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Baseline plasma total homocysteine and adenoma recurrence: results from a double blind randomized clinical trial of aspirin and folate supplementation

A. Joan Levine[1], **Maria V. Grau**[2], **Leila A. Mott**[2], **Per Magne Ueland**[3], and **John A. Baron**[2]

[1]Department of Preventive Medicine, University of Southern California, Los Angeles California, 90033

[2]Departments of Medicine and Community and Family Medicine Biostatistics and Epidemiology, Dartmouth Medical School, Hanover, NH 03756

[3]Section of Pharmacology, Institute of Medicine, University of Bergen and Haukeland University Hospital, Bergen, Norway

Abstract

Background—Elevated plasma total homocysteine (tHcy) is an accepted marker of functional folate deficiency but may have independent effects on colorectal neoplasia risk. It is uncertain whether plasma tHcy is associated with risk at the low levels common in a folate-fortified population.

Methods—Study subjects, about half of which were recruited after fortification of grain products with folic acid in the US and Canada, were 871 individuals with a recent history of one or more colorectal adenomas who were randomized to receive either a 1 mg/day folic acid supplement or a placebo within one of three randomly assigned aspirin treatment groups (placebo, 81 or 325 mg/d). Non-fasting plasma tHcy was determined by a GC-MS method. We estimated adjusted risk ratios and 95% confidence intervals for one or more adenoma recurrences for each quartile of baseline plasma tHcy using generalized linear regression with an overdispersed poisson approximation to the binomial.

Results—The Q4/Q1 adjusted RR for any adenoma was 0.98; 95% CI 0.70-1.38, p-trend =0.17 in the placebo group, and 0.81; 95% CI 0.58-1.12, p-trend=0.17 in the folic acid group. Results were similar for adenomas with advanced features. There was no modification by sex, aspirin treatment group or *MTHFR 677C>T* genotype.

Conclusions—Plasma tHcy is not an independent marker for an increase in colorectal adenoma recurrence risk in post-fortification populations where plasma tHcy levels are in the lower range of values.

Impact—Controlling plasma tHcy levels is unlikely to favorably modify adenoma recurrence risk in folate fortified populations.

Address of Corresponding Author: A. Joan Levine USC Keck School of Medicine, Department of Preventive Medicine, Genetic Epidemiology, NRT 1450 Biggy Street Room 1509A, Los Angeles CA 90033, ajoanlev@usc.edu.
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Keywords

Homocysteine; Colorectal Adenoma; Folic Acid; One Carbon Metabolism; Randomized Clinical Trial; MTHFR

Introduction

Plasma total homocysteine (tHcy) is a sulfur containing amino acid and a product of S-adenosylmethionine-dependent methylation reactions. Plasma tHcy is a marker of overall folate dependent one-carbon metabolism because it reflects the status of several of the B-vitamins and methyl donors involved. Although plasma folate is the main determinant of plasma tHcy, the B-vitamins B_{12} ,B_6 and B_2 are also significant determinants (1-4) as is the *MTHFR* genotype (5-8).

Interest in plasma tHcy initially stemmed from its association with cardiovascular disease risk (9). Much less is known about the association between plasma tHcy and cancer. Plasma tHcy levels are associated with potential cancer risk factors, such as cigarette smoking and body mass index, only in part because of their association with plasma folate (1-4,10). An effect of plasma tHcy on carcinogenesis independent of B-vitamin status is also possible, given that it is in equilibrium with S-adenosylhomocysteine (SAH), a potent inhibitor of intracellular methylation reactions (11-13) and that elevated plasma tHcy may be associated with increased DNA damage even after controlling for plasma folate (14,15).

An association between increased plasma tHcy and risk of colorectal neoplasia is suggested by four studies all of which reported similarly increased risks for colorectal cancer (CRC) or adenomas in subjects with the highest plasma tHcy (12,16-18). However, only one study (12) controlled for plasma folate and other plasma tHcy determinants, and all studies involved subjects with relatively low folate intake. Thus, it is not clear whether the association between plasma tHcy and colorectal neoplasia is independent of folate availability or whether the association is still present when plasma tHcy is in the lower range. Plasma tHcy was not associated with CRC risk in three more recent studies (19-21).

