

MTHFR Genotype and Colorectal Adenoma Recurrence: Data from a Double-blind Placebo-controlled Clinical Trial

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Abstract

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism. We assessed the association between two common MTHFR variants, 677C>T and 1298A>C, and adenoma recurrence in the context of a randomized double-blind clinical trial of aspirin use and folate supplementation. We used generalized linear regression to estimate risk ratios and 95% confidence intervals (95% CI) for recurrence, adjusting for age, sex, clinical center, follow-up time, and treatment status. Neither MTHFR polymorphism was associated with overall or advanced adenoma recurrence.

Compared with those with two wild-type alleles, the relative risk for advanced adenoma was 0.75 (95% CI, 0.36-1.55) for the MTHFR 677 TT genotype and 1.16 (95% CI, 0.58-2.33) for the MTHFR 1298 CC genotype. The effect of folate supplementation on recurrence risk did not differ by genotype. Our findings indicate that the MTHFR genotype does not change adenoma risk in a manner similar to its effect on colorectal cancer, and does not modify the effect of folate supplementation on metachronous adenoma risk. (Cancer Epidemiol Biomarkers Prev 2008;17(9):2409-15)

Introduction

One-carbon groups are essential for many physiologic reactions including nucleotide synthesis and DNA methylation (1-4). In humans, virtually all one-carbon groups are carried by folates. Data from human studies support an association between folate deficiency and an increased risk of colorectal neoplasia (5).

Methylenetetrahydrofolate reductase (MTHFR) irreversibly reduces 5,10-methylene-tetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate (5-MTHF) in a flavin adenine dinucleotide-dependent reaction that shunts one-carbon groups from DNA synthesis toward the synthesis of the primary methyl donor S-Adenosylmethionine (SAM; ref. 6). Two common polymorphisms in the *MTHFR* gene, 677C>T (*rs1801133*; Ala222Val) and 1298A>C (*rs11801131*; Glu429Ala), have been frequently studied. The 677 Val/Val enzyme has been associated with a 65% to 70% reduction in enzyme activity *in vitro* (7-9) and an approximately 20% decrease in colorectal cancer risk among folate-replete individuals

in vivo (10-12). The published studies, however, suggest that the *MTHFR* 677 TT genotype does not have the same association with colorectal adenoma risk as it does for colorectal cancer, with a summary odds ratio (OR) of approximately 1.0 in meta-analyses (10-12), although some studies have suggested an increased adenoma risk in those with a low folate status (13-17), or among subjects not using multivitamins (18).

The *MTHFR* 1298A>C polymorphism has been associated with a reduction in enzyme activity relative to the wild-type enzyme (AA genotype) in some (8, 19) but not all (9) *in vitro* studies. Epidemiologic data regarding associations of this polymorphism with the risk of colorectal cancer or adenomas are inconsistent, and do not provide strong support for an independent effect (10, 11).

Although there is now a reasonable body of data assessing how genetic variation in *MTHFR* is associated with the risk of colorectal neoplasia (10, 11, 20), the assessment of interactions with folate intake is hampered by the limitations of nutritional epidemiology, most prominently measurement error in the assessment of folate intake. Investigations of associations with adenoma risk are further limited by the fact that most studies have focused on prevalent rather than incident adenomas. We recently reported data from a randomized double-blind clinical trial of a 1 mg/day folic acid supplement versus a placebo on the risk of adenoma recurrence (21). In the current analysis, we assess the association of the *MTHFR* genotype with the risk of adenoma recurrence in the

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Table 1. Selected subject characteristics at baseline and year 3

