No association of the MTHFR gene A1298C polymorphism with the risk of prostate cancer: A meta-analysis

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Abstract. Various studies have demonstrated that the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene polymorphism contributes to the risk of prostate cancer, while other studies have provided conflicting findings. In the present study, we carried out a comprehensive meta-analysis with the aim of determining whether there is a significant association of the MTHFR gene A1298C polymorphism with the susceptibility of prostate cancer. Studies on the MTHFR gene A1298C polymorphism and prostate cancer were retrieved using the electronic PubMed database without any restriction on language through Aug 21, 2011. Data were abstracted by a standardized protocol. Crude odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the strength of association. The analyses were conducted with Review Manager software version 4.2. Nine case-control studies were identified, including 2,723 prostate cancer patients and 3,442 controls. Overall, no significant associations were found between the MTHFR gene A1298C polymorphism and prostate cancer (codominant models: CC vs. AA, OR=1.03, 95% CI 0.79-1.34, P=0.84; AC vs. AA, OR=1.04, 95% CI 0.93-1.16, P=0.46; dominant model: AC + CC vs. AA, OR=1.04, 95% CI 0.94-1.15, P=0.48; recessive model: CC vs. AC + AA, OR=1.02, 95% CI 0.76-1.35, P=0.91; allele model: C vs. A, OR=1.04, 95% CI 0.90-1.19, P=0.61). Similarly, in the subgroup analyses by DNA source, ethnicity, control source, pathological stage and Hardy-Weinberg equilibrium, no significant associations were observed. Our meta-analysis suggests that the MTHFR gene A1298C polymorphism is not associated with the risk of prostate cancer.

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Introduction

Prostate cancer (PC) is the most common malignancy and the second leading cause of cancer-related death in men in industrialized countries (1,2). Its incidence is at a relatively low rate in the Asian population (3), but is increasing rapidly (4). It is supposed that complex elements, such as hormones, age, family history of PC, cultural and enviromental factors and genetic background (3), contribute to the cancerization and progression of PC. However, the specific mechanism remains undetermined.

Folate is indispensably required for DNA synthesis and methylation of DNA and histones. Epidemiological studies have shown an effective association between low folate intake and an increased cancer risk (5,6). MTHFR plays a vital role in the metabolism of folates by irreversibly converting 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate (7), which donates a methyl group for the remethylation of homocysteine to methionine used for DNA synthesis and repair (8). Therefore, MTHFR deficiency may lead to DNA hypomethylation to initialize cancerization and affect the progression of malignant tumors (9,10).

The human MTHFR gene, composed of 11 exons, is located at chromosome 1p36.3, codes cDNA of 2.2-kb in length and produces a protein of 656 amino acids (11). The 1298A>C polymorphism, marked as rs1801131 in the NCBI database, is located at exon 7 and results in a glutamate-to-valine substitution at codon 429 (8). Alterations in genomic bases result in single-nucleotide polymorphisms (SNPs), which may subsequently affect the genetic instability, amino acid sequence and function of protein. Recently, SNPs have been used as a tool for predicting diseases (12) in addition to carcinogenesis (13,14). The MTHFR gene A1298C polymorphism has been implicated in several diseases (15,16), including various types of cancer (17,18), and has been investigated in relation to the risk of PC but with inconclusive results (19-27). Among the nine eligible case-control studies, three considered the MTHFR gene A1298C polymorphism as a genetic marker for PC (19,24,26), while six reported negative associations between the two (20-23,25,27). Hence, we carried out a meta-analysis concerning the association between the MTHFR gene A1298C polymorphism and PC susceptibility by pooling data from the identified studies to obtain a more conclusive estimation.

Materials and methods

Identification of relevant studies. Publications were identified by a systematic electronic search in the PubMed database with the following keywords: 'methylenetetrahydrofolate reductase', 'MTHFR', 'polymorphism', 'variation', 'mutation', 'prostate' and 'prostatic', as well as their combinations. The last search was updated on Aug 21, 2011. We did not set any restriction on the language of the published literature. Additional studies were searched by manually screening references in review articles and original papers.

Inclusion and exclusion criteria. The inclusion criteria used for the article selection in this meta-analysis were as follows: i) case-control study with PC and control groups; ii) study focusing on the association between the MTHFR gene A1298C polymorphism and the susceptibility of PC; iii) frequencies of the various genotypes in the publications were available.

The major exclusion criteria were studies that were duplication of a previous publication, studies without detailed information, or not case-control studies, such as review articles, case reports, editorials, conference abstracts and letters.

