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Association of Folate-Pathway Gene Polymorphisms with the Risk of Prostate Cancer: a Population-Based Nested Case-Control Study, Systematic Review, and Meta-analysis

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Abstract

Folate-pathway gene polymorphisms have been implicated in several cancers and investigated inconclusively in relation to prostate cancer. We conducted a systematic review, which identified nine case-control studies (eight included, one excluded). We also included data from four genome-wide association studies and from a case-control study nested within the UK population–based Prostate Testing for Cancer and Treatment study. We investigated by meta-analysis the effects of eight polymorphisms: *MTHFR C677T* (rs1801133; 12 studies; 10,745 cases; 40,158 controls), *MTHFR A1298C* (rs1801131; 5 studies; 3,176 cases; 4,829 controls), *MTR A2756G* (rs1805087; 8 studies; 7,810 cases; 37,543 controls), *MTRR A66G* (rs1801394; 4 studies; 3,032 cases; 4,515 controls), *MTHFD1 G1958A* (rs2236225; 6 studies; 7,493 cases; 36,941 controls), *SLC19A1/RFC1 G80A* (rs1051266; 4 studies; 6,222 cases; 35,821 controls), *SHMT1 C1420T* (rs1979277; 2 studies; 2,689 cases; 4,110 controls), and *FOLH1 T1561C* (rs202676; 5 studies; 6,314 cases; 35,190 controls). The majority (10 of 13) of eligible studies had 100% Caucasian subjects; only one study had <90% Caucasian subjects. We found weak evidence of dominant effects of two alleles: *MTR 2756A>G* [random effects pooled odds ratio, 1.06 (1.00-1.12); $P = 0.06$ ($P = 0.59$ for heterogeneity across studies)] and *SHMT1 1420C>T* [random effects pooled odds ratio, 1.11 (1.00-1.22); $P = 0.05$ ($P = 0.38$ for heterogeneity across studies)]. We found no effect of *MTHFR 677C>T* or any of the other alleles in dominant, recessive or additive models, or in comparing *a/a* versus *A/A* homozygous. Neither did we find any difference in effects on advanced or localized cancers. Our meta-analysis suggests that known common folate-pathway single nucleotide polymorphisms do not have significant effects on susceptibility to prostate cancer. (Cancer Epidemiol Biomarkers Prev 2009;18(9):2528–39)

Introduction

There is a growing body of evidence that *MTHFR* and other folate-pathway genes are associated with some cancers, most notably colorectal and gastric cancers (1-3). The putative mechanisms behind these associations are manifold because folate and related metabolites, including vitamins B6 and B12, are fundamental to DNA synthesis, repair, and methylation (Fig. 1; ref. 4). Aberrant DNA methylation in particular seems to play a role in prostate cancer (5, 6). The mechanisms are complex and interdependent, and their possible roles in cancer etiology are likely to be complicated by interactions with dietary intakes and by the different stages of tumorogenesis (7).

The majority of dietary intake and blood level studies have indicated no association with prostate cancer risk of folate, vitamin B6, methionine, and homocysteine (8-16). A recent study reported a positive association between folic

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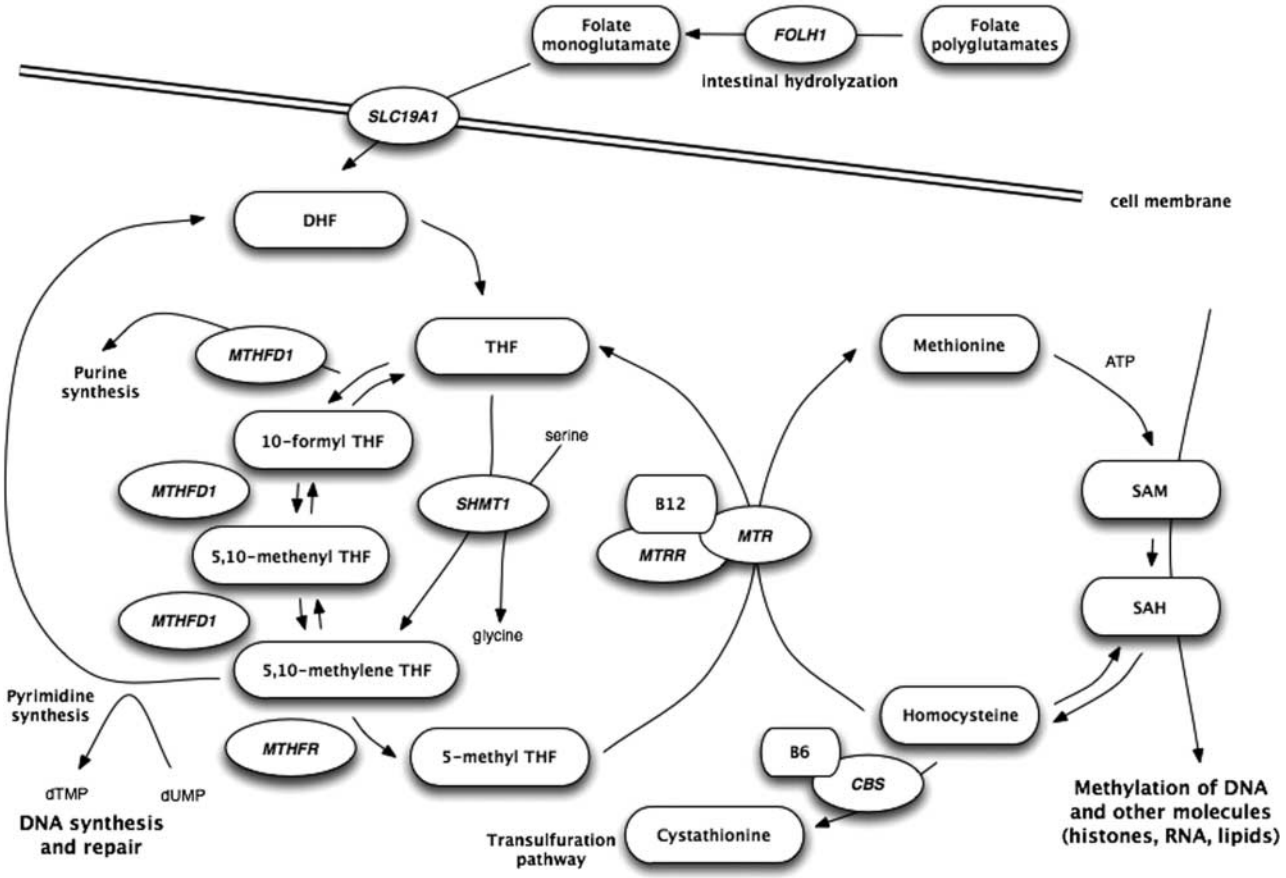

Figure 1. Simplified overview of folate-mediated one-carbon metabolism, highlighting enzymes with polymorphisms investigated in this study. *Ovals,* enzymes; *oblongs,* substrates; *CBS,* cystathionine beta synthase; *DHF,* dihydrofolate; *FOLH1,* folate hydrolase 1; *MTHFD1,* methylene tetrahydrofolate dehydrogenase; *MTHFR,* methylene tetrahydrofolate reductase; *MTR,* methionine synthase; *SAH,* S-adenosylhomocysteine; *SAM,* S-adenosylmethionine; *MTRR,* methionine synthase reductase; *SHMT1,* serine hydroxymethyltransferase 1; *SLC19A1,* solute carrier family 19; *THF,* tetrahydrofolate.

acid supplementation and prostate cancer risk (17). However, the same study suggested an inverse association with baseline plasma folate, which is consistent with two earlier studies that reported inverse associations with folate intake (11) and plasma folate (18). By contrast, there is some evidence that vitamin B12 intake and blood levels are positively associated with risk of prostate cancer (8, 10, 13, 14).