Characteristic	Placebo (<i>n</i> = 461)	Folic acid (<i>n</i> = 462)	<i>P</i>
Mean age in y (SD)	57.5 (9.6)	57.7 (9.6)	0.726
Male sex <i>n</i> (%)	297 (64.4)	296 (64.1)	0.910
Race/Ethnicity <i>n</i> (%)			
White	405 (87.9)	410 (88.7)	0.695
African American	25 (5.4)	20 (4.3)	
Hispanic	17 (3.7)	22 (4.8)	
Other	14 (3.0)	10 (2.2)	
Current cigarette smoker <i>n</i> (%)	60 (13.1)	70 (15.2)	0.615
Mean dietary intake in kcal/d (SD)	1,642.3 (649.6)	1,616.2 (656.8)	0.552
Mean dietary folate intake in µg/d (SD)	329.0 (165.3)	317.2 (145.1)	0.260
Mean baseline plasma folate in nmol/L (SD)	23.7 (16.8)	23.9 (18.1)	0.873
Mean year-3 plasma folate in nmol/L (SD)	30.0 (14.1)	74.9 (35.9)	<0.0001
Mean baseline plasma T-homocysteine in µmol/L (SD)	9.8 (2.9)	9.8 (2.9)	0.806
Mean year-3 plasma T-homocysteine in µmol/L (SD)	9.2 (2.5)	8.9 (2.2)	0.074
MTHFR 677 C>T genotype			
CC <i>n</i> (%)	207 (45.0)	213 (46.2)	0.614
CT <i>n</i> (%)	201 (43.7)	205 (44.5)	
TT <i>n</i> (%)	52 (11.3)	43 (9.3)	
MTHFR 1298 A>C genotype			
AA <i>n</i> (%)	234 (50.8)	220 (47.6)	0.283
AC <i>n</i> (%)	193 (41.9)	195 (42.2)	
CC <i>n</i> (%)	34 (7.4)	47 (10.2)	
Joint genotypes (677/1298)			
CC/AA (baseline) <i>n</i> (%)	72 (15.7)	71 (15.4)	
CC/AC <i>n</i> (%)	101 (22.0)	96 (20.8)	
CC/AA <i>n</i> (%)	34 (7.4)	46 (10.0)	0.670
CT/AA <i>n</i> (%)	110 (23.9)	106 (23.0)	
CT/AC <i>n</i> (%)	91 (19.8)	99 (21.5)	
CT/CC <i>n</i> (%)	0	0	
TT/AA <i>n</i> (%)	52 (11.3)	43 (9.3)	
TT/AC <i>n</i> (%)	0	0	
TT/CC <i>n</i> (%)	0	0	

NOTE: Of the 1,021 subjects randomized to folate treatment or placebo, MTHFR 677C>T and MTHFR 1298A>C genotype data were available for for 923 subjects (90%) and of these subjects 23 did not have a year-3 colonoscopy and are not included in further analyses. Missing data: Smoking status, *n* = 4; Dietary folate, *n* = 36; Dietary intake, *n* = 36; Plasma folate, *n* = 67; Baseline HCY, *n* = 64; Year-3 plasma folate and HCY, *n* = 119.

context of this trial (21, 22) and investigate whether genetic variation in MTHFR modifies the effect of a folic acid supplement on recurrence. Our clinical trial design allowed us largely to avoid the difficulties of nutritional assessment, because we provided a fixed amount of folate as a study treatment. Our study also allows us to assess the effect of genotype on incident rather than prevalent adenomas, making clear the temporal relationships between intake and adenoma occurrence.

Materials and Methods

Design. These data were collected as part of a randomized, double-blind, placebo-controlled trial of the efficacy of oral aspirin (ASA), folic acid, or both to prevent colorectal adenomas as described in (21, 22). Briefly, this was a three-by-two factorial design in which subjects were randomized to receive 81 mg/d ASA, 325 mg/d ASA or placebo. Within each ASA/placebo group, subjects were additionally randomized to receive supplemental 1 mg folic acid/d or a placebo (22). The study initially focused only on aspirin; 100 subjects who were randomized only to aspirin are not included in this analysis.

