

## RESEARCH COMMUNICATION

# The MTHFR C677T Polymorphism and Prostate Cancer Risk: New Findings from a Meta-analysis of 7306 Cases and 8062 Controls

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### Abstract

Methylenetetrahydrofolate reductase (MTHFR) is an essential enzyme involved in folate metabolism; a single nucleotide polymorphism (SNP) C677T has been reported to be linked with altered incidences of several diseases. We here conducted a meta-analysis of 15 published epidemiological studies with a total of 7306 cases and 8062 controls to evaluate its association with prostate cancer risk with overall and subgroup analyses. No statistical relationship was found overall with any genetic model (TT vs. CC: OR = 0.80, 95% CI = [0.62, 1.04], P = 0.094; CT vs. CC: OR = 0.97, 95% CI = [0.84, 1.12], P = 0.667; Dominant: OR = 0.94, 95% CI = [0.82, 1.07], P = 0.343; Recessive: OR = 0.81, 95% CI = [0.64, 1.04], P = 0.104), but after the exclusion of several studies, we could observe the homozygote TT to confer less susceptibility to prostate cancer in carriers; moreover, different effects of the polymorphism on prostate cancer risk was detected from subgroup analysis stratified by participants' residential region: significant reduced prostate cancer risk was found to be associated with the polymorphism from Asian studies (TT vs. CC: OR = 0.47, 95% CI = [0.33, 0.67], P < 0.001; CT vs. CC: OR = 0.73, 95% CI = [0.60, 0.90], P = 0.002; Dominant: OR = 0.67, 95% CI = [0.56, 0.82], P < 0.001; Recessive: OR = 0.55, 95% CI = [0.40, 0.76], P < 0.001) while studies from Europe indicated a slight increased risk under dominant model with marginal significance (OR = 1.14, 95% CI = [0.99, 1.30], P = 0.064). Moreover, the protective effect of the polymorphism against prostate cancer was also shown by studies performed in yellow Asians (TT vs. CC: OR = 0.48, 95% CI = [0.31, 0.75], P = 0.001; CT vs. CC: OR = 0.68, 95% CI = [0.51, 0.90], P = 0.006; Dominant: OR = 0.63, 95% CI = [0.48, 0.82], P < 0.001; Recessive: OR = 0.57, 95% CI = [0.39, 0.84], P = 0.004). We propose that these phenomena should be viewed with the consideration of folate metabolism profile and different gene background as well as living habits of different populations, and more relevant studies should be conducted to confirm our hypothesis and provide a comprehensive and clear picture concerning this topic.

**Keywords:** Meta-analysis - MTHFR - polymorphism - prostate cancer risk - ethnic groups

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### Introduction

Prostate cancer is one of the most commonly diagnosed male malignancies, and it remains a leading cause of death in most Western countries, especially in elderly men (Detchukul et al., 2011; Jemal et al., 2011; Siegel et al., 2011). 240,890 new cases and 33,720 deaths of prostate cancer are estimated to occur in the US during 2011. It is accepted that age, race/ethnicity and family history of prostate cancer are the only well-established risk factors for the disease (American Cancer Society, 2011; Hoffman, 2011; Mori et al., 2011; Schröder, 2011). However, diet pattern and some variants were reported to be associated with altered risk of prostate cancer: Lin Yan (Yan et al., 2009) found consumption of soy foods could reduce prostate cancer risk and such protection may be associated with the type and quantity of soy foods; Ben Liu (Liu et al., 2012) suggested significant relationship

between cruciferous vegetables intake and decreased risk of prostate cancer. As a common and major nutrient from daily vegetables, folate was believed to decrease risk of many cancer types, such as colon cancer, esophageal squamous cell carcinoma, esophageal adenocarcinoma and pancreatic cancer (Larsson et al., 2006; Kim et al., 2010), however, meta-analysis of six randomized controlled trials indicated association between a 24% increased risk of prostate cancer with folic acid intake (Wien et al., 2012).

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate-involved one carbon metabolism, which catalyzes the conversion of 5,10-methylenetetrahydrofolate (5,10-methylene-THF) to 5-methyltetrahydrofolate (5-methyl-THF). 5-Methyl-THF, the major form of folate in plasma, acts as the methyl-donor for methionine synthesis through homocysteine. Moreover, folate is also involved in the formation of purine and thymidine,