Data extraction. Two investigators (D.L. and C.G.) reviewed and extracted the information from all included publications independently by a standardized protocol, according to the inclusion and exclusion criterias. Characteristics, such as year of publication, name of first author, country of origin, ethnicity, source of control group, methods for detecting the MTHFR gene A1298C polymorphism, C allele percentage in controls and frequencies of AA, AC and CC genotypes in the case and control groups, were respectively extracted from the included studies. In the case of disagreement, discrepancies of included studies were resolved by discussion.

Statistical analysis. We predicted the contribution of the MTHFR gene A1298C polymorphism to the risk of PC by adopting the Review Manager software version 4.2 developed by the Cochrane Collaboration. The strength of association was estimated by calculating summary crude odds ratios (ORs) and the corresponding 95% confidence intervals (CIs). We evaluated the risk of the dominant (CC + AC vs. AA), recessive (CC vs. AA + AC), allele (C vs. A) and codominant models (AC vs. AA; CC vs. AA), respectively. Hardy-Weinberg equilibrium (HWE) for the control groups in each study was checked by the goodness-of-fit test. Heterogeneity assumption was assesed by the Chi-square-based Q test and was regarded to be statistically significant at P<0.10. The random-effects model was used when the test of heterogeneity was significant, otherwise the fixed-effects model was applied in the analysis. Potential publication bias was primarily appraised by the funnel plot. An asymmetric plot suggested a possible publication bias. Funnel plot asymmetry was further evaluated by Egger's linear regression and Begger's rank correlation tests with STATA software, version 7.0. All P-values were two-tailed.

Results

Study characteristics. Nine published studies were eligible for this meta-analysis on the association between MTHFR

Table I. Main characteristics of all studies included in the meta-analysis.

Ref.	Year	First author	Method	Country	Ethnicity	Study design	Cases			Controls			Allele C % in controls	HWE	
							AA	AC	CC	AA	AC	CC		χ^2-test	P-value
19	2004	Cicek	PCR-RFLP	USA	Mixed	F	195	205	39	233	201	44	30.23	0.00	0.95
20	2004	Singal	PCR-RFLP	USA	Mixed	H	29	43	9	18	17	7	36.90	0.72	0.40
21	2006	Van Guelpen	TaqMan	Sweden	Caucasian	P	87	108	27	176	203	55	36.06	0.09	0.77
22	2008	Marchal	TaqMan	Spain	Caucasian	H	98	62	17	108	79	22	29.43	1.69	0.19
23	2008	Stevens	TaqMan	USA	Mixed	P	481	518	105	491	493	125	33.50	0.01	0.94
24	2009	Muslumanoglu	PCR-RFLP	Turkey	Caucasian	H	31	16	44	77	45	44	40.06	31.49	0.00
25	2010	Cai	PCR-RFLP	China	Asian	H	150	63	4	144	71	5	18.41	1.21	0.27
27	2010	Wu	PCR-RFLP	China	Asian	P	138	70	10	287	135	14	18.69	0.15	0.70
26	2010	Safarinejad	PCR-RFLP	Iran	Caucasian	P	90	70	14	158	150	40	33.05	0.23	0.63

RFLP, restriction fragment length polymorphism; H, hospital-based case-control study; F, family-based case-control study; P, population-based case-control study; HWE, Hardy-Weinberg equilibrium.

