Polymorphism in the Thymidylate Synthase Promoter Enhancer Region and Risk of Colorectal Adenomas

Jia Chen,1 Charles Kyte,1 Wendy Chan,2 James G. Wetmur,2 Charles S. Fuchs,3 and Edward Giovannucci4,5

Abstract

Thymidylate synthase (TS), a key one-carbon metabolizing gene, encodes an enzyme that converts dUMP to dTMP, the rate-limiting nucleotide in DNA synthesis. We recently reported that a promoter polymorphism in TS modified the risk of colorectal cancer as well as the survival rate after the disease. To explore whether TS may play an important role in colorectal carcinogenesis early in the multistaged pathogenic pathway, we investigated the relation between the TS promoter polymorphism and risk of colorectal adenoma in a nested case-control study within the prospective Health Professionals Follow-up Study. We ascertained the TS genotype from 373 incident colorectal adenoma cases and 720 control subjects. Although there was no overall association between the TS promoter polymorphism and adenoma risk, we observed a significant TS-alcohol interaction (P for interaction = 0.009); relative to low alcohol consumers with the 2R/2R genotype, those with high alcohol consumption (>30 g/d) were not at elevated risk if they had the 2R/2R genotype [relative risk (RR), 0.80; 95% confidence interval (95% CI), 0.34-1.90], but were at higher risk if they had the 2R/3R genotype (RR, 1.70; 95% CI, 0.87-3.31), and at the highest risk (RR, 3.16; 95% CI, 1.50-6.63) if they had the 3R/3R genotype. In addition, a significant interaction was observed between the TS promoter polymorphism and the 677C > T polymorphism of methylenetetrahydrofolate reductase (MTHFR; P for interaction = 0.007). These findings lend additional support that one-carbon metabolism is an important process in pathogenesis of colorectal cancer. (Cancer Epidemiol Biomarkers Prev 2004;13(12):2247–50)

Introduction

The importance of one-carbon metabolism in the etiology of colorectal cancer has been implicated in numerous studies (reviewed in ref. 1). Low dietary one-carbon (methyl) supply, which is largely dependent upon intakes of folate and methionine (as well as choline) and disrupted by alcohol, which is a folate antagonist, has been shown to increase risk of colorectal cancer (2) as well as colorectal adenomas (3) in cohort studies. Functional polymorphisms in one-carbon metabolizing genes, such as methylenetetrahydrofolate reductase (MTHFR; ref. 4) and alcohol dehydrogenase 3 (ADH3; ref. 5), have been shown to modify the risk of colorectal cancer independently and in particular, the risk associated with dietary methyl supply (6-8). We recently reported that a promoter polymorphism in another key gene in one-carbon-metabolism, thymidylate synthase (TS), modified colorectal cancer risk as well as survival rate after the disease (9). TS competes with MTHFR for 5,10-methylenetetrahydrofolate as the substrate for intracellular conversion of dUMP to dTMP, the only de novo source of thymidine for DNA biosynthesis (10). A tandem repeat polymorphism in the promoter enhancer region of the TS (TS*3R or TS*2R) has been shown to influence gene expression; the TS*3R allele was correlated with a 3.6-fold increase in the TS mRNA levels (11). In this study using colorectal adenoma as the disease end point, we explored whether the effect of TS occurred early in the pathogenic pathway of colorectal cancer.

Materials and Methods

A nested case-control study was carried out within the Health Professionals Follow-up Study, an ongoing prospective study of the causes of chronic diseases in 51,529 U.S. male health professionals aged 40 to 75 years (12). Blood samples were provided by 18,018 cohort members from 1993 to 1995. All participants in the current study have undergone sigmoidoscopy or colonoscopy after the date of return of the 1986 (baseline) dietary questionnaire. A detailed study design including methods of case-control identification...
for the nested case-control study was described previously (8). In brief, two controls were matched to each case on year of birth, year of endoscopy, and whether they had had a previous endoscopy. The study was approved by the Institutional Review Boards of the Harvard School of Public Health and Brigham and Women’s Hospital.

Dietary intake was assessed from validated semiquantitative food frequency questionnaires (13). The correlation coefficients between intakes determined by two 1-week diet records and the dietary questionnaires among a sample of 127 cohort members was 0.77 for folate. In this sample, total folate intake from food and supplements also correlated with erythrocyte folate levels ($r = 0.56$). The Spearman correlation between alcohol from dietary records and that from the diet questionnaire was 0.86. Alcohol intake also correlated inversely with erythrocyte folate levels ($P = 0.01$).

