Research Article

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Methylenetetrahydrofolate Reductase (MTHFR) Gene rs1801133 C>T Polymorphisms and Lung Cancer Susceptibility: An Updated Meta-analysis

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Abstract: Objective: The aim of this study was to investigate the methylenetetrahydrofolate reductase (*MTHFR*) gene rs1801133 C>T polymorphisms and lung cancer susceptibility by pooling openly published data.

Methods: Electronic databases of Medline, Web of Science, Embase, Google scholar, CBM and CNKI were systematic searched to find the relevant studies related to *MTHFR* gene rs1801133 C>T polymorphisms and lung cancer susceptibility. The odds of TT, CT and CC alleles in lung cancer patients compared with healthy controls was pooled by the effect size of odds ratio (OR) and corresponding 95% confidence interval (95%CI) under random or fixed effect model. Publication bias was analyzed by Begg's funnel plot and Egger's line regression test.

Results: Overall, twenty-one studies relevant to *MTHFR* gene rs1801133 C>T polymorphisms and lung cancer susceptibility were included. The pooled data showed subject with T allele had significant increased risk of developing lung cancer in dominant (OR=1.14, 95%CI: 1.01-1.28, $p<0.05$), recessive (OR=1.26, 95%CI: 1.08-1.48, $p<0.01$) and homologous (OR=1.36, 95%CI: 1.12-1.65, $p<0.01$) genetic model. Begg's funnel plot and Egger's line regression test showed significant publication bias in all genetic models.

Conclusion: Based on present data, subjects with TT or CT alleles may have increased susceptibility to lung cancer. However, due to significant publication bias, the conclusion should be drawn with caution and should be proved by further well-designed case-control or cohort studies relevant to *MTHFR* gene rs1801133 C>T polymorphisms and lung cancer risk.

Keywords: Lung cancer; *MTHFR*; polymorphisms; susceptibility; meta-analysis

Introduction

Global cancer statistical analysis has found that lung cancer, including non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC), was the leading cause of malignant carcinoma related death in males and females worldwide in 2016 [1]. In females, the incidence of lung cancer is second only to breast cancer but is the first in males [2-4]. Cigarette smoking was confirmed as the leading cause of carcinogenesis in lung cancer patients. However, genetic factors including oncogenes, tumor suppressor genes and metabolic enzyme gene polymorphisms also play an important role in the development of lung cancer.

Methylenetetrahydrofolate reductase is a key enzyme in the folate metabolism pathway coded by *MTHFR* gene [5, 6]. Methylenetetrahydrofolate reductase irreversibly converts folic acid metabolite 5, 10-methylenetetrahydrofolic acid to 5-methyltetrahydrofolic acid and further provides the materials for DNA synthesis. *MTHFR* rs1801133 C>T is one the most common polymorphism SNP sites which cause alanine to be replaced with valine and reduces enzyme activity and heat resistance. Correlations between *MTHFR* gene rs1801133 C>T polymorphisms and cancer susceptibility has been widely discussed in lung cancer [7, 8], esophageal cancer [9, 10], colorectal cancer [11, 12], breast cancer [13], and other cancers. *MTHFR* gene rs1801133 C>T polymorphisms and lung cancer risk was evaluated by several case-control studies with inconclusive results [7, 8]. Therefore, we performed this updated meta-analysis.

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Material and Methods

Search strategy

Electronic databases of Medline, Web of Science, Embase, Google scholar, CBM and CNKI were systematic searched to find the relevant studies related to *MTHFR* gene rs1801133 C>T polymorphisms and lung cancer susceptibility. The publication identification process was performed according to the Cochrane handbook and demonstrated by **Figure 1**. The terms “lung cancer OR carcinoma of the lung OR non-small cell lung cancer OR NSCLC” AND “methylenetetrahydrofolate reductase OR *MTHFR*”, were used to identify relevant studies.