In the current study we assessed the association between plasma tHcy and subsequent colorectal adenoma recurrence risk in subjects who were randomized to receive either a 1 mg/day folic acid supplement or a placebo for up to 3 years. In our study population, subjects were randomized and followed during a period of increasing folic acid intake in the source population, during which time the mean plasma tHcy levels in the study population decreased. We sought to assess how any associations with baseline plasma tHcy were modified by folic acid treatment assignment. We hypothesized that if plasma tHcy was a risk marker for adenoma recurrence it would be evident only in the placebo group which maintained higher mean plasma tHcy levels during follow-up than those in the folic acid group in which plasma tHcy remained uniformly low.

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 (22,23). Briefly, this was a three-by-two factorial design in which subjects were randomized to receive 81 mg /day ASA, 325 mg/day ASA or placebo. Within each ASA /placebo group, subjects were additionally randomized to receive 1 mg supplemental folic acid/day or a

placebo. The study initially focused only on aspirin; 100 subjects who were randomized only to aspirin were not included in this analysis.

Recruitment, Randomization, Treatment and Follow-up

Details of subject eligibility, recruitment, randomization, treatment and follow-up and study outcomes have been described (22,23). Briefly, subjects were recruited from July 1994 until March 1998 from 9 clinical centers. Eligible subjects were between 21 and 80 years of age, in good health and had received a recommendation for a 3 year follow-up colonoscopy by their regular medical practitioner. Each eligible subject met at least one of the following three criteria: a) one or more histologically confirmed colorectal adenomas removed within 3 months of their recruitment, b) one or more histologically confirmed adenomas removed within 16 months of their enrollment as well as a history of two or more confirmed adenomas or c) an adenoma greater than 1 cm in diameter. Individuals were ineligible if they had a history compatible with a familial colorectal cancer syndrome, invasive colorectal cancer (CRC), 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, non-aspirin NSAIDs or folate. Before entering the trial all subjects were required to have had a complete colonoscopy with removal of all polyps within 3 months of their entry into the trial. Each subject underwent a 3 month run-in period on 325 mg ASA prior to randomization into ASA and folic acid treatment groups. Only subjects with at least 80% compliance and no other contraindications were randomized. By protocol, all subjects had an anticipated follow-up complete surveillance colonoscopy 34 to 40 months after the qualifying examination.

In the initial study design, subjects in each treatment group were followed for 3-years and adenoma recurrence was determined at the end of that 3-year follow-up interval (the first follow-up). Due to concern that a longer follow-up interval may be necessary to observe the effect of folic acid supplementation, subjects were invited to remain on the study supplements until the next surveillance examination (typically 3 or 5 years later. The current analysis reflects plasma tHcy measurements made at study enrollment and adenomas found during the first follow-up period, i.e. through 36-40 months after the baseline colonoscopy. To assess the change in plasma tHcy levels over the follow-up interval, a second measurement was taken at the beginning of the second follow-up.

Study outcomes

The primary study outcome was the proportion of patients in whom one or more colorectal adenomas were detected in the period starting one 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 one year after randomization. Adenomas were classified as neoplastic (adenomatous) or non-neoplastic 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 centimeter or greater. Patients were considered to have “multiple adenomas” when there were a total of 3 or more follow-up adenomas by the end of the year 3 exam.

Plasma total Hcy, folate, vitamin B_2, B_6 and B_{12} determination

Plasma tHcy and other nutrients were determined in non-fasting blood samples taken at baseline and approximately three years later. Blood samples were collected into 7 mL Vacutainer brand tubes containing EDTA. After collection, specimens were immediately put on ice and then centrifuged at 1100 G for 10 min. Whole blood, plasma, and buffy coat fractions were stored at −20° C and then transferred to Dartmouth Medical School where they were stored at −80° C until analysis. Plasma tHcy was determined by a gas chromatograph mass

chromatography method as described (24). Plasma totalfolate and vitamin B_{12} were determined by microbiological assay using published methods (25,26). Vitamins B_6 (as PLP, the main active form of vitamin B_6) and B_2 were determined in plasma by liquid chromatography-tandem mass spectrometry (27). All B-vitamin assays were conducted at the laboratory of Bevital AS, Bergen Norway.

