Lack of Association between the C677T *MTHFR* Polymorphism and Colorectal Hyperplastic Polyps[1]

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Abstract

Colorectal hyperplastic polyps are benign lesions that share many risk factors with colorectal adenomas and cancers. Low folate intakes are associated with an increased risk of colon cancer. The enzyme 5,10-methylene-tetrahydrofolate reductase (MTHFR) may be linked to DNA methylation and nucleotide synthesis and thus play a role in the etiology of colorectal neoplasia. We investigated an association between the common *MTHFR* polymorphism (C677T) and colorectal hyperplastic polyps within the Minnesota Cancer Prevention Research Unit case-control study. Cases ($n = 200$) were diagnosed with colonoscopically confirmed hyperplastic polyps; controls ($n = 645$) were derived from the same gastroenterology practice and were polyp-free at colonoscopy. Dietary intakes were estimated from a self-administered food-frequency questionnaire prior to colonoscopy. Multivariate adjusted odds ratios (ORs) and 95% confidence intervals for *MTHFR* status were 0.8 (0.6–1.2; *CT versus CC wild-type*) and 0.9 (0.5–1.6; *TT versus CC*). In subgroup analyses stratified on dietary intakes of folate, vitamin B_{12}, vitamin B_6, or methionine, those with the *TT* genotype and either low intakes of folate or vitamin B_6 were at increased risk relative to those with normal or high vitamin intake. However, most 95% confidence intervals included 1.0, and no consistent trends were observed. In contrast to our findings on colorectal adenomas, increasing alcohol consumption was associated with an elevated risk of colorectal hyperplastic polyps, regardless of genotype. The *MTHFR* (C677T) variant genotype does not appear to be related to risk of colorectal hyperplastic polyps, and there is no convincing evidence that *MTHFR* shows a different relation to risk, dependent on dietary intakes of nutrients related to its pathway.

Introduction

Colorectal carcinogenesis is a multistage process involving alterations in DNA methylation, hyperproliferation, adenoma formation, and growth, specific somatic genetic changes, and malignant transformation (1). The role of hyperplastic polyps in this carcinogenic process is uncertain. Whereas these polyps are often considered as benign lesions without carcinogenic potential, mixed polyps showing histological characteristics of both hyperplastic polyps and adenomas have been encountered (2–4). However, the frequency of the mixed polyp is not established because pathologists usually describe polyps by their most abnormal feature. Little research on the role of hyperplastic polyps in colon carcinogenesis is available. Many studies have focused on the possibility that rectal hyperplastic polyps are indicator lesions for the presence of more proximal adenomas or carcinomas but have reported disparate findings (5–9).

Hyperplastic polyps have molecular features indicative of neoplastic change that are intermediate between normal mucosa and adenomas or carcinomas (10–18). Furthermore, they share a number of common risk factors with colorectal adenomas and carcinomas: high alcohol intake, cigarette smoking, and a high body mass index have been found to be associated with an increased risk of hyperplastic polyps; use of aspirin or NSAIDs[3] and fiber intake are inversely associated (19, 20). Unlike adenomatous polyps, the number and size of hyperplastic polyps does not appear to increase with age (21, 22). Overall, it is reasonable to hypothesize that hyperplastic polyps may constitute an early event during colon carcinogenesis, and, if so, that only a small proportion progress to malignant lesions.

In epidemiological observational studies, low-folate diets have been found to increase the risk of adenomatous polyps and colon cancer (23–27) and the recurrence of adenoma (28). Other dietary factors, including the vitamins B_{12} and B_6, as well as methionine, play a role in folate metabolism (29, 30). Furthermore, alcohol intake may be related to folate availability, and some investigators have reported that a combination of low-folate, low-methionine, and high-alcohol intake resulted in a greater increase in risk (31, 32). The extent to which these dietary factors are related to the risk of hyperplastic polyps is largely unexplored.

The enzyme MTHFR is a central enzyme in folate metabolism (30). MTHFR plays a key role in the provision of methyl

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[3] The abbreviations used are: NSAID, nonsteroidal anti-inflammatory drug; MTHFR, 5,10-methylene-tetrahydrofolate reductase; THF, 5,10-methylene tetrahydrofolate; HRT, hormone replacement therapy; BMI, body mass index.

