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MTHFR C677T polymorphism contributes to prostate cancer risk among Caucasians: A meta-analysis of 3511 cases and 2762 controls

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ABSTRACT

Published data regarding the association between 5,10-methylenetetrahydrofolate reductase (*MTHFR*) polymorphisms and prostate cancer risk have been conflicting. To derive a more precise estimation of the relationship, a meta-analysis was performed. Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated to assess the association between *MTHFR* C677T and A1298C polymorphisms and prostate cancer risk. Six studies including 3511 cases and 2762 controls described C677T genotypes, among which four articles totalling 838 cases and 1121 controls described A1298C genotypes, were involved in this meta-analysis. Overall meta-analysis indicated 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. No association was found for the 677CT genotype and the 677TT mutant homozygote with prostate cancer risk compared with 677CC, with OR = 1.13 (95% CI: 0.88–1.45) and OR = 0.85 (95% CI: 0.71–1.03), respectively. No evidence of an association of *MTHFR* A1298C polymorphism with prostate cancer was found. This meta-analysis supports that the C677T of the *MTHFR* gene is a low-penetrance susceptibility gene for prostate cancer, and might provide protective effects against prostate cancer risk.

1. Introduction

Prostate cancer is one of the most common malignant diseases among men. The mechanism of its carcinogenesis, like other cancers, still remains unclear. Folate metabolism is thought to play an important role in tumourigenesis through its involvement in both DNA methylation and DNA repair.

The 5,10-methylenetetrahydrofolate reductase (*MTHFR*) catalyses the irreversible conversion of 5,10-methylenetetrahydrofolate (required for purine and thymidine synthesis) to 5-methyltetrahydrofolate; this serves as the methyl donor for the remethylation of homocysteine to methionine (the precursor of S-adenosyl methionine, which is necessary for DNA methylation).[1]

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Two common *MTHFR* polymorphisms may be implicated in the cancer development, via alteration of *MTHFR* enzyme activity: C677T (Ala222Val) and A1298C (Glu429Ala). The mutant homozygous genotype 677TT has been reported to have about 30% of the *in vitro MTHFR* enzyme activity of the 677CC wild-type genotype, whereas the heterozygote 677CT genotype has about 65% of normal enzyme activity.[2] The *MTHFR* A1298C polymorphism also influences the specific activity of the enzyme, but to a lesser extent than the C677T polymorphism.[3,4]

It is speculated that lower *MTHFR* enzyme activity may increase cancer susceptibility via genome-wide hypomethylation. However, there is another postulation that activating the tumour suppressor gene *GSTP1* (glutathione-S-transferase) by reducing the hypermethylation of the CpG promoter sequences may have a protective effect against prostatic tumourigenesis, because the *GSTP1* gene is frequently silenced in prostate cancer cells.[5,6] In addition, a high concentration of 5,10-methylenetetrahydrofolate could maintain genetic stability by maintaining sufficient thymidylate, thus reducing DNA misrepair and chromosome breakage by uracil misincorporation.[7]

Previous studies had reported the role of *MTHFR* C677T and A1298C polymorphisms in prostate cancer risk but the observed associations were inconsistent and a single study may be too underpowered to detect a possible small effect of the polymorphisms on prostate cancer, especially when the sample size is relatively small. Hence, we performed a meta-analysis of all eligible studies to derive a more precise estimation of the associations of *MTHFR* C677T and A1298C polymorphisms with prostate cancer.

2. Materials and methods

2.1. Publication search

PubMed was searched (the last search update on the 10th July 2008) using the search terms: '*MTHFR*', 'polymorphism' and 'prostate or prostatic'. All studies matching the eligible criteria were retrieved, and bibliographies checked for other relevant publications. Review articles and bibliographies of other relevant studies identified were hand searched to identify additional studies. Only published studies with full text were included. When the same patient population was included in several publications, only the most recent or complete study was included in this meta-analysis.

2.2. Inclusion criteria

The inclusion criteria were (a) evaluation of the *MTHFR* C677T or A1298C polymorphism and prostate cancer risk, (b) case-control studies and (c) the size of the sample, odds ratios (ORs) and their 95% confidence intervals (CIs) or the information that can help infer the results in the papers.