Questionnaires

Subjects completed a questionnaire at baseline that queried smoking history, height, weight and personal medical history. Dietary variables as well as use of multivitamins, coffee and tea consumption and alcohol use were derived from the Block food frequency questionnaire (28) and reflected average intake over the prior 12 months. Daily nutrient intakes were estimated using a nutrient data base and subject estimates of the number of servings per day multiplied by the estimated serving size for each food.

MTHFR genotype

The *MTHFR* polymorphisms, *677C>T* and *1298A>C*, were genotyped with the 5'nuclease TaqMan allelic discrimination assay using the ABI7900 (Applied Biosystems, Foster City, CA). Polymerase chain reaction primers and dual-labeled allele discrimination probes were designed using the Primer Express software package (PE Biosystems) as described in (29). 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

Statistical significance was defined as a two sided p-value ≤ 0.05. To compare those with an adenoma recurrence to those without a recurrence, we used t-tests or Wilcoxon rank-sum tests for continuous variables and χ^2 tests for categorical variables. The main exposure variable was quartile of baseline plasma tHcy. Baseline plasma tHcy quartiles were determined with reference to the whole study population.

In the analysis of the change in plasma tHcy over calendar time we calculated means and standard deviations by recruitment year and estimated a p for trend using orthogonal linear contrasts and Wald tests. For the analysis of the association between plasma tHcy quartile and number of polyps at the baseline examination we used a proportional odds model, where the outcome was the number of baseline adenomas (1, 2, 3, 4 or 5+) (30). The output of this analysis can be interpreted as the odds ratio for the higher versus lower adenoma number, whatever the high/low cut-point. We controlled for a priori potential confounders by adding to the model variables that have been associated with both plasma tHcy and adenoma recurrence in the literature or in the current trial population: age, cigarette smoking (never, former current), alcohol use (continuous, as drinks/day), body mass index (BMI; continuous), plasma total folate, plasma B_2, plasma B_6 (as PLP), and plasma B_{12} (all as continuous values). Race/ethnic group was not a confounder in this population, which was over 85% non-Hispanic white. We did not measure serum creatinine. We used the baseline measures for all of the potential confounders in this analysis.

In the analysis of the effect of plasma tHcy on adenoma recurrence we estimated risk ratios (and 95% confidence intervals) for one or more adenomas after randomization with a generalized linear regression analysis using an overdispersed poisson approximation to the binomial. We obtained p-values for trend using orthogonal linear contrasts. In the analysis we controlled for age, sex, study center, alcohol, BMI, smoking, plasma levels of plasma total folate, B_2, B_6, B_{12}, and aspirin treatment group, as defined above. Separate models were used to assess the association between plasma tHcy and recurrence risk within groups defined by

folate and aspirin treatment assignment, sex and the *MTHFR 677C>T* genotype. We used multiplicative interaction terms and Wald tests to test for interaction. STATA version 9.2 was used for all analyses.

Results

There were 1021 subjects randomized to folic acid or placebo. A total of 871 subjects (85.0%), had baseline and follow-up plasma tHcy and provided adenoma data; 392 had one or more recurrent adenomas and 479 had no recurrent adenomas. The baseline characteristics of the study population by adenoma recurrence status are shown in Table 1. Those with an adenoma recurrence were significantly older, more likely to be male, have a higher BMI, drink alcohol and had lower plasma vitamin B_6 levels. Baseline plasma total folate was inversely associated with recurrence risk in the total study population (p=0.013). Baseline plasma tHcy was significantly higher in those with an adenoma recurrence in the crude analysis (p= 0.04).

