

Folic acid supplementation and risk of colorectal neoplasia during long-term follow-up of a randomized clinical trial

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ABSTRACT

Background: The Aspirin/Folate Polyp Prevention Study previously found folic acid increased risk of advanced and multiple colorectal adenomas during a surveillance colonoscopy interval starting about 3 y after randomization.

Objective: We conducted secondary analyses to evaluate folic acid effects with additional follow-up after treatment was stopped.

Methods: In total, 1021 participants recently diagnosed with colorectal adenomas were randomly assigned to 1 mg/d of folic acid ($n = 516$) or placebo ($n = 505$), with or without aspirin, beginning 6 July 1994. The original 3-y treatment period was extended into a subsequent colonoscopy interval, but eventually stopped prematurely on 1 October 2004. With additional post-treatment follow-up, a total of 663 participants who extended treatment completed a second colonoscopic surveillance interval after the initial 3-y follow-up. In addition, 490 participants provided information regarding a subsequent surveillance colonoscopy occurring before completion of follow-up on 31 May 2012, including 325 who had agreed to extended treatment. Study endpoints included conventional adenomas, sessile serrated adenomas/polyps (SSA/Ps), or colorectal cancer, and RRs with 95% CIs were adjusted for baseline characteristics associated with availability of follow-up.

Results: Among those who extended treatment, any colorectal neoplasia was found in 118 (36%) participants assigned to placebo and 146 (43%) assigned to folic acid during the second surveillance interval (RR: 1.21; 95% CI: 0.99, 1.47; $P = 0.06$). Increased risk of SSA/P with extended folic acid supplementation was statistically significant during the second surveillance interval (RR: 1.94; 95% CI: 1.02, 3.68; $P = 0.04$). There was no evidence of post-treatment effects for any colorectal neoplasia (RR: 1.01; 95% CI: 0.80, 1.28; $P = 0.94$), and the post-treatment effect for SSA/P was no longer statistically significant (RR: 1.38; 95% CI: 0.59, 3.19; $P = 0.46$).

Conclusions: Delayed treatment effects were not observed, but folic acid may increase SSA/P risk. This trial was registered at clinicaltrials.gov as NCT00272324. *Am J Clin Nutr* 2019;110:903–911.

Keywords: folic acid, colorectal adenoma, sessile serrated adenoma/polyp, colorectal cancer, clinical trial

Introduction

Folate has been a focus of colorectal cancer (CRC) chemoprevention research for several decades because of its role in DNA methylation, repair, and nucleotide synthesis (1). Together, preclinical and clinical studies suggest a complex and possible dual role for folate in colorectal tumorigenesis, suppressing carcinogenesis before lesion initiation but promoting tumor progression after lesion initiation (2–7).

The Aspirin/Folate Polyp Prevention Study previously evaluated whether supplementation with 1 mg/d folic acid, with or without aspirin, could prevent new colorectal adenomas in a placebo-controlled randomized trial of 1021 individuals with a recent adenoma diagnosis (8, 9). Data that emerged shortly after the trial began suggested that longer exposure to folic acid may be necessary to observe any effect (10). Investigators decided to extend the original 3-y treatment period for a second

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colonoscopic surveillance interval with participant re-consent. Folic acid treatment was ultimately stopped prematurely because of a possible increase in adenoma risk, specifically of advanced adenomas and ≥ 3 adenomas during the second interval. Findings for the first and second intervals based on follow-up through the date treatment was stopped for all participants were previously reported (9). Folic acid supplementation also appeared to increase the risk of prostate cancer (11).

Here, we re-evaluated colorectal neoplasia risk in the second surveillance interval with the addition of participants who extended treatment but had not completed their second surveillance interval before treatment was stopped (9). Additionally, we assessed possible delayed folic acid treatment effects in an analysis of neoplasia occurring after cessation of folic acid supplementation. These post-treatment analyses were prompted by a previous clinical trial of calcium supplementation for adenoma prevention, in which we found that treatment effects were more striking after treatment ended than during the active treatment period (12).

Methods

Study design

The Aspirin/Folate Polyp Prevention Study (NCT00272324) was a multicenter, double-blind, placebo-controlled, randomized clinical trial that used a 3×2 factorial design to compare low-dose (81 mg/d) and high-dose (325 mg/d) aspirin with an aspirin placebo and 1 mg/d of folic acid to a folic acid placebo. Study methods and inclusion/exclusion criteria have been previously documented (8, 9). Briefly, participants were recruited between 6 July 1994 and 20 March 1998 from 9 centers in the United States and Canada. Participating medical centers were affiliated with Dartmouth-Hitchcock Medical Center (Lebanon, NH); University of North Carolina (Chapel Hill, NC); University of Southern California (Los Angeles, CA); University of Colorado (Denver, CO); Henry Ford Health System (Detroit, MI); University of Toronto (Toronto, ON); University of Iowa (Iowa City, IA); Cleveland Clinic Foundation (Cleveland, OH); and University of Minnesota (Minneapolis, MN).

