Published in final edited form as:

Cancer Epidemiol Biomarkers Prev. 2010 January ; 19(1): 89–100. doi:10.1158/1055-9965.EPI-09-0727.

Genetic Variability in the *MTHFR* gene and colorectal cancer risk using the Colorectal Cancer Family Registry

A. Joan Levine¹, Jane C. Figueiredo¹, Won Lee¹, Jenny N. Poynter², David Conti¹, David J Duggan³, Peter T. Campbell⁴, Polly Newcomb⁵, Maria Elena Martinez⁶, John L. Hopper⁷, Loic Le Marchand⁸, John A. Baron⁹, Paul J. Limburg¹⁰, Cornelia M Ulrich¹¹, and Robert W Haile¹

¹ Department of Preventive Medicine, University of Southern California, Los Angeles California

² Division of Pediatric Epidemiology and Clinical Research, University of Minnesota, Minneapolis, MN

³ Translational Genomics Research Institutes, Phoenix Arizona

⁴ Department of Epidemiology, American Cancer Society, Atlanta, GA

⁵ Fred Hutchinson Cancer Research Center, Seattle, Washington

⁶ Arizona Cancer Center, University of Arizona, Tucson, AZ and the Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson AZ

⁷ Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, University of Melbourne, Melbourne, Australia

⁸ Cancer Research Center of Hawaii, University of Hawaii, Honolulu, HI

⁹ Departments of Medicine and Community and Family Medicine, Dartmouth Medical School, Hanover, NH

¹⁰ Mayo Clinic, Rochester, MN

¹¹ German Cancer Research Center, Heidelberg Germany

Abstract

Background—The *MTHFR* C677T TT genotype is associated with a 15%–18% reduction in colorectal cancer (CRC) risk but it is not clear if other variants of the gene are associated with CRC risk.

Methods—We used a tagSNP approach to comprehensively evaluate associations between variation in the *MTHFR* gene and CRC risk using a large family-based case control study of 1,750 population-based and 245 clinic-based families from the Colon Cancer Family Registry (CCFR). We assessed 22 TagSNPs, selected based on pairwise $r^2 > 95\%$, using Haploview Tagger and genotyped on the Illumina GoldenGate or Sequenom platforms. The association between SNPs and colorectal cancer was assessed using log additive, co-dominant, and recessive models.

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.

Authors Contributions: AJL and RWH conceived of the study, participated in its design and in drafting the manuscript. JCF, WL, JNP and DC structured the statistical analyses. JCF and WL performed the statistical analyses. DJD conducted SNP selection and genotyping and assisted in drafting the manuscript. JCF, JNP, PTC, PN, MEM, JLH, LLM, JAB, PJL, CMU and RWH participated in the design of the study and made substantive comments in drafting the manuscript. All authors read and approved the final manuscript.

Potential Conflicts of Interest: D. Conti is a consultant for Pfizer Inc and P. Limburg is a consultant for Genomic Health Inc.

Results—From studying the population-based families, the C677T (rs1801133) and A1298C (rs1801131) polymorphisms were associated with a decreased CRC risk overall (OR=0.81, 95% CI=0.63–1.04 and OR=0.82, 95% CI=0.64–1.07, respectively). The 677 TT genotype was associated with a decreased risk of microsatellite stable/microsatellite low tumors (OR=0.69, 95% CI=0.49–0.97) and an increased risk of microsatellite high tumors (OR= 2.22, 95% CI=0.91–5.43) (interaction p-value = 0.01), as well as an increased risk of proximal cancers and a decreased risk of distal and rectal cancers (interaction p-value = 0.02). No other SNP was associated with risk overall or within subgroups.

Conclusion—The 677 TT and 1298 CC genotypes may each be associated with a decrease in CRC risk. We observed little evidence of additional genetic variability in the *MTHFR* gene relevant to CRC risk.

Keywords

MTHFR; Colorectal Cancer; TagSNP; Folate Supplementation; Multivitamins; Microsatellite Instability; Colon subsite

Background

5,10-methylenetetrahydrofolate reductase (*MTHFR*) is a key enzyme in folate associated one carbon metabolism (FOCM). The *MTHFR* enzyme is a flavin adenine dinucleotide (FAD)-dependent enzyme that irreversibly reduces 5,10-methyltetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate (5-MTHF), acting at the junction of two critical uses for folate-associated one carbon groups, nucleotide synthesis and synthesizing the universal methyl donor S-adenosylmethionine (SAM). The *MTHFR* gene is polymorphic and two common non-synonymous SNPs, C677T (A222V; rs1801133) and A1298C (E429A; rs1801131), have been extensively studied for associations with colorectal cancer (CRC). Both genotypes have been associated with decreased enzyme function *in vitro*, with reductions of approximately 60% for the 677 TT genotype (1,2) and 30% for the 1298 CC genotype (3,4). Four recent meta-analyses of these *MTHFR* genotypes and CRC risk overall, utilizing data from 22 studies, reported a modest but statistically significant 15–18% decrease in risk for the 677 TT genotype (5–8). A similar inverse association was also reported for the 1298 CC genotype on CRC risk (5,7). Many studies reported that the decreased CRC risk associated with the 677 TT genotype was observed mainly in those with higher folate availability (9–14). However, all of these studies were conducted in populations not exposed to supplementation of the food supply with folic acid.

More recently, studies have assessed associations between an increased number of polymorphic loci in the *MTHFR* gene and other health outcomes using tagging SNP or haplotype-based approaches for SNP selection (15,16). Liu et al (15) reported statistically significant associations between two SNPs and lung cancer risk while, in another study, 3 *MTHFR* SNPs were significantly associated with lean body mass after correction for multiple testing (16). These data suggest that there may be additional functionally significant variants in the *MTHFR* gene but whether such variants are important determinants of CRC risk is unknown.

In the present study, we used a tagSNP approach to comprehensively evaluate associations between variation in the *MTHFR* gene, including the two known functional polymorphisms, and CRC risk in a large family-based case control study based on the Colon Cancer Family Registry (C-CFR). We also assessed whether associations between SNPs and CRC risk were modified by dietary or total folate intake, folate supplement or multivitamin use and assessed potential heterogeneity of the *MTHFR* SNP – CRC associations by selected tumor characteristics.

Methods

Data for this study were obtained through the Colon Cancer Family Registry (C-CFR), a National Cancer Institute funded registry of CRC cases, unaffected family members, and population-based controls, which utilizes comprehensive and standardized methods for data collection and genotyping. Detailed information about the C-CFR can be found in a recent report by Newcomb et al (17) and at <http://epi.grants.cancer.gov/CFR/>.

Case and control ascertainment

The C-CFR is an international collaborative study initiated in 1997 with the goal of creating a resource for the study of the genetic epidemiology of colorectal cancer. Subject recruitment at the different C-CFR sites is described in detail in Newcomb et al (17) and is described only briefly here. Participants were recruited from six centers including centers in the USC Consortium (Arizona, Cleveland Clinic, Colorado, Dartmouth, Minnesota, North Carolina, and USC), Hawaii (Honolulu), Fred Hutchinson Cancer Research Center (FHCRC, Seattle, WA), Mayo Clinic (Rochester, MN), Cancer Care Ontario (Toronto, Canada), and University of Melbourne (Victoria, Australia) using population-based and clinic-based ascertainment strategies. All centers except FHCRC oversampled cases with multiple first degree relatives reporting CRC or CRC cases diagnosed under age 50 to target families with excess CRC risk. First degree and some second degree relatives with CRC were also recruited from families with multiple CRC cases. For all centers, unaffected siblings or, when necessary, second degree relatives were recruited as controls. The clinic-based sample represents multiple-case families at high risk of HNPCC or other familial CRC phenotypes.

