Dietary intake of folate and alcohol, MTHFR C677T polymorphism, and colorectal cancer risk in Korea1–3

Jeongseon Kim, Young Ae Cho, Dong-Hyun Kim, Bong-Hwa Lee, Dae-Yong Hwang, Jinyoung Jeong, Hun-Jae Lee, Keitaro Matsuo, Kazuo Tajima, and Yoon-Ok Ahn

ABSTRACT
Background: The incidence of colorectal cancer (CRC) is increasing sharply in Korea, and evidence has suggested the role of dietary methyl supply and related polymorphisms on colorectal carcinogenesis.
Objective: We investigated the association between folate and alcohol intake, methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism, and CRC risk in Koreans.
Design: A total of 787 cases and 656 controls were recruited from 2 university hospitals. Multiple logistic regression models were used to estimate ORs and corresponding 95% CIs.
Results: MTHFR 677T homozygotes were at a lower risk of CRC (OR: 0.60; 95% CI: 0.46, 0.78 for TT compared with CC/CT). High folate intake was associated with reduced CRC risk (OR: 0.64; 95% CI: 0.49, 0.84 for high compared with low intake), and high alcohol consumption was associated with increased risk of CRC (OR: 1.76; 95% CI: 1.26, 2.46 for high compared with low intake). When data were stratified by the amount of dietary methyl (combined intake of folate and alcohol), those with low-methyl diets had higher risk of CRC (OR: 2.32; 95% CI: 1.18, 4.56) than did those with high-methyl diets among CC/CT carriers, whereas the amount of dietary methyl did not affect the CRC risk among carriers with the TT homozygous variant. This association was stronger in patients with colon cancer than in patients with rectal cancer.
Conclusion: We found that the effect of dietary methyl supply on colorectal carcinogenesis may differ according to MTHFR C677T genotype and the subsite of origin in a Korean population. Am J Clin Nutr 2012;95:405–12.

INTRODUCTION
CRC4 is the third most common cancer in men and the second in women, and the fourth most common cause of death from cancer worldwide (1). Even though the incidence of CRC is stabilizing or even declining in most developed countries, it is increasing sharply in some Asian countries, such as China and Korea (1, 2). The CRC incidence rate in South Korea is currently one of the highest in the world (1).

It has been proposed that the complex interplay between environmental factors and genetics has an important role in colorectal carcinogenesis (3). A recent pooled analysis of 13 prospective cohort studies found a significantly lower risk of CRC among participants with relatively high folate intake compared with those with low folate intake (4), and a meta-analytic study also reported similar findings in both cohort and case-control studies (5). Folate functions as a donor of one-carbon unit for nucleotide synthesis and DNA methylation (6). MTHFR is an important enzyme in folate metabolism, which mediates the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (6). A common MTHFR C677T polymorphism is associated with decreased enzyme activity, and thus increases the availability of 5,10-methyltetrahydrofolate for DNA synthesis, which partially explains the reduced risk of CRC in subjects carrying the TT genotype (7, 8). Alcohol is also known as a folate antagonist that is responsible for folate malabsorption and increased excretion, as well as abnormalities in folate-mediated one-carbon metabolism (9).

Findings from recent studies, however, suggest that the role of folate in carcinogenesis may differ by dose, source, and timing of folate administration during the carcinogenic process (10, 11). In addition, various genetic background and nutrient status factors could modify the association between folate and carcinogenesis (7, 12, 13). To date, most studies concerning the MTHFR C677T polymorphism and CRC risk according to folate and alcohol status have been reported in the United States (14, 15), where folate intake is higher than in other countries due to a higher intake of multivitamin supplements with folic acid and US Food Supplement Program (USFP) consumptions (16, 17), which could modify the association between folate and carcinogenesis (18, 19). Therefore, studies of CRC in Asia are necessary to provide new insights into the complex interplay between folate intake and the genetic background in colorectal carcinogenesis.