Eligible participants were between 21 and 80 y of age, diagnosed with ≥ 1 adenoma within 3 mo of recruitment, or diagnosed with ≥ 1 large adenoma (≥ 1 cm in diameter) or ≥ 2 adenomas within 16 mo of recruitment provided that a colonoscopy within 3 mo of recruitment confirmed no remaining polyps. Those with a personal history of invasive CRC, inflammatory bowel disease, or familial polyposis syndromes were ineligible, as were those with health conditions that could be treated or exacerbated by aspirin or folic acid, such as recurrent arthritis and atherosclerosis. Potential participants with deficient plasma vitamin B12 concentrations (<162 pg/mL) were also excluded. One hundred participants who had been randomized to only aspirin/placebo and did not receive a folic acid/placebo treatment assignment were not included in this analysis.

Randomization was stratified by age (≤ 60 and >60 y), sex, and clinical center using blocks of size 6 following a 3-mo run-in period to assess adherence and tolerance to treatments. During the study, participants were advised to avoid using nonprotocol supplements that contained folic acid as well as nonsteroidal anti-inflammatory drugs. Institutional review boards

at all clinical centers approved the study protocol, and all participants provided informed consent. Re-consent was obtained for extended follow-up beyond the originally planned treatment period. An independent data- and safety-monitoring committee reviewed the study semiannually.

Baseline assessment

A baseline questionnaire assessed demographics, general health behaviors, medical history, use of common medications and nutritional supplements, and included a dietary assessment using a semiquantitative food frequency questionnaire (13). Participants were considered baseline users of nutritional supplements, including multivitamins, if they reported use at least once per week on average in the 4 mo preceding enrollment. Plasma folate concentrations were measured by a microbiological assay using a chloramphenicol-resistant strain of *Lactobacillus casei* (14) from blood specimens collected at baseline and again at the time of the 3-y colonoscopy.

Follow-up

Both the aspirin and folic acid components were originally planned to end at the time of a follow-up colonoscopy scheduled for ~ 3 y after the colonoscopy qualifying for study eligibility. Shortly after the study began, investigators decided to extend the folic acid/placebo treatment period for a second surveillance interval, expected to be about 3 or 5 y, as recommended by each participant's gastroenterologist. Participants had the option to discontinue folic acid/placebo at the end of the first surveillance interval but continue to be followed observationally for study endpoints. Treatment with aspirin/placebo was not extended.

Continued folic acid/placebo was expected to end by 31 December 2006, but was terminated early on 1 October 2004 because of concerns regarding increased adenoma risk from folic acid. All participants were unblinded to assignment on 11 April 2005. Observational follow-up with subsequent surveillance colonoscopies scheduled at the direction of each participant's gastroenterologist continued until 31 May 2012. Our previous report only included follow-up through the early termination of study treatment on 1 October 2004 (9). The inclusion of additional follow-up here necessitated a modified description of colonoscopic surveillance intervals. Examples illustrating how intervals were incorporated in the 2 analyses are displayed in **Supplemental Figure 1**.

Participants were asked to complete questionnaires every 4 mo while on study treatment and annually during observational follow-up to assess adherence to study pills, use of prescription and over-the-counter medications, nutritional supplements, and the occurrence of medical events. Annual questionnaires ended 31 December 2006, and a final questionnaire was completed between 1 February 2010 and 31 May 2012 in order to update information since the previous contact.

Study endpoints

All colorectal tissue slides underwent a standardized pathology review by a single study pathologist (DCS). For the primary endpoint, we investigated the occurrence of ≥ 1 colorectal

neoplastic lesion after the first year of follow-up after randomization. These included conventional adenomas, sessile serrated adenomas/polyps (SSA/Ps), and invasive CRC. Conventional adenomas were characterized as tubular, tubulovillous (25–75% villous features), or villous ($\geq 75\%$ villous features). Secondary endpoints included advanced conventional adenomas (tubulovillous or villous adenomas, or ≥ 1 cm in diameter, or with high-grade dysplasia or CRC), ≥ 3 conventional adenomas, and SSA/Ps. Understanding of SSA/Ps was still evolving when the study was initiated, and only the conventional adenoma was the focus of the original primary and secondary study endpoints. SSA/Ps were evaluated post hoc in the previous report of study findings (9), but excluded SSA/Ps with cytological dysplasia (considered “mixed lesions” according to the terminology used at the time) (15, 16). Here, SSA/Ps include SSA/Ps with and without cytological dysplasia, according to the currently accepted definitions (17).