2.3. Data extraction

Information was carefully extracted from all eligible publications independently by two investigators (Bai J and Xia X) according to the inclusion criteria listed above. The following data were collected from each study: first author's surname, publication date, country, ethnicity, study design, characteristics of controls, genotyping methods, total number of cases and controls, and numbers of cases and controls with *MTHFR* C677T and A1298C genotypes, respectively. For those studies that included subjects of different ethnic groups, data were extracted separately for each of the ethnic groups, categorised as Caucasians, Asian, African or Mixed (for those that included more than one ethnic group, such as both Caucasians and Asians); however, no Africans or Asians were identified in these studies. We did not define any minimum number of patients for including a study in our meta-analysis.

2.4. Statistical methods

Crude ORs with 95% CIs were calculated, according to the method of Woolf,[8] to assess the association of *MTHFR* C677T and A1298C polymorphisms with prostate cancer risk. The pooled ORs for *MTHFR* C677T genotypes CC, CT and C-allele carriers (CC + CT) against the TT genotype were calculated under a recessive model. Estimates for *MTHFR* A1298C genotypes CA, AA and A-allele carriers (CA + AA) against the CC genotype were also calculated. Heterogeneity assumption was checked by a chi-square-based Q-test.[9] A *P*-value of more than 0.10 for the Q-test indicated a lack of heterogeneity across the studies, so the pooled estimation of the ORs of each study was calculated by the fixed effects model (Mantel–Haenszel method). Otherwise, the random effects model (DerSimonian and Laird method) was used.[10] The significance of the pooled OR was determined by the *Z*-test, and $P < 0.05$ was considered as statistically significant. To evaluate the ethnic-specific effect, subgroup analysis was conducted on the basis of different ethnicities. One-way sensitivity analysis was performed to assess the stability of the results, namely, a single study in the meta-analysis was deleted each time to reflect the influence of the individual data set to the pooled OR.[11,12] An estimate of the potential publication bias was carried out by funnel plot, in which the standard error (SE) of log(OR) of each study was plotted against its log(OR). An asymmetric plot suggested a possible publication bias. The funnel plot asymmetry was assessed by Egger's test, a linear regression approach to measure funnel plot asymmetry on the natural logarithmic scale of the OR. The significance of the intercept was determined by the *t*-test suggested by Egger, $P < 0.05$ was considered representative of statistically significant publication bias.[13] Hardy–Weinberg equilibrium in the control group was tested by the chi-square test for goodness of fit, and a *P*-value of <0.05 was considered significant. All the statistical tests for our meta-analysis were performed with STATA version 9.2 (Stata Corporation, College Station, TX). All *P*-values were two-sided.

3. Results

3.1. Study characteristics

Seven papers were retrieved based on the search criteria for prostate cancer, and all of them met our inclusion criteria.[14–20] The basic information including authors and published years, country, ethnicity of the study populations,

study design, and the numbers of cases and controls of each study is listed in Table 1. Six papers stated matching conditions between the case and control populations. All the papers used blood samples for genotyping except one,[16] which used frozen tissue samples. PCR-RFLP was used to validate genotype in four papers, and Taqman SNP genotyping assay was used for the other three papers. All the papers provided data on Caucasians while Singal and colleagues reported on mixed ethnicities (noted as Black and White), but no detailed genotype distribution among these ethnicities was sought, thus ethnic subgroup analysis was not performed. Because of the inability to distinguish between racial/ethnic-specific allele frequencies/genotyping counts from 'Mixed' study populations, only Caucasians were analysed. Six eligible papers including 3511 cases and 2672 controls described C677T genotypes, among which four papers totalling 838 cases and 1121 controls described A1298C genotypes. The genotype frequencies of C667T and A1298C in the individual studies are also shown in Table 1. Overall, the prevalence of 677TT and 1298CC homozygosity was 10.03% and 10.79% in control subjects, respectively. Genotype distributions in the control populations in two studies significantly deviated from HWE.[18,20]

