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Plasma folate, methylenetetrahydrofolate reductase (MTHFR), and colorectal cancer risk in three large nested case-control studies

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Abstract

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Few prospective studies have examined the associations between blood levels of folate, in conjunction with methylenetetrahydrofolate reductase (MTHFR) polymorphisms, and colorectal cancer. We evaluated the associations between plasma folate, MTHFR C677T and A1298C, and colorectal cancer in three large prospective studies: the Nurses' Health Study, the Health Professionals Follow-up Study, and the Physicians' Health Study. A total of 602 incident cases were identified and individually matched to controls who provided blood specimens. We used conditional logistic regression to calculate the relative risk (RR) and 95% confidence interval (95% CI) and then pooled the estimates using a random effects model. We found a lower risk of colorectal cancer among participants with low plasma folate levels: compared with the lowest quartile, RRs (95% CIs) for each successively higher quartile of plasma folate levels were 1.55 (1.14–2.11), 1.37 (1.00–1.88), and 1.47 (1.07–2.01; P for trend = 0.10). For the MTHFR polymorphisms, RRs (95% CIs) were 0.62 (0.44–0.90) for the 677TT vs. CC/CT and 0.68 (0.31–1.51) for the 1298CC vs. AC/AA, and these lower risk genotypes were associated with lower circulating plasma folate levels. When we partitioned the variation in plasma folate levels, variation due to folate intake was not positively associated with colorectal cancer risk. We found that low plasma folate levels were associated with lower risk of colorectal cancer. The reasons underlying a lower risk of colorectal cancer with low plasma folate levels require elucidation because plasma folate levels can reflect dietary intake, genetic influences, and other factors.

Keywords

Folate; methylenetetrahydrofolate reductase (MTHFR); colorectal cancer

Introduction

Folate is a key B vitamin in one-carbon mechanism, and is critical for DNA methylation, and DNA synthesis and repair [1]. Deficiency of folate has been suggested to increase the risk of colorectal cancer through aberrations in DNA methlyation and imbalance of DNA pre-cursors [1–2]. Although low dietary folate intake has been associated with increased risk of colorectal cancer [3–4], the results from a few prospective studies that examined the associations between blood folate levels and colorectal cancer were not consistent [5–11]. In a recent U.S. randomized trial, folic acid supplementation (1 mg/d) did not prevent the recurrence of colorectal adenomas, a precursor of colorectal carcinoma, and tended to increase greater adenoma multiplicity [12]. Two other recent randomized trials of folic acid (1 mg/d in a smaller U.S. trial and 0·5 mg/d in the U.K. trial) found no overall influence of folic acid supplementation on colorectal adenoma recurrence [13–14], but in the latter U.S. trial, folic acid supplementation was associated with lower recurrence of adenomas among those with low plasma folate at baseline [14].

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the prevailing form of circulating folate and the methyl donor for the conversion of homocysteine to methionine. Two common MTHFR polymorphisms, MTHFR C677T (rs1801133) and A1298C (rs1801131), have been most studied. Those with TT variant or CC variant have lower enzyme activity of MTHFR [15–16], producing lower circulating levels of 5-methyltetrahydrofolate, and the MTHFR 677TT genotype was associated with a lower risk of colorectal cancer risk compared to the homozygous CC genotype [17].

We previously reported a non-significant increased risk of colorectal cancer among those with folate deficiency (<3 ng/ml) compared with men with adequate folate levels [18] among participants followed up through 1995 in the Physicians' Health Study. In the present analysis, including three cohort studies (the Nurses' Health Study with follow-up to 2000,

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the Health Professionals Follow-up Study with follow-up to 2002, and the Physicians' Health Study with follow-up to 2000), we prospectively examined the associations between plasma folate levels, two common functional polymorphisms (MTHFR C677T and A1298C), and colorectal cancer risk.

