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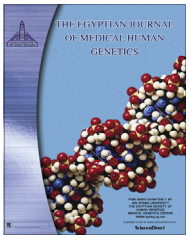

REVIEW

Role of *MTHFR* A1298C gene polymorphism in the etiology of prostate cancer: A systematic review and updated meta-analysis

Upendra Yadav, Pradeep Kumar, Vandana Rai *

Human Molecular Genetics Laboratory, Department of Biotechnology, VBS Purvanchal University, Jaunpur 222 003, UP, India

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Meta-analysis

Abstract Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme of folate/homocysteine pathway and is essential for synthesis, repair and methylation of DNA. Various studies have performed to evaluate the role of *MTHFR* A1298C gene polymorphism to the risk of prostate cancer and the results were inconclusive and inconsistent. A meta-analysis of published case-control studies, up to December 2014, was performed to investigate the association between *MTHFR* A1298C gene polymorphism and the susceptibility of prostate cancer. PubMed, Science direct, Springer link and Google scholar databases were searched for case-control studies and crude odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to estimate the strength of association. The analyses were conducted with Open Meta-Analyst and MIX softwares. Total thirteen case-control studies with 4673 prostate cancer patients and 6982 controls were included in this meta-analysis. No associations were observed between *MTHFR* A1298C gene polymorphism and prostate cancer in any genetic model (allele contrast (C vs. A): OR = 1.01; 95% CI: 0.91–1.13; $p = 0.73$; dominant model (CC + AC vs. AA): OR = 0.98, 95% CI = 0.91–1.06, $p = 0.73$; homozygote model (CC vs. AA): OR = 0.96, 95% CI = 0.83–1.10, $p = 0.55$; co-dominant model (AC vs. AA): OR = 0.98, 95% CI = 0.91–1.07, $p = 0.76$; and recessive model (CC vs. AC + AA): OR = 0.96, 95% CI = 0.84–1.10, $p = 0.61$). Moreover, when the data were stratified on the basis of ethnicity no significant associations were observed. The results of the present meta-analysis suggest that the *MTHFR* A1298C gene polymorphism has no effect on the etiology of prostate cancer.

* Corresponding author. Tel.: +91 05452 252538; fax: +91 05452 252244.
E-mail address: raivandana@rediffmail.com (V. Rai).
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Contents

1. Introduction

Prostate cancer (PCa) is the second most common cancer in men with an estimated 1.1 million new PCa cases and 0.30 million deaths in 2012 [1]. The estimated age standardized rate for PCa is 30.7 per 100,000 and it is more prevalent in developed regions (68.0) then less developed regions (14.5) [1]. PCa is a slow-growing cancer and remains localized at first and later due to the abnormal proliferation of prostatic tissue cells it may extend as it spreads to nearby tissues and organs and then metastasizes. Although it is one of the most common types of cancer its causes are least understood. Several risk factors have been reported for PCa like age, race, family history of cancer, smoking, alcohol intake etc [2].

Genome–environment interaction and genetic susceptibility were evaluated for cancer risk. Folic acid is essential for DNA synthesis, repair and methylation and several studies reported enzyme variants of folate–methionine pathway as risk factor for carcinogenesis. Methylenetetrahydrofolate reductase (MTHFR) plays an important role in the metabolism of folic acid/homocysteine pathway by converting 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, which donates a methyl group for remethylation of homocysteine to methionine [3]. *MTHFR* gene has 11 exons and located at chromosome 1p36.3. A number of SNPs are reported in this gene. Clinically important one of them is A1298C (rs1801131) which is located at exon 7 and results in a glutamate to alanine substitution [4]. The frequency of mutant allele (C) greatly differs in different populations. The prevalence of the A1298C homozygote genotype (CC) ranges from 1% to 4% in Chinese, 4% to 5% in Hispanics, and 7% to 12% in North American and European populations [5,6].