3.2. *Meta-analysis results*

No significant heterogeneity existed between the six studies upon comparing the *MTHFR* C677TT to the C allele in prostate cancer ($P = 0.38$). There was no evidence that the T allele resulted in an altered susceptibility to prostate cancer. The overall OR was 1.00 with 95% CI (0.92–1.08) by fixed effects (Fig. 1). Whereas there was heterogeneity among the six studies upon comparing the 677CT genotype versus the 677CC genotype ($P = 0.01$), thus a random effect model was adopted to pool the results. No association was found for the 677 CT genotype with prostate cancer risk (OR = 1.13, 95% CI: 0.88–1.45, Fig. 2). The 677TT mutant homozygote decreased the risk of prostate cancer compared with 677CC wild-type homozygote, but no significant association with prostate cancer susceptibility was observed using a fixed effects model ($P = 0.10$, OR = 0.85, 95% CI: 0.71–1.03; $P = 0.29$ for heterogeneity). No significant heterogeneity was found between the studies upon comparing the 677TT to 677CT together with 677CC (TT versus CT + CC) in prostate cancer ($P = 0.10$). The recessive genetic model by fixed effects suggested that the 677T allele was more likely to exert a protective effect on prostate cancer risk ($P = 0.03$, OR = 0.81, 95% CI: 0.68–0.98). The results of meta-analysis of four *MTHFR* C677T genetic models indicated that only the recessive genetic model was significant (Table 2).

Data on the *MTHFR* A1298C C to the A allele in prostate cancer were pooled with a fixed effects model ($P = 0.548$ for heterogeneity). We found no relationship between the C allele and the prostate cancer susceptibility ($P = 0.68$, OR = 1.03, 95% CI: 0.90–1.18, figure not shown). No heterogeneity was found among the studies when comparing the 1298AC or the 1298CC to the 1298AA genotype ($P = 0.42$ and 0.88, respectively). No association was found when fixed models were used to perform the comparisons, the OR was 1.10 ($P = 0.35$, 95% CI: 0.91–1.33) and 0.99 ($P = 0.95$, 95% CI: 0.72–1.35), respectively. The results of meta-analysis of four *MTHFR* A1298C

Table 1 – Main characteristics of all studies included in the meta-analysis.

Author, published year	Country	Ethnicity	Study design	Characteristics of controls	Genotyping methods	No. of cases/controls	C677T							A1298C						
							Cases (%)			Controls (%)			P_{HWE}	Cases (%)			Controls (%)			P_{HWE}
							CC	CT	TT	CC	CT	TT		AA	AC	CC	AA	AC	CC	
Kimura 2000	Germany	Caucasians	H	No matching	PCR-RFLP	132/150	49 (37.1)	67 (50.8)	16 (12.1)	26 (39.4)	36 (54.5)	4 (6.1)	0.06	–	–	–	–	–	–	
Cicek 2004	United States	Caucasians	F	Sibling	PCR-RFLP	439/479	214 (48.7)	182 (41.5)	43 (9.8)	219 (45.7)	199 (41.6)	61 (12.7)	0.14	195 (44.4)	205 (46.7)	39 (8.9)	233 (48.7)	201 (42.1)	44 (9.2)	0.94
Singal 2004	US	Mixed	H	BPH	PCR-RFLP	81/42	49 (60.5)	25 (30.9)	7 (8.6)	20 (47.6)	20 (47.6)	2 (4.8)	0.28	29 (35.8)	43 (53.1)	9 (11.1)	18 (42.8)	17 (40.5)	7 (16.7)	0.40
Van 2006	Sweden	Caucasians	P	1:2 matching	TaqMan	223/435	111 (49.8)	100 (44.8)	12 (5.4)	243 (55.9)	156 (35.9)	36 (8.3)	0.13	87 (39.2)	108 (48.6)	27 (12.2)	176 (40.5)	203 (46.8)	55 (12.7)	0.77
Marchal 2007	Spain	Caucasians	H	BPH	TaqMan	182/205	67 (36.8)	104 (57.1)	11 (6.1)	96 (47.1)	77 (37.7)	31 (15.2)	**0.02**	98 (55.4)	62 (35.0)	17 (9.6)	108 (51.7)	79 (37.8)	22 (10.5)	0.19
Johansson 2007	Sweden	Caucasians	P	Matching age region	TaqMan	2777/1639	1229 (50.4)	1025 (42.0)	209 (7.6)	801 (52.0)	612 (39.7)	128(8.3)	0.47	–	–	–	–	–	–	
Reljic 2007	Croatia	Caucasians	H	Matching age	PCR-RFLP	95/37	38 (40.0)	48 (50.5)	9 (9.5)	8 (21.6)	25 (67.6)	4 (10.8)	**0.03**	–	–	–	–	–	–	

