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Review Article

Methylenetetrahydrofolate Reductase (MTHFR) A1298C Polymorphism and Risk of Lung Cancer

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Abstract

Recent epidemiological studies have reported association between Methylenetetrahydrofolate Reductase (MTHFR) gene polymorphism and lung cancer. The aim of the present study to perform a meta-analysis of published studies to validate the association between MTHFR A1298C polymorphism and risk of lung cancer.

PubMed, Springer Link. Elsevier and Google Scholar databases were searched for eligible studies. Of the 78 initially identified studies, 11 case–control studies with 5,996 patients and 7,404 healthy controls were finally included in the present meta-analysis. Odds ratios (ORs) with 95% Confidence Intervals (CIs) were estimated to assess the association, and meta-analysis was performed using MIX software (Version 1.7).

No statistically significant associations were found between the MTHFR A1298C polymorphism and lung cancer risk in the genetic additive, co-dominant, homozygote, dominant and recessive models (C *vs.* A: OR= 0.95, 95% CI= 0.83-1.08; CC *vs.* AA: OR= 1.13, 95% CI= 0.83-1.5; AC *vs.* AA: OR= 0.86, 95% CI= 0.70-1.02; AC+CC *vs.* AA: OR= 0.89, 95% CI= 0.75-1.05; CC *vs.* AA+AC: OR= 1.20, 95% CI= 0.89-1.40). A significant heterogeneity between individual studies was evident in all five models. In conclusion, present meta-analysis results indicated that there in no significant association between MTHFR A1298C polymorphism and risk of lung cancer.

Keywords: Methylenetetrahydrofolate Reductase; Lung Cancer; MTHFR; A1298C; Meta-analysis; Polymorphism

Introduction

Lung cancer is the leading cause of cancer-related death worldwide. The incidence and mortality of lung cancer have been significantly and constantly increasing [1-3]. Lung cancer is still the most common cancer in men worldwide (1.1 million cases, 16.5% of the total), with high rates in Central-eastern and Southern Europe, Northern America and Eastern Asia. Very low rates are still estimated in Middle and Western Africa (2.8 and 3.1 per 100,000, respectively) [4]. Lung cancer is a common disease that results from a complex interplay of genetic and environmental risk factors [5]. Many epidemiological studies have provided evidence that high consumption of vegetables and fruits is associated with a reduced risk of lung cancer [6]. Folate is one of the constituents found in vegetables and fruits, and dietary folate may be one of the micronutrients that provide protection against lung carcinogenesis [6].

5,10-Methyl Enetetrahydrofolate Reductase (MTHFR) gene (OMIM*607093; chromosome 1p36.3) is an important enzyme involved in folate metabolism and is thought to influence DNA methylation and nucleotide synthesis. The low enzymatic activity of the MTHFR C677T genotypic variant is associated with DNA hypomethylation, which may induce genomic instability or randomly reactivates the proto-oncogenes to oncogenes [7]. Two common and clinically important polymorphisms (C677T and A1298C) identified in the MTHFR gene [8-10]. Substitution at nucleotide 1,298 (exon 7) results in an amino acid substitution of glutamate for alanine at codon 429 [11]. A1298C (glutamate to alanine) polymorphism, has been associated with decreased enzyme activity (40%), although to a lesser extent than C677T [9]. A1298C allele frequency differs greatly in various ethnic groups of the world. The prevalence of the A1298C homozygote variant genotype ranges from 7 to 12% in White populations from North America and Europe. Lower frequencies have been reported in Hispanics (4 to 5 %), Chinese (1 to 4 %) and Asian populations (1 to 4%) [12,13].

To date, several studies have shown that the MTHFR A1298C polymorphism are associated with either increased or decreased risk of lung cancer, whereas others observed no association between the MTHFR A1298C genotype and lung cancer. Small sample size, various ethnic groups, diet, environment, and methodologies might be responsible for the discrepancy. Therefore, a meta-analysis is required to evaluate MTHFR A1298C polymorphism as risk factor for lung cancer.

Methods

Present meta-analysis was conducted according to Moose guidelines. PubMed, Google Scholar, Springer Link and Elsevier database s were searched for eligible studies. The last search was conducted on January 20, 2014. Following terms were used for search: 'Methylenetertahydrofolate reductase', 'MTHFR', 'A1298C', and 'lung cancer'.