Materials and Methods

Study population

The Nurses' Health Study (NHS) was established in 1976, when 121,700 female registered nurses who were 30 to 55 years of age returned a mailed questionnaire. The Health Professionals Follow-up Study (HPFS) was initiated in 1986, when 51,529 male health professionals aged 40 to 75 years returned a mailed questionnaire. Participants in these two cohorts provided information about medical history, lifestyle, and various risk factors for chronic diseases on biennial follow-up questionnaires. The Physicians' Health Study (PHS) was a randomized, double-blind, placebo-controlled trial of aspirin and β-carotene for the primary prevention of cancer and cardiovascular disease among 22,071 US male physicians aged 40 to 84 in 1982. Participants provided information about medical history, lifestyle, and various risk factors for chronic diseases at baseline.

Blood samples were provided by 32,826 women from 1989 to 1990 in the NHS, by 18,225 men from 1993 to 1995 in the HPFS, and by 14,916 men from 1982 to 1984 in the PHS. Thus, all blood samples were collected prior to FDA-mandated fortification of grain products with folate, which began in 1998. Collection kits contained heparin tubes in the NHS and EDTA tubes in the HPFS and PHS. In the PHS, the blood specimens were immediately centrifuged and separated for plasma by the participants and were sent with chill packs by overnight courier and stored in at −82° C (now at −130° C). The specimens of the NHS and the HPFS were sent as whole blood with chill packs by overnight courier. On receipt, the samples were centrifuged, aliquoted, and stored in liquid nitrogen freezers at −130°C. The NHS and the PHS were approved by the Institutional Review Boards (IRB) of the Brigham and Women's Hospital (Boston, MA); the HPFS was approved by the IRB of the Harvard School of Public Health (Boston, MA).

Case ascertainment and control selection

Cases were identified by annual (PHS) or biennial (NHS and HPFS) follow-up questionnaires and then confirmed by review of medical records by the study investigators (NHS, HPFS) or an end-point committee (PHS).

Among those who provided baseline blood samples and had plasma folate available, we included 189 incident colorectal cancer cases confirmed through May 31, 2000, and 377 controls matched on age, month and year of blood collection, and fasting status at time of blood collection in the NHS; 173 cases confirmed through January 31, 2002, and 345 controls matched on age, month and year of blood collection in the HPFS; and 240 cases confirmed through March 31, 2000, and 408 controls matched on age and smoking status in the PHS. Thus, a total of 602 incident colorectal cancer cases and their matched 1130 controls were included in this analysis.

Laboratory analyses

Plasma folate was measured using a radioassay kit (Bio-Rad, Richmond, CA) in the NHS and HPFS or a microbiological method [19] in the PHS. All assays were conducted at the laboratory of Dr. Selhub at the Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University. Cases and their matched controls were analyzed in the same batch, and laboratory personnel were blinded to case-control status,

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and quality-control samples, which were randomly interspersed among the real samples. The mean intraassay coefficients of variation (CVs) from these blinded quality controls were ≤10% in each study.

DNA was extracted from blood using a commercially available process based on the absorption of DNA to a silica membrane after lysis with a proprietary agent (Qiagen, Chatsworth, CA). The TaqMan assay was used to genotype methylenetetrahydrofolate reductase (MTHFR) C677T (rs1801133) and A1298C (rs1801131).

Dietary and other information

Information on weight, physical activity, multivitamin use, smoking status, aspirin use, endoscopy history, and postmenopausal hormone use (NHS) have been updated every 2–4 years in the NHS and HPFS. Family history of colorectal cancer in parents and siblings were elicited on the 1982 and 1988 questionnaires in the NHS and in 1986, 1990, and 1992 in the HPFS. Height was assessed at baseline. We used the non-dietary information and multivitamin use closest but prior to blood collection. We calculated average daily intake of food and nutrient up to the year of blood collection using validated food frequency questionnaire (FFQ)s assessed every 2 to 4 years in the NHS and HPFS. In the PHS, information on height, weight, physical activity, alcohol intake, multivitamin use, and smoking habits was collected by self-administered questionnaires at baseline. We calculated the average daily intake of red meat and dairy calcium based on the frequencies of one standard serving of specified food groups on the 18-week or 12-month questionnaires.