BPH: benign prostatic hyperplasia; RFLP: restriction fragment length polymorphism; H: hospital based; F: family based; P: population based; P_{HWE}: P-value of Hardy–Weinberg equilibrium.

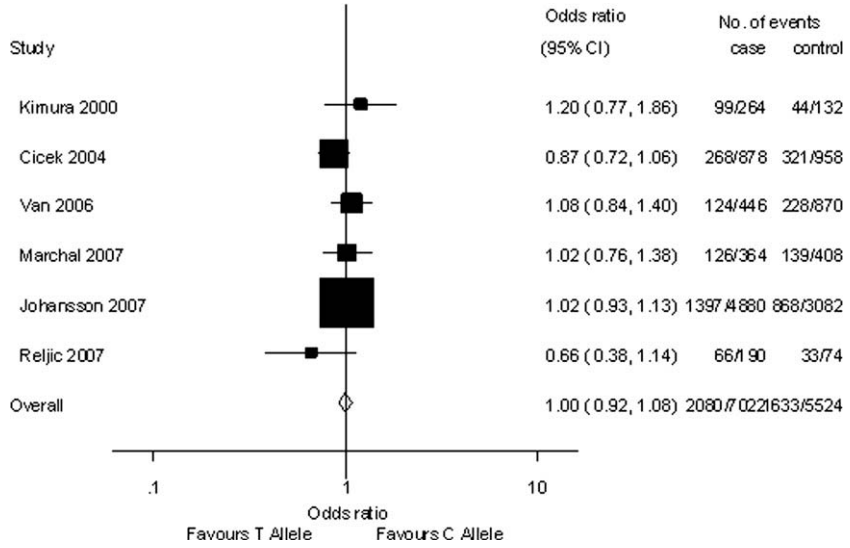

Fig. 1 – Forest plot of the odds ratios (ORs) and 95% confidence intervals (CIs) of studies of the association between the prostate cancer risk and the *MTHFR* C677T polymorphism (T allele versus C allele).

genetic models indicated that none of them were significant (Table 2).

3.3. *Sensitivity analysis*

Sensitivity analysis was performed by sequential omission of individual studies from various contrasts. For all the C677T genotype comparisons, the pooled 95% CIs were consistently covering 1.0 with sensitivity analysis. When individual studies were sequentially omitted under recessive contrasts, the pooled ORs were consistently below 1.0, indicating that the protective role exhibited by 677T against the prostate cancer risk when using a recessive genetic model is affected by this study. The associations of the A1298C polymorphisms with prostate cancer did not change during the sensitivity analysis.

3.4. *Bias diagnostics*

Begg's funnel plot and Egger's test were performed to assess the publication bias of the literature. The shapes of the funnel plots for the comparison of the 677T allele and the 677C allele seemed symmetrical in all comparing models (Fig. 3 shows Begg's funnel plot for T allele versus C allele). Furthermore, Egger's test was used to provide statistical evidence for funnel plot symmetry ($P = 0.63$). The results still did not suggest any evidence of publication bias ($P = 0.92$ for CT versus CC, $P = 0.55$ for TT versus CC, and $P = 0.70$ for TT versus CC + CT, respectively). Similarly, no publication bias was detected for associations of the A1298C polymorphisms with prostate cancer.