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groups by reducing THF to 5-methyl-THF. As substrate for the remethylation of homocysteine to methionine, 5-methyl-THF is linked to the production of *S*-adenosyl-methionine. *S*-Adenosyl-methionine is the universal methyl donor in humans and required for DNA methylation. The methylation of homocysteine is catalyzed by the enzyme methionine synthase (requiring vitamin B_{12} as a cofactor). Through its links to the production of dTMP via thymidylate synthase and to purine synthesis, MTHFR also plays a role in the provision of nucleotides essential for DNA synthesis.

We hypothesized that a defect in MTHFR could impact both DNA methylation and DNA synthesis and predicted interactions between such a defect, the nutritional cofactors vitamin B_{12}, vitamin B_6, and the substrate folate, and colorectal carcinogenesis. There is substantial experimental evidence that deficiencies in folate or vitamin B_{12} can affect DNA methylation (33, 34), result in deoxynucleotide pool disturbances (35–37), in uracil misincorporation into human DNA, and in genomic DNA strand breaks (38–40). Chromosome breaks are characteristic of nearly all human cancers, including colorectal cancer (41).

A common polymorphism in the *MTHFR* gene (C677T) has been identified. Individuals with the variant *MTHFR TT* genotype show ~30% of the enzyme activity found among those with the wild-type (*CC*) enzyme (42). Individuals who are heterozygous for the mutation (*CT*) have ~65% of wild-type enzyme activity (42). Individuals with the *TT* genotype, particularly if combined with a diet low in folate, have elevated plasma homocysteine levels, thus illustrating the physiological importance of this genotype (43–49).

Previous studies of colorectal cancer (50, 51) found a significantly decreased risk of colorectal cancer associated with the variant *TT* genotype that was not observed among those with low folate intakes or serum levels. Our own study of adenomas (52) showed an increased risk with *TT* genotype and low intakes of nutrients in the MTHFR pathway. We report here on the association between the *MTHFR* genotype and colorectal hyperplastic polyps within a clinic-based case-control study and explore possible modifications in risk with differing levels of intakes of folate, vitamin B_{12}, vitamin B_6, methionine, and alcohol.

Materials and Methods

Study Subjects. Subject recruitment for this case-control study has been described elsewhere (53). In brief, cases and controls were recruited through a large multiclinic private gastroenterology practice, Digestive Healthcare, which conducts colonoscopies in 10 hospitals, and, at the time of this study, undertook ~60% of all colonoscopies in metropolitan Minneapolis. All patients of ages 30–74 years who were scheduled for colonoscopy at all 10 Digestive Healthcare clinics between April 1991 and April 1994 were screened for the eligibility criteria described below and recruited prior to colonoscopy. Recruitment was initiated at the time of scheduling of the colonoscopy. The intent was to recruit subjects with both patient and recruiter blind to the final diagnosis. Indications for colonoscopy included bleeding, follow-up to sigmoidoscopy or barium enema, family history, and screening; indications by case/control status have been described previously (52).

Eligibility criteria for all participants were: resident of Twin Cities metropolitan area; age 30–74 years; English speaking; no known genetic syndromes associated with predisposition to colonic neoplasia; no individual history of cancer (except nonmelanoma skin cancer); and no history of inflammatory bowel disease. In addition, cases were eligible if their visit resulted in the diagnosis of colon or rectal hyperplastic polyps; carcinoma patients were excluded; controls were defined as being free of all polyps (hyperplastic or adenomatous) at colonoscopy.

The questionnaires used were self-administered, and patients received all study material (including food frequency questionnaires) before their clinic visit. At colonoscopy, the signed consent form and completed questionnaires were collected, and blood was drawn. The colonoscopy findings were recorded on standardized forms. Polyp size was measured *in vivo* by comparison of the polyp with a fully opened standard-sized flexible colonoscopy forceps. Upon removal, polyps were examined histologically by the study pathologist. Only participants with a complete colonoscopy reaching the cecum were eligible. The presence or absence of pathology was determined and, based on colonoscopy and pathology findings, participants were assigned to one of the following three groups: (*a*) adenomatous polyp group (defined as either adenomatous or mixed pathology, $n = 575$); (*b*) hyperplastic polyp-only group ($n = 219$); and (*c*) colonoscopy-negative group (controls, $n = 708$). The adenomatous polyp group was considered as a separate group and is not discussed further in this report (52). The participation rate for all colonoscoped patients was 68%.

