Methylenetetrahydrofolate Reductase, Alcohol Dehydrogenase, Diet, and Risk of Colorectal Adenomas

Abstract

An increased occurrence of colorectal cancer and its adenoma precursor is observed among individuals with low intakes or circulating levels of folate, especially if alcohol intake is high, although results have not been statistically significant in all studies. We examined folate and alcohol intake and genetic polymorphisms in methylenetetrahydrofolate reductase [MTHFR 667→T (ala→val) and MTHFR 1298A→C (gln→ala)] (associated with reduced MTHFR activity) and in alcohol dehydrogenase 3 [ADH3 (2-2) associated with decreased alcohol catabolism] in relation to risk of colorectal adenoma in the Health Professionals Follow-Up Study. Among 379 cases and 726 controls, MTHFR genotypes were not appreciably related to risk of adenoma, but a suggestive interaction (P = 0.09) was observed between MTHFR 677C→T and alcohol intake; men with TT homozygotes who consumed 30+ g/day of alcohol had an odds ratio (OR) of 3.52 [95% confidence interval (CI), 1.41–8.78] relative to drinkers of ≤5 g/day with the CC/CT genotypes. ADH3 genotype alone was not appreciably related to risk, but its influence was modified by alcohol intake. Compared with fast alcohol catabolizers [ADH3(1-1)] with low intakes of alcohol (≤5 g/day), high consumers of alcohol (30+ g/day) had a marked increase in risk if they had the genotype associated with slow catabolism [ADH3(2-2); OR, 2.94; 95% CI, 1.24–6.92] or intermediate catabolism [ADH3(1-2)] of alcohol (OR, 1.83; 95% CI, 1.03–3.26) but not if they were fast catabolizers [ADH3(1-1); OR = 1.27; 95% CI = 0.63–2.53]. In addition, an increased risk of colorectal adenoma (OR, 17.1; 95% CI, 2.1–137) was observed for those with the ADH3(2-2) genotype and high alcohol-low folate intake compared with those with low alcohol-high folate intake and the ADH3(1-1) genotype (P for interaction = 0.006).

Our results indicate that high intake of alcohol is associated with an increased risk of colorectal adenoma, particularly among MTHFR 677TT and ADH3(2-2) homozygotes. The findings that alcohol interacts with a folate-related gene (MTHFR) and that the interaction between alcohol and ADH3 is stronger among those with low folate intake support the hypothesis that the carcinogenic influence of alcohol in the large bowel is mediated through folate status.

Introduction

An increased occurrence of colorectal adenomas, precursors of colorectal cancer, is observed fairly consistently among individuals with low intakes (1–5) or circulating levels of folate (2, 6). In case-control and cohort studies of folate intake and levels and colorectal cancer risk, inverse associations were seen in most (1, 7–18), although several studies provided equivocal results (5, 19) or were supportive (20, 21). In addition, almost all of these studies found higher alcohol intake, which antagonizes folate (22), is associated with higher risk of colorectal neoplasia (1–5, 7, 9, 11, 12, 14, 17, 23, 24). The higher risk associated with lower folate or higher alcohol has not always been statistically significant and has been relatively moderate in magnitude. However, a 2–5-fold elevation in colorectal cancer or adenoma risk is relatively consistently observed among individuals with high intake of alcohol and low intake of folate relative to those with low alcohol and high folate intakes (3, 7, 11–14, 17, 18, 24, 25), although some studies do not show this (20, 21). The association has been less clear for women (7, 14, 17, 20, 21) but has been statistically significant in essentially every study that included men (3, 24, 25) or examined men separately (7, 11, 12, 14), possibly because of higher alcohol consumption in men.

The specific mechanisms whereby folate and alcohol influence colorectal carcinogenesis are unclear, but folate is required in the form of 5-methyl-THF to produce methionine required for DNA methylation and as 5,10-methylene THF to convert dUMP into dTMP, a relatively consistently observed ingredient for DNA synthesis (Fig. 1). Various experiments indicate that deficiency of folate in either form, leading to abnormalities in DNA synthesis (and repair) or methylation, could enhance carcino-
Fig. 1. Competing pathways in folate metabolism. MTHFR = 5,10-methylene THF reductase.
ical activity, and diet. We updated exposure and medical history information every 2 years. Blood samples were provided by 18,018 cohort members from 1993 to 1995. The study was approved by the Institutional Review Boards of the Harvard School of Public Health and Brigham and Women’s Hospital.

**Assessment of Diet.** A semiquantitative food-frequency questionnaire, described in detail previously (48), was administered in 1986, as well as every 4 years thereafter. The questionnaires contained a list of ~130 food and beverage items, each with a specified commonly used unit or portion size, and an open-ended section for unlisted foods. Each participant reported how often, on average, over the past year, he consumed the specified amount of each item and reported on brand of breakfast cereal, and brand, duration, and frequency of vitamin supplements. Nutrient intakes were based on composition values from United States Department of Agriculture sources, supplemented with other data.