Baseline plasma tHcy decreased significantly over the 5-year recruitment period (p for trend < 0.001; data not shown). Mean baseline plasma tHcy was 10.7 ± 3.6 μmol/L among subjects recruited in 1995 and decreased to a mean of 8.7 ± 2.0 μmol/L in among subjects recruited in 1998. By the time of the year 3 measurement mean plasma tHcy for those in the placebo group had leveled off at approximately 9.2 μmol/L (p for trend 0.142).

Table 2 shows the univariate associations between selected baseline factors and plasma tHcy. Those in the highest quartile of tHcy were older, more likely to be male, current smokers, and to drink alcohol. They also had a higher BMI, lower plasma folate, B_2, B_{12}, and B_6, and a higher number of adenomas at the baseline examination. Table 3 shows the association between baseline plasma tHcy and the number of adenomas at the baseline examination. There was a borderline significant direct association between increasing plasma tHcy and the number of adenomas at the baseline examination after adjustment for age, sex, recruitment center, current smoking, BMI and alcohol intake (p for trend = 0.06). Subjects with the highest plasma tHcy were about 50% more likely to be in a higher baseline adenoma group. The proportional odds ratio (POR) was 1.49 (95% CI = 0.95–2.31; p for trend=0.06). After additional control for plasma folate and vitamins B_2, B_6 and B_{12} the adjusted POR was attenuated: POR = 1.18 (95% CI = 0.74-1.86; p for trend=0.43).

Table 4 shows the association between baseline plasma tHcy and subsequent adenoma risk after stratifying on folic acid treatment group. The Quartile 4/Quartile 1 adjusted RRs were 0.98 (95% CI = 0.70-1.38; p for trend = 0.17) and 0.81 (95% CI = 0.58-1.12; p for trend = 0.17) in the placebo and supplemented groups respectively (p for heterogeneity between treatment groups = 0.53). There was also no material difference in the RR's for advanced lesions (Table 4). There was no modification of RR's for either endpoint by sex, aspirin treatment group or *MTHFR 677C>T* genotype (data not shown).

Discussion

In this analysis of subjects participating in a randomized clinical trial of folate and/or aspirin for the prevention of colorectal adenomas there was no association between baseline plasma tHcy and adenoma recurrence risk in either the placebo or folic acid supplementation groups. The lack of association between plasma tHcy and recurrence risk was similar for all adenoma endpoints. Baseline plasma tHcy was associated with the number of adenomas at the baseline examination but this association was attenuated and no longer statistically significant after control for potential confounders including plasma total folate and other B vitamins.

Homocysteine is formed from S-adenosylhomocysteine (SAH), a product of transmethylation reactions using S-adenosylmethionine (SAM) as methyl donor. Hcy can be remethylated to

methionine or, alternatively, irreversibly transsulfurated to cystathionine by the vitamin B_6-dependent enzyme cystathionine-β-synthase (CBS). Any Hcy which is not remethylated or converted to cystathionine is rapidly exported to plasma where it can be measured as a combination of related protein-bound thiols and free Hcy, referred to as plasma total Hcy (plasma tHcy) (31) .

Plasma tHcy has been suggested to be a direct measure of intracellular methylation capacity (11,13,32) due to its equilibrium with SAH, a potent inhibitor of SAM-dependent methylation reactions. In the study by Yi et al, (13) the relationship between plasma tHcy and SAH appeared to be linear for plasma tHcy values from 5 to 18 μmol/L suggesting a positive association even for very low values of plasma tHcy. That this association may be functionally relevant is suggested by several studies which have reported significantly less global DNA methylation in circulating lymphocytes or colorectal epithelium in those with higher plasma tHcy (13,17, 33), and in human umbilical vein endothelial cells (HUVEC) (32).However, none of the human studies have shown this association to be independent of plasma folate levels. Plasma tHcy may also be associated with increased DNA damage. Fenech et al (15) reported that plasma tHcy greater than 10 μmol/L was associated with increased numbers of micronuclei in circulating lymphocytes, an association independent of circulating folate. Additionally, plasma tHcy is a potentially more inclusive marker of compromised one-carbon metabolism than is plasma folate alone because of its association with B-vitamins other than folate (1-4), as well as the *MTHFR 677C>T* genotype (5-8). In one study plasma tHcy was a more sensitive index of colonic mucosal folate than was plasma folate (34).

