

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

Simon M. Collin,¹ Chris Metcalfe,¹ Luisa Zuccolo,^{1,2} Sarah J. Lewis,^{1,2} Lina Chen,^{1,2} Angela Cox,³ Michael Davis,¹ J. Athene Lane,¹ Jenny Donovan,¹ George Davey Smith,² David E. Neal,⁴ Freddie C. Hamdy,⁶ Julius Gudmundsson,⁷ Patrick Sulem,⁷ Thorunn Rafnar,⁷ Kristrun R. Benediktsdottir,⁸ Rosalind A. Eeles,^{9,10} Michelle Guy,⁹ Zsafia Kote-Jarai,⁹ UK Genetic Prostate Cancer Study Group,^{9,10} Jonathan Morrison,⁵ Ali Amin Al Olama,⁵ Kari Stefansson,⁷ Douglas F. Easton,⁵ and Richard M. Martin^{1,2}

¹Department of Social Medicine, University of Bristol, Canynge Hall and ²MRC Centre for Causal Analyses in Translational Epidemiology, Department of Social Medicine, University of Bristol, Oakfield House, Oakfield Grove, Bristol, United Kingdom; ³Institute for Cancer Studies, University of Sheffield, G Floor Medical School, Beech Hill Road, Sheffield, United Kingdom; ⁴Department of Oncology, University of Cambridge, Addenbrooke's Hospital and ⁵Cancer Research UK Genetic Epidemiology Unit, University of Cambridge, Strangeways Laboratory, Worts Causeway, Cambridge, United Kingdom; ⁶Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Headley Way, Headington, Oxford, United Kingdom; ⁷deCODE genetics and ⁸Department of Pathology, Landspítali-University Hospital, 101 Reykjavik, Iceland; ⁹The Institute of Cancer Research and ¹⁰The Royal Marsden NHS Foundation Trust, Sutton, Surrey, United Kingdom

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 tumorigenesis (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

Received 3/9/09; revised 6/9/09; accepted 7/9/09; published OnlineFirst 8/25/09.

Grant support: World Cancer Research Fund UK (grant numbers 2004/18 and 2007/07), with additional support from the National Cancer Research Institute (administered by the Medical Research Council), which provided funding through the Prostate Mechanisms of Progression and Treatment collaborative. The Prostate Testing for Cancer and Treatment (ProtecT) study is funded by the UK National Institute for Health Research Health Technology Assessment Programme (projects 96/20/06, 96/20/99). Funding for the ProtecT biorepository in Cambridge is provided by National Institute for Health Research through the Biomedical Research Centre. DNA extraction in ProtecT was supported by U.S. Department of Defense award W81XWH-04-1-0280 and Yorkshire Cancer Research.

Note: Supplementary data for this article are available at Cancer Epidemiology Biomarkers and Prevention Online (<http://cebp.aacrjournals.org/>).

A. Cox, M. Davis, J.A. Lane, J. Donovan, G.D. Smith, D.E. Neal, F.C. Hamdy, J. Gudmundsson, P. Sulem, T. Rafnar, K.R. Benediktsdottir, R.A. Eeles, M. Guy, Z. Kote-Jarai, J. Morrison, A.A. Al Olama, K. Stefansson, and D.F. Easton contributed equally to this work. The list of names of the members of the UK Genetic Prostate Cancer Study Group is available upon request.

Requests for reprints: Simon M. Collin, University of Bristol, Canynge Hall, 39 Whatley Road, Bristol, BS8 2PS United Kingdom. Phone: 0117-3313934; Fax: 0117-9287292. E-mail: simon.collin@bristol.ac.uk

Copyright © 2009 American Association for Cancer Research.

