Null Results in Brief

No Association of Single Nucleotide Polymorphisms in One-Carbon Metabolism Genes with Prostate Cancer Risk

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Abstract

One-carbon metabolism mediates the interconversion of folates for the synthesis of precursors used in DNA synthesis, repair, and methylation. Inadequate folate nutrition or compromised metabolism can disrupt these processes and facilitate carcinogenesis. In this study, we investigated associations of 39 candidate single nucleotide polymorphisms (SNP) in 9 one-carbon metabolism genes with risk of prostate cancer using 1,144 cases and 1,144 controls from the Cancer Prevention Study-II Nutrition Cohort. None of these SNPs were significantly associated with prostate cancer risk, either overall or in cases with advanced prostate cancer. Thus, our findings do not support the hypothesis that common genetic variation in one-carbon metabolism genes influences prostate cancer risk. (Cancer Epidemiol Biomarkers Prev 2008;17(12):3612–4)

Introduction

Perturbation in one-carbon metabolism caused by inadequate folate nutrition or genetic variation in the enzymes in this pathway can compromise the synthesis of DNA precursors, disturb DNA repair and methylation, and facilitate carcinogenesis. Disruption of these basic processes could potentially influence tumor development in any tissue. However, the only cancer for which an association with altered one-carbon metabolism has been consistently found to date is colorectal cancer (1, 2). A number of studies have examined whether risk of prostate cancer, the most frequent cancer among men in the United States (3), is affected by changes in folate nutrition or metabolism. The results of studies of dietary folate (4-7), blood folate levels (8-11), and common genetic variants in *methylenetetrahydrofolate reductase* (*MTHFR*; refs. 12-16) have been mixed. In this study, we investigated the association of the genes *MTHFR, methionine synthase* (*MTR*), *methionine synthase reductase* (*MTRR*), *cystathionine β-synthase* (*CBS*), *serine hydroxymethyltransferase* (*SHMT1*), *thymidine synthase* (*TYMS*), *dihydrofolate reductase* (*DHFR*), *methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthase* (*MTHFD1*), and *formyltetrahydrofolate dehydrogenase* (*FTHFD*) with prostate cancer using cases and controls nested in the American Cancer Society Cancer Prevention II Nutrition Cohort.

Materials and Methods

Study Population. Prostate cancer cases and controls were participants in the Cancer Prevention II Nutrition Cohort, a prospective study of cancer incidence of ~184,000 U.S. adults that begun in 1992. The recruitment and characteristics of this cohort have been described previously (17). Incident cases reported by response to follow-up questionnaires, which were sent to participants in 1997 and every 2 y afterwards, or by linkage with the National Death Index were verified through medical records or state cancer registries (17). Blood samples were collected from a subset of Nutrition Cohort participants (21,965 women and 17,411 men) between June 1998 and June 2001.

We identified 1,144 men with a blood sample who had been diagnosed with prostate cancer between 1992 and 2003 and had no previous history of cancer (except nonmelanoma skin cancer). An equal number of controls were matched to the cases on age (±6 mo), race/ethnicity, and date of blood collection (±6 mo) using risk set sampling (18). Among the cases, 272 men had advanced prostate cancer, defined as prostate cancer with Gleason score of ≥8, grades 3 to 4, or stage D at diagnosis, or who had prostate cancer as their underlying cause of death.

Single Nucleotide Polymorphism Selection and Genotyping. Single nucleotide polymorphisms (SNP) were selected from the dbSNP or Celera databases in October, 2004 if they (*a*) had a minor allele frequency in Caucasians (>5%), and (*b*) caused changes in the amino acid sequence, or (*c*) had been previously found to be associated with disease. For some genes for which only one SNP was identified using these criteria, one or two additional SNPs for which only frequency information was available were added. Seven additional tagging SNPs were selected for the *FTHFD* gene using early

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HapMap data (Release 16, 3/1/2005) to allow haplotype analysis of this gene.

All SNPs were genotyped using TaqMan at Applied Biosystems, Inc. The genotyping success rate was >96%, and the genotype distributions of the controls for all SNPs were in Hardy-Weinberg equilibrium ($P > 0.01$).

Statistical Analysis. The association with prostate cancer risk was evaluated using a per allele odds ratio determined using unconditional logistic regression in which a continuous variable for the number of minor alleles (0, 1, or 2) was entered into the regression model. All models were adjusted for age (single year categories), race (White, other), and date of blood draw (single year categories). Haplotypes for blocks defined through Haploview (19) were estimated using the expectation-maximization algorithm implemented in the TAGSNPS program (20).