The objective of the present study was to investigate the association between folate and alcohol intake, MTHFR C677T polymorphism, and CRC risk in Koreans. We used a total of 787 cases and 656 controls who were recruited from 2 university hospitals in South Korea. Multiple logistic regression models were used to estimate ORs and corresponding 95% CIs. We found that the effect of dietary methyl supply on colorectal carcinogenesis may differ according to MTHFR C677T genotype and the subsite of origin in a Korean population.
and Drug Administration–mandated folate fortification since 1998 (16). Therefore, it is meaningful to investigate these associations in other populations with a low folate intake and a different genetic background. In the present study, we aimed to examine whether the MTHFR C677T polymorphism and folate and alcohol intake are associated with the risk of CRC stratified by cancer subsite (colon/rectum) and whether the MTHFR C677T polymorphism may modulate cancer risk depending on the amount of dietary methyl—ie, combined folate and alcohol intake—among Koreans.

SUBJECTS AND METHODS

Study participants

This study conducted analyses by using the extended data of a case-control study in Korea, which was published previously (17). In the present study, cases consisted of 849 patients, aged 30–79 y, who were consecutively admitted to 2 university hospitals and with confirmed diagnoses of CRC between 1998 and 2004 in Seoul, Korea. The cases were reconfirmed from both pathology report and chart review. The International Classification of Diseases-10 codes C18 and C20 were used to identify colon and rectal cancer, respectively (18). Controls consisted of 744 patients, aged 30–79 y, who had been hospitalized during the same period as the cases for a wide spectrum of nonneoplastic conditions. After excluding subjects with a history of malignant neoplasms and those with missing information on dietary intake and genetic information, 793 cases and 664 controls were eligible for inclusion. Participants who reported implausible daily energy intake (<600 or >3500 kcal) were also excluded, and the final analysis included 787 cases and 656 controls. All protocols and consent forms were approved by the institutional review board of the participating institution, and participants provided consent following recommendations by their attending physicians.

Data collection

A trained nurse interviewer collected information on diet and other lifestyle characteristics by using a structured questionnaire. Alcohol intake was measured in terms of the reported frequencies and amounts before symptom appearance. Daily ethanol intake of individual alcoholic beverages was calculated in grams based on the ethanol content of the beverage. Total ethanol intake was calculated and categorized into 3 amounts (<5, 5 to <30, ≥30 g/d); 30 g of ethanol corresponds to ~2 drinks in Western countries. Dietary intake was assessed by using an FFQ. The reliability and validity of this questionnaire has been reported previously (19, 20). Participants were queried about their individual average frequency of eating and typical portion sizes before symptom appearance. Daily ethanol intake of alcoholic beverages was calculated in grams based on the ethanol content of the beverage. Total ethanol intake was calculated and categorized into 3 amounts (<5, 5 to <30, ≥30 g/d). To investigate the combined effect of folate and alcohol consumption, we categorized subjects by their amount of dietary methyl: high-methyl diets (high folate and low alcohol intake), low-methyl diets (low folate and high alcohol intake), and moderate-methyl diets. The multivariate model was adjusted for age, sex, family history, smoking, physical activity, BMI, multivitamin use, and total energy intake. Folate intake and alcohol consumption were added to the model if necessary. Interactions between the MTHFR C677T genotype and methyl-group diets were assessed with the likelihood ratio test by comparing the model with interaction term, with one model containing only the main effects. To test for linear trends across tertiles of folate and alcohol consumption, we assigned a median value for the study population to each category and used these values as continuous variables. Colon and rectal cancer were analyzed separately. SAS 9.1 software (SAS Institute Inc) was used to perform the calculations, and a 2-sided P value <0.05 was considered significant.

RESULTS

Characteristics of the study population are shown in Table 1. Compared with controls, cases were older, had a lower BMI, were less likely to be physically active, were more likely to have a family history of CRC, and were more likely to be current smokers and drinkers. In addition, dietary folate intake was significantly lower among cases.

The risks of CRC according to MTHFR C677T polymorphism are presented in Table 2. Frequencies of the CC, CT, and TT genotypes were 31.3%, 44.0%, and 24.7%, respectively, among...
controls and 33.7%, 49.9%, and 16.4%, respectively, among cases. The MTHFR C677T polymorphism was inversely related to CRC risk (OR: 0.60; 95% CI: 0.46, 0.78 for TT compared with CC/CT). High folate consumption was associated with a reduced risk (OR: 0.64; 95% CI: 0.49, 0.84 for high compared with low intake; P-trend = 0.002), and high alcohol consumption was associated with an increased risk (OR: 1.76; 95% CI: 1.26, 2.46 for high compared with low intake; P-trend = 0.001). The distribution of genotype did not differ by cancer site, but the effect of folate and alcohol was slightly stronger among patients with colon cancer than among those with rectal cancer.