MTHFR A1298C gene polymorphism is associated with a number of diseases like Down syndrome [7], schizophrenia [8], neural tube defects [9], orofacial clefts [10], nonsyndromic cleft lip and palate [11] etc. Contrary reports were published regarding *MTHFR* polymorphism as a risk factor for cancer, i.e. positive association [12] and negative association [13–15] have been continuously reported in different types of cancers. *MTHFR* A1298C polymorphism has also been investigated to assess the risk of PCa but the results were inconclusive [16–21]. These inconsistent results might be due to differences in ethnicity, genotyping methods and small sample sizes in individual studies. Hence we decided to carry out an updated meta-analysis to shed some light on this controversial association.

2. Materials and methods

2.1. Selection of studies

PubMed, Science direct, Springer link and Google scholar databases were searched for "prostate cancer" with the combination of following keywords – "methylenetetrahydrofolate reductase", "MTHFR", and "rs1801131". Included papers were further searched manually for additional studies. The databases were searched up to March, 2015.

2.2. Inclusion and exclusion criteria

The inclusion criteria were as follows – (i) published studies; (ii) case-control study; (iii) distribution of genotypes in cases and controls were reported in the publication. The following exclusion criteria were studies – (i) not in English language; (ii) that contained duplicate data; and (iii) case reports, letter to editor, book chapters or reviews.

2.3. Data extraction

The following data were extracted from each study-year of publication, first author's name, country of study, ethnicity, and frequency of C allele in controls for detection of Hardy–Weinberg equilibrium. The AA, AC and CC genotype numbers in the case and control groups were extracted from each included study to calculate odds ratio (OR) and 95% confidence interval (95% CI).

2.4. Meta-analysis

We calculated the pooled OR with 95% CI to investigate the association between *MTHFR* A1298C gene polymorphism and risk of PCa for the allele contrast (C vs. A), dominant (CC and AC vs. AA), homozygote (CC vs. AA), co-dominant (AC vs. AA), and recessive (CC vs. AC and AA) models. The Q statistic was used to test for heterogeneity ($p < 0.05$ was considered as statistically significant heterogeneity) and I^2 statistic was used to quantify the inconsistency between study estimates. I^2 ranges between 0% and 100%, and I^2 values of 25%, 50% and 75% were defined as low, moderate and high estimates of heterogeneity, respectively. When a significant Q-test ($p < 0.05$) or $I^2 > 50\%$, indicated heterogeneity across studies, the random effects model [22] was used or else fixed effects model was applied [23]. Subgroup analysis was conducted on the basis of ethnicity. Publication bias was assessed by the symmetry of the funnel plot. An asymmetrical funnel plot suggested publication biasness. Funnel plot asymmetry was further evaluated by the Egger's linear regression method [24]. Statistical analyses were done with Open Meta-Analyst [25] and MIX 1.7 [26]. All p-values were two-tailed with a significance level at 0.05.

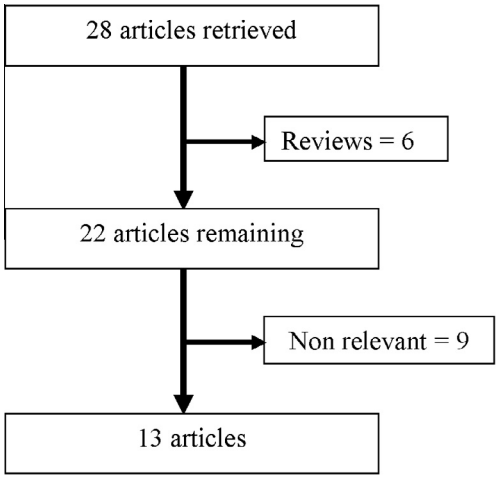

Figure 1 Flow diagram of study search and selection process.

3. Results

3.1. Eligible studies

Fig. 1 presents the flow diagram of the retrieved studies and the studies excluded, with specifying reasons and the information extracted from the studies included in the meta-analysis is provided in Table 1. On the basis of our predefined eligibility criteria thirteen studies were found eligible to include in this meta-analysis [16–21,27–33] which consists of 4673 and 6982 cases and controls respectively.