We used a case/unaffected sibling control design with data from both population-based and clinic-based families in the main effect analyses. There were too few clinic-based case/control pairs for stratified analyses so all stratified analyses used the population-based families only. Cases were probands and siblings diagnosed with CRC and controls were siblings without CRC at the time of ascertainment. All cases were interviewed within 5 years of diagnosis (73% within 2 years). We excluded monozygous twins. In addition, we also genotyped a random set of unrelated population-based controls (n=265) from one of the Colon CFR sites (FHCRC). All subjects signed an informed consent before providing data to the C-CFR.

SNP selection

TagSNPs were selected using Haploview Tagger (18) using the following criteria: MAF>5%, pairwise $r^2>0.95$, and distance from closest SNP greater than 60 base pairs on the Illumina platform. 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. In this analysis, we report results for 22 tagSNPs and 3 candidate nonsynonymous SNPs in one gene central to regulation of the folate pathway, *MTHFR*.

SNP Genotyping

SNPs were genotyped on the Illumina GoldenGate platform (19). We implemented a series of quality control checks based on Illumina metrics, and SNPs were excluded from analysis based on the following criteria: GenTrain Score <0.4, 10%GC Score <0.25, AB T Dev >0.1239, call rate <0.95, more than 2 P-P-C errors > 2 or discordance with HapMap >3. Inter- and intra-plate replicates were included, and SNPs were excluded from the analysis if there were greater than 2 errors on replicate genotypes. In addition, genotype data from 30 CEPH trios (Coriell Cell

Repository, Camden, NJ) were used to confirm reliability and reproducibility of the genotyping. SNPs were excluded from the analysis if more than 3 discordant genotypes were discovered in comparison with genotypes from the International HapMap Project (20). We performed additional genotyping using Sequenom's iPLEX Gold for 2 SNPs (rs17376328 and rs2050265) that were not successfully genotyped on the Illumina platform. Polymerase chain reaction (PCR) and extension primers for these 2 SNPs were designed using the MassARRAY Assay Design 3.0 software (Sequenom, Inc) and are available upon request. PCR amplification and single-base-extension reactions were performed according to the manufacturer's instructions. Extension product sizes were determined by mass spectrometry using Sequenom's Compact MALDI-TOF mass spectrometer. The resulting mass-spectra were converted to genotype data using Spectro TYPER-RT software.

Two SNPs were excluded because they failed genotyping (rs17375901 and rs3753582), one non-synonymous SNP (rs2274976, R593Q) was excluded due to missing genotypes for over 900 individuals and one SNP (rs7533315) was excluded because it was significantly out of Hardy-Weinberg equilibrium at a significance level <0.001 ($p=4.74E-08$). This analysis reports on the remaining 22 SNPs.

MSI testing

All available tumors from the C-CFR's Jeremy Jass Memorial Pathology Bank were assayed for instability at the following 10 microsatellites: BAT25, BAT26, BAT40, BAT34C4, D5S346, D17S250, D18S55, D10S197, ACTC and MYCL as described previously (17). Only subjects with clear results for at least 4 markers were included. MSI data were available for 1,200 (66.4%) of cases. Instability at $\geq 30\%$ of the tested loci was defined as MSI-H, instability at $\geq 10\%$ of loci but less than 30% of loci was defined as MSI-L and those with instability at 0 loci were categorized as MSS. Due to small numbers and the lack of evidence for a separate effect in these data, the MSI-L cases were combined with the MSS cases in the analysis.

Folate supplement use, Multivitamin use and Colon Subsite

A standard risk factor questionnaire, was administered to all participants in both populations at the time of recruitment and was available for 1,782 cases and 2,815 controls, approximately 98% of the study population for this analysis. The questionnaire collected information on demographic factors; personal and family cancer histories, including personal history of colorectal polyps, colorectal and other cancers; and other risk factors including selected medication use, use of a folate supplement and multivitamin at least twice a week for more than a month in the two years before recruitment; tobacco and alcohol use history; physical activity and selected dietary preferences (e.g. cooking preference for red meat); and reproductive history (in women). Alcohol use was queried for three time periods (20's and 30's, 40's and since turning 50). In this analysis, we summed across all three periods to get an estimate of lifetime use. Weekly alcohol intake was calculated as the sum of drinks per week from beer, wine and liquor.

Dietary folate intake

Estimated dietary folate intake during the same two-year time period, available for 585 cases, 837 controls (approximately 1/3 of the study population) was estimated from a validated food frequency questionnaire developed at the University of Hawaii (21) and available only for subjects recruited in North America, excluding those from the Mayo clinic and Fred Hutchinson Cancer Research Center (which did not administer a food frequency questionnaire). All food frequency data were collected after 1998, when supplementation of the food supply with 140 μg folic acid per 100 g of cereal products became mandatory. Dietary folate intake was estimated using a food composition table that included that supplementation in one analysis and also, in a separate analysis, where we ignored this supplementation. For the post-

supplementation analysis dietary and total folate were estimated using dietary folate equivalents (DFE) to allow for the different bioavailability of folic acid.

Tumor subsite

Tumor subsite was obtained from the pathology report and was available for 1,734 (96.0%) of cases. 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.

Statistical Analysis

Minor allele frequency was estimated from genotype data from unrelated population-based controls. Pairwise linkage disequilibrium between SNPs was estimated using the square of the correlation coefficient (R^2) between markers. In the analysis of main effects, the population- and clinic-based data were analyzed separately. We used multivariable conditional logistic regression with sibship as the matching factor to estimate main effects and stratum-specific odds ratios. We controlled for age and sex in all analyses. Additional control for folic acid supplement use (yes/no), multivitamin use (yes/no), and alcohol intake (none, 1–7 drinks/week and > 7 drinks/week) did not change the results and we present only the age- and sex-adjusted models here. Except for the C677T and A1298C polymorphisms, for which there are data supporting a recessive model, we assumed a log additive model to assess genotype/CRC associations. Since prior data suggest specific effects for C677T and A1298C, p-values for these two SNPs were not corrected for multiple testing. For the log-additive model, p-values for the remaining SNPs were adjusted for multiple testing taking into account correlated tagSNPs using a modified test of Conneely and Boehnke (22), which is valid only for one degree of freedom tests. As a secondary analysis we assessed all genotype effects in a co-dominant model comparing heterozygotes and homozygotes for the minor allele to those homozygous for the major allele using dummy variables to obtain the OR and 95% confidence interval for each genotype and a 2 df likelihood ratio to test to estimate p-values for each comparison. Third, we assessed the possibility for recessive effects for all SNPs, comparing those homozygous for the minor allele to those with one or no minor alleles. The results using the recessive models were the same as those using the co-dominant models and only the co-dominant model results are included here. For multiple degree of freedom likelihood ratio tests the Bonferroni method was used to reset the significance level to 0.00227.

All analyses within exposure strata were specified in advance based on indications for potential effect modification in the literature. Stratum-specific OR's were estimated among population-based families to evaluate heterogeneity by MSI (MSS/MSI-L and MSI-H), tumor subsite (right, left, rectum/rectosigmoid junction), regular use of a folate or multivitamin supplement (yes or no) and dietary folate intake (dichotomized at the median of the control population). Since not all centers recruited subjects from populations with folic acid fortification of the food supply (i.e. Australia and New Zealand) we assessed potential heterogeneity by center. Finally, we considered whether inclusion of cases recruited more than 2 years after diagnosis resulted in biased estimates by comparing SNP odds ratio estimates for cases diagnosed under and over 2 years after diagnosis. There was no heterogeneity for either variable. For all stratified analyses, we included interaction terms in the regression models to get the interaction p-values and used a 2- (MSI status) or 3-df (tumor subsite) likelihood ratio test to assess heterogeneity. All statistical analyses were conducted using the R programming language and SAS v9.1.