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Table 1: Characteristics of eleven studies included in the present meta-analysis.

Study	Ethnicity	Control	Case	Reference
Shen et al., 2001	Non-Hispanic White	554	550	Can Epidemiol Biomarkers Prev 10:397-401
Siemianowicz et al., 2003	Europen	44	146	Oncol Rep, 10: 1341-4
Shen et al., 2005	Asian	49	114	Lung Cnacer, 49:299-309
Shi et al., 2005	Non-Hispanic White	1141	1051	Can Epidemiol Biomarkers Prev 14:1477-84
Zhang et al., 2005	Asian	400	505	Acta Acad Med Sin 27:700-703
Hung et al., 2007	Europen	2865	2209	Carcinogenesis 28:1334–1340
Suzuki et al., 2007	Asian	1019	485	Carcinogenesis 28:1718–1725
Liu et al., 2009	Asian	716	358	Can. Geno & Proteo 6: 325-330
Arslan et al., 2011	Europen	61	64	Mol Biol Rep (2011) 38:991–996
Kiyoaha et al., 2011	Asian	379	462	BMC Cancer 2011, 11:459
Ozen et al., 2013	European	176	52	Asian Pac J Cancer Prev, 14 (9):5449-5454

Table 2: The distributions of MTHFR A1298C genotypes and alleles number for lung cancer cases and controls.

Study ID	Genotype						Alleles			
	AA		AC		CC		A		C	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Shen, 2001	261	265	246	249	43	40	768	779	332	329
Siemianowicz, 2003	32	12	76	24	38	8	140	48	152	40
Shen, 2005	71	9	41	34	2	6	183	52	45	46
Shi, 2005	480	554	462	496	109	91	1422	1604	680	678
Zhang, 2005	355	245	141	150	9	5	851	640	159	160
Hung, 2007	1031	1285	960	1268	218	312	3022	3838	1396	1892
Suzuki, 2007	314	652	149	322	22	45	777	1626	193	412
Liu, 2009	228	467	115	226	15	23	571	1160	145	272
Arslan, 2011	29	28	29	29	6	4	87	85	41	37
Kiyoaha, 2011	278	239	154	122	30	18	710	600	214	158
Ozen, 2013	31	72	15	104	6	0	77	248	27	104

Inclusion criteria

The following inclusion criteria were used: (i) study should be case control and should evaluate MTFR A1298C polymorphism, (ii) study should be published, (iii) study should be in English language, (iv) study should contained sufficient data to calculate Odds Ratio (OR) with 95% Confidence Interval (CI), and (v)study should not contained duplicated data.

Data Extraction

The following information was extracted from each included study: first author's name, journal name, year of publication, country name, number of cases and controls. Number of alleles or genotypes in both cases and controls were extracted or calculated from published data to recalculate ORs.

Statistical analysis

The associations were indicated as a pooled Odd Ratio (OR) with the corresponding 95% Confidence Interval (CI). The heterogeneity between studies was tested using the Q-statistic, which is a weighted sum of the squares of the deviations of individual study OR estimates from the overall estimate [14]. When the ORs are homogeneous, Q follows a chi-squared distribution with r – 1 (r is the number of studies) degrees of freedom (df). When P<0.05 then the heterogeneity was considered to be statistically significant. Heterogeneity was quantified with the I2 metric (I2 = (Q – df)/Q), which is independent of the number of studies in the meta-analysis. I2 takes values of between 0 and 100%, with higher values denoting a greater degree of heterogeneity [15-16]. The pooled OR was estimated using Fixed Effect (FE) [17] and Random Effect (RE) [18] models. Random effect modelling assumes a genuine diversity in the results of various studies, and it incorporates a between-study variance into the calculations. Hence, when there is heterogeneity between studies then the pooled OR is preferably estimated using the RE model [19,16]. Genetic models were chosen based on the method described by briefly calculating and comparing the ORs of C *vs* A (allele contrast), CC *vs.* AA (homozygote), AC *vs.* AA (co-dominant) and CC+AC *vs.* AA (dominant) and CC *vs.* AC+AA (recessive), checking the heterogeneity and significance, then determining the best model [20,21]. The Hardy–Weinberg equilibrium of genotypes of controls was tested and if P >0. 05, then it suggests that the controls followed the Hardy–Weinberg Equilibrium (HWE) balance.