4. Discussion

In the recent years, interest in the genetic susceptibility to cancers has led to a growing attention to the study of polymorphisms of genes involved in tumourigenesis. The 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene has been widely studied, and the 677TT genotype was observed to reduce the risk of colorectal cancer,[21] acute lymphocytic leukaemia,[22] childhood leukaemia[23] and malignant lymphoma.[24] However, there has been a continuing debate over the protective effect of *MTHFR* 677T allele carrier against prostate cancer risk. Supportive evidence comes from a study of 81 patients with prostate cancer and 42 controls with benign prostatic hypertrophy,[16] though the differences fell within the realm of chance variation ($P > 0.05$). Interestingly, Heijmans and colleagues[25] reported a significant risk increase for 677TT genotype carriers in cancer overall (149 cancer cases, including 21 prostate cancer cases). Johansson and colleagues investigated 2777 incident prostate cancer cases and 1639 population controls from the CAncer Prostate in Sweden study (CAPS) and observed an increase in prostate cancer risk among men younger than 65 years, but the association was attributed to a deviation from Hardy–Weinberg equilibrium in the young controls. Most of these studies have generally been underpowered to draw a convincing conclusion.

In the present study, the mutant homozygote 677TT decreased the risk of prostate cancer but with borderline non-significance (OR = 1.03, 95% CI: 0.70–1.01), and the 677T allele exerted the reverse effect on prostate cancer risk in a recessive genetic model (OR = 0.81, 95% CI: 0.68–0.96). These results were similar to that observed by Hubner et al. in another meta-analysis, showing significantly reduced risk of colorectal cancer for the 677TT genotype (OR = 0.83, 95% CI = 0.75–0.93), but not for the 677CT genotype (OR = 0.99, 95% CI = 0.94–1.04).[26] Functional data revealed that 677CT and 677TT genotypes reduce enzymatic activity to 65% and 30%, respectively, compared to wild type,[2] and assuming a threshold of reduced enzyme activity somewhere between 30% and 65% at which the phenotype emerges, it implicates that the recessive genetic model seems biologically plausible. Our results indicated that the *MTHFR* 677TT genotype is associated with a modest, but significantly decreased risk of prostate cancer. Though the studies on *MTHFR* C677T and prostate