The mean correlation coefficients between intakes determined by two 1-week diet records and the dietary questionnaire (adjusting for week-to-week variation in the diet records) among a sample of 127 cohort members were 0.65 for nutrients (adjusting for week-to-week variation in the diet records) mined by two 1-week diet records and the dietary questionnaire, described in detail previously (48), was administered to the 17 men who had at least two drinks (~30 g) of alcohol daily, who all had erythrocyte folate levels at or below the median, although their folate intake was comparable with the other men (3).

**Identification of Adenoma Cases and Controls.** Methods for identifying and confirming adenoma cases have been described in detail previously (3). Briefly, when a participant reported a diagnosis of colorectal polyps on the follow-up questionnaire, we asked him for permission to access the relevant medical records. All cases of adenomatous polyps in this analysis were identified to be cases, so the final analysis included 379 cases and 727 controls. Among the 379 cases, 79 had at least one adenoma in the proximal colon, 225 in the distal colon, and 95 in the rectum [Note: these numbers sum to >379 because an individual could have an adenoma(s) in more than one subsite.]

For the cases, we identified 148 as having large or tubulovillous/villous adenomas.

**MTHFR and ADH1 Genotypes.** Genotyping for MTHFR 677C→T polymorphism was carried out based on methods described by Chen et al. (30). In brief, two primers are designed from the cDNA sequence to generate a 198-bp fragment. The primer sequences are: 5'-TGAAGGACAGTTGTGTGTCGGGAA-3' and 5'-AGGACGGGTCGGGAGGTG-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 included 20 mM Tris (pH 8.8), 10 mM (NH4)2SO4, 10 mM KCl, 7.5 mM MgSO4, and 0.1% Triton X-100. The PCR products were digested with Hinnl and size fractionated on 6% polyacrylamide gels. For genotyping for the MTHFR 1298A→C polymorphism, two primers were designed from the cDNA sequence to generate a 138-bp fragment (38). The primer sequences are: 5’-GGGGAGCTGACCTAGTGCGAG-3’ and 5’-GGGGCTAGGCGAGGCGGCAG-3’. The PCR products were digested with Fnu4HI into 119- and 19-bp fragments and size fractionated on 6% polyacrylamide gels.

Genotyping for ADH1 was carried out using a PCR-RFLP-based method modified from that of Freudenheim et al. (50). In brief, primers with the sequences 5’-GGCTAAGAAGTTCTCACCTGATGC-3’ and 5’-ACCCCTTCTCCAGGCGAGC-3’ were used in PCR reactions. Genomic DNA was predigested with NalIII for 1.5 h and then amplified with AmpliTaq Gold, using initial denaturation at 95°C for 9 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s with a final extension at 72°C for 10 min. PCR products were digested with SstI and size fractionated on 3.5% low-melting agarose gels.

Laboratory personnel were blind to case-control status. We were unable to genotype 4 men for MTHFR 677C→T, 3 men for MTHFR 1298A→C, and 2 men for ADH1. Blinded quality control samples were added for each genotype. The error rate for reproducibility was 0% for the MTHFR 677 and the ADH1 polymorphisms and was <5% for the MTHFR 1298 polymorphism. All three genotypes were in agreement with Hardy-Weinberg equilibrium.

**Statistical Analyses.** We considered total colorectal adenoma and advanced adenoma (large or tubulovillous/villous) in relation to the genotypes. For MTHFR 677C→T, we combined the CC and the CT genotypes because most previous studies (30, 31, 33–37) generally indicate that these have a similar risk level and because the risk for colorectal adenoma was similar in this population (Table 1). The MTHFR 1298A→C polymorphism was categorized into three genotypes, AA, AC, and CC, because functionality of these, if any, is uncertain. We also examined whether the combined heterozygosity of the two MTHFR polymorphisms (677C→T and 1298A→C) influenced risk of colorectal adenoma. For ADH1, we examined the three genotypes ADH1(1-1) (fast metabolizers), ADH1(1-2) (intermediate), and ADH1(2-2) (slow).

We then considered these genotypes in combination with alcohol and folate intakes and for combinations of alcohol and folate. For these analyses, the men were categorized into tertiles of energy-adjusted folate and categories of alcohol intake (<5, 5–30, and >30 g) based on 1986 intakes. When alcohol and folate were combined, high risk was considered as alcohol intakes of ≥20 g/day in combination with folate intake in the low tertile. For the combined analysis, we used 20 g for the cutpoint as done previously (3, 12) because if 30 g was used, the numbers were insufficient. Low risk was considered as
alcohol intakes ≤ 5 g/day and folate in the top tertile of intake. Men were jointly categorized by genotype and intake of alcohol, folate, and their combination. We also considered the influence of genotype on risk of adenoma across strata of alcohol and folate intakes and conversely alcohol and folate intake across strata of genotype. Our primary analysis was based on the 1986 rather than the updated diet because prior analyses suggest folate and alcohol act relatively early in colorectal carcinogenesis (12, 13).