doi:10.1158/1055-9965.EPI-09-0223

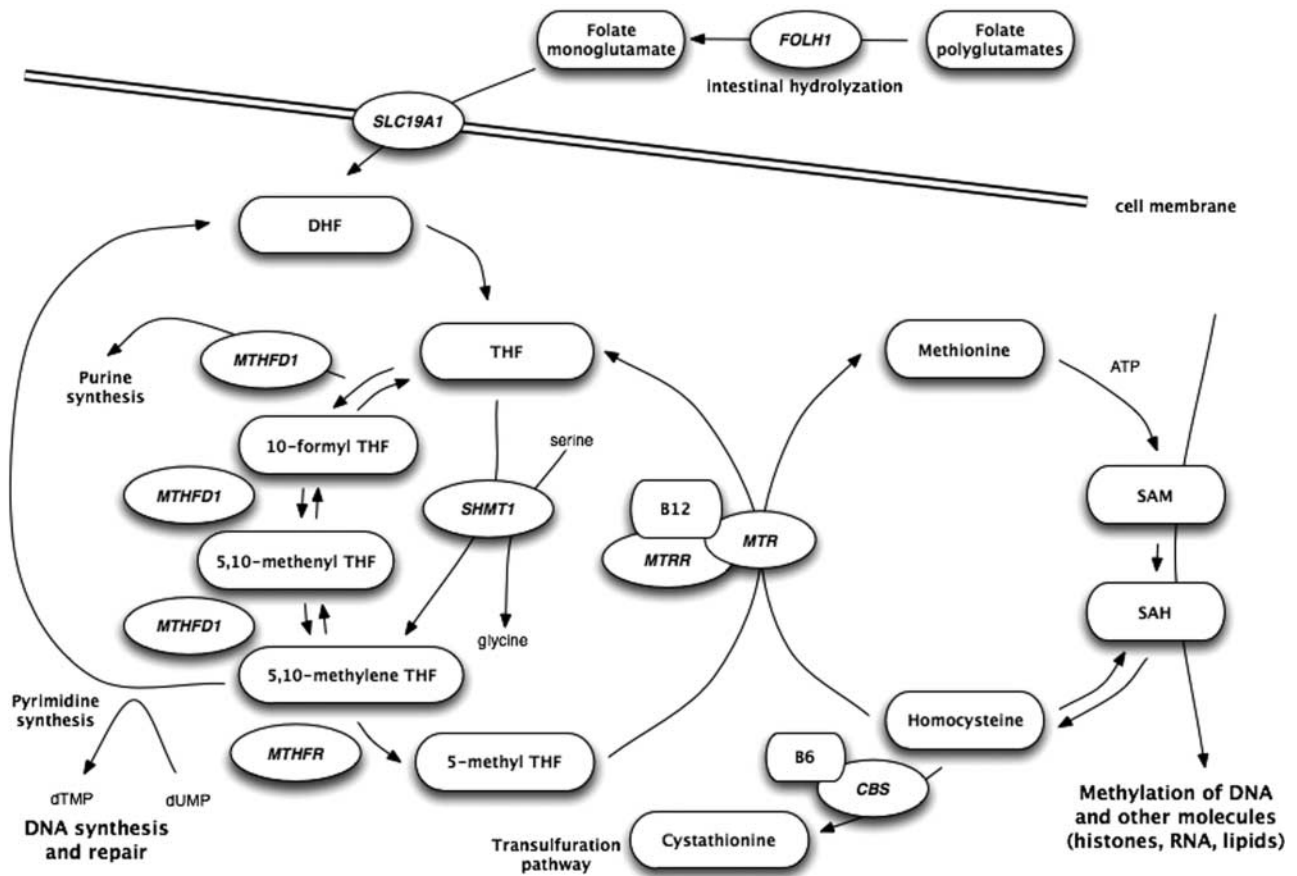


Figure 1. Simplified overview of folate-mediated one-carbon metabolism, highlighting enzymes with polymorphisms investigated in this study. Ovals, enzymes; oblongs, substrates; CBS, cystathionine beta synthase; DHF, dihydrofolate; FOLH1, folate hydrolase 1; MTHFD1, methylene tetrahydrofolate dehydrogenase; 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., "methylene tetrahydrofolate 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

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 $\geq 7/8$) 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-Specific 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 T₁-T₂, N_x, M₀; advanced prostate cancer was defined as positive biopsy, clinical stage T₃-T₄ or N₁ or M₁. 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 Teplnel 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 $\geq 7/8$); 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 I² 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 Off-spring 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.