Study endpoints involving colorectal neoplasia were timed according to the colonoscopic surveillance intervals and included diagnoses of lesions made during any interim colorectal endoscopies occurring before the planned surveillance exams. Outcomes of repeated colonoscopies performed within 6 mo were combined and counted as occurring on the date of the latest exam in order to avoid considering re-evaluations for persistent issues as new events. At each colonoscopy, endpoints were treated as missing if colorectal tissue pathology was unavailable. For a limited number of participants colonoscopy outcomes were available from a third surveillance interval before study treatment was stopped, but these were not considered in analyses. Also, when outcomes from multiple post-treatment colonoscopic surveillance intervals were available, only the first was considered.

Medical events

Predefined adverse events monitored during the study included death, cancer, myocardial infarction, coronary revascularization, and stroke. Medical records were requested to confirm major medical events self-reported on any postrandomization questionnaire. Pathology records were requested to verify cancer diagnoses and histology. This adjudication was performed centrally by study physicians blinded to treatment assignment.

Statistical analysis

In a previous analysis outcomes in the first and second follow-up intervals were evaluated for the intention-to-treat population with follow-up ending 1 October 2004 (9). New to this report, we summarize findings for SSA/Ps using current definitions of these lesions and present 2 post hoc analyses not prespecified in the original study protocol. The first analysis focuses on the second surveillance interval among only those who agreed to extend folic acid/placebo. This extended treatment interval began after the initial 3-y colonoscopy and ended at the next surveillance colonoscopy planned to occur 3 or 5 y later. Our analysis for the second interval differs from Cole et al. (9) in 2 ways (Supplemental Figure 1). Whereas Cole et al. (9) included both those who did and did not agree to extended treatment as

part of the second interval in accordance with an intention-to-treat approach, our main focus is on those who agreed to extended treatment. Also, whereas Cole et al. (9) only included follow-up from colonoscopies that took place before study treatment was terminated on 1 October 2004, here we include follow-up beyond that date until the intended end of the second colonoscopic interval. The second analysis focuses on neoplastic lesions found during the surveillance interval that began after the end of study treatment for both participants who extended treatment and those who did not.

Treatment effects were always defined according to randomized assignment. Each endpoint was contrasted with those free of any conventional adenoma, SSA/P, and/or CRC. We estimated RRs with 95% CIs from log-linear Poisson regression with robust standard errors (18). We preferred to estimate RRs rather than ORs because ORs differ substantially from RRs for common events, as recurrent adenomas are in this population.

The RR estimates in each interval were adjusted for a common set of baseline characteristics found to be associated with agreement to extend treatment and the availability of post-treatment follow-up in addition to the randomization strata (age, sex, and center). To identify relevant adjustment variables, each baseline characteristic from **Table 1** was included in 4 separate log-linear models with a binary outcome variable defined by having completed: 1) the first follow-up interval (**Supplemental Table 1**); 2) the second follow-up interval for those who agreed to extended treatment (**Supplemental Table 2**); 3) the post-treatment colonoscopy for those who agreed to extended treatment (**Supplemental Table 3**); and 4) the post-treatment colonoscopy for those who did not agree to extended treatment (**Supplemental Table 3**). For each of the 4 models, 2 variations were considered: 1) a model with the baseline characteristic as a main effect adjusted for age, sex, and center; and 2) a model that additionally included an interaction between the baseline characteristic and folic acid treatment assignment. Any baseline characteristic with $P \leq 0.05$ as a main effect or with $P \leq 0.05$ for the treatment interaction was included as an adjustment variable in the primary analysis for each interval.

Distributions of major medical events by treatment assignment were evaluated using Fisher's exact test. Unlike for adenoma endpoints, which could only be ascertained during colonoscopies marking the end of surveillance intervals, the incidence of other medical events could be diagnosed at any time during follow-up as reported by participants and centrally adjudicated by review of medical records. Accordingly, treatment effects of these medical events were evaluated using time-to-event analyses. To visualize changes to the folic acid treatment effect over all follow-up, we plotted a smoothed estimate of the time-dependent HR derived from trends in scaled Schoenfeld residuals (19) from Cox proportional hazards regression models adjusted for the randomization strata and an interaction with untransformed time since randomization. Adverse events for which there were fewer than 10 events per treatment group were not considered in time-dependent analyses.