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making the enzyme vital in maintaining metabolic balance (Molloy, 2012). A single nucleotide polymorphism (SNP) C677T in MTHFR locates in exon 4 at the folate binding site of the gene, leading to the substitution of alanine to valine at codon 222, which further results in the reduction of enzyme activity (Frosst et al., 1995; Kim, 1999; Friso et al., 2002). Such change would disturb the homeostasis of folate metabolism, which was believed to be associated with the occurrence and development of several chronic diseases. Systematic reviews and meta-analysis revealed the MTHFR SNP C677T could increase the risk of developing schizophrenia, congenital heart defects, esophageal cancer, gastric cancer and breast cancer (Lewis et al., 2005; Langevin et al., 2009; Dong et al., 2010; Zhang et al., 2010; Yin et al., 2012) while probably playing as a preventive factor against colorectal cancer and children acute lymphoblastic leukemia (Hubner et al., 2007; Taioli et al., 2009; Yan et al., 2012). There were also many studies concerning the association between prostate cancer risk and SNP C677T, however, no conclusiveness was achieved: Heijmans (Heijmans et al., 2003) suggested MTHFR SNP C677T be a risk factor of prostate cancer in Dutch population, but HSI-CHIN WU (Wu et al., 2010) found the polymorphism conferred a significant decreased risk of the disease; moreover, some studies showed no statistical relationship between them (Stevens et al., 2008). Through pooling studies together according to their characteristics, we conducted this meta-analysis based on published literature to make a more comprehensive and compelling evaluation of the connection between MTHFR SNP C677T and prostate cancer risk, in order to explore some evidences concerning the influence of folate metabolism on the etiology of prostate cancer.

## Materials and Methods

### Literature search

We searched Pubmed and MEDLINE databases online to identify potential relevant epidemiological publications through February 2012. We used the key term “MTHFR” as well as “prostate cancer” in the search with no other restrictions for a completed cover of candidate studies. Those fulfilling the following criteria were considered eligible for further analysis: 1. Examining the exact topic of our concern; 2. Published work with access; 3. Case-control or cohort study providing the individual numbers of all three genotypes from both case and control groups, or providing gene frequencies and sample sizes, or sufficient data for measuring OR and corresponding 95%CI; 4. Publication in English. The reference lists of retrieved publications were also reviewed in case any relevant study was missed.

### Identified publications and data extraction

The preliminary search resulted in 18 publications from Pubmed and 15 from MEDLINE, and all the results from MEDLINE were contained in Pubmed candidates. We identified 14 eligible publications out of the 18 and reference screening found one more study relevant. Thus, a total of 15 publications were included in our meta-analysis. The search workflow was shown in Figure 1.

Then the following information was extracted from each study: 1, the family name of first author and the year of publication; 2, study design based on the background of control individuals; 3, residential region of participants in studies; 4, race/ethnicity of participants in studies; 5, total sample size and distribution of each genotype in case and control group respectively. Literature search and data extraction was finished by two independent investigators.

### Statistical analysis

Pooled crude ORs and 95% CIs were calculated to assess the association between MTHFR SNP C677T and prostate cancer risk. Both fixed-effects model and random effects model were applied in measurements; inverse variance weighting method was used in former model while DerSimonian-Laird method (DerSimonian, 1986) for latter one. Comprehensive tests were conducted under co-dominant model (TT vs. CC; CT vs. CC), dominant model ((CT+TT) vs. CC) and recessive model (TT vs. (CT+CC)). We performed overall as well as subgroup analysis, stratified by study type or participants' region or race, to evaluate the association in different aspects. We assessed heterogeneity between studies by chi-square-based Q-test and  $I^2$  statistics (Higgins, 2003;), and a P value less than 0.05 for the Q-test or  $I^2$  value greater than 25% in  $I^2$  statistics indicates the existence of heterogeneity between studies, then random-effects model was better for interpretation, otherwise, we chose the fixed-effects model results. In sensitivity tests for assessing the stability of the results, every single study was deleted each time from the analysis to evaluate the study influence on pooled ORs. The estimate of publication bias was assessed by Egger's linear regression test along with funnel plot. Egger's test is based on a weighted linear regression of the treatment effect on its standard error, and  $P < 0.05$  as well as an asymmetric plot suggests significant publication bias. All statistical tests were performed with software R and the meta-package for R ([www.r-project.org](http://www.r-project.org)).