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)*	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)				
MTR	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,

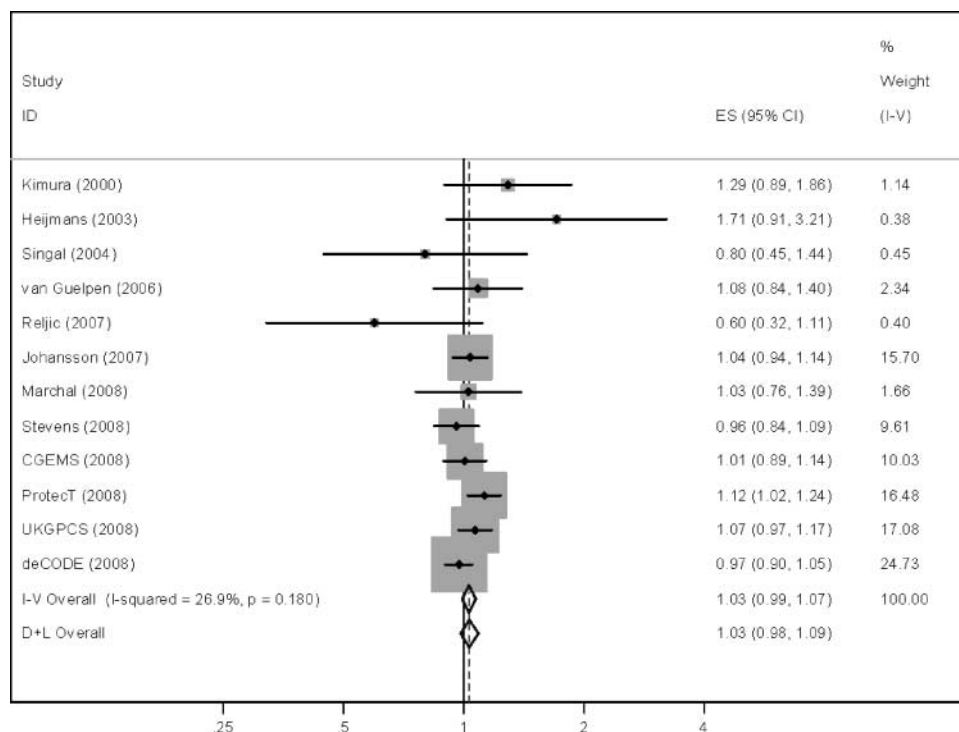


Figure 2. Association of *MTHFR* C677T with risk of prostate cancer: additive model comparing T/T, C/T versus C/C genotypes [ES, effect size (per allele odds ratio); I-V overall, inverse-variance fixed effects estimate; D+L overall, DerSimonian and Laird random effects estimate].

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) [†]
<i>MTHFR</i> (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		<i>P</i> = 0.8	<i>P</i> = 0.4	<i>P</i> = 0.4	<i>P</i> = 0.8
<i>MTHFR</i> (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		<i>P</i> = 0.8	<i>P</i> = 0.8	<i>P</i> = 0.8	<i>P</i> = 0.7
<i>MTR</i> (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		<i>P</i> = 0.1	<i>P</i> = 0.7	<i>P</i> = 0.6	<i>P</i> = 0.2
<i>MTRR</i> (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		<i>P</i> = 0.7	<i>P</i> = 0.4	<i>P</i> = 0.8	<i>P</i> = 1.0
<i>MTHFD1</i> (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		<i>P</i> = 0.7	<i>P</i> = 0.6	<i>P</i> = 0.6	<i>P</i> = 0.6
<i>SLC19A1</i> (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		<i>P</i> = 0.6	<i>P</i> = 0.7	<i>P</i> = 0.6	<i>P</i> = 0.5
<i>SHMT1</i> (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		<i>P</i> = 0.9	<i>P</i> = 0.2	<i>P</i> = 0.3	<i>P</i> = 0.6
<i>FOLH1</i> (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		<i>P</i> = 0.5	<i>P</i> = 0.4	<i>P</i> = 0.5	<i>P</i> = 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%.