We used both conditional and unconditional logistic regression for the analyses to compute ORs, 95% CIs, tests for trends and interactions, and to control for potentially confounding variables. Although the dataset was initially based on matched cases and controls, some of the stratified analyses required breaking the match. When we used unconditional logistic regression, we controlled for the matching variables, including age, history of previous endoscopy, and year of endoscopy. For all analyses, we also controlled for other risk factors for adenomas, including family history of colorectal cancer, aspirin use, tobacco use (never, current, and past), BMI, and intakes of red meat and methionine. Conditional and unconditional logistic regression yielded very similar results so only those for unconditional logistic regression are presented. For trends for trend, we used the medians of the categories for alcohol and folate modeled as continuous variables (Wald test).

To test for interactions between the dietary exposure and genotype, we computed a cross-product term using the ordinal value of genotype [three-category ordinal value for ADH, and MTHFR 1298 (A→C) and a two-category ordinal term for MTHFR (677C→T)] and the medians of the categories for alcohol and folate. For interaction was based on the Wald test for this cross-product term added to the model. All Ps are based on two-sided tests. All statistical analyses were done using the SAS 6.12 statistical package (SAS Institute).

### Results

#### Diet and Lifestyle Factors.

We first examined whether the risk factors for colorectal adenoma were similar in this sample of cases and controls that provided blood samples compared with previous observations in the whole cohort. For total colorectal adenoma, family history of colorectal cancer was associated with increased risk (multivariate OR, 1.67; 95% CI, 1.03–2.69), current smoking (OR, 1.84; 95% CI, 1.09–3.12), and past smoking (OR, 1.49; 95% CI, 1.12–1.96) were associated with an increased risk relative to never smokers, regular aspirin use was associated with lower risk relative to nonusers (OR, 0.72; 95% CI, 0.54–0.96), and alcohol intake was associated with increased risk, although primarily in those drinking 30+ g/day [OR, 1.75; 95% CI, 1.16–2.63, relative to infrequent (<5g/day) drinkers]. As we previously observed (51), physical activity and BMI were not associated with risk of total adenoma, but these were nonsignificantly associated with risk of advanced adenoma (large and/or tubulovillous/villous; OR, 0.66; 95% CI, 0.35–1.22 for high versus low METs quintile of activity) and BMI (OR, 1.24; 95% CI, 0.65–2.39 for high versus low category of BMI). Folate intake was not related to risk of total adenoma but was inversely associated with advanced adenoma (OR, 0.64; 95% CI, 0.34–1.18 for high versus low quintile), although this association was not statistically significant. The risk patterns observed for this sample were largely similar to that for the entire set of HPFS adenoma cases, as reported previously (3, 12, 51–53).

#### MTHFR Polymorphisms.

Table 1 shows the main results for the two MTHFR polymorphisms, 677C→T and 1298A→C individually and in combination. A formal test based on Terwiliger and Ott (54) revealed that the two polymorphisms were in strong linkage disequilibrium (P < 0.001). Only 5 individuals were homozygous variant at one locus and heterozygous at the other (677TT/1298AC or 677CT/1298CC), and no individual was homozygous variant at both loci (677TT/1298CC). In regards to risk of adenoma, neither MTHFR genotype showed a statistically significant association. In addition, heterozygotes for either MTHFR polymorphism individually or both together, were at similar risk as the wild-type homozygotes (677CC or 1298AA).

We next examined each MTHFR polymorphism separately in combination with alcohol and folate intakes (Table 2). For alcohol intake, we found a suggestion of an interaction (P = 0.09), with the highest risk observed for alcohol drinkers of 30+ g/day who were also TT homozygotes. Although the TT homozygotes comprised only 14% of the population (controls), a significant trend between alcohol intake and colorectal adenoma risk was observed in these men (P = 0.03) but not among men with the CC or CT genotypes. In an analysis restricted to the TT homozygotes, the multivariate OR for ≤30 versus ≤5 g alcohol was 4.94; 95% CI, 1.48–16.51. Conversely, when stratified by alcohol intake, MTHFR TT homozygotes had a slightly reduced risk if they were low or moderate drinkers but a higher risk if heavy drinkers (OR, 2.70; 95% CI, 0.93–7.87). For folate or combinations of alcohol and folate, MTHFR did not show the same pattern as with alcohol (Table 2).