Publication bias

Egger's test [22] and Begg's test [23] described for funnel plot

Table 3: Summary estimates for the odds ratio (OR) of MTHFR A1298C in various allele/genotype contrasts, 95% confidence limits, the significance level, p value of heterogeneity test (Q test), and the I2 metric, and publication bias.
p-value (Egger Test).

Genetic Models	Fixed effect OR (95% CI), p value	Random effect OR (95% CI), p value	Heterogeneity p-value (Q test)	I^2 (%)	Publication bias (p value of Begg's test)	Publication bias (p of Egger's test)
Additive (C *vs* A)	0.99(0.93-1.04), 0.62	0.95(0.83-1.08), 0.44	<0.0001	75.12	0.24	0.44
Co-dominant (AC *vs* AA)	0.94(0.87-1.0), 0.07	0.86(0.7-1.02), 0.9	<0.0001	73.77	0.07	0.13
Homozygote (CC *vs* AA)	1.05(0.92-1.2), 0.5	1.13(0.83-1.5), 0.42	0.002	64.03	0.75	0.51
Dominant (CC+AC *vs* AA)	0.95(0.88-1.01), 0.16	0.89(0.75-1.05), 0.19	<0.001	75.05	0.19	0.23
Recessive (AA+AC *vs* CC)	1.1(0.93-1.2), 0.35	1.2(0.89-1.4), 0.27	0.02	53.36	0.63	0.25

asymmetry were applied to evaluate the evidence for publication bias. All p values are two tailed with a significance level at 0.05. All statistical analyses were undertaken by MIX version 1.7 [24].

Results

Characteristics of included studies

Information extracted from the studies included in the meta-analysis is provided in (Tables 1 and 2). Total 78 articles were retrieved using search strategies, but 57 articles did not meet the inclusion criteria after reviewing full articles. Out of remaining twenty-one articles, ten studies were also excluded because reported only C677T polymorphism details. Eleven articles were suitable for the inclusion in the meta-analysis [25-32] [5-7]. Out of eleven studies five studies were from Asian population [26,29,31] [5,6] and remaining studies were from Caucasian population [25,27,28,30,32,7].

Overall, eleven studies provided 5,996/7404 cases/controls for MTHFR A1298C polymorphism with AA (3,110), AC (2,388) and CC (498) genotypes in cases, and with AA (3,828), AC (3,024), and CC (552) genotypes in controls. In total cases, genotype percentage of AA, AC, and CC was 51.67%, 38.83% and 8.30% respectively. In controls genotypes, percentage of AA, AC and CC were 51.70%, 40.84%, and 7.45% respectively. The frequencies of the genotypes AA and AC were the highest in both cases and controls, and allele A was the most common (Table 3). In all the studies, distribution of genotypes in the control group was in Hardy Weinberg Equilibrium.

Meta-analysis

Meta-analysis with allele contrast (C *vs* A) showed no significant association with both fixed effect (ORC *vs* A= 0.99; 95% CI= 0.93-1.04; p= 0.062; PPb= 0.44) and random effect model (ORC *vs* A = 0.95; 95% CI= 0.83-1.08; p= 0.44) (Table 3, Figure 1 and 2).

Table 3 summarizes the ORs with corresponding 95% CIs for association between mutant A1298C polymorphism and risk of lung cancer in homozygote, co-dominant, dominant, and recessive models. Genotype meta-analysis did not report any association with lung cancer (CC *vs* AA (homozygote model): ORCC *vs* AA = 1.13, 95% CI= 0.83-1.5, p= 0.42 (Figure 3); AC *vs*. AA (co-dominant model): ORAC *vs* AA = 0.86, 95% CI= 0.70-1.02, p= 0.90; CC+AC *vs*. AA (dominant model): ORCC+AC *vs* AA = 0.89, 95% CI= 0.75-1.05, p= 0.19 (Figure 3); CC *vs* AC+AA (recessive model): ORCC *vs* AC+AA = 1.2, 95% CI= 0.89-1.4 , p= 0.27) .