Effect modification of the folic acid treatment effect by aspirin treatment was evaluated with Wald tests of interaction for the overall colorectal neoplasia endpoint, but not for other endpoints, because of the limited sample sizes. All P values were 2-sided with $P \leq 0.05$ considered statistically significant. Analyses were

TABLE 1 Baseline characteristics of 1021 study participants randomly assigned to 1 mg/d folic acid or placebo according to treatment assignment¹

Baseline characteristic	Placebo (n = 505)	Folic acid (n = 516)
Age, y	57 ± 10	57 ± 10
Male sex	321 (64)	330 (64)
Race/ethnicity		
Non-Hispanic white	431 (85)	443 (86)
Non-Hispanic black	35 (7)	28 (5)
Hispanic	22 (4)	30 (6)
Asian, Pacific Islander, or other	17 (3)	15 (3)
BMI, kg/m ²	27 ± 4	27 ± 5
Current cigarette smoking	68 (14)	79 (15)
Alcohol consumption, drinks/d	0.6 ± 1.1	0.6 ± 1.1
CRC in first-degree relatives	154 (30)	159 (31)
Using multivitamins	191 (38)	176 (34)
Dietary energy intake, kcal/d	1632 ± 654	1637 ± 674
Dietary folate intake, µg/d	325 ± 163	320 ± 147
Daily use of supplements containing folic acid	118 (23)	101 (20)
Plasma folate, ng/mL	10.4 ± 7.5	10.5 ± 7.9
≥3 adenomas on examinations qualifying for study entry	67 (13)	67 (13)
Advanced adenoma on examinations qualifying for study entry	142 (31)	151 (32)
Aspirin randomization assignment		
Placebo	169 (33)	170 (33)
81 mg/d	169 (33)	175 (34)
325 mg/d	167 (33)	171 (33)

¹Values are n (%) or means ± SDs. Measurements with missing values are as follows: n = 2 for BMI (n = 1 placebo, n = 1 folic acid); n = 3 for smoking status (n = 3 placebo); n = 48 for alcohol consumption (n = 26 placebo, n = 22 folic acid); n = 46 for dietary intake and dietary folate (n = 24 placebo, n = 22 folic acid); n = 118 for plasma folate (n = 59 placebo, n = 59 folic acid); n = 4 for number of adenomas on qualifying exam (n = 1 placebo, n = 3 folic acid); and n = 87 for advanced pathology on qualifying exam (n = 49 placebo, n = 38 folic acid). CRC, colorectal cancer.

performed using SAS version 9.4 (SAS Institute) and R version 3.3.0 (R Foundation for Statistical Computing).

Results

Randomization and adherence

A total of 1409 participants entered the run-in period, and 1021 were randomly assigned to 1 mg/d of folic acid or placebo (**Figure 1**). Distributions of baseline characteristics were similar in the folic acid and placebo groups (**Table 1**). Of 1021 randomly assigned participants, 987 (97%) completed the first surveillance interval. As previously documented, 87% of participants reported taking study pills ≥6 d/wk during the first surveillance interval. During the second surveillance interval, use for ≥6 d/wk was reported for 86% of participants who consented to extended treatment and 71% of participants when also including those who did not consent to extended treatment (**9**).

Selection of adjustment variables

Baseline characteristics of participants who completed the first surveillance interval, the second surveillance interval, and the post-treatment follow-up are described in Supplemental Tables 1–3. Baseline characteristics that met our criteria of inclusion as common adjustment variables for all intervals were age, sex, center, race/ethnicity, BMI, cigarette smoking, first-degree family history of CRC, and number of advanced adenomas on examinations qualifying for study entry.

First follow-up interval

Results for the first surveillance interval have been previously reported (**9**), and conclusions are unchanged in this analysis with the updated endpoint definitions for SSA/P and different adjustment variables (**Supplemental Table 4**). In summary, we documented 206 participants (42%) assigned to placebo and 221 participants (44%) assigned to folic acid with a conventional adenoma, SSA/P, or CRC during the first surveillance interval (RR: 1.04; 95% CI: 0.90, 1.21). Folic acid was unrelated to the risk of advanced or ≥3 conventional adenomas during the first interval, but there was a nonstatistically significant suggestion of increased risk of SSA/P (RR: 1.41; 95% CI: 0.96, 2.08; *P* = 0.08).

Second follow-up interval

Of the 987 participants who completed the first surveillance interval, 729 (74%) agreed to extend treatment with folic acid/placebo, 197 participants (20%) chose not to extend treatment but agreed to be followed observationally, and 61 participants (6%) refused further participation or were lost to follow-up (**Figure 1**). The second surveillance interval was completed by 663 participants who had agreed to extended treatment. In all, 161 (24%) participants completed the interval after the termination of study treatment on 1 October 2004. Outcomes for these 161 participants are included here but were not included in our previous analysis (**9**). The duration of follow-up (mean ± SD) for those who extended treatment was 7.3 ± 2.5 y since randomization and the duration on treatment was 6.2 ± 1.1 y.