Study selection criteria

The inclusion criteria used to screen all identified articles were: (1) lung cancer, including non-small cell lung cancer and small cell lung cancer, was confirmed by pathology or clinical diagnosis; (2) the controls were healthy subjects without any malignant diseases; (3) the genotype of the lung cancer and controls could be extracted from original studies; (4) the study type was either a case-control study or a cohort study; (5) the studies were published in English or Chinese.

Data extraction and critical appraisal

Two reviewers carefully read the included full-text papers and extracted the eligible information and data independently according to the Cochrane Handbook. The extracted information and data included: (1) first and corresponding authors; (2) year of publication; (3) region of the work performed; (4) ethnicity of the included subjects; (5) control type (hospital-based or population-based); (6) Hardy–Weinberg equilibrium (HWE) of the control group; (7) TT, CT and CC allele frequency in original study of the case and control groups.

Statistical analysis

The odds of TT, CT and CC alleles in lung cancer patients compared with healthy controls were pooled by the effect size of odds ratio (OR) and corresponding 95% confidence interval (95%CI) under random or fixed effect model. The statistical heterogeneity of the included publications in each genetic model was assessed by I^2 test. The OR

Figure 1: The study search strategy.

was pooled by random or fixed effect model according to statistical heterogeneity. The publication bias was evaluated by Begg’s funnel plot and Egger’s line regression test[14]. All the data analysis was performed using STATA/SE 11.0 software (<http://www.stata.com>; Stata Corporation, College Station, TX). A two-tailed p-value less than 0.05 was considered statistically significant.

Results

General characteristics of the included publications

After systematically searching the electronic databases, a total of 21 studies were included in the present meta-analysis. The main characteristics of the studies were shown in **Table 1**.

Statistical heterogeneity analysis

The statistical heterogeneity of the effect size in each different genetic model was assessed by I^2 test. Significant statistical heterogeneity was found in the dominant, recessive and homologous genetic models (**Table 2**).

Table 1: General information of the included original publications.

First author	Year	Region	Control type	Case				Control				HWE	Race
				n	TT	CT	CC	n	TT	CT	CC		
Sun[15]	2018	China	Population-based	225	52	113	60	225	24	124	77	Yes	East Asia
Cai[16]	2014	China	Population-based	202	46	102	54	202	21	112	69	Yes	East Asia
Ma[17]	2012	China	Hospital-based	120	46	54	20	60	10	28	22	Yes	East Asia
Cheng[18]	2011	China	Hospital-based	178	71	58	49	180	45	88	47	Yes	East Asia
Cui[8]	2011	China	Hospital-based	438	140	240	58	641	195	325	121	Yes	East Asia
Arslan[19]	2011	Turkey	Population-based	64	7	27	30	61	3	29	29	Yes	Caucasians
Cui[7]	2011	Korea	Hospital-based	3938	668	1909	1361	1700	298	862	540	Yes	East Asia
Yao[20]	2010	China	Hospital-based	93	20	46	27	106	19	51	36	Yes	East Asia
Yang[21]	2010	China	Hospital-based	120	19	52	49	165	28	75	62	Yes	East Asia
Liu[22]	2009	Taiwan	Population-based	358	29	124	205	716	63	291	362	Yes	East Asia
Liu[23]	2008	China	Hospital-based	500	98	245	157	517	103	265	149	Yes	East Asia
Jin[24]	2007	China	Population-based	100	24	52	24	100	13	48	39	Yes	East Asia
Huang[25]	2007	France	Hospital-based	2169	231	929	1009	2803	259	1147	1397	Yes	Caucasians
Suzuki[26]	2007	Japan	Hospital-based	515	77	256	182	1048	177	474	397	Yes	East Asia
Shi[27]	2005	US	Population-based	1051	100	468	483	1141	124	519	498	Yes	Mixed
Zhang[28]	2005	China	Population-based	505	155	230	120	500	109	231	160	Yes	East Asia
Shen[29]	2005	China	Hospital-based	116	18	65	33	111	16	42	53	Yes	East Asia
Siemianowicz [30]	2003	Poland	Population-based	146	48	60	38	44	6	20	18	Yes	Caucasians
Jeng[31]	2003	Taiwan	Hospital-based	59	1	22	36	232	14	95	123	Yes	East Asia
Heijmans[32]	2003	Netherland	Population-based	44	4	17	23	793	65	329	399	Yes	Caucasians
Shen[33]	2001	US	Population-based	550	57	252	241	554	57	252	245	Yes	Mixed

Table 2: Statistical heterogeneity analysis of different genetic models.