3.2. Characteristics of included studies

Among the thirteen studies, three were from Asian populations [18,19,32], five were form Caucasian populations [16,17,29–31], and the remaining five were of mixed ethnicities [20,21,27,28,33]. In thirteen studies included in the present meta-analysis, the smallest case sample was 55 [33] and the highest sample size was 1599 [17]. Control populations in all the studies were in Hardy–Weinberg equilibrium except three studies [20,21,31].

In all thirteen studies the numbers of cases were 4673 with AA (2347), AC (1899), and CC (427) and controls were AA (3,461), AC (2856), and CC (665). In cases the percentages for genotypes were 50.22% for AA, 40.64% for AC and 9.14% for CC. Similarly for controls these values were 49.57%, 40.91% and 9.52% for AA, AC and CC genotype. All included studies have sufficient information to calculate the five genetic models-allele contrast (C vs. A), dominant (CC + AC vs. AA), homozygote (CC vs. AA), co-dominant (AC vs. AA), and recessive (CC vs. AC + AA) to evaluate A1298C polymorphism as PCa risk (Table 2).

3.3. Meta-analysis

Meta-analysis with allele contrast showed insignificant association with random effect model ($OR_{CvsA} = 1.01$; 95% CI: 0.91–1.13; $p = 0.73$; $I^2 = 54\%$; $P_{heterogeneity} = 0.01$) (Fig. 2). Moderate heterogeneity was found so random effect model was applied. Low heterogeneity was found in all the other four genetic models, so fixed effect model was applied. No association between the *MTHFR* A1298C gene polymorphism and

Table 1 Characteristics of the eligible studies considered in the meta-analysis.

SN	Author	Ethnicity	Country	No. of controls	No. of cases	Years
1	Cicek et al. (2004)	Mixed	USA	479	439	2004
2	Singal et al. (2004)	Mixed	USA	42	81	2004
3	Van Guelpen et al. (2006)	Caucasian	Sweden	617	299	2006
4	Marchal et al. (2008)	Caucasian	Spain	205	182	2008
5	Stevens et al. (2008)	Caucasian	USA	1107	1100	2008
6	Collin et al. (2009)	Caucasian	UK	2084	1599	2009
7	Muslumanoglu et al. (2009)	Caucasian	Turkey	166	93	2009
8	Cai et al. (2010)	Asian	China	220	217	2010
9	Safarinejad et al. (2010)	Asian	Iran	348	174	2010
10	Wu et al., 2010	Asian	Taiwan	436	218	2010
11	Vidal et al. (2012)	Caucasian	USA	192	55	2012
12	Jackson et al. (2013)	Mixed	West Indies	273	243	2013
13	López-Cortés et al. (2013)	Mixed	Equador	110	104	2013

Table 2 The distributions of *MTHFR* A1298C genotypes and allele frequencies for prostate cancer cases and controls.

Study ID	Genotype						Alleles				HWE
	AA		AC		CC		A		C		
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	
Cicek et al. (2004)	195	233	205	201	39	44	595	667	283	289	0.94
Singal et al. (2004)	29	18	43	17	9	7	101	53	61	31	0.39
Van Guelpen et al. (2006)	87	176	108	203	27	55	282	555	162	313	0.76
Marchal et al. (2008)	98	108	62	79	17	22	258	295	96	123	0.19
Stevens et al. (2008)	481	491	518	493	105	125	1480	1475	728	743	0.94
Collin et al. (2009)	775	1407	673	1339	144	289	2223	4153	961	1917	0.24
Muslumanoglu et al. (2009)	31	77	16	45	44	44	78	199	104	133	0.00
Cai et al. (2010)	150	144	63	71	4	5	363	359	71	81	0.27
Safarinejad et al. (2010)	90	158	70	150	14	40	250	466	98	230	0.62
Wu et al. (2010)	138	287	70	135	10	14	346	709	90	163	0.69
Vidal et al. (2012)	36	103	17	79	2	11	89	285	21	101	0.40
Jackson et al. (2013)	137	151	52	43	10	8	326	345	72	59	0.03
Lopez-Cortes et al. (2013)	100	108	2	1	2	1	202	217	6	3	0.00
Total	2347	3461	1899	2856	427	665	6593	9778	2753	4186	
Percentage	50.22	49.57	40.64	40.91	9.14	9.52					