Statistical analyses

We compared several baseline risk factors for colorectal cancer according to the quartiles of plasma folate levels among controls. Means or proportions of risk factors were age-standardized for this comparison. To estimate the relative risks (RRs) and 95% confidence intervals (95% CIs), we categorized participants in groups based on the quartiles of plasma folate levels among the controls determined separately from each laboratory batch in each study, and used a conditional logistic regression model to account for the matched case-control study design. In the multivariate analyses, we adjusted for body mass index (BMI; continuous, kg/m^2), physical activity (quartiles, METs/wk), postmenopausal hormone use (NHS; premenopausal women, among post-menopausal women, never use, past use, and current use), fasting status (HPFS; <5, ≥5 hours), family history of colorectal cancer (yes, no), pack years of smoking (continuous), height (continuous), aspirin use (yes, no), history of endoscopy (yes, no), red meat intake (quartiles), alcohol intake (continuous), vitamin D intake from food (continuous), and calcium intake from food (continuous) in the NHS and HPFS, and BMI (<23, 23–<25, 25–<27, ≥27 kg/m^2), vigorous exercise (< 2/wk, ≥ 2 times/wk), fasting status (≤8, >8 hours), aspirin assignment (yes, no), red meat intake (<1/d, ≥1 servings/d), alcohol intake (<1/wk, 1/wk–<1/d, ≥ 1 drinks /d) and dairy calcium intake (continuous) in the PHS. To test for trend across quartiles, participants were assigned the median value of their quartile level. This variable was entered as a continuous term in the model, the pooled estimate for which was evaluated by the Wald test.

After obtaining RRs from each cohort, we combined $\log_e$ RRs using a random effects model [20]. Two-sided 95% CIs were obtained for the RRs. Heterogeneity between the two studies was assessed by the Q statistic [21]. To test for heterogeneity due to sex, we used a mixed effects meta-regression model [22].

Geometric means and 95% CIs were calculated based on exponentiation of logtransformed plasma folate levels according to MTHFR genotypes. To test for trend for plasma folate

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levels across MTHFR genotypes, MTHFR variable was entered as an additive term in the model.

To examine whether dietary or genetic factors (MTHFR genotypes) associated with increased levels of folate explained any association between plasma folate and risk of colorectal cancer, we obtained the predicted plasma folate and residual by regressing plasma folate on natural folate, synthetic folic acid, and two MTHFR genotypes in a combined data of NHS and HPFS. We did not include PHS population because of the absence of folate intake from diet. To evaluate whether the residual and each predicted plasma folate level was associated with colorectal cancer, we multiplied the regression coefficients by the variables for folic acid, natural folate, MTHFR677 (TT vs. CC/CT) and MTHFR1298 (CC vs. AA/AC) and fitted these predicted plasma folate explained by each component (natural folate, synthetic folic acid, two MTHFR genotypes, and other factors) into the conditional logistic regression.

We examined whether the associations between plasma folate levels and colorectal cancer risk differed by MTHFR C677T (CC/CT, TT), MTHFR A1298C (AA/AC, CC), alcohol intake (<1 or ≥ 1 drink/d), multivitamin use (nonuser, user), BMI (<25, ≥25 kg/m^2), and aspirin use (yes, no) in the pooled analyses of three studies. We also examined whether the associations varied by family history of colorectal cancer (yes, no), total folate intake (<400, ≥400 mcg/d), smoking status (never smoker, ever smoker), or history of endoscopy (yes, no) in the pooled analyses of the NHS and HPFS. To test the null hypothesis that there was no interaction by the potential effect modifiers, we used a Wald test based on the pooled cross-product term as a dichotomous variable of the plasma folate levels (quartiles 1 and 2–4) with the modifier variable modeled as a dichotomous variable. The study was analyzed with the SAS 9.1 statistical package (SAS institute, Cary, NC). P<0.05 was considered statistically significant.