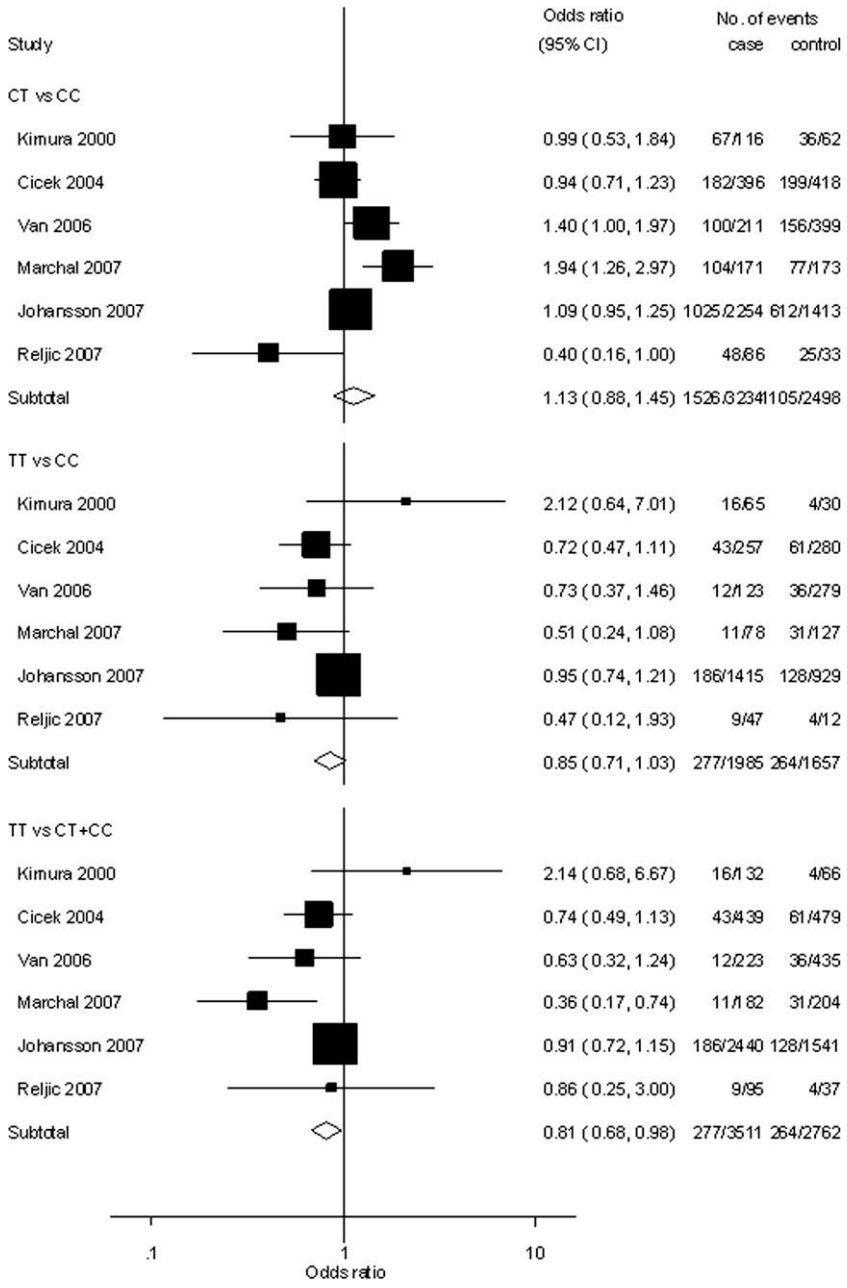

Fig. 2 – Forest plot of the odds ratios (ORs) and 95% confidence intervals (CIs) of studies of the association between prostate cancer and the *MTHFR* C677T polymorphism (CT versus CC, TT versus CC and TT versus CT + CC).

cancer are not rich enough to date, the functional data and findings observed on colorectal cancer support our results.

The tumour suppressor gene *GSTP1* is most frequently silenced in prostate cancer cells, and reducing the hypermethylation status of the CpG island sequences in the promoter region of *GSTP1* gene may have a protective effect against prostatic tumourigenesis.[5] The 677TT genotype, by reducing *MTHFR* enzyme activity, might reduce the synthesis of SAM, the only methyl donor for DNA methylation, thus allowing the expression of tumour suppressor genes and shrinking the risk of cancer development. Meanwhile, it would also decrease the possibility of insufficient thymidylate synthesis and uracil misincorporation, ensuring the stability of chromosome structure. Furthermore, *in vitro* experiments proved that *MTHFR* inhibition arrests the growth of cancer cells because of limited methionine supply.[27]

In this pooled analysis, a total of six studies with 3511 cases and 2762 controls were included. The genotypes among controls were in Hardy–Weinberg equilibrium (HWE) for four studies. A test for HWE often assumes that the genotypes are a random sample from the large, randomly mating populations. Genotyping errors can distort genotype distribution and can lead to departure from HWE. But for the two studies of HWE,[18,20] genotyping error was minimised by strict quality control. Therefore, in the final analysis, we did not exclude these two studies. Furthermore, we further performed sensitivity analysis to detect the stability of the meta-analysis, and the results did not alter the pattern of association and

Table 2 – The results of meta-analysis of four genetic models of the association between prostate cancer and the MTHFR polymorphisms (odds ratio (OR) and confidence interval (CI)).

SNPs	Genetic models		OR 95% CI	P	P(Q-test) for heterogeneity
C677T					
	Co-dominant model	CT versus CC	1.13(0.88, 1.45)	0.33	0.01
		TT versus CC	0.85(0.71, 1.03)	0.10	0.29
	Dominant model	TT + CT versus CC	1.07(0.88, 1.30)	0.50	0.06
	Recessive model	TT versus CT + CC	0.81(0.68, 0.98)	**0.03**	0.10
	Additive model	CC is reference	1.00(0.92, 1.08)	0.98	0.33
A1298C					
	Co-dominant model	AC versus AA	1.10(0.91, 1.33)	0.35	0.42
		CC versus AA	0.99(0.72, 1.35)	0.95	0.88
	Dominant model	CC + AC versus AA	1.08(0.90, 1.29)	0.44	0.42
	Recessive model	CC versus AC + AA	0.95(0.70, 1.28)	0.72	0.99
	Additive model	AA is reference	1.03(0.90, 1.18)	0.67	0.55