A true heterogeneity existed between studies for allele contrast (Pheterogeneity <0.0001, Q= 40.19, I2=67.22%, t2=0.030, z =0.78) and genotype homozygote (Pheterogeneity =0.002, Q= 27.83, I2=64.07%,

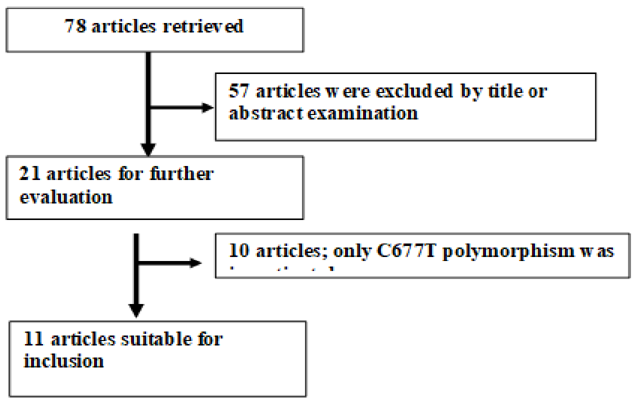

Figure 1: Flowchart of exclusion and inclusion studies in meta-analysis.

t2=0.12, z = 0.79), dominant (Pheterogeneity <0.0001, Q= 40.08, I2= 75.05%, t2=0.05, z = 1.30) and recessive (Pheterogeneity =0.02, Q= 21.44, I2= 53.36%, t2=0.07, z = 1.1) comparisons. The 'I2' value of more than 50% for between studies comparison in both allele and genotype analysis shows high level of true heterogeneity.

Publication bias

Funnel plots using standard error and precision values for allele and genotypes using random effect model were generated (Figure 4). Symmetrical distribution of studies in the funnel plots suggests absence of publication bias. This is also supported by Beggs and Eggers test (Begg's p= 0.24, Egger's p= 0.44 for C *vs*. A; Begg's p= 0.75, Egger's p= 0.51 for CC *vs* AA; and Begg's p= 0.07, Egger's p= 0.13 for AC *vs*. AA; Begg's p= 0.19, Egger's p= 0.23 for CC+AC *vs*. AA; Begg's p= 0.63, Egger's p= 0.25 for CC *vs*. AC+AA) (Table 3).

Discussion

MTHFR plays a central role in balancing DNA synthesis (which involves 5,10-methylentetrahydrofolate) and DNA methylation (which involves 5,10-methyltetrahydrofolate). Specifically, the 677T allele contributes to DNA hypomethylation, which in turn may lead to altered gene expression; at the same time, this polymorphism might exert a protective effect, as observed for colorectal cancer [12], by increasing the levels of the MTHFR substrate, essential for DNA synthesis. Folate deficiency and metabolism disorders may cause DNA hypomethylation, and A to C substitution at nucleotide 1298 in MTHFR, which alters enzyme activity, affecting DNA methylation or DNA synthesis, thereby increasing susceptibility to cancer [33-35]. Present meta-analysis included eleven studies with a total of 5996

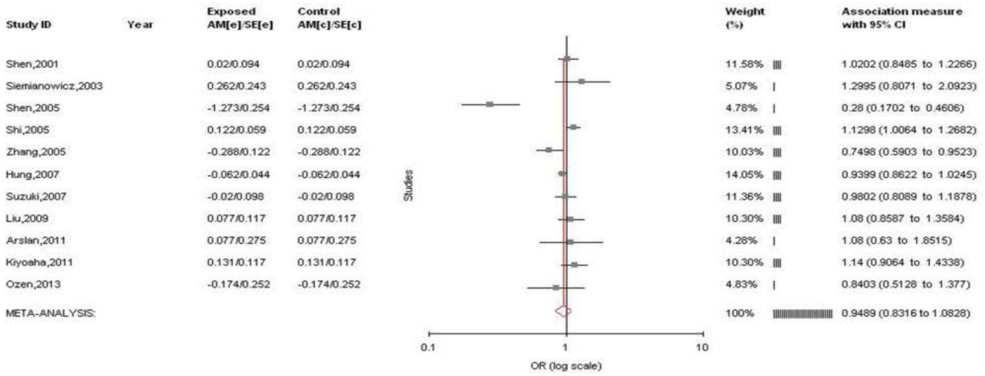

Figure 2: Forest plots for the association between MTHFR A1298C polymorphism and lung cancer for allele contrast model (C *vs* A) with random effect model.