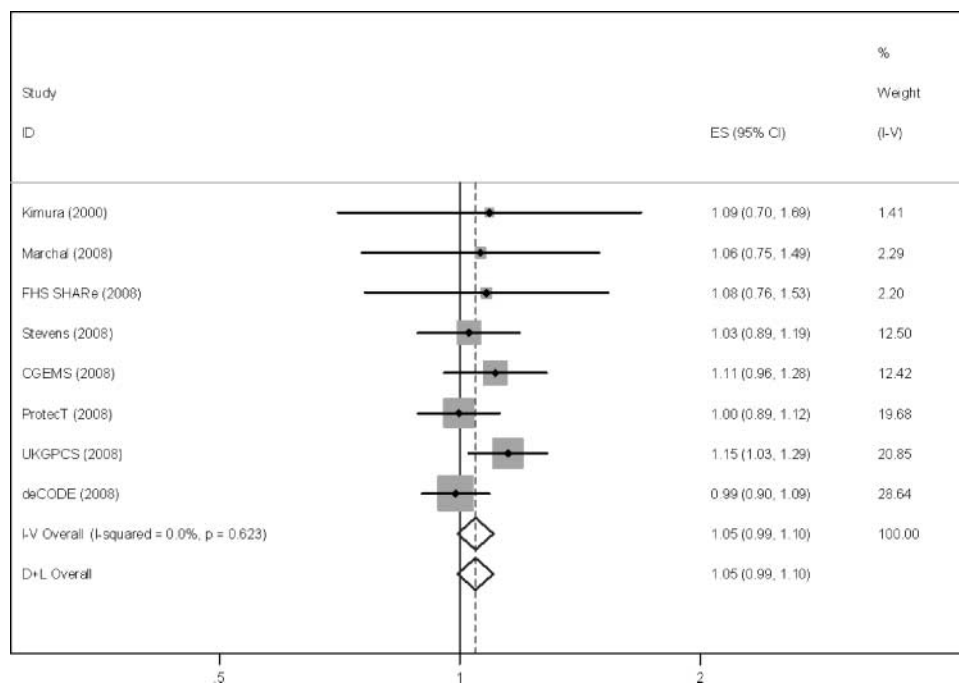


Figure 3. Association of *MTR* A2756G with risk of prostate cancer: additive model comparing G/G, A/G versus A/A genotypes [ES, effect size (per allele odds ratio); I-V overall, inverse-variance fixed effects estimate; D+L overall, DerSimonian and Laird random effects estimate].

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 hydroxymethyltransferase-1

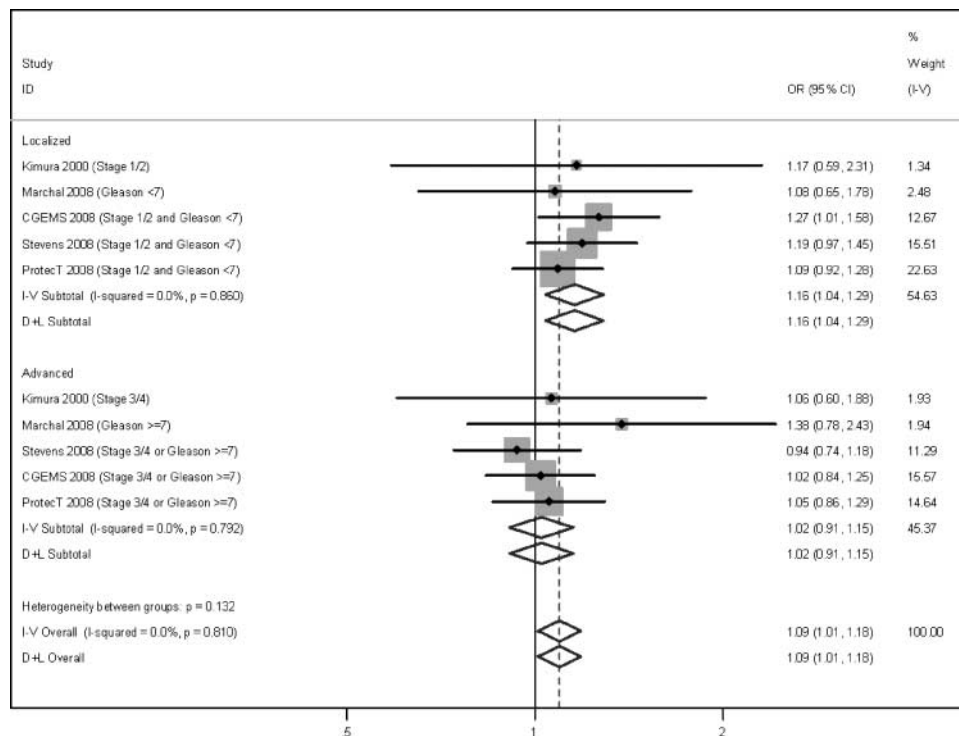


Figure 4. Association of *MTR* A2756G with risk of localized and advanced prostate cancer: dominant model comparing (G/G and A/G) versus A/A genotypes (I-V, inverse-variance fixed effects estimate; D+L, DerSimonian and Laird random effects estimate).

