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# A Candidate Gene Study of Folate-Associated One Carbon Metabolism Genes and Colorectal Cancer Risk

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## Abstract

**Background—**Folate-associated one carbon metabolism (FOCM) may play an important role in colorectal carcinogenesis. Variation in FOCM genes may explain some of the underlying risk of colorectal cancer.

**Methods—**This study utilized data from 1,805 population-based colorectal cancer cases and 2,878 matched sibling controls from the Colon Cancer Family Registry (C-CFR). We used a comprehensive tagSNP approach to select 395 tagSNPs in 15 genes involved in folate and vitamin $B_{12}$ metabolism. Genotyping was performed using the Illumina GoldenGate or Sequenom platforms. Risk factor and dietary data were collected using self-completed questionnaires. MSI status was determined using standard techniques and tumor subsite was obtained from pathology reports. The association between

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SNPs and colorectal cancer was assessed using conditional logistic regression with sibships as the matching factor and assuming a log additive or co-dominant model.

**Results—**In the log additive model, two linked ($r^2$=0.99) tagSNPs in the *DHFR* gene (rs1677693 and rs1643659) were associated with a significant decrease in CRC risk after correction for multiple testing (OR=0.87; 95% CI=0.71 – 0.94; P=0.029 and OR=0.87 95% CI=0.71 – 0.95, P=0.034 for rs1677693 and rs1643659 respectively. These two linked ($r^2$=0.99) tagSNPs and one tagSNP in the *MTR* gene (rs4659744) were significantly associated with reduced CRC risk only among individuals not using multivitamin supplements.

**Conclusions—**Overall, we found only moderate evidence that genetic variation in 15 folate pathway genes may affect CRC risk except in non multivitamin users.

**Impact—**This study suggests that multivitamin supplement use may modify the association between folate pathway genes and CRC risk in a post folic acid supplemented population.

### Keywords

Colorectal Cancer; TagSNP; Folate Supplementation; Multivitamins; Microsatellite Instability; Colon subsite; ADA; ADH1C; AHCY; AMD1; CBS; DHFR; GIF; CUBN; MAT2A; MTHFD1; MTR; MTRR; SHMT1; TCN2; TYMS

## Introduction

Folates carry most of the one carbon groups essential for hundreds of intracellular transmethylation reactions including those involved in DNA methylation and DNA synthesis. A role for these reactions and the carcinogenic changes important in colorectal cancer has been defined, both *in vitro* and *in vivo*, in both humans and animals (1–3).

Folates are a family of molecules that have a common structure based on a pteridine ring conjugated to one or more glutamate side chains. This basic structure is modified by the binding of various single carbon groups to the pteridine ring. Folate-associated one carbon metabolism (FOCM) provides the 1-carbon groups for numerous critical intracellular reactions including methionine synthesis, the de novo synthesis of dTMP from dUMP and de novo purine synthesis (2). Methionine synthesis is the first step in the synthesis of S-Adenosylmethionine (SAM). SAM is the primary methyl donor for hundreds of intracellular methylation reactions including the methylation of DNA. Aberrant DNA methylation, either global hypomethylation or hypermethylation of tumor suppressor genes, plays a key role in colorectal carcinogenesis (4). Global hypomethylation is associated with chromosomal instability and aneuploidy (5,6) while hypermethylation is associated with loss of transcription which can affect the activity of tumor suppressor genes (4). Low folate conditions have also been associated with increased uracil in DNA (7,8) and increased DNA damage (9).

Dietary folates have been associated with decreased colorectal neoplasia risk (1). However, their role in populations exposed to folic acid fortification of grain products is less clear (10, 11). Folic acid is the monoglutamated folate form, not found in nature, that is used in supplements and for fortifying cereal grain products in North America. In experimental preclinical studies the effect of folic acid supplementation on the risk for colon tumor formation depended upon the timing of its administration. Increased dietary folic acid decreased the number of intestinal tumors when given to APC $^{min}/^{-}$ and APC $^{min}$/MSH2$^{-}/^{-}$ mice before the onset of pre-neoplastic foci but the number of tumors was increased significantly when given after the establishment of pre-neoplastic foci (12,13). Some recent human studies also suggest that neoplasia risk may be increased in those taking high doses of folic acid from supplements (14–18) in the context of a folic acid-supplemented diet. In this regard Mason et al documented

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a significant trend toward increasing CRC incidence in the US and Canada co-incident with the fortification of grain products with folic acid (19). The present study population was recruited after fortification of the US and Canadian food supplies and so represents one of the first post-fortification study cohorts in which to assess associations between folate associated one carbon metabolism (FOCM)-associated genes and CRC risk.

Several studies have reported on the potential significance of polymorphic variation in FOCM-associated genes and colorectal cancer risk but all of these studies have been restricted to a few variants in a limited number of genes (3,20). We conducted a systematic investigation of genetic variation in 15 folate pathway genes involved in nucleotide synthesis, methionine metabolism, uptake and distribution of vitamin $B_{12}$ and polyamine synthesis (Table 1) and CRC risk using a comprehensive tagSNP approach. Previous analyses in this population include variation in MTHFR (21), where we reported an association only for the *C677T TT* and *A1298C CC* genotypes in non-multivitamin supplement users, and four genes involved in folate uptake and distribution; the reduced folate carrier 1 (*RFC1/SLC19A1*), folate receptor 1 (*FOLR1*), γ-glutamyl hydrolase (*GGH*), and folylpolyglutamate synthase (*FPGS*). None of these genes were associated with CRC risk in any subgroup (22).

## Materials and Methods

### Study Population

The methods used for this study are described in detail in Levine et al (21): 1,806 cases and their sibling controls (n=2,879) were enrolled in the six sites of the Colon Cancer Family Registry (C-CFR), an NCI-supported consortium, initiated in 1997 (23). Details of subject recruitment can be found in Newcomb et al (24). Briefly, cases were recruited in two phases, from 1998 – 2002 (Phase 1) and from 2002 – 2007 (Phase 2). Phase 2 subjects were enriched in cases more likely to have a family history of CRC. In this study we used only cases that were identified through population-based cancer registries as described by Newcomb et al. (24). Controls were the unaffected siblings of cases. All subjects signed an informed consent before providing data to the C-CFR.

### SNP selection

TagSNPs were selected using Haploview Tagger (25) for the CEU population as described (21). The linkage disequilibrium blocks were determined using data from HapMap data release #16c.1, June 2005, on NCBI B34 assembly, dbSNP b124. For each gene, we extended the 5'- and 3'-UTR regions to include the 5'- and 3'-most SNP within the LD block (approximately 10 kb upstream and 5 kb downstream). In regions of no- or low-LD, SNPs with MAF > 5% at a density of approximately 1 per kb were selected from either HapMap or dbSNP. Finally, non-synonymous SNPs and expert-curated SNPs regardless of MAF were included. A total of 395 SNPs met our criteria of a MAF ≥ 0.05 and a p-value for Hardy Weinberg equilibrium ≥ 0.00013.