## Results

### Selected studies and main characteristics

We included 15 independent studies (Kimura et al.,

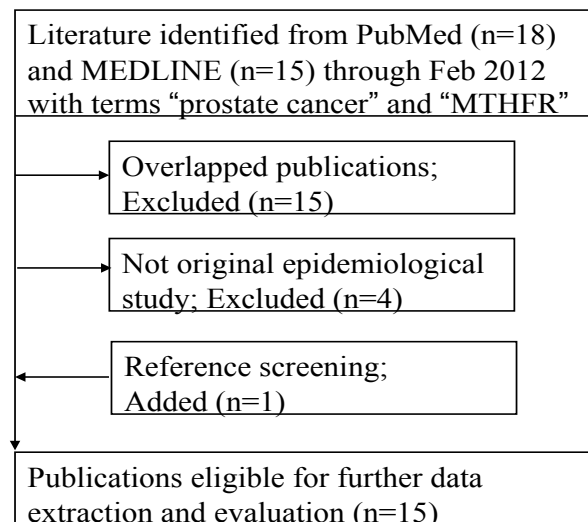
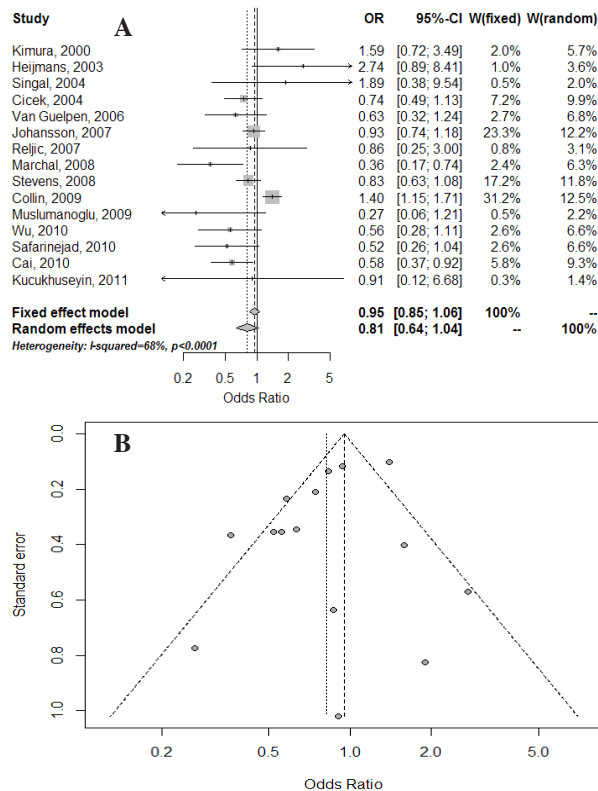


Figure 1. Workflow of the Literature Search

**Table 1. Main Characteristics of the 15 Studies Included in the Meta-analysis**

Study (First Author, Year)	Study design	Region	Race/Ethnicity	Sampe Size		Genotype Distribution					
				Case	Control	CC		CT		TT	
Kimura, 2000	Hospital-based case-control study	Dusseldorf/Dortmund, Germany	Caucasian	132	150	49	65	67	73	16	12
Heijmans, 2003	Population-based cohort study	Zutphen, Netherlands	Caucasian	21	772	8	391	9	320	4	61
Cicek, 2004	Population-based case-control study	Cleveland, Ohio, USA	Caucasian, African-American, Latino	439	479	214	219	182	199	43	61
Singal, 2004	Hospital-based case-control study	Shreveport, LA, USA	Caucasian, African-American	81	42	49	20	25	20	7	2
Van Guelpen, 2006	Population-based nested case-control study	Sweden	Caucasian	223	435	111	243	100	156	12	36
Johansson, 2007	Population-based case-control study	Six health-care regions in Sweden	Caucasian	2677	1541	1340	801	1128	612	209	128
Reljic, 2007	Population-based case-control study	Zagreb, Croatia	Caucasian	95	37	38	8	48	25	9	4
Marchal, 2008	Hospital-based case-control study	Ma'laga, Spain	Caucasian	182	204	67	96	104	77	11	31
Stevens, 2008	Population-based nested case-control study	USA	Caucasian	1100	1107	472	474	517	501	111	132
Collin, 2009	Population-based nested case-control study	Nine UK cities	Caucasian	1599	2084	676	917	697	948	226	219
Musulmanoglu, 2009	Hospital-based case-control study	Eskisehir, Turkey	Caucasian	93	157	53	80	38	65	2	12
CAI, 2010	Hospital-based case-control study	Northeast China	Yellow Asian	217	220	58	45	121	116	38	59
Safarinejad, 2010	Population-based case-control study	Tehran,Iran	Caucasian	174	348	86	153	77	155	11	40
WU, 2010	Hospital-based case-control study	Taichung, Taiwan, China	Yellow Asian	218	436	139	221	68	177	11	38
Kucukhuseyin, 2011	Population-based case-control study	Istanbul, Turkey	Caucasian	55	50	32	18	21	30	2	2



**Figure 2. Overall Association Between MTHFR SNP C677T and Prostate Cancer Risk.** A) Forest plot of the association under recessive model; B) Funnel plot of publication bias of overall studies (OR, odds ratio; 95% CI, 95% confidential interval)