Questionnaire. Questionnaires included information on dietary intake, physical activity, smoking habits, anthropometric measurements, medical information, demographic information, reproductive history (women), and family history of polyps and cancer (especially history of colon, breast, endometrial, or ovarian cancers). Study staff followed up with the participants when data were incomplete.

The dietary questionnaire was adapted from the Willett semiquantitative food frequency questionnaire, which has been evaluated for validity and repeatability within this study (54, 55), as well as the Nurses' Health Study cohort (56), the Iowa Women's Health Study cohort (57), and the Health Professionals Follow-up Study cohort (58). Participants were asked to recount their average consumption, over the past year, of 127 food items. For each food, a commonly used portion size was defined and participants selected one of nine categories ranging from "never or less than once per month" to "6 or more per day" to describe their frequency of consumption. Data on the brand of breakfast cereal and the brand and frequency of multivitamin and individual vitamin supplement use were collected. Daily intakes of nutrients were computed using composition values from United States Department of Agriculture sources (59), which are continually supplemented by other published sources and personal communications from laboratories and manufacturers. Among a similar study population, correlation coefficients of this instrument on repeat administration were $r = 0.62$ for dietary folate, $r = 0.67$ for vitamin B_{12} intake, and $r = 0.99$ for alcohol consumption (57). Giovannucci *et al.* (31) compared Willett questionnaire values with RBC folate levels (an indicator of long-term folate status) and reported correlations between folate intake and erythrocyte folate levels of $r = 0.55$ for women and $r = 0.56$ for men.

Blood Collection and Processing. Venous blood was collected from each participant in two 20-ml ACD vacutainer tubes. White cells, red cells, and plasma were separated according to a standardized protocol. White cells were stored, in appropriate cell culture medium, as multiple 0.5-ml aliquots at −70°C for DNA extraction or preparation of cell lines. White cells were shipped in frequent batches to the University of Utah for extraction of DNA. DNA was extracted using the PureGene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN).

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Table 1 *MTHFR* genotype and hyperplastic polyps: characteristics and dietary intakes of the study population

	Cases (n = 200)	Controls (n = 645)	P
Age (yr)			
<40	11%	13%	
40–49	20%	26%	
50–59	39%	32%	
60–69	24%	23%	
70+	6%	6%	0.10[a]
Sex			
Men	57%	38%	
Women	43%	62%	0.001[a]
Race/Ethnicity			
White	97%	97%	
Other	3%	3%	0.89[a]
Smoking status			
Current	30%	15%	
Ex-smoker	48%	37%	
Never	22%	48%	0.001[a]
MTHFR genotype			
CC (wild-type)	51%	47%	
CT (heterozygous)	37%	42%	
TT (homozygous variant)	13%	11%	0.41[a]
Dietary intakes			
Intake from diet (not including supplements)[b]			
Folate (μg/day)	295 ± 115 (107–699)	316 ± 152 (84–1161)	0.24[d]
Vitamin B_{12} (μg/day)	6.39 ± 4.93 (1.53–51.00)	6.72 ± 5.21 (0.40–61.16)	0.64[d]
Vitamin B_6 (mg/day)	2.00 ± 0.69 (0.64–4.14)	2.13 ± 0.90 (0.62–6.52)	0.13[d]
Methionine (g/day)	1.89 ± 0.66 (0.46–3.86)	1.94 ± 0.80 (0.27–6.25)	0.76[d]
Alcohol (g/day)	10.6 ± 17.1 (0–123.6)	6.6 ± 13.7 (0–96.3)	0.0001[d]
Total intakes (including supplements)			
Folate (μg/day)	404 ± 223 (107–1287)	412 ± 243 (84–1457)	0.88[d]
Vitamin B_{12} (μg/day)	9.57 ± 10.96 (1.53–102.99)	9.24 ± 9.23 (0.40–105.34)	0.67[d]
Vitamin B_6 (mg/day)	6.04 ± 15.67 (0.64–102.55)	7.12 ± 18.33 (0.62–103.7)	0.42[d]
Proportion of individuals with dietary intakes (including supplements) <75% RDA[c]			
Folate	42%	43%	0.69
Vitamin B_{12}	4%	6%	0.17
Vitamin B_6	7%	9%	0.38
Current alcohol intake			
None	33%	45%	
≤7 g/day	27%	29%	
>7 g/day	40%	26%	0.001[d]

[a] P based on χ^2 or t test.
[b] Mean ± SD (range).
[c] 1998 recommended dietary allowances for men and women age 51 and older (63): folate, 400 μg/day; vitamin B_{12}, 2.4 μg/day (most of this amount to be obtained from foods fortified with B_{12} or from supplements); vitamin B_6, men 1.7 mg/day, women 1.5 mg/day.
[d] t test (two-tailed). Not-normally-distributed nutrient intakes were log transformed, alcohol intake square-root transformed.

