Study. Comparing high to low quintiles of folate intake, the relative risk (RR) was 0.66 for adenoma in women and 0.63 in men, adjusting for age, family history of colorectal cancer, total energy, saturated fat, dietary fiber and body mass index. Additional control for intakes of α-carotene and vitamins C, D and E, did not alter the benefit of folate. A subsequent analysis of various components of vegetables and fruits in the Health Professionals Follow-up Study showed only soluble fiber and vegetables high in folate independently related to lower risk of adenoma (13).

These findings have been replicated in several other studies. In a multivariate analysis of colonoscopy patients in the University of North Carolina (11), folate was the micronutrient that showed the strongest inverse association with adenoma in women [odds ratio (OR) = 0.35, controlling for many vitamins]. However, in men the OR was only 0.84, and a stronger inverse association was observed for vitamin E. In a sigmoidoscopy-based study in Southern California (9), a 30–40% lower risk was observed with higher folate intake in men; however, no relationship was seen in women. In a study in France (12), an inverse association was observed with folate intakes [OR = 0.5; 95% confidence interval (CI) = 0.3–1.0 in men, and OR = 0.5; 95% CI = 0.3–1.0 in women, between high and low quintiles]. A study in Majorca, Spain, which used a general population control group, found an OR of 0.27 for colorectal adenoma comparing low and high quartiles of folate intake (14).

One case-control study (15) did not find an overall difference between intakes of folate and risk of colorectal adenoma, but among individuals bearing the thymine thymine (*TT*) genotype for *MTHFR*, those with low folate intake were at twofold increased risk compared with those with high folate intakes. A study of recurrent adenomas (16) indicated a modest inverse relationship with higher folate intakes, but this did not persist in multivariate models. However, high alcohol and low folate combined was associated with an increased risk of recurrent adenoma. The relevance of MTHFR and alcohol is discussed below.

Only a few studies have compared blood levels of folate in individuals found to have an adenoma at endoscopy to endoscopied individuals without adenoma. The same endoscopy-based study from Southern California described above found that for red blood cell folate levels 160 μg/L or more, compared with lower levels, the OR of colon adenoma was 0.76 overall and 0.53 in men (9). This result was statistically

TABLE 1

Summary of the studies of folate and risk of colorectal adenoma

Study	Country	Year	RR[1]	
Benito et al. (14)	Spain	1993	0.27*	
Giovannucci et al. (10)	U.S.	1993	0.66*	Women
Giovannucci et al. (10)	U.S.	1993	0.63*	Men
Bird et al. (9)	U.S.	1995	0.76	Men/women (diet)
			0.53*	Men (red blood cell)
Boutron-Ruault et al. (12)	France	1996	0.50*	Women/men
Tseng et al. (11)	U.S.	1996	0.35*	Women
			0.84	Men

[1] Relative risk for high vs. low category for folate in each study.
* Results were statistically significant ($P \leq 0.05$).

significant only in men, paralleling the findings for folate intake. A smaller study of 112 individuals in Greece found that the mean erythrocyte folate level in patients with colonic adenoma was 536 μg/L in cases and 743.8 μg/L in controls ($P < 0.01$). Serum folate levels were also slightly lower in cases than controls (17).

Folate and colorectal cancer risk

Both case-control and prospective cohort studies have examined folate intake in relation to colorectal cancer risk (**Table 2**). Six case-control studies have reported on this association. In Spain (8) men and women with higher folate intake had a multivariate OR of 0.56 relative to those with lower intake; these results were similar for rectal and colon cancer and for men and women. In western New York state (18) higher folate intake was associated with a lower OR for rectal cancer in women (OR = 0.50) and men (OR = 0.31), and for colon cancer in women (OR = 0.69) but not men (OR = 1.03). In an Italian study (19) high folate intake was related to a lower risk of colon (OR = 0.55) and rectal cancer (OR = 0.75); however, similar if not stronger associations were observed for β-carotene and ascorbic acid, making it difficult to determine whether folate had an independent effect. In a case-control study conducted in Washington state (20), folate was inversely related to colon cancer risk in women (OR = 0.54), although the OR was attenuated to 0.73 when adjusted for fiber intake. No relationship was seen in men. In this study population, daily multivitamin use averaged over a 10-y period was related to a lower risk of colon cancer in men (OR = 0.55) and women (OR = 0.43) (overall OR = 0.49) (21). Folic acid supplementation for a decade was also related to about a halving of the risk. Almost all of supplemental folic acid comes from multivitamin supplements, so it is possible that other components of multivitamins accounted for this association. However, although vitamin E supplementation also was related inversely to risk, supplemental vitamin A, vitamin C and calcium were unrelated to risk.