Results

Baseline characteristics among controls according to the quartiles of plasma folate levels are presented in Table 1. Participants who had higher plasma folate levels were more likely to be older and to take multivitamins and less likely to consume red meat. The median levels of plasma folate were 7.9 ng/ml among cases and 7.5 ng/ml among controls in the NHS, 5.7 ng/ml among cases and 6.0 ng/ml among controls in the HPFS, and 5.4 ng/ml among cases and 5.1 ng/ml among controls in the PHS.

Overall, we found a positive association between plasma folate levels and colorectal cancer risk. There was evidence of a threshold at the second quartile (Table 2). The pooled age and matching factor-adjusted RR comparing the highest quartile with the lowest quartile (95% CI) was 1.30 (0.97–1.74; P for trend = 0.30), but the association became strengthened after we adjusted for non-dietary and dietary factors; the pooled multivariate RR (95% CI) comparing the highest quartile with the lowest quartile was 1.47 (1.07–2.01). Additional adjustment for MTHFR C677T (CC/CT, TT) and MTHFR A1298C (AA/AC, CC) did not appreciably change the results (RR=1.45). When we adjusted for total folate intake in addition to non-dietary and dietary factors, the pooled multivariate RRs (95% CIs) were 1.57 (1.16–2.15), 1.43 (1.04–1.97) and 1.58 (1.14–2.20) comparing each successively higher quartile with the lowest quartile (data not shown).

The positive association was similar in each study. In the NHS, the multivariate RRs (95% CIs) comparing each successive quartile with the lowest quartile of plasma folate, adjusted for non-dietary and dietary factors, were 1.47 (0.84–2.57), 1.61 (0.93–2.78), 1.54 (0.87–2.74; P for trend = 0.23). In the HPFS, for the same comparison, the multivariate RRs (95%

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CIs) were 1.76 (1.00–3.11), 1.30 (0.71–2.38), 1.46 (0.80–2.65; P for trend = 0.49). In the PHS, the corresponding multivariate RRs (95% CIs) were 1.46 (0.90–2.37), 1.26 (0.76–2.07), and 1.43 (0.88–2.32; P for trend = 0.36). There was no significant heterogeneity among the three studies (P for heterogeneity = 0.98).

We examined the associations by including only cases that were diagnosed in the prefolic acid fortification period (before March 1996), in the transition period (March 1996 to January 1998), and in the post-folic acid fortification period (after January 1998) and their matched controls (Table 2). When we included only cases diagnosed after January 1998, when folic acid fortification became mandatory in the US, the RRs were higher (RR = 2.56) than those from the analyses in which we included cases diagnosed in the pre- fortification period (RR = 1.27) or in the transition period (RR = 1.02).

Our group was the first to report the inverse association between the common polymorphism in the MTHFR C677T (CC, CT, TT) and risk of colorectal cancer [18,23]. With additional samples in combined data of three studies and longer follow-up, we reevaluated this SNP, as well as and MTHFR A1298C (AA, AC, CC) with colorectal cancer risk (Table 3). Geometric means of plasma folate levels were lower among those with MTHFR 677TT genotypes or MTHFR 1298CC genotypes compared to those with MTHFR 677CC/CT or MTHFR 1298AA/AC, respectively.

We found a RR of 0.62 (95% CI = 0.44–0.90) for TT genotype compared to CC genotype of the MTHFR 677. We found no overall statistically significant association for MTHFR A1298C in men and women combined, but a significantly lower risk among men with MTHFR 1298CC genotype compared men with MTHFR 1298AA (RR = 0.45, 0.26–0.78; P for trend = 0.12).