DNA was quantitated and examined for purity by UV absorption at 260 and 280 nm (60), and extracted DNA was shipped to Seattle for genotyping analyses.

***MTHFR* Genotyping.** The *MTHFR* polymorphism at bp 677 was determined using the PCR/RFLP method of Frosst *et al.* (42). PCR reactions were done on a Deltacycler II thermal cycler in 96-well plates (Ericomp, San Diego, CA). Primers (5′-TGA AGG AGA AGG TGT CTG CGG GA-3′) and (5′-AGG ACG GTG CGG TGA GAG TG-3′) were used to amplify a portion of the *MTHFR* sequence from 100 ng of genomic DNA in a 30-μl reaction containing 3 μl of 10× PCR buffer [100 mM Tris-HCl (pH 8.3) at 25°C, 500 mM KCl, 15 mM $MgCl_2$, and 0.01% (w/v) gelatin; Perkin-Elmer], 50 μg/ml BSA, 0.2 mM deoxynucleotide triphosphates, 0.2 μM each primer, and 1 unit of Taq DNA polymerase. Cycling conditions were: initial melting at 93°C for 5 min, then 30 amplification cycles of 93°C for 60 s, 58°C for 60 s, and 72°C for 60 s. Upon amplification, the 198-bp *MTHFR* fragment was digested with *Hin*fI in a 20-μl reaction containing 10 μl of PCR fragment, 2 μl of 10× buffer H (Amersham Life Science; supplied by the manufacturer), and 4 units of *Hin*fI at 37°C for 1 h. The resulting digestion products were separated on a 3% NuSieve agarose gel (FHC Corp.), and the ethidium bromide-stained bands were photographed on a UV transilluminator.

Wild-type (*CC*) individuals were characterized by a 198-bp fragment only, heterozygotes (*CT*) by fragments of 198, 175, and 23 bp, and homozygote variants (*TT*) by fragments of 175 and 23 bp. Blinded repeat genotyping was undertaken for 20 DNA samples. Reproducibility of the genotype was 100%. DNA quality or quantity was insufficient for *MTHFR* genotyping in 19 cases and 63 controls, which resulted in a final sample size of 200 cases and 645 controls.

Statistical Data Analysis. Standard techniques for unmatched case-control studies were used. Odds ratios and 95% confidence intervals were estimated by logistic regression analysis. The association between *MTHFR* genotype and hyperplastic polyps was estimated first in the entire study population and then in subsets based on sex, age, polyp characteristics, and

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Table 2 *MTHFR* genotype and hyperplastic polyps (n = 822)

(n cases/n controls)	Genotype			P^a (interaction)
	CC (cases/controls)	*CT* (cases/controls)	*TT* (cases/controls)	
All subjects (multivariate adjusted)[b]	1.0 (ref) (98/297)	0.8 (0.6–1.2) (72/258)	0.9 (0.5–1.6) (26/71)	
Men	1.0 (ref) (56/110)	0.7 (0.4–1.2) (39/102)	1.2 (0.6–2.5) (16/27)	
Women	1.0 (ref) (42/187)	0.9 (0.5–1.5) (33/156)	0.7 (0.3–1.6) (10/44)	
Number of hyperplastic polyps				
One	1.0 (ref) (43/297)	0.9 (0.6–1.4) (37/258)	1.1 (0.6–2.0) (15/71)	
Two or more	1.0 (ref) (55/297)	0.7 (0.4–1.3) (35/258)	0.7 (0.3–1.9) (11/71)	
Age[c]				
<60 years	1.0 (ref) (68/198)	0.8 (0.5–1.2) (53/188)	0.8 (0.4–1.6) (16/55)	
≥60 years	0.9 (0.5–1.6) (30/99)	0.8 (0.4–1.4) (19/70)	1.6 (0.7–3.9) (10/16)	0.37
Family history of colon cancer (first degree)				
No	1.0 (ref) (81/216)	0.7 (0.5–1.1) (53/183)	0.8 (0.4–1.5) (19/54)	
Yes	0.6 (0.3–1.1) (16/78)	0.8 (0.5–1.5) (19/74)	1.1 (0.4–2.9) (6/17)	0.27
Smoking status				
Never	1.0 (ref) (21/146)	0.9 (0.4–1.7) (16/121)	1.2 (0.4–3.6) (6/28)	
Former	2.4 (1.3–4.4) (50/107)	2.1 (1.2–4.0) (36/95)	1.7 (0.7–4.2) (10/32)	
Current	4.2 (2.1–8.4) (27/44)	3.0 (1.4–6.3) (20/42)	6.4 (2.3–17.2) (10/11)	0.85