Here, we have done a meta-analysis of associations between polymorphisms in the folate metabolic pathway and prostate cancer using data obtained from the UK population–based Prostate Testing for Cancer and Treatment (ProtecT) study, from studies identified by systematic review of the literature, and from genome-wide association studies. Our aim was to determine whether the folate metabolic pathway has a role in prostate cancer. Food fortification and use of dietary supplements make this a research question imperative to public health.

Materials and Methods

Candidate Genes. Candidate genes were identified by reviewing published literature and public access metabolic pathway and gene-disease association databases. We reviewed published studies that had investigated associations of folate and one-carbon metabolic pathway genetic variants with disease and with blood levels of folate and related metabolites (homocysteine, methionine, B6, and B12).

Systematic Review and Extraction of Published Data. Eligible studies were identified by searching the Medline and Embase databases using text search terms for the folate-pathway genes: "*MTHFR*," "*MTR/MS*," "*MTRR/MSR*," "*MTHFD1*," "*SLC19A1*"/"*RFC*," "*SHMT1*," "*FOLH1*," "*CBS*," "*FUT2*," and "*TCN2*"; or their full names, e,g., "methylenetetrahydrofolate reductase"; or single nucleotide polymorphism (SNP) identifiers, e.g., "rs1801133," "rs1801131," "rs1805087," "rs1801394," "rs2236225," "rs1051266," "rs1979277," "rs202676," "rs492602," and "rs1801198"; each in conjunction with the MeSH term "prostatic neoplasm" and text terms "prostate cancer" and "prostatic carcinoma." No language or publication date restrictions were imposed. HuGENet was also searched for genetic associations with prostatic diseases. All databases were last searched on 19/12/2008. References of retrieved articles were screened. Nonfamilial case-control studies were eligible

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in which genotype frequencies had been determined by molecular methods among cancer cases and prostate cancer–free controls. Where available, we also extracted genotype frequencies by cancer stage and grade, i.e., advanced/high-risk (stage III/IV or Gleason of ≥7/8) or localized/low-risk cases (stage I/II and Gleason of <7/8). Studies in which controls had Benign Prostatic Hyperplasia (BPH) or which included female controls were accepted. Data were extracted independently by two investigators.

Data from Genome-Wide Association Studies. Eligible genome-wide association studies were identified by searching the Medline and Embase databases using text search terms for "gene/genetic/genome-wide association" in conjunction with the MeSH term "prostatic neoplasm" and text terms "prostate cancer" and "prostatic carcinoma." Genotype count data were obtained via public access data portals or by submitting requests for data via the principal investigators.

Data from the ProtecT Study

Study Population. The aim of the ProtecT study is to evaluate, in a randomized controlled trial, treatments for localized prostate cancer. Recruitment to the study occurred between 2001 and 2008. Men ages 50 to 69 y in general practices located around nine UK cities were invited to attend a nurse-led prostate check clinic and, if they consented, to have a Prostate-Specifc Antigen (PSA) test. Participants with a single raised PSA test result between 3.0 and 19.9 ng/mL were invited to attend the center's urology department for digital rectal examination, repeat PSA test, and 10-core transrectal ultrasound-guided biopsy. Men with clinically localized disease were eligible to participate in the treatment trial. Men with a PSA level of ≥20 ng/mL were referred as a matter of urgency to a urologist, and were eligible to participate in the treatment trial only if localized cancer was confirmed. A diagnosis of localized prostate cancer was defined as a positive biopsy, clinical stage T_1-T_2, N_X, M_0; advanced prostate cancer was defined as positive biopsy, clinical stage T_3-T_4 or N_1 or M_1. All men provided written informed consent. Trent Multicentre Research Ethics Committee approved the study.

DNA Extraction and Genotyping. In an allied (nested case-control) study, blood samples from 1,600 men with and 1,855 men without prostate cancer who had consented to DNA extraction and analysis were genotyped for 72 candidate gene polymorphisms, including 10 folate pathway genes. DNA was extracted by Tepnel Life Sciences PLC and genotyping was done by KBiosciences Ltd. using their own competitive allele-specific PCR (KASPar) and Taqman. Samples that produced seven or more genotype failures were considered to have poor DNA quality (2.5% of samples) and were discarded. Genotyping was carried out in duplicate in approximately 1 in 10 of the samples; of these repeats, 98.1% were in exact agreement.

Statistical Methods. We tested whether genotype frequencies of controls were in Hardy-Weinberg Equilibrium (HWE) using the Pearson χ^2 test. We calculated unadjusted odds ratios of prostate cancer risk from genotype counts using all cases versus controls, and odds ratios of advanced and localized prostate cancer defined by cancer stage and/or grade: advanced (stage III/IV or Gleason of ≥7/8); localized (stage I/II and Gleason of <7/8). We estimated effects in four models: dominant [(a/a | a/A) versus A/A], recessive [a/a versus (a/A | A/A)], a/a homozygous versus A/A homozygous, and additive [in which genotype was coded A/A = 0 (reference), a/A = 1, a/a = 2]. Meta-analysis was by fixed (Mantel-Haenszel) and random effects models, the latter using the method of DerSimonian and Laird (19). Heterogeneity was quantified by the χ^2-based Q statistic and the I^2 measure of percentage between-studies variation attributable to heterogeneity. Small-study bias was assessed by inspection of funnel plots and tested by the Egger meta-regression method (20). Differences in effects on advanced and localized cancer subgroups were assessed using the χ^2-based Q statistic (test for heterogeneity). All statistical analyses were done using Stata Release 10 (StataCorp. 2007. *Stata Statistical Software: Release 10*).

Results

Eligible Studies. Our systematic review identified nine published studies (Table 1) of which eight were eligible (21-28) and one family study was excluded (29). Unpublished data were obtained from four genome-wide association studies (30-33) and from the ProtecT study (Table 2). The majority (10 of 13) of eligible studies had 100% Caucasian subjects; of the remainder, only one study had <90% Caucasian subjects (23). Six studies recruited prostate cancer cases from hospitals (21, 23, 25-27, 33), five from prospective longitudinal cohorts or cancer registries (22, 24, 28, 30, 32), and the CGEMS (31) and ProtecT studies by population-based PSA testing. Two of the hospital case-control studies recruited men with BPH to the control group (23, 27). ProtecT and four other studies (24, 25, 28, 31) purposively matched controls on age and at least one other characteristic. The mean age of cases and controls was reported to be different in one study (23). Two studies included women among their control groups (21, 30), which affects only the power of the study if genotype frequencies are not associated with sex (verified by the Icelandic study group). The Icelandic study reported an inflation factor (1.12) due to relatedness among cases and controls (30). ProtecT controls that had also been used in the UK Genetic Prostate Cancer Study (33) were removed from the ProtecT control group when both studies were included in a meta-analysis.