Previous studies of the association between plasma tHcy and risk of colorectal neoplasia have had mixed results. Four studies reported increased risk of colorectal cancer (12,16-18), colorectal adenomas (17) or adenoma recurrence (12) for those with higher plasma tHcy. However,, the increase was statistically significant in only two of these studies (12,18) and only one (12) controlled for plasma folate. Our results are concordant with those of three other recent studies that did not observe a significant increase in CRC risk with increasing plasma tHcy (19-21).

We did not observe any association between plasma tHcy and adenoma recurrence even among subjects assigned to placebo. About half the subjects in our study were recruited after voluntary folate fortification of the US food supply began in 1996, and the first 3-year observation period overlapped a time of gradually increasing folic acid availability in the US and Canadian diets, with consequently decreasing tHcy levels. It is possible that our negative results are due to the progressively lower plasma tHcy, which may have fallen to levels below a threshold for an association with adenoma risk. In this regard, it is of interest that one of the studies that did not find an association of tHcy with CRC risk (19) relied on bloods collected from 2001 – 2006, after fortification of grain products with folic acid, as in the current study. However, this would not explain the null results of two other studies (20,21) which involved non-fortified European populations with comparatively higher plasma tHcy levels.

Plasma tHcy levels were highest at the baseline examination. The number of adenomas at the baseline examination was significantly associated with baseline plasma tHcy in univariate analysis. When we pursued this finding further, we found that after controlling for other plasma tHcy determinants (e.g., plasma folate and other B vitamins) the association between baseline plasma tHcy and adenomas was substantially attenuated and no longer statistically significant. This suggests that even at the higher plasma tHcy levels at the baseline examination there was little independent effect of plasma tHcy in this study population.

This study has several limitations, including 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, and the progressively higher folate levels our study subjects experienced over the period of follow-up, even for those randomized to placebo. We were not able to control for serum creatinine, a well known determinant of plasma tHcy. However, we do not think this affected the main result of no association between plasma tHcy and adenoma recurrence since creatinine is not a risk factor for either adenoma occurrence or recurrence and therefore is not likely to be a confounder by definition. For the analysis of the association of baseline plasma tHcy and the number of baseline adenomas we would not expect confounding a priori but in the worst case scenario, where increased serum creatinine is positively associated with baseline adenoma number as well as higher plasma tHcy, the bias would be away from the null toward an increased risk for multiple adenomas in those with higher plasma tHcy as we saw in the minimally adjusted model. However, our results were null in the fully adjusted model suggesting that this was not a major source of confounding in the final analysis. Additionally, since baseline plasma tHcy was measured after the occurrence of prior adenomas we cannot exclude the possibility that the presence of adenomas affected baseline plasma tHcy, though this seems unlikely.

The advantages of our study include the large sample size and the high follow-up rate for subjects. Additionally, we had data on many known determinants of plasma tHcy and could control for them in our analysis. Finally, because of the prospective design and the fact that all subjects were cleared of polyps at their baseline exam, we were able to assess the effect of baseline plasma tHcy on incident as well as prevalent adenomas.

In summary, we did not see an association between higher baseline plasma tHcy and adenoma recurrence risk in this prospective study of folate and aspirin supplementation. These results do not support those of some earlier studies, which suggested that plasma tHcy may be a marker for increased risk of CRC, adenoma and adenoma recurrence at higher plasma tHcy values but are concordant with three other studies that did not observe any association between plasma tHcy and colorectal cancer risk. Our data suggest one of two possibilities: that there is no independent association between plasma tHcy and adenoma recurrence risk or that any association between plasma tHcy and adenoma recurrence may be limited to plasma tHcy levels higher than those characterizing the current, largely folic acid-fortified, study population.