[a] P for interaction, testing for different slopes with variable of interest across genotypes.
[b] Multivariate adjustment for: age; sex; BMI; hormone replacement therapy (yes/no); smoking (pack-years); percentage kilocalories from fat, dietary fiber, and folate; vitamin B_{12}; vitamin B_6; methionine; and alcohol. ref, reference value.
[c] Not adjusted for age.

dietary intakes. We also undertook a polytomous regression analysis with four possible outcomes: clean colon; hyperplastic polyp(s) only; hyperplastic plus adenomatous polyps; and adenoma only.

Effect modification was evaluated by stratification on the variable of interest, and ORs in each stratum were compared. Potential confounders were evaluated in the following manner. An age- and sex-adjusted OR was computed for each association. Subsequently, a full multivariate model with all potential confounders was fitted, and the log likelihood and the ORs were compared with the age-sex-adjusted model. Then all factors were eliminated separately from the model to evaluate its effect on the OR of interest. Only covariates that altered any OR of interest by at least 10% were maintained in the model. The following potential confounding factors were evaluated: age, sex, race/ethnicity, HRT (ever/never), BMI, waist:hip ratio, pack-years of smoking, regular use of aspirin (at least 1 per week), regular use of NSAIDs (at least 1 per week), hours of physical activity, and the dietary intake variables kilocalories, dietary fiber, percentage of kilocalories from fat, vitamin B_6, vitamin B_{12}, folate, and alcohol.

The subset of these variables maintained for multivariate adjustment was: age, sex, BMI, percentage of kilocalories from fat, dietary fiber intake (g), HRT use (ever/never), pack-years of smoking, and dietary intakes of folate, vitamin B_{12}, vitamin B_6, methionine, and alcohol. All adjustment variables were included in the model as continuous variables, with the exception of HRT use and sex. In general, the confounding effects were small and often only apparent in the analyses stratified by dietary intakes. With the exception of number of pack years and folate intake (both greater among those with TT genotype), confounding factors were related more to diet than the MTHFR genotype. For consistency, we report the multivariate-adjusted estimates throughout.

Tertiles of consumption of nutrients were determined based on the distribution in the controls. Alcohol intake was divided into three groups by separating nondrinkers from drinkers and dividing those who drank alcohol by their median intake.

Statistical significance testing was conducted on multiple levels. Differences in nutrient intakes and other population characteristics between cases and controls were assessed with t tests and χ^2 tests. Nutrient intakes that were not normally distributed were log transformed (square root transformed for alcohol intake). To evaluate the dose-response relationship between nutrient intakes or other variables and colorectal hyperplastic polyps within each of the three genotypes, a test for trend was used. Effect modification of the relation between nutrients and other variables and risk of polyps by genotype was assessed by testing for different slopes with nutrient intake across genotype. All tests of statistical significance were two-sided.