In a large United States case-control study (22), women who consumed more folate and vitamin B-6 were at lower risk for colon cancer than women who consumed less of these nutrients after adjustment for total energy intake, body mass index, physical activity, smoking and calcium. However, further adjustment for dietary fiber slightly altered these associations as follows for the upper relative to lower quintile of intake: folate (dietary fiber unadjusted) for all women, OR = 0.8 (95% CI = 0.6–1.1), and for women < 67 y, OR = 0.7 (95% CI = 0.4–1.0); and adjusted for fiber, OR = 0.9 (95% CI = 0.6–1.4) and for women < 67 y, OR = 0.7 (95% CI = 0.4–1.2). Associations were stronger for cancers of the distal colon, even adjusting for fiber (for all women, OR = 0.7; 95% CI = 0.5–1.1). Similar but weaker trends existed for men.

A French study (12), which found an inverse association with higher folate intakes and adenomas (see above), did not find an association with colorectal cancer risk (OR = 1.0; 95% CI = 0.5–2.0, between high and low quintiles).

Evidence from five prospective studies provides information on this relation; four of the studies had dietary information (23–26). In the Alpha-Tocopherol Beta-Carotene Study cohort of male Finnish smokers, men in the low quartile of dietary folate had approximately double the risk of colon cancer, although a monotonic gradient was not observed. However, no association was observed for rectal cancer or between baseline plasma folate and risk of colorectal cancer (23). In the Health Professionals Follow-Up Study, total folate was not appreciably related to risk of colon cancer; but for 10 y or more of multivitamin use, there was a suggestive inverse association (RR = 0.74; 95% CI = 0.47–1.17) (24). In the Nurses' Health Study (25), women who consumed >400 μg/d folate had a multivariate RR of 0.69 (95% CI = 0.52–0.93) relative to those consuming <200 μg/d. Also women who had used multivitamins containing folic acid for at least 15 y had a 75% reduction in risk for colon cancer, controlling for known and suspected risk factors for colon cancer. In data based on the National Health and Nutrition Examination Survey I Epidemiologic Follow-Up Study, dietary folate was significantly inversely associated with colon cancer risk in men (RR = 0.40; 95% CI = 0.18–0.88), although the association did not reach statistical significance in women (26).

Another study based on United States male physicians (27), which did not have comprehensive dietary data, showed an inverse association between plasma folate and risk of colon cancer, particularly in men with the *TT* genotype for *MTHFR* (see below). In the New York University Women's Health Study cohort (28), prediagnostic serum folate was inversely related to risk of colorectal cancer (OR = 0.52; 95% CI = 0.27–0.97; *P* (trend) = 0.04 for high versus low quartile of serum folate).

The optimal dose of folate to minimize risk of colorectal cancer is not established. Preliminary evidence based on pooled results from nine prospective studies suggests that intakes of ~400–500 μg/d may be required to minimize risk (29). Higher intake may not confer additional benefits.

TABLE 2

Studies of folate intake and colorectal cancer risk

Study	Country	Year	Study type[1]	RR[2]	Site	
Freudenheim et al. (18)	U.S.	1991	CC	0.50	Rectal	Women
				0.31[3]	Rectal	Men
				0.69	Colon	Women
				1.03	Colon	Men
Benito et al. (14)	Spain	1993	CC	0.56[3]		
White et al. (21)	U.S.	1993	CC	0.54		Women[4]
				Null		Men[4]
Ferraroni et al. (19)	Italy	1994	CC	0.55[3]	Colon	
				0.75	Rectal	
Giovannucci et al. (24)	U.S.	1995	P	0.74		Men
Boutron-Ruault et al. (12)	France	1996	CC	1.0		
Glynn et al. (23)	Finland	1996	P	0.51		Men
				0.94		
Slattery et al. (22)	U.S.	1997	CC	0.80		Women
				Null		Men
Giovannucci et al. (25)	U.S.	1998	P	0.69[3]		Women
Kato et al. (28)	U.S.	1999	P	0.52[3]		Women[5]
Su & Arab (26)	U.S.	2001	P	0.40[3]		Men
				0.74		Women

[1] CC, case-control study; P, prospective study.
[2] Relative risk (RR) for high vs. low category for folate.
[3] Results were statistically significant ($P \leq 0.05$).
[4] Multivitamins 10 + y; odds ratio (OR) = 0.43 men, 0.55 women (statistically significant).
[5] Plasma based; other studies dietary based.