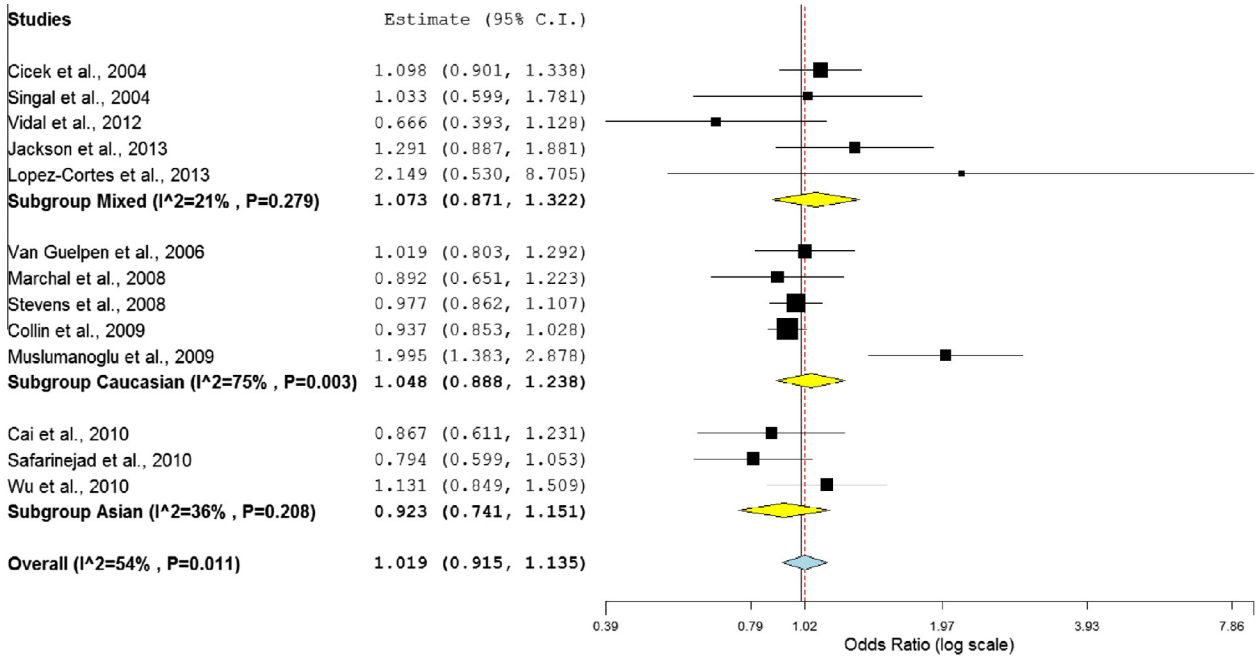

Figure 2 Random effect Forest plot of allele contrast model (C vs. A) of *MTHFR* A1298C polymorphism.

PCa was found in any other genetic models (Table 3). In homozygote model (CC vs. AA): OR = 0.96, 95% CI = 0.83–1.10, $p = 0.55$ (Fig. 3); for dominant model (CC + AC vs. AA) OR = 0.98, 95% CI = 0.91–1.06, $p = 0.73$ (Fig. 4); for AC vs. AA (co-dominant model): OR = 0.98, 95% CI = 0.91–1.07, $p = 0.76$; for CC vs. AC + AA (recessive model): OR = 0.96 (95% CI = 0.84–1.10, $p = 0.61$).

The sub-group analyses were conducted on the basis of ethnicity (Asian, Caucasian and Mixed). Low heterogeneity was observed in Asian and mixed studies but high heterogeneity was found in Caucasian studies. No significant results were found in any sub-group in any genetic models (Table 3).

