RESEARCH ARTICLE

Association between MTHFR C677T Polymorphism and Risk of Prostate Cancer: Evidence from 22 Studies with 10,832 Cases and 11,993 Controls

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Abstract

Background: The MTHFR C677T polymorphism is a genetic alteration affecting an enzyme involved in folate metabolism, but its relationship to host susceptibility to prostate cancer remains uncertain. The aim of this study was to investigate the association between MTHFR C677T polymorphism and prostate cancer by performing a meta-analysis. Materials and Methods: Pubmed and Web of Science databases were searched for case-control studies investigating the association between MTHFR C677T polymorphism and prostate cancer. Odds ratios (OR) and 95% confidence intervals (95%CI) were used to assess any link. Results: A total of 22 independent studies were identified, including 10,832 cases and 11,993 controls. Meta-analysis showed that there was no obvious association between MTHFR C677T polymorphism and risk of prostate cancer under all five genetic models. There was also no obvious association between MTHFR C677T polymorphism and risk of prostate cancer in the subgroup analyses of Caucasians. In contrast, MTHFR C677T polymorphism was associated with increased risk for prostate cancer in Asians with the allele model (C *vs* G: OR=1.299, 95 %CI =1.121-1.506, P=0.001, $P_{heterogeneity}$ =0.120, I^2=45%), additive genetic model (CC *vs* TT: OR =1.925, 95 % CI= 1.340-2.265, P=0.00, $P_{heterogeneity}$ =0.587, I^2=0.00%), recessive model (CC *vs* TT+TC: OR= 1.708, 95 % CI= 1.233-2.367, P=0.001, $P_{heterogeneity}$ =0.716, I^2=0.00%), and heterozygote genetic model (CT *vs* TT: OR=2.193, 95 % CI =1.510-3.186, P=0.000, $P_{heterogeneity}$ =0.462, I^2=0.00%). Conclusions: These results suggest that the MTHFR C677T polymorphism does not contribute to the risk of prostate cancer from currently available evidence in populations overall and Caucasians. However, the meta analysis indicates that it may play a role in prostate cancer development in Asians.

Keywords: MTHFR C677T - polymorphism - prostate cancer - meta analysis

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Introduction

Prostate cancer is the most common malignancy and the second leading cause of cancer related death in men in indus¬trialized countries. Its incidence is at a relatively low rate in the Asian population, but is increasing rapidly (Li et al., 2012). The mechanism of its carcinogenesis, like other cancers, still remains unclear (Bai et al., 2009). It is known that age, ethnicity and family history are the well established risk factors for prostate cancer (Zhang et al., 2014). About 99% of cases occur in those over the age of 50. Having a first degree relative with the disease increases the risk 2 to 3 fold (Albright et al., 2015). No single gene is responsible for prostate cancer; many different genes have been implicated. However, genome-wide association studies have identified several genetic variants that each slightly increases prostate cancer risk (Eeles 2t al, 2008).

MTHFR plays a pivotal role in the folate metabolism, it can catalyze the irreversible conversion of 5, 10-methylenetrahydrofolate to 5- methylenetrahydrofolate, which participates in the remethylation of homocysteine to methionine (Yilmaz et al., 2014; Kreile et al., 2014). 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 (Ozen et al., 2014). The 1298A>C polymorphism, marked as rs1801131 in the NCBI database, is located at exon 7 and results in a glutamate to valine substitu¬tion at codon 429 (Rai et al., 2014). Two functional polymorphisms in the MTHFR gene have been identified C677T and A1298C, which both result in amino acid substitutions in the MTHFR protein (Yang et al., 2014). These MTHFR polymorphisms have been associated with reduced enzyme activity of MTHFR, which lead to an accumulation of 5,

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10-methylenetetrahydrofolate and DNA hypomethylation (Rai et al., 2014).