Results

Details of the 39 one-carbon metabolism SNPs studied are listed in Table 1. Based on the per-allele odds ratios

Table 1. Gene locations, resulting amino acid changes, minor allele frequencies, and associations with prostate cancer risk for the one-carbon metabolism gene SNPs investigated in this study

Gene/SNP	Location	AA change	Minor allele	Minor allele frequency		OR (95% CI)*	P †
				Cases	Controls		
MTHFR							
rs2066470, T118C	Exon 2	None	T	210 (9.6)	207 (9.5)	1.01 (0.82-1.23)	0.94
rs1801133, C677T	Exon 5	Val → Ala	T	739 (33.6)	765 (34.6)	0.96 (0.85-1.09)	0.56
rs1801131, A1298C	Exon 8	Ala → Glu	C	728 (33.0)	743 (33.5)	0.97 (0.86-1.10)	0.64
rs2274976	Exon 12	Gln → Arg	A	95 (4.3)	120 (5.4)	0.78 (0.59-1.03)	0.08
MTR							
rs1806505	Intron 13	N/A ‡	T	877 (39.6)	856 (38.6)	1.05 (0.93-1.18)	0.44
rs1805087, A2756G	Exon 26	Asp → Gly	G	435 (19.9)	430 (19.5)	1.03 (0.89-1.20)	0.69
rs1050993	3′ UTR	N/A ‡	A	837 (37.8)	880 (39.6)	0.92 (0.82-1.04)	0.18
MTRR							
rs1801394, A66G	Exon 2	Met → Ile	A	1,021 (46.1)	1,005 (45.5)	1.02 (0.91-1.15)	0.70
rs1532268	Exon 5	Leu → Ser	T	815 (36.9)	798 (36.0)	1.04 (0.92-1.17)	0.54
rs162036	Exon 7	Lys → Arg	G	273 (12.4)	251 (11.3)	1.11 (0.92-1.33)	0.28
rs10380	Exon 14	Tyr → His	T	231 (10.4)	221 (10.0)	1.05 (0.86-1.27)	0.65
CBS							
rs234706, Y233Y	Exon 8	None	A	745 (33.7)	780 (35.1)	0.94 (0.83-1.06)	0.32
rs1801181, C1261T	Exon 12	None	T	774 (36.9)	739 (35.1)	1.08 (0.95-1.23)	0.23
SHMT1							
rs64333	5′ UTR	N/A ‡	A	677 (30.7)	636 (28.7)	1.10 (0.97-1.25)	0.15
rs2273028	Intron 7	N/A ‡	T	725 (32.9)	691 (31.2)	1.08 (0.96-1.23)	0.22
rs1979277, C1420T	Exon 12	Leu → Phe	A	702 (31.8)	675 (30.6)	1.06 (0.93-1.20)	0.39
TYMS							
rs502396	Intron 1	N/A ‡	C	1,078 (49.0)	1,057 (47.9)	1.04 (0.93-1.17)	0.50
rs1001761	Intron 2	N/A ‡	T	1,067 (49.3)	1,016 (47.0)	1.09 (0.97-1.23)	0.15
rs699517	3′ UTR	N/A ‡	T	733 (33.2)	693 (31.2)	1.09 (0.96-1.23)	0.20
DHFR							
rs836821	Intron 2	N/A ‡	T	552 (25.0)	594 (26.8)	0.91 (0.79-1.04)	0.16
rs1677693	Intron 3	N/A ‡	A	550 (25.0)	581 (26.5)	0.93 (0.81-1.06)	0.26
rs1643638	Intron 4	N/A ‡	C	547 (24.9)	589 (26.6)	0.92 (0.80-1.05)	0.20
MTHFD1							
rs1076991	5′ UTR	N/A ‡	G	1,017 (46.1)	1,022 (46.5)	0.98 (0.87-1.11)	0.78
rs1950902, R134K	Exon 6	Arg → Lys	T	413 (18.7)	411 (18.5)	1.01 (0.87-1.17)	0.91
rs2236225, R653Q	Exon 20	Arg → Gln	T	1,039 (47.3)	1,006 (46.0)	1.05 (0.93-1.19)	0.39
rs2236224	Intron 21	N/A ‡	T	919 (41.5)	911 (41.0)	1.02 (0.90-1.15)	0.78
FTHFD (ALDH1L1)							
rs7617733	Intron 1	N/A ‡	A	379 (17.1)	360 (16.2)	1.06 (0.91-1.24)	0.46
rs4646701	Intron 2	N/A ‡	A	854 (38.7)	868 (39.4)	0.98 (0.86-1.10)	0.69
rs1823213	Intron 2	N/A ‡	A	737 (33.4)	765 (34.5)	0.96 (0.85-1.09)	0.50
rs2276731	Intron 4	N/A ‡	C	411 (18.7)	418 (18.8)	0.99 (0.85-1.15)	0.90
rs10934751	Intron 8	N/A ‡	G	1,066 (48.1)	1,066 (48.0)	1.00 (0.89-1.13)	0.94
rs1965848	Intron 8	N/A ‡	T	896 (40.7)	906 (41.1)	0.98 (0.87-1.11)	0.80
rs2886059	Exon 9	Val → Phe	T	357 (16.1)	352 (15.9)	1.01 (0.86-1.19)	0.88
rs11923466	Intron 9	N/A ‡	C	920 (42.1)	911 (41.3)	1.03 (0.91-1.16)	0.64
rs2002287	Intron 13	N/A ‡	C	766 (34.6)	771 (34.8)	0.99 (0.88-1.12)	0.88
rs2365004	Intron 14	N/A ‡	A	768 (34.6)	773 (34.9)	0.99 (0.87-1.12)	0.85
rs6774437	Intron 16	N/A ‡	A	1,065 (48.0)	1,051 (47.3)	0.97 (0.86-1.09)	0.65
rs2290053	Intron 18	N/A ‡	A	1,085 (49.2)	1,099 (49.9)	1.03 (0.91-1.16)	0.63
rs1127717, A2380C	Exon 21	Asp → Gly	G	490 (22.2)	452 (20.4)	1.12 (0.97-1.29)	0.13