3.4. Sensitivity analyses

Sensitivity analysis was performed on the basis of deviation of the control samples from HWE ($p < 0.05$). The control samples of three studies [20,21,31] were deviated from the HWE. Sensitivity analysis was performed after removal of these studies and no significant association was found in the main analysis ($OR_{CvsA} = 0.95$; 95% CI: 0.90–1.01; $p = 0.16$; $I^2 = 0\%$) or in any sub-groups-Asian ($OR_{CvsA} = 0.92$; 95% CI: 0.77–1.09; $p = 0.36$; $I^2 = 36\%$); Caucasian ($OR_{CvsA} = 0.95$; 95% CI: 0.88–1.02; $p = 0.17$; $I^2 = 0\%$); and mixed ($OR_{CvsA} = 1.02$; 95% CI: 0.86–1.22; $p = 0.75$; $I^2 = 34\%$). Moreover,

Table 3 Summary estimates for the odds ratio (OR) of *MTHFR* A1298C in various allele/genotype contrasts, the significance level (*p* value) of heterogeneity test (*Q* test), and the I^2 metric: overall analysis, and subgroup analyses.

	Genetic contrast	Fixed effect	Random effect OR (95% CI), *p*	Heterogeneity *p*-value (*Q* test)	I^2 (%)	Publication bias (*p* of Egger's test)
All (13 studies)	Allele contrast (C vs. A)	0.98 (0.92–1.04), 0.62	1.01 (0.91–1.13), 0.73	0.01	54	0.3
	Dominant (CC + AC vs. AA)	0.98 (0.91–1.06), 0.73	1.00 (0.90–1.12), 0.86	0.15	28	0.29
	Homozygote (CC vs. AA)	0.96 (0.83–1.10), 0.55	0.99 (0.82–1.19), 0.94	0.20	24	0.49
	Co-dominant (AC vs. AA)	0.98 (0.91–1.07), 0.76	0.99 (0.91–1.07), 0.81	0.42	2	0.58
	Recessive (AA + AC vs. CC)	0.96 (0.84–1.10), 0.61	1.00 (0.82–1.22), 0.98	0.10	34	0.6
Asian (3 studies)	Allele contrast (C vs. A)	0.92 (0.77–1.09), 0.36	0.92 (0.74–1.15), 0.47	0.20	36	0.9
	Dominant (CC + AC vs. AA)	0.91 (0.74–1.13), 0.40	0.91 (0.73–1.14), 0.43	0.32	11	0.52
	Homozygote (CC vs. AA)	0.83 (0.51–1.34), 0.46	0.86 (0.48–1.55), 0.63	0.26	25	0.83
	Co-dominant (AC vs. AA)	0.92 (0.73–1.14), 0.46	0.92 (0.74–1.14), 0.46	0.53	0	0.36
	Recessive (AA + AC vs. CC)	0.87 (0.54–1.39), 0.56	0.88 (0.54–1.44), 0.63	0.35	4	0.81
Caucasian (5 studies)	Allele contrast (C vs. A)	0.97 (0.91–1.04), 0.52	1.04 (0.88–1.23), 0.57	0.003	75	0.26
	Dominant (CC + AC vs. AA)	0.96 (0.88–1.06), 0.50	0.99 (0.86–1.13), 0.93	0.18	35	0.26
	Homozygote (CC vs. AA)	0.96 (0.82–1.12), 0.61	1.04 (0.77–1.41), 0.77	0.02	64	0.36
	Co-dominant (AC vs. AA)	0.96 (0.87–1.06), 0.47	0.96 (0.87–1.06), 0.47	0.59	0	09
	Recessive (AA + AC vs. CC)	0.98 (0.84–1.13), 0.79	1.07 (0.77–1.48), 0.65	0.006	72	0.42
Mixed (5 studies)	Allele contrast (C vs. A)	1.08 (0.92–1.26), 0.33	1.07 (0.87–1.32), 0.50	0.27	21	0.99
	Dominant (CC + AC vs. AA)	1.15 (0.94–1.40), 0.16	1.13 (0.86–1.49), 0.34	0.25	25	0.97
	Homozygote (CC vs. AA)	1.03 (0.71–1.51), 0.84	1.04 (0.71–1.52), 0.82	0.79	0	0.9
	Co-dominant (AC vs. AA)	1.17 (0.95–1.45), 0.13	1.16 (0.89–1.52), 0.26	0.29	19	0.99
	Recessive (AA + AC vs. CC)	0.94 (0.65–1.36), 0.77	0.94 (0.65–1.36), 0.77	0.79	0	0.96