The associations between folate intake and alcohol consumption and CRC risk were examined according to MTHFR C677T genotypes. Dietary folate intake was associated with a lower risk of CRC among CC/CT carriers, with a dose-response relation (OR: 0.60; 95% CI: 0.46, 0.78 for high compared with low intake; P-trend = 0.002), but a reduced risk in subjects with TT homozygotes was independent of folate status (Table 3). Alcohol consumption was associated with increased CRC risk among CC/CT carriers (OR: 1.87; 95% CI: 1.29, 2.71 for high compared with low intake; P-trend < 0.001). The protective effect of the TT genotype disappeared when alcohol consumption was ≥5 g/d (Table 4). These associations were slightly stronger among patients with colon cancer than in those with rectal cancer.

The interactive effect of methyl-group diets and MTHFR C677T polymorphisms on the risk of CRC is shown in Table 5. Low-methyl diets were associated with a higher risk of CRC compared with high-methyl diets among CC/CT carriers (OR: 2.32; 95% CI: 1.18, 4.56) but not among TT carriers (OR: 0.95; 95% CI: 0.38, 2.40). This association was observed only among patients with colon cancer. Among subjects with the TT homozygous variant, no association was observed between methyl-group diet and CRC risk.

**DISCUSSION**

In the present study, the homozygous variant (TT) of MTHFR C677T and high folate intake were associated with a lower risk of CRC, and high alcohol consumption was associated with an increased risk of CRC in a Korean population. When data were stratified by the amount of dietary methyl (combined intake of folate and alcohol), low-methyl diets were associated with increased risk of CRC compared with high-methyl diets among CC/CT carriers but not among TT carriers. These associations were stronger among patients with colon cancer than among those with rectal cancer.

Numerous epidemiologic and experimental studies have investigated the role of diet on the risk of CRC. Several studies have suggested a role of folate and alcohol on colorectal carcinogenesis (5, 6) and have implied a combinatorial effect between folate intake and alcohol drinking on the risk of CRC (14, 22, 23). Several mechanisms have been proposed to explain the association between folate and alcohol consumption. Folate is required as 5-methyltetrahydrofolate to produce methionine for DNA synthesis (6, 15). Therefore, folate deficiency may induce higher mutation rates and reduced stability of DNA methylation patterns (24). Alcohol may impede the bioavailability of dietary folate and is known to inhibit folate-dependent one-carbon metabolism are associated with CRC risk (32–34), and MTHFR has received the most attention. The MTHFR C677T polymorphism is known to reduce enzyme activity (7), thereby increasing the availability of folate for the production of...
### TABLE 2

Risks of colorectal, colon, and rectal cancer according to MTHFR C677T genotype and folate and alcohol consumption

<table>
<thead>
<tr>
<th></th>
<th>Colorectal cancer</th>
<th>Colon cancer</th>
<th>Rectal cancer</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td></td>
<td>( n (% ) )</td>
<td>Crude OR (95% CI)</td>
<td>Multivariate OR (95% CI)</td>
</tr>
<tr>
<td><strong>MTHFR C677T</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CC</strong></td>
<td>205 (31.3)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>CT</strong></td>
<td>289 (44.1)</td>
<td>1.05 (0.83, 1.33)</td>
<td>1.02 (0.80, 1.30)</td>
</tr>
<tr>
<td><strong>TT</strong></td>
<td>162 (24.7)</td>
<td>0.62 (0.46, 0.83)</td>
<td>0.60 (0.45, 0.82)</td>
</tr>
<tr>
<td><strong>TT vs CC/CT</strong></td>
<td></td>
<td>0.60 (0.46, 0.78)</td>
<td>0.60 (0.46, 0.78)</td>
</tr>
<tr>
<td><strong>Folate consumption</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>&lt;209.69 ( \mu g/d )</td>
<td>218 (33.2)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>209.69 to &lt;282.72 ( \mu g/d )</td>
<td>219 (33.4)</td>
<td>0.65 (0.50, 0.83)</td>
<td>0.69 (0.53, 0.90)</td>
</tr>
<tr>
<td>≥282.72 ( \mu g/d )</td>
<td>219 (33.4)</td>
<td>0.64 (0.50, 0.82)</td>
<td>0.64 (0.49, 0.84)</td>
</tr>
<tr>
<td><strong>P-trend</strong></td>
<td>0.001</td>
<td>0.002</td>
<td></td>
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<tr>
<td><strong>Alcohol consumption</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 ( g/d )</td>
<td>422 (64.3)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>5 to &lt;30 ( g/d )</td>
<td>121 (18.5)</td>
<td>1.22 (0.93, 1.61)</td>
<td>1.22 (0.88, 1.69)</td>
</tr>
<tr>
<td>≥30 ( g/d )</td>
<td>113 (17.2)</td>
<td>1.81 (1.39, 2.36)</td>
<td>1.76 (1.26, 2.46)</td>
</tr>
<tr>
<td><strong>P-trend</strong></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 Tests of association from logistic regression were used. To test for trends across folate and alcohol tertiles, the median intake of each tertile category was used as a continuous variable. MTHFR, methylenetetrahydrofolate reductase.