The MTHFR C677T polymorphism (also known as rs1801133 , Ala222Val, and A222V) have been linked to increased risk for various types of cancer (Rai et al., 2014), and has been investigated in relation to the risk of PC but with inconclusive results (Küçükhüseyin et al., 2011; Zhang et al., 2012). Compared to the 677CC genotype, the 677TT genotype takes up about 30 % of the MTHFR enzyme activity, but the 677CT genotype is higher, accounting for 65 % (Zhang et al., 2012; Rai et al., 2014). Reports of C677T polymorphism as a carcinogen for multiple cancers promote many investigations to explore its genetic effects on prostate cancer.

Materials and Methods

Literature and search strategy

A computerized literature search was conducted for the relevant available studies published in English in PubMed, Web of Science, and EMBASE, on January 5, 2015. The search strategy identified all possible studies using combinations of the following keywords: ''methylenetetrahydrofolate reductase'', ''MTHFR'', ''MTHFR C677T'', ''folate'', ''one-carbon metabolism'', ''rs1801133'', ''polymorphism'', ''genotype'', and ''prostate cancer'' All references cited in the included studies were also hand-searched and reviewed to identify additional published articles not indexed in common databases. Of the studies with overlapping data published by the same authors, only the most recent or complete study was included in this meta-analysis. Two authors conducted all searches independently.

Inclusion and exclusion criteria

Studies included in this meta-analysis had to meet the following criteria: (1) evaluate the MTHFR C677T polymorphism and prostate cancer risk, (2) only cohort studies and case control studies were included in this meta-analysis; (3) Provision of information on genotype frequencies of the MTHFR C677T polymorphism and sufficient data for the calculation. The exclusion criteria were as follows: (1) none case–control studies including review, case report, editorial, or comment; (2) A duplicated study; (3) Laboratory molecular or animal studies. If studies contained overlapping cases and/or controls, the largest study with extractable data was preferred.

Data extraction

Information was independently extracted from all eligible publications by two authors according to the inclusion and exclusion criteria listed above. The following data were collected from each study: first author's surname, year of publication, ethnicity, the numbers of cases and controls, and the frequencies of CC, CT and TT genotypes. Different ethnicity descents were categorized as Caucasian, Asian, and African. When studies included subjects of more than one ethnicity and were able to separate, data were extracted separately for each ethnic group. We did not define any minimum number of patients to include a study in our meta-analysis.

Statistical analysis

Summary odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were estimated for each polymorphism in different comparison models, including allele model C *vs* T; homozygote (CC *vs* TT), recessive model (CC *vs* CT+ TT) and dominant model (CC+CT *vs* TT). Subgroup analyses were stratified by ethnicity. Both fixed-effects model using the Mantel– Haenszel method and random-effects model using the DerSimonian and Laird method were used to pool the results (DerSimonian

Table 1. Characteristics of 22 Studies Included in the Meta-analysis

Author	Country	Case/Control	Case			Allele		Control			Allele		P value
			CC	CT	TT	C	T	CC	CT	TT	C	T	
Kimura, 2000	Germany	132/150	49	67	16	165	99	65	73	12	203	97	0.16
Heijmans, 2003	Netherlands	21/772	8	9	4	25	17	391	320	61	1102	442	0.68
Cicek, 2004	USA	439/479	214	182	43	610	268	219	199	61	637	321	0.13
Singal, 2004	USA	81/42	49	25	7	123	39	20	20	2	60	24	0.28
Van Guelpen, 2006	Sweden	223/435	111	100	12	322	124	243	156	36	642	228	0.12
Johansson, 2007	Sweden	2677/1541	1340	1128	209	3808	1546	801	612	128	2214	868	0.46
Reljic, 2007	Croatia	95/37	38	48	9	124	66	8	25	4	41	33	0.02
Marchal, 2008	Spain	182/204	67	104	11	238	126	96	77	31	269	139	0.02
Stevens, 2008	USA	1100/1107	472	517	111	1461	739	474	501	132	1449	765	0.98
Collin, 2009	UK	1599/2084	676	697	226	2046	1149	917	948	219	2782	1386	0.25
Muslumanoglu, 2009	Turkey	93/157	53	38	2	144	42	80	65	12	225	89	0.8
Cai, 2010	China	217/220	58	121	38	237	197	45	116	59	206	234	0.38
Safarinejad, 2010	Iran	174/348	86	77	11	246	99	153	155	40	461	235	0.93
Wu, 2010	China	218/436	139	68	11	346	90	221	177	38	619	253	0.76
Kucukhuseyin, 2011	Turkey	50/55	18	30	2	66	34	32	21	2	85	25	0.51
Fard-Esfahani, 2012	Iran	67	29	33	5	91	43	-	-	-	-	-	-
Raju K, 2012	Indian	195/250	156	35	4	347	45	210	36	4	456	44	0.1
Kobayashi, 2012	Canada	80/334	22	19	2	63	23	72	86	12	230	110	0.04
Vidal, 2012	USA	55/192	36	19	0	91	19	103	89	0	295	89	0
López-Cortés, 2013	Ecuadorian	104/110	30	73	1	133	75	52	57	1	161	59	0
de Vogel, 2013	Norway	3000/3000	1407	820	295	3634	1410	1334	929	344	3597	1617	0
Ghasemi, 2014	Iran	30/40	27	3	0	57	3	34	6	0	74	6	0.6