(*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 *SHMT2* 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*

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.

Acknowledgments

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Protect study: We thank the tremendous contribution of all members of the Protect study research group, and especially the following who were involved in this research: Prasad Bollina, Sue Bonnington, Debbie Cooper, Andrew Doble, Alan Doherty, Emma Elliott, David Gillatt, Pippa Herbert, Peter Holding, Joanne Howson, Liz Down, Mandy Jones, Roger Kockelbergh, Howard Kynaston, Teresa Lennon, Norma Lyons, Hilary Moody, Philip Powell, Stephen Prescott, Liz Salter, and Pauline Thompson.

UKGPCS: The UK Genetic Prostate Cancer Study Group includes Lynne O'Brien, Rosemary Wilkinson, Amanda Hall, and Emma Sawyer. Rosalind Eeles is supported by a National Institute for Health Research grant to the Biomedical Research Centre at the Institute of Cancer Research and Royal Marsden NHS Foundation Trust. This work was supported by Cancer Research UK Grant C5047/A3354. Douglas Easton is a Principal Research Fellow of Cancer Research UK. We thank the following for funding support: The Institute of Cancer Research and The Everyman Campaign, The Prostate Cancer Research Foundation, Prostate Research Campaign UK, The National Cancer Research Network UK, The National Cancer Research Institute UK. We thank the collaboration of the UK Genetic Prostate Cancer Study Collaborators and The British Association of Urological Surgeons' Section of Oncology.

Framingham Heart Study: We thank Shih-Jen Hwang [The National Heart, Lung, and Blood Institute (NHLBI) Framingham Heart Study (FHS), Framingham, MA, and NHLBI Bethesda, MD], Joanne M Murabito (NHLBI's FHS, Framingham, MA, and Department of Medicine, Boston University School of Medicine, Boston, MA), and Carol L Rosenberg (Department of Medicine, Boston University School of Medicine, Boston, MA), for data from the FHS (NHLBI contract numbers N01-HC-25195, N02-HL-6-4278), and FHS investigators participating in the SNP Health Association Resource (SHARe)¹¹ project for resource development. We thank the FHS participants for their ongoing participation and dedication to that study.

Others: We thank the CGEMS project for making their results available online to registered users,¹² and to Dr. Victoria Stevens (American Cancer Society, Atlanta, GA) for providing additional data.

References

- Choi SW, Friso S. Interaction between folate and methylene-tetrahydrofolate reductase gene in cancer. In: Choi SW, Friso S, editors. *Nutrient-Gene Interactions in Cancer*. CRC Press; 2006. p. 57-74.
- Dong LM, Potter JD, White E, et al. Genetic susceptibility to cancer: the role of polymorphisms in candidate genes. *JAMA* 2008;299:2423-36.
- Ulrich CM. Genetic variability in folate-mediated one-carbon metabolism and cancer risk. In: Choi SW, Friso S, editors. *Nutrient-Gene Interactions in Cancer*. CRC Press; 2006. p. 75-91.
- Choi SW, Mason JB. Folate and carcinogenesis: an integrated scheme. *J Nutr* 2000;130:129-32.
- Dobosy JR, Roberts JL, Fu VX, Jarrard DF. The expanding role of epigenetics in the development, diagnosis and treatment of prostate cancer and benign prostatic hyperplasia. *J Urol* 2007;177:822-31.
- Murphy TM, Perry AS, Lawler M. The emergence of DNA methylation as a key modulator of aberrant cell death in prostate cancer. *Endocr Relat Cancer* 2008;15:11-25.
- Kim YI. Role of folate in colon cancer development and progression. *J Nutr* 2003;133:3731-95.
- Vlajinac HD, Marinkovic JM, Ilic MD, Kocev NI. Diet and prostate cancer: a case-control study. *Eur J Cancer* 1997;33:101-7.
- Weinstein SJ, Hartman TJ, Stolzenberg-Solomon R, et al. Null association between prostate cancer and serum folate, vitamin B(6), vitamin B(12), and homocysteine. *Cancer Epidemiol Biomarkers Prev* 2003;12:1271-2.
- Huldtin J, Van GB, Bergh A, Hallmans G, Stattin P. Plasma folate, vitamin B12, and homocysteine and prostate cancer risk: a prospective study. *Int J Cancer* 2005;113:819-24.
- Pelucchi C, Galeone C, Talamini R, et al. Dietary folate and risk of prostate cancer in Italy. *Cancer Epidemiol Biomarkers Prev* 2005;14:944-8.