2 Adjusted for age, sex, family history, multivitamin use, BMI, smoking status, and total energy. Alcohol consumption and folate intake were added to the model if necessary.

3 Measurements of folate were adjusted for total energy intake by using the linear residual regression method (21).
thymidylate and purine for DNA synthesis and repair (26). We also observed a decrease in the risk of CRC among homozygous variant (TT) carriers of MTHFR C677T. The association between the MTHFR polymorphism and dietary methyl supply has been investigated, but findings remain inconsistent. Several studies reported that the protective effect of the TT genotype of the MTHFR C677T polymorphism was limited to subjects with high-methyl diets such as those with a high folate intake (35–38) and low alcohol consumption (38–40). It is plausible that 5,10-methylenetetrahydrofolate amounts associated with a high folate intake are sufficient to overcome the reduced enzyme activity, thus resulting in adequate methylation and enhanced DNA synthesis and repair (14, 24). But mutations may reduce an already low methyl supply for methylation reactions in those with low-methyl diets and may increase the cancer risk in these patients (14, 24). However, we found that the protective effect of the TT homozygous variants compared with the CC/CT genotype was not affected by folate status and that low-methyl diets were associated with the risk of CRC among CC/CT carriers but not among TT carriers. In a large prospective cohort study in Sweden, Van Guelpen et al (41) also found that reduced CRC risk in TT homozygotes of MTHFR C677T was independent of plasma folate status and was even present in subjects with an inadequate folate intake. The folate intake amount from our study and the Swedish cohort study (41) were quite lower compared with those from previous studies conducted in the United States (35–38). The relation between folate and CRC may be more complicated than initially expected, but these findings support a role of methyl-group availability in the pathway of colorectal carcinogenesis.

Studies on folate and CRC have reported contradictory findings and have implied that various factors could affect the association between folate and colorectal carcinogenesis. The protective role of folate in carcinogenesis may be dependent on dose, source, and timing of folate administration during the carcinogenic process (41, 42). It has been reported that dietary folate may be protective, but the effect of folic acid supplements and fortification is questionable (5, 42, 43). A meta-analysis of cohort studies reported the protective effect of dietary folate but not of total folate (5). It is plausible that different biological properties of dietary and supplemental folate sources may have a different effect on carcinogenesis, and other components that coexist with folate in the diet may result in the true beneficial effect (44, 45). In addition, the timing of folate consumption may influence the carcinogenic process, and folate consumption after precancerous lesions have become established may even increase progression because of the nucleotide synthesis function of folate in rapidly proliferating tissues (46). In addition, different populations possess various genetic backgrounds (eg, differences in allelic distribution) and nutrient status (eg, methionine, riboflavin, vitamin B-6, vitamin B-12) (12, 13), and these could modify the association between folate and carcinogenesis (7). Furthermore, CRC involves distinct carcinogenic pathways from cancer subsites, possibly due to physiologic circumstances and genetic abnormalities (47, 48). Several risk factors have been reported to have different effects in specific cancer subsites (7, 44, 49). The present study observed a stronger association between folate and alcohol consumption and cancer risk among patients with colon cancer than among patients with rectal cancer. A prospective Danish cohort studies reported similar findings of a beneficial

### TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Folate consumption (tertiles)</th>
<th></th>
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<th></th>
<th>P-trend</th>
<th>P-interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>167/298</td>
<td>156/176</td>
<td>171/184</td>
<td>0.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT (cases/controls)</td>
<td>1.0 (ref)</td>
<td>0.68 (0.51, 0.92)</td>
<td>0.62 (0.46, 0.84)</td>
<td>0.002</td>
<td></td>
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</tr>
<tr>
<td>Adjusted OR (95% CI)</td>
<td>51/46</td>
<td>63/47</td>
<td>48/56</td>
<td>0.341</td>
<td></td>
<td></td>
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<tr>
<td>Colonic cancer</td>
<td>167/147</td>
<td>156/82</td>
<td>171/77</td>
<td>0.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT (cases/controls)</td>
<td>1.0 (ref)</td>
<td>0.64 (0.44, 0.92)</td>
<td>0.51 (0.35, 0.74)</td>
<td>&lt;0.001</td>
<td></td>
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</tr>
<tr>
<td>Adjusted OR (95% CI)</td>
<td>51/20</td>
<td>63/19</td>
<td>48/18</td>
<td>0.435</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectal cancer</td>
<td>167/117</td>
<td>156/72</td>
<td>171/84</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT (cases/controls)</td>
<td>1.0 (ref)</td>
<td>0.74 (0.51, 1.08)</td>
<td>0.72 (0.49, 1.05)</td>
<td>0.051</td>
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<tr>
<td>Adjusted OR (95% CI)</td>
<td>51/23</td>
<td>63/18</td>
<td>48/16</td>
<td>0.526</td>
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</tbody>
</table>

1 Tests of association were from logistic regression, adjusted for age, sex, family history, multivitamin use, BMI, smoking status, physical activity, total energy, and alcohol consumption. MTHFR, methylenetetrahydrofolate reductase; ref, reference.
2 Measurements of folate were adjusted for total energy intake by using the linear residual regression method (21).
3 To test for trends across folate tertiles, the median intake of each tertile category was used as a continuous variable.
4 Tests of interaction from the likelihood ratio test.

Cutoffs for tertile of folate intake: 209.69 and 282.72 µg/d.
Further research that integrates studies of nutrient status, relevant genotypes, and cancer subsite may help clarify and resolve these questions.

The present study possesses several limitations that should be considered. First, this study was a hospital-based, case-control study; thus, selection and recall biases may have affected the results. The hospital-based control group may not represent community-
based counterparts, and cases and controls may differ in their recall of dietary habits. Second, intakes of folate and alcohol were collected via FFQ and thus may not be accurate measures of true consumption amounts. However, the data were gathered through face-to-face interviews by a trained nurse who was unaware of the specific hypotheses of this study, thus lessening the potential for differential misclassification and measurement errors. Third, folate consumption was generally low in this study population and thus limited our ability to evaluate the combined effects of deficient one-carbon nutrient intake and high alcohol intake. Finally, subgroup analyses stratified by dietary factors and genotype may have lacked the power to reach statistical significance. In the present study, the interaction between MTHFR C677T genotype and dietary methyl amount did not reach statistical significance. However, our results suggest that the effect of the amount of dietary methyl could differ according to an individual’s genotype. Therefore, the null results in the interaction should be interpreted cautiously because of our limited power.

It was found in the present study that the amount of dietary methyl was associated with the risk of CRC among CC/CT carriers of MTHFR C677T but not among TT homozygous variants, which differs from previous studies conducted in populations with a high folate intake. In consideration of the change in dietary habit and the sharp increase of CRC incidence in Korea, findings from this study may help to identify high-risk groups and provide an appropriate intervention to prevent CRC.

The authors’ responsibilities were as follows—JK and YAC: data analysis and manuscript preparation; D-HK and Y-OA: study design and supervision, data collection, and manuscript revision; B-HL and D-YW: study design and data collection; H-JL and JJ: data cleaning and preliminary data analysis; and KM and KT: study design and genotyping. None of the authors declared a conflict of interest.

REFERENCES