Genetic model	Comparison	I ²	p-value	Statistical heterogeneity	Data pooling
Dominant	TT+CT vs CC	67.9%	<0.01	Yes	Random
Recessive	TT vs CT+CC	66.4%	<0.01	Yes	Random
Homologous	TT vs CC	72.5%	<0.01	Yes	Random

Meta-analysis

Dominant genetic model (TT+CT vs CC)

In the dominant genetic model (TT+CT vs CC), subjects with TT or CT alleles had an elevated risk of developing lung cancer (OR=1.14, 95%CI:1.01-1.28, $p<0.05$) by pooling data with random effect method (**Figure 2**). Significant

publication bias was observed in the dominant genetic model evaluated by Begg's funnel plot (**Figure 3**) and Egger's line regression test ($t=2.44$, $p=0.025$).

Recessive genetic model (TT vs CT+CC)

Under the recessive genetic model (TT vs CT+CC), lung cancer risk was significantly increased in subjects with

Figure 2: Forrest plot of *MTHFR* gene rs1801133 C>T polymorphisms and lung cancer susceptibility in the dominant genetic model (TT+CT vs CC).

Figure 3: Funnel plot of *MTHFR* gene rs1801133 C>T polymorphisms and lung cancer susceptibility in the recessive genetic model (TT vs CT+CC).

Figure 4: Forrest plot of *MTHFR* gene rs1801133 C>T polymorphisms and lung cancer susceptibility in the recessive genetic model (TT vs CT+CC).

TT allele compared to CC/CT (OR=1.26, 95%CI:1.08-1.48, $p<0.01$) in random effect model (**Figure 4**). Begg’s funnel plot (**Figure 5**) and Egger’s line regression test ($t=2.67$, $p=0.015$) demonstrated significant publication bias.

Homologous model (TT vs CC)

In the homologous model (TT vs CC), increased lung cancer risk was found in the population with TT allele compared to CC with statistical difference (OR=1.36, 95%CI:1.12-1.65, $p<0.01$) in random effect model. Significant publication bias was determined by Begg’s funnel plot (**Figure 7**) and Egger’s line regression test ($t=2.93$, $p=0.009$).

Discussion

In 2001, the relationship between *MTHFR* gene polymorphism and susceptibility to lung cancer was examined for the first time in non-Spanish Caucasians by Shen et al. [33]. It was concluded that *MTHFR* gene C677T (rs1801133 C>T) and A1298C polymorphisms were not associated with lung cancer. Jeng et al. [31] in 2003 also

suggested that C677T locus of *MTHFR* gene had no risk effect on lung cancer susceptibility. Mao et al. [34] in 2008 and Boccia et al. [35] in 2009 conducted a meta-analysis of the association of *MTHFR* gene C677T and A1298C polymorphisms with lung cancer susceptibility. However, the correlation between *MTHFR* gene C677T (rs1801133 C>T) polymorphisms has not been identified as yet. In 2012, Hou et al [36] performed an up-date meta-analysis relevant to *MTHFR* gene C677T polymorphisms and lung cancer risk and found the correlation. In Hou’s study, the authors found that the *MTHFR* 677T null genotype (T vs C) may increase NSCLC risk. However, 6 years had passed since Hou et al., reported their work and several new studies had been published. Therefore, we performed this updated meta-analysis by including the new publications relevant to *MTHFR* gene C677T polymorphisms and lung cancer risk in order to further elucidate the correlation.

Here, the data from 21 publications relating to *MTHFR* gene C677T polymorphisms