RESEARCH ARTICLE

Folate Pathway Gene MTHFR C677T Polymorphism and Risk of Lung Cancer in Asian Populations

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Abstract

Background: Previous studies concerning the association between the 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism with lung cancer in Asian populations have provided inconclusive findings. Aim: A meta-analysis was performed to investigate a more reliable association between MTHFR C677T polymorphism and lung cancer in Asians. Materials and Methods: A comprehensive search was conducted to identify all case-control studies of MTHFR polymorphisms and lung cancer in Asia, using odds ratios (ORs) with 95% confidence intervals (CIs) to assess the strength of any association. Results: Meta-analysis results suggested that the MTHFR C677T polymorphism contributed to an increased lung cancer risk in Asian populations (for T *vs* C: OR=1.11, 95%CI=1.0-1.23; for CT *vs* CC: OR= 1.1, 95%CI= 0.95-1.2 ; for TT+CT *vs* CC: OR=1.13, 95%CI=1.0-1.30; for TT *vs* CC: OR=1.25, 95%CI=1.01-1.30; for TT *vs* CT+CC: OR=1.16, 95%CI=1.0-1.36). Conclusions: MTHFR C677T polymorphism is significantly associated with lung cancer in Asians.

Keywords: Lung cancer - meta-analysis - methylenetetrahydrofolate reductase - C677T - Asian populations

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Introduction

Lung cancer has been the most common cancer in the world for several decades and there were an estimated 1.61 million new cases, representing 12.7% of all new cancers. It was also the most common cause of death from cancer, with 1.38 million deaths (18.2% of the total). The majority of the cases now occur in the developing countries (55%), a large increase since the estimates in 1980, when it was estimated that only 31% of lung cancer cases occurred in developing countries (Parkin et al., 2005; Ferlay et al., 2010). Numerous epidemiological studies have pointed out that low dietary folate intake is an important factor in development of cancer including lung, breast, colorectum, bladder and pancreas. Folate and methionine metabolism plays crucial roles in DNA synthesis and methylation (Sharp and Little, 2004). Folate deficiency may cause uracil misincorporation and subsequent DNA instability, retarded DNA repair capacity for oxidative or alkylating damage, and global and proto-oncogenic DNA hypomethylation (Duthie, 1999; Kim, 1999).

Methylenetetrahydrofolate reductase (MTHFR) is the key enzyme in folate metabolism, and functional polymorphisms in the MTHFR gene have been seen with a highly variable prevalence in different ethnic populations and geographical areas (Sharp and Little, 2004). The human MTHFR gene contains 11 exons, located on chromosome 1p36.3 (Goyette et al., 1994, 1998), and encodes methylenetetrahydrofolate reductase (MTHFR) a key enzyme in folate and homocysteine metabolism. MTHFR catalyzes the biologically irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which provides the methyl group for the remethylation of homocysteine to methionine (Bailey and Gregory, 1999). The MTHFR enzyme is constituted by dimers in humans, each of which contains an N-terminal catalytic domain and a C-terminal regulatory domain. The monomer arrangement might be head to tail, with each catalytic domain flanked by a regulatory domain. Two common polymorphisms in the MTHFR gene have been described: C677T (A222V) in exon 4 and A1298C (E429A) in exon 7 (Frosst et al., 1995; Weisberg et al., 1998). The frequency of the MTHFR 677T allele varies in different ethnic and regional world populations for example, the allele frequency is 0.07 is Sub-Saharan Africans and 0.06 in Canadian Inuit, whereas in Asians the allele frequencies are 0.04-0.54 (Hegele et al., 1997; Pepe et al., 1998; Rai et al., 2010; 2012). MTHFR functions in dimeric form and FAD works as a co-factor, but variant MTHFR (222V) dissociates into monomers and its enzymatic activity reduces. It is established by docking study that the mutant enzyme (222V) shows less affinity towards FAD than the wild enzyme (222A) (Yadav et al., 2011). The C677T polymorphism has been proven to affect the enzymatic activity and homocysteine level. Considering that methylation abnormalities appear to be important for the pathogenesis of many cancer types, many authors have examined the association between

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the genotype of the MTHFR C677T polymorphism with various cancers. Case-control studies investigating the association between the MTHFR C677T polymorphism and lung cancer have given controversial results. However, the results of these studies remain conflicting rather than conclusive. In consideration of the extensive role of MTHFR, a meta-analysis of all eligible case-control studies was carried out to estimate the overall lung cancer risk of these two polymorphisms and to quantify the potential between-study heterogeneity.