Table 2. Meta-analysis Results for the MTHFR C677T Polymorphism and Prostate Cancer Risk

Contrasts	Studies (cases/controls)	OR[95 % CI]	POR	I^2 (%)	PH
Overall	10832/11993				
C *vs* T		1.005[0.965-1.047]	0.796	67	<0.001
CC *vs* TT		1.095[0.995-1.206]	0.065	59	<0.001
CT *vs* CC		1.078[0.977-1.189]	0.138	68	<0.001
CC+CT *vs* TT		0.996[0.921-1.078]	0.928	63	<0.001
CT+TT *vs* CC		0.984[0.931-1.040]	0.572	87	<0.001
Caucasian	9931/10699				
C *vs* T		0.984[0.943-1.027]	0.466	63	<0.001
CCvs.TT		1.049[0.949-1.160]	0.348	56	0.003
CT *vs* CC		1.022[0.923-1.132]	0.675	62	0.001
CC+CT *vs* TT		1.049[0.949-1.160]	0.348	61	0.001
CT+TT *vs* CC		0.974[0.920-1.032]	0.37	68	<0.001
Asian	901/1294				
C *vs* T		1.299[1.121-1.506]	0.001	45	0.12
CCvs.TT		1.925[1.340-2.265]	<0.001	0	0.587
CT *vs* CC		2.193[1.510-3.186]	<0.001	0	0.462
CC+CT *vs* TT		0.963[0.888-1.045]	0.369	61	0.001
CT+TT *vs* CC		1.708[1.233-2.367]	0.001	0	0.716

et al, 1986). The distribution of the genotypes in the control population was tested for Hardy-Weinberg equilibrium using Chi-square test. The Q test and I^2 statistics were used to assess the statistical heterogeneity among studies. The result of the Q test was PQ<0.1 or I^2>50%, indicating the presence of heterogeneity. Sensitivity analysis was performed by sequential omission of individual studies. Publication bias was evaluated using a funnel plot and Egger's regression asymmetry test. If publication bias existed, the Duval and Tweedie nonparametric "trim and fill" method was used to adjust for it. All analyses were performed using Comprehensive Meta Analysis (CMA) software, version 2.2.064 (NIH, USA). To ensure the reliability and the accuracy of the results, two authors entered the data into the statistical software programs independently with the same results. A P value <0.05 was considered statistically significant, except where otherwise specified.