2000; Heijmans et al., 2003; Cicek et al., 2004; Singal et al., 2004; Van 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) published during 2000 to 2011 with a total of 7306 cases and 8062 controls in our meta-analysis. According to the background of control population, 6 were hospital-based case-control studies (Kimura et al., 2000; Singal et al., 2004; Marchal et al., 2008; Muslumanoglu et al., 2009; Cai et al., 2010; Wu et al., 2010), 5 were population-based studies (Cicek et al., 2004; Johansson et al., 2007; Reljic et al., 2007; Safarinejad et al., 2010; Kucukhuseyin et al., 2011) and the rest 4 were population-

based studies nested in cohort (Heijmans et al., 2003; Van et al., 2006; Stevens et al., 2008; Collin et al., 2009). One study (Cicek et al., 2004) used sibling as control population. Two of the 15 studies were conducted in mixed ethnicities (Cicek et al., 2004; Singal et al., 2004) (Cicek: Caucasian/African-American/Latino; Singal: Caucasian/African-American), two in yellow Asian (Cai et al., 2010; Wu et al., 2010) and the rests in Caucasian (Kimura et al., 2000; Heijmans et al., 2003; Van 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; Safarinejad et al., 2010; Kucukhuseyin et al., 2011). In terms of the residential region of the participants, the most recent 5 studies were all conducted in Asia (Muslumanoglu et al., 2009; Cai et al., 2010; Safarinejad et al., 2010; Wu et al., 2010; Kucukhuseyin et al., 2011), 3 of the rest 10 were in the USA (Cicek et al., 2004; Singal et al., 2004; Stevens et al., 2008) and 7 in Europe (Kimura et al., 2000; Heijmans et al., 2003; Van et al., 2006; Johansson et al., 2007; Reljic et al., 2007; Marchal et al., 2008; Collin et al., 2009). All studies extracted DNA from blood/serum samples for further test except one (Singal et al., 2004), in which DNA was isolated from the archived paraffin blocks. The main characteristics of identified publications were shown in Table 1.

**Overall analysis**

We pooled all the studies together to assess the overall association between the MTHFR SNP C677T and prostate cancer risk; marginal protective effect of the polymorphism was found under all 4 genetic models without significance, Table 2 (TT vs. CC: OR = 0.80, 95%CI = [0.62, 1.04], P = 0.094; CT vs. CC: OR = 0.97, 95%CI = [0.84; 1.12], P = 0.667; Dominant: OR = 0.94, 95%CI = [0.82; 1.07], P = 0.343; Recessive: OR = 0.81, 95%CI = [0.64; 1.04], P = 0.104). However, sensitivity tests revealed significant association between the homozygotes TT and reduced prostate cancer risk after exclusion of the study from Kimura, Heijmans and Collin (Kimura et al., 2000; Heijmans et al., 2003; Collin et al., 2009) respectively (TT vs. CC: Omitting Kimura: OR = 0.77, 95%CI = [0.59; 1.00], P = 0.048,  $I^2 = 66.5%$ ; Omitting Heijmans: OR = 0.77, 95%CI = [0.60; 0.99], P = 0.043,  $I^2 = 65.1%$ ; Omitting Collin: OR = 0.75, 95%CI

**Table 2. Overall and Subgroup Results of the Association Between MTHFR SNP C677T and Prostate Cancer Risk under Different Genetic and Mathematical Models**