Results

Table 1 describes the *MTHFR* SNPs included in this study. Characteristics of the study population are presented in Table 2. There were 1,806 population-based cases with 2,879

sibling controls. For the clinic-based population, there were 269 cases with 475 sibling controls. Both study populations were mainly Caucasian: 87.5% of cases and 87.3% of controls in the population-based sample and 97.3% of cases and 97.5% of controls in the clinic-based sample reported their race/ethnic group as White. In the population-based sample 17% were from Ontario, Canada; 64% were from the four US sites and 19% were from Australia or New Zealand. For the Clinic-based population none of the participants were from Canada, 14% of cases were from the USC consortium in the US, 41% were from Australia or New Zealand, 45% of the cases were from the Mayo Clinic in the US and no cases or controls were from the Hawaii or Seattle sites. Sixty five percent of population-based cases reported no family history of CRC, 30% reported at least one first degree relative with a family CRC history and family history data was missing for 4.6%. In the clinic-based cases 37.5% reported no first degree relative with CRC, 26% reported at least one first degree relative with CRC and family history was missing for 36.4%.

Approximately 47% of subjects with risk factor data in each population reported drinking a moderate amount of alcohol (1–7 drinks per week) and approximately 10% of each study population reported taking a folic-acid supplement. In the population-based families, 54% of cases and 47% of controls reported taking a multivitamin regularly in the 2 years prior to enrollment in this study ($p < 0.01$). Population based cases were significantly younger ($p < 0.01$), more likely to be male ($p < 0.01$) and had a significantly higher BMI ($p < 0.01$). Among the subjects with food frequency questionnaire data (32.4% of cases and 29.1% of controls), cases were less likely to take calcium supplements ($p = 0.04$), had lower total folate intake ($p = 0.02$), and lower total B₁₂ intake (0.019). In the clinic-based families, cases were significantly younger ($p < 0.01$), more likely to be smokers ($p < 0.01$), and more likely to be very active ($p < 0.01$). Cases in the clinic-based population were more likely to have tumors with MSI-H (20.4% and 9.9% in the clinic-based and population-based samples respectively) and less likely to have MSS tumors (22.7% and 47.3%, respectively).

We evaluated 22 SNPs in *MTHFR* with minor allele frequencies (MAF) ranging from 0.05 to 0.41 (Table 1). The results of the main effects analyses are presented in Table 3. We observed a borderline statistically significant decrease in CRC risk (OR=0.81, 95% CI 0.63–1.04; $p = 0.10$) for the 677 TT genotype (rs1801133) relative to the CC and CT genotypes in the population-based series. There was a non-statistically significant inverse association for the 677 TT relative to the CT and TT genotypes in the clinic-based population (OR 0.59, 95% CI 0.31–1.12; $p = 0.11$). The 1298 CC genotype (rs1801131) relative to the AC and AA genotypes was also associated with a borderline statistically significant decrease in CRC risk among the population-based families (OR 0.82, 95% CI=0.64–1.07; $p = 0.14$) but not among the clinic-based families (OR 1.02, 95% CI=0.53–1.98; $p = 0.95$). Under the assumption of a log-additive model, no other SNP was statistically significantly associated with CRC risk in either study population after correction for multiple testing (Table 3). When we assessed associations assuming a co-dominant model, homozygosity for the minor allele was nominally associated with CRC risk for 5 SNPs in the population-based cases (rs12404124, rs1994798, rs4846048, rs4846049 and rs6541003) but no p-value was significant at the Bonferroni-adjusted p-value (Table 3). In the clinic-based cases, no SNPs were associated with risk in the co-dominant models. The results for the recessive models were essentially the same as those for the co-dominant models.

Among those with food frequency questionnaire data, we found no evidence of heterogeneity for *MTHFR* genotypes when stratified by dietary or total (food + supplemental) folate intake using either pre- or post-fortification estimates (data not shown). Folate and multivitamin supplement use (yes/no) in the two years prior to diagnosis or recruitment was available for the 98% of the study population with completed risk factor questionnaire data. The 677 TT genotype was associated with a decrease in CRC risk only in non-users of a folic-acid

supplement (OR=0.78, 95% CI=0.60—1.01; $p=0.06$ and OR=1.16, 95% CI=0.65—2.08; $p=0.40$ in folic acid supplement non-users and users, respectively) although the group of folic acid supplement users was small and neither the interaction term ($p=0.55$) nor the p for heterogeneity ($p=0.08$) were statistically significant. The results were similar when we stratified on multivitamin use (OR= 0.68, 95% CI=0.49—0.95; $p=0.02$ and OR=0.97, 95% CI=0.72—1.32; $p=0.87$ in multivitamin non-users and users respectively). Again neither the p for the interaction ($p=0.48$) nor heterogeneity ($p=0.07$) were statistically significant. The same pattern was evident for the 1298 CC genotype. There were no other interactions with folate or multivitamin supplement use for any SNP.

We also assessed heterogeneity of the *MTHFR* effect by tumor microsatellite instability among the population-based families (Table 4). The 677 TT genotype was associated with decreased risk of MSS/MSI-L CRC (OR=0.69; 95% CI= (0.50—0.97; $p=0.03$) and increased risk of an MSI-H tumor (OR=2.22; 95% CI=0.91—5.43; $p=0.08$; interaction p -value = 0.02; heterogeneity p -value = 0.01). The 1298 CC genotype was associated with a non-statistically significant decreased risk of MSS/MSI-L (OR=0.77; 95% CI=0.54—1.08; $p=0.13$) and MSI-H (OR=0.51; 95% CI=0.24—1.08; $p=0.08$) tumors. There was no heterogeneity by MSI status for the other SNPs.

The overall test for interaction between the three subsites for the 677 TT genotype was statistically significant (interaction $p=0.01$; 3df likelihood ratio p -value for heterogeneity = 0.02). The *MTHFR* 677 TT genotype was associated with an increase in the risk of proximal tumors (OR=1.40, 95% CI=0.88—2.23; $p=0.16$) and a decrease in the risk for distal (OR=0.69, 95% CI=0.43—1.10; $p=0.12$) and rectal (OR=0.62; 95% CI=0.41—0.93; $p=0.02$) tumors. There was no evidence for heterogeneity in risk by tumor subsite for the *MTHFR* 1298 CC genotype (OR=0.70, 95% CI=0.45—1.07 for proximal colon; OR=1.22, 95% CI=0.74—1.99 for distal colon; and OR=0.69, 95% CI=0.44—1.10 for rectal tumors; p for interaction=0.95). No other SNP was differentially associated with tumor subsite.

We also conducted a stratified analysis to examine possible modification by age (< 65/ $\geq$ 65) or sex. There was no modification by age or sex for any SNP (data not shown).

Discussion

In the current study, we assessed a comprehensive set of SNPs that characterized the genetic variation of the *MTHFR* gene, including 10 kb 5' of the transcription start site and 5 kb into the 3' UTR. To our knowledge, this is the most comprehensive analysis of genetic variation in the *MTHFR* gene in relation to colorectal cancer risk completed to date. Our data from both the population-based and clinic-based series are consistent with inverse associations for the 677 TT (rs1801133; A222V) and the 1298 CC genotypes (rs1801131; E429A). Stratification by folate- and multivitamin supplement-use suggested that 677 TT and 1298 CC genotypes may be associated with decreased CRC risk only among non-users of folate or multivitamin supplements, but neither interaction was statistically significant. Our data also support a positive association between the 677 TT genotype and the MSI-H phenotype and tumors of the proximal colon (wherein most MSI-H tumors are located); conversely, the results suggested an inverse association between this SNP and MSS/MSI-L tumors and tumors in the distal colon and rectum. No other SNPs were significantly associated with risk overall or in any subgroup.