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Table 1

Baseline characteristics of study subjects by adenoma recurrence

Characteristic	Adenoma Recurrence	No Adenoma Recurrence	P-value
No. Participants	392 (45.0)	479 (55.0)	
Age (y) at baseline mean (SD)	59.1 (9.4)	56.2 (9.5)	<0.001†
Male sex N (%)	270 (68.9)	289 (60.3)	0.01‡
Colorectal cancer in 1st degree relative N (%)	117 (37.6)	148 (38.1)	0.91‡
NSAID use at baseline N (%)	319 (81.4)	379 (79.1)	0.41‡
Multivitamin use at baseline N (%)	134 (34.2)	183 (38.2)	0.22‡
Race/Ethnicity N (%): White African American Hispanic Other	346 (88.3) 23 (5.9) 16 (4.1) 7 (1.8)	408 (85.2) 25 (5.2) 27 (5.6) 19 (4.0)	0.18‡
BMI kg/m^2 <25 25-<30 >=30	104 (26.6) 191 (48.9) 96 (24.6)	161 (33.7) 212 (44.4) 105 (22.0)	0.08‡
Current cigarette smoker N (%)	72 (18.5)	51 (10.7)	0.00‡
Dietary intake kcal/d mean (SD)	1645 (697)	1614 (612)	0.49†
Dietary folate intake† µg /d mean (SD)	316 (144)	327 (158)	0.31†
Total folate intake µg /d mean (SD)	452 (256)	466 (249)	0.41†
Baseline Plasma folate nmol/L mean (SD)	22.1 (15.3)	25.3 (19.4)	0.01§
Baseline Plasma B_{12} pmol/L mean (SD)	322 (144)	337 (179)	0.15§
Baseline Plasma B_6 nmol/L mean (SD)	77.8 (90.8)	84.1 (90.6)	0.01§
Plasma Hcy at baseline mean µmol/L (SD)	10.1 (3.1)	9.6 (2.8)	0.04§
Alcohol use at baseline N (%)	278 (72.8)	297 (64.7)	0.01‡
Caffeine use at baseline N(%)	366 (95.8)	428 (93.3)	0.11‡
History of high Cholesterol at baseline N (%)£	142 (36.3)	147 (30.8)	0.08‡
History of Hypertension at baseline N (%)£	97 (24.7)	120 (25.1)	0.92‡
Lifetime adenomas at baseline mean (SD)	2.8 (2.6)	2.0 (1.7)	<0.001§
Number of baseline adenomas mean (SD)	1.7 (1.1)	1.5 (0.9)	<0.001§
Advanced adenomas at baseline N (%)¶ 0 ≥ 1	248 (70.5) 104 (29.6)	315 (70.3) 133 (29.7)	0.97‡
MTHFR 677 C>T Genotype CC CT TT	178 (47.2) 166 (44.0) 33 (8.8)	197 (44.0) 199 (44.4) 52 (11.6)	0.35‡
MTHFR 1298 A>C Genotype AA	174 (46.0) 169 (44.7)	234 (52.1) 179 (39.9)	0.22‡

Characteristic	Adenoma Recurrence	No Adenoma Recurrence	P-value
AC CC	35 (9.3)	36 (8.0)	