Classification	Genetic model	Fixed effects model		Random effects model			Heterogeneity		Publication bias
		OR	95%CI	P	OR	95%CI	P	I <sup>2</sup>	P
Overall studies	TT vs. CC	0.96 [0.85; 1.08]	0.479	<b>0.80 [0.62; 1.04]</b>	0.094	65.8%	< 0.001	0.102	
	CT vs. CC	1.02 [0.95; 1.09]	0.629	<b>0.97 [0.84; 1.12]</b>	0.667	62.2%	< 0.001	0.216	
	Dominant	1.00 [0.94; 1.07]	0.895	<b>0.94 [0.82; 1.07]</b>	0.343	62.6%	< 0.001	0.104	
	Recessive	0.95 [0.85; 1.06]	0.371	<b>0.81 [0.64; 1.04]</b>	0.104	68%	< 0.001	0.178	
Study design									
Hospital-based	TT vs. CC	0.61 [0.44; 0.84]	0.003	<b>0.63 [0.38; 1.05]</b>	0.074	49.2%	0.080	0.711	
	CT vs. CC	0.93 [0.76; 1.12]	0.424	<b>0.92 [0.62; 1.37]</b>	0.688	75.6%	0.001	0.948	
	Dominant	0.85 [0.71; 1.02]	0.089	<b>0.86 [0.61; 1.23]</b>	0.417	71.2%	0.004	0.887	
	Recessive	0.62 [0.46; 0.83]	0.002	<b>0.64 [0.39; 1.05]</b>	0.080	54%	0.054	0.737	
Population-based	TT vs. CC	<b>0.85 [0.70; 1.04]</b>	0.116	0.80 [0.61; 1.04]	0.098	19.3%	0.292	0.097	
	CT vs. CC	1.02 [0.91; 1.14]	0.776	<b>0.85 [0.64; 1.12]</b>	0.245	65.7%	0.020	0.003	
	Dominant	0.99 [0.89; 1.10]	0.811	<b>0.81 [0.62; 1.07]</b>	0.142	68.1%	0.014	< 0.001	
	Recessive	<b>0.85 [0.70; 1.03]</b>	0.095	0.85 [0.70; 1.03]	0.09	5 0%	0.559	0.390	
Cohort-nested	TT vs. CC	1.16 [0.98; 1.36]	0.076	<b>1.12 [0.73; 1.72]</b>	0.609	75.5%	0.007	0.965	
	CT vs. CC	<b>1.05 [0.94; 1.16]</b>	0.379	1.06 [0.94; 1.21]	0.353	18.7%	0.297	0.234	
	Dominant	<b>1.07 [0.97; 1.18]</b>	0.184	1.07 [0.97; 1.18]	0.18	4 0%	0.426	0.212	
	Recessive	1.15 [0.98; 1.34]	0.084	<b>1.08 [0.69; 1.69]</b>	0.741	79.9%	0.002	0.855	
Study region									
Asia	TT vs. CC	<b>0.47 [0.33; 0.67]*</b>	< 0.001	0.47 [0.33; 0.67]	< 0.001	1 0%	0.948	0.399	
	CT vs. CC	<b>0.73 [0.60; 0.90]*</b>	0.002	0.73 [0.58; 0.92]	0.007	18.6%	0.296	0.564	
	Dominant	<b>0.67 [0.56; 0.82]*</b>	< 0.001	0.67 [0.56; 0.82]	< 0.001	1 0%	0.467	0.550	
	Recessive	<b>0.55 [0.40; 0.76]*</b>	< 0.001	0.55 [0.40; 0.76]	< 0.001	1 0%	0.879	0.641	
America	TT vs. CC	<b>0.82 [0.64; 1.03]</b>	0.087	0.82 [0.64; 1.03]	0.087	0%	0.668	0.686	
	CT vs. CC	0.98 [0.85; 1.14]	0.813	<b>0.95 [0.76; 1.18]</b>	0.638	36.4%	0.208	0.027	
	Dominant	<b>0.95 [0.82; 1.09]</b>	0.437	0.94 [0.82; 1.09]	0.433	4.1%	0.352	0.101	
	Recessive	<b>0.82 [0.65; 1.02]</b>	0.074	0.82 [0.65; 1.02]	0.074	0%	0.537	0.478	
Europe	TT vs. CC	1.15 [0.99; 1.33]	0.063	<b>1.06 [0.76; 1.48]</b>	0.742	63.8%	0.011	0.615	
	CT vs. CC	1.10 [1.01; 1.20]	0.038	<b>1.17 [0.96; 1.42]</b>	0.111	61.7%	0.016	0.618	
	Dominant	1.11 [1.02; 1.20]	0.018	<b>1.14 [0.99; 1.30]</b>	0.064	36.7%	0.148	0.669	
	Recessive	1.12 [0.97; 1.29]	0.116	<b>0.99 [0.68; 1.43]</b>	0.957	74%	< 0.001	0.564	
Population race/ethnicity									
Caucasian	TT vs. CC	1.04 [0.91; 1.18]	0.570	<b>0.90 [0.68; 1.20]</b>	0.467	64.7%	0.002	0.208	
	CT vs. CC	1.06 [0.98; 1.15]	0.121	<b>1.06 [0.92; 1.23]</b>	0.424	57.4%	0.009	0.675	
	Dominant	1.06 [0.98; 1.13]	0.144	<b>1.04 [0.91; 1.18]</b>	0.581	50.7%	0.027	0.422	
	Recessive	1.02 [0.90; 1.15]	0.781	<b>0.87 [0.65; 1.17]</b>	0.354	70.3%	< 0.001	0.257	
Yellow	TT vs. CC	0.48 [0.31; 0.75]	0.001	<b>0.48 [0.31; 0.75]*</b>	0.001	—	—	—	
	CT vs. CC	0.68 [0.51; 0.90]	0.006	<b>0.68 [0.51; 0.90]*</b>	0.006	—	—	—	
	Dominant	0.63 [0.48; 0.82]	0.001	<b>0.63 [0.48; 0.82]*</b>	< 0.001	—	—	—	
	Recessive	0.57 [0.39; 0.84]	0.004	<b>0.57 [0.39; 0.84]*</b>	0.004	—	—	—	

\*Effect is significant statically; Results in bold were chosen for analysis according to the heterogeneity among studies