### SNP Genotyping

SNPs were genotyped on the Illumina 1536 GoldenGate platform (26). We implemented a series of quality control checks based on Illumina metrics, as described previously (21). We performed additional genotyping using Sequenom's iPLEX Gold for 13 SNPs that were not successfully genotyped on the Illumina platform as described (21). Polymerase chain reaction (PCR) and extension primers for these SNPs are available upon request.

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### MSI testing

All available tumors were assayed for instability at as many as 10 microsatellite markers: BAT25, BAT26, BAT40, BAT34C4, D5S346, D17S250, D18S55, D10S197, ACTC and MYCL as described (24). MSI data were available for 1,200 (66.4%) cases including all phase one tumors in probands and their relatives with tumors that had clear results for at least 4 markers, and a sample of phase 2 tumors as described by Newcomb et al (24). Instability at ≥30% of the tested loci was defined as MSI-H, instability at ≥10% but less than 30% of loci was defined as MSI-L and those with instability at 0 loci were categorized as MSS. MSI-L and MSS cases were combined in the analysis.

### Colon Subsite

Colon subsite was obtained by reviewing pathology reports. Right colon was defined as occurring in the cecum through the splenic flexure, left colon included the descending colon through the sigmoid colon and rectal tumors included the recto-sigmoid junction and the rectum.

### Folate supplement use, Multivitamin use and family history of colorectal cancer

A standard risk factor questionnaire, described in Newcomb et al (24), was administered to all participants at recruitment and available for approximately 98% of the study population. Family history of CRC was defined as any $1^{st}$-degree relative with CRC. Folate- and multivitamin-supplement use was defined as ever use at least twice a week for more than a month.

### Dietary folate intake

Estimated dietary folate intake, available for 585 cases, 837 controls (about 1/3 of the study population) was estimated from a validated food frequency questionnaire developed at the University of Hawaii (27) and available only for subjects recruited in Ontario, Hawaii and the USC Consortium. All food frequency data were collected after folic acid fortification of the North American food supply in 1998 and analysed, in separate analyses, assuming either pre- or post-fortification folate values as dietary folate equivalents. In the analysis of total dietary folate intake we assumed that both multivitamin and folic acid supplements contained 400 µg of folic acid per pill or tablet.

### Statistical Analysis

We used multivariable conditional logistic regression with sibship as the matching factor to estimate main effects assuming a log-additive model. As a secondary analysis we assessed all genotype effects in a co-dominant model and a 2 df likelihood ratio test to estimate p-values for each comparison. We controlled for age and sex in all analyses. Additional control for other variables did not change the results by more than 10%. For the log-additive model and within each gene, p-values for all SNPs were adjusted for multiple testing taking into account correlated tagSNPs using a modified test of Conneely and Boehnke ($P_{act}$) (28). We report both the observed likelihood ratio p-value and $P_{act}$. For a test of a single gene, a α-level of 0.05 for $P_{act}$ was used to determine statistical significance. To define significance across all the genes tested, a Bonferroni corrected p-value of ($\alpha = 0.05/15 = 0.0033$) may also be considered. For the 2 degree of freedom co-dominant model and all stratified analyses we used the Bonferroni significance level of 0.00013 (0.05/395 tagSNPs).

## Results

Table 2 shows the demographic characteristics of the study population. The study population was over 87% non-Hispanic White. The mean age of cases was 53.5 (± 10.9) and the mean

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age of controls was 54.0 (±11.8). Fifty one percent of cases and 44% of controls were male and 81% of the cases were from sites in North America. Thirty percent of cases reported a family history of CRC. Multivitamin supplement use was reported for 52.8% of cases and 46.8% of controls while 11% of cases and 9.5% of controls reported taking a folic acid supplement.

The results of the single SNP analysis, log additive model, are shown in Figure 1 and Supplementary Table 1. Only 2 tagSNPs were associated significantly with CRC risk after correction for multiple testing. Table 3 shows the associations for the 10 tagSNPs with nominally significant associations with risk of CRC. The two significant tagSNPs, DHFR rs1677693 and rs1643659, were strongly linked ($r^2$=0.99) and associated with a decrease in CRC risk (per minor allele OR= 0.87; 0.71–0.94, $P_{act}$=0.029 and 0.87; 0.71–0.95, $P_{act}$=0.034 for rs1677693 and rs1643659 respectively).

We assessed possible heterogeneity of effects by MSI-status, tumor subsite (proximal, distal, rectal), folate and multivitamin supplement use (yes or no), dietary folate intake (continuous), time to interview ($\geq$ 2 years or > 2 years), ascertainment site (Australia or North America) and family history of CRC (yes or no). There was no significant heterogeneity except with multivitamin use (Table 4). The two *DHFR* tagSNPs (rs1677693 and rs1643659) and one tagSNP in *MTR* (rs4659744) were significantly associated with risk only in individuals not using multivitamin supplements. In individuals who did not use multivitamin supplements the per allele OR for both rs1677693 and rs1643659 was 0.69 (95% CI 0.58–0.82; p = 0.00002 and 0.00003 respectively). For individuals using multivitamin supplements these OR's were 0.91 (0.78–1.07) for both tagSNPs. The p-values for heterogeneity between the two strata were 0.000899 and 0.000986 respectively. For *MTR* rs4659744 the OR in non-multivitamin supplement users was 0.76 (0.67–0.88; p=0.00011) and 0.97 (0.85–1.11) in supplement users (p for heterogeneity = 0.000265). There was also significant heterogeneity between non-multivitamin and multivitamin supplement users for 4 tagSNPs in *MAT2A* (rs1446669,rs699664, rs10179195, rs6739015) and one tagSNP in *MTRR* (rs716537). In each case the per allele OR was non-significantly decreased in non-users and slightly increased in users of multivitamin supplements (Table 4).

## Discussion

In this large family-based case-control study of CRC risk we found that common variants in *DHFR* and *MTR* may be associated with a decrease in CRC risk in non-users of multivitamin supplements while there was no association in multivitamin users. No other significant associations were observed for common variants in these 15 FOCM-associated genes involved in nucleotide synthesis, methylation, vitamin $B_{12}$ transport or polyamine synthesis. There was significant heterogeneity by multivitamin use for 4 tagSNPs in *MAT2A* and 1 tagSNP in *MTRR* although neither association was statistically significant.