Recruitment, Randomization, Treatment, and Follow-up. The details of subject eligibility, recruitment, randomization, treatment and follow-up, and study outcomes have been described (22, 23). Briefly, the subjects were recruited from July 1994 until March 1998 from 9

clinical centers. Eligible subjects were between 21 and 80 y of age, in good health, and had received a recommendation for follow-up colonoscopy by their regular medical practitioner. Each eligible subject had at least one or more histologically confirmed colorectal adenomas removed within 3 mo of recruitment, or one or more histologically confirmed adenomas removed within 16 mo of enrollment as well as a history of two or more confirmed adenomas or an adenoma greater than 1 cm in diameter removed within 16 mo of enrollment. Individuals were ineligible if they had a history compatible with a familial colorectal cancer syndrome, invasive colorectal cancer, any malabsorption syndrome, a medical condition that could be worsened by use of aspirin or folic acid, or any medical condition commonly treated with aspirin or folate. A complete colonoscopy with removal of all polyps within 3 mo of enrollment was required for all subjects. Each subject underwent a 3-mo run-in period on 325 mg ASA prior to randomization into ASA and folate treatment groups. Only subjects with at least 80% compliance and no other contraindications were randomized. By protocol, all subjects had an anticipated follow-up surveillance complete colonoscopy 34 to 40 mo after the qualifying examination.

To assess whether a longer follow-up was needed to observe an effect of folate treatment, participants completing their first follow-up colonoscopy were invited to continue their blinded folate treatment for another colonoscopic surveillance cycle (generally 3-5 y).

In this analysis, we defined the second follow-up interval from the time after the year-3 colonoscopy through the subsequent second colonoscopy or October 1, 2004, whichever came first. Of the 1,021 participants initially randomized to folate or placebo, 729 (71.4%) continued with their randomized treatment and 607 (59.5%) completed their second follow-up examination with a mean (SD) follow-up time of 41.8 (11.8) mo. The current analysis was limited to adenomas that had been found by the time of the protocol year-3 follow-up colonoscopy (36-40 mo after the baseline colonoscopy).

Study Outcomes. The primary study outcome was the proportion of patients in whom one or more colorectal adenomas were detected in the period starting 1 y year after randomization to the end of the year-3 surveillance follow-up examination. If a year-3 colonoscopy was not performed, we used the last examination at least 1 y after randomization. Polyps were classified as neoplastic (adenomatous) or nonneoplastic by the study pathologist, who also assessed the degree of dysplasia and the extent of villous component in each adenoma. We defined advanced lesions as invasive carcinoma or adenomas with at least 25% villous component, high-grade dysplasia, or an estimated size of ≥ 1 cm. Patients were considered to have had "multiple adenomas" when there were a total of 3 or more follow-up adenomas by the end of the year-3 exam. The plasma levels of folate were determined by microbiological assays using a colistin sulphate resistant strain of *Lactobacillus leichmannii*, and RBC folate was determined by the ACS:180 folate assay, a competitive immunoassay using direct chemiluminescent technology (Bayer Corporation).

We assessed the *MTHFR*/recurrence association within the strata of post-follow-up RBC and plasma folate levels, age, sex, and subsite of the large bowel (right colon and left colorectum). Median values for folate measurements, taken at baseline and the end of follow-up, and age were defined by reference to the entire population of subjects.

Genotyping. The *MTHFR* polymorphisms, 677C>T and 1298A>C, were genotyped with the 5'nuclease TaqMan allelic discrimination assay using the ABI7900 (Applied Biosystems). PCR primers and dual-labeled allele discrimination probes were designed using the Primer Express software package (PE Biosystems) as described in (24). Each 384-well assay contained internal quality controls for homozygous wild-type, heterozygous, and homozygous variant alleles for the respective polymorphisms along with no template controls. Genotype calls were determined by SDS 2.1 analysis software.

Statistical Analysis. The statistical analyses were as described in Cole 2007 (21). All analyses of folic acid treatment were done using an intention-to-treat strategy, which included all randomized participants who had a follow-up colonoscopy whether or not they had continued using their supplement. To measure the association between folate status and genetic variants and adenoma risk, we estimated genotype-specific risk ratios (RR) and 95% confidence intervals (95% CI) for one or more adenomas after randomization, calculated with generalized linear regression analyses using a logarithmic linkage and a binomial distribution correcting for over- and under-dispersion. Genotype was coded by the number of variant alleles: 0, 1, or 2. We obtained the RRs and the *P* for trend using orthogonal linear contrasts. Interactions between genotype and treatment group were evaluated using product interaction terms. We used Wald tests to assess the significance of these terms. All effect estimates were adjusted for age, sex, study center, aspirin treatment group, and follow-up time. We assessed Hardy-Weinberg equilibrium by using a contingency table χ^2 test to compare observed genotype frequencies to those expected under Hardy-Weinberg equilibrium. All tests of statistical significance are two-sided. We considered the joint genotypes to assess possible effects of compound heterozygosity (8, 25, 26).