12. Stevens VL, Rodriguez C, Pavluck AL, et al. Folate nutrition and prostate cancer incidence in a large cohort of US men. *Am J Epidemiol* 2006;163:989–96.
13. Weinstein SJ, Stolzenberg-Solomon R, Pietinen P, et al. Dietary factors of one-carbon metabolism and prostate cancer risk. *Am J Clin Nutr* 2006;84:929–35.
14. Johansson M, Appleby PN, Allen NE, et al. Circulating concentrations of folate and vitamin B12 in relation to prostate cancer risk: results from the European prospective investigation into cancer and nutrition study. *Cancer Epidemiol Biomarkers Prev* 2008;17:279–85.
15. Lu QY, Hung JC, Heber D, et al. Inverse associations between plasma lycopene and other carotenoids and prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2001;10:749–56.
16. McCann SE, Ambrosone CB, Moysich KB, et al. Intakes of selected nutrients, foods, and phytochemicals and prostate cancer risk in western New York. *Nutr Cancer* 2005;53:33–41.
17. Figueiredo JC, Grau MV, Haile RW, et al. Folic acid and risk of prostate cancer: results from a randomized clinical trial. *J Natl Cancer Inst* 2009;101:432–5.
18. Rossi E, Hung J, Beilby JP, et al. Folate levels and cancer morbidity and mortality: prospective cohort study from Busselton, Western Australia. *Ann Epidemiol* 2006;16:206–12.
19. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;7:177–88.
20. Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;315:629–34.
21. Kimura F, Franke KH, Steinhoff C, et al. Methyl group metabolism gene polymorphisms and susceptibility to prostatic carcinoma. *Prostate* 2000;45:225–31.
22. Heijmans BT, Boer JM, Suchiman HE, et al. A common variant of the methylenetetrahydrofolate reductase gene (1p36) is associated with an increased risk of cancer. *Cancer Res* 2003;63:1249–53.
23. Singal R, Ferdinand L, Das PM, Reis IM, Schlesselman JJ. Polymorphisms in the methylenetetrahydrofolate reductase gene and prostate cancer risk. *Int J Oncol* 2004;25:1465–71.
24. Van Guelpen BR, Wren SM, Bergh AR, et al. Polymorphisms of methylenetetrahydrofolate reductase and the risk of prostate cancer: a nested case-control study. *Eur J Cancer Prev* 2006;15:46–50.
25. Johansson M, Van GB, Hultdin J, et al. The MTHFR 677C → T polymorphism and risk of prostate cancer: results from the CAPS study. *Cancer Causes Control* 2007;18:1169–74.
26. Reljic A, Simundic AM, Topic E, et al. The methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and cancer risk: the Croatian case-control study. *Clin Biochem* 2007;40:981–5.
27. Marchal C, Redondo M, Reyes-Engel A, et al. Association between polymorphisms of folate-metabolizing enzymes and risk of prostate cancer. *Eur J Surg Oncol* 2008;34:805–10.
28. Stevens VL, Rodriguez C, Sun J, et al. No association of single nucleotide polymorphisms in one-carbon metabolism genes with prostate cancer risk. *Cancer Epidemiol Biomarkers Prev* 2008;17:3612–4.
29. Cicek MS, Nock NL, Li L, et al. Relationship between methylenetetrahydrofolate reductase C677T and A1298C genotypes and haplotypes and prostate cancer risk and aggressiveness. *Cancer Epidemiol Biomarkers Prev* 2004;13:1331–6.
30. Amundadottir LT, Sulem P, Gudmundsson J, et al. A common variant associated with prostate cancer in European and African populations. *Nat Genet* 2006;38:652–8.
31. Yeager M, Orr N, Hayes RB, et al. Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. *Nat Genet* 2007;39:645–9.
32. Murabito JM, Rosenberg CL, Finger D, et al. A genome-wide association study of breast and prostate cancer in the NHLBI's Framingham Heart Study. *BMC Med Genet* 2007;8 Suppl 1:S6.
33. Eeles RA, Kote-Jarai Z, Giles GG, et al. Multiple newly identified loci associated with prostate cancer susceptibility. *Nat Genet* 2008;40:316–21.
34. Hazra A, Kraft P, Selhub J, et al. Common variants of FUT2 are associated with plasma vitamin B12 levels. *Nat Genet* 2008;40:1160–2.
35. von Castel-Dunwoody KM, Kauwell GP, Shelnutz KP, et al. Transcobalamin 776C → G polymorphism negatively affects vitamin B-12 metabolism. *Am J Clin Nutr* 2005;81:1436–41.
36. Banerjee RV, Matthews RG. Cobalamin-dependent methionine synthase. *FASEB J* 1990;4:1450–9.
37. Goulding CW, Postigo D, Matthews RG. Cobalamin-dependent methionine synthase is a modular protein with distinct regions for binding homocysteine, methyltetrahydrofolate, cobalamin, and adenosylmethionine. *Biochemistry* 1997;36:8082–91.
38. Matthews RG, Sheppard C, Goulding C. Methylenetetrahydrofolate reductase and methionine synthase: biochemistry and molecular biology. *Eur J Pediatr* 1998;157 Suppl 2:S54–9.
39. Chen J, Stampfer MJ, Ma J, et al. Influence of a methionine synthase (D919G) polymorphism on plasma homocysteine and folate levels and relation to risk of myocardial infarction. *Atherosclerosis* 2001;154:667–72.
40. Fredriksen A, Meyer K, Ueland PM, et al. Large-scale population-based metabolic phenotyping of thirteen genetic polymorphisms related to one-carbon metabolism. *Hum Mutat* 2007;28:856–65.