We evaluated whether the predicted plasma folate explained by folate intake and MTHFR genotypes was associated with colorectal cancer risk. The simple matched RRs (95% CIs) were 0.79 (0.61–1.04) for 1 ng/mL increase in plasma folate explained by synthetic folic acid, 0.82 (0.56–1.20) for 1 ng/mL increase in plasma folate explained by natural folate, 7.49 (0.97–57.8) for 1 ng/mL increase in plasma folate explained by two MTHFR genotypes, and 1.09 (0.94–1.26) for 1 ng/mL increase in plasma folate explained by other factors (residual).

We examined whether the associations between plasma folate intake and colorectal cancer varied by the two common functional polymorphisms of the MTHFR gene, alcohol intake, aspirin use (aspirin assignment in the PHS), BMI, multivitamin use, total folate intake from foods and supplements, family history of colorectal cancer, smoking status, and history of endoscopy. We found higher risk of colorectal cancer among participants who took multivitamins and whose plasma folate levels fell into the quartiles 2–4 (RR=1.48), compared to those with low plasma folate levels who took multivitamins (RR=0.61; P for interaction = 0.08). The associations did not vary significantly by the other factors (data not shown).

Discussion

In this nested case-control study of women and men in three prospective studies, we found that lower plasma folate levels were associated with a lower risk of colorectal cancer with the suggestion of a threshold effect. For participants with longer follow-up times (i.e., cancers diagnosed after January 1998), a more pronounced positive association was observed. In agreement with previous review of the MHTFR C677T and A1298C in relation to colorectal cancer risk [24], we found an inverse association for MTHFR 677TT genotype and a nonsignificant association for MTHFR 1298CC genotype.

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There are several possible explanations for our finding that lower plasma folate levels were associated with a lower risk of colorectal cancer. First, the men carrying the MTHFR 677 TT genotype, compared to those with the wild-type genotype, had lower circulating folate, but higher proportion of cellular levels of formylated tetrahydrofolate (THF) [25–26], the conversion to which from 5,10-methyleneTHF is necessary for DNA synthesis and repair. Decreased 5-methyltetrahydrofolate (5-methylTHF), but increased formylTHF among those with a common variant in the MTHFRs and/or certain genetic features associated with low risk of colorectal cancer could explain the association we observed in this study. Also, in the analysis of predicted plasma folate, we found a non significant lower risk of colorectal cancer with high predicted plasma folate explained by either synthetic folic acid or natural folate, suggesting that greater intake of folate from diet does not contribute to increased risk of colorectal cancer. This observation is consistent with our finding that the association between plasma folate and colorectal cancer risk increased when adjusted for folate intake. However, the positive association between plasma folate and colorectal cancer risk persisted even after controlling for the MTHFR genotype, suggesting other unknown genetic determinants might exist, but this warrants further study.

Second, if those who consumed high folate at baseline also tended to consume high folate consistently across time, those with consistently high folate levels may increase the risk of colorectal cancer among those who had existing neoplastic lesions. Some in vitro and animal studies suggest that folic acid administration promotes the growth of existing cancer [27–28]. However, we did not find any harmful effect of total folate intake in the pooled analysis of NHS and HPFS cohort studies, but rather that total folate intake 12–16 years prior to diagnosis was associated with lower risk of colorectal cancer [29].

Thirdly, unmetabolized folic acid, which was included in our measurement of plasma folate, could play a role [30]. In our data, 47–77% of the participants in the highest quartile of circulating folate took multivitamins, the main source of folate. Given that folic acid fortification began in 1996 (and became mandatory in 1998), a combination of increased levels of unmetabolized folic acid from folic acid fortification and consistent supplementation could saturate the enzymatic reduction to 5-methyltetrahydrofolate; a metabolic study showed that unmetabolized folic acid was detected after the 14-week folic acid supplementation of more than 400 mcg/d among healthy folate replete adults [31]. Additional provision of folate above adequate levels could tip the balance of DNA precursors enough to lead to hypermethylation in cancerous cells. However, we did not find a dose-response increase in risk or a threshold effect among participants in the fourth quartile of plasma folate levels. The association between unmetabolized folic acid and colorectal cancer in post-folic acid fortification period warrants further investigation.