Results on MTHFR and ADH3 polymorphisms have been published previously (8). Genotyping for the TS promoter polymorphism was carried out based on a modified method of Villafranca et al. (14). Primers with the sequences 5'-TCCGAGCCGGCCACAGGCATGGCGCGG-3' (sense) and 5'-CTGGCAGCCGGCCACAGGCATGGCGCGG-3' (antisense) were used in PCR reactions. PCR products were size-fractionated on 4% agarose gels. About 10% of samples were duplicate samples for the purpose of quality control. Laboratory personnel were blinded to samples. Genotype distributions from 373 cases and 720 controls. Frequencies for the 2R/2R, 2R/3R, and 3R/3R were 21%, 49%, and 30%, respectively (cases) in comparison with 22%, 51%, and 27%, respectively (controls). Genotype distributions were in agreement with Hardy-Weinberg Equilibrium in both cases ($P = 0.99$) and controls ($P = 0.91$). Using the 2R/2R genotype as reference, no modification in adenoma risk seemed associated with the 2R/3R or 3R/3R

### Results

There were 377 cases and 726 controls who were eligible for the study. We were able to ascertain the TS genotype from 373 cases and 720 controls. Frequencies for the 2R/2R, 2R/3R, and 3R/3R were 21%, 49%, and 30%, respectively (cases) in comparison with 22%, 51%, and 27%, respectively (controls). Genotype distributions were in agreement with Hardy-Weinberg Equilibrium in both cases ($P = 0.99$) and controls ($P = 0.91$). Using the 2R/2R genotype as reference, no modification in adenoma risk seemed associated with the 2R/3R or 3R/3R

### Table 1. Interactions between the TS promoter polymorphism and intakes of alcohol and folate in relation to risk of colorectal adenoma in the Health Professionals Follow-up Study

<table>
<thead>
<tr>
<th>Variable</th>
<th>All</th>
<th>2R/2R</th>
<th>3R/2R</th>
<th>3R/3R</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS polymorphism</td>
<td>—</td>
<td>1.0 (reference)</td>
<td>0.96 (0.69-1.34)</td>
<td>1.15 (0.80-1.66)</td>
</tr>
<tr>
<td></td>
<td>[373, 720]</td>
<td>[78, 157]</td>
<td>[182, 367]</td>
<td>[113, 196]</td>
</tr>
<tr>
<td>Alcohol intake (g/d)</td>
<td>&lt;5</td>
<td>1.0 (reference)</td>
<td>1.05 (0.63-1.73)</td>
<td>0.77 (0.43-1.38)</td>
</tr>
<tr>
<td></td>
<td>[137, 315]</td>
<td>[34, 74]</td>
<td>[72, 149]</td>
<td>[31, 91]</td>
</tr>
<tr>
<td></td>
<td>5-30</td>
<td>1.27 (0.70-2.33)</td>
<td>0.89 (0.54-1.46)</td>
<td>1.30 (0.76-2.22)</td>
</tr>
<tr>
<td></td>
<td>[172, 331]</td>
<td>[34, 58]</td>
<td>[81, 184]</td>
<td>[55, 87]</td>
</tr>
<tr>
<td></td>
<td>&gt;30</td>
<td>0.80 (0.34-1.90)</td>
<td>1.70 (0.87-3.31)</td>
<td>3.16 (1.50-6.63)</td>
</tr>
<tr>
<td></td>
<td>[66, 80]</td>
<td>[10, 25]</td>
<td>[29, 34]</td>
<td>[27, 18]</td>
</tr>
<tr>
<td>$P$ for interaction = 0.009</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total folate (g/d)</td>
<td>≥492</td>
<td>1.0 (reference)</td>
<td>0.94 (0.55-1.62)</td>
<td>0.66 (0.36-1.22)</td>
</tr>
<tr>
<td></td>
<td>[128, 246]</td>
<td>[33, 56]</td>
<td>[64, 113]</td>
<td>[31, 75]</td>
</tr>
<tr>
<td></td>
<td>338-491</td>
<td>0.60 (0.30-1.20)</td>
<td>0.72 (0.42-1.25)</td>
<td>1.29 (0.72-2.31)</td>
</tr>
<tr>
<td></td>
<td>[127, 241]</td>
<td>[19, 54]</td>
<td>[57, 124]</td>
<td>[48, 62]</td>
</tr>
<tr>
<td></td>
<td>&lt;338</td>
<td>0.84 (0.43-1.63)</td>
<td>0.71 (0.41-1.22)</td>
<td>0.94 (0.51-1.74)</td>
</tr>
<tr>
<td></td>
<td>[122, 239]</td>
<td>[26, 47]</td>
<td>[61, 130]</td>
<td>[34, 59]</td>
</tr>
<tr>
<td>$P$ for interaction = 0.18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total folate and alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High folate, low alcohol</td>
<td>1.0 (reference)</td>
<td>1.22 (0.69-2.17)</td>
<td>0.91 (0.47-1.74)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>[116, 263]</td>
<td>[25, 63]</td>
<td>[62, 121]</td>
<td>[29, 77]</td>
</tr>
<tr>
<td>Low folate, high alcohol</td>
<td>1.17 (0.79-1.73)</td>
<td>0.99 (0.59-2.35)</td>
<td>1.23 (0.65-2.35)</td>
<td>1.66 (0.78-3.51)</td>
</tr>
<tr>
<td></td>
<td>[71, 122]</td>
<td>[11, 23]</td>
<td>[38, 66]</td>
<td>[22, 30]</td>
</tr>
<tr>
<td>$P$ for interaction = 0.24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: RR (95% CI) adjusted for age, previous endoscopy, year of endoscopy, family history, smoking history, aspirin use, body mass index, physical activity, and intakes of red meat and methionine.