Dihydrofolate reductase (DHFR) reduces dihydrofolate to tetrahydrofolate (THF). Failure of this reaction can trap folate moieties in an oxidized form that cannot be used for further one-carbon transfers. Inhibition of the DHFR reaction has been exploited for chemotherapy by the drug methotrexate (MTX) and increased DHFR activity is a common cause for resistance to MTX (29). Reduction by DHFR is also involved in the utilization of folic acid, the fully oxidized folate form used for folate supplementation (30).

Variation in DHFR seems to affect folate metabolism. Homozygosity for a 19bp deletion polymorphism in the 1st intron of the *DHFR* gene has been associated with increased DHFR mRNA (31,32), a 14% decrease in plasma homocysteine (33), increased red blood cell and plasma folate in women (34), increased circulating folic acid in those with high folic acid intake

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(>500 μg/day) and decreased red blood cell folate in those consuming less than 250 μg/day (35). A study that assessed the association between this polymorphism and CRC risk reported no association for either CpG island methylator positive (CIMP) or negative tumors (36). Other promoter region (37) or 3'UTR SNPs (38) have been identified in DHFR but no SNPs in the coding region of the gene have been reported (33).

The vitamin $B_{12}$-dependent enzyme methionine synthase (MTR) catalyses the transfer of a methyl group from 5-methyltetrahydrofolate (5-MTHF) to homocysteine to make methionine and tetrahydrofolate (THF). Interference with this process traps folates as 5-MTHF, which cannot be recycled for further one-carbon transfer reactions (39). The de novo synthesis of methionine is the first step in the synthesis of the universal methyl donor S-adenosylmethionine (SAM) suggesting a possible mechanism by which genetic *variation* in the *MTR* gene may influence CRC risk. In this population one intronic tagSNP in *MTR* (rs4659744) was associated with decreased CRC risk in non-multivitamin supplement users. The association between one non-synonymous SNP in the *MTR* gene (*A2756G*, D919G, rs1805087) and CRC risk has been studied by several groups (40–46). The reported associations were inconsistent although a recent meta-analysis reported a small but significant decrease in CRC risk for the *GG* genotype in European populations (47). The D919G genotype (not linked to rs4659744) was not associated with risk in this population.

TagSNPs in two other genes, *MTRR* and *MAT2A* were also modified significantly by multivitamin use, although the individual associations were not statistically significant in either multivitamin use group. MTRR reduces oxidized MTR and $B_{12}$ after transfer of the methyl group from the $B_{12}$ cofactor to homocysteine, while the MAT2A enzyme transfers the methyl group from methionine to SAM in an ATP-dependent reaction. The *MTRR* SNPs rs2303080 and rs2287780 were significantly associated with CRC risk in a recent study of 24 non-synonymous SNPs in 13 folate pathway genes (42) but were not associated with risk overall or in any subgroup in our study.

Martinez et al reported that the association between the *MTHFR C677T TT* genotype and adenoma recurrence was limited to non-multivitamin supplement users with risk increased in that largely post-fortification population (48). In the current study population, on the other hand, the *MTHFR-677 TT* genotype was associated with a significantly decreased CRC risk in individuals not using a multivitamin supplement but an OR of approximately 1.0 in those using a multivitamin supplement, results that are similar to those reported here for the other folate pathway gene variants (21).

Numerous other studies of the *MTHFR C677T* and *A1298C* polymorphisms and other FOCM-associated genes have assessed interactions between genotypes and indices of folate, alcohol, or other B-vitamins, although most of these studies were undertaken in populations not exposed to fortification of foods with folic acid (36,41–46,49–64). All but 5 of these studies (36,46, 52,57,63) support the existence of such interactions. For the *MTHFR C677T* genotype the majority of studies reported that the *TT* genotype is more protective in those with a diet high in folate or other methyl-group nutrients or low in alcohol (42–44,49,50,[53]–55,60,64,65) although other studies have reported no such trends or trends in the opposite direction (45, 51,59). Although none of these studies looked specifically at multivitamin supplement use, such supplement use is the major source of folates for those in the highest category (66).

Clearly, we do not know for certain which nutrients or what combination of nutrients in multivitamins may modify the association between these FOCM-associated genetic variants and colorectal cancer risk. However, folic acid, $B_6$ and $B_{12}$ are likely to play the dominant roles. Additionally, in this study we used a tagSNP approach to assess genetic effects so it is not possible to predict how the variations tagged by these SNPs might interact with methyl-

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group nutrient status. It is notable, however, that over 80% of our study population was recruited after fortification of the North American food supply with folic acid, the synthetic folate used in fortified foods and supplements.

In our population, those not taking multivitamin supplements may have had folate levels more similar to those taking multivitamin supplements than to the nonmultivitamin supplement users in pre-fortification populations. The significant increase in plasma and red blood cell folate in both supplement users and non-users after fortification is well documented (67–69). Thus, for genotype effects that are more relevant at higher folate levels we would expect to see associations in non-multivitamin supplement users in this population.

For the multivitamin supplement users the interaction between genotype and CRC risk may be more complex. First, genotype effects may become less important when mucosal folate levels are maximized, consistent with the OR's close to 1.0 that we observed in multivitamin supplement users. Additionally, unmetabolized folic acid levels become measurable in the circulation at folic acid levels over 400 μg/d, due to saturation of the DHFR enzyme (30,70, 71). Such high folic acid intakes may be more prevalent in multivitamin users eating folic acid fortified foods (72). The effect of this on cancer risk is unknown. One *in vivo* study (73) reported that increased unmetabolized folic acid was associated with a decrease in natural killer cell activity, suggesting a possible decrease in tumor surveillance. Additionally, sustained exposure to high levels of folic acid was associated with a significant decrease in folate uptake in human colon cancer cells *in vitro* (74). Whether circulating folic acid levels among multivitamin users in fortified populations are high enough to have this effect *in vivo* is not known but the possibility should be studied further as it suggests a paradoxical decrease in tissue folate stores among those with the highest folic acid intakes. Decreased folate uptake in those with higher folic acid intakes may nullify any protective effect of genotype. Alternatively, the lack of any effect of genotype on CRC risk in multivitamin users may be due to a higher progression risk in multivitamin users with pre-clinical lesions at the time fortification was instituted in 1998. Such an effect would be consistent with the increased neoplasia risk observed in recent studies of post folic acid fortified populations (14–18), the dual effect of folic acid supplementation observed in animal studies (12,13) and the co-incident rise in CRC incidence in the US and Canada around the time of fortification (19). In this regard it may be of interest that the prevalence of multivitamin use was significantly higher in cases than controls in this study population (Table 2, $p<0.01$) suggesting increased CRC risk in supplement users. It is possible that this increase in CRC risk among supplement users ameliorated any protective effects attributable to genotype. Whether multivitamin use is a CRC risk factor in post-fortification populations requires further study.