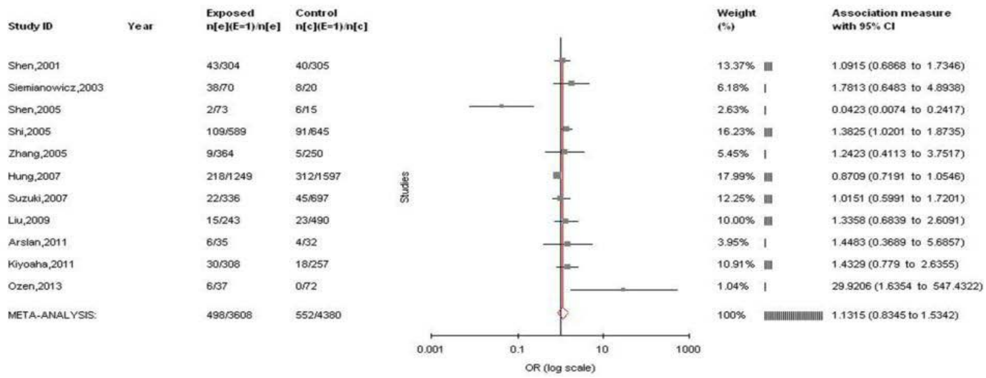

Figure 3: Forest plots for the association between MTHFR A1298C polymorphism and lung cancer for homozygote model (CC *vs* AA) with random effect model.

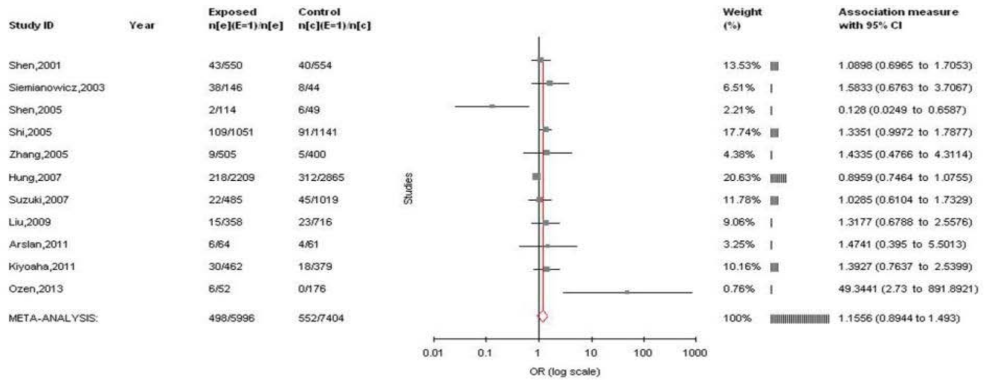

Figure 4: Forest plots for the association between MTHFR A1298C polymorphism and lung cancer for dominant model (CC +AC *vs* AA) with random effect model.

cases and 7404 controls have investigated the A1298C polymorphism with lung cancer.

Meta-analysis is a powerful tool for analyzing cumulative data of studies wherein the individual sample sizes are small and the disease can be easily masked by other genetic and environmental factors [36]. A meta-analysis potentially investigates a large number of individuals and can estimate the effect of a genetic factor on the risk of the disease [36].

Several meat-analyses were published to asses effect of MTHFR on the risk of several diseases [20, 36-43]. Six meta-analyses were published on related subject [44-49], out of which only two groups investigated A1298C polymorphism [44,45]. [44] included six studies in their meta-analysis and reported no association with lung cancer (OR = 1.00; 95% CI= 0.92–1.08). [45] included seven studies with 5098 cases and 6243 controls and also did not find significant association with lung cancer (OR= 1.07; 95% CI= 0.83–1.38). Present meta-analysis included the largest number of studies (11 studies) with largest sample size (13,400). This meta-analysis further confirmed the results of previous meta-analyses and reported that MTHFR A1298C polymorphism is not risk factor for lung cancer.