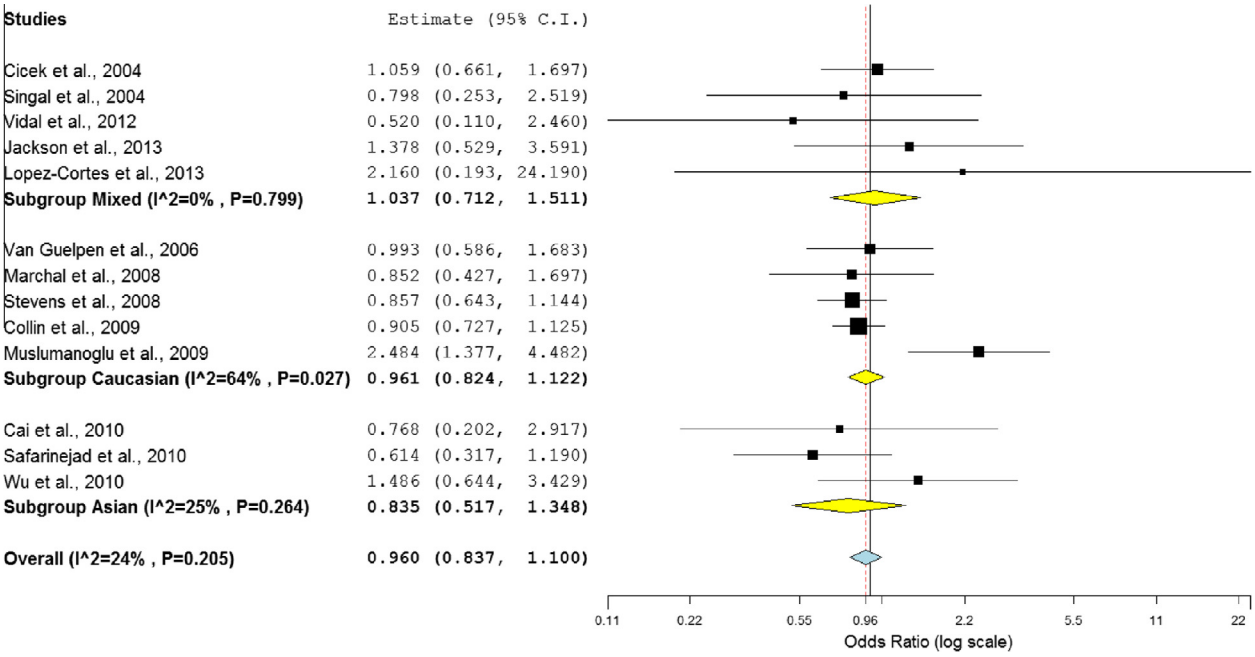

Figure 3 Fixed effect Forest plot of homozygote model (CC vs. AA) of *MTHFR* A1298C polymorphism.

when these studies (deviated from HWE) were removed from the analysis then the between study heterogeneity decreases both in the overall and sub-group meta-analysis.

3.5. Publication bias

The funnel plots are symmetrical for all genetic models in either overall or sub-group meta-analyses. Moreover, Egger's test reveals no evidence of publication bias in any genetic model in both the main and sub-group meta-analysis (Fig. 5).