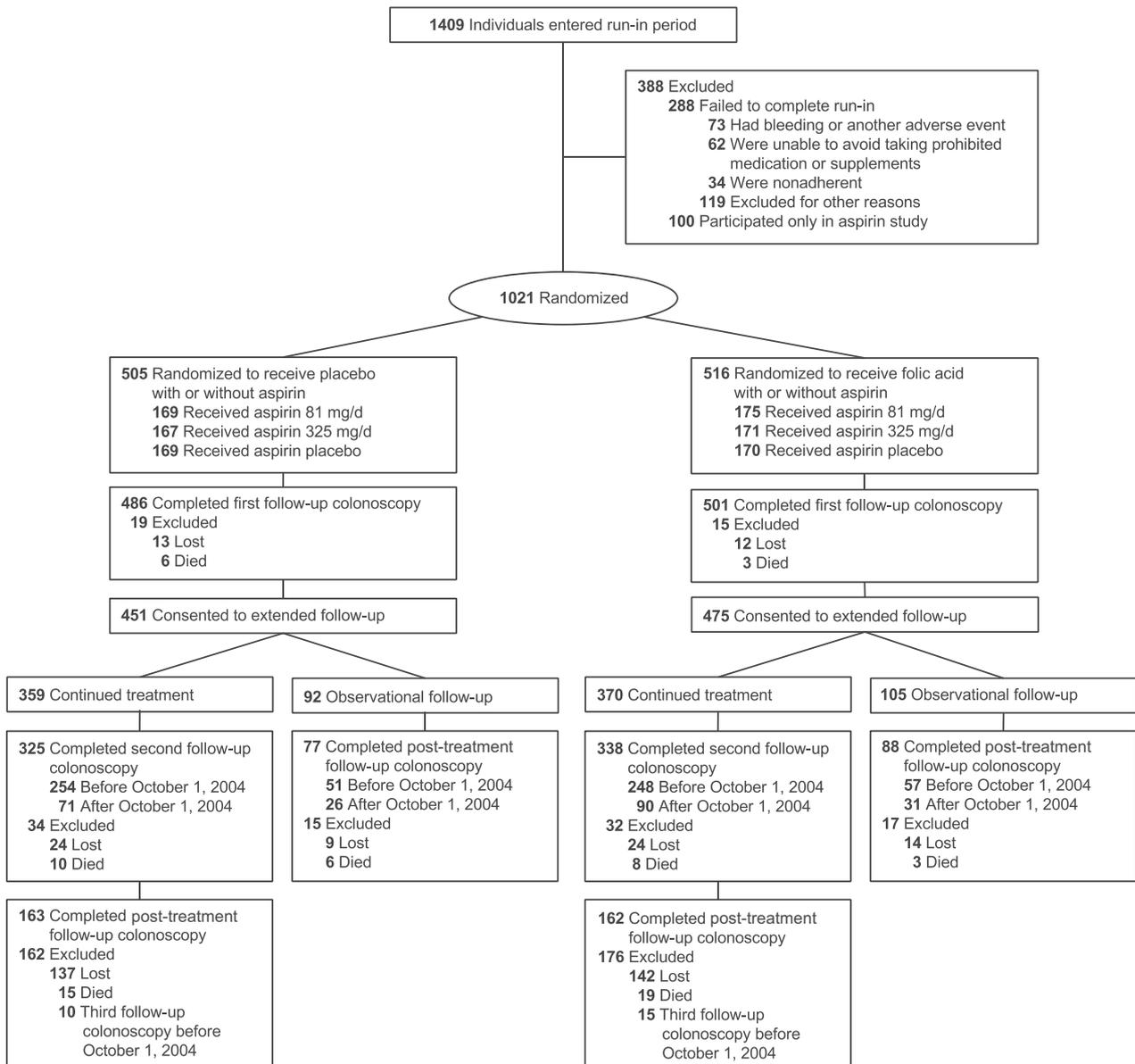


FIGURE 1 Flow of study participants.

Among those participants who extended treatment, any colorectal neoplasia was found during the second follow-up interval in 118 of 325 (36%) participants in the placebo group and 146 of 338 (43%) in the folic acid group (RR: 1.21; 95% CI: 0.99, 1.47; **Table 2**). The RRs of advanced and ≥ 3 conventional adenomas for the folic acid group were not statistically significant (RRs: 1.20 and 1.58; 95% CIs: 0.73, 1.97 and 0.87, 2.86, respectively). Folic acid supplementation was associated with an increased risk of SSA/P during the second follow-up interval (RR: 1.94; 95% CI: 1.02, 3.68).