Materials and Methods

Search criteria

For electronic searches, the comprehensive search strategy was used to find eligible studies for present meta-analysis. Published studies were searched through PubMed, Google scholar, Elsevier and Springer Link databases, using following keywords "MTHFR", "methylenetetrahydrofolate reductase", and "Lung Cancer", in combination with "C677T", "mutation", "polymorphism". Furthermore, references of all relevant articles were retrieved to search for additional eligible studies.

Inclusion and exclusion criteria

The studies meeting the following criteria were included: (1) concerning the relationship between MTHFR C677T polymorphism and lung cancer; (2) case-control studies from Asian population; (3) providing complete data of cases and controls for calculating odd ratio (OR) with 95% confidence interval (CI); and (4) the distribution of the genotypes in control groups should be in Hardy-Weinberg equilibrium (HWE). The studies not reported the genotype/ allele numbers were excluded.

Data extraction

Following data were collected from included studies: first author name, year of publication, journal name, ethnicity, numbers of genotyped cases and controls. If studies contained overlapping cases and/or controls, the largest study was preferred.

Statistical analysis

For the control group of each study, the observed genotype frequencies of the MTHFR C677T polymorphism was assessed for Hardy-Weinberg equilibrium (HWE) using the x^2 test. The strength of association was accessed odds ratios (ORs) and 95% confidence intervals (CIs). The pooled ORs were performed for allele contrast (T *vs* C), codominant (CT *vs* CC), homozygote (TT vs CC), dominant (TT+CT *vs* CC), and recessive (TT *vs* CT+CC and CC) models. Heterogeneity assumption was evaluated by a chi-square based Q-test. A p-value of >0.05 for the Q-test indicated a lack of heterogeneity among the studies, the summary OR estimate was calculated by the fixed or random effects models (Mantel and Haenszel,1959; DerSimonian and Laird,1986). Heterogeneity was quantified with the I^2 metric (I^2=(Q-df)/Q), which is independent of the number of studies in the meta-analysis. I^2 takes values of between 0 and 100%, with higher values denoting a greater degree of heterogeneity (Zintzaras and Hadjigeorgiou 2005; Zintzaras and Ioannidis, 2005; Zintzaras, 2007). Random effects modeling assume 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 (Whitehead 2002).

Publication bias

The potential for publication bias was examined by a Begg's test (funnel plot method) and Egger's linear regression test (Begg and Mazumdar, 1994; Egger et al., 1997). $p<0.05$ was considered representative of statistical significance. All analyses were performed using the computer program MIX version 1.7 (Bax et al., 2006). A p value less than 0.05 was considered statistically significant, and all the p values were two sided.

Results

Eligible studies

The full articles of the retrieved studies were read to assess their appropriateness for meta-analysis. Data from 14 articles that investigated the association between MTHFR C677T gene polymorphism and lung cancer in Asian population were included in the meta-analysis (Jeng et al., 2003; Zhang et al., 2005; Shen et al., 2005; Suzuki et al., 2007; Jin et al., 2007; Hung et al., 2007; Liu et al., 2008; 2009; Yang et al., 2010; Yao et al., 2010; Kiyohara et al., 2011; Cui et al., 2011a; 2011b; Cheng et al., 2011).

All these fourteen studies were performed in different countries-China (Shen et al., 2005; Zhang et al., 2005; Hung et al., 2007; Jin et al., 2007; Liu et al., 2008; Yang et al., 2011; Yao et al., 2010; Cui et al., 2011; Cheng et al., 2011), Korea (Cui et al., 2011), Japan (Suzuki et al., 2007; Kiyohara et al., 2011), Taiwan (Jeng et al., 2003; Liu et al., 2009), In all studies, the polymorphism C677T was genotyped using validated genotyping methods like-polymerase chain reaction analysis followed by restriction digestion (PCR-RFLP).