4. Discussion

MTHFR enzyme is crucial for folate metabolic pathway because it is involved in two important pathways-DNA methylation and purines and thymidine synthesis. *MTHFR* gene

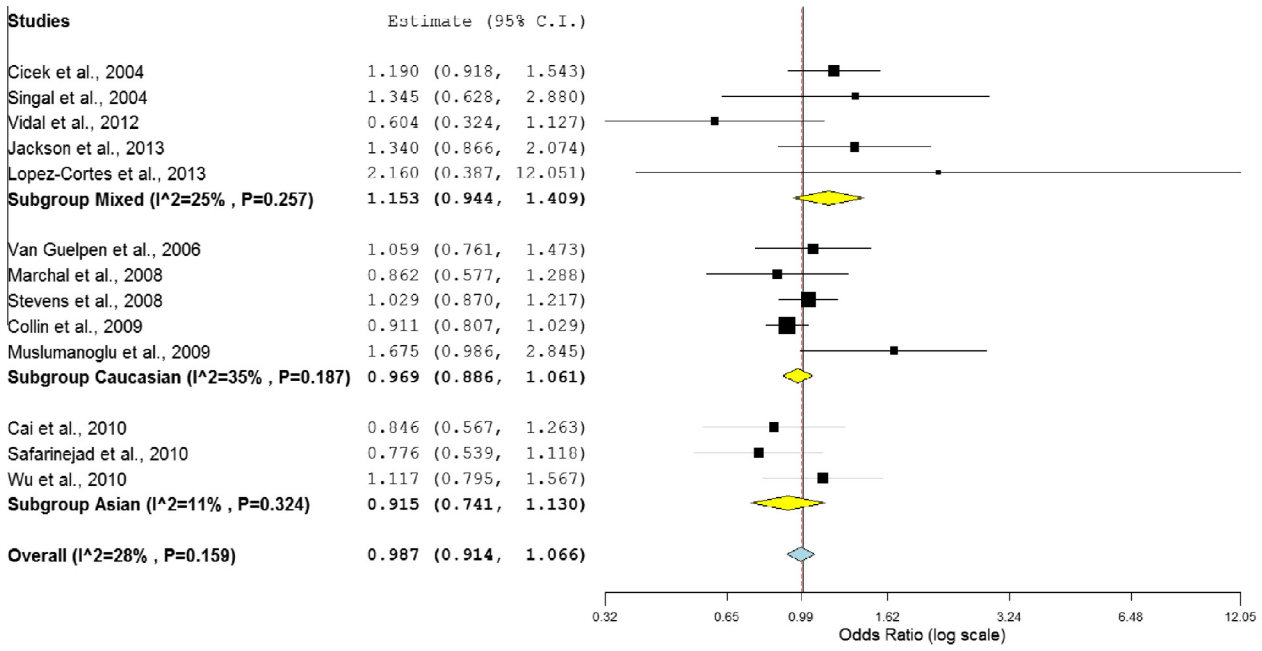

Figure 4 Fixed effect Forest plot of homozygote model (CC + AC vs. AA) of *MTHFR* A1298C polymorphism.

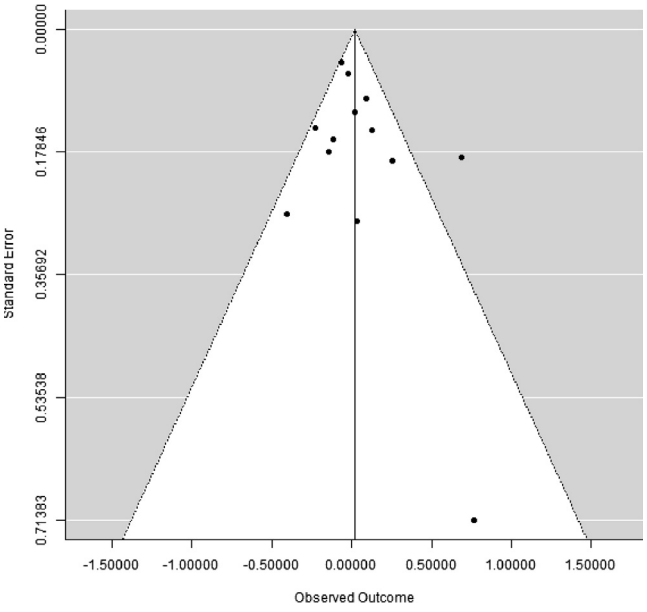

Figure 5 Funnel plots of standard error by OR of *MTHFR* A1298C allele contrast model (C vs. A).