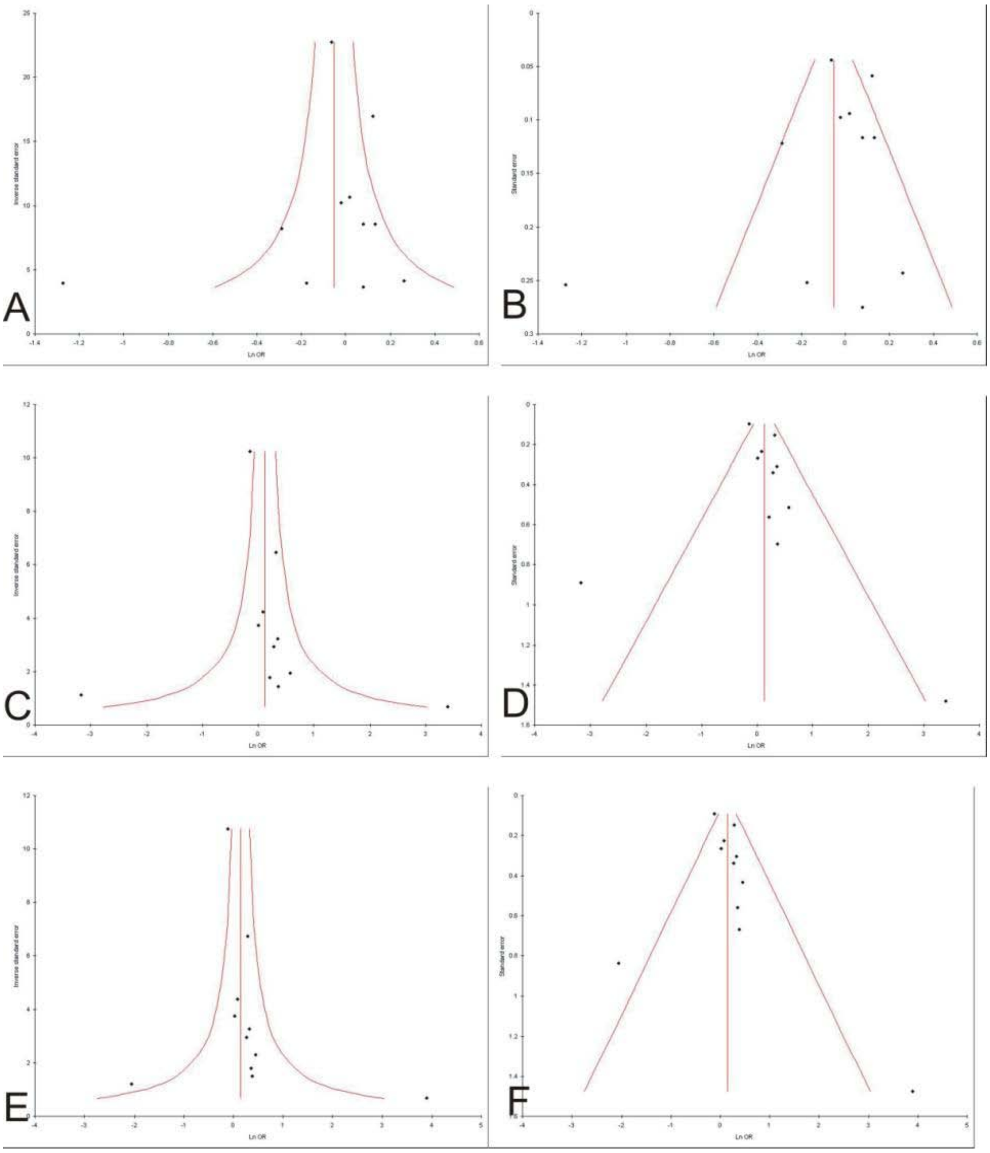

Figure 5: Funnel plots. **A:** Precision versus OR (C *vs* A), **B:** Standard error versus OR (C *vs* A), **C:** Precision versus OR (CC *vs* AA), **D:** Standard error versus OR (CC *vs* AA), **E:** Precision versus OR (CC+AC *vs* AA), **F:** Standard error versus OR (CC+AC *vs* AA).

Limitations

(i) sample size in two studies were small [7,32], (ii)controls were not uniform in all studies, in some studies hospital based patients of other diseases were considered, (iii) other important factors like smoking and folate intake were not considered in the present meta-analysis and (iv) present review is restricted only one folate pathway gene polymorphism. Further the main strength of the present meta-analysis is absence of publication bias and larger pooled sample size. Present meta-analysis suggested that A1298C polymorphism did not play any role in the etiology of lung cancer.

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