[no. cases, no. controls]

Alcohol: categorized by the median of <7.6 versus ≥7.6 g/d; Total folate: categorized by low: ≤338 μg/d; high: >338 μg/d.
genotypes (Table 1). When we restricted our analyses to large or tubulovillous/villous adenomas (n = 148), the T5-adenoma relationship was similar to that of total adenoma (data not shown). We next examined joint effects of the T5 polymorphism and intakes of alcohol and folate in relation to adenoma risk (Table 1). Overall, alcohol consumption increased risk of colorectal adenoma; individuals who consumed >30 g/d of alcohol had an 81% (95% CI, 1.21-2.70) increase in risk (P for trend = 0.009). We also observed a significant TS-alcohol interaction (P for interaction = 0.009), with the highest risk observed for alcohol drinkers of >30 g/d with the 3R/3R genotype (RR, 3.16; 95% CI, 1.50-6.63). Among the 3R/3R individuals who comprised 28% of the population, there was a significant trend between alcohol intake and colorectal adenoma risk (P for trend <0.0001). Total folate or the combination of low folate and high alcohol was not associated with risk of colorectal adenoma in this population, nor were these relations modified by the TS polymorphism (Table 1).

As TS competes with MTHFR for 5,10-methylene-tetrahydrofolate as substrate for thymidylate synthesis, we examined the association of compound TS-MTHFR genotypes in relation to risk of colorectal adenoma (Fig. 1). A significant TS-MTHFR interaction was observed (P for interaction = 0.007). Individuals who were compound homozygous for TS 2R/2R and MTHFR 677CC (2R/2R-CC) had the lowest risk compared with individuals with other compound genotypes. Using this group (26 cases and 75 controls) as reference, there was a dose-dependent TS-alcohol interaction (P for trend = 0.02) as well as a TS-adenoma relationship only among the MTHFR 677CC individuals (P for trend = 0.02). Increase in risk of colorectal adenoma was significant in 3R/3R-CC (RR, 2.18; 95% CI, 1.22-3.92) and 2R/2R-CT (RR, 1.95; 95% CI, 1.06-3.59) individuals and borderline significant in 2R/2R-CC individuals (RR, 2.35; 95% CI, 0.97-5.69). Individuals with compound heterozygosity (i.e., 2R/3R-CT) had a nonsignificant elevation in adenoma risk (RR, 1.42; 95% CI, 0.84-2.41). There was no indication of an interaction between TS and the alcohol-metabolizing gene, ADH3.