polymorphisms have been extensively studied as increased/reduced risk factor for different types of cancers and it was observed that these polymorphisms increased the risk for breast [34], cervical [35], and esophageal cancer [36] as well as reduced the risk of colorectal cancer [37], non-Hodkin's lymphoma [38] and childhood acute leukemia [39]. *MTHFR* gene polymorphisms confer increased/reduced risk of cancer by two mechanisms, both mechanisms oppose one another. Low activity of MTHFR variant enzyme causes DNA hypomethylation and uracil misincorporation into DNA [40,41]. These two events resulted into abnormal gene expression, increased DNA break/damage and reduced DNA repair and consequently increased risk of carcinogenesis. On the other hand, reduced MTHFR activity increased the availability of 5,10-methylenetetrahydrofolate which donates the methyl group for the synthesis of deoxythymidylate (dTMP) from deoxyuridylate (dUMP). Increased 5,10-methylenetetrahydrofolate increased the synthesis of dTMP and reduced incorporation of uridine in DNA and protects cell against carcinogenesis [19,42].

During past decade several meta-analyses were published assessing *MTHFR* A1298C gene polymorphism as a risk factor to various diseases/disorders like NTD [43], recurrent pregnancy loss [44], Down syndrome [45], nonsyndromic cleft lip and palate [46], and cancer [47,48] etc. Four meta-analyses were published regarding *MTHFR* A1298C polymorphism and PCa risk [17,47,49,50]. Bai et al. [49] performed a meta-analysis with only four studies including 838 cases and 1121 controls and found no statistical significant association. In another meta-analysis published in the same year Collin et al. [17] reported no association of *MTHFR* A1298C gene polymorphism with PCa. This meta-analysis included 5 studies comprising 3176 cases and 4829 controls. Out of the 5 studies three were Caucasian and two were of mixed ethnicity. The major limitation of both these meta-analyses was that they included only Caucasian and mixed ethnicity samples as up to 2010 no study was published from the Asian population. In a recent meta-analysis performed by Li et al. [47] a total of nine case-control studies were included consisting of 2723 cases and 3442 controls. Out of nine studies four were from Caucasians, three from mixed and two from Asian ethnicities but they did not find any statistically significant association. No association was found in another study published in 2012 by Li and Xu [50] with the same papers as in Li et al. [47].

The last meta-analyses were published in 2012 after that several case-control studies were published. These studies were

not included in the previous meta-analyses. So we conducted a comprehensive meta-analysis with the largest number of studies (13 studies) and the highest number of samples (11,655) to date to investigate the possible association between *MTHFR* A1298C polymorphism and the risk of PCa. No statistically significant association was observed in either main analysis or sub-group analysis which was based on ethnicity. The results of the present meta-analysis support the results of the previous meta-analyses that *MTHFR* A1298C gene polymorphism has no role in the etiology of PCa. Also on the stratification of data on the basis of ethnicity, no significant association was observed between the *MTHFR* A1298C gene polymorphism and PCa either in overall or in any sub-group (Asian, Caucasian or mixed) populations.

The main strengths of our meta-analysis were absence of publication bias and pooled number of cases and controls from different studies which significantly increased the power of the study. Present meta-analysis also has some limitations which must be acknowledged like – (i) crude odds ratio was used, (ii) meta-analysis was restricted to only one polymorphism (A1298C), and (iii) except genetic polymorphism other important environmental factors were not considered.

5. Conclusion

In conclusion, the present study did not support any association between *MTHFR* A1298C gene polymorphism and PCa in total and stratified populations. For future case-control studies gene–gene and gene–environment interactions should also be considered which might well elucidate genetics of PCa.

Conflict of interest

The authors have no conflict of interest to declare.

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