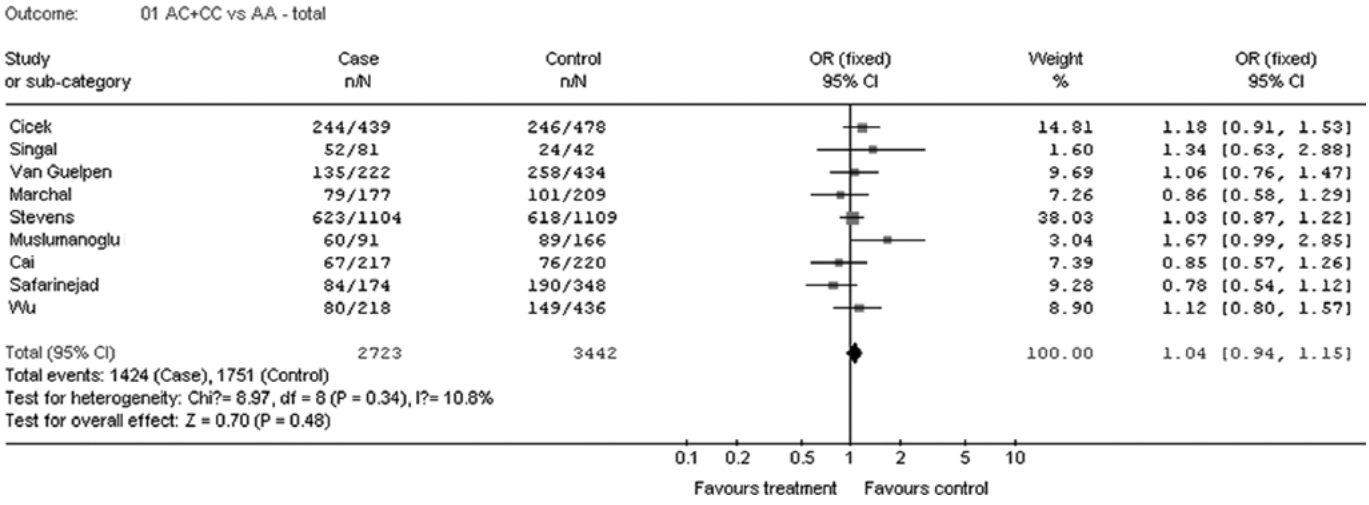

Figure 1. Association of the MTHFR gene A1298C polymorphism with the risk of prostate cancer in the dominant model: AC + CC vs. AA.

gene A1298C polymorphism and PC susceptibility in the electronic PubMed database (19-27). All of the qualified articles were case-control designed studies, consisting of a total of 2,723 PC cases and 3,442 controls. The detailed characteristics of the studies, such as year of publication, name of first author, country of origin, ethnicity, source of control groups, genotyping methods, C allele percentage in controls, HWE and the genotype distribution of the MTHFR gene A1298C polymorphism, are documented in Table I. Among the nine studies, two involved Asian populations (25,27), four involved Caucasian populations (21,22,24,26), and the remaining three were of mixed ethnicities (19,20,23). All studies, but one (24), were consistent with HWE. As to the source of control groups, four were population-based studies (21,23,26,27), four were hospital-based studies (20,22,24,25) and the other one (19) was a family-based study. Only four studies provided data on disease stage, including advanced and localized PC (19,20,22,26). PCR-RFLP was used to distinguish genotype in six studies (19,20,24-27), and TaqMan SNP genotyping assay was chosen for the other three (21-23). All of the research studies made use of DNA samples extracted from peripheral blood cells for genotyping, except one, which employed fixed tissue samples (20). Thus, subgroup analysis was conducted in this meta-analysis.

Test of heterogeneity and main results of the meta-analysis. Significant heterogeneity between studies (P<0.10) was observed in several comparisons, and the data are listed in Table II. The random-effects model (R) was chosen in the analysis when the P-value for the heterogeneity test was <0.10, otherwise the fixed-effects model (F) was applied.

The results of the association between the MTHFR gene A1298C polymorphism and PC risk are also shown in Table II. Overall, when all the qualified studies were pooled into the meta-analysis, no evidence of significant association was found between PC risk and MTHFR gene 1298A>C polymorphism in any genetic model (codominant models: CC vs. AA, OR=1.03, 95% CI 0.79-1.34, P=0.84; AC vs. AA, OR=1.04, 95% CI 0.93-1.16, P=0.46; dominant model: AC + CC vs. AA, OR=1.04, 95% CI 0.94-1.15, P=0.48; recessive model: CC vs. AC + AA, OR=1.02, 95% CI 0.76-1.35, P=0.91; allele model: C vs. A, OR=1.04, 95% CI 0.90-1.19, P=0.61) (Fig. 1).

In the stratified analyses by ethnicity, no significant results were found for Asian and Caucasian subjects in the different statistical models (all P>0.05). Moreover, meta-analyses of studies illustrating advanced and localized PC were conducted, and these analyses again found no significant correlations in any type of statistical model (all P>0.05). Furthermore, insignificant statistical conclusions were found for hospital- and population-based subjects in various statistical models in the subgroup analyses according to source of controls (all P>0.05).

Sensitivity analyses. In the study by Muslumanoglu *et al* (24), genotype frenquencies of the MTHFR gene A1298C polymorphism in the control group deviated from HWE (P=0.00). Sensitivity analyses were carried out by excluding the above study and no evident changes were found for the pooled ORs. Similarly, the pooled ORs were not qualitatively influenced after exclusion of one heavily weighted study by Stevens *et al* (23). Sensitivity analyses suggested that the results of this meta-analysis were stable.