Our data are consistent with four recent meta-analyses that suggested 15%–18% reductions in CRC risk for the 677 TT genotype (5–8). The A1298C polymorphism has been also well studied. Although the functional effects of the C allele are unclear (2), two meta-analyses (5, 7) suggest that homozygosity for the enzyme with a C at nucleotide 1298 is associated with an approximately 10–15% decrease in CRC risk, consistent with the estimate in the current study.

Several studies published after the meta-analyses were completed have reported increased risk associated with the 677 TT genotype (23–25) while others reported no association (26–28) or a decreased risk for those with the variant genotype (29). Chang et al. reported a significant increase in risk for the 677 TT genotype in those with low folate intake (9). For the 1298 CC genotype, 2 studies reported an increase in risk (27,28) one reported a decreased risk (29) and one reported no association with risk (9). Additionally, one recent study of a Hereditary Non-Polyposis Colon Cancer (HNPCC) cohort reported an increase in the age of CRC onset among those with the 1298 CC or joint 677T/1298C genotypes, suggesting a protective effect of the these variant alleles in HNPCC as well (30). Variability in folate availability in these different source populations may explain the diverse findings, suggesting that future meta-analyses should account for such differences.

Data from observational studies suggest that the phenotype of the *MTHFR* valine protein (677 TT genotype) depends significantly on folate availability (31–34). A recent *in vitro* study in HCT116 colon carcinoma cells reported that the valine protein (TT genotype) was associated with increased genomic DNA methylation in the setting of adequate folate but a significant decrease in genomic methylation when folate was deficient (35), supporting the impression that folate availability is a modifier of genotype effect. This is consistent with the biochemical changes in the valine-containing enzyme, which show that the enzyme is stabilized by the addition of 5-MTHF to the culture medium (2). Therefore, we considered whether folate availability might modify associations between other SNPs and CRC risk as well. While there was no heterogeneity by dietary or total folate consumption in the subgroup of participants with food frequency data, in data from the whole study population homozygosity for the *MTHFR* 677 T allele was significantly associated with a decreased risk only in non-multivitamin users, a result that was suggested for non-users of folate supplements as well. These results conflict with most previous reports of non-folate-supplemented populations, which suggest that the 677 TT genotype is protective mainly for those with higher folate availability (9–14), although other studies have not observed this difference (29,36–38). However, in the current study neither the interactions nor the tests for heterogeneity were statistically significant. Therefore this finding may be due to chance. On the other hand, one difference between our study population and those of the earlier studies is the likely higher folic acid levels to which most of our study population had been exposed during the 2-years preceding their recruitment, due to fortification of the food supply. It is likely that non-supplement users in the current population have a greater folate intake than subjects in pre-fortification study populations (39) with levels more similar to those with higher folate intakes in previous studies. Subjects taking supplements in the current population showed little association with CRC risk, as one would expect if there was stabilization of the enzyme in the presence of high folate availability. Whether post-supplement folate levels are relevant to the lower risk we observed in 677 TT homozygotes not taking folate or multivitamin supplements is unclear and must be assessed in future studies of populations with similar levels of fortification.

In stratified analyses, we found that the 677 TT genotype was associated with a decreased risk of MSS tumors and tumors in the distal colon or rectum while being associated with an increased risk for MSI-H tumors and tumors in proximal colon. The literature on the relationship between the 677 TT genotype and MSI status is mixed. Most studies have reported an increased risk for MSI-H tumors in those homozygous for the 677 TT genotype (9,40–42) but some studies have reported no difference between MSI subgroups (43,44) or a decrease in the risk of MSI-H tumors in those homozygous for the 677 T allele (45,46). Our data are also consistent with those of a recent study of Australian CRC patients, which reported a significant increase in the risk of proximal and decreased risk of distal colorectal cancers in those with the 677 TT genotype (47) adding to the general consensus that those with the 677 TT genotype may have an increased risk for tumors with the MSI-H phenotype and for tumors of the

proximal colon. These two findings are likely to reflect the same association since data strongly suggest that the majority of MSI-H tumors develop in proximal colon and rarely in distal colon or rectum (47,48). We did not observe any modification by MSI status for the 1298 CC genotype, a result that is consistent with those of other reports (41,45,46). It is unclear why the association between the 1298 genotype and MSI status should be different from that of the 677 genotype.

This study has several strengths. In addition to the large sample size, we had a comprehensive approach to identifying genetic variation in the *MTHFR* gene, a family-based design which minimized the probability of population stratification, included detailed risk factor information on all our subjects and the ability to assess possible heterogeneity of genotype effects by folate nutrition, multivitamin use, MSI status and tumor subsite. The ability to assess genotype associations in two separate samples is another strength.

Weaknesses of this study include the possibility that we have missed an important source of genetic variability since we used public data bases to define SNPs and these data bases are incomplete. Additionally, although the case-unaffected sibling design is more powerful for assessing gene-environment interactions, it is less powerful for detecting main effects (49). Thus our study may have been underpowered in the main effects analyses. Also, we did not have dietary data from all the participants and not all cases provided tumor tissue for MSI analysis, limiting statistical power for analyses involving diet and MSI status; however, it is unlikely that the availability of dietary or MSI data is associated with genotype, so this should not have resulted in any bias in the observed odds ratios. As in any case-control study, dietary intake information was assessed after the diagnosis in cases and so may be affected by recall bias. We were unable to genotype the rare R593Q (G1793A, rs2274976) polymorphism and so could not assess whether this SNP was associated with risk overall or in any subgroup. The functional consequences of the R593Q polymorphism are not completely clear but some studies have suggested associations with various outcomes (50–54). Several studies have shown the A allele for this SNP to be in cis with the 1298 C allele (53,55,56). Finally, many of the protocols used in the C-CFR were designed to over-sample cases with a greater risk for a family history of CRC which may decrease the generalizability of our findings. Additionally, in the population-based sample almost 90% of our study population was Caucasian and from the US or Canada while this percentage was nearly 100% in the clinic-based subjects. Only 19% of the population-based sample was not of North American origin and none of the subjects in either population came from Latin America, South America or Western Europe, all countries with significantly lower folate availability. To the extent that *MTHFR* genotype modifies risk differently in different countries, perhaps associated with differences in folate intake and the prevalence of smoking and alcohol use, our results may not generalize to all relevant populations.

Conclusion

In this study, using a tagSNP approach, we did not find strong evidence for additional functional genetic variation in the *MTHFR* gene for CRC risk. Our data suggest that the well known C677T and A1298C variants are the most relevant *MTHFR* variants for CRC risk and that there may be heterogeneity in the risk associated with the C677T TT variant by MSI status, tumor subsite and, possibly, by folate or multivitamin supplement use in folic acid supplemented populations.

Acknowledgments

This work was supported by the National Cancer Institute, National Institutes of Health under RFA # CA-95-011 and through cooperative agreements with the Australasian Colorectal Cancer Family Registry (U01 CA097735), the USC Familial Colorectal Neoplasia Collaborative Group (U01 CA074799), the Mayo Clinic Cooperative Family Registry

for Colon Cancer Studies (U01 CA074800), the Ontario Registry for Studies of Familial Colorectal Cancer (U01 CA074783), the Seattle Colorectal Cancer Family Registry (U01 CA074794), and the University of Hawaii Colorectal Cancer Family Registry (U01 CA074806) as well as NCI T32 CA009142 (JNP), NCI R01 CA112237 (RWH), NCI 1K07CA10629-01A1 (ETJ) and NCI P01 CA41108CA-23074 and CA 956060 (M.E.M).P.T.C. and J.C.F. were supported in part by National Cancer Institute of Canada post-PhD Fellowships (#18735 and #17602).