Genotype Frequencies. Genotype counts and frequencies for all cases and controls and for advanced and localized cases are presented in Supplementary Tables S1 and S2, respectively. There was strong evidence ($P < 0.001$) of a departure from HWE among the control group in the Icelandic study for rs2236225 and rs202676, and marginal evidence ($0.03 < P < 0.08$) among the control groups in three studies for rs1801133 (23, 24, 26), in one study for rs1805087 (28), in the ProtecT study for rs1979277, and in the Framingham Heart Study SNP Health Association Resource study for rs202676.

Meta-analysis

MTHFR C677T (rs1801133). Twelve eligible studies included data from 10,745 cases and 40,158 controls. The T allele was not associated with risk of prostate cancer in dominant or recessive modeling, in comparing T/T versus C/C genotypes, or in an additive (per allele) model (Table 3; Fig. 2). There was substantial between-study

Table 1. Studies identified by systematic review of the literature

First author	Year	Country	Cases	Controls	Population	SNPs	Included	Reference
Kimura	2000	Germany	132 patients with histopathologically confirmed prostatic carcinoma from the Department of Urology of Heinrich Heine University (Düsseldorf, Germany). Mean age, 65.6 ± 6.0 y (range, 52-89 y).	150 patients without malignant disease treated for various abdominal diseases at the Department of Surgery of the Städtische Kliniken (Dortmund, Germany). Sixty-six patients were male and 84 were female. Their mean age was 62.0 ± 11.4 (SD) y (range, 16-90 y).	Caucasian (100%)	rs1801133 rs1805087	Yes	(21)
Heijmans	2003	the Netherlands	21 incident prostate cancer cases among 793 men initially without any cancer who were recruited to a population-based longitudinal study to investigate risk factors for chronic diseases in elderly men. Mean age, 71 ± 5 y (range 65-84 y).	772 men from the same cohort who did not develop prostate cancer during the 10-y follow-up (but who may have developed other diseases, including other cancers).	Caucasian (100%)	rs1801133	Yes	(22)
Cicek	2004	United States	439 cases (diagnosed at age ≤73 y) from 413 families recruited from the major medical institutions in the greater Cleveland, OH area and from the Henry Ford Health System (Detroit, MI). Mean age, 61.0 ± 6.7 y.	479 sibling controls <8 y younger than their brother's age at diagnosis. The disease status of unaffected brothers was further confirmed through testing of prostate-specific antigen levels. Mean age, 62.7 ± 9.1 y.	Caucasian (91%) African-American (8%) Latino (1%)	rs1801133 rs1801131	No (sibling controls)	(29)
Singal	2004	United States	81 prostate cancer patients who had radical prostatectomy at the Overton Brooks VA Medical Centre (Shreveport, CA). Mean age, 64 y (range, 49-80 y).	42 patients with benign prostatic hyperplasia who had trans-urethral resection of the prostate. Mean age, 70 y (range, 53-88 y).	Caucasian (67%) African-American (33%)	rs1801133 rs1801131	Yes	(23)
van Guelpen	2006	Sweden	299 histopathologically confirmed incident prostate cancer cases among 37,776 men recruited to the Northern Swedish Health and Disease Cohort study. Mean age at recruitment was 58.7 ± 4.6 y.	617 healthy and cancer-free controls randomly selected from the same cohort and matched 2:1 on age and date of blood sampling. Mean age at recruitment was 58.6 ± 4.6 y.	Caucasian (100%)	rs1801133 rs1801131	Yes	(24)
Johansson	2007	Sweden	2,777 histopathologically confirmed cases, diagnosed through a rapid ascertainment scheme at four regional oncological centers, were recruited to a population-based case-control study. Mean age, 65.9 ± 7.2 y.	1,639 controls, randomly selected from the Swedish population register and frequency matched to the predicted distribution of cases by age (5-year intervals) and geographic region. Mean age, 67.2 ± 7.4 y.	Caucasian (100%)	rs1801133	Yes	(25)
Reljic	2007	Croatia	95 patients diagnosed with histopathologically confirmed prostate cancer at Sestre milosrdnice University hospital, Croatia. Mean age, 69 ± 7 y.	37 cancer-free controls recruited as part of a community health screening program in the same geographic region. Controls were given a digital rectal examination and underwent a prostate-specific antigen test. Mean age, 69 ± 7 y.	Caucasian (100%)	rs1801133	Yes	(26)
Marchal	2008	Spain	182 patients diagnosed with histopathologically confirmed prostate cancer at the Urology Department of the Hospital Clínico Universitario in Málaga, Spain. Mean age, 70.7 ± 7.3 y.	205 patients diagnosed with benign prostatic hyperplasia. These patients were given a digital rectal examination and underwent a prostate-specific antigen test. Mean age, 70.3 ± 7.8 y.	Caucasian (100%)	rs1801133 rs1801131 rs1805087 rs1801394	Yes	(27)
Stevens	2008	United States	1,144 incident cases among a subset of men participating in the Cancer Prevention II Nutrition Cohort study who had provided blood samples.	1,144 controls from the same cohort matched on age, race, and date of blood sample collection.	Caucasian (97%)	rs1801133 rs1801131 rs1805087 rs1801394 rs1979277 rs2236225	Yes	(28)

Table 2. Sources of unpublished genotype frequency data

Project	Country	Cases	Controls	Population	SNPs	Included	Reference
deCODE	Iceland	1,619 cases identified from a list maintained by the Icelandic Cancer Registry that contains all 4,144 Icelandic prostate cancer patients diagnosed from January 1, 1955, to June 31, 2006.	30,779 controls (43.3% male) from genetic research projects at deCODE: some diagnosed with common diseases (cardiovascular, endocrine, autoimmune, psychiatric, and neurologic); others randomly selected from the Icelandic genealogic database.	Caucasian (100%)	rs1801133 rs1805087 rs2236225 rs1051266 rs202676	Yes	(30)
CGEMS*	United States	1,188 cases diagnosed among men enrolled in the screening arm of the PLCO cancer screening trial who had no history of prostate, lung or colon cancer at randomization.	1,110 controls selected by incidence-density sampling from the screening arm of the PLCO trial.	Caucasian (100%)	rs1801133 rs1805087 rs2236225 rs1051266 rs202676	Yes	(31)
FHS SHARe†	United States	172 cases identified at routine examinations or by health history updates among 5,209 Original and 5,124 Offspring Cohort participants in the FHS.	231 unrelated (to each other and to cases) cancer-free controls randomly selected from among the same FHS cohorts.	Caucasian (100%)	rs1805087 rs1801394 rs2236225 rs202676	Yes	(32)
UKGPCS	United Kingdom	1,854 cases diagnosed in the United Kingdom through clinical symptoms, selected from the UK Genetic Prostate Cancer Study on the basis of age ≤60 y (64%) or first or second-degree family history of prostate cancer (36%).	1,894 controls selected from among men invited to participate in the ProtecT study who had a PSA level of <0.5 ng/mL.	Caucasian (100%)	rs1801133 rs1805087 rs2236225 rs1051266 rs202676	Yes	(33)
ProtecT study	United Kingdom	1,600 cases diagnosed during recruitment of men age 50-69 y to a UK population–based trial of treatments for localized prostate cancer. Men underwent a biopsy if they had a PSA level of ≥3 ng/mL.	1,855 controls selected at random from among men age 50-69 y invited to participate in the ProtecT study who had a PSA level of <3 ng/mL or PSA of ≥3 ng/mL and a negative biopsy, plus 1,203 "low PSA" controls (PSA level, <0.5 ng/mL). Of the controls in these two groups, 344/1,855 and 622/1,203 were also used by the UKGPCS.	Caucasian (>98%)	rs1801133 rs1801131 rs1805087 rs1801394 rs2236225 rs1051266 rs202676 rs1979277	Yes	—