Table 2. Association of MTHFR genotype and risk of recurrent adenomas

Genotype	Any adenoma		Advanced adenoma*	
	No.	RR [†] (95% CI)	No.	RR [†] (95% CI)
677C>T (n)				
CC (405)	182	1.0	45	1.0
CT (400)	177	0.97 (0.83-1.14)	37	0.81 (0.36-1.55)
TT (93)	36	0.89 (0.68-1.16)	8	0.75 (0.36-1.55)
<i>P</i> for trend [‡]		0.42		0.28
1298A>C (n)				
AA (444)	182	1.0	43	1.0
AC (379)	178	1.14 (0.98-1.33)	38	1.03 (0.68-1.57)
CC (77)	36	1.14 (0.87-1.50)	9	1.16 (0.58-2.33)
<i>P</i> for trend [‡]		0.12		0.71
MTHFR 677/1298 genotype	No.	RR [†] (95% CI)	No.	RR [†] (95% CI)
CC/AA (137)	55	1.0	14	1.0
CC/AC (192)	91	1.17 (0.90-1.51)	22	1.08 (0.56-2.07)
CC/CC (76)	36	1.17 (0.85-1.60)	9	1.05 (0.47-2.37)
CT/AA (214)	91	1.03 (0.80-1.33)	21	0.89 (0.47-1.72)
CT/AC (186)	86	1.14 (0.88-1.47)	16	0.79 (0.40-1.58)
TT/AA (93)	36	0.98 (0.72-1.35)	8	0.78 (0.34-1.81)

*Advanced adenoma defined as >25% tubulovillous or villous adenoma, large adenoma (≥ 1 cm), advanced dysplasia, carcinoma *in situ*, or invasive cancer.

[†]RRs were adjusted for age, sex, clinical center, treatment assignment, and follow-up time.

[‡]Wald test used to generate *P* values.

Table 3. Effect of folate treatment on the risk of adenomas, by MTHFR genotype

Genotype	Any adenoma		Advanced adenoma*	
	Ns [†]	Folate/Placebo RR [‡] (95% CI)	Ns [†]	Folate/Placebo RR (95% CI)
677C>T (n) Folate/Placebo [†]	Folate /Placebo [†]		Folate/Placebo	
CC (204/201)	96/86	1.09 (0.87-1.36)	21/22	0.96 (0.54-1.68)
CT (203/197)	93/84	1.06 (0.85-1.33)	22/15	1.48 (0.78-2.79)
TT (43/50)	16/20	0.93 (0.57-1.53)	4/4	1.30 (0.34-4.96)
1298A>C (n)				
AA (218/226)	91/91	1.01 (0.81-1.26)	22/21	1.11 (0.62-1.98)
AC (189/190)	94/84	1.11 (0.89-1.39)	21/17	1.22 (0.66-2.27)
CC (44/33)	20/16	1.02 (0.62-1.68)	6/3	1.40 (0.36-5.38)
MTHFR 677/1298 Genotype [†]				
CC/AA (69/68)	27/28	0.90 (0.60-1.35)	4/10	0.39 (0.13-1.19)
CC/AC (92/100)	49/42	1.25 (0.92-1.72)	13/9	1.41 (0.62-3.22)
CC/CC (43/33)	20/16	1.04 (0.63-1.72)	6/3	1.44 (0.37-5.54)
CT/AA (106/108)	48/43	1.12 (0.82-1.53)	14/7	2.05 (0.85-4.95)
CT/AC (97/89)	45/41	0.99 (0.72-1.37)	8/8	0.99 (0.38-2.55)
TT/CC (43/50)	16/20	0.93 (0.56-1.53)	4/4	1.30 (0.34-4.95)

*Advanced adenoma defined as tubulovillous or villous adenoma, large adenoma (≥ 1 cm), carcinoma *in situ*, or invasive cancer.