Discussion

Our results lend consistent support to the notion that one-carbon metabolism plays an important role in pathogenesis of colorectal cancer. Thymidylate synthase catalyzes the transformation of dUMP to dTMP, the only de novo source of thymidine for DNA biosynthesis (10). The 28-bp tandem repeat polymorphism near the initiation start site of the TS promoter (15), which acts as a cis-acting enhancer element of the TS gene (16), was shown to be correlated with gene expression in terms of mRNA levels of the gene (11). We previously reported that individuals with the TS 3R/3R genotype (associated with higher mRNA levels) had significantly increased risk of colorectal cancer from an unrelated prospective study, the Physicians’ Health Study (9). However, diet and alcohol consumption was not comprehensively assessed in the Physicians’ Health Study, thus we could not systematically examine the TS-folate and TS-alcohol interactions. Although in the current study, we failed to observe a main effect of the TS in colorectal adenomas, presumed precursors for colorectal cancer, presence of the TS-alcohol interaction was similar to that of the MTHFR-alcohol interaction which was present in both colorectal cancer (6, 7) and adenomas (8). These observations suggest that TS plays an important role along the multistaged pathogenic pathway of colorectal cancer, but its impact may become more prominent in the later stage of carcinogenesis. Perturbation of TS may result in resetting the deoxynucleotide triphosphate pool (17) and possibly in increased DNA replication errors (18) leading to genomic instability. The resulting genomic instability is a hallmark of progression to invasive cancer and metastatic progression of tumors (19). It is worth mentioning that we previously reported similar findings with the MTHFR 677C > T polymorphism in terms of presence of the main gene effect in colorectal cancer (6, 7) but absence of this effect in adenoma (20), whereas gene-environment interactions were present in both diseases (6-8).

Lack of main TS effect in colorectal adenoma but presence of a significant interaction with folate intake has been reported previously in a case-control population undergoing screening by colonoscopy in Minnesota (21). The lack of interaction with folate but a stronger interaction with alcohol in the current study (8) may have reflected the fact that the participants in the Health Professionals Follow-up Study (and the U.S. population in general) were in general well nourished and few were folate deficient (2.7% of men in this study had daily folate intake below the recommended daily allowance of 200 g/d for male adults). A “functional” folate deficiency may become relevant only when an individual consumes moderate amounts of alcohol. Alcohol may impair folate metabolism by inhibiting DNA methyltransferase or methionine synthase, thus trapping folate as 5-methyltetrahydrofolate (thereby depleting cellular 5,10-methylene tetrahydrofolate; ref. 22). The synergistic effect of alcohol and TS corroborates the fact that 5,10-methylenetetrahydrofolate, as a substrate for TS, may be

**Figure 1.** Interactions of the MTHFR 677C > T polymorphism with the TS promoter polymorphism in relation to risk of colorectal adenoma from the Health Professionals Follow-up Study. The RRs were adjusted for age, previous endoscopy, year of endoscopy, family history, smoking history, aspirin use, body mass index, physical activity, and intakes of red meat and methionine. P for interaction = 0.007. *, P < 0.05. [no. cases, no. controls].
lowered even further by TS overexpression associated with the 3R/3R genotype. Importantly, the significant elevation in adenoma risk among the 5R/5R individuals at the alcohol level of >30 g/d is consistent with most epidemiologic literature in which alcohol consumption conferred higher risk of cancer only when the level is substantial (i.e., >20 g/d; ref. 23).

Based on the notion that the TS carried out reactions in favor of DNA synthesis, whereas the MTHFR is in favor of methylation, the apparent TS-MTHFR interactions in this study implicate that one-carbon metabolism functions as a "cross-talk" between genetic and epigenetic processes, both of which are important in colorectal carcinogenesis. Furthermore, this adverse effect is more pronounced when the biological folate supply is stressed by alcohol consumption. In a previous study by Ulrich et al. (21), an increased risk (OR, 1.81) was associated with 3R/3R-CC (when folate intake was low), a similar finding to our study. However, no elevation in risk was apparent among individuals with the 2R/2R-TT genotype. Interactions of TS-MTHFR warrant replication by other studies.

Although only about one third of eligible men in the cohort provided blood samples, major findings with respect to risk factors of colorectal adenoma reported in the overall cohort were similar in this nested, case-control study (8). The relatively homogeneous nature of the study population may limit generalizability, whereas it may reduce residual confounding by factors related to socioeconomic status. Another limitation is that the promoter polymorphism studied here may be one of many functional polymorphisms in the TS gene. A 6-bp deletion in the 3'-untranslated region of the TS gene (TS 1494del6) has been identified but results from our previous study does not support any functionality of this polymorphism (9). Recently, a G → C polymorphism in the second repeat of 3R alleles has been shown to alter the transcriptional activation of the gene (24). Due to budgetary constraint, this polymorphism was not analyzed in our study population. The prospective design of this study is of importance because bias related to differential reporting between cases and controls is unlikely. Finally, data on one-carbon metabolism and colorectal neoplasia are relatively sparse for women, so these findings need to be replicated in women.

Acknowledgments

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References


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