Abbreviations: CGEMS, Cancer Genetic Markers of Susceptibility; PLCO, Prostate, Lung, Colon, and Ovarian; FHS SHARe, Framingham Heart Study SNP Health Association Resource; UKGPCS, UK Genetic Prostate Cancer Study.
*http://cgems.cancer.gov/data/ accessed 10th July 2009.
†http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000007.v6.p3 accessed 10th July 2009.

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Table 3. Meta-analysis results (all cases vs controls)

Gene	Variant	SNP	Studies	Cases	Controls	Dominant effect (a/a | a/A) vs AA	Recessive effect aa vs (a/A | A/A)	Homozygous a/a vs A/A	Additive effect a/a, a/A vs A/A (ref)
						Pooled odds ratio (95% CI)*	Pooled odds ratio (95% CI)*	Pooled odds ratio (95% CI) *	Pooled odds ratio (95% CI) *
MTHFR	*677C>T*	rs1801133	12	10,745	40,158	1.04 (0.97-1.12)	1.03 (0.86-1.22)†	1.05 (0.89-1.23)‡	1.03 (0.98-1.09)
MTHFR	*1298A>C*	rs1801131	5	3,176	4,829	0.96 (0.87-1.05)	0.90 (0.77-1.05)	0.89 (0.76-1.05)	0.95 (0.89-1.02)
MTR	*2756A>G*	rs1805087	8	7,810	37,543	1.06 (1.00-1.12)	1.02 (0.88-1.19)	1.04 (0.90-1.21)	1.05 (0.99-1.10)
MTRR	*66A>G*	rs1801394	4	3,032	4,515	0.95 (0.86-1.05)	0.93 (0.76-1.13)	0.93 (0.80-1.08)	0.96 (0.90-1.03)
MTHFD1	*1958G>A*	rs2236225	6	7,493	36,941	1.01 (0.95-1.08)	1.00 (0.93-1.07)	1.01 (0.92-1.09)	1.00 (0.96-1.05)
SLC19A1	*80G>A*	rs1051266	4	6,222	35,821	0.98 (0.92-1.05)	1.02 (0.94-1.10)	1.00 (0.91-1.09)	1.00 (0.95-1.04)
SHMT1	*1420C>T*	rs1979277	2	2,689	4,110	1.11 (1.00-1.22)	1.10 (0.93-1.28)	1.15 (0.97-1.35)	1.08 (1.00-1.16)
FOLH1	*1561T>C*	rs202676	5	6,314	35,190	1.00 (0.93-1.08)	0.99 (0.86-1.13)	0.99 (0.85-1.15)	1.00 (0.93-1.07)

*DerSimonian and Laird random effects model (DerSimonian et al., 1986).
†Q statistic $P = 0.001$; $I^2 = 66\%$.
‡Q statistic $P = 0.01$; $I^2 = 56\%$.

heterogeneity in the recessive and T/T versus C/C models. In sensitivity analyses, exclusion of the three studies (Marchal, Reljic, deCODE) that showed weak evidence of departure from HWE among the control group (respectively, $P = 0.03$, $P = 0.05$, and $P = 0.07$) yielded pooled (random effects) odds ratios [95% confidence interval (95% CI)] for the dominant, recessive, T/T versus C/C, and additive models of the following: 1.05 (0.99-1.11), $P = 0.14$ ($P = 0.53$ for heterogeneity across studies); 1.11 (0.93-1.34), $P = 0.26$ ($P = 0.01$ for heterogeneity across studies); 1.12 (0.95-1.33), $P = 0.18$ ($P = 0.05$ for heterogeneity across studies); and 1.05 (1.00-1.11), $P = 0.05$ ($P = 0.34$ for heterogeneity across studies), respectively. For the recessive and T/T versus C/C models, the pooled fixed-effects odds ratios (95% CI) with these exclusions were, respectively: 1.13 (1.02-1.24), $P = 0.02$; and 1.13 (1.02-1.26), $P = 0.02$.

In a meta-analysis based on the seven studies that provided genotype count data for advanced ($n = 2,959$) and localized ($n = 3,763$) prostate cancer cases (seven studies; 6,002 controls), we found no differences in effects of the T allele on these two subgroups (Table 4). However, in comparing T/T versus C/C genotypes of *MTHFR*, the pooled (random effects) odds ratio for advanced cancer was 1.14 (0.98-1.33), $P = 0.09$ ($P = 0.70$ for heterogeneity across studies). Excluding the Marchal study, for which there was some evidence of Hardy Weinberg disequilibrium ($P = 0.03$), changed this odds ratio to 1.16 (95% CI, 0.99-1.35), $P = 0.07$ ($P = 0.83$ for heterogeneity across studies).

***MTR A2756G* (rs1805087).** Eight eligible studies included data from 7,810 cases and 37,543 controls. We found weak evidence that the G allele was associated with increased risk of prostate cancer in dominant and additive models (Table 3; Fig. 3), but not in a recessive model or in comparing G/G versus A/A genotypes. There was no heterogeneity in any of the models. Exclusion of the Stevens study (HWE $P = 0.03$) yielded pooled odds ratios for the dominant and additive models of 1.06 (0.99-1.13), $P = 0.10$, and 1.05 (0.99-1.11), $P = 0.09$, respectively. We found no differences in effects of the G allele on advanced ($n = 1,780$) and localized ($n = 2,187$) cancers (5 studies; 4,414 controls), although dominant (Fig. 4) and additive effects were evident for the localized, but not for the advanced, subgroup: dominant effect pooled odds ratio, 1.16 (1.04-1.29); $P = 0.008$ ($P = 0.79$ for heterogeneity across studies); additive effect pooled odds ratio, 1.10 (1.01-1.21); $P = 0.03$ ($P = 0.77$ for heterogeneity across studies; Table 4). These effects were slightly weakened if the Stevens study was excluded [dominant effect pooled odds ratio, 1.14 (1.01-1.30); $P = 0.04$; additive effect pooled odds ratio, 1.10 (0.99-1.23); $P = 0.07$].