We thank the following individuals for their support in data collection and management: Margreet Luchtenborg, Maj Earle, Barbara Saltzman, Kathy Kennedy, Darin Taverna, Chris Edlund, Matt Westlake, Paul Mosquin, Darshana Daftary, Douglas Snazel, Allyson Templeton, Terry Teitsch, Helen Chen and Maggie Angelakos and Paul Mosquin. We also thank all the individuals who participated in the Colon CFR.

References

1. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995;10:111–3. [PubMed: 7647779]
2. Yamada K, Chen Z, Rozen R, et al. Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. *Proc Natl Acad Sci U S A* 2001;98:14853–8. [PubMed: 11742092]
3. van der Put NM, Gabreels F, Stevens EM, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *Am J Hum Genet* 1998;62:1044–51. [PubMed: 9545395]
4. Weisberg IS, Jacques PF, Selhub J, et al. The 1298A-->C polymorphism in methylenetetrahydrofolate reductase (MTHFR): in vitro expression and association with homocysteine. *Atherosclerosis* 2001;156:409–15. [PubMed: 11395038]
5. Huang Y, Han S, Li Y, et al. Different roles of MTHFR C677T and A1298C polymorphisms in colorectal adenoma and colorectal cancer: a meta-analysis. *J Hum Genet* 2007;52:73–85. [PubMed: 17089070]
6. Hubner RA, Houlston RS. MTHFR C677T and colorectal cancer risk: A meta-analysis of 25 populations. *Int J Cancer* 2007;120:1027–35. [PubMed: 17131337]
7. Kono S, Chen K. Genetic polymorphisms of methylenetetrahydrofolate reductase and colorectal cancer and adenoma. *Cancer Sci* 2005;96:535–42. [PubMed: 16128738]
8. Little J, Sharp L, Duthie S, et al. Colon cancer and genetic variation in folate metabolism: the clinical bottom line. *J Nutr* 2003;133:3758S–66S. [PubMed: 14608111]
9. Chang SC, Lin PC, Lin JK, et al. Role of MTHFR polymorphisms and folate levels in different phenotypes of sporadic colorectal cancers. *Int J Colorectal Dis* 2007;22:483–9. [PubMed: 16941173]
10. Chen J, Giovannucci E, Kelsey K, et al. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res* 1996;56:4862–4. [PubMed: 8895734]
11. Le Marchand L, Wilkens LR, Kolonel LN, et al. The MTHFR C677T polymorphism and colorectal cancer: the multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev* 2005;14:1198–203. [PubMed: 15894672]
12. Ma J, Stampfer MJ, Giovannucci E, et al. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. *Cancer Res* 1997;57:1098–102. [PubMed: 9067278]
13. Slattery ML, Potter JD, Samowitz W, et al. Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev* 1999;8:513–8. [PubMed: 10385141]
14. 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;95:908–13. [PubMed: 15546509]
15. Liu H, Jin G, Wang H, et al. Association of polymorphisms in one-carbon metabolizing genes and lung cancer risk: a case-control study in Chinese population. *Lung Cancer*. 2008
16. Liu X, Zhao LJ, Liu YJ, et al. The MTHFR gene polymorphism is associated with lean body mass but not fat body mass. *Hum Genet* 2008;123:189–96. [PubMed: 18180959]
17. 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;16:2331–43. [PubMed: 17982118]

18. Barrett JC, Fry B, Maller J, et al. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263–5. [PubMed: 15297300]
19. Shen R, Fan JB, Campbell D, et al. High-throughput SNP genotyping on universal bead arrays. *Mutat Res* 2005;573:70–82. [PubMed: 15829238]
20. A. A haplotype map of the human genome. *Nature* 2005;437:1299–320. [PubMed: 16255080]
21. 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;151:358–70. [PubMed: 10695594]
22. Conneely KN, Boehnke M. So Many Correlated Tests, So Little Time! Rapid Adjustment of P Values for Multiple Correlated Tests. *Am J Hum Genet* 2007;81.
23. Cao HX, Gao CM, Takezaki T, et al. Genetic polymorphisms of methylenetetrahydrofolate reductase and susceptibility to colorectal cancer. *Asian Pac J Cancer Prev* 2008;9:203–8. [PubMed: 18712959]
24. Gallegos-Arreola MP, Garcia-Ortiz JE, Figuera LE, et al. Association of the 677C → T polymorphism in the MTHFR gene with colorectal cancer in Mexican patients. *Cancer Genomics Proteomics* 2009;6:183–8. [PubMed: 19487547]
25. 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;88:1413–8. [PubMed: 18996879]
26. Derwinger K, Wettergren Y, Odin E, et al. A study of the MTHFR gene polymorphism C677T in colorectal cancer. *Clin Colorectal Cancer* 2009;8:43–8. [PubMed: 19203896]
27. Kury S, Buecher B, Robiou-du-Pont S, et al. Low-penetrance alleles predisposing to sporadic colorectal cancers: a French case-controlled genetic association study. *BMC Cancer* 2008;8:326. [PubMed: 18992148]
28. Lightfoot TJ, Barrett JH, Bishop T, et al. Methylene tetrahydrofolate reductase genotype modifies the chemopreventive effect of folate in colorectal adenoma, but not colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 2008;17:2421–30. [PubMed: 18703816]
29. 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;99:379–89. [PubMed: 18053312]
30. Reeves SG, Meldrum C, Groombridge C, et al. MTHFR 677 C>T and 1298 A>C polymorphisms and the age of onset of colorectal cancer in hereditary nonpolyposis colorectal cancer. *Eur J Hum Genet* 2009;17:629–35. [PubMed: 19156174]
31. Friso S, Choi SW, Girelli D, et al. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. *Proc Natl Acad Sci U S A* 2002;99:5606–11. [PubMed: 11929966]
32. Jacques PF, Bostom AG, Williams RR, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* 1996;93:7–9. [PubMed: 8616944]
33. Malinow MR, Nieto FJ, Kruger WD, et al. The effects of folic acid supplementation on plasma total homocysteine are modulated by multivitamin use and methylenetetrahydrofolate reductase genotypes. *Arterioscler Thromb Vasc Biol* 1997;17:1157–62. [PubMed: 9194768]
34. Nelen WL, Blom HJ, Thomas CM, et al. Methylenetetrahydrofolate reductase polymorphism affects the change in homocysteine and folate concentrations resulting from low dose folic acid supplementation in women with unexplained recurrent miscarriages. *J Nutr* 1998;128:1336–41. [PubMed: 9687553]
35. Sohn KJ, Jang H, Campan M, et al. The methylenetetrahydrofolate reductase C677T mutation induces cell-specific changes in genomic DNA methylation and uracil misincorporation: a possible molecular basis for the site-specific cancer risk modification. *Int J Cancer* 2009;124:1999–2005. [PubMed: 19123462]
36. 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;29:146–54. [PubMed: 15829374]
37. 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;3:353–9. [PubMed: 12716294]