The strengths of our study include the large number of subjects, the comprehensive approach to tagSNP selection and the use of a family-based design which limits the possibility of population stratification. This study has several limitations. In the main analysis we had limited statistical power for SNPs with a minor allele frequency (MAF) of 5% or less (60% power to detect an OR of 1.4). For SNPs with an MAF of 10% we had 80% power to detect an OR of 1.35. We did not have data on all potentially relevant FOCM pathway genes and may have missed some relevant tagSNPs. However, gene coverage ranged from 71% of all identified SNPs for CBS to 98% for SHMT1. The mean coverage was 89%. Although we did take multiple testing into account some possibility of a false positive result remains given the large number of parameters estimated. Similarly the stringent Bonferroni correction may have resulted in some false negatives in stratified analyses. Our study sample was selected to enrich the subject pool for those at higher CRC risk and recruited largely from populations with mandatory supplementation of foods with folic acid, potentially limiting the generalizability of our results.

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In conclusion, in these data from a large population-based study we found significant associations between tagSNPs in *DHFR* and CRC risk in a study of 395 tagSNPs in 15 folate-pathway genes. Our data suggest that two linked tagSNPs in *DHFR* and one in *MTR* may mark genotypes that decrease CRC risk in non-multivitamin supplement users in this folate fortified population. Future studies of similarly fortified populations are required to replicate these results.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## References

1. Giovannucci E. Epidemiologic studies of folate and colorectal neoplasia: a review. J Nutr 2002;132:2350S–2355S. [PubMed: 12163691]
2. Kim YI. Role of folate in colon cancer development and progression. J Nutr 2003;133:3731S–3739S. [PubMed: 14608107]
3. Ulrich CM. Nutrigenetics in cancer research--folate metabolism and colorectal cancer. J Nutr 2005;135:2698–2702. [PubMed: 16251633]
4. Jones PA, Baylin SB. The epigenomics of cancer. Cell 2007;128:683–692. [PubMed: 17320506]
5. Ehrlich M. DNA methylation in cancer: too much, but also too little. Oncogene 2002;21:5400–5413. [PubMed: 12154403]
6. Pogribny IP, Beland FA. DNA hypomethylation in the origin and pathogenesis of human diseases. Cell Mol Life Sci 2009;66:2249–2261. [PubMed: 19326048]
7. Blount BC, Mack MM, Wehr CM, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A 1997;94:3290–3295. [PubMed: 9096386]
8. Duthie SJ, Narayanan S, Sharp L, Little J, Basten G, Powers H. Folate, DNA stability and colo-rectal neoplasia. Proc Nutr Soc 2004;63:571–578. [PubMed: 15831129]
9. Beetstra S, Thomas P, Salisbury C, Turner J, Fenech M. Folic acid deficiency increases chromosomal instability, chromosome 21 aneuploidy and sensitivity to radiation-induced micronuclei. Mutat Res 2005;578:317–326. [PubMed: 16005909]
10. Kim YI. Will mandatory folic acid fortification prevent or promote cancer? Am J Clin Nutr 2004;80:1123–1128. [PubMed: 15531657]
11. Ulrich CM, Potter JD. Folate supplementation: too much of a good thing? Cancer Epidemiol Biomarkers Prev 2006;15:189–193. [PubMed: 16492904]
12. Song J, Medline A, Mason JB, Gallinger S, Kim YI. Effects of dietary folate on intestinal tumorigenesis in the apcMin mouse. Cancer Res 2000;60:5434–5440. [PubMed: 11034085]
13. Song J, Sohn KJ, Medline A, Ash C, Gallinger S, Kim YI. Chemopreventive effects of dietary folate on intestinal polyps in Apc+/−Msh2−/− mice. Cancer Res 2000;60:3191–3199. [PubMed: 10866310]

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14. Charles D, Ness AR, Campbell D, Davey Smith G, Hall MH. Taking folate in pregnancy and risk of maternal breast cancer. BMJ 2004;329:1375–1376. [PubMed: 15591563]
15. Cole BF, Baron JA, Sandler RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. JAMA 2007;297:2351–2359. [PubMed: 17551129]
16. Figueiredo JC, Grau MV, Haile RW, et al. Folic acid and risk of prostate cancer: results from a randomized clinical trial. J Natl Cancer Inst 2009;101:432–435. [PubMed: 19276452]
17. Hirsch S, Sanchez H, Albala C, et al. Colon cancer in Chile before and after the start of the flour fortification program with folic acid. Eur J Gastroenterol Hepatol 2009;21:436–439. [PubMed: 19190501]
18. Stolzenberg-Solomon RZ, Chang SC, Leitzmann MF, et al. Folate intake, alcohol use, and postmenopausal breast cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Am J Clin Nutr 2006;83:895–904. [PubMed: 16600944]
19. Mason JB, Dickstein A, Jacques PF, et al. A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. Cancer Epidemiol Biomarkers Prev 2007;16:1325–1329. [PubMed: 17626997]
20. Xu, X.; Liu, AY.; Ulrich, CM.; Chen, J. Folate and Cancer: Epidemiological Perspecitve. New York: Taylor and Francis; 2009.
21. Levine AJ, Figueiredo JC, Lee W, et al. Genetic variability in the MTHFR gene and colorectal cancer risk using the colorectal cancer family registry. Cancer Epidemiol Biomarkers Prev 2010;19:89–100. [PubMed: 20056627]
22. Figueiredo JC, Levine AJ, Lee WH, et al. Genes involved with folate uptake and distribution and their association with colorectal cancer risk. Cancer Causes Control. 2009
23. http://epi.grants.cancer.gov/CFR/.
24. Newcomb PA, Baron J, Cotterchio M, et al. Colon Cancer Family Registry: an international resource for studies of the genetic epidemiology of colon cancer. Cancer Epidemiol Biomarkers Prev 2007;16:2331–2343. [PubMed: 17982118]
25. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–265. [PubMed: 15297300]
26. Shen R, Fan JB, Campbell D, et al. High-throughput SNP genotyping on universal bead arrays. Mutat Res 2005;573:70–82. [PubMed: 15829238]
27. Stram DO, Hankin JH, Wilkens LR, et al. Calibration of the dietary questionnaire for a multiethnic cohort in Hawaii and Los Angeles. Am J Epidemiol 2000;151:358–370. [PubMed: 10695594]
28. Conneely KN, Boehnke M. So Many Correlated Tests, So Little Time! Rapid Adjustement of P Values for Multiple Correlated Tests. Am J Hum Genet 2007;81:1158–1168.
29. Banerjee D, Mayer-Kuckuk P, Capiaux G, Budak-Alpdogan T, Gorlick R, Bertino JR. Novel aspects of resistance to drugs targeted to dihydrofolate reductase and thymidylate synthase. Biochim Biophys Acta 2002;1587:164–173. [PubMed: 12084458]
30. Bailey SW, Ayling JE. The extremely slow and variable activity of dihydrofolate reductase in human liver and its implications for high folic acid intake. Proc Natl Acad Sci U S A 2009;106:15424–15429. [PubMed: 19706381]
31. Parle-McDermott A, Pangilinan F, Mills JL, et al. The 19-bp deletion polymorphism in intron-1 of dihydrofolate reductase (DHFR) may decrease rather than increase risk for spina bifida in the Irish population. Am J Med Genet A 2007;143A:1174–1180. [PubMed: 17486595]
32. Xu X, Gammon MD, Wetmur JG, et al. A functional 19-base pair deletion polymorphism of dihydrofolate reductase (DHFR) and risk of breast cancer in multivitamin users. Am J Clin Nutr 2007;85:1098–1102. [PubMed: 17413111]
33. Gellekink H, Blom HJ, van der Linden IJ, den Heijer M. Molecular genetic analysis of the human dihydrofolate reductase gene: relation with plasma total homocysteine, serum and red blood cell folate levels. Eur J Hum Genet 2007;15:103–109. [PubMed: 16969375]
34. Stanislawska-Sachadyn A, Brown KS, Mitchell LE, et al. An insertion/deletion polymorphism of the dihydrofolate reductase (DHFR) gene is associated with serum and red blood cell folate concentrations in women. Hum Genet 2008;123:289–295. [PubMed: 18247058]