***SHMT1 C1420T* (rs1979277).** Two eligible studies included data from 2,689 cases and 4,110 controls. We found weak evidence that the T allele was associated with increased risk of prostate cancer in dominant and additive models (Table 3), but not in a recessive model or in comparing T/T versus C/C genotypes. There was no heterogeneity in any of the models. We found no differences in effects of the T allele on advanced ($n = 960$) and localized ($n = 1,540$) cancers (2 studies; 4,110 controls), although effects were evident for the localized, but not for the advanced,

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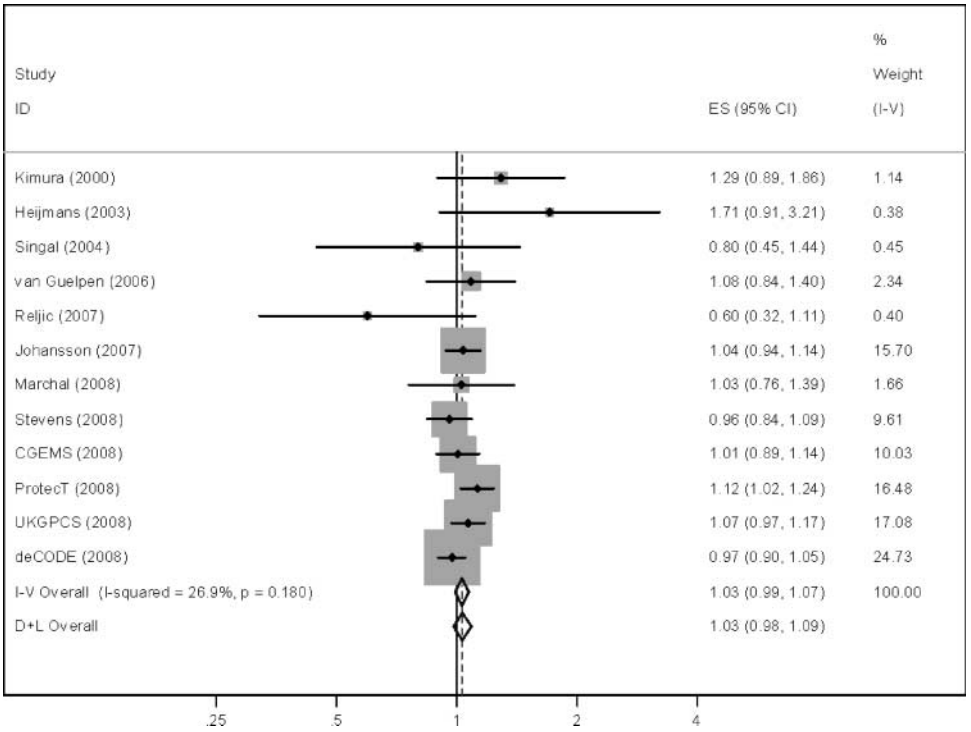

Figure 2. Association of *MTHFR C677T* with risk of prostate cancer: additive model comparing T/T, C/T versus C/C genotypes [*ES,* effect size (per allele odds ratio); *I-V overall,* inverse-variance fixed effects estimate; *D+L overall,* DerSimonian and Laird random effects estimate].

subgroup in a recessive model [pooled odds ratio, 1.20 (95% CI, 1.00-1.45); $P = 0.05$ ($P = 0.57$ for heterogeneity across studies)], an additive model [pooled odds ratio, 1.10 (95% CI 1.01-1.20); $P = 0.04$ ($P = 0.96$ for heterogeneity across studies)], and in comparing T/T versus C/C genotypes [pooled odds ratio, 1.24 (95% CI, 1.02-1.51); $P = 0.03$ ($P = 0.43$ for heterogeneity across studies; Table 4)].

Null Findings. We found no associations with prostate cancer in dominant, recessive, or additive modeling, or in comparing homozygous genotypes for *MTHFR A1298G* (rs1801131), *MTRR A66G* (rs1801394), *MTHFD1 G1958A* (rs2236225), *SLC19A1 G80A* (rs1051266), and *FOLH1 T1561C* (rs202676; Table 3). Neither did we find any differential association with advanced versus localized prostate cancer (Table 4) for these alleles. For *MTHFD1 G1958A* and *FOLH1 T1561C*, exclusion of the studies that had evidence of departure from HWE made no difference to these null findings.

Bias Diagnostics. We found no evidence of small-study bias for measures of effect (dominant, recessive, a/a versus A/A) derived from genotype count data (all cases versus controls) for any of the SNPs. Neither did we detect any small-study bias for the two SNPs (rs1801133, rs1801131) for which more than three studies had been published. For rs1805087, there was weak evidence of a trend with study size toward a protective effect of the 2756G allele in recessive modeling (Egger's test, $P = 0.07$) and in comparing a/a versus A/A genotypes (Egger's test, $P = 0.05$). However, these apparent trends were based on only three studies [Kimura et al. (21), Marchal et al. (27), and Stevens et al. (28)]. The Kimura and Stevens studies (21, 28) reported null findings for this SNP, and the Marchal study (27) reported only that the 2756G allele was more common in advanced cases (χ^2 test, $P = 0.02$). We found no differences in pooled measures of effect from published versus unpublished (genome-wide and ProtecT) studies.

Other Folate-Pathway Genes. Two of the studies identified by systematic review had investigated cystathionine β synthase (*CBS*) polymorphisms in relation to prostate cancer risk: Kimura et al. (21), the *844ins68* polymorphism; and Stevens et al. (28), the *C699T* (rs234706) and *C1261T* (rs1801181) polymorphisms. Neither study found any association with risk of prostate cancer. The ProtecT study genotyped polymorphisms rs492602 in the *FUT2* gene and rs1801198 in the *TCN2* gene, the homozygous G/G variants of which are associated with altered vitamin B12 metabolism (34, 35). In the ProtecT data, there were no associations between these alleles (rs492602 HWE in controls $P = 0.7$; rs1801198 HWE in controls $P = 0.4$) and prostate cancer in dominant modeling [rs492602 odds ratio, 1.06 (95% CI, 0.92-1.22); rs1801198 odds ratio, 1.04 (95% CI, 0.91-1.19)] or in comparing G/G versus A/A genotypes [rs492602 odds ratio, 1.04 (95% CI, 0.87-1.23); rs1801198 odds ratio, 1.15 (95% CI, 0.97-1.37)]. In recessive modeling, *TCN2* rs1801198 showed a positive association [odds ratio, 1.15 (95% CI, 0.99-1.34); $P = 0.06$], and *FUT2* rs492602 showed no association [odds ratio, 0.99 (95% CI, 0.86-1.14)]. There were no differences in effects of these alleles on advanced and localized cancer subgroups. Stevens et al. (28) reported no associations for a further 31 SNPs in 9 one-carbon metabolic pathway genes.