Abbreviations: AA, amino acid; UTR, untranslated region; OR, odds ratio; 95% CI, 95% confidence interval.
*Per allele odds ratio, adjusted for gender, age, and draw date.
† P for trend, adjusted for gender, age, and draw date.
‡ N/A: nonapplicable because SNP is not located in a coding region.

and related *P* values shown in this table, which assume an additive genetic model, none of the SNPs were significantly associated with an altered risk of prostate cancer. Additionally, no significant association was seen when the analysis was restricted to advanced prostate cancer. To consider the possibility that some SNPs may act through a recessive model, we also assessed the association of the homozygous variant genotype with prostate cancer. Again, no statistically significant associations were found, either with all prostate cancer cases or with advanced prostate cancer cases.

The genotyped SNPs included seven tagging SNPs that defined three separate haplotype blocks in the *FTHFD* gene. None of the haplotype blocks [(*a*) defined by rs2365004 and rs11923466; (*b*) defined by rs2886059, rs10934751, and rs2276731; (*c*) third was defined by rs1823213 and rs4646701] were associated with altered risk of all prostate cancer or advanced prostate cancer.

Discussion

This study is the first to investigate 9 one-carbon metabolism genes in prostate cancer and uses one of the largest study populations for this purpose to date. With 1,144 cases and 1,144 controls, this study has ≥80% power to detect odds ratios as low as 1.3 for SNPs with minor allele frequencies of ≥25% and as low as 1.45 for SNPs with minor allele frequencies of ≥10%. Thus, this study was adequately powered to detect associations with common genetic variants of the magnitude typically observed.

Our null findings are consistent with the results of all (12, 14-16) but one (13) of the previous studies. The study that reported a significant association of prostate cancer risk with the *MTHFR* C677T SNP was based on only 21 cases of prostate cancer (13). Besides the *MTHFR* gene, only two other genes in one-carbon metabolism have been investigated in relation to prostate cancer. Similar to our findings, neither the nonsynonymous A2756G SNP in *MTR* (12, 16) nor an insertion/deletion polymorphism in *CBS* (12) were significantly associated with altered prostate cancer risk.

Our findings of no significant association for 39 SNPs in 9 genes involved in one-carbon metabolism suggest that perturbations in the enzymes in this pathway have little or no effect on prostate cancer incidence. Coupled with previous null findings for folate intake (4, 6, 7) or blood levels (8, 11), these results argue that altered folate nutrition or metabolism do not contribute to risk for prostate cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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