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35. Kalmbach RD, Choumenkovitch SF, Troen AP, Jacques PF, D'Agostino R, Selhub J. A 19-base pair deletion polymorphism in dihydrofolate reductase is associated with increased unmetabolized folic acid in plasma and decreased red blood cell folate. J Nutr 2008;138:2323–2327. [PubMed: 19022952]
36. Curtin K, Slattery ML, Ulrich CM, et al. Genetic polymorphisms in one-carbon metabolism: associations with CpG island methylator phenotype (CIMP) in colon cancer and the modifying effects of diet. Carcinogenesis 2007;28:1672–1679. [PubMed: 17449906]
37. Dulucq S, St-Onge G, Gagne V, et al. DNA variants in the dihydrofolate reductase gene and outcome in childhood ALL. Blood 2008;111:3692–3700. [PubMed: 18096764]
38. Goto Y, Yue L, Yokoi A, et al. A novel single-nucleotide polymorphism in the 3'-untranslated region of the human dihydrofolate reductase gene with enhanced expression. Clin Cancer Res 2001;7:1952–1956. [PubMed: 11448909]
39. Lucock M. Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. Mol Genet Metab 2000;71:121–138. [PubMed: 11001804]
40. de Vogel S, Wouters KA, Gottschalk RW, et al. Genetic variants of methyl metabolizing enzymes and epigenetic regulators: associations with promoter CpG island hypermethylation in colorectal cancer. Cancer Epidemiol Biomarkers Prev 2009;18:3086–3096. [PubMed: 19843671]
41. Guerreiro CS, Carmona B, Goncalves S, et al. Risk of colorectal cancer associated with the C677T polymorphism in 5,10-methylenetetrahydrofolate reductase in Portuguese patients depends on the intake of methyl-donor nutrients. Am J Clin Nutr 2008;88:1413–1418. [PubMed: 18996879]
42. Koushik A, Kraft P, Fuchs CS, et al. Nonsynonymous polymorphisms in genes in the one-carbon metabolism pathway and associations with colorectal cancer. Cancer Epidemiol Biomarkers Prev 2006;15:2408–2417. [PubMed: 17164363]
43. Le Marchand L, Wilkens LR, Kolonel LN, Henderson BE. The MTHFR C677T polymorphism and colorectal cancer: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev 2005;14:1198–1203. [PubMed: 15894672]
44. Ma J, Stampfer MJ, Christensen B, et al. A polymorphism of the methionine synthase gene: association with plasma folate, vitamin B12, homocyst(e)ine, and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 1999;8:825–829. [PubMed: 10498402]
45. Matsuo K, Ito H, Wakai K, et al. One-carbon metabolism related gene polymorphisms interact with alcohol drinking to influence the risk of colorectal cancer in Japan. Carcinogenesis 2005;26:2164–2171. [PubMed: 16051637]
46. Ulvik A, Vollset SE, Hansen S, Gislefoss R, Jellum E, Ueland PM. Colorectal cancer and the methylenetetrahydrofolate reductase 677C -> T and methionine synthase 2756A -> G polymorphisms: a study of 2,168 case-control pairs from the JANUS cohort. Cancer Epidemiol Biomarkers Prev 2004;13:2175–2180. [PubMed: 15598777]
47. Yu K, Zhang J, Dou C, et al. Methionine synthase A2756G polymorphism and cancer risk: a meta-analysis. Eur J Hum Genet 2010;18:370–378. [PubMed: 19826453]
48. Martinez ME, Thompson P, Jacobs ET, et al. Dietary factors and biomarkers involved in the methylenetetrahydrofolate reductase genotype-colorectal adenoma pathway. Gastroenterology 2006;131:1706–1716. [PubMed: 17087956]
49. Chang SC, Lin PC, Lin JK, Yang SH, Wang HS, Li AF. Role of MTHFR polymorphisms and folate levels in different phenotypes of sporadic colorectal cancers. Int J Colorectal Dis 2007;22:483–489. [PubMed: 16941173]
50. Chen J, Giovannucci E, Kelsey K, et al. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. Cancer Res 1996;56:4862–4864. [PubMed: 8895734]
51. Curtin K, Bigler J, Slattery ML, Caan B, Potter JD, Ulrich CM. MTHFR C677T and A1298C polymorphisms: diet, estrogen, and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 2004;13:285–292. [PubMed: 14973104]
52. Jiang Q, Chen K, Ma X, et al. Diets, polymorphisms of methylenetetrahydrofolate reductase, and the susceptibility of colon cancer and rectal cancer. Cancer Detect Prev 2005;29:146–154. [PubMed: 15829374]
53. Keku T, Millikan R, Worley K, et al. 5,10-Methylenetetrahydrofolate reductase codon 677 and 1298 polymorphisms and colon cancer in African Americans and whites. Cancer Epidemiol Biomarkers Prev 2002;11:1611–1621. [PubMed: 12496052]

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54. Le Marchand L, Donlon T, Hankin JH, Kolonel LN, Wilkens LR, Seifried A. B-vitamin intake, metabolic genes, and colorectal cancer risk (United States). Cancer Causes Control 2002;13:239–248. [PubMed: 12020105]