38. 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;26:2164–71. [PubMed: 16051637]
39. 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;24:266–74. [PubMed: 16093404]
40. Clarizia AD, Bastos-Rodrigues L, Pena HB, et al. Relationship of the methylenetetrahydrofolate reductase C677T polymorphism with microsatellite instability and promoter hypermethylation in sporadic colorectal cancer. *Genet Mol Res* 2006;5:315–22. [PubMed: 16819711]
41. Hubner RA, Lubbe S, Chandler I, et al. MTHFR C677T has differential influence on risk of MSI and MSS colorectal cancer. *Hum Mol Genet* 2007;16:1072–7. [PubMed: 17350979]
42. Shannon B, Gnanasampanthan S, Beilby J, et al. A polymorphism in the methylenetetrahydrofolate reductase gene predisposes to colorectal cancers with microsatellite instability. *Gut* 2002;50:520–4. [PubMed: 11889073]
43. Plaschke J, Schwanebeck U, Pistorius S, et al. Methylenetetrahydrofolate reductase polymorphisms and risk of sporadic and hereditary colorectal cancer with or without microsatellite instability. *Cancer Lett* 2003;191:179–85. [PubMed: 12618331]
44. Ulrich CM, Curtin K, Potter JD, et al. Polymorphisms in the reduced folate carrier, thymidylate synthase, or methionine synthase and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev* 2005;14:2509–16. [PubMed: 16284371]
45. Eaton AM, Sandler R, Carethers JM, et al. 5,10-methylenetetrahydrofolate reductase 677 and 1298 polymorphisms, folate intake, and microsatellite instability in colon cancer. *Cancer Epidemiol Biomarkers Prev* 2005;14:2023–9. [PubMed: 16103455]
46. Toffoli G, Gafa R, Russo A, et al. Methylenetetrahydrofolate reductase 677 C→T polymorphism and risk of proximal colon cancer in north Italy. *Clin Cancer Res* 2003;9:743–8. [PubMed: 12576444]
47. Iacopetta B, Heyworth J, Girschik J, et al. The MTHFR C677T and DeltaDNMT3B C-149T polymorphisms confer different risks for right- and left-sided colorectal cancer. *Int J Cancer* 2009;125:84–90. [PubMed: 19326430]
48. Iacopetta B. Are there two sides to colorectal cancer? *Int J Cancer* 2002;101:403–8. [PubMed: 12216066]
49. Witte JS, Gauderman WJ, Thomas DC. Asymptotic bias and efficiency in case-control studies of candidate genes and gene-environment interactions: basic family designs. *Am J Epidemiol* 1999;149:693–705. [PubMed: 10206618]
50. Huemer M, Vonblon K, Fodinger M, et al. Total homocysteine, folate, and cobalamin, and their relation to genetic polymorphisms, lifestyle and body mass index in healthy children and adolescents. *Pediatr Res* 2006;60:764–9. [PubMed: 17065574]
51. Melo SS, Persuhn DC, Meirelles MS, et al. G1793A polymorphisms in the methylene-tetrahydrofolate gene: effect of folic acid on homocysteine levels. *Mol Nutr Food Res* 2006;50:769–74. [PubMed: 16865747]
52. Winkelmayr WC, Huber A, Wagner OF, et al. Associations between MTHFR 1793G>A and plasma total homocysteine, folate, and vitamin B in kidney transplant recipients. *Kidney Int* 2005;67:1980–5. [PubMed: 15840047]
53. Winkelmayr WC, Sunder-Plassmann G, Huber A, et al. Patterns of co-occurrence of three single nucleotide polymorphisms of the 5,10-methylenetetrahydrofolate reductase gene in kidney transplant recipients. *Eur J Clin Invest* 2004;34:613–8. [PubMed: 15379760]
54. Kebert CB, Eichner JE, Moore WE, et al. Relationship of the 1793G-A and 677C-T polymorphisms of the 5,10-methylenetetrahydrofolate reductase gene to coronary artery disease. *Dis Markers* 2006;22:293–301. [PubMed: 17264399]
55. Rady PL, Szucs S, Grady J, et al. Genetic polymorphisms of methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) in ethnic populations in Texas; a report of a novel MTHFR polymorphic site, G1793A. *Am J Med Genet* 2002;107:162–8. [PubMed: 11807892]
56. Wakutani Y, Kowa H, Kusumi M, et al. A haplotype of the methylenetetrahydrofolate reductase gene is protective against late-onset Alzheimer's disease. *Neurobiol Aging* 2004;25:291–4. [PubMed: 15123333]

Table 1

Primary SNP data for the 22 SNPs

SNP	Location in Gene/ protein change	Position	Base change in assay	MAF [†] Population based families
rs11121832	Intron	11782707	A/G	0.25
rs12121543	Intron	11777258	A/G	0.24
rs12404124	Intron	11796456	C/T	0.41
rs13306556	Intron	11774697	A/G	0.10
rs1476413	Intron	11774887	A/G	0.27
rs17037390	Intron	11783430	A/G	0.16
rs17037396	Intron	11784634	C/T	0.11
rs17037425	Intron	11792970	C/T	0.14
rs17421462	Flanking 3'-UTR	11779434	A/G	0.08
rs17421511	Intron	11780375	A/C	0.16
rs1801131	E428A	11777063	A/G	0.31
rs1801133	A221V	11778956	C/T	0.32
rs1994798	Intron	11777342	A/G	0.41
rs3737964	Intron	11789631	C/T	0.25
rs3737965	Intron	11789038	C/T	0.06
rs4846048	Intron	11768839	C/T	0.29
rs4846049	Intron	11772952	C/T	0.32
rs4846054	Intron	11791817	G/T	0.40
rs6541003	3'-UTR	11778454	G/T	0.40
rs9651118	Intron	11784801	A/G	0.23
rs17376328	5' UTR	11799249	G/A	0.05
rs2050265	5'UTR	11802286	T/C	0.16

[†] Minor allele frequency was estimated from genotype data from unrelated population-based controls

Table 2

Selected Characteristics of the Study Population

Person Characteristic	Population-based Families		Clinic-based Families		P-value [†]	P-value [‡]
	Cases (n=1,806)	Sibling Controls (n=2,879)	Cases (n=269)	Sibling Controls (n=475)		
Person Characteristic						
Mean Age ± SD	53.5 ± 10.9	54.0 ± 11.8	49.1 ± 11.4	51.4 ± 11.8	<0.01	<0.01
Sex, No. (%)						
Male	927 (51.3)	1278 (44.4)	133 (49.4)	204 (42.9)	<0.01	0.19
Female	879 (48.7)	1601 (55.6)	136 (50.6)	271 (57.1)		
Race, No. (%)						
Non-Hispanic White	1580 (87.5)	2512 (87.3)	262 (97.4)	463 (97.5)	1.00	1.00
Black	32 (1.8)	42 (1.5)	1 (0.4)	2 (0.4)		
Asian	69 (3.8)	113 (3.9)	0 (0)	0 (0)		
Other [§]	104 (5.8)	189 (6.6)	5 (1.9)	9 (1.9)		
Unknown/Missing	21 (1.2)	23 (0.8)	1 (0.4)	1 (0.2)		
Center, No. (%)						
Ontario, Canada	308 (17.1)	515 (17.9)	0 (0)	0 (0)	-	1.00
USC Consortium, U.S.	384 (21.3)	519 (18.0)	38 (14.1)	48 (10.1)		
Melbourne, Australia	344 (19.0)	611 (21.2)	110 (40.9)	213 (44.8)		
Hawaii, U.S.	63 (3.5)	103 (3.6)	0 (0)	0 (0)		
Mayo Foundation, U.S.	282 (15.6)	526 (18.3)	121 (45.0)	214 (45.1)		
Seattle, U.S.	425 (23.5)	605 (21.0)	0 (0)	0 (0)		
Family History of CRC, No. (%)						
No 1 st degree relative	1177 (65.2)	-	101 (37.5)	-	-	-
At least 1 st degree relative	546 (30.2)		70 (26.0)			
Unknown/Missing	83 (4.6)		98 (36.4)			
BMI (kg/m²)[§]						
15–18 (underweight)	22 (1.2)	25 (0.9)	6 (2.2)	12 (2.5)	<0.01	0.82
18–25 (normal)	629 (34.8)	1155 (40.1)	97 (36.1)	174 (36.6)		
25–30 (overweight)	670 (37.1)	1036 (36.0)	100 (37.2)	174 (36.6)		
30+ (obese)	422 (23.4)	594 (20.6)	53 (19.7)	92 (19.4)		