41. Harmon DL, Shields DC, Woodside JV, et al. Methionine synthase D919G polymorphism is a significant but modest determinant of circulating homocysteine concentrations. *Genet Epidemiol* 1999;17:298–309.
42. Tsai MY, Bignell M, Yang F, et al. Polygenic influence on plasma homocysteine: association of two prevalent mutations, the 844ins68 of cystathionine β-synthase and A(2756)G of methionine synthase, with lowered plasma homocysteine levels. *Atherosclerosis* 2000;149:131–7.
43. Yates Z, Lucock M. Interaction between common folate polymorphisms and B-vitamin nutritional status modulates homocysteine and risk for a thrombotic event. *Mol Genet Metab* 2003;79:201–13.
44. Brunaud L, Alberto JM, Ayav A, et al. Vitamin B12 is a strong determinant of low methionine synthase activity and DNA hypomethylation in gastrectomized rats. *Digestion* 2003;68:133–40.
45. Ma J, Stampfer MJ, Christensen B, et al. A polymorphism of the methionine synthase gene: association with plasma folate, vitamin B12, homocyst(e)ine, and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev* 1999;8:825–9.
46. Yamaji T, Iwasaki M, Sasazuki S, et al. Methionine synthase A2756G polymorphism interacts with alcohol and folate intake to influence the risk of colorectal adenoma. *Cancer Epidemiol Biomarkers Prev* 2009;18:267–74.
47. Ulvik A, Vollset SE, Hansen S, et al. Colorectal cancer and the methylenetetrahydrofolate reductase 677C → T and methionine synthase 2756A → G polymorphisms: a study of 2,168 case-control pairs from the JANUS cohort. *Cancer Epidemiol Biomarkers Prev* 2004;13:2175–80.
48. Lissowska J, Gaudet MM, Brinton LA, et al. Genetic polymorphisms in the one-carbon metabolism pathway and breast cancer risk: a population-based case-control study and meta-analysis. *Int J Cancer* 2007;120:2696–703.
49. Gemmati D, Ongaro A, Scapoli GL, et al. Common gene polymorphisms in the metabolic folate and methylation pathway and the risk of acute lymphoblastic leukemia and non-Hodgkin's lymphoma in adults. *Cancer Epidemiol Biomarkers Prev* 2004;13:787–94.
50. Lincz LF, Scorgie FE, Kerridge I, et al. Methionine synthase genetic polymorphism MS A2756G alters susceptibility to follicular but not diffuse large B-cell non-Hodgkin's lymphoma or multiple myeloma. *Br J Haematol* 2003;120:1051–4.
51. Lima CS, Ortega MM, Ozelo MC, et al. Polymorphisms of methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), and thymidylate synthase (TYMS) in multiple myeloma risk. *Leuk Res* 2008;32:401–5.
52. Matsuo K, Suzuki R, Hamajima N, et al. Association between polymorphisms of folate- and methionine-metabolizing enzymes and susceptibility to malignant lymphoma. *Blood* 2001;97:3205–9.
53. Skibola CF, Forrest MS, Coppede F, et al. Polymorphisms and haplotypes in folate-metabolizing genes and risk of non-Hodgkin lymphoma. *Blood* 2004;104:2155–62.
54. Koushik A, Kraft P, Fuchs CS, et al. Nonsynonymous polymorphisms in genes in the one-carbon metabolism pathway and associations with colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 2006;15:2408–17.
55. Goode EL, Potter JD, Bigler J, Ulrich CM. Methionine synthase D919G polymorphism, folate metabolism, and colorectal adenoma risk. *Cancer Epidemiol Biomarkers Prev* 2004;13:157–62.
56. Le Marchand L, Donlon T, Hankin JH, et al. Association between polymorphisms of folate-metabolizing genes and risk of non-Hodgkin lymphoma. *Blood* 2004;104:2155–62.
57. Shi Q, Zhang Z, Li G, et al. Polymorphisms of methionine synthase and methionine synthase reductase and risk of lung cancer: a case-control analysis. *Pharmacogenet Genomics* 2005;15:547–55.
58. Skibola CF, Smith MT, Hubbard A, et al. Polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and risk of adult acute lymphocytic leukemia. *Blood* 2002;99:3786–91.
59. Heil SG, Van der Put NM, Waas ET, et al. Is mutated serine hydroxymethyltransferase (SHMT) involved in the etiology of neural tube defects? *Mol Genet Metab* 2001;73:164–72.
60. Chen J, Kyte C, Valcin M, et al. Polymorphisms in the one-carbon metabolic pathway, plasma folate levels and colorectal cancer in a prospective study. *Int J Cancer* 2004;110:617–20.
61. Hishida A, Matsuo K, Hamajima N, et al. Associations between polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and susceptibility to malignant lymphoma. *Haematologica* 2003;88:159–66.
62. Moore LE, Malats N, Rothman N, et al. Polymorphisms in one-carbon metabolism and trans-sulfuration pathway genes and susceptibility to bladder cancer. *Int J Cancer* 2007;120:2452–8.
63. Wang L, Lu J, An J, et al. Polymorphisms of cytosolic serine hydroxymethyltransferase and risk of lung cancer: a case-control analysis. *Lung Cancer* 2007;57:143–51.
64. Kelemen LE, Sellers TA, Schildkraut JM, et al. Genetic variation in the one-carbon transfer pathway and ovarian cancer risk. *Cancer Res* 2008;68:2498–506.