55. Ma J, Stampfer MJ, Giovannucci E, et al. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res 1997;57:1098–1102. [PubMed: 9067278]

56. Matsuo K, Hamajima N, Hirai T, et al. Methionine Synthase Reductase Gene A66G Polymorphism is Associated with Risk of Colorectal Cancer. Asian Pac J Cancer Prev 2002;3:353–359. [PubMed: 12716294]

57. Murtaugh MA, Curtin K, Sweeney C, et al. Dietary intake of folate and co-factors in folate metabolism, MTHFR polymorphisms, and reduced rectal cancer. Cancer Causes Control 2007;18:153–163. [PubMed: 17245555]

58. Otani T, Iwasaki M, Hanaoka T, et al. Folate, vitamin B6, vitamin B12, and vitamin B2 intake, genetic polymorphisms of related enzymes, and risk of colorectal cancer in a hospital-based case-control study in Japan. Nutr Cancer 2005;53:42–50. [PubMed: 16351505]

59. Sharp L, Little J, Brockton NT, et al. Polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene, intakes of folate and related B vitamins and colorectal cancer: a case-control study in a population with relatively low folate intake. Br J Nutr 2008;99:379–389. [PubMed: 18053312]

60. Slattery ML, Potter JD, Samowitz W, Schaffer D, Leppert M. Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 1999;8:513–518. [PubMed: 10385141]

61. Steck SE, Keku T, Butler LM, et al. Polymorphisms in methionine synthase, methionine synthase reductase and serine hydroxymethyltransferase, folate and alcohol intake, and colon cancer risk. J Nutrigenet Nutrigenomics 2008;1:196–204. [PubMed: 19776626]

62. Ulrich CM, Curtin K, Potter JD, Bigler J, Caan B, Slattery ML. Polymorphisms in the reduced folate carrier, thymidylate synthase, or methionine synthase and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 2005;14:2509–2516. [PubMed: 16284371]

63. Van Guelpen B, Hultdin J, Johansson I, et al. Low folate levels may protect against colorectal cancer. Gut 2006;55:1461–1466. [PubMed: 16638790]

64. Yin G, Kono S, Toyomura K, et al. Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and colorectal cancer: the Fukuoka Colorectal Cancer Study. Cancer Sci 2004;95:908–913. [PubMed: 15546509]

65. Wang J, Gajalakshmi V, Jiang J, et al. Associations between 5,10-methylenetetrahydrofolate reductase codon 677 and 1298 genetic polymorphisms and environmental factors with reference to susceptibility to colorectal cancer: a case-control study in an Indian population. Int J Cancer 2006;118:991–997. [PubMed: 16152599]

66. Yeung L, Yang Q, Berry RJ. Contributions of total daily intake of folic acid to serum folate concentrations. JAMA 2008;300:2486–2487. [PubMed: 19050191]

67. Dietrich M, Brown CJ, Block G. The effect of folate fortification of cereal-grain products on blood folate status, dietary folate intake, and dietary folate sources among adult non-supplement users in the United States. J Am Coll Nutr 2005;24:266–274. [PubMed: 16093404]

68. Ganji V, Kafai MR. Trends in serum folate, RBC folate, and circulating total homocysteine concentrations in the United States: analysis of data from National Health and Nutrition Examination Surveys, 1988–1994, 1999–2000, and 2001–2002. J Nutr 2006;136:153–158. [PubMed: 16365075]

69. Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. N Engl J Med 1999;340:1449–1454. [PubMed: 10320382]

70. Kelly P, McPartlin J, Goggins M, Weir DG, Scott JM. Unmetabolized folic acid in serum: acute studies in subjects consuming fortified food and supplements. Am J Clin Nutr 1997;65:1790–1795. [PubMed: 9174474]

71. Sweeney MR, Staines A, Daly L, et al. Persistent circulating unmetabolised folic acid in a setting of liberal voluntary folic acid fortification. Implications for further mandatory fortification? BMC Public Health 2009;9:295. [PubMed: 19689788]

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72. Kalmbach RD, Choumenkovitch SF, Troen AM, D'Agostino R, Jacques PF, Selhub J. Circulating folic acid in plasma: relation to folic acid fortification. Am J Clin Nutr 2008;88:763–768. [PubMed: 18779294]

73. Troen AM, Mitchell B, Sorensen B, et al. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. J Nutr 2006;136:189–194. [PubMed: 16365081]

74. Ashokkumar B, Mohammed ZM, Vaziri ND, Said HM. Effect of folate oversupplementation on folate uptake by human intestinal and renal epithelial cells. Am J Clin Nutr 2007;86:159–166. [PubMed: 17616776]

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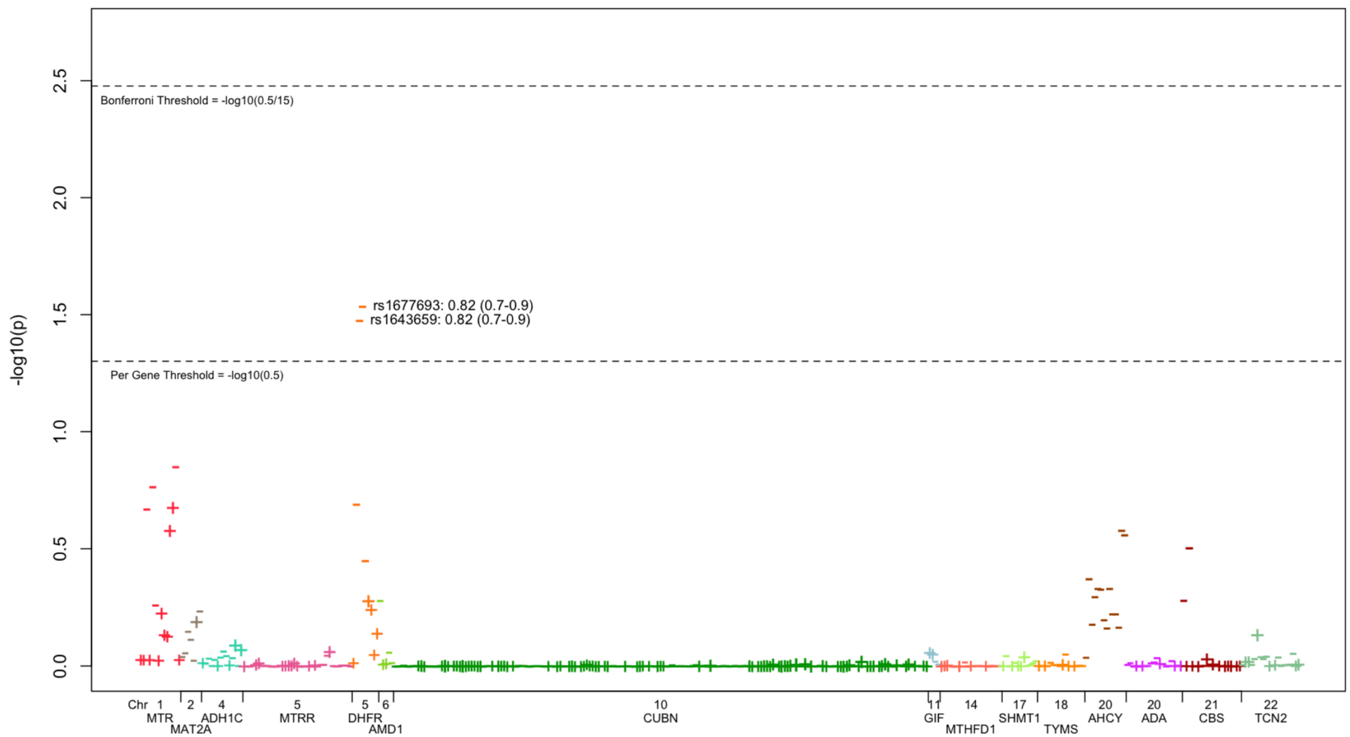