Potential interacting dietary factors: methionine and alcohol

Methionine. Dietary methionine could be beneficial either through DNA methylation or by sparing folate required for other pathways (DNA synthesis, repair) (Fig. 1). Dietary methionine has rarely been evaluated in epidemiologic studies, but dietary protein tends to be correlated highly with methionine. Most prospective studies of colorectal cancer or adenoma have reported either significant or nonsignificant inverse associations with one or more groups of high protein foods (e.g., poultry, fish) or with total protein (30). However, this relationship may be complex because red meat has often been related to a higher risk, possibly through other mechanisms (31). Only a few studies have reported directly on methionine intake; the results are mixed, either supporting an inverse association (10,24,25) or not (19,22). Most important have been studies that have examined methionine intakes concurrently with folate and alcohol status (as discussed in the next section).

Alcohol. The acute and chronic effects of alcohol on folate metabolism have been reviewed in depth by Hillman and Steinberg (32) and are summarized briefly here. In rapidly proliferating tissues such as the bone marrow, alcohol impairs hematopoiesis causing megaloblastic anemia as well as neutropenia and thrombocytopenia. Alcohol accelerates the induction of megaloblastic anemia in subjects fed low folate diet and can prevent the hematologic response to folic acid in subjects with folate-deficient anemia (32). Suppression of the hematologic response to folate by alcohol can be overcome with larger doses of folate (32). Impairment of erythropoiesis by alcohol is at least partly related to blockage of release of folate from the hepatocyte. In addition the alcohol metabolite acetaldehyde may inactivate methyltetrahydrofolate (33) or inhibit methionine synthase (5-methyltetrahydrofolate–homocysteine *S*-methyltransferase; EC 2.1.1.13) (34), which may trap folate as 5-methyltetrahydrofolate, depleting 5,10-methylenetetrahydrofolate. Moreover, cellular depletion of overall folate occurs because 5-methyltetrahydrofolate is a poor substrate for polyglutamation, which is required to retain folate in cells. Additionally, chronic high intakes of alcohol can deplete folate stores by causing malabsorption (32).

Alcohol may have specific adverse effects in the highly proliferative colonic mucosa. The colonic bacteria are capable of oxidizing ethanol in the colon to produce substantial levels of acetaldehyde at ethanol concentrations that typically occur in the colonic mucosa in alcohol drinkers (35). In rats ethanol consumption increases the concentration of acetaldehyde in the colonic mucosa while decreasing the colonic mucosal folate level by 48% (36). In the rat colonic mucosa, chronic alcohol consumption induces DNA hypomethylation (37).

In one animal study, the carcinogenicity of methyl-deficient diets is enhanced by ethanol (38), suggesting that a cancer-enhancing influence of alcohol is because of its adverse effect on methyl-group metabolism (39,40). An association between alcohol intake and colon cancer risk has been observed in many ecologic, cohort and population-based case-control studies of colorectal cancer, and alcohol has been consistently related to higher risk of colorectal adenoma (30). The association is less consistent in case-control studies that used hospital-based controls, but these studies may be biased because alcohol intake is related to many conditions requiring hospitalization. Almost all of the epidemiologic studies of folate and colorectal adenoma or cancer that also contained data on alcohol found alcohol to be associated independently with higher risk (8–12,14,16,18,20,23,24,26,28).