Gene-Gene and Gene-Nutrient Interactions. Four of the included studies assessed haplotype effects on prostate cancer: one study found no pairwise interactions between *MTHFR C677T, MTR A2756G*, and *CBS 844ins68* (21); two studies found no interaction between *MTHFR C677T* and *MTHFR A1298C* (23, 24); and one study found that the *MTR 2756* A/A genotype

Table 4. Meta-analysis results (advanced or localized cases vs controls)

Gene	Variant	SNP	Cases*	*n*	Dominant effect (a/a | a/A) vs AA	Recessive effect aa vs (a/A | A/A)	Homozygous a/a vs A/A	Additive effect a/a, a/A vs A/A (Reference)
					Pooled odds ratio (95% CI)[†]	Pooled odds ratio (95% CI)[†]	Pooled odds ratio (95% CI)[†]	Pooled odds ratio (95% CI)[†]
MTHFR (7 studies; 6,002 controls)	677C>T	rs1801133	Advanced	2,959	1.05 (0.94-1.18)	1.12 (0.94-1.33)	1.14 (0.98-1.33)	1.05 (0.98-1.13)
			Localized	3,763	1.06 (0.95-1.18)	0.99 (0.69-1.41)[‡]	1.03 (0.73-1.44)[§]	1.03 (0.93-1.15)[‖]
			Test for heterogeneity		$P = 0.8$	$P = 0.4$	$P = 0.4$	$P = 0.8$
MTHFR (4 studies; 3,196 controls)	1298A>C	rs1801131	Advanced	1,053	0.97 (0.84-1.12)	0.97 (0.77-1.23)	0.96 (0.76-1.23)	0.98 (0.88-1.09)
			Localized	1,694	0.94 (0.84-1.06)	0.93 (0.77-1.14)	0.92 (0.74-1.13)	0.95 (0.87-1.04)
			Test for heterogeneity		$P = 0.8$	$P = 0.8$	$P = 0.8$	$P = 0.7$
MTR (5 studies; 4,414 controls)	2756A>G	rs1805087	Advanced	1,780	1.02 (0.91-1.15)	0.90 (0.67-1.20)	0.91 (0.68-1.22)	1.00 (0.91-1.11)
			Localized	2,187	1.16 (1.04-1.29)	0.97 (0.73-1.28)	1.02 (0.76-1.37)	1.10 (1.01-1.21)
			Test for heterogeneity		$P = 0.1$	$P = 0.7$	$P = 0.6$	$P = 0.2$
MTRR (3 studies; 3,115 controls)	66A>G	rs1801394	Advanced	1,028	0.98 (0.84-1.14)	1.11 (0.93-1.32)	1.07 (0.87-1.31)	1.01 (0.91-1.11)
			Localized	1,633	1.02 (0.90-1.16)	1.01 (0.87-1.17)	1.02 (0.86-1.22)	1.01 (0.94-1.08)
			Test for heterogeneity		$P = 0.7$	$P = 0.4$	$P = 0.8$	$P = 1.0$
MTHFD1 (3 studies; 4,053 controls)	1958G>A	rs2236225	Advanced	1,648	0.97 (0.86-1.10)	0.99 (0.80-1.21)	0.97 (0.80-1.17)	0.98 (0.91-1.07)
			Localized	2,022	1.03 (0.84-1.28)[¶]	1.04 (0.91-1.18)	1.03 (0.89-1.21)	1.02 (0.93-1.12)
			Test for heterogeneity		$P = 0.7$	$P = 0.6$	$P = 0.6$	$P = 0.6$
SLC19A1 (2 studies; 2,937 controls)	80G>A	rs1051266	Advanced	1,185	0.96 (0.83-1.11)	0.99 (0.81-1.21)	0.96 (0.75-1.24)	0.98 (0.87-1.10)
			Localized	1,374	1.01 (0.88-1.17)	1.05 (0.89-1.23)	1.05 (0.87-1.26)	1.02 (0.93-1.12)
			Test for heterogeneity		$P = 0.6$	$P = 0.7$	$P = 0.6$	$P = 0.5$
SHMT1 (2 studies; 4,110 controls)	1420C>T	rs1979277	Advanced	960	1.11 (0.96-1.28)	0.99 (0.78-1.25)	1.05 (0.82-1.34)	1.06 (0.95-1.18)
			Localized	1,540	1.10 (0.98-1.24)	1.20 (1.00-1.45)	1.24 (1.02-1.51)	1.10 (1.01-1.20)
			Test for heterogeneity		$P = 0.9$	$P = 0.2$	$P = 0.3$	$P = 0.6$
FOLH1 (2 studies; 2,922 controls)	1561T>C	rs202676	Advanced	1,180	0.95 (0.83-1.10)	1.08 (0.80-1.47)	1.06 (0.75-1.50)	0.98 (0.85-1.13)
			Localized	1,361	1.01 (0.84-1.22)	0.90 (0.66-1.22)	0.91 (0.67-1.25)	1.00 (0.90-1.12)
			Test for heterogeneity		$P = 0.5$	$P = 0.4$	$P = 0.5$	$P = 0.7$

*See Supplementary Table S2 for definitions of "advanced" case within each study.
[†]DerSimonian and Laird random effects model (DerSimonian et al., 1986).
[‡]Q statistic $P < 0.001$; $I^2 = 78\%$.
[§]Q statistic $P = 0.001$; $I^2 = 74\%$.
[‖]Q statistic $P = 0.04$; $I^2 = 54\%$.
[¶]Q statistic $P = 0.05$; $I^2 = 67\%$.

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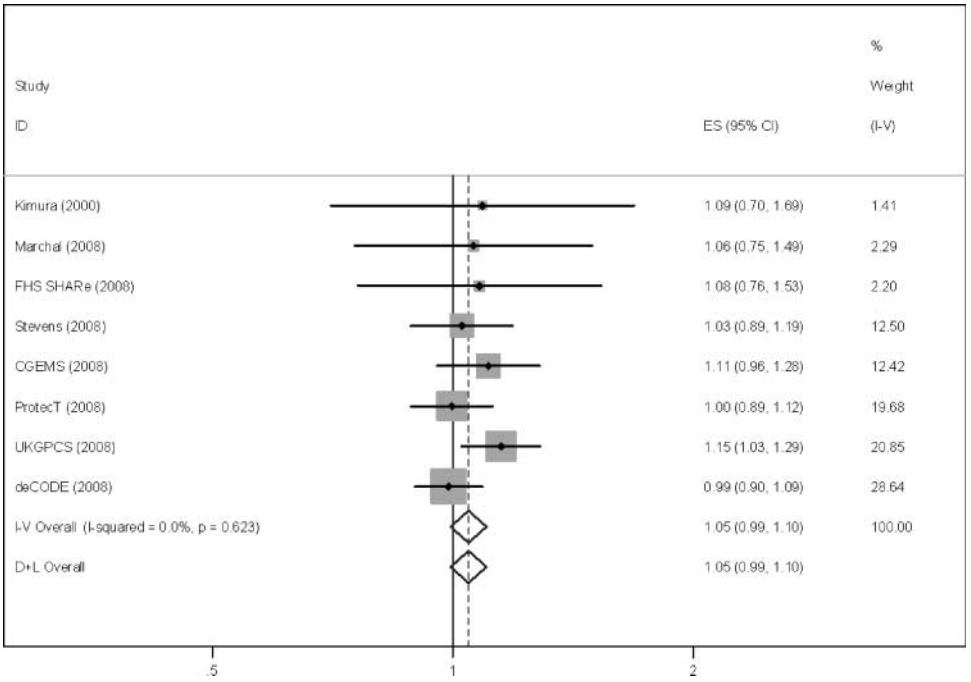