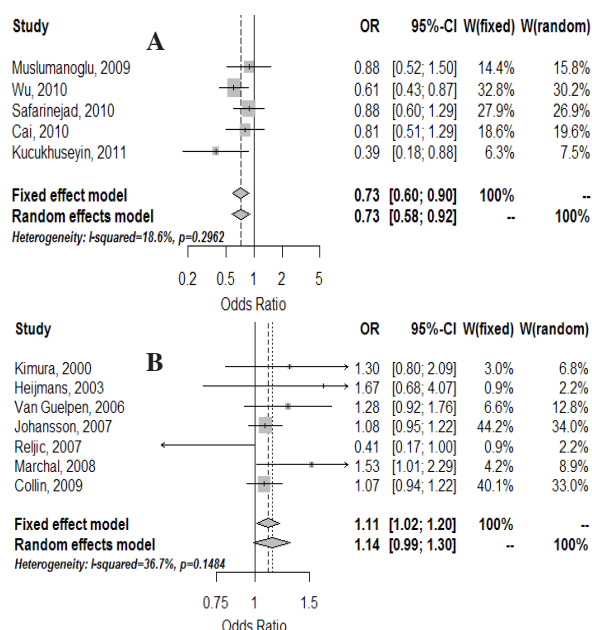
= [0.59; 0.94], P=0.014, I<sup>2</sup>=43.5%; Recessive: Omitting Kimura: OR = 0.78, 95%CI = [0.60; 1.01], P = 0.059, I<sup>2</sup> = 69.1%; Omitting Heijmans: OR = 0.78, 95%CI = [0.61; 1.00], P = 0.049, I<sup>2</sup> = 67.7%; Omitting Collin: OR = 0.76, 95%CI = [0.61; 0.94], P = 0.011, I<sup>2</sup> = 41.7%). Substantial heterogeneity was detected in all calculations; therefore, the results from random effects model were used. We found no publication bias in any pool of studies (TT vs. CC: P = 0.102; CT vs. CC: P = 0.216; Dominant: P = 0.104; Recessive: P = 0.178), Figure 2.

### Subgroup analysis

We stratified study subgroups by study design (background of controls), participants' region or population race, Table 2.

From hospital-based studies, we found no significant overall relationship between prostate cancer and the polymorphism under 4 models and great heterogeneity between studies; then sensitivity test detected the study

of Kimura et al. (2000) influenced the pooled OR substantially, and protective effect of homozygote TT against prostate cancer risk was shown to be significant as well as no heterogeneity found in the rest studies when omitting the above one (TT vs. CC: OR = 0.50, 95%CI = [0.35; 0.71], P < 0.001, I<sup>2</sup> = 0.0%; Recessive: OR = 0.52, 95%CI = [0.37; 0.75], P < 0.001, I<sup>2</sup> = 10.3%), which is similar to the overall results. No significant relationship was found between SNP C677T and prostate cancer risk from population-based studies or nested case-control studies. However, when the study of Johansson (Johansson et al., 2007) was excluded from the pool of population-based studies, significant protective effect could be detected under 3 genetic models (TT vs. CC: OR = 0.63, 95%CI = [0.45; 0.90], P = 0.012, I<sup>2</sup> = 0.0%; Dominant: OR = 0.71, 95%CI = [0.51; 0.98], P = 0.040, I<sup>2</sup> = 46.3%; Recessive: OR = 0.70, 95%CI = [0.50; 0.97], P = 0.034, I<sup>2</sup> = 0%). Publication bias was only found from two models (CT vs. CC, dominant) examining population-



**Figure 3. Different Effects of MTHFR SNP C677T on Prostate Cancer Risk: Forest Plot of Association Between the Polymorphism and Prostate Cancer Risk.** A) Under co-dominant model in Asian studies and B) Under dominant model in European studies (OR, odds ratio; 95% CI, 95% confidential interval)

based studies.

When subgroup analysis was applied according to the residential region of the participants from studies, we observed compelling significance in the association between the polymorphism and a reduced prostate cancer risk under all 4 genetic models from Asian studies (TT vs. CC: OR = 0.47, 95%CI = [0.33; 0.67], P < 0.001; CT vs. CC: OR = 0.73, 95%CI = [0.60; 0.90], P = 0.002; Dominant: OR = 0.67, 95%CI = [0.56; 0.82], P < 0.001; Recessive: OR = 0.55, 95%CI = [0.40; 0.76], P < 0.001), Figure 3. Every single study from Asia was detected to affect the pooled meta-analysis significantly; however, such influence would not alter the conclusive protective role of the polymorphism against prostate cancer even after the exclusion of every single study. No significance was found in studies from America. Nevertheless, the dominant model examining studies from Europe revealed marginal significant association between an increased risk of prostate cancer and SNP C677T (OR = 1.14, 95%CI = [0.99; 1.30], P = 0.064), Figure 3; moreover, sensitivity test indicated the T allele would increase the susceptibility to prostate cancer in European after the exclusion of the study from Reljic (CT vs. CC: OR = 1.20, 95%CI = [1.01; 1.42], P = 0.036, I<sup>2</sup> = 54.1%; Dominant: OR = 1.12, 95%CI = [1.03; 1.21], P = 0.010, I<sup>2</sup> = 0%) (Reljic et al., 2007). We detected publication bias only from CT vs. CC model of American studies.