[†]Number of subjects in folate/placebo treatment groups.

[‡]RR, risk ratio for effect of 1 mg/day folate supplement versus placebo on adenoma recurrence risk; RRs were adjusted for age, sex, clinical center, and aspirin treatment assignment.

Results

A total of 505 patients were randomized to receive placebo and 516 patients were randomized to 1 mg of folic acid per day. A total of 987 patients (96.7%) underwent a follow-up colonoscopy at least 1 year following randomization. The two treatment groups were similar with regard to all baseline characteristics (Table 1). Genotypes were distributed randomly across folate treatment groups. Both loci were in the Hardy-Weinberg equilibrium, and in strong linkage disequilibrium. There were no subjects with the 677TT/1298AC, 677CT/1298CC, or 677TT/1298CC genotypes.

Association between MTHFR Genotype and Adenoma Recurrence. The associations of *MTHFR* genotypes with the risk of one or more recurrent adenomas are shown in Table 2. Relative to those with two wild-type alleles, the adjusted RR and 95% CI for any adenoma were 0.89 (0.68-1.16) for the *MTHFR* 677TT genotype (P for trend over number of variant alleles = 0.42) and 1.14 (0.87-1.50) for the *MTHFR* 1298CC genotype (P for trend = 0.12). The RR and 95% CI for any advanced lesion were 0.75 (0.36-1.55) for the 677TT genotype (P for trend = 0.28) and 1.16 (0.58-2.33) for the 1298CC genotype (P for trend = 0.71). Heterozygotes did not have materially different risks than those who were wild-type homozygotes. Consideration of the combined 677/1298 genotypes also did not suggest an association (Table 2).

Because the association between *MTHFR* genotype and adenoma risk is known to interact with folate availability, we also assessed the main effect of genotype after stratifying on the folate treatment group (data not shown). Treatment group did not modify the effects of genotype on adenoma risk. For the 677TT genotype, the RRs for any adenoma were 0.82 (95% CI, 0.55-1.22) and 0.96 (95% CI, 0.66-1.38) among subjects randomized to folate and placebo, respectively (P for interaction = 0.85). For the 1298CC genotype, these RR's were 1.15 (95% CI, 0.79-1.66) and 1.14 (95% CI, 0.79-1.71) for the folate and placebo groups, respectively (P = 0.83). Genotype RRs for

advanced adenoma risk were also essentially the same in both the supplement and placebo groups. There was some suggestion that the association of the *MTHFR* 677TT genotype with the risk of any adenoma was modified by sex. The adjusted RRs for any adenoma were 1.07 (95% CI, 0.79-1.45) in males but 0.48 in females (95% CI, 0.25-0.90; P for interaction = 0.05). Control for baseline folate levels did not change either the sex-specific RR's or the interaction P value. There was no evidence of modification of genotype associations with all adenomas by age or any baseline or post-treatment folate measure, and the findings in bowel subsites were comparable (data not shown). There was no interaction with aspirin treatment (data not shown). The RRs for advanced adenomas were not modified by age, sex, baseline or post-treatment folate levels. The RRs using 3 or more recurrent adenomas as an endpoint were essentially identical to those for advanced adenomas (data not shown). When we expanded the analysis to consider recurrences occurring within the second surveillance cycle, the findings were similar (data not shown).

Modification of the Effects of Folate Supplementation. There was no evidence that the effects of folate supplementation were modified by *MTHFR* genotypes for any endpoint (Table 3). The folate/placebo RR for all adenomas was 0.93 (95% CI, 0.57-1.53) among those with the *MTHFR* 677TT genotype and 1.02 (95% CI, 0.62-1.68) for those carrying the *MTHFR* 1298CC genotype. Both estimates were essentially the same as the overall result. When we limited the endpoints to adenomas with advanced features, these RRs were 1.30 (95% CI, 0.34-4.96) and 1.40 (95% CI, 0.36-5.38), respectively.

Discussion

We studied the association between *MTHFR* 677C>T and 1298A>C genotypes and the risk of recurrent adenomas in subjects participating in a randomized clinical trial of aspirin and folic acid supplementation. Overall, *MTHFR* genotype was not associated with recurrence risk for all

adenomas or for advanced adenomas, regardless of folate treatment group. Neither MTHFR genotype appeared to alter the effects of folate supplementation on adenoma risk.