Results

Study characteristics

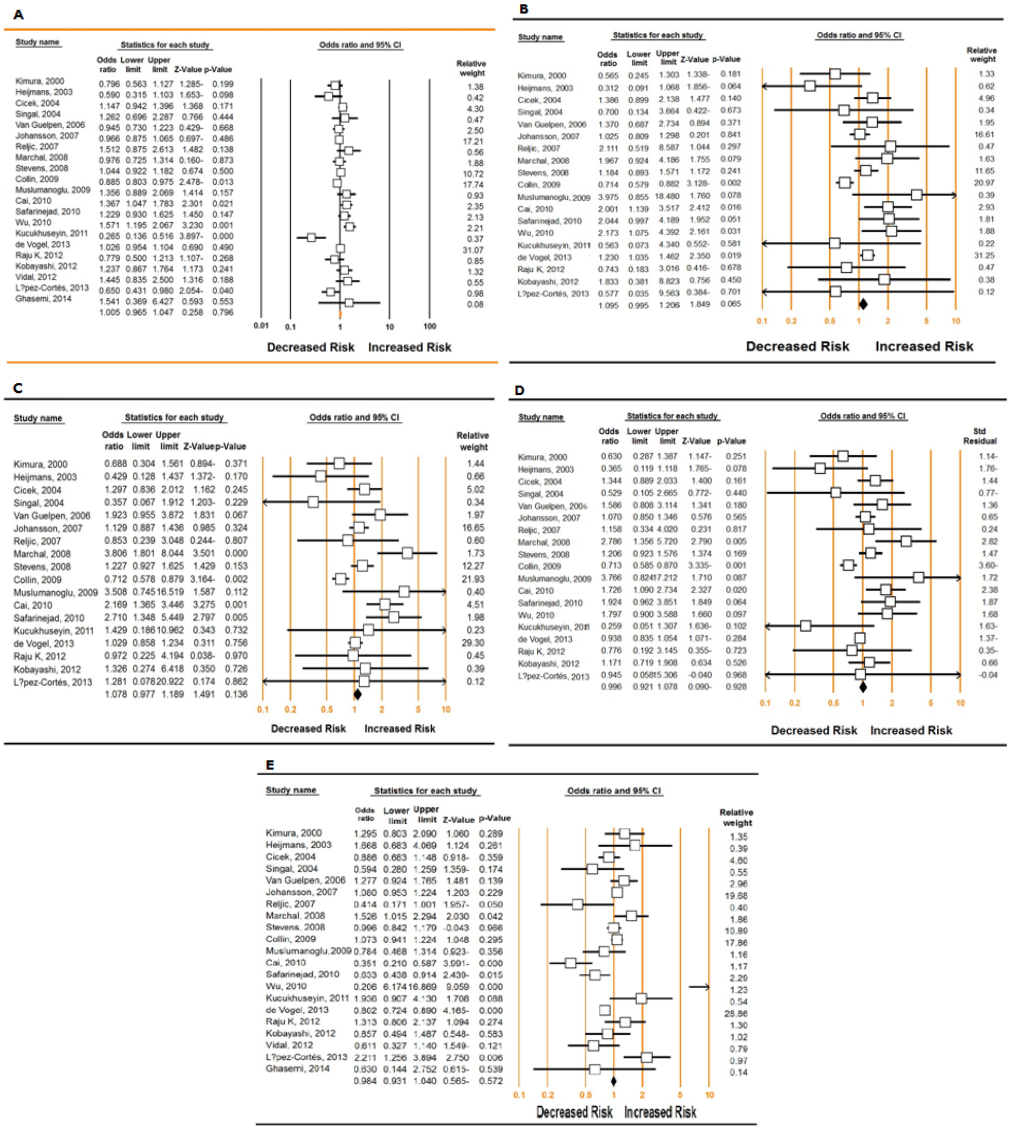

Figure 1. Meta-analysis of the Association between MTHFRC677T Polymorphism and Susceptibility to Prostate Cancer (A: C *vs* T; B: CC *vs* TT; C: CT *vs* TT; D: CC+CT *vs* TT; E: CC *vs* CT+TT)

Through the systematic computer-based search, we derived 157 references in total. One hundred forty-eight articles concerning topics irrelevant to the association between MTHFR C677T polymorphism and risk of prostate cancer were initially removed. Then, we examined the full texts of the remaining nine papers, in which two were further removed, because of being published as a review article and subsequently updated by a recent study.