We then examined the MTHFR 1298A→C polymorphism...
MTHFR, ADH, Diet, and Risk of Colorectal Adenomas

in combination with alcohol and folate in relation to adenoma risk. Unlike the MTHFR 677C→T polymorphism, interactions between alcohol intake and the MTHFR 1298A→C polymorphism were not apparent (Table 3). Neither was there an interaction with folate intake or combinations of high alcohol and low folate, although the power was limited (data not shown).

ADH3 Polymorphism. Compared with the ADH3 (1-1) genotype (fast alcohol metabolizers), the multivariate OR, 0.93 (95% CI, 0.70–1.22) for the ADH3 (1-2) genotype (intermediate metabolizers) and OR, 0.94 (95% CI, 0.64–1.40) for the ADH3 (2-2) genotype (slow metabolizers) were then examined. The joint association between ADH3 genotype with alcohol intake, folate intake, and with the combination of alcohol and folate intakes (Table 4). Alcohol was not appreciably related to risk of colorectal adenoma among those with the ADH3 (1-1) genotype, but consumption of 30 g of alcohol daily was associated with a higher risk among men with the ADH3 (1-2) genotype, and risk was even higher among frequent drinkers with the ADH3 (2-2) genotype. The test for interaction for increasing level of alcohol intake across levels (1–3) of ADH3 genotype was not significant (P = 0.17). Also, among men with the ADH3 (2-2) genotype, those in the lowest tertile of folate intake were at higher risk compared with those in the middle or upper tertiles of folate, whereas no clear pattern was apparent.

### Table 2 Relationships of alcohol, folate and their combinations to colorectal adenoma risk stratified by MTHFR 677 genotype in the HPFS (1986–1995)

<table>
<thead>
<tr>
<th>MTHFR 677</th>
<th>≤5 Alcohol (g/day)</th>
<th>5–30</th>
<th>&gt;30</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC/CT OR (95% CI)a</td>
<td>1.0 (ref) [123; 272]b</td>
<td>1.11 (0.82–1.49) [149; 280]</td>
<td>1.50 (0.97–2.32) [53; 72]</td>
</tr>
<tr>
<td>TT OR (95% CI)a</td>
<td>0.79 (0.42–1.49) [15; 43]</td>
<td>0.85 (0.48–1.51) [20; 50]</td>
<td>3.52 (1.41–8.78) [14; 8]</td>
</tr>
</tbody>
</table>

### Table 3 Relationships of alcohol and folate intake to colorectal adenoma risk stratified by MTHFR 1298 genotype in the HPFS (1986–1995)

<table>
<thead>
<tr>
<th>MTHFR 1298</th>
<th>Alcohol ≤5 g/day Folate &gt;497 μg/day</th>
<th>Alcohol/Folate Middle</th>
<th>Alcohol ≥20 g/day Folate &lt;338 μg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1298 AA OR (95% CI)a</td>
<td>1.0 [48; 92]</td>
<td>0.84 (0.57–1.24) [242; 491]</td>
<td>1.40 (0.77–2.54) [35; 41]</td>
</tr>
<tr>
<td>1298 AC OR (95% CI)a</td>
<td>0.45 (0.12–1.69) [3; 13]</td>
<td>0.82 (0.49–1.40) [39; 83]</td>
<td>2.08 (0.61–7.07) [7; 5]</td>
</tr>
</tbody>
</table>

### Table 4 Relationships of alcohol and folate intake to colorectal adenoma risk stratified by MTHFR 1298 genotype in the HPFS (1986–1995)

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<th>Alcohol ≥20 g/day Folate &lt;338 μg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1298 AA OR (95% CI)a</td>
<td>1.0 [55; 126]</td>
<td>1.43 (0.92–2.23) [68; 122]</td>
<td>1.37 (0.87–2.15) [63; 119]</td>
</tr>
<tr>
<td>1298 AC OR (95% CI)a</td>
<td>1.35 (0.83–2.16) [56; 97]</td>
<td>1.40 (0.87–2.25) [52; 97]</td>
<td>1.33 (0.85–2.15) [57; 105]</td>
</tr>
<tr>
<td>1298 CC OR (95% CI)a</td>
<td>1.41 (0.61–3.27) [11; 18]</td>
<td>0.82 (0.32–2.07) [7; 22]</td>
<td>0.93 (0.34–2.55) [6; 17]</td>
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</table>

### Table 5 Relationships of alcohol and folate intake to colorectal adenoma risk stratified by MTHFR 677 genotype in the HPFS (1986–1995)

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<thead>
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<th>Alcohol (g/day)</th>
<th>5–30</th>
<th>&gt;30</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC/CT OR (95% CI)a</td>
<td>1.0 (ref) [16; 35]</td>
<td>1.49 (0.78–2.89) [20; 50]</td>
<td>2.43 (1.24–4.75) [14; 8]</td>
</tr>
<tr>
<td>TT OR (95% CI)a</td>
<td>0.98 (0.53–1.82) [10; 26]</td>
<td>0.93 (0.52–1.67) [15; 26]</td>
<td>2.08 (0.94–4.60) [7; 5]</td>
</tr>
</tbody>
</table>