Summary statistics

In all sixteen studies, total cases were 9,468 with CC

Table 1. Characteristics of Fourteen Studies Included in the Present Meta-Analysis

Study	Country	Case	Control
Cheng et al., 2011	China	94	78
Cui et al., 2011	China	438	641
Cui et al., 2011	Korea	3939	1700
Kiyohara et al., 2011	Japan	462	379
Yang et al., 2010	China	120	165
Yao et al., 2010	Asian	93	106
Liu et al., 2009	Taiwan	358	716
Liu et al., 2008	China	500	517
Hung et al., 2007	China	2169	2803
Jin et al., 2007	China	100	100
Suzuki et al., 2007	Japan	515	1030
Shen et al., 2005	China	116	111
Zhang et al., 2005	China	505	500
Jeng et al., 2003	Taiwan	59	232

(3441), CT (4404) and TT (1623), and controls were 9,078 with CC (3,600), CT (4,115), and TT (1,363) genotypes. The number of cases varied from 59 to 3,939, with a mean of 676, and the numbers of controls varied from 78 to 2,803, with a mean of 648 (Table 2). In controls genotypes, percentage of CC, CT and TT were 39.66%, 45.33%, and 15.01% respectively. In total cases, genotype percentage of CC, CT, and TT was 36.34%, 46.15% and 17.14% respectively. Frequencies of CC and CT genotypes were highest in both cases and controls (Table 2). In cases and controls, the allele C was the most common. In seven studies OR is above one (Shen et al., 2005; Zhang et al., 2005; Hung et al., 2007;Yao et al., 2010; Cui et al., 2011; Kiyohara et al., 2011; Cheng et al., 2012).

Meta-analysis

An association was detected between the MTHFR C677T polymorphism and the susceptibility to lung cancer in Asian population in all the genetic models except co-dominant model using random effect model (for T *vs* C: OR=1.11, 95%CI=1.0-1.23; CT *vs* CC: OR=1.1, 95%CI=0.95-1.2; for TT+CT *vs* CC: OR=1.13, 95%CI=1.0-1.30; for TT *vs* CC: OR=1.25, 95%CI=1.01-1.54; for TT *vs* CT+CC: OR=1.16, 95%CI=1.0-1.36). (Table 3; Figures 1-3).

Significant association was also found in fixed effect models using all genetic models except co-dominant (for T *vs* C: OR=1.1, 95%CI=1.01-1.10; for TT+CT *vs* CC: OR=1.1, 95%CI=1.0-1.13; for TT *vs* CC: OR=1.14, 95%CI=1.03-1.25; for TT *vs* CT+CC: OR=1.10, 95%CI=1.02-1.20; for CT *vs* CC: OR=1.04, 95%CI=0.97-1.11)(Table 3).

A true heterogeneity existed between studies for allele contrast ($P_{heterogeneity}$ <0.0001, Q=55.60, I^2=76.60%, t^2=0.026, z=0.88), genotype homozygote ($P_{heterogeneity}$<0.0001, Q=45.23, I^2=71.26%, t^2=0.09, z=2.09), dominant ($P_{heterogeneity}$<0.0001, Q=48.63, I^2=73.27%, t^2=0.05, z=1.62) and recessive ($P_{heterogeneity}$=0.002, Q=32.01, I^2=59.39%, t^2=0.04, z=1.93) comparisons. The 'I²' value of more than 50% for between studies comparison in both allele and genotype analysis shows high level of true heterogeneity.

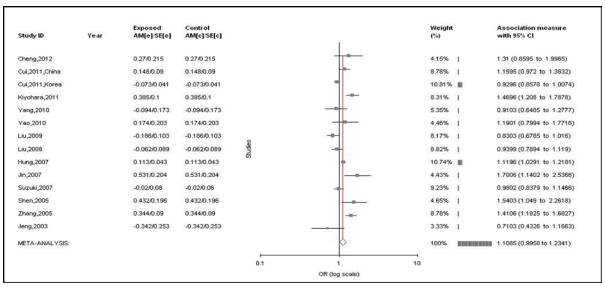

Figure 1. Forest Plot for the Association between MTHFR C677T Polymorphism and Lung Cancer for Allele Contrast Model (T *vs* C) with Random Effect Model in Asian Population