Results

Characteristics of the study population are shown in Table 1. Cases and controls were similar with respect to race/ethnicity

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Table 3 *MTHFR* genotype and hyperplastic polyps: risks associated with dietary variables ($n = 822$)[a]

Nutrient intakes from both diet and supplements (*n* cases/*n* controls)	Genotype			
	CC (98/297)	*CT* (72/258)	*TT* (26/71)	
Folate				
Low[b]	1.0 (ref)	1.1 (0.6–2.1)	1.8 (0.6–4.9)	
Medium	1.5 (0.8–2.8)	1.2 (0.6–2.3)	1.7 (0.7–4.2)	
High	1.7 (0.9–3.4)	1.0 (0.5–2.2)	1.1 (0.4–2.9)	
P for inter-tertile slope	0.10	0.81	0.43	
P for interaction[c]				0.19
Vitamin B_{12}				
Low[b]	1.0 (ref)	1.5 (0.8–2.9)	1.3 (0.4–3.8)	
Medium	2.2 (1.2–4.2)	1.4 (0.7–2.7)	2.6 (1.0–6.8)	
High	1.5 (0.7–3.1)	0.8 (0.3–1.8)	1.2 (0.4–3.1)	
P for inter-tertile slope	0.30	0.11	0.76	
P for interaction[c]				0.09
Vitamin B_6				
Low[b]	1.0 (ref)	1.2 (0.6–2.3)	2.0 (0.8–5.5)	
Medium	1.9 (1.0–3.7)	1.4 (0.7–2.8)	1.5 (0.6–3.7)	
High	1.2 (0.5–2.6)	0.7 (0.3–1.6)	0.9 (0.3–2.7)	
P for inter-tertile slope	0.51	0.28	0.30	
P for interaction[c]				0.19
Methionine				
Low[b]	1.0 (ref)	1.2 (0.7–2.2)	1.0 (0.3–2.7)	
Medium	0.9 (0.5–1.7)	0.6 (0.3–1.2)	1.1 (0.4–2.6)	
High	0.8 (0.4–1.6)	0.5 (0.2–1.0)	0.7 (0.3–1.8)	
P for inter-tertile slope	0.53	0.01	0.62	
P for interaction[c]				0.24
Alcohol				
None[b]	1.0 (ref)	1.3 (0.7–2.4)	1.1 (0.4–2.8)	
Medium	1.6 (0.8–2.9)	1.4 (0.7–2.6)	1.9 (0.7–5.2)	
High	3.1 (1.7–5.6)	1.4 (0.7–2.6)	2.6 (1.0–6.3)	
P for inter-tertile slope	0.0004	0.93	0.14	
P for interaction[c]				0.05

[a] Multivariate adjustment for: age; sex; BMI; hormone replacement therapy (yes/no); smoking (pack-years); percentage kilocalories from fat, dietary fiber, and folate; vitamin B_{12}; vitamin B_6, methionine; and alcohol, where appropriate.
[b] Cutpoints for tertiles of dietary intakes: folate, 268 μg/440 μg; vitamin B_{12}, 5.18 μg/9.77 μg; vitamin B_6, 1.94 mg/3.18 mg; methionine, 1.54 g/2.16 g; and alcohol, 0 g/7 g per day.
[c] Testing for different slopes with nutrient intake across genotypes.

and age. They also did not differ on use of aspirin, NSAIDs, and HRT (data not shown). Cases were more likely to be male and to be current smokers. Prevalences of the *MTHFR* genotype did not differ between cases and controls; however, among cases, the *MTHFR* genotype was not distributed according to a Hardy-Weinberg Equilibrium. The *CT* genotype was relatively underrepresented. With respect to dietary intakes, there were few differences between cases and controls. A larger proportion of the cases reported consuming alcohol, and the average alcohol intake was higher in cases than in controls.

Intakes of folate, vitamin B_{12}, vitamin B_6 (including supplements), and methionine were not significantly associated with risk of colorectal hyperplastic polyps, although there were trends toward lower risk with higher dietary intakes of vitamin B_{12} and B_6. Alcohol intake was associated with an increased risk [compared with nondrinkers: >0–7 g/day, OR, 1.2 (0.8–1.9); >7 g/day, OR, 1.7 (1.1–2.6)].

Associations between the *MTHFR* genotype and risk of hyperplastic polyps are shown in Table 2. Overall, there was no significant association between the *MTHFR* genotype and risk of hyperplastic polyps, and multivariate adjustment did not change these results. There were also no consistent trends in risk across genotypes within subgroups, *e.g.*, based on sex.

Associations between the *MTHFR* genotype and hyperplastic polyps stratified on dietary intakes of folate, vitamin B_{12}, vitamin B_6, methionine, and alcohol are shown in Table 3. Among those with the *TT* genotype, there were some nonsignificant trends in risk by folate intake and in risk by vitamin B_6 intake, but the numbers generally were too small to allow firm conclusions. An increase in risk of hyperplastic polyps associated with increasing alcohol intake was observed regardless of *MTHFR* genotype.