The MTHFR enzyme plays a key role in intra-cellular folate distribution, irreversibly moving one-carbon groups away from nucleotide synthesis and toward maintenance of the universal methyl-donor S-adenosylmethionine (see Fig. 1). When one-carbon groups are not limiting, individuals with the 677 TT genotype appear to retain more folates for nucleotide synthesis at the expense of DNA and protein methylation reactions (6, 27). In the context of these well-described functional effects, the decreased colorectal cancer risk observed in 677T homozygotes in epidemiologic studies (10, 11, 20) emphasizes the importance of imbalanced nucleotide pools over disturbed methylation reactions in folate-associated colorectal carcinogenesis (3).

Regarding the 677 T allele, our clinical trial data are generally consistent with those from adenoma case-control and cohort studies, none of which have reported a significant association between the MTHFR 677TT genotype and adenoma prevalence (11, 13, 14, 16, 28-32), even after stratifying on adenoma type (13, 28, 31, 32). Our findings are also consistent with an analysis of incident adenomas in a similar clinical trial of 500 µg folic acid/day with or without 300 mg/day aspirin (33). However, the results of both these trials differ from those of the observational analysis of incident adenomas in another trial (18), which reported an increased risk for any recurrent adenoma and an approximate doubling in the risk of advanced adenomas in subjects with the 677 TT genotype, but only in those not taking a multivitamin supplement.

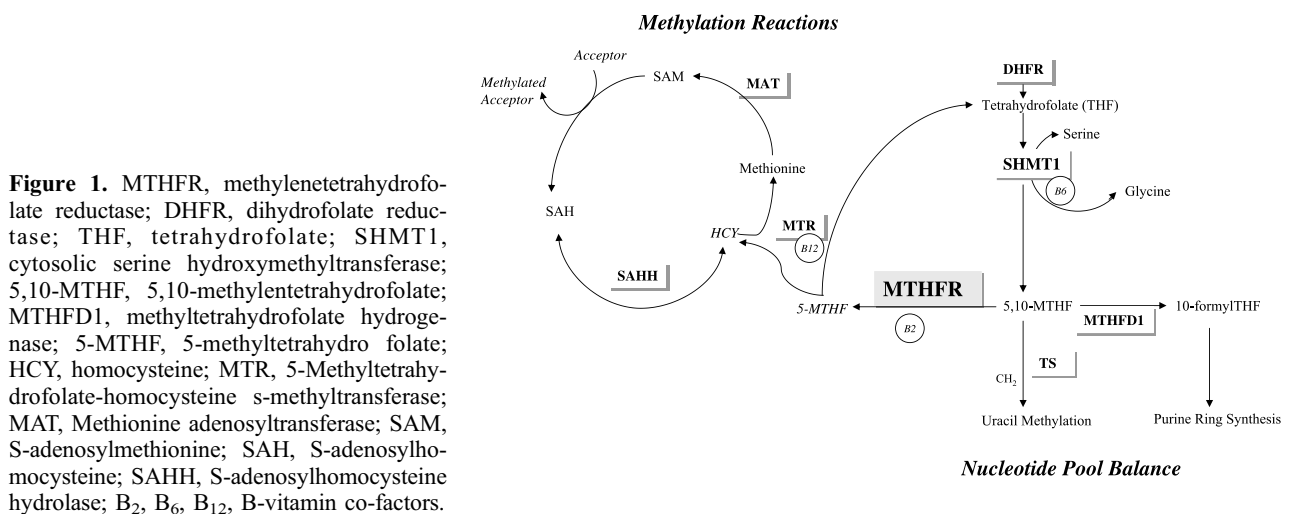
The aggregate data from case-control, cohort studies and clinical trials suggest that overall, the MTHFR 677C>T genotype has at most a weak association with adenoma risk. However, some (13, 16, 30, 31, 34) though not all (17, 29) studies have reported lower MTHFR 677TT RRs among those with higher folate status, and increased RRs have been reported in subjects who use alcohol, a known folate antagonist (13, 17, 28-30, 32). All these findings suggest that folate availability may modify adenoma risk in 677 TT homozygotes. To reduce the