Findings from an observational study based ontwo randomized trials of wheat bran fiber and ursodeoxycholic acid showed that the highest quartile of plasma folate, compared to the lowest quartile, was associated with a 35–44 % lower risk of colorectal adenoma recurrence only among multivitamin nonusers, but no association among multivitamin users [32]. The Northern Sweden Health and Disease Cohort, where there was no mandatory folic acid fortification policy, found that higher plasma folate levels were associated with a higher risk of colorectal cancer among participants with follow-up times greater than the median of 4.2 years, but not among those with shorter follow-up [7]. Other prospective studies found no association [8,10–11] or inverse associations between plasma folate levels [5–6, 9] and cancers of colorectum or colon.

Our analysis has several limitations. We had only a baseline measure of plasma folate level, which did not allow us to examine changes in levels across time. Plasma folate levels in the U.S. increased after 1996, because of an increase in supplement use [33] and mandatory

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folic acid fortification. The National Health and Nutrition Examination Survey (NHANES) showed that serum folate levels among U.S. adults increased by 14–21 nmol/L (116–153% increase) and erythrocyte folate levels increased by 187–244 nmol/L (49–66% increase) between NHANES III (1988–1994) and NHANES (1999–2000) [34]. Information on plasma folate levels during the post-fortification era was not available in our study. To date, no prospective studies have reported associations between plasma folate levels during the post-fortification era and colorectal cancer risk. Although there were the possibility of measurement error in the laboratory assays or different responses of folate vitamers in either radioassay or micobiological procedure, this type of error or difference responses should not be related to case and control status because sets of case and matched control were measured at the same time in random blinded order using the same methods, and therefore, measurement error or difference responses cannot explain the positive association we observed. Our study had limited power to allow for conclusive interaction in the cross-tab analyses.

The important strengths of our study include a prospective design, in which blood samples were collected before colorectal cancer diagnosis, large sample size, and high follow-up rates. Because of the comprehensiveness of our questionnaires, we were able to adjust for most of the established risk factors for colorectal cancer.

In conclusion, our data suggest that low plasma folate levels may be associated with lower risk of colorectal cancer, but that this association may not be due to folate intake. Our data also confirm an inverse association between the MTHFR 677TT genotype and colorectal cancer risk. However, adjustment for functionally important variants in this gene did not account for the lower risk of colorectal cancer associated with lower plasma folate levels. Further investigations are needed to better understand 1) the genetic determinant of low folate in a folate replete population; 2) the long-term relationships between folate intake and plasma folate levels and colorectal cancer before and after folic acid fortification in the US; and 3) the impact of longterm synthetic folic acid supplementation on colorectal cancer risk.

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Table 1

Age-standardized baseline characteristics by plasma folate levels in controls in each study

Characteristics*	Plasma Folate†			
	1 Quartile	2 Quartile	3 Quartile	4 Quartile
Nurses' Health Study (NHS) N=377				
MTHFR 677 TT, %	22	8	13	15
MTHFR 1298 CC, %	17	7	7	9
Age at blood draw, y, mean ǂ	59	59	60	62
Total folate intake, mcg/d, mean§	333	374	421	548
BMI, kg/m^2, mean	26	25	25	25
Current smokers, %	23	7	11	10
Family history of colorectal cancer, %	12	16	16	16
Physical activity 4th quartile, %	21	22	26	29
Red meat 4th quartile, %	26	24	22	16
Alcohol, g/d, mean	6	6	6	6
Multivitamin use, %	15	15	37	77
Health Professionals Follow-up Study (HPFS) N=345				
MTHFR 677 TT, %	16	19	13	14
MTHFR 1298 CC, %	11	8	17	5
Age at blood draw, y, mean ǂ	64	66	68	69
Total folate intake, mcg/d, mean§	401	448	581	668
BMI, kg/m^2, mean	26	26	25	26
Current smokers, %	8	5	3	2
Family history of colorectal cancer, %	17	11	11	8
Physical activity 4th quartile, %	22	28	30	20
Red meat 4th quartile, %	32	29	18	18
Alcohol, g/d, mean	12	14	10	11
Multivitamin use, %	31	37	61	74
Physicians' Health Study (PHS) N=408				
MTHFR 677 TT, %	21	14	12	14
MTHFR 1298 CC, %	13	14	12	9
Age at randomization, y, mean ǂ	54	58	58	57
BMI, kg/m^2, mean	25	25	24	25
Current smokers, %ǂ	11	9	7	12
Vigorous exercise ≥2 times/wk, %	56	56	55	64
Red meat consumption ≥ 1 servings/day, %	29	27	22	24
Alcohol use ≥ 1/day, %	36	28	23	34
Multivitamin use, %	11	5	19	47