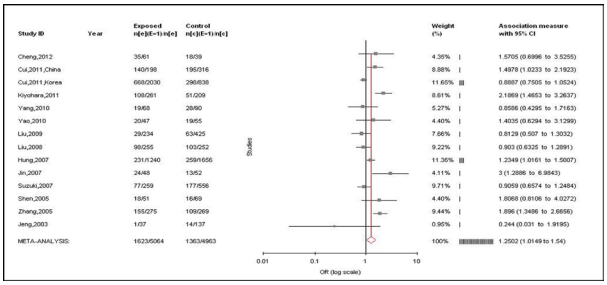

Figure 2. Forest Plot for the Association between MTHFR C677T Polymorphism and Lung Cancer for Homozygote Model (TT *vs* CC) with Random Effect Model in Asian Population

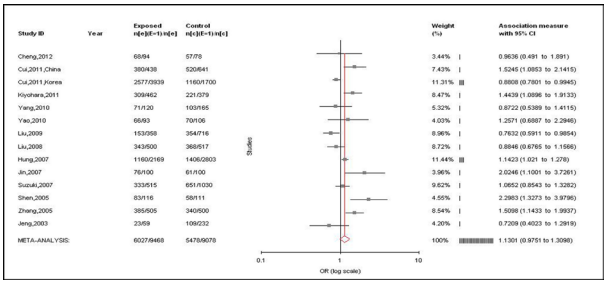

Figure 3. Forest Plot for the Association between MTHFR C677T Polymorphism and Lung Cancer for Dominant Model (TT+CT *vs* CC) with Random Effect Model in Asian Population

Table 2. Genotype and Allele Findings in Published Studies

Study ID	Genotype						Alleles			
	CC		CT		TT		C		T	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Cheng et al., 2012	26	21	33	39	35	18	85	81	103	75
Cui et al., 2011	58	121	240	325	140	195	356	567	520	715
Cui et al., 2011	1362	540	1909	862	668	298	4633	1942	3245	1458
Kiyohara et al., 2011	153	158	201	170	108	51	507	486	417	272
Yang et al., 2010	49	62	52	75	19	28	150	199	90	131
Yao et al., 2010	27	36	46	51	20	19	100	123	86	89
Liu et al., 2009	205	362	124	291	29	63	534	1015	182	417
Liu et al., 2008	157	149	245	265	98	103	559	563	441	471
Hung et al., 2007	1009	1397	929	1147	231	259	2947	3941	1391	1665
Jin et al., 2007	24	39	52	48	24	13	100	126	100	74
Suzuki et al., 2007	182	379	256	474	77	177	620	1232	410	828
Shen et al., 2005	33	53	65	42	18	16	131	148	101	74
Zhang et al., 2005	120	160	230	231	155	109	470	551	540	449
Jeng et al., 2003	36	123	22	95	1	14	94	341	24	123

Table 3. Summary Estimates for the Odds Ratio (OR) of MTHFR C677T in Various Allele/Genotype Contrasts, the Significance Level (p value) of Heterogeneity Test (Q test), and the I^2 Metric and Publication Bias p-value (Egger Test) in Asian Population

Genetic Models	Fixed effect OR (95% CI), p	Random effect OR (95% CI), p	Heterogeneity p-value (Q test)	I^2 (%)	Publication Bias (p of Egger's test)
Allele Contrast (T *vs* C)	1.1 (1.01-1.10) 0.01	1.11 (1.0-1.23) 0.05	<0.0001	76.60	0.30
Co-dominant (CT *vs* CC)	1.04 (0.97-1.11) 0.26	1.1 (0.95-1.2) 0.25	0.0003	65.56	0.39
Homozygoote (TT *vs* CC)	1.14 (1.03-1.25) 0.005	1.25 (1.01-1.54) 0.03	<0.0001	71.26	0.33
Dominant (TT+CT *vs* CC)	1.1 (1.0-1.13) 0.05	1.13 (1.0-1.30) 0.03	<0.0001	73.27	0.29
Recessive (TT *vs* CT+CC)	1.1 (1.02-1.20) 0.01	1.16 (1.0-1.36) 0.05	0.002	59.39	0.46