	Population-based Families			Clinic-based Families		
	Cases (n=1,806)	Sibling Controls (n=2,879)	P-value [†]	Cases (n=269)	Sibling Controls (n=475)	P-value [†]
<i>Unknown/Missing</i>	63 (3.5)	69 (2.4)		13 (4.8)	23 (4.8)	
Alcohol use (drinks/wk)						
None	467 (25.9)	829 (28.8)	0.08	76 (28.3)	132 (27.8)	0.43
1–7 (moderate)	857 (47.5)	1353 (47.0)		124 (46.1)	226 (47.2)	
8+ (heavy)	229 (12.7)	362 (12.6)		39 (14.5)	61 (12.8)	
<i>Unknown/Missing</i>	253 (14.0)	335 (11.6)		30 (11.2)	56 (11.8)	
Smoking						
Never	781 (43.2)	1309 (45.5)	0.24	138 (51.3)	226 (47.6)	<0.01
Former	632 (35.0)	1001 (34.8)		58 (21.6)	153 (32.2)	
Current	343 (19.0)	509 (17.7)		66 (24.5)	87 (18.3)	
<i>Unknown/Missing</i>	50 (2.8)	60 (2.1)		7 (2.6)	9 (1.9)	
Folate supplements[‡]						
No	1586 (87.8)	2557 (88.8)	0.14	233 (86.6)	411 (91.3)	0.25
Yes	196 (10.9)	274 (9.5)		31 (11.5)	39 (8.7)	
<i>Unknown/Missing</i>	24 (1.3)	48 (1.7)		5 (1.9)	11 (2.3)	
Multivitamins[‡]						
No	820 (45.4)	1497 (52.0)	<0.01	138 (51.3)	267 (56.2)	0.27
Yes	971 (53.8)	1346 (46.8)		129 (48.0)	200 (42.1)	
Calcium Supplements[‡]						
No	1335 (73.9)	2063 (71.7)	0.03	208 (77.3)	345 (76.7)	0.33
Yes	459 (25.4)	785 (27.3)		56 (20.8)	105 (23.3)	
<i>Unknown/Missing</i>	12 (0.7)	31 (1.1)		5 (1.9)	2 (0.4)	
Dietary folate (µg) (Mean ± SD)[*]	327.4 ± 118.7	334.1 ± 126.8	0.32	349.6 ± 154.9	346.0 ± 145.0	0.95
Total folate DFE (Mean ± SD)[*]	477 ± 265.6	525.4 ± 439.7	<0.01	606.5 ± 463.1	549.6 ± 322.2	0.29
Dietary B₁₂ (Mean ± SD)[*]	3.0 ± 1.2	2.9 ± 1.3	0.67	3.1 ± 1.4	3.0 ± 1.4	0.36
Total B₁₂ (Mean ± SD)[*]	6.2 ± 6.4	7.4 ± 11.8	<0.01	10.0 ± 17.6	8.8 ± 10.0	0.41
Dietary B₆ (Mean ± SD)[*]	1.1 ± 0.4	1.1 ± 0.4	0.26	1.1 ± 0.5	1.1 ± 0.4	0.64
Total B₆ (Mean ± SD)	1.9 ± 2.0	2.3 ± 3.8	<0.01	3.0 ± 6.0	2.6 ± 3.3	0.48
Tumor Characteristics						

Site, No. (%)	Population-based Families		Clinic-based Families		P - value [†]
	Cases (n=1,806)	Sibling Controls (n=2,879)	Cases (n=269)	Sibling Controls (n=475)	
Right Colon	598 (33.1)	-	85 (31.6)	-	
Left Colon	525 (29.1)		44 (16.4)		
Rectum	593 (32.8)		77 (28.6)		
Unknown/Missing	90 (5.0)		63 (23.4)		
MSI, No. (%)					
MSS	855 (47.3)	-	61 (22.7)	-	
MSI-L	151 (8.4)		14 (5.2)		
MSI-H	179 (9.9)		55 (20.4)		
Unknown/Missing	621 (34.4)		139 (51.7)		

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

† p-values using a 1-degree of freedom likelihood test from a conditional logistic regression model.

§ Self-reported weight and height two years prior to questionnaire completion date used to calculate body mass index.

¶ Ever use of supplements regularly during the two years prior to recruitment (at least 2x/week for more than a month)

* Calorie adjusted calculated from food frequency questionnaire using post-fortification food composition tables (N cases=585; N controls=837).

† Met-hours per week

Table 3

Single SNP analysis: colorectal cancer risk by SNP, analysis model and study population

Population based sample [§]						
SNP	Log Additive Model [†]	Corrected P-value	Co-dominant Model: Heterozygotes [§]	P-value	Co-dominant Model: Homozygotes	2df LRT P-value
	OR (95% CI)		OR (95% CI) [‡]		OR (95% CI)	
rs11121832	0.95 (0.82–1.10)	0.93	0.96 (0.81–1.14)	0.63	0.89 (0.64–1.25)	0.51
rs12121543	0.94 (0.81–1.08)	0.94	0.89 (0.75–1.05)	0.17	1.00 (0.71–1.41)	1.00
rs12404124	0.90 (0.79–1.02)	0.54	0.96 (0.81–1.14)	0.62	0.77 (0.60–1.00)	0.05
rs13306556	1.01 (0.82–1.23)	0.99	0.98 (0.79–1.22)	0.86	1.22 (0.63–2.39)	0.56
rs1476413	0.95 (0.82–1.09)	0.95	0.93 (0.78–1.10)	0.38	0.93 (0.67–1.30)	0.68
rs17037390	0.86 (0.73–1.02)	0.56	0.86 (0.72–1.04)	0.13	0.75 (0.45–1.25)	0.26
rs17037396	1.00 (0.83–1.22)	0.98	0.94 (0.76–1.17)	0.59	1.34 (0.71–2.53)	0.37
rs17037425	0.90 (0.75–1.07)	0.80	0.91 (0.75–1.10)	0.34	0.74 (0.42–1.28)	0.27
rs17421462	1.01 (0.81–1.26)	1.00	0.94 (0.74–1.18)	0.59	1.97 (0.83–4.67)	0.12
rs17421511	0.93 (0.79–1.10)	0.95	0.93 (0.77–1.12)	0.46	0.88 (0.54–1.45)	0.63
rs1801131 [*]	0.82 (0.64–1.07)	0.14 [¶]	0.90 (0.76–1.07)	0.23	0.76 (0.56–1.02)	0.07
rs1801133 [*]	0.81 (0.63–1.04)	0.10 [¶]	1.10 (0.93–1.31)	0.26	0.88 (0.66–1.17)	0.36
rs1994798	0.89 (0.78–1.00)	0.44	0.95 (0.80–1.14)	0.59	0.76 (0.59–0.98)	0.03
rs3737964	0.95 (0.83–1.09)	0.93	0.96 (0.81–1.13)	0.61	0.89 (0.64–1.24)	0.50
rs3737965	0.90 (0.68–1.17)	0.95	0.90 (0.68–1.19)	0.45	0.77 (0.22–2.70)	0.69
rs4846048	0.87 (0.76–1.00)	0.42	0.93 (0.78–1.10)	0.37	0.71 (0.52–0.96)	0.03
rs4846049	0.87 (0.76–0.99)	0.37	0.89 (0.75–1.05)	0.16	0.74 (0.56–0.99)	0.04
rs4846054	0.91 (0.80–1.03)	0.64	0.97 (0.82–1.14)	0.75	0.79 (0.61–1.03)	0.08
rs6541003	0.89 (0.79–1.01)	0.52	0.96 (0.81–1.14)	0.62	0.77 (0.59–0.99)	0.05
rs9651118	1.15 (1.00–1.33)	0.45	1.26 (1.06–1.50)	0.01	1.09 (0.76–1.55)	0.65
rs17376328	1.04 (0.78–1.38)	1.00	1.06 (0.80–1.42)	0.67	0.43 (0.05–3.43)	0.43
rs2050265	0.87 (0.73–1.03)	0.58	0.88 (0.73–1.07)	0.21	0.68 (0.41–1.15)	0.15
Clinic-based Families [‡]						
rs11121832	1.09 (0.77–1.54)	1.00	1.28 (0.81–2.02)	0.30	0.91 (0.41–2.03)	0.83
rs12121543	1.12 (0.81–1.56)	0.99	1.05 (0.70–1.59)	0.81	1.41 (0.64–3.10)	0.39
rs12404124	1.00 (0.74–1.36)	1.00	0.94 (0.61–1.46)	0.79	1.02 (0.55–1.89)	0.95