*Values except age are standardized according to the age distribution of cohort at baseline.

†Quartiles were based on distribution among controls

[‡] Matching factors

[§] Cumulative average intake from baseline

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Table 2

Relative risks (RR) and 95% confidence intervals (CIs) of colorectal cancer according to plasma folate levels

Plasma Folate*	NHS		HPFS		PHS		All	All
	RR (95% CI)†	IQR	RR (95% CI)†	IQR	RR (95% CI)†	IQR	RR (95% CI)‡	RR (95% CI)§
No. of cases/controls	189 / 377		173 / 345		240 / 408		602 / 1130	
Quartile 1	1.00	3.1–4.4	1.00	2.3–3.3	1.00	1.8–3.4	1.00	1.00
Quartile 2	1.28 (0.76–2.14)	5.5–6.8	1.33 (0.80–2.24)	4.2–5.3	1.49 (0.92–2.40)	2.9–5.4	1.47 (1.09–1.99)	1.55 (1.14–2.11)
Quartile 3	1.42 (0.85–2.35)	8.3–10.5	1.00 (0.58–1.72)	6.6–7.9	1.22 (0·75–1.99)	4.2–7.3	1.31 (0.97–1.79)	1.37 (1.00–1.88)
Quartile 4 ‖	1.26 (0.75–2.12)	14.3–21.2	1.22 (0.71–2.10)	9.5–13.7	1.40 (0.88–2.24)	8.5–12.9	1.39 (1.02–1.89)	1.47 (1.07–2.01)
P for trend¶	0.51		0.69		0.43		0.17	0.10
Cases diagnosed during#								
Prefortification period								
No. of cases/controls	109 / 217		42 / 83		193 / 324			
Quartile 1	1.00		1.00		1.00		1.00	-**
Quartile 2–4	1.21 (0.69–2.12)		0.89 (0.35–2.28)		1.26 (0.82–1.95)		1.27 (0.90–1.78)	-**
Transition period								
No. of cases/controls	36 / 72		38 / 76		21 / 36			
Quartile 1	1.00		1.00		1.00		1.00	-**
Quartile 2–4	0.72 (0.23–2.20)		1.55 (0.61–3.93)		0.92 (0.23–3.62)		1.02 (0.48–2.20)	-**
Post-fortification period								
No. of cases/controls	44 / 88		93 / 186		26 / 48			
Quartile 1	1.00		1.00		1.00		1.00	-**
Quartile 2–4	2.11 (0.91–4.88)		1.19 (0.66–2.14)		8.34 (0.98–71.0)		2.56 (1.09–6.02)	-**

IQR; interquartile range

*Median levels of plasma folate in each quartile when we included all the cases were as follows; 3.8, 6.4, 10.0, and 17.6 ng/ml for batch 1, 3.6, 5.9, 9.0, and 15.7 ng/ml for batch 2 in the NHS; 2.9, 4.8, 7.3, and 11.3 ng/ml in the HPFS; 3.2, 5.1, 7.1, and 11.0 ng/ml for batch 1, 1.7, 2.6, 3.9, and 6.6 ng/ml for batch 2 in the PHS