Correction

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/GCP11 (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

1. Collin SM, Metcalfe C, Zuccolo L, Lewis SJ, Chen L, Cox A, Davis M, Lane JA, Donovan J, Smith GD, Neal DE, Hamdy FC, Gudmundsson J, Sulem P, Rafnar T, Benediktsdottir KR, Eeles RA, Guy M, Kote-Jarai Z, UK Genetic Prostate Cancer Study Group, Morrison J, Al Olama AA, Stefansson K, Easton DF, and Martin RM. Association of folate-pathway gene polymorphisms with the risk of prostate cancer: a population-based nested case-control study, systematic review, and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2009;18:2528–39.

©2010 American Association for Cancer Research.
doi: 10.1158/1055-9965.EPI-10-0762

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association
for Cancer Research

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

Simon M. Collin, Chris Metcalfe, Luisa Zuccolo, et al.

Cancer Epidemiol Biomarkers Prev 2009;18:2528-2539. Published OnlineFirst August 25, 2009.

Updated version	Access the most recent version of this article at: doi: 10.1158/1055-9965.EPI-09-0223
Supplementary Material	Access the most recent supplemental material at: http://cebp.aacrjournals.org/content/suppl/2009/08/25/1055-9965.EPI-09-0223.DC1

Cited articles	This article cites 62 articles, 23 of which you can access for free at: http://cebp.aacrjournals.org/content/18/9/2528.full#ref-list-1
Citing articles	This article has been cited by 7 HighWire-hosted articles. Access the articles at: http://cebp.aacrjournals.org/content/18/9/2528.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org .
Permissions	To request permission to re-use all or part of this article, use this link http://cebp.aacrjournals.org/content/18/9/2528 . Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.