Population based sample [‡]						
SNP	Log Additive Model [†]	Corrected P-value	Co-dominant Model: Heterozygotes [§]	P-value	Co-dominant Model: Homozygotes	2df LRT P-value
	OR (95% CI)		OR (95% CI) [†]		OR (95% CI)	
rs13306556	1.12 (0.68–1.83)	0.93	1.12 (0.68–1.87)	0.65	1.14 (0.11–11.8)	0.91
rs1476413	1.03 (0.74–1.44)	1.00	1.02 (0.68–1.52)	0.93	1.09 (0.50–2.38)	0.98
rs17037390	0.90 (0.59–1.38)	1.00	0.90 (0.56–1.43)	0.65	0.83 (0.23–3.07)	0.89
rs17037396	1.07 (0.66–1.75)	1.00	1.11 (0.67–1.84)	0.69	0.73 (0.06–8.58)	0.89
rs17037425	0.99 (0.64–1.53)	1.00	1.01 (0.63–1.61)	0.97	0.79 (0.14–4.59)	0.95
rs17421462	1.32 (0.78–2.24)	0.87	1.16 (0.67–2.00)	0.59	0.91 (1.25–78.4)	0.12
rs17421511	0.98 (0.64–1.50)	1.00	1.19 (0.77–1.83)	0.43	0.27 (0.05–1.48)	0.13
rs1801131 [*]	1.02 (0.53–1.98)	0.95 [¶]	0.98 (0.65–1.47)	0.92	1.01 (0.48–2.10)	0.98
rs1801133 [*]	0.59 (0.31–1.12)	0.10 [¶]	1.21 (0.82–1.80)	0.33	0.69 (0.33–1.44)	0.16
rs1994798	1.08 (0.78–1.49)	1.00	1.04 (0.67–1.61)	0.86	1.19 (0.62–2.27)	0.85
rs3737964	1.06 (0.76–1.47)	1.00	1.23 (0.78–1.93)	0.37	0.90 (0.43–1.87)	0.49
rs3737965	2.04 (1.07–3.88)	0.43	2.04 (1.07–3.88)	0.03	NA [€]	0.17
rs4846048	1.02 (0.72–1.44)	1.00	1.27 (0.81–1.98)	0.30	0.85 (0.42–1.74)	0.33
rs4846049	0.96 (0.69–1.34)	1.00	0.98 (0.65–1.48)	0.93	0.91 (0.44–1.88)	0.96
rs4846054	1.07 (0.78–1.45)	1.00	1.03 (0.67–1.60)	0.88	1.15 (0.62–2.15)	0.89
rs6541003	1.06 (0.76–1.46)	1.00	0.93 (0.60–1.43)	0.73	1.17 (0.61–2.25)	0.69
rs9651118	0.95 (0.68–1.33)	1.00	1.08 (0.70–1.68)	0.73	0.71 (0.35–1.48)	0.64
rs17376328	0.64 (0.32–1.32)	0.81	0.64 (0.32–1.32)	0.23	NA	0.46
rs2050265	0.92 (0.60–1.40)	1.00	0.92 (0.57–1.46)	0.71	0.85 (0.23–3.12)	0.93

[‡]Based on a minimum of 1702 cases and 2726 controls from 1647 discordant sibships.

[†]OR's estimated assuming a log additive model and controlling for age and sex.

[¶]P-value not corrected for multiple testing.

^{*}ORs estimate assuming a recessive genotype effect and controlling for age and sex.

[§]ORs estimated assuming a co-dominant model and controlling for age and sex.

[‡]Based on a minimum of 262 cases and 459 controls from 239 discordant sibships.

[€]OR could not be estimated because there were 0 subjects in the case or control group or the number was too small for a valid estimate.

Table 4

Colorectal cancer risk by *MTHFR* genotype and MSI status

SNP	MSS + MSI-L [‡]	P	MSI-H [†]	P	p-interaction [§]
rs11121832	1.04 (0.86–1.27) [¶]	0.66	1.05 (0.66–1.69)	0.23	0.97
rs12121543	0.94 (0.78–1.14)	0.54	0.97 (0.59–1.60)	0.83	0.91
rs12404124	0.96 (0.81–1.13)	0.61	0.90 (0.59–1.37)	0.61	0.77
rs13306556	1.03 (0.80–1.33)	0.83	0.93 (0.47–1.87)	0.85	0.79
rs1476413	0.97 (0.81–1.17)	0.75	1.05 (0.65–1.71)	0.83	0.74
rs17037390	0.88 (0.70–1.09)	0.25	0.76 (0.44–1.33)	0.34	0.64
rs17037396	0.97 (0.76–1.25)	0.84	1.11 (0.60–2.07)	0.74	0.69
rs17037425	0.95 (0.76–1.19)	0.65	0.82 (0.47–1.42)	0.48	0.63
rs17421462	1.25 (0.92–1.70)	0.16	1.00 (0.53–1.87)	0.99	0.56
rs17421511	0.97 (0.77–1.20)	0.75	1.08 (0.63–1.85)	0.78	0.71
rs1801131 [*]	0.77 (0.54–1.08)	0.13	0.51 (0.24–1.08)	0.08	0.36
rs1801133 [*]	0.69 (0.50–0.97)	0.03	2.22 (0.91–5.43)	0.08	0.01
rs1994798	0.94 (0.80–1.11)	0.50	0.85 (0.55–1.31)	0.47	0.65
rs3737964	1.01 (0.83–1.22)	0.92	1.08 (0.68–1.72)	0.75	0.80
rs3737965	0.98 (0.70–1.39)	0.92	0.81 (0.33–2.00)	0.66	0.69
rs4846048	0.94 (0.78–1.13)	0.52	0.92 (0.59–1.43)	0.71	0.93
rs4846049	0.91 (0.76–1.08)	0.27	0.85 (0.54–1.35)	0.49	0.79
rs4846054	0.99 (0.84–1.17)	0.88	0.91 (0.59–1.40)	0.68	0.74
rs6541003	0.98 (0.83–1.16)	0.86	0.87 (0.57–1.34)	0.54	0.61
rs9651118	1.07 (0.88–1.30)	0.48	0.68 (0.41–1.12)	0.13	0.12
rs17376328	0.86 (0.59–1.25)	0.43	1.41 (0.58–3.45)	0.45	0.32
rs2050265	0.87 (0.69–1.08)	0.21	0.90 (0.51–1.58)	0.71	0.91

[‡] Based on a minimum of 958 cases and 1532 controls from 931 discordant sibships.

[†] Based on a minimum of 175 cases and 255 controls from 166 discordant sibships.

[§] p-value for the multiplicative interaction term (MSI status * genotype (coded as 0, 1 or 2 for the presence of the minor allele)).

[¶] Except as noted all OR's were estimated assuming a log-additive model and controlling for age and sex.

* ORs estimated from a recessive model and controlling for age and sex.