In total, 22 publications with 10,832 cases and 11,993 controls met the selection criteria (Kimura et al., 2000; Heijmans et al., 2003; Cicek et al., 2004; Singal et al., 2004; Van Guelpen et al., 2006; Johansson et al., 2007; Reljic et al., 2007; Marchal et al., 2008; Stevens et al., 2008; Collin et al., 2009; Muslumanoglu et al., 2009; Cai et al., 2010; Safarinejad et al., 2010; Wu et al., 2010; Kucukhuseyin et al., 2011; Fard-Esfahani et al., 2012; Raju K et al., 2012; Kobayashi et al., 2012; Vidal et al., 2012; López-Cortés et al., 2013; de Vogel et al., 2013; Ghasemi et al., 2014) published during 2000 to 2011 with a total of 7306 cases and 8062 controls in our meta-analysis. The characteristics of the studies included in this meta-analysis are listed in Table 1. Among the 22 studies, 16 studies of Caucasians, 6 studies of Asians, and all studies of population-based controls and hospital based. The distribution of the MTHFR C677T genotype in the control groups of 16 studies was consistent with HWE (all PHWE values were more than 0.05, Table 1). According to the quality criteria, all the 22 studies were high quality.

Meta-analysis (overall and ethnicity)

Meta-analyses of total 22 studies suggested that MTHFR C677T polymorphism was not associated with an increased risk of prostate cancer risk under all genetic models in overall population (Table 2).

According to ethnicity, 16 publications with 9,931 cases and 10,699 controls were carried out among Caucasians, whereas six publications including 901cases and 1,294 controls were among Asians. In the stratified analysis by ethnicity, increased risks were not found among Caucasians for all genetic models, whereas significantly increased risks were found among Asians for Allele model (C *vs* G: OR=1.299, 95%CI =1.121-1.506, P=0.001, $P_{heterogeneity}$ =0.120, I^2=45%), Additive genetic model (CCvs. TT: OR =1.925, 95%CI= 1.340-2.265, P=0.00, $P_{heterogeneity}$ =0.587, I^2=0.00%), Recessive model (CC *vs* TT+TC: OR= 1.708, 95%CI= 1.233-2.367, P=0.001, $P_{heterogeneity}$ =0.716, I^2=0.00%), and Heterozygote genetic model (CT *vs* TT: OR=2.193, 95%CI =1.510-3.186, P=0.000, $P_{heterogeneity}$ =0.462, I^2=0.00%). Therefore, subgroup meta-analysis by ethnicity suggested that MTHFR C677T polymorphism was associated with an increased risk of PC in Asians, but not in Caucasians.

Sensitivity analysis

A single study involved in the meta-analysis was deleted each time to reflect the influence of the individual data-set to the pooled ORs, and the corresponding pooled ORs were not materially altered (data not shown), indicating that our results were statistically robust. Although the genotype distribution in two studies of C677T polymorphism and one study of A1298C polymorphism was not in accordance with HWE, the corresponding pooled ORs were not qualitatively altered with or without including these studies.

Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias of literatures in all comparison models. The shape of the funnel plot did not reveal any evidence of obvious asymmetry (Figure 1). Then, the Egger's test was used to provide statistical evidence of funnel plot symmetry. The results still did not suggest any evidence of publication bias in C677T (P=0.900 for TT *vs* CC; P=0.804 for CT *vs* CC; P=0.834 for recessive model TT *vs* CT+CC; and P=0.365 for dominant model TT+CT *vs* CC) and A1298C (P=0.508 for CC *vs* AA; P=0.717 for AC *vs* CC; P=0.458 for recessive model CC *vs* AC+AA; and P=0.409 for dominant model AC+CC *vs* AA) polymorphisms.

Discussion

MTHFR is a central enzyme involved in the regulation of folate metabolism. Folate plays a pivotal role in synthesis, repair, and methylation of DNA. Deceased levels of dietary folate, via a futile cycle of uracil misincorporation and removal, may cause dysfunction of DNA methylation, activation of proto-oncogene, and/or instability in the DNA molecules (Berger et al., 2008). C677T polymorphism in the MTHFR gene with a transition of alanine to valine at position 222 may alter the activity of its enzyme (Ashton et al., 2009).

Our results were consistent with three previously published meta-analyses by Zhang et al. (Zhang et al., 2012), Bai et al (Bai et al., 2009) not showed an increased risk of prostate cancer associated with the MTHFR C677T polymorphism. However, this meta-analysis included a larger number of studies than the 2 previous meta analysis did. Our study involved 22 studies related to C677T polymorphism and provided 10832/11993 cases/controls.