TABLE 3

Studies of methyl poor versus methyl rich diets and colorectal cancer or adenoma

Study[1]	RR[2]	95% CI	
Freudenheim et al., 1991 (18)	5.07	2.17–11.9	Men
	1.28	NS	Women
Giovannucci et al., 1993 (A) (10)	3.17	1.69–5.75	Men/women
Giovannucci et al., 1995 (24)	3.3	1.58–6.88	Men
Glynn et al., 1996 (23)	4.79	1.36–16.9	Men
Giovannucci et al., 1998 (25)	4.0	$P < 0.01$	Women
Baron et al., 1998 (A) (16)	1.85	1.15–2.97	Men/women
Kato et al., 1999 (28)	1.99	0.92–4.29	Women
Su & Arab, 2001 (26)	2.67	1.16–6.16	Men
	1.20	0.61–2.36	Women

[1] A, adenoma study; CI, confidence interval.
[2] Relative risk (RR) for high alcohol/low folate versus low alcohol/high folate (studies by Giovannucci et al. and Su et al. also considered methionine).

Combinations of alcohol, folate and methionine. If a methyl-deficient diet enhances colorectal carcinogenesis, specific dietary combinations might be particularly deleterious. **Table 3** summarizes results of studies of colorectal cancer or adenoma that have compared "methyl-poor" to "methyl-rich" diets based on combinations of alcohol and folate and sometimes methionine. For example, diets high in alcohol and low in folate (and methionine) are considered "methyl-poor," whereas diets low in alcohol and high in folate (and methionine) are considered "methyl-rich." In general, individuals with methyl-poor diets are at markedly higher risk of colorectal adenoma compared with those with methyl-rich diets (Table 3). In a large case-control study, compared with a low risk diet based on alcohol, methionine and folate intake, those fed a high risk diet had approximately twice the risk of colorectal cancer (estimated from data presented separately by *MTHFR* genotypes) (41).

Genetic susceptibility

Methylenetetrahydrofolate reductase catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (see Fig. 1). The activity of this enzyme is upregulated when cellular methionine level is low and homocysteine level is high (42). A polymorphism of the *MTHFR* gene [cytosine-to-thymine transition at position 677 (C677T); alanine-to-valine], which correlates with reduced enzyme activity (43), has an allele prevalence of approximately 35% in Caucasians. The C677T substitution may result in a relative deficiency of methionine (44), and homozygotes for this allele (TT) have genomic hypomethylation in peripheral leukocytes (45). Because either methyltetrahydrofolate or methylenetetrahydrofolate may influence colorectal neoplasia, it is possible that polymorphic forms of *MTHFR* are differentially related to risk of colorectal adenoma or cancer, and this risk could be modified by levels of folate and other methyl factors.

Three studies to date have reported on interactions between folate, alcohol and sometimes methionine and risk of colorectal cancer (27,41,46), and 3 have reported on adenoma risk (15,47,48). The data are inconclusive thus far, possibly because relatively few individuals possess the TT genotype (~11%) but suggest several patterns. Among individuals with the cytosine cytosine or cytosine thymine genotypes, only

modest associations are observed between dietary methyl-related factors and risk of colorectal cancer (**Table 4**) or adenoma (**Table 5**). Also, individuals with the TT genotype appear to be at relatively low risk (compared with those with cytosine cytosine or cytosine thymine genotypes) if they have a low risk diet (high folate or methionine, low alcohol), but no apparent protection if they have a high risk diet. This pattern is most striking for alcohol, for which statistically significant interactions with *MTHFR* are observed in three of the six studies (27,46,48). Although further study is required to reach firmer conclusions, the apparent relationship between a functional polymorphism of a specific folate-metabolizing gene and colon cancer risk provides independent evidence of a role for folate.

Various lines of evidence summarized here strongly suggest that folate has a role in colorectal cancer prevention. Folate is critical for both DNA synthesis and DNA methylation, and various mechanisms have been hypothesized through which folate may influence carcinogenesis. Moderate folate deficiency enhances carcinogenicity in animal models. The majority of epidemiologic studies suggest a moderately lower risk of colorectal cancer or adenoma associated with higher folate intakes. No studies suggest an increased risk. Because currently insufficient data from randomized studies address the relationship between folate and colorectal neoplasia, the evidence for a causal association must be considered carefully from the epidemiologic data.