Figure 3. Association of *MTR A2756G* with risk of prostate cancer: additive model comparing G/G, A/G versus A/A genotypes [*ES,* effect size (per allele odds ratio); *I-V overall,* inverse-variance fixed effects estimate; *D+L overall,* DerSimonian and Laird random effects estimate].

strengthened slightly the protective effect of the *MTHFR 677* T/T genotype, but this was reported without a statistical test of interaction (27). Only one of the included studies assessed modification of SNP genotype effects on prostate cancer by nutrients, reporting an increased risk of prostate cancer associated with the highest versus lowest plasma folate levels in men with the *MTHFR 677* C/T or T/T genotypes but not in men with the C/C genotype; no interactions with plasma levels of homocysteine or B12 were found (24).

Discussion

Our meta-analysis suggests that the methionine synthase (*MTR*) *A2756G* and serine hydroxymethlytransferase-1

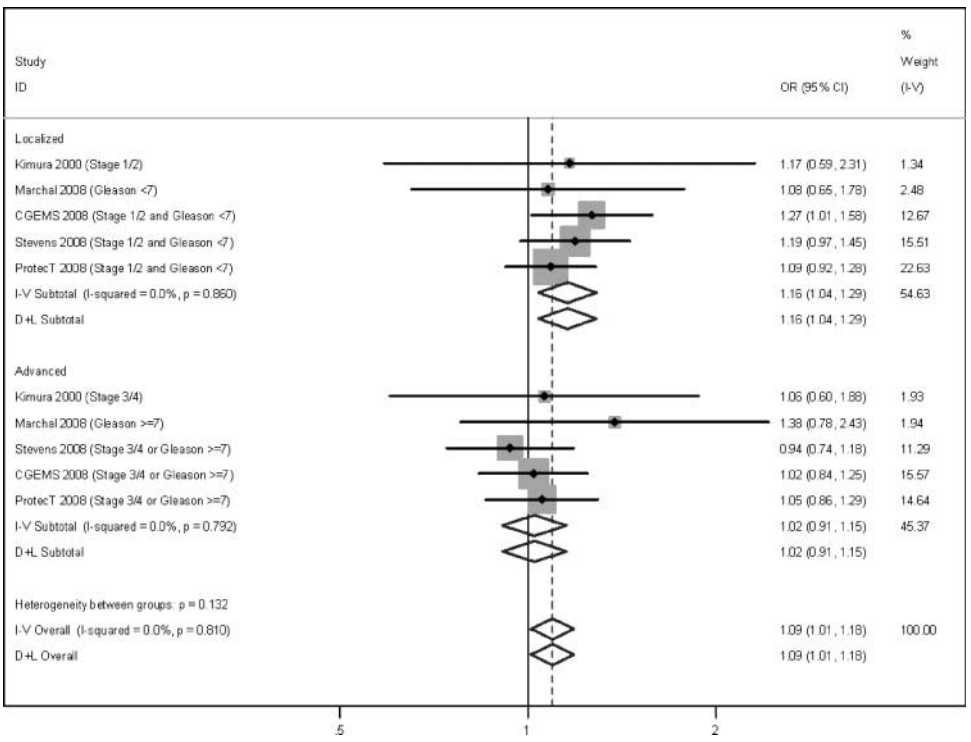

Figure 4. Association of *MTR A2756G* with risk of localized and advanced prostate cancer: dominant model comparing (G/G and A/G) versus A/A genotypes (*I-V,* inverse-variance fixed effects estimate; *D+L,* DerSimonian and Laird random effects estimate].

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(*SHMT1*) *C1420T* single-nucleotide polymorphisms are associated with increased risk of prostate cancer. The *MTR 2756G* allele was associated with 6% higher odds of prostate cancer [eight studies, pooled odds ratio, 1.06 (1.00-1.12); $P = 0.06$] and 16% higher odds of localized prostate cancer [five studies; pooled odds ratio, 1.16 (1.04-1.29); $P = 0.008$] in a dominant model (comparing G/A and G/G genotypes with the A/A genotype). The *SHMT1 1420T* allele was associated with 11% higher odds of prostate cancer [two studies; pooled odds ratio, 1.11 (1.00-1.22); $P = 0.05$], also in a dominant model. This allele was also associated with 20% higher odds of localized prostate cancer in a recessive model [two studies; pooled odds ratio, 1.20 (1.00-1.45); $P = 0.05$) and with 24% higher odds of localized prostate cancer in a model comparing T/T versus C/C genotypes [two studies; pooled odds ratio, 1.24 (1.02-1.51); $P = 0.03$]. We found no convincing evidence that other known folate-pathway polymorphisms, including *MTHFR C677T*, were associated with prostate cancer risk.

***MTR A2756G* (rs1805087).** Methionine synthase (*MTR*) catalyzes the demethylation of 5-methyl-tetrahydrofolate to tetrahydrofolate and the remethylation (using the methyl group donated by 5-methyl-tetrahydrofolate) of homocysteine to methionine (Fig. 1). *MTR* is therefore a key enzyme linking the folate and methionine cycles within the one-carbon metabolic pathway (36). Vitamin B12 (cobalamin) is an essential cofactor of *MTR*, acting in its methyl-cobalamin(III) form as an intermediate carrier of the donated methyl group. After transferring this methyl group to homocysteine, the resultant cobalamin (I) form is either remethylated by *MTR* or oxidized to an inactive cobalamin (II) form (37). Cobalamin (II) is remethylated to the active methyl-cobalamin(III) form by the enzyme methionine synthase reductase (*MTRR*) using a methyl group donated by S-adenosylmethionine (AdoMet). The *A2756G* polymorphism occurs in the activation domain of *MTR* (containing the binding site for AdoMet) in close proximity to the adjacent cobalamin-binding domain (38).

Evidence from dietary and blood-level studies suggests that B12 is positively associated with risk of prostate cancer (8, 10, 13, 14). Our results would be consistent with this evidence if the *MTR A2756G* polymorphism were to encode an enzyme whose activity was equivalent to elevated intake or plasma levels of B12. Given that the polymorphism occurs in the activation domain of *MTR*, and has been associated with depleted levels of homocysteine (39-43), this "activating polymorphism" hypothesis is not implausible (39). In a rat model, deficient B12 intake was associated with low *MTR* activity, and these factors together were associated with DNA hypomethylation (44). It is conceivable that high B12 or hyperactive *MTR* could be associated with DNA hypermethylation, hence increased prostate cancer risk through methylation of tumor suppressor genes (6).