Post-treatment follow-up

Post-treatment follow-up refers to observation after the initial 3-y colonoscopy for those who did not extend treatment and

observation after the second interval for those who did extend treatment. Among 663 participants who extended treatment and completed the second surveillance interval, a total of 325 (49%) reported ≥ 1 post-treatment colonoscopy. Among 197 participants who declined extended treatment but consented to observational follow-up, 165 (85%) reported ≥ 1 post-treatment colonoscopy during observational follow-up after the first interval (**Figure 1**). Those who extended treatment had the post-treatment colonoscopy an average of 11.5 y after randomization and had been taking folic acid/placebo for 6.7 y on average, whereas those who did not agree to extend treatment had the post-treatment colonoscopy an average of 7.9 y after randomization and had been taking folic acid/placebo for an average of 2.3 y.

During post-treatment follow-up, ≥ 1 neoplastic lesions were found in 91 of 240 (38%) participants in the placebo group

TABLE 2 Colorectal neoplasia RR for folic acid treatment assignment during the second surveillance interval for those who consented to extended treatment and completed the interval¹

Endpoint	Placebo (<i>n</i> = 325)	Folic acid (<i>n</i> = 338)	RR ² (95% CI)	<i>P</i>
Any conventional adenoma, SSA/P, CRC	118 (36)	146 (43)	1.21 (0.99, 1.47)	0.06
Advanced conventional adenoma, CRC	27 (8)	32 (9)	1.20 (0.73, 1.97)	0.47
≥3 conventional adenomas, CRC	16 (5)	30 (9)	1.58 (0.87, 2.86)	0.13
SSA/P	16 (5)	28 (8)	1.94 (1.02, 3.68)	0.04

¹Values are *n* (%) unless otherwise indicated. Only those participants who agreed to extended treatment after the first surveillance interval are included. Interval starts after the first follow-up colonoscopy and ends at the follow-up colonoscopy planned ~3–5 y later, regardless of whether it occurred before or after treatment was stopped for all participants on 1 October 2004. Participants with the given endpoint are separately compared with those without conventional adenoma, SSA/P, or CRC (*n* = 197 for placebo, *n* = 181 for folic acid). A total of *n* = 21 are missing data for endpoints (*n* = 10 for placebo, *n* = 11 for folic acid). CRC, colorectal cancer; SSA/P, sessile serrated adenoma/polyp.

²RR for folic acid relative to placebo adjusted for age, sex, center, race/ethnicity, BMI, cigarette smoking, first-degree family history of CRC, and number of advanced adenomas on examinations qualifying for study entry (all measured at baseline).

and in 92 of 250 (37%) participants in the folic acid group (RR: 1.01; 95% CI: 0.80, 1.28; **Table 3**). There were no statistically significant treatment effects for the other colorectal endpoints in the post-treatment period. Stratification of the post-treatment period by agreement to extend treatment did not suggest differences for those who did and did not agree to extend, but there was limited information on potential heterogeneity because of the small sample size (**Supplemental Table 5**). We also found no evidence of aspirin–folic acid interaction for the primary endpoint during any interval (**Supplemental Table 6**), consistent with our previous reports for the active treatment period (9, 20).

Medical events

In general, cancer and cardiovascular events were uncommon, and none was associated with treatment assignment (**Table 4**). With additional follow-up, the increased risk of prostate cancer for those assigned to folic acid previously identified during the active treatment period (11) was attenuated and no longer statistically significant. Specifically, a total of 35 men (11%) assigned to folic acid developed prostate cancer compared with 22 men (7%) assigned to placebo (Fisher's exact test, *P* = 0.10). Time-dependent analyses supported the conclusion that any harmful effect on prostate cancer incidence was specific to on-treatment follow-up. Only the pattern for myocardial infarction

seemed similar to that for prostate cancer (**Supplemental Figure 2**).

Discussion

In the Aspirin/Folate Polyp Prevention Study, supplementation with 1 mg/d of folic acid did not alter the overall risk of colorectal neoplasia within ~3 y, but increased risk of SSA/Ps emerged with extended treatment for ~7 y in total. There were also nonsignificant suggestions of an increased risk of any neoplasia during this second surveillance interval. There was no evidence that the treatment effect persisted after supplementation was stopped. A previously identified increased risk of prostate cancer with folic acid was attenuated and no longer statistically significant with follow-up beyond the end of study treatment.