In population ethnicity subgroup analysis, we found no statistically relationship between the polymorphism and prostate cancer risk in Caucasian subjects while the summary of two studies revealed a protective effect of SNP C677T from Yellow Asian population (TT vs. CC: OR = 0.48, 95%CI = [0.31; 0.75], P = 0.001; CT vs. CC: OR = 0.68, 95%CI = [0.51; 0.90], P = 0.006; Dominant: OR = 0.63, 95%CI = [0.48; 0.82], P < 0.001; Recessive:

OR = 0.57, 95%CI = [0.39; 0.84], P = 0.004). Due to the mixed ethnicities used in studies by Cicek and Singal, we did not include their data in the pool of Caucasian analysis. No publication bias was detected.

## Discussion

Our meta-analysis suggested no significant overall association between MTHFR SNP C677T and prostate cancer risk. Nevertheless, two models examining hospital-based studies indicated that significant reduced prostate cancer risk was related to the homozygote TT when the study of Kimura (Kimura et al., 2000) was omitted and such effect of SNP C677T was also detected in 3 models assessing population-based studies after the exclusion of Johansson's study (Johansson et al., 2007). The most important and interesting finding of the meta-analysis was the inverse results of the association between the two when measurement was conducted in studies from Asia and Europe respectively. Significant protective role of the polymorphism in prostate cancer was shown from studies performed in Asia no matter what kind of genetic model was used while one model examining the studies from Europe suggested a marginal increased risk with the polymorphism. No relationship was found from Caucasian population, but two studies from Yellow Asian population revealed a protective effect of SNP C677T.

We identified two meta-analysis (Bai et al., 2009; Collin et al., 2009) published in 2009 concerning similar topic as we did during the literature search; both of them examined the effect of MTHFR SNP C677T on prostate cancer risk, but no consistent conclusion was achieved. Collin found no effect of MTHFR C677T or any of the other alleles under all models while Bai stated that "the 677T allele was more likely to exert a protective effect on prostate cancer risk (OR = 0.81, 95% CI: 0.68–0.98) with a recessive genetic model". Our work included more studies, which were published during the past 3 years, than the previous two; however, we did not contain unpublished data from GWAS as Collin did. Interestingly, the newly published 5 studies (Muslumanoglu et al., 2009; Cai et al., 2010; Safarinejad et al., 2010; Wu et al., 2010; Kucukhuseyin et al., 2011) were all conducted in Asia, which allowed us to conduct a subgroup analysis, and no such study was included in the previous analysis.

In our calculation, we found similar results to Collin's work from overall studies; however, when we stratified studies by the residential region of participants, we detected a protective effect of the polymorphism on prostate cancer in Asian studies; but on the contrary, the same polymorphism was shown to be a risk factor of prostate cancer in European populations. The relatively low incident rate of prostate cancer in Asian population (American Cancer Society, 2011) and small sample sizes in the recent Asian studies could be the very reason why the protective effect of the polymorphism was diluted in overall studies; it is supported by the sensitivity tests which removed three European studies (Kimura et al., 2000; Heijmans et al., 2003; Collin et al., 2009) respectively from overall studies and detected such protective effect again. However, we still doubt whether