The most important consideration regarding the causality of the association is the potential of confounding by other protective factors that are associated with folate intake. Although confounding cannot be entirely excluded, particularly since the magnitude of the relative risk is modest, several facts argue against this. Both dietary and supplemental folate appear protective, and when controlling for the most plausible confounders (other vitamins, fiber, other dietary and lifestyle variables) in many of the studies, folate remained inversely associated with risk. Also the findings in diverse populations in multiple countries argue against confounding. The evidence supporting a role for folate is more convincing when other factors that influence methylation reactions interactively with folate status are considered. Low methyl diets defined by various combinations of high alcohol and low folate (and methionine) have consistently yielded two- to fivefold higher risks of colorectal adenoma or cancer in comparison with high methyl diets in nine populations that assessed this. Relative risks of this magnitude are unlikely to be caused entirely by residual confounding. Further suggesting a causal role for folate is the apparent association with *MTHFR* polymorphisms, which is unlikely to be caused by confounding by the same factors that may be correlated with folate intake. Given the high incidence rates of colorectal cancer, the potential beneficial role of folate should be evaluated further.

TABLE 4

Studies of interactions between dietary methyl group related factors and polymorphisms in MTHRF in relation to risk of colorectal cancer

	Genotype			
	CC or CC/CT		TT	
	Low-risk diet	High-risk diet	Low-risk diet	High-risk diet
Chen et al. (46)				
Folate	0.86[1] (0.54–1.39)	1.0 —	0.44 (0.13–1.53)	0.73 (0.29–1.89)
Alcohol	1.0 —	1.35 (0.82–2.24)	0.11 (0.01–0.85)	1.56 (0.65–3.81)
Methionine	0.70 (0.43–1.13)	1.0 —	0.27 (0.06–1.2)	0.95 (0.41–2.15)
Ma et al. (27)				
Folate	1.0 —	1.49 (0.76–2.94)	0.32 (0.15–0.68)	1.33 (0.34–5.17)
Alcohol	1.0 —	0.72 (0.37–1.42)	0.12 (0.03–0.57)	1.31 (0.48–3.58)
Slattery et al.(41)				
Folate	0.8 (0.6–1.2)	1.0 —	0.6 (0.4–1.0)	0.8 (0.5–1.3)
Alcohol	1.0 —	0.8 (0.6–1.1)	1.0 (0.7–1.4)	1.0 (0.6–1.6)
Methionine	0.8 (0.6–1.0)	1.0 —	0.8 (0.5–1.3)	0.9 (0.5–1.4)
Alcohol/Folate/ Methionine	0.6 (0.4–1.0)	1.0 —	0.4 (0.1–0.9)	1.0 (0.4–2.4)

[1] CC, cytosine cytosine genotype; CC/CT, cytosine cytosine genotype/cytosine thymine genotype; MTHFR, methylenetetrahydrofolate reductase; TT, thymine thymine genotype.
[2] Relative risk or odds ratio (95% confidence interval).

TABLE 5

Studies of interactions between dietary methyl group related factors and polymorphisms in MTHRF in relation to risk of colorectal adenoma

	Genotype			
	CC or CC/CT		TT	
	Low-risk diet	High-risk diet	Low-risk diet	High-risk diet
Chen et al. (47)				
Folate	0.78[1] (0.53–1.15)	1.0 —	1.31 (0.58–2.96)	0.99 (0.45–2.16)
Alcohol	1.0 —	1.03 (0.71–1.49)	2.43 (1.14–5.19)	1.91 (0.76–4.84)
Methionine	0.74 (0.50–1.09)	1.0 —	1.03 (0.38–2.78)	1.36 (0.66–2.82)
Ulrich et al. (15)				
Folate	1.0 —	0.9 (0.5–1.4)	0.7 (0.3–1.3)	1.5 (0.6–3.5)
Alcohol	1.0 —	1.9 (1.2–3.0)	1.6 (0.9–2.9)	0.8 (0.3–1.8)
Methionine	1.0 —	0.9 (0.5–1.8)	0.6 (0.3–1.3)	1.4 (0.6–3.3)
Levine et al. (48)				
Folate	0.8 (0.51–1.28)	1.0 —	0.8 (0.33–1.78)	2.04 (0.6–6.56)
Alcohol	1.0 —	1.26 (0.89–1.78)	0.69 (0.35–1.35)	3.13 (1.34–7.34)
Methionine	1.17 (0.81–1.7)	1.0 —	1.8 (0.47–2.48)	1.03 (0.46–2.27)

[1] CC, cytosine cytosine genotype; CC/CT, cytosine cytosine genotype/cytosine thymine genotype; MTHFR, methylenetetrahydrofolate reductase; TT, thymine thymine genotype.
[2] Relative risk or odds ratio (95% confidence interval).

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