### Table 6 Relationships of alcohol and folate intake to colorectal adenoma risk stratified by MTHFR 1298 genotype in the HPFS (1986–1995)

<table>
<thead>
<tr>
<th>MTHFR 1298</th>
<th>Alcohol ≤5 g/day Folate &gt;497 μg/day</th>
<th>Alcohol/Folate Middle</th>
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</tr>
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<td>0.93 (0.34–2.55) [6; 17]</td>
</tr>
</tbody>
</table>
for folate intake for the other genotypes (Table 4). In alternative analyses where we stratified the cohort by \( ADH_3 \) genotype, alcohol became an increasingly stronger risk factor across the slower metabolizing genotypes [OR for \( 30+ \) versus <5 g/day alcohol = 1.43; 95% CI, 0.68–3.01] for \( ADH_3 \) (1-1), (OR, 1.95; 95% CI, 1.09–3.49) for \( ADH_3 \) (1-2), and OR, 3.14; 95% CI, 0.84–11.76 for \( ADH_3 \) (2-2)]. Across strata of alcohol, the association between \( ADH_3 \) and colorectal adenoma risk varied. In never/infrequent drinkers, \( ADH_3 \) was unrelated to risk [OR, 1.00; 95% CI, 0.51–1.96 for \( ADH_3 \) (2-2) versus \( ADH_3 \) (1-1)]; in moderate drinkers having the slower genotype tended to be associated (nonsignificantly) with lower risk (OR, 0.62; 95% CI, 0.34–1.14), and among high drinkers, the slow genotype tended to be associated (nonsignificantly) with greater risk (OR, 2.46; 95% CI, 0.82–7.41).

A marked increased risk of colorectal adenoma (OR, 17.1; 95% CI, 2.1–137) was observed for those with \( ADH_3 \) (2-2) genotype and high alcohol-low folate intake. Although the CIs were wide (2.1–137), the test for interaction for increasing high alcohol and low folate simultaneously across levels of \( ADH_3 \) genotype was statistically significant (\( P = 0.006 \)).

**Large and Tubulovillous/Villous Adenomas.** We conducted all analyses described above for large and tubulovillous/villous adenomas. Although the number of cases in some strata were small and thus confidence intervals were wider, the results were generally in concordance to those with total adenomas (results not shown).

**Discussion**

Our results show some consistencies with previous studies regarding the \( MTHFR 677C→T \) polymorphism. In all four studies for cancer (16, 30–32) and for four (33, 35–37) of six (33–37, 55) studies of adenomas, the lowest risk has been associated with the \( TT \) genotype and low-risk diet (high folate and/or low alcohol). In contrast, among those with low folate or high alcohol intakes, this benefit becomes attenuated, and the risk in the \( TT \) homozygotes may even exceed that of all others. Thus, \( MTHFR TT \) homozygotes appear to be hyperresponders to folate status, which are relatively resistant to colorectal neoplasia when folate is high and alcohol is low but are at high risk when folate is low or alcohol is high. In addition, we found that men who have an \( ADH_3 \) genotype associated with slower metabolism of alcohol are at considerably higher risk of colorectal adenoma if they consume substantial alcohol and have a low folate diet.

These findings may provide some insights into colorectal carcinogenesis. If folate intake is adequate (and alcohol intake not excessive), \( MTHFR 677 TT \) homozygotes tend to accumulate appreciable levels of 5,10-methylene THF intracellularly, whereas \( CC \) and \( CT \) individuals have 5-methyl THF predominantly (Ref. 29; Fig. 1). The retention of folate in these different forms may have phenotypic advantages or disadvantages that depend on folate status. For example, under conditions of high folate, the \( TT \) genotype, presumably by allowing the accumulation of folate in the forms required for DNA and RNA synthesis (29), may improve fetal health (56) and hematopoiesis (57). When levels of 5,10-methylene THF are low, misincorporation of uracil for thymidine may occur during DNA synthesis (58), leading to increased frequency of chromosomal breaks (59). Thus, under high folate conditions, the \( TT \) genotype may be beneficial by enhancing the pool of 5,10-methylene THF. However, when folate is deficient, the \( TT \) genotype

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**Table 4** Relationships of alcohol, folate and their combinations to colorectal adenoma risk stratified by \( ADH_3 \) genotype in the HPFS (1986–1995)