**Figure 1.**

Single SNP analysis of the total study population. Odds ratios were estimated assuming a log additive model for all SNPs. Each gene is a different color. The + symbol means the per minor allele OR was greater than 1.0 and the – symbol means the OR was less than 1.0. P-values were corrected for multiple comparisons by the method of Connelly and Boehnke (18). We show a Bonferroni adjustment to account for the inclusion of 15 genes in the analysis.

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**Table 1**

Genes involved in Folate and Vitamin $B_{12}$ Metabolism

| Gene | Function |
|---|---|
| *ADA* | Catalyses the deamination of adenosine and 2'deoxyadenosine to their inosine derivatives. Linked to ACHY on Chr. 20. Adenosine inhibits AHCY activity. |
| *ADH1C* | Metabolizes alcohol to acetaldehyde, a folate antagonist, in the colon |
| *AHCY* (*SAHH*) | Catalyses the reversible hydrolysis of SAH, HCY and Adenosine. Linked to ADA on Chr. 20. Activity is linked to the presence of ADA. |
| *AMD1* | Catalyses SAM decarboxylation to ornithine – the rate-limiting substrate for polyamine synthesis. |
| *CBS* | Catalyses the transsulfuration of homocysteine to cysteine, the precursor for glutathione synthesis and an important mechanism for controlling intracellular homocysteine. |
| *DHFR* | Converts dihydrofolate into tetrahydrofolate preventing the trapping of folates as dihydrofolates, which cannot be used in subsequent methyl group transfers. |
| *GIF* | Binds cobalamin ($B_{12}$) for transport from the gut to the circulation. |
| *IFCR* (CUBN) | Transfers cobalamin ($B_{12}$) from GIF to TC II (TCN2). |
| *MAT2A* | In non-liver tissues, MAT2A catalyses the biosynthesis of SAM from methionine and ATP. |
| *MTHFD1* | Catalyses the transfer of methylene and formyl groups for the initial steps of purine synthesis. |
| *MTR* | With $B_{12}$, catalyses the transfer of a methyl group to HCY to form methionine, the first step in SAM synthesis. This reaction can trap folates as MTHF which cannot be recycled via the SHMT reaction or can oxidize $B_{12}$ also preventing additional reactions (the "folate trap"). |
| *MTRR* | Catalyses reduction of oxidized $B_{12}$ releasing MTR and $B_{12}$ for further reactions. |
| *SHMT1* | Catalyses the rate limiting step in 5–10MTHF synthesis, itself the initial step of folate metabolism in cytoplasm. 5–10MTHF is the methyl group donor for pyrimidine and the substrate for the methyl groups involved in purine synthesis. |
| *TC* II (*TCN2*) | The primary transport protein for cobalamin (vitamin $B_{12}$) in plasma. |
| *TYMS* (*TS*) | Catalyses transfer of a methyl group from 5,10-MTHF to dUMP to form dTMP to maintain balanced nucleotide pools. |

**Abbreviations**: ADA, adenosine deaminase; ADH1C, alcohol dehydrogenase-1C; AHCY, adenosylhomocysteine hydrolase; AMD1, S-adenosylmethionine decarboxylase; CBS, cystathionine-β-synthase; DHFR, dihydrofolate reductase; GIF, Gastric intrinsic factor; IFCR (CUBN), intrinsic factor cobalamin receptor (AKA cubulin); MAT2A, methionine adenosyl-transferase isoform 2 (non-liver); MTHFD1, methylenetetrahydrofolate dehydrogenase/methynltetrahydrofolate- cyclohydrolase/formyltetrahydrofolate synthetase; MTR, methionine synthase, MTRR, methionine synthase reductase; SHMT, serine hydroxymethyltransferase; TC II, (AKA TCN2) Transcobalamin transporter 2; TYMS, thymidylate synthase.

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## Table 2

Selected Characteristics of the Study Population

| | Cases (n=1,806) | Sibling Controls (n=2,879) |
|---|---|---|
| **Person Characteristic** | | |
| **Mean Age ± SD** | 53.5± 10.9 | 54.0 ± 11.8 |
| **Sex, No. (%)** | | |
| Male | 927 (51.3) | 1278 (44.4) |
| Female | 879 (48.7) | 1601 (55.6) |
| **Race, No. (%)** | | |
| Non-Hispanic White | 1580 (87.5) | 2512 (87.3) |
| Black | 32 (1.8) | 42 (1.5) |
| Asian | 69 (3.8) | 113 (3.9) |
| Other† | 104 (5.8) | 189 (6.6) |
| *Unknown/Missing* | 21 (1.2) | 23 (0.8) |
| **Center, No. (%)** | | |
| Ontario, Canada | 308 (17.1) | 515 (17.9) |
| USC Consortium, U.S. | 384 (21.3) | 519 (18.0) |
| Melbourne, Australia | 344 (19.0) | 611 (21.2) |
| Hawaii, U.S. | 63 (3.5) | 103 (3.6) |
| Mayo Foundation, U.S. | 282 (15.6) | 526 (18.3) |
| Seattle, U.S. | 425 (23.5) | 605 (21.0) |
| **Family History of CRC, No. (%)** | | |
| No 1st degree relative | 1177 (65.2) | - |
| At least 1 1st degree relative | 546 (30.2) | |
| *Unknown/Missing* | 83 (4.6) | |
| **Alcohol use (drinks/wk)** | | |
| None | 467 (25.9) | 829 (28.8) |
| 1–7 (moderate) | 857 (47.5) | 1353 (47.0) |
| 8+ (heavy) | 229 (12.7) | 362 (12.6) |
| *Unknown/Missing* | 253 (14.0) | 335 (11.6) |
| **Folate supplements‡** | | |
| No | 1586 (87.8) | 2557 (88.8) |
| Yes | 196 (10.9) | 274 (9.5) |
| *Unknown/Missing* | 24 (1.3) | 48 (1.7) |
| **Multivitamins‡** | | |
| No | 820 (45.4) | 1497 (52.0) |
| Yes | 971 (53.8) | 1346 (46.8) |
| **Dietary folate (μg/d) (Mean ± SD)§** | 327.4 ± 118.7 | 334.1 ± 126.8 |
| **Total folate (DFE/d) (Mean ± SD)¶** | 477 ± 265.6 | 525.4 ± 439.7 |