A polytomous regression analysis with four outcome groups (clean colon; adenoma; hyperplastic polyp plus adenoma; and hyperplastic polyp only) showed little variation in the associations reported here for hyperplastic polyps (data not shown).

Discussion

To our knowledge, this is the first study to report on the risk of colorectal hyperplastic polyps associated with the *MTHFR* genotype. The results presented here on hyperplastic polyps differ from those obtained on adenomatous polyps in the same population (52), as well as those obtained from other studies of colon cancer and adenoma (50, 51, 61, 62). In the study of adenomatous polyps, we observed elevated risks associated with the variant *MTHFR* genotype (*TT*) among those with low intakes of folate, vitamin B_{12}, and vitamin B_6. These increases in risk were particularly pronounced among the elderly (>60 years of age). However, in this study of hyperplastic polyps and *MTHFR* genotype, little association with the *MTHFR* genotype and no consistent trends across nutrient intakes were seen.

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Furthermore, increasing alcohol consumption was associated with an increased risk of hyperplastic polyps, irrespective of genotype. In contrast, for adenomatous polyps we previously observed a significant gene-environment interaction between alcohol intake and *MTHFR* genotype; surprisingly, in that study, lower risks were found among those with the *TT* genotype who drank any alcohol.

Hyperplastic polyps appear to be subject to fewer mutagenic events than colorectal adenomas or carcinomas. For example, mutations in the *p53* tumor suppressor gene occur frequently in carcinoma and in some adenomas but have not been observed in hyperplastic polyps (16, 17). A similar relationship has been reported for adenomatous polyposis coli mutations (18). In contrast, K-*ras* activation appears to occur quite frequently (about 12–50%) in hyperplastic polyps, comparable, perhaps, with that seen in adenomas (37–56%; Refs. 16 and 18). On the basis of our study of adenomas, we hypothesized that the *MTHFR TT* genotype in combination with low intakes of folate or vitamins B_{12} and B_6 will result in mutagenesis, both because of disturbances in DNA synthesis and DNA methylation changes. Disturbances in DNA synthesis can result in misincorporation of uracil instead of thymidine into DNA, resulting in DNA strand breaks. Because hyperplastic polyps show fewer mutagenic events than adenomas and carcinomas, it is plausible that there would be no increase in risk of hyperplastic polyps in those with a variant *MTHFR* genotype. Reduced DNA methylation could alter gene expression, but its effects on mutagenesis are less well understood.

Cases with hyperplastic polyps were significantly younger (mean age, 53.7 years) than those with adenomatous polyps (mean age, 58.1 years). Among the latter, most of the associations between *MTHFR* genotype and adenomas associated with low dietary intakes of B vitamins were evident among older individuals (>60 years of age; Ref. 52). This may be explained by a decline in the ability to absorb certain vitamins with increasing age, thus exacerbating risks associated particularly with low intakes. Consistently, the relative risk for hyperplastic polyps associated with the *TT* genotype was higher among individuals >60 years (Table 2), yet the number of cases in this age group was small. The younger age of the hyperplastic polyp patients and the different biology, therefore, may both contribute to the difference in findings compared with adenomatous polyps.

Another possible explanation for the absence of an association in this study may be study size. The number of cases in this study was smaller ($n = 200$) than in the adenoma study, and only 26 cases were carriers of the variant *TT* genotype. Within such small strata, chance plays a larger role, and the statistical power to detect a gene-environment interaction is small. Our ability to evaluate low B vitamin intakes, which comprised the strata at greatest risk in the adenoma study, was therefore restricted, and our results should be considered exploratory. Another limitation of this study was the reliance on dietary data from questionnaires. Strengths of this study are its specific focus on hyperplastic polyps, its design (patients were drawn from the same clinic, and both cases and controls had complete colonoscopies), and the comprehensive information obtained on dietary intakes.

In conclusion, in contrast to previous findings on adenomatous polyps, our data do not provide evidence of associations between the *MTHFR* genotype, nutrients involved in folate metabolism, and colorectal hyperplastic polyps. These observations add to the accumulating evidence of the differing biology of adenomatous and hyperplastic polyps.

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