Evidence of associations of *MTR A2756G* with other cancers is inconsistent. Reduced risk of colorectal (45-47) and breast (48) cancer, and reduced (49, 50) or increased (51-53) risk of hematopoietic malignancies have been reported, but the majority of studies have reported no associations.

Some of the studies that investigated associations between *MTR A2756G* and cancer or adenoma risk reported interactions with intakes of folate, methionine and B vitamins (including B12; refs. 46, 48, 54, 55), and alcohol (45, 46, 55), and with other folate-pathway gene polymorphisms (46, 49, 55-58). With regard to interactions with B12 status, one study reported increased risk of colorectal cancer associated with the *MTR A2756G* polymorphism in men with the lowest versus highest B12 intakes (54), another a protective effect of the *MTR A2756G* polymorphism against breast cancer only in women with the highest B12 intakes (48).

Our finding (from a meta-analysis of data from 7,810 cases and 37,543 controls in eight studies) of an increased risk of prostate cancer associated with the *MTR A2756G* polymorphism warrants further research, to include investigations of phenotypic effects (particularly on plasma homocysteine and B12), haplotypic effects, and interactions with dietary intakes of folate, B vitamins, and alcohol.

***SHMT1 C1420T* (rs1979277).** Our finding that the serine hydroxymethyltransferase-1 (*SHMT1*) *C1420T* polymorphism is associated with increased risk of prostate cancer should be interpreted with some caution because our meta-analysis included only two studies. *SHMT1* catalyzes the conversion of tetrahydrofolate to 5,10-methylenetetrahydrofolate and serine to glycine, a reversible reaction that requires vitamin B6 as a cofactor (Fig. 1). *SHMT1* exists in the cytoplasm; an isoenzyme *SHMT*2 catalyzes the same reactions in the mitochondrion. The *SHMT1 1420T* allele was associated with elevated plasma and RBC levels of folate in one (59) but not another study (60). In studies of associations with cancer risk, the *SHMT1 1420T* allele was reported to be associated with reduced risk of acute lymphocytic leukemia (58) and malignant lymphoma (61), but null findings have been reported for colorectal (60), bladder (62), breast (48), lung (63), and ovarian cancers (64). In the absence of a phenotypic effect of *SHMT1 C1420T*, and given the generally null findings of dietary and blood level studies with regard to vitamin B6 (8, 9, 11, 13), our result could be attributable to chance. However, haplotypic approaches to analysis of *SHMT* gene-cancer associations have proven more fruitful (63, 64), and could be justified on the basis of our finding.

***MTHFR C677T* and other Folate-Pathway Gene Polymorphisms.** That we found no associations of other folate-pathway gene polymorphisms with prostate cancer is consistent with the predominantly null findings from dietary and blood-level studies. Our null result for *MTHFR C677T* (based on a meta-analysis of 10,745 cases and 40,158 controls from 12 studies) is an important finding, given the considerable research interest in this important functional polymorphism and its associations with colorectal and gastric cancer (2). Our findings suggest that the aberrant DNA methylation implicated by epigenetic studies of prostate cancer cells is not caused primarily by genetically determined enzymatic defects in the folate metabolic pathway, and may differ from the methylation processes postulated for colorectal and gastric carcinogenesis. That we found no differential effects of folate-pathway gene polymorphisms on localized versus advanced prostate cancer suggests that the role, if any, of the folate metabolic pathway in prostatic carcinogenesis does not change as the cancer progresses. The localized versus advanced subgroup results for *MTR A2756G* and *SHMT1 C1420T* should be interpreted with some caution, given that the large deCODE study did not contribute data for *MTR A2756G* and the result for *SHMT1*

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C1420T was based on only two studies. In neither case was there statistical evidence of heterogeneity between the subgroups.

Limitations. The included study groups were almost entirely Caucasian with little or no population variation in minor allele frequency; hence, subgroup meta-analyses by race were not possible. The only study that had a significant proportion (33%) of Black men found no difference in the effects of *MTHFR C677T* or *A1298C* by race, but the study was small (n = 123; ref. 23). We made no correction for the known relatedness of individuals in the deCODE study population, but a sensitivity analysis in the additive effects model showed that this correction would make no difference to our pooled measures of effect (due to the small inflation factor, large study size, and null findings from the deCODE study). The large size of the control group in the deCODE study would also have contributed to the departure from HWE for two SNPs in this study. These deviations would have had little or no effect on the null findings from this study for these two SNPs. The test for heterogeneity between subgroups may be invalid when there is substantial heterogeneity within subgroups, as observed within advanced and localized subgroups for some of the SNPs (Table 4). Gene effects may have been underestimated in studies that used patients with BPH as controls, but these control groups were used only by two of the smaller studies (23, 27). Only one of the included studies investigated interactions with dietary factors (24), a mechanism of effect modification that has been shown to be of importance in studies of other cancers. It is also possible that unknown folate-pathway polymorphisms could affect prostate cancer risk, and a tag SNP approach may yet yield evidence for the involvement of folate-pathway genetic variation in susceptibility to prostate cancer.

Our meta-analyses were based on very large numbers of prostate cancer cases and controls, and the inclusion of unpublished data from genome-wide association studies protected against publication bias. The observed or hypothetical phenotypic effects of folate-pathway single-nucleotide gene polymorphisms readily accommodate the two principal mechanisms postulated for a role of one-carbon metabolism in carcinogenesis, namely disturbances to DNA synthesis and methylation. Our study revealed potential effects of methionine synthase and serine hydroxymethyltransferase polymorphisms, the former consistent with observations that vitamin B12 may have a role in prostatic carcinogenesis. However, convincing evidence of associations of folate-pathway gene polymorphisms with prostate cancer has yet to emerge, suggesting either that different approaches, e.g., haplotype analyses for nonfunctional variants, are required, or that genetic variation in the folate metabolic pathway is not a detectable determinant of prostate cancer risk.

Disclosure of Potential Conflicts of Interest

Dr. R. Eeles receives educational support from Illumina Inc. and from Tepnel Life Sciences PLC. The other authors disclosed no potential conflicts of interest.

[11] http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000007.v6.p3 accessed 10th July 2009.
[12] http://cgems.cancer.gov/data/ accessed 10th July 2009.

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Correction: Association of Folate-Pathway Gene Polymorphisms with the Risk of Prostate Cancer: a Population-Based Nested Case-Control Study, Systematic Review, and Meta-analysis

In this article (1), which was published in the September 2009 issue of *Cancer Epidemiology, Biomarkers & Prevention*, the FOLH1 SNP rs202676 was referred to incorrectly as FOLH1 "1561T>C." The correct designation is as follows:

rs202676 occurs in exon 2 of FOLH1/GCPII (referred to as 223T>C or 484T>C), corresponding to amino acid substitution Y75H. The 1561C>T polymorphism (also referred to as 1684C>T) occurs in exon 13 of FOLH1, is identified as rs61886492, and corresponds to amino acid substitution H475Y.

Reference

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