<table>
<thead>
<tr>
<th>( ADH_3 )</th>
<th>( \leq 5 )</th>
<th>Alcohol (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5-30</td>
</tr>
<tr>
<td>1-1 OR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0 (ref)</td>
<td>1.33 (0.85–2.07)</td>
</tr>
<tr>
<td>1-2 OR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>[55; 127]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>[72; 116]</td>
</tr>
<tr>
<td>2-2 OR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.99 (0.64–1.54)</td>
<td>1.02 (0.67–1.57)</td>
</tr>
<tr>
<td></td>
<td>[65; 149]</td>
<td>[76; 162]</td>
</tr>
<tr>
<td></td>
<td>1.06 (0.55–2.02)</td>
<td>0.83 (0.45–1.53)</td>
</tr>
<tr>
<td></td>
<td>[21; 53]</td>
<td>[16; 11]</td>
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<sup>P for interaction = 0.17</sup>

<table>
<thead>
<tr>
<th>( ADH_3 )</th>
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<tbody>
<tr>
<td></td>
<td>338–490</td>
</tr>
<tr>
<td></td>
<td>497</td>
</tr>
<tr>
<td>1-1 OR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0</td>
</tr>
<tr>
<td>1-2 OR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>[41; 88]</td>
</tr>
<tr>
<td>2-2 OR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.04 (0.63–1.70)</td>
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<tr>
<td></td>
<td>[61; 131]</td>
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<tr>
<td></td>
<td>1.97 (0.97–4.02)</td>
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<td>[21; 24]</td>
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<sup>P for interaction = 0.55</sup>

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<tr>
<td></td>
<td>Alcohol &lt;20 g/day or Folate ≥338 µg/day</td>
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<td></td>
<td>Alcohol ≥20 g/day and Folate &lt;338 µg/day</td>
</tr>
<tr>
<td>1-1 OR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0</td>
</tr>
<tr>
<td>1-2 OR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>[136; 251]</td>
</tr>
<tr>
<td>2-2 OR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.85 (0.64–1.13)</td>
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<tr>
<td></td>
<td>[152; 326]</td>
</tr>
<tr>
<td></td>
<td>0.79 (0.52–1.19)</td>
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<tr>
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<td>[45; 103]</td>
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</table>

<sup>P for interaction = 0.006</sup>

<sup>a</sup> OR and 95% CI controlling for matching variables (age, previous endoscopy, year of endoscopy), and smoking history, aspirin use, BMI, physical activity, and intakes of red meat and alcohol or folate.

<sup>b</sup> [cases; controls].
may be deleterious. Low folate status and the TT genotype interact to increase risk of neural tube defects (60, 61) and hyperhomocysteinemia (62), which is a risk factor for cardiovascular disease, and decreases DNA methylation (63), which may enhance carcinogenesis (26, 64). Interestingly, both TT genotype and poor folate status appear to be required to adversely influence DNA methylation because those with other genotypes (CT or CC) do not experience DNA hypomethylation even if they have low folate levels (29). In a recent small study, leukocyte and colonic hypomethylation were associated with a significantly increased risk of adenoma (65).

The haplotype frequency of the MTHFR 677 and the 1298 polymorphism were examined recently in a meta-analysis of 16 studies that provided reliable data on combined MTHFR genotypes in general populations (n = 5389; Ref. 66). The percentage for MTHFR 677/1298 from our population and from (the meta-analysis) are as follows: AA/AA, 0.13(0.15); CC/AC, 0.21(0.22); CC/CC, 0.08(0.085); CT/AA, 0.25(0.22); CT/AC, 0.20(0.20); CT/CC, 0.0018(0.0025); TT/AA, 0.135(0.11); TT/AC, 0.0041(0.0046); and TT/CC, 0.00032. Our results, even for the rare haplotypes (CT/CC, TT/AC, and TT/CC), are quite comparable. However, small errors in genotyping can influence the prevalence of the rarer genotypes substantially.

The relation between the MTHFR 1298A→C polymorphism and risk of colorectal cancer was investigated in two previous studies. In the Physicians’ Health Study (67), which was based on 211 cases and 343 controls, a nonsignificant lower risk was observed with the 1298 CC genotype (relative risk = 0.73; 95% CI = 0.37–1.43). Moreover, the association was not modified by plasma folate status, but alcohol intake was not examined. A case-control study of African Americans and whites found that the 1298 CC genotype was inversely associated with risk overall (OR, 0.6; 95% CI, 0.4–0.9) and for whites (OR, 0.5; 95% CI, 0.3–0.9; Ref. 55). Our study did not indicate an appreciable role of the CC polymorphism versus the AA polymorphism in relation to adenoma risk (OR, 0.84; 95% CI, 0.50–1.41), even in combination with folate and alcohol intakes (the OR was ∼0.8 compared with AA/AC genotype). Because the data are limited, a firm conclusion regarding the 1298 CC polymorphism cannot be made; the evidence is consistent with no appreciable association or a moderate reduction in risk.