†Matched on age, month of blood draw, and fasting status at time of blood collection (only NHS) in the NHS and HPFS; age and smoking status in the PHS

‡In addition to matching factors, models were adjusted for body mass index, family history of colorectal cancer, physical activity, pack years of smoking, postmenopausal hormone use and fasting status (only HPFS), aspirin use, height, and history of endoscopy in the NHS and HPFS; models were adjusted for body mass index, physical activity, fasting status, and aspirin assignment in the PHS.

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[§] In addition to covariates included in Multivariate RR‡, alcohol intake, red meat intake, vitamin D from food and calcium from food in the NHS and HPFS; red meat intake, dairy calcium and alcohol intake in the PHS.

[‖] All P values, test for heterogeneity > 0.90

[¶] P for trend (two-sided) was calculated using the Wald test statistic.

[#] Pre-fortification period: baseline to March 1996; Transition period: April 1996 to January 1998; Post-fortification period: February 1998 to end of follow-up period

[**] Small number of cases did not allow further adjustment for other factors.

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Table 3

Geometric mean of plasma folate, relative risks (RR)* and 95% confidence intervals (CIs) of colorectal cancer according to MTHFR genotype

	NHS			HPFS			PHS			Pooled
MTHFR	**No of cases / controls**	**Plasma folate, ng/ml†**	**RR (95% CI)**	**No. of cases / controls**	**Plasma folate, ng/ml†**	**RR (95% CI)**	**No. of cases / controls**	**Plasma folate, ng/ml†**	**RR (95% CI)**	**RR (95% CI)**
C677T										
CC	89 / 165	8.3 (7.5–9.2)	1.00	72 / 140	6.0 (5.4–6.7)	1.00	89 / 159	4.5 (4.0–5.0)	1.00	1.00
CT	66 / 140	8.0 (7.2–8.9)	0.85 (0.57–1.27)	69 / 127	5.6 (5.0–6.2)	1.13 (0.74–1.70)	94 / 124	4.3 (3.8–4.8)	1.39 (0.94–2.05)	1.10 (0.83–1.46)
TT	20 / 48	6.6 (5.5–7.9)	0.75 (0.41–1.37)	17 / 51	5.3 (4.5–6.3)	0.63 (0.33–1.18)	15 / 50	3.8 (3.2–4.5)	0.50 (0.27–0.96)	0.62 (0.44–0.90)
P for trend		0.05	0.29		0.17	0.34		0.09	0.35	0.09
A1298C										
AA	72 / 181	8.3 (7.5–9.1)	1.00	73 / 147	5.5 (5.0–6.1)	1.00	101 / 167	4.3 (3.9–4.8)	1.00	1.00
AC	82 / 136	7.7 (6.9–8.6)	1.57 (1.05–2.34)	73 / 133	5.9 (5.3–6.5)	1.05 (0.69–1.58)	100 / 154	4.4 (4.0–4.9)	1.15 (0.81–1.65)	1.24 (0.98–1.56)
CC	21 / 38	6.8 (5.6–8.3)	1.41 (0.74–2.70)	7 / 32	5.2 (4.2–6.5)	0.44 (0.18–1.07)	12 / 44	4.0 (3.3–4.8)	0.46 (0.23–0.93)	0.68 (0.31–1.51)‡
P for trend		0.08	0.07		0.91	0.28		0.64	0.26	0.90

*Models were adjusted for matching factors

†Geometric mean (95% CI) among controls in each study. Models were adjusted for age, batch, and cigarette smoking (only PHS) in the NHS, HPFS, and PHS.

‡P for heterogeneity due to sex = 0.008. Pooled RR (95% CI) among men (only HPFS and PHS) was 0.45 (0.26–0.78; P for trend = 0.12).