As for the MTHFR C677T, most evidence points to decrease in the susceptibility to prostate cancer (Wu et al., 2010). However, but the effect on the prostate cancer susceptibility was not consistent. In this meta-analysis, no statistically significant difference was found in the frequency of the MTHFR C677T polymorphism in the patients with prostate cancer when compared with the controls. This finding was consistent with that of one previous meta-analysis (Bai et al., 2009; Zhang et al., 2012).

It has been well known that cancer occurrence and mortality varied by ethnicity and geographic location (Goovaerts et al., 2011). Since the genotypic frequency of MTHFR C677T differs markedly across different ethnicities, population stratification is a major concern in all gene association studies as a source of bias (Zhang et al., 2012). We meta-analyzed the eligible case-control studies for C677T by geographic regions. No association was found between the C677T polymorphism and the prostate cancer in the Asian. However, a significant inverse association was found in the European population. Different genetic backgrounds or environmental

conditions could explain the discrepancy.

There was significant heterogeneity for MTHFR C677T among the 22 studies. Many factors may contribute to such heterogeneity, and ethnicity may be one of them because allele and genotype distribution of MTHFR C677T locus was different in different ethnicities (Li et al., 2012). We sub-grouped the 22 studies based on ethnicity. In the Asian group, the heterogeneity disappeared, and the results indicated significant association. However, heterogeneity still existed in the Caucasian group. Heterogeneity analysis of C677T polymorphism suggested significant heterogeneity in additive model TT *vs* CC, recessive model TT *vs* CT+CC, and dominant model TT+CT *vs* CC only in the Asian ethnicity. To explore the sources of heterogeneity, we performed meta regression and subgroup analyses. Meta regression analysis of data showed that the ethnicity might substantially influence the initial heterogeneity. Subgroup analyses by ethnicity indicated that heterogeneity still existed in Asians in the above mentioned genetic comparison models.

According to study characteristics, subgroup analysis and sensitivity analysis were performed. The results showed that the T allele in overall population, Caucasian and Asian population had not significant effect on the risk of prostate cancer. A wide variation of T allele frequencies observed between the controls across all studies (Bai et al., 2009; Fard-Esfahani et al., 2012). The result of X^2 indicated that the T allele frequencies were significant difference in overall population. When meta-analysis was performed to assess association between MTHFR C677T polymorphism and different ethnicities, the T allele of MTHFR C677T polymorphism had not significant association with prostate cancer susceptibility in Asians (Table 1).

However, this meta-analysis had several limitations that must be considered when interpreting the findings. Some limitations of our meta-analysis should be addressed. Firstly, the numbers of published studies collected in our analysis were not large enough for the comprehensive analysis of subgroups such as sex, and ethnicity. Secondly, lacking the original data of the included studies limited our study to further evaluate the potential interactions, since gene environment and gene-gene interactions may modulate prostate cancer risk. Finally, gene–gene and gene–environment factors interactions were not fully addressed in this meta-analysis for the lack of sufficient data. Future studies may further assess the possible gene–gene and gene–environment interactions. Another limitation was that significant heterogeneity in the studies was mainly present in overall analyses and subgroup analyses. Though several possible sources of the between-study heterogeneity were investigated, including ethnicity, geographic region, source of controls, and pathological history, none of them could sufficiently explain the heterogeneity. So, a more precise analysis needs to be conducted if individual data such as age and sex are available.

Nevertheless, advantages in our meta analysis should also be acknowledged. A systematic review of the association of MTHFR C677T polymorphism with prostate cancer risk is statistically more powerful than any single study. Furthermore, the studies included in our meta-analysis strictly and satisfactory met our selection criteria.

In conclusion, despite the above-mentioned limitations, this meta analysis provides evidence that the MTHFR C677T polymorphism may not increase the susceptibility to prostate cancer risk. However, the present meta-analysis reveals a negative association between the MTHFR C677T mutations and prostate cancer risk, especially in the European populations.

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