it is proper to pool all studies together here to evaluate the association between a polymorphism and prostate cancer risk without the consideration of gene background, living habits and baseline metabolism profile of population in studies. Therefore, the subgroup assessments in our meta-analysis seemed to be more valuable in the interpretation. We propose to evaluate the effect of SNP C677T on prostate cancer risk with the involvement of folate metabolism profile and dietary structure of individuals in the studies. It is well accepted of the favorable role of folate in the prevention of cardiovascular disease and neural tube defects (Kim, 1999; Ulrich et al., 2006), but there was still no conclusiveness of folate's effect on cancer risks. The vital role of folate in daily life brought about many investigations, and Ulrich, an expert of this field, pointed out the effect of folate on carcinogenesis could be more complex than we thought (Ulrich, 2007). She hypothesized an U-shape relation between folate status and breast cancer risk, in which individuals with relatively low folate status would benefit from extra folate intake, but after the folate status was increased to a certain level, more folate intake would exert no further influence on cancer prevention, instead, excessive folate may play as a risk factor to breast cancer, which means folate could act as a double-edged sword with a tolerable upper level. It is supported by animal studies from Kim, in which excessive supplementation of folate was likely to increase tumor growth (Kim, 2004). This could probably be the case not only in breast cancer but also in many other cancer types. And such concern on the differences induced by dose of folate was put forward in many publications (Choi et al., 2000; Ulrich et al., 2006; Ulrich, 2008). Moreover, a large chemoprevention trial finished by Cole and colleagues indicated that folic acid exerted different effects on cancer risk during different phases of follow-up, which raised another variable besides dose of folate that should be taken into consideration, and that is timing (Cole, 2007;). Kim's experiments also supported such concept. He found administration of folic acid after lesions increased colorectal neoplasia (Kim, 2004). These probably could explain the disparity of effects of folate on different cancer types, especially when different sensitivities of cancer types to folate and distinctive etiology of a certain cancer were considered. Cancers that more susceptible to folate intake are likely to be prevented at a relatively low folate level, and those cancers developing lesions at an early stage were probably more likely to be deteriorated by extra folic acid supplementation. A summary of six randomized controlled trials (Wien et al., 2012) showed folic acid intake provided a 24% increased risk of prostate cancer, and this mentioned us that prostate cancer is probably a kind of cancer with high sensitivity to folic acid. SNP C677T in MTHFR, a major enzyme in folate metabolic pathway, weakens its activity in catalyzing 5,10-methylene-THF to 5-methyl-THF, and 5-Methyl-THF is just the primary form of folate in plasma. Therefore, the polymorphism decreased the accumulation of 5-Methyl-THF in metabolism, further impaired the risk effect of folate intake on prostate cancer. Such protective role of the polymorphism was what we observed from Asian studies. Moreover, Stankova

suggested antisense inhibition of MTHFR could reduce cancer cell survival in vitro and tumor growth in vivo (Stankova et al., 2005), and such inhibition produced similar effect as the polymorphism. But why did opposite effect exist in studies from Europe. This may be attributed to the dietary habits of participants in different regions. WHO data indicated the per capita consumption of alcohol from total adults in Europe was more than 12.50 liters in 2005, which was the highest level in the world, on the other hand, Asian population consumed less than 7.5 liters (Iran: <2.5; Turkey: 2.5-5) of alcohol per capita while American less than 10 (World Health Organization, 2011). And it is well accepted that alcohol could influence the absorption and metabolism of folate (Halsted et al., 2002), therefore, excessive high amount of alcohol intake reduces the uptake of folate to a badly low level, at which the deficiency of folate would increase the prostate cancer risk just like the U-shape model mentioned, meanwhile, the polymorphism decreased the level of 5-Methyl-THF again, and further enhanced such risk effect on prostate cancer. Interestingly, American participants consumed moderate amount of alcohol, and the polymorphism would not alter their folate status to an extreme level, thus it seemed to be no association between SNP C677T and prostate cancer from American studies. These could be supported by a meta-analysis published in 2007 (Larsson et al., 2007), in which Larsson found no overall association between folate intake and breast cancer risk, but significant protective effect of high folate intake was detected in population with high alcohol consumption, which means higher folate intake alleviates the substantial influence of alcohol in folate absorption, so that the folate status would not be decreased to an badly low level. As to the protective effect observed from the sensitivity test of hospital-based studies, control participants recruited from hospital were more likely to be health-conscious and consume more folate (vegetables/supplementation) as well as less alcohol; however, such increase in folate seemed to be too much for prostate cancer prevention, therefore, the reduction of 5-Methyl-THF by the polymorphism acted as a favorable factor here. Due to the lack of studies from the Yellow population, we could not conclude that the effect of MTHFR SNP C677T varies in different races, although we found a preventive effect in Yellow but not in Caucasian. More studies performed in Yellow population out of Asia are needed to form a more comprehensive assessment.

We found in our meta-analysis that the SNP C677T in MTHFR reduced prostate cancer risk in population living in Asia while probably increasing such risk in people from Europe, notwithstanding no significant relation could be detected from overall studies. Such phenomenon probably could be ascribed to the folate metabolism profile and dietary structure of different regions. There were several limitations in our study: 1. We used crude ORs in the pooled analysis without adjustment; 2. The robustness of every single study would be affected by the technique they used; 3. The relatively small sample sizes of some studies we included in the analysis, especially those from Asia. However, our analysis and hypothesis were supported by many existing publications, although more specific and thorough investigations should be performed

to confirm the conclusion. And our work revealed that the effects of polymorphisms should be considered in a more comprehensive way; it is more reasonable and compelling to evaluate a polymorphism along with its related metabolic pathway, participants' gene background and living habits.

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