Publication bias. The shape of the funnel plots did not show any evidence of obvious asymmetry for all genetic models in either overall or stratified meta-analyses. Subsequently, Begger's funnel plot, Begger's test and Egger's test were performed to assess the publication bias of the eligible studies. Still, the results did not reveal obvious evidence of publication bias (P=0.767 for Egger's test in the dominant model; Fig. 2).

Discussion

MTHFR is involved in the one-carbon cycle, which is of importance for nucleotide synthesis and methylation of DNA, membranes, proteins and lipids. The MTHFR gene A1298C polymorphism, one of the most popular sites, is associated with a 30% decreased enzymatic activity without thermolability (28,29). Thus, the MTHFR gene A1298C polymorphism is considered to produce the potentially functional site rs1801131, which was extensively studied.

Table II. Main results of the meta-analysis in codominant, dominant, recessive and alleles models.

Genetic models	No. of studies	PC (n)	OR	95% CI	I^2 (%)	P_h	Statistical model	P-value
Codominant models								
AC vs. AA								
Total	9	2,454	1.04	0.93-1.16	0	0.67	F	0.46
PBC DNA	8	2,382	1.03	0.93-1.15	0	0.68	F	0.55
Caucasian	5	612	0.94	0.77-1.16	0	0.68	F	0.59
Asian	2	421	0.97	0.75-1.27	0	0.39	F	0.85
H-based	4	492	0.92	0.71-1.19	0	0.60	F	0.51
P-based	4	1,562	1.04	0.91-1.19	0	0.61	F	0.58
HWE	8	2,407	1.05	0.94-1.17	0	0.59	F	0.42
Advanced PC	4	395	1.12	0.87-1.44	39.9	0.17	F	0.40
Localized PC	4	355	0.95	0.73-1.24	0	0.67	F	0.69
CC vs. AA								
Total	9	1,568	1.03	0.79-1.34	41.7	0.09	R	0.84
PBC DNA	8	1,530	1.04	0.78-1.39	48.4	0.06	R	0.78
Caucasian	5	432	1.01	0.59-1.73	66.4	0.02	R	0.98
Asian	2	302	1.22	0.60-2.49	0	0.41	F	0.58
H-based	4	382	1.18	0.60-2.33	58.9	0.06	R	0.63
P-based	4	952	0.88	0.70-1.10	0	0.41	F	0.26
HWE	8	1,493	0.90	0.74-1.09	0	0.84	F	0.27
Advanced PC	4	233	0.78	0.50-1.20	44.5	0.14	R	0.26
Localized PC	4	242	1.03	0.68-1.54	0	0.89	F	0.90
Dominant model								
CC + AC vs. AA								
Total	9	2,723	1.04	0.94-1.15	10.8	0.34	F	0.48
PBC DNA	8	2,642	1.03	0.93-1.15	19.0	0.28	F	0.53
Caucasian	5	720	0.99	0.82-1.20	36.7	0.18	F	0.94
Asian	2	435	0.99	0.77-1.29	6.8	0.30	F	0.96
H-based	4	566	1.02	0.81-1.30	44.2	0.15	F	0.84
P-based	4	1,718	1.01	0.89-1.15	0	0.48	F	0.88
HWE	8	2,632	1.02	0.92-1.13	0	0.56	F	0.72
Advanced PC	4	431	1.04	0.82-1.33	49.0	0.12	F	0.74
Localized PC	4	400	0.97	0.75-1.24	0	0.86	F	0.79
Recessive model								
CC vs. AA + AC								
Total	9	2,723	1.02	0.76-1.35	53.4	0.03	R	0.91
PBC DNA	8	2,642	1.05	0.77-1.42	57.5	0.02	R	0.77
Caucasian	5	720	1.00	0.57-1.76	73.5	0.01	R	0.99
Asian	2	435	1.22	0.60-2.47	0	0.46	F	0.58
H-based	4	566	1.15	0.55-2.41	68.7	0.02	R	0.71
P-based	4	1,718	0.86	0.69-1.07	0	0.51	F	0.17
HWE	8	2,632	0.87	0.73-1.04	0	0.89	F	0.14
Advanced PC	4	431	0.71	0.47-1.09	32.4	0.22	F	0.12
Localized PC	4	400	1.05	0.71-1.56	0	0.74	F	0.80
Allele model								
C vs. A								
Total	9	5,446	1.04	0.90-1.19	57.7	0.02	R	0.61
PBC DNA	8	5,284	1.04	0.90-1.20	63.0	0.01	R	0.62
Caucasian	5	1,440	1.05	0.77-1.43	76.2	0.00	R	0.76
Asian	2	870	1.02	0.81-1.27	24.6	0.25	F	0.89
H-based	4	1,132	1.12	0.75-1.69	78.0	0.00	R	0.58
P-based	4	3,436	0.98	0.89-1.08	8.9	0.35	F	0.62
HWE	8	5,264	0.98	0.91-1.07	0	0.62	F	0.69
Advanced PC	4	862	0.96	0.80-1.15	50.8	0.11	F	0.65
Localized PC	4	800	1.01	0.84-1.23	0	0.97	F	0.88