The interaction between the MTHFR 677C→T polymorphism and colorectal neoplasia has been striking for alcohol (16, 30, 35). In the current study, the test for interaction was borderline statistically significant (P = 0.08), and men with TT homozygotes who drank 30+ g alcohol/day had a particularly high risk. In contrast, one study of adenomas found that alcohol increased risk among those with the CC genotype (33). It is striking that four studies found statistically significant or borderline significant interactions with alcohol intake because most studies have had low power to examine this.

Of note, an interaction between MTHFR 677C→T with folate in regards to colorectal neoplasia intake has been less evident than the interaction between MTHFR and alcohol. This finding may possibly be related to the relatively good folate status in the United States where most of the studies were conducted. For example, in the current study, very few men had folate intakes < 200 μg, and about half had levels exceeding 400 μg/day, and in a subsample, the mean erythrocyte level was well within the normal range (325 ng/ml) in men in the lowest quintile of folate intake. In contrast, in a study of advanced adenomas in Norway (36), a strong interaction between erythrocyte folate status and MTHFR was observed, as well as a 6-fold elevated risk of advanced adenomas among men with TT homozygotes with poor folate status relative to TT homozygotes with good folate levels. This finding suggests that the potential interaction with folate could possibly be quite strong in populations with poor folate status. In United States studies, interactions with folate alone, without considering alcohol concurrently, may be difficult to elicit, especially with current fortification with folic acid.

The strong interaction between alcohol and MTHFR 677C→T may reflect the potent effects of alcohol on folate metabolism in proliferative tissues such as the intestines and bone marrow (22). Heavy alcohol consumption impairs hematopoesis, especially in those with poor folate status, causing megaloblastic anemia, as well as neutropenia and thrombocytopenia and can prevent the hematological response to folic acid supplementation in these patients (22). Recent evidence also suggests an interaction between alcohol and the MTHFR 677C→T genotype in regards to homocysteine levels. In one study, the prevalence of hyperhomocysteinemia in heavy drinkers was 84.2% in TT homozygotes, 54.3% in CT heterozygotes, and 31.6% in CC homozygotes (68). Among heavy drinkers who were TT homozygotes, homocysteine levels were remarkably elevated if folate levels were low (up to 10-fold above normal in some individuals) but were not markedly increased if folate levels were high.

Alcohol’s impairment of folate metabolism may be caused by blocking release of folate from the hepatocyte, inhibiting DNA methyltransferase or methionine synthase, trapping folate as 5-methyl THF (thereby depleting cellular 5,10-methylene THF), and inducing intestinal malabsorption of folate (22, 69, 70). Additionally, acetaldehyde at high concentrations cleaves folate at the C9-N10 bond (71), although concentrations of acetaldehyde that far exceed typical concentrations in the blood and most tissues in the body may be required for this effect. However, intestinal bacteria are capable of oxidizing ethanol in the colon to produce substantial levels of acetaldehyde locally (72). In rats, ethanol consumption increases the concentration of acetaldehyde in the colonic mucosa while decreasing the colonic mucosal folate level by 48% (73). The concentrations of acetaldehyde produced by the large bowel bacteria when alcohol is consumed, perhaps 1000-fold higher than circulating levels, may be sufficiently high to break down folate (72, 73). In rats, chronic alcohol consumption induces genomic DNA hypomethylation in the intestinal mucosa (74), which may contribute to carcinogenesis. Although bacterial catabolism of ethanol have been studied mostly in rats, incubation of human colonic contents with various ethanol concentrations in vitro at 37°C results in significant production of acetaldehyde (46).

The adverse influence of alcohol on colorectal neoplasia suggests a modifying role of alcohol-metabolizing enzymes. Alcohol is oxidized mainly by ADH into acetaldehyde, which is then catalyzed by ALDH. A well-known variant of ALDH causes slow breakdown of acetaldehyde and hence high levels of this compound. Interestingly, Japanese alcoholics with the slow-metabolizing ALDH variant have a much higher risk of alcohol-related cancers, including colon cancer (75–77), consistent with an adverse effect of acetaldehyde. The ALDH variant cannot be studied in a predominantly white population such as the HPFS because it occurs exclusively in Asian populations. A common polymorphism in alcohol metabolism identified in Caucasian populations is in ADH1, where the γ1 allele differs from the γ2 by two amino acids at positions 271 and 349. Pharmacokinetic studies show a 2.5-higher rate of maximal velocity of ethanol oxidation for the homodimeric γ1 compared with homodimeric γ2 enzyme (45). Although an influence on short-term blood alcohol levels has not been...
clearly demonstrated, studies have associated the ADH1 polymorphism with risk of alcoholism (78) and cirrhosis (79). Moreover, moderate alcohol drinkers who are homozygous for the slow-oxidizing ADH1 allele have higher high-density lipoprotein cholesterol levels and a lower risk of myocardial infarction (80), suggesting that a lower clearance rate of alcohol enhances the benefit of moderate alcohol intake on high-density lipoprotein cholesterol and coronary disease. The ADH1 genotype also appears to modify the response to some plasma steroid hormones to alcohol (81). These relationships suggest that this polymorphism is functional and influences the human physiological response to alcohol consumption.