These findings should be interpreted in the context of our initial report of study results based on follow-up through the end of the active folic acid treatment period (9). The number of participants who completed the first surveillance interval (*n* = 987) is unchanged here, but as a result of late reports of colon procedures to the study coordination center, we identified 3 additional participants who completed the second follow-up interval before 1 October 2004, but were not included previously (9). In intention-to-treat analyses, which included those who did not extend treatment in the second surveillance interval, we previously observed a higher risk of advanced and multiple

TABLE 3 Colorectal neoplasia RR for folic acid treatment assignment during post-treatment follow-up¹

Endpoint	Placebo (<i>n</i> = 240)	Folic acid (<i>n</i> = 250)	RR ² (95% CI)	<i>P</i>
Any conventional adenoma, SSA/P, CRC	91 (38)	92 (37)	1.01 (0.80, 1.28)	0.94
Advanced conventional adenoma, CRC	18 (8)	23 (9)	1.25 (0.68, 2.30)	0.46
≥3 conventional adenomas, CRC	12 (5)	13 (5)	1.18 (0.50, 2.77)	0.71
SSA/P	11 (5)	13 (5)	1.38 (0.59, 3.19)	0.46

¹Values are *n* (%) unless otherwise indicated. Both those who agreed to extended treatment and those who agreed to observational follow-up after the first surveillance interval are included. For those who declined extended treatment, the post-treatment follow-up starts after the first surveillance interval and ends before the conclusion of follow-up on 31 May 2012 (*n* = 77 for placebo, *n* = 88 for folic acid). For those who agreed to extended treatment, the post-treatment follow-up starts after the second surveillance interval and ends at the next surveillance colonoscopy occurring before the conclusion of follow-up on 31 May 2012 (*n* = 163 for placebo, *n* = 162 for folic acid). Participants with the given endpoint are separately compared with those without conventional adenoma, SSA/P, or CRC (*n* = 145 for placebo, *n* = 146 for folic acid). A total of *n* = 16 are missing data for endpoints (*n* = 4 for placebo, *n* = 12 for folic acid). CRC, colorectal cancer; SSA/P, sessile serrated adenoma/polyp.

²RR for folic acid relative to placebo adjusted for age, sex, center, race/ethnicity, BMI, cigarette smoking, first-degree family history of CRC, and number of advanced adenomas on examinations qualifying for study entry (all measured at baseline).

TABLE 4 Medical events after randomization¹

Event	Placebo (n = 505)	Folic acid (n = 516)	P
Death	48 (10)	52 (10)	0.83
CRC	6 (1)	6 (1)	>0.99
Non-CRC malignancy	68 (13)	91 (18)	0.07
Prostate	22 (4)	35 (7)	0.10
Lung	8 (2)	10 (2)	0.81
Breast	8 (2)	9 (2)	>0.99
Leukemia/lymphoma	7 (1)	10 (2)	0.63
Coronary revascularization	24 (5)	32 (6)	0.34
Myocardial infarction	13 (3)	19 (4)	0.37
Stroke	10 (2)	14 (3)	0.54

¹ Values are *n* (%) unless otherwise indicated. Cancers exclude keratinocyte carcinoma (nonmelanoma skin cancer). CRC, colorectal cancer.

colorectal adenomas in the folic acid group than in the placebo group during the second, but not the first, surveillance interval (9). As presented here, specific to those who extended treatment, but with more participants followed until the completion of their second surveillance intervals, RRs for advanced and multiple conventional adenomas were in the same direction as previously reported, but attenuated and not statistically significant.

Our initial report (9) documented a treatment effect for folic acid in relation to SSA/P in secondary analyses that was in the direction of increased risk, but not statistically significant. Given the current understanding of the importance of SSA/Ps, thought to give rise to ~20% of sporadic CRC (17), we were able to more carefully incorporate these lesions into all analyses. Participants without conventional adenoma, SSA/P, or CRC served as a common comparison group for RRs for the treatment effect across all lesion types. Inclusion of polyps formerly known as “mixed lesions” resulted in 7 additional participants with SSA/P during the first interval (6 of these 7 participants were assigned to folic acid). In terms of absolute risks, the increased risks of SSA/P were modest, with 12% compared with 9% in the folic acid and placebo groups, respectively, having SSA/P after ~3 y of use, and 8% compared with 5% having SSA/P in a subsequent surveillance interval among those who agreed to be treated for ~7 y in total.

Unlike a similar adenoma chemoprevention trial investigating daily calcium supplementation (12), our trial did not provide evidence of a greater treatment effect of folic acid after treatment stopped. We did not present results in the intention-to-treat population during the second surveillance interval because this has been previously documented (9). Instead, focusing on extended-treatment effects required consideration of treatment after the first surveillance interval as determined by participant choice and not by randomization. In our analysis of the post-treatment period, both those who did and those who did not choose to extend treatment are included. A potential drawback of this approach is that results for the post-treatment follow-up combine effects for some shorter-term users with those of longer-term users. Analyses that stratified post-treatment follow-up according to whether the participant agreed to extend treatment did not suggest differences, but suffered from relatively poor statistical power.