† two sample t-test

‡ Chi-squared test

§ Non parametric Wilcoxon rank sum test

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

£ Self reported from the risk factor questionnaire

Table 2

Univariate Associations between selected risk factors and baseline plasma Hcy

Characteristic[†]	**Baseline Plasma Homocysteine μmol/L**[‡]				
	≤ 7.95	**7.96-9.26**	**9.27-11.25**	**> 11.25**	**P** [*]
Age mean (SD)	54.6 (9.3)	58.1 (9.1)	58.4 (9.6)	58.9 (9.7)	<0.001
Male (%)	41.0	62.2	76.9	77.7	<0.001
Current smoker (% yes)	12.2	11.7	11.9	21.0	0.01
BMI (kg/m^2) % /category 1 (≤ 25 kg/m^2) 2 (25 - < 30 kg/m^2) 3 (≥ 30 kg/m^2)	37.8 38.7 23.4	34.7 43.2 22.1	25.1 49.3 25.6	23.8 54.7 21.5	0.01
Alcohol drinker (% yes)	62.0	70.1	67.7	73.9	0.06
Alcohol mean (SD) drinks /d among drinkers	0.6 (0.8)	0.9 (1.3)	0.9 (0.9)	1.2 (1.4)	<0.001
Baseline Plasma Folate nmol/L mean (SD)	31.0 (18.5)	26.2 (16.6)	20.7 (14.0)	17.3 (17.6)	<0.001
Baseline Plasma B_{12} pmol /L mean (SD)	371 (145)	346 (210)	309 (113)	295 (163)	<0.001
Baseline Plasma B_6 nmol/L mean (SD)	107 (118)	84 (87)	73 (84)	61 (57)	<0.001
Baseline Plasma B_2 nmol/L mean (SD)	36 (50)	33 (61)	27 (51)	24 (43)	<0.001
Number of adenomas at baseline exam mean (SD)	1.4 (0.8)	1.5 (0.8)	1.7 (1.2)	1.7 (1.0)	0.001
MTHFR *677 C>T* Genotype (% yes) *CC* *CT* *TT*	49.5 44.1 6.4	45.2 42.9 12.0	49.0 40.4 10.6	37.9 50.0 12.1	0.11

[†]All risk factor values were taken from the baseline questionnaire.

[‡]Quartile cutpoints based on the distribution of plasma tHcy in the whole study population.

[*]P for trend for continuous variables (age, number of alcohol drinks, plasma levels, number of baseline adenomas) or p for heterogeneity for categorical variables (sex, current smoker, BMI, alcohol drinker, *MTHFR 677*).

Table 3

Association between baseline plasma tHcy and number of adenomas at the baseline exam

	Adjustor set A†	Adjustor set B‡
Baseline Homocysteine (μmol/L)	**OR (95% CI)** §	**OR (95% CI)** §
<= 7.95	Reference	Reference
7.96-9.26	1.15 (0.74-1.79)	1.02 (0.63-1.66)
9.27-11.25	1.37 (0.87-2.16)	1.17 (0.73-1.88)
>11.25	1.49 (0.95-2.31)	1.18 (0.74-1.86)
	p-trend=0.06	p-trend=0.43

† Adjustor set A = age, sex, study center, alcohol (continuous), BMI (continuous), current smoking.

‡ Adjustor set B = age, sex, study center, alcohol (continuous), BMI (continuous), current smoking, plasma B_2, plasma B_6, plasma B_{12}, plasma folate.

§ OR calculated using a proportional odds model where the outcome is number of baseline adenomas (1, 2, 3, 4,or 5+).

Table 4

Association between baseline plasma tHcy and adenoma recurrence risk by folate treatment group and adenoma type

	Placebo Group					Folate Group				
All Adenomas										
plasma tHcy μmol/L	**≤ 7.95**	**7.96-9.26**	**9.27-11.25**	**> 11.25**	**P_{trend}**	**≤ 7.95**	**7.96-9.26**	**9.27-11.25**	**> 11.25**	**P_{trend}**
recurrences/total	41/109	57/118	40/102	49/102		44/113	53/104	55/110	53/113	
Adjusted RR†	1.0	1.15 (0.84-1.58)	0.84 (0.60-1.19)	0.98 (0.70-1.38)	0.17	1.0	1.05 (0.76-1.44)	0.95 (0.69-1.32)	0.81 (0.58-1.12)	0.17
P- heterogeneity					0.53					
Advanced Adenomas ‡										
recurrences/total	7/103	13/115	10/101	8/101		13/111	13/102	7/110	19/110	
Adjusted RR †	1.0	1.39 (0.56-3.42)	1.20 (0.46-3.13)	1.00 (0.36-2.75)	0.83	1.0	0.86 (0.40-1.86)	0.41 (0.17-1.03)	0.98 (0.47-2.07)	0.21
P- heterogeneity					0.30					

† Adjusted for age, sex, study center, alcohol (continuous), BMI (continuous), smoking status (never, former, current), plasma B_2, plasma B_6, plasma B_{12}, plasma folate and aspirin treatment assignment.

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