| | Cases (n=1,806) | Sibling Controls (n=2,879) |
|---|---|---|
| **Dietary $B_{12}$(μg/d) (Mean ± SD)*** | 3.0 ± 1.2 | 2.9 ± 1.3 |
| **Total $B_{12}$(μg/d) (Mean ± SD)£** | 6.2 ± 6.4 | 7.4 ± 11.8 |
| **Dietary $B_6$ (mg/d) (Mean ± SD)*** | 1.1 ± 0.4 | 1.1 ± 0.4 |
| **Total $B_6$ (mg/d) (Mean ± SD)£** | 1.9 ± 2.0 | 2.3 ± 3.8 |
| **Tumor Characteristics** | | |
| **Site, No. (%)** | | |
| Right Colon | 598 (33.1) | - |
| Left Colon | 525 (29.1) | |
| Rectum | 593 (32.8) | |
| *Unknown/Missing* | 90 (5.0) | |
| **MSI, No. (%)** | | |
| MSS | 855 (47.3) | - |
| MSI-L | 151 (8.4) | |
| MSI-H | 179 (9.9) | |
| *Unknown/Missing* | 621 (34.4) | |

† includes individuals who self-identified themselves as Hispanic, Native, Hawaiian/Pacific Islander and Mixed Race.

‡ Ever use of supplements at least 2x/week for more than a month.

§ Calorie adjusted, calculated from food frequency questionnaire as dietary folate equivalents using post-fortification food composition tables (N cases=585; N controls=837).

¶ Calorie adjusted, sum of dietary and supplement use (as dietary folate equivalents) from food frequency questionnaire (N cases=585; N controls=837).

* Calorie adjusted, calculated from food frequency questionnaire.

£ Calorie adjusted, sum of dietary and supplement use from food frequency questionnaire.



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**Table 3**

CRC tagSNP associations with nominally significant P-values

| SNP | Gene | Cases | Controls | Sibships | OR† | L95 | U95 | LRT P | $P_{act}$‡ |
|---|---|---|---|---|---|---|---|---|---|
| rs1677693 | DHFR | 1805 | 2878 | 1750 | 0.818 | 0.710 | 0.942 | 0.0041 | 0.0293 |
| rs1643659 | DHFR | 1805 | 2876 | 1749 | 0.821 | 0.713 | 0.945 | 0.0048 | 0.0336 |
| rs3788050 | CBS | 1796 | 2864 | 1742 | 0.814 | 0.686 | 0.965 | 0.0205 | 0.3137 |
| rs4659744 | MTR | 1805 | 2878 | 1749 | 0.867 | 0.769 | 0.977 | 0.0225 | 0.1409 |
| rs12563688 | MTR | 1804 | 2877 | 1748 | 0.871 | 0.773 | 0.982 | 0.0287 | 0.1718 |
| rs1650723 | DHFR | 1803 | 2875 | 1747 | 0.836 | 0.705 | 0.991 | 0.0361 | 0.2040 |
| rs12060264 | MTR | 1799 | 2862 | 1743 | 0.877 | 0.778 | 0.989 | 0.0381 | 0.2148 |
| rs10737812 | MTR | 1806 | 2879 | 1750 | 1.137 | 1.008 | 1.282 | 0.0393 | 0.2112 |
| rs2281649 | CUBN | 1806 | 2879 | 1750 | 1.176 | 1.006 | 1.375 | 0.0440 | 0.9591 |
| rs719037 | CBS | 1804 | 2877 | 1749 | 0.882 | 0.779 | 0.997 | 0.0444 | 0.5264 |

† OR's and 95% confidence limits computed using conditional logistic regression with sibship as the matching factor and controlling for age and sex.

‡ Adjusted for multiple testing taking into account correlated tagSNPs using a modified test of Conneely and Boehnke ($P_{act}$) (28).

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## Table 4

Interactions between folate-associated genotypes by multivitamin supplement use

| | | No Multivitamin Supplement use† | | | Multivitamin Supplement use | | | |
|---|---|---|---|---|---|---|---|---|
| **Gene** | **SNP** | **Sibships** | **OR (95% CI)‡** | **P-value§** | **Sibships** | **OR (95% CI)‡** | **P-value** | **P-interaction** |
| DHFR | rs1677693 | 565 | 0.69 (0.58–0.82) | 0.000021 | 637 | 0.91 (0.78–1.07) | 0.299 | 0.000899 |
| DHFR | rs1643659 | 564 | 0.69 (0.58–0.82) | 0.000026 | 637 | 0.91 (0.78–1.07) | 0.223 | 0.000986 |
| MTR | rs4659744 | 565 | 0.76 (0.67–0.88) | 0.000108 | 637 | 0.97 (0.85–1.11) | 0.624 | 0.000265 |
| MTRR | rs16537 | 565 | 0.79 (0.68–0.91) | 0.0017 | 637 | 1.04 (0.91–1.20) | 0.547 | 0.000057 |
| MAT2A | rs1446669 | 564 | 0.81 (0.69–0.95) | 0.0084 | 634 | 1.13 (0.98–1.32) | 0.103 | 0.000033 |
| MAT2A | rs6739015 | 562 | 0.83 (0.72–0.96) | 0.0137 | 633 | 1.09 (0.95–1.25) | 0.238 | 0.000030 |
| MAT2A | rs10179195 | 565 | 0.84 (0.73–0.97) | 0.0142 | 637 | 1.07 (0.94–1.22) | 0.338 | 0.000063 |
| MAT2A | rs699664 | 564 | 0.80 (0.68–0.93) | 0.0041 | 635 | 1.06 (0.92–1.23) | 0.428 | 0.000107 |

†Multivitamin supplement users were defined as those reporting ever use of supplements regularly (at least 2x/week for more than a month).

‡OR's and 95% confidence limits computed using conditional logistic regression with sibship as the matching factor and controlling for age and sex.

§The Bonferroni-adjusted p-value based on an analysis of 395 tagSNPs is 0.00013.