incidence of neural tube defects, fortification of the North American food supply with 140 µg folic acid per 100 g cereal-grain product began on a voluntary basis in 1996 and became mandatory on January 1, 1998. Recruitment in this study overlapped a time period of increasing supplementation of the US and Canadian food supply, so our study population was relatively folate-replete at the start of the study (21). We also observed a small, non-significant decrease in recurrence risk for those with the TT genotype. Thus, our finding of a small, non-significant decrease in recurrence risk for subjects with the TT genotype are consistent with those from some prior studies, indicating that this genotype may decrease adenoma risk modestly in those with adequate folate availability.

It is possible that longer-term folate supplementation may modify the association between the risk of incident adenomas in those with the MTHFR 677 TT genotype. We considered this, in part, by extending our analysis to adenomas that occurred in all subjects who were followed for an additional colonoscopic surveillance cycle. This analysis, however, did not lead to any change in our conclusions.

In general, our findings regarding the 1298A>C variant are similar to those seen in the other aspirin/folate trial (33) and the literature (10). There is significant heterogeneity in the biological (9, 35) and epidemiologic data for the MTHFR 1298A>C CC genotype (10) and colorectal cancer risk. The lack of consistency in the association with the 1298 A>C polymorphism may reflect a lack of true functional effect or it may imply the presence of an important modifier of such an effect.

Our results and those of Hubner et al. (33), showing that the MTHFR C677T TT genotype did not significantly modify the effect of folic acid supplementation in a similar trial, may be a reflection of the interaction between genotype and folate availability. Yamada et al. showed experimentally that the enzyme coded for by the 677 T allele becomes indistinguishable from the wild-type enzyme in the presence of folic acid (9). It is possible that 677TT subjects in the supplement group (1700 µg folate equivalents/day in our study) had a functionally wild-type MTHFR enzyme during the follow-up period, suggesting that our ability to assess a genotype effect



may have been limited to subjects in the placebo group. The fact that we did not observe an association even here, suggests additional support for the conclusion that the *MTHFR* genotype does not substantially affect adenoma recurrence risk. Nonetheless, all our subjects were relatively folate-replete at the beginning of the trial, and so it is possible that these associations may be different in populations with lower folate intake.

The advantages of our study include the high follow-up rate for subjects and the randomization to folate treatment, a design that largely avoids the measurement error inherent in nutritional epidemiology and minimizes the potential for confounding in the assessment of the main effects of folate. Additionally, almost all studies of *MTHFR* and adenomas to date have been studies of prevalent adenomas, complicating inferences regarding the timing of the neoplasm and the folate intake. Because all subjects in this clinical trial were cleared of polyps at baseline, the adenomas we detected at follow-up were incident lesions (missed polyps aside). This design thus allows us to estimate the association of genotype with incidence rather than prevalence of adenomas, further strengthening the impression that the *MTHFR* 677 TT genotype does not modify the risk of adenoma development. Disadvantages include the requirement that all subjects in the study cohort had at least one adenoma before randomization, a design feature that limits the generalizability of our results. Also, the study population had high baseline folate levels, and it is unclear how this might have modified the functional effects of the *MTHFR* 677 T allele. Finally, despite the substantial size of the study, the small genotype-specific Ns, especially for stratified estimates, limits the inferences that are possible from the data.

In summary, we assessed the association between *MTHFR* genotype and the risk of recurrent colorectal adenomas and did not observe any significant association with recurrence risk for either genotype. These findings were the same for any adenomas and adenomas with advanced features and were not modified by baseline folate, post-follow-up folate or folate supplementation. Our results are consistent with numerous previous studies and extend to incident recurrent adenomas the finding that *MTHFR* genotype is not substantially associated with adenoma risk.

Disclosure of Potential Conflicts of Interest

J.A. Baron: Wyeth provided folate and folate placebo tablets for the Aspirin/Folate Polyp Prevention Study; P. Ueland: received consulting fees from Nycomed and is a member of the steering board. The other authors disclosed no potential conflicts of interest.

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