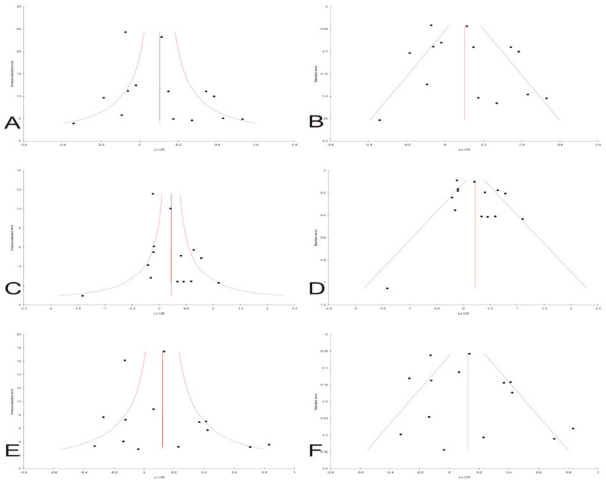

Figure 4. Funnel Plots. A) precision versus OR (T *vs* C), **B)** standard error versus OR (T *vs* C) **C)** precision versus OR(TT *vs* CC), **D)** standard error versus OR (TT *vs* CC), **E)** precision versus OR (TT+CT *vs* CC), **F)** standard error versus OR (TT+CT *vs* CC)

Publication bias

Funnel plot and Egger's test were performed to quantitatively evaluate the publication bias of literature on lung cancer. Funnel plots' shape of all contrasts did not reveal obvious evidence of asymmetry, and all the P values of Begg's and Egger's tests were more than 0.05 (Begg's p=0.29, Egger's p=0.30 for T *vs* C; Begg's p=0.23, Egger's p=0.33 for TT *vs* CC; and Begg's p=0.43, Egger's p=0.39 for CT *vs* CCA; Begg's p=0.27, Egger's p=0.29 for TT+AC *vs* CC; Begg's p=0.36, Egger's p=0.46 for TT *vs* CT+CC) (Table 3; Figure 4).

Discussion

The results from the meta-analysis of 14 studies highlighted a higher risk of developing lung cancer for subjects carrying the MTHFR 677 TT genotype. Impaired MTHFR activity might influence cancer risk is determined by the level of S-adenosyl-L-methionine, the common donor of methyl that is necessary for maintenance of the methylation patterns in DNA. Changes in methylation modify DNA conformation and gene expression. A less active form of MTHFR leads to lower S-adenosyl-L-methionine levels and consequently to hypomethylation; this phenomenon would be expected to increase the risk of some cancers (Stern et al., 2000). Similarly, low folate intake may modify cancer risk by inducing uracil misincorporation during DNA synthesis, leading to chromosomal damage, DNA strand breaks and impaired DNA repair, and DNA hypomethylation (Blount et al., 1997; Kim et al., 1997; Duthie, 1999; Kim, 1999).

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 (Botto and Yang, 2000), by increasing the levels of the MTHFR substrate, essential for DNA synthesis.

Meta-analysis is a powerful tool for analyzing cumulative data of studies where the individual sample sizes are small and the statistical power low (Guan et al., 2011; Rai, 2011; 2014; Liao et al., 2012; Yadav et al., 2014). Several meta-analyses were published to assess the role of MTHFR polymorphism in cancer development like: breast cancer (Liang et al., 2013), lung cancer (Boccia et al., 2009; Pan et al., 2011), colorectal cancer (Hubner and Houlston, 2007), pancreatic cancer (Tu et al., 2012), esophageal cancer (Liu et al., 2011), and cervical cancer (Mei et al., 2012).

There are few limitations in present meta-analysis like- *i)* crude OR was used, *ii)* higher heterogeneity was observed, *iii)* controls were not uniform, *iv)* other genes of folate pathway was not considered and (iv) other risk factors among the subjects in the available studies, such as folate intake and smoking status etc were not considered. There is a need for larger and wider case-control studies to explore the role of other factors that are likely to cause Lung cancer.

This is a meta-analysis with sufficient individual data to stratify results by ethnicity. This analysis supports conclusions that the T carriers genotype had increased risk of Lung cancer (TT *vs* CC: OR=1.25, 95%CI: 1.01-1.54, p=0.03) in the case of Asian population; this suggests the MTHFR C677T polymorphism may be associated with the risk of lung cancer. Future well designed large studies might be necessary to validate this association in different populations incorporated with micronutrient factors in the susceptibility of lung cancer.

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