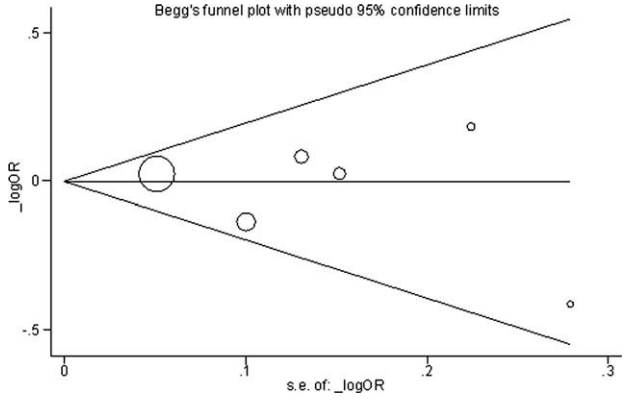

Fig. 3 – Begg's funnel plot (using OR of prostate cancer risk in 677T allele when compared to 677C allele). The horizontal line represents the meta-analysis summary estimate, and the diagonal lines pseudo-95% CI limits about the effect estimate. In the absence of publication bias, studies will be distributed symmetrically above and below the horizontal line. Asymmetry on the top of the graph indicates evidence of publication bias towards studies reporting a positive log OR (increased prostate cancer risk with T allele). log OR, natural logarithm of the OR; s.e. of log OR, standard error of the log OR.

revealed that the protective effect of 677TT genotype was stable. In addition, publication bias was not detected for both C677T and A1298C, which indicated that our findings seemed not to be due to biased publications.

Prostate cancer, as a complex disease, was considered as the result of combined effects of multi-factors, including inherited and environmental factors. Some studies observed significant interactions between *MTHFR* C677T genotypes and other factors on cancer risk, such as alcohol drinking.[28] Unfortunately, no study focused on the gene–gene and gene–environment interactions with prostate cancer risk. In light of the potentially combined effects of the folate intake factors and other genes involved in the folate metabolism pathway, further studies should include these factors and detect the potential interactions between *MTHFR* C677T and these factors.

We did not find any relation between the risk of prostate cancer and *MTHFR* A1298C polymorphism, either due to the minor effect of A1298C on the enzyme activity, or to the relatively small sample size.

Some limitations of this meta-analysis should be acknowledged. Firstly, the controls were not uniform. Healthy populations, as well as benign prostatic hyperplasia patients and siblings, were included. Some individuals in the control group are likely to develop prostate cancer (hereditary, familial or sporadic) in subsequent years though they had no clinical symptoms at the time of investigation. Misclassification bias results in deviation of genotype distribution in the controls. Secondly, disease progression status was not taken into account. Cicek and colleagues[15] found no association overall, but an increased risk for less advanced prostate cancers and a decreased risk for more advanced ones. Nevertheless, not every single study provided disease degrees. Thirdly, folate intake, which is another condition that is not available from the included studies, is probably an important factor affecting the function of the *MTHFR* 677 polymorphism on prostate cancer risk. Heijmans and colleagues investigated dietary folate intake as a potential effect modifier in overall cancer—but not prostate cancer exclusively—and found a higher relative risk for TT heterozygotes in the group of low dietary intake compared to the group with high folate intake. Finally, only studies on Caucasians (except for one study on mixed populations) were obtained via our search strategy, which is not representative of all ethnicities.

Despite some limitations, this meta-analysis supports that the *MTHFR* 677T allele exerts a protective effect in the development of prostate cancer in Caucasians. However, large trials using standardised unbiased methods, enrolling precisely defined prostate cancer patients and well-matched controls, with more detailed individual data are needed. Moreover, gene–gene and gene–environment interactions should also be considered.

Conflict of interest statement

None declared.

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