The relationship of the ADH1 genotype to various alcohol-related cancers, including breast (50, 81) and oral-pharyngeal cancer (82, 83), has been equivocal. Only two studies have been reported for colorectal neoplasia (84, 85). The results for ADH1 from a study based on 211 incident cases of colorectal cancer and 1113 controls from the Physicians’ Health Study were not statistically significant, although there were some limitations (84). The top cutpoint for alcohol consumption was >5 drinks/week, and no overall relationship was observed between alcohol intake and colorectal cancer risk (P trend = 0.25). In the current study, we observed a clear association for alcohol only at levels exceeding 30 g/day (~15 drinks or more/week), which is consistent with most prospective data on alcohol and colorectal cancer. Moreover, in an analysis of colorectal cancer limited to slow metabolizers [ADH1(2-2)] in the Physicians’ Health Study, a 3.7-fold increase with higher alcohol consumption was observed (P trend = 0.04), similar to the 3.2-fold increased risk we observed with higher alcohol consumption in the ADH1(2-2) homozygotes. Thus, both studies suggest that slow-metabolizers are more susceptible to the effects of alcohol. In contrast, a case-control study of 433 adenoma cases and 436 polyp-free controls conducted in the Netherlands had apparently opposing results; similar to our study, alcohol increased risk, but risk associated with alcohol appeared to be stronger for men with the ADH1(1-1) genotype than for men with other genotypes (85). However, the interaction term was not statistically significant (P = 0.4). Thus, the limited results from the literature to date are not consistent, and larger additional studies are required to resolve this. A theoretical complexity is that decreased ADH1 activity may have a dual role for folate in the intestinal mucosa. Lower ADH1 activity in the liver could reduce the rate of acetaldehyde production in the liver (beneficial for overall folate status) but could increase ethanol reaching the large intestine, which is then available for acetaldehyde conversion by the microflora (adverse for intestinal folate status).

Several potential limitations of this study should be discussed. Only about one-third of eligible men of the cohort provided blood samples. However, it is unlikely that the genotypes are related to the probability of providing a blood sample. The dietary data were collected prospectively, so bias based on differential reporting between cases and controls is unlikely. The major findings we have reported in the overall cohort were similar in this nested, case-control study. Our dietary questionnaire was reasonably valid, although measurement error would be unrelated to genotype and tend only to attenuate true associations. It is possible that some of the heavy drinkers (>30 g/day) could be underreporting this alcohol consumption, so it is plausible that risk begins to increase at some level >30 g/day. The relatively homogeneous nature of the highly educated study population helps ensure valid information and reduces the likelihood of residual confounding by factors related to socioeconomic status but may limit generalizability of findings. An important limitation is that the sample size was relatively small, especially to examine interactions. The statistically significant or near significant interactions are even more impressive in this regard, but the null results (e.g., for MTHFR 1298 A → G polymorphism) should be interpreted cautiously because of our limited power.

Another limitation is that few in this population would be expected to suffer from very poor folate status. However, this limitation would tend to diminish our ability to observe associations, and we may have underestimated the full influence of these polymorphisms among those with poor folate status. These results may be most generalizable to the United States, where high rates of vitamin supplementation (and more recently fortification with folic acid) have made severe folate deficiency rare (86). Of note, in a sample from this cohort, alcohol intake was a stronger predictor of low erythrocyte folate levels than dietary folate itself (3), suggesting a prominent role of alcohol in generally folate-adequate populations. Finally, data on MTHFR and colorectal neoplasia are relatively sparse for women, so these findings need to be replicated in women.

In conclusion, our results support the hypothesis that high intakes of alcohol, at least two drinks daily, are associated with an increased risk of colorectal adenoma, particularly among MTHFR 677TT homozygotes and among slow metabolizers of ethanol based on ADH1 genotypes. The findings that alcohol interacts with a folate-related enzyme (MTHFR) and that the interaction between alcohol and ADH1 genotype is stronger among those with low folate intakes support the hypothesis that the carcinogenic effect of alcohol in the large bowel is mediated through its adverse effects on folate status.

References
MTHFR, ADH, Diet, and Risk of Colorectal Adenomas


Methylenetetrahydrofolate Reductase, Alcohol Dehydrogenase, Diet, and Risk of Colorectal Adenomas

Edward Giovannucci, Jia Chen, Stephanie A. Smith-Warner, et al.