PC, prostate cancer; P_h, P-value of Q test for heterogeneity test; H-based, hospital-based case-control study; P, population-based case-control study; F, fixed-effects model; R, random-effects model; HWE, Hardy-Weinberg equilibrium; PBC, peripheral blood cell.

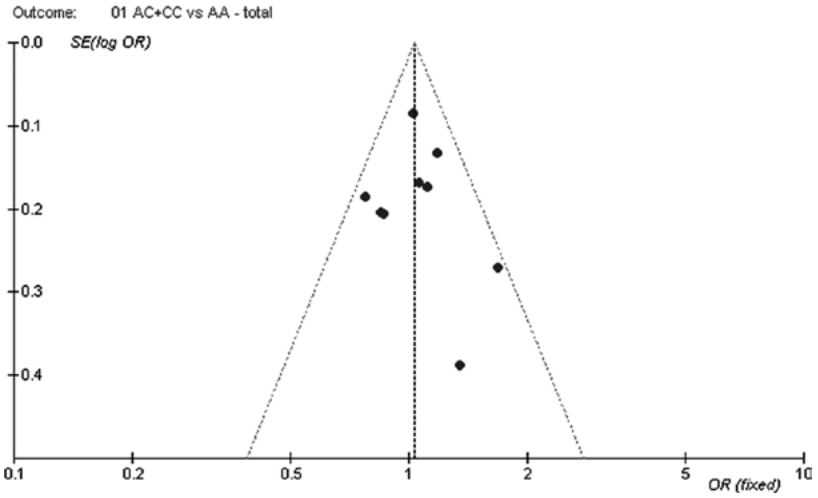

Figure 2. Funnel plot for the relationship of the MTHFR gene A1298C polymorphism with the risk of prostate cancer in the dominant model: AC + CC vs. AA.

The published literature regarding the association between the MTHFR gene A1298C polymorphism and the risk of PC consists of small studies mainly in Caucasian populations, which show conflicting findings. No consensus has yet been reached. To date, there have been two meta-analyses (30,31) focusing on the association between the two, both of which found that the MTHFR gene A1298C polymorphism did not contribute to the susceptibility of PC. However, the two previous meta-analyses included only a small number of case-control studies, with limited Caucasian subjects. Therefore, we performed a meta-analysis of all eligible studies, in order to derive a more conclusive estimation of the relationship. A total of nine case-control studies were selected in our meta-analysis, consisting of 2,723 PC cases and 3,442 controls. In the total population, we did not find an association between the MTHFR gene A1298C polymorphism and PC risk in the codominant, dominant, recessive and allele models (all P>0.05).

In the eligible studies, the percentage of the C allele was 0.1841 (25) and 0.1869 (27) in Asian population, while it was >0.25% for other populations. Hence, the different genetic background may have affected the results of the meta-analysis. In the stratified analysis by ethnicity, no significant association between the MTHFR gene A1298C polymorphism and PC risk was found for either Caucasian or Asian populations in the codominant, dominant, recessive and allele models (all P>0.05). Furthermore, no statistically significant results were found between the MTHFR gene A1298C polymorphism and PC development in the subgroup analyses of HWE, PBC DNA, advanced PC, localized PC, hospital-based and population-based case control studies (all P>0.05).

The results of the present study, along with those of other meta-analyses regarding the MTHFR gene A1298C polymorphism (32-34), reached the same conclusions, which found no association between the polymorphism site and the disease. It may not be uncommon that the results of the funciional study were not coincident with the epidemiological results. The mentioned discrepancy may be due to complex genetic background and multi-genetic interaction (35). On the other hand, one recent study highlighted that the MTHFR gene A1298C polymorphism is not associated with the modification of MTHFR activity (36). As a result, the specific mechanism of how the MTHFR gene A1298C polymorphism influences the MTHFR function warrants further investigation.

In conclusion, this meta-analysis suggests that the MTHFR gene A1298C polymorphism is not associated with prostate cancer susceptibility in either total or stratified populations. Furthermore, gene-gene and gene-environment interactions should also be considered in the analysis, which may contribute to a better understanding of the possible genetic risk of prostate carcinoma.

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