In contrast to the general direction of findings from our study, many observational studies suggest an inverse association

between folate and CRC risk (21, 22). There have been inconsistent findings from other placebo-controlled randomized clinical trials of folic acid supplementation for adenoma prevention, which generally have had a shorter total duration of follow-up than our study. To our knowledge, the Aspirin/Folate Polyp Prevention Study is unique among adenoma prevention trials of folic acid to have evaluated long-term follow-up after cessation. Among clinical trials evaluating incident adenomas in those with previous adenomas, 1 mg/d of folic acid had no effect in both the United Kingdom Colorectal Adenoma Prevention study (23) and a clinical trial among participants of the Health Professionals Follow-Up Study and Nurses' Health Study (24). The former investigated 3 y of treatment, and the latter, like our study, extended an originally planned 3-y treatment period. In contrast, a small clinical trial of 94 patients concluded that 5 mg/d of folic acid for 3 y was effective at reducing new adenomas, suggesting that higher doses may be required for chemoprevention (25).

It has been thought that SSA/Ps give rise to CRC with the CpG island methylator phenotype (17), and the increased risk of SSA/P we found for folic acid seems consistent with folate-induced DNA methylation in lesion development (26–28). Short-term folic acid supplementation can increase promoter-region DNA methylation in colorectal mucosa (29–32). Epigenome-wide effects of folic acid intake have recently been characterized (33). A small epigenome-wide study suggested randomized supplementation with folic acid and vitamin B12 can induce complex patterns of methylation in leukocytes in those over 65 y of age (34).

Unlike findings for conventional adenomas, findings suggestive of increased risk of SSA/P with folic acid began in early follow-up and stronger evidence emerged with longer use. The overall risk of SSA/P during the initial surveillance interval of our study was slightly higher than the proportion detected (8%) in a more recent study of asymptomatic average-risk patients (35), but declined to 5% in the subsequent follow-up. Contrary to our findings for SSA/P, findings of a case-control study showed a reduced risk of SSA/P with high dietary folate consumption, although not after adjustment for other risk factors (36). Although SSA/Ps are known to be more common in patients with a higher adenoma-burden (37), we did not formally test whether folic acid independently alters risk of SSA/P and of multiple lesions. We also did not evaluate anatomic locations of lesions, but our previous analysis suggested that the effect of folic acid during the

first surveillance interval may be stronger for right-sided serrated neoplasia (38).

Our previous analyses suggested high circulating methylated folates after folate supplementation (5-methyl-tetrahydrofolate and 4- α -hydroxy-5-methyl-tetrahydrofolate) were associated with increased risk of ≥ 3 or advanced adenomas in the second surveillance interval, but with an inverse association for serrated lesions during this interval (39). Plasma folate concentrations were not available after the 3-y colonoscopy, and so we were unable to consider these trends in post-treatment follow-up. After a median follow-up of 7 y, we reported that folic acid may increase the risk of prostate cancer (11), consistent with evidence of higher prostate cancer risk in men with higher circulating folate concentrations (40). As addressed here, this excess risk did not persist after stopping treatment. There was also no evidence that folic acid was associated with risk of cancers at other sites, consistent with aggregated evidence from 13 randomized controlled studies (41).

The impact of losses to follow-up is particularly important in studies of delayed treatment effects. In the Aspirin/Folate Polyp Prevention Study, participants were required to re-consent to extended treatment after the first follow-up interval and again for surveillance after the active treatment period. As expected, most attrition occurred once participants were instructed to stop taking study pills. Statistical power was diminished and adjustment was needed to help control for confounding when separately considering those who did and those who did not agree to extended treatment.

The timing of nationwide dietary folate fortification, which in the United States began in 1996 and became mandatory in 1998, may have affected our results (42). Randomization began in 1994 in a folate-replete population, and the fortification policy may have changed the distribution of participants most likely to benefit or be harmed by supplementation by increasing baseline folate status in both treatment and control groups or by increasing the maximum attained folate levels in the treatment group. We expect, however, that this was less of an issue during the post-treatment follow-up when postfortification folate status stabilized in the population (43).

In summary, results of extended follow-up in the Aspirin/Folate Polyp Prevention Study suggest that any increased risk of advanced conventional adenoma, multiple conventional adenomas, and prostate cancer evident with ~ 7 y of folic acid supplementation at 1 mg/d did not persist after treatment discontinuation. Folic acid may increase the risk of SSA/P, which has important clinical implications given the increased risk of subsequent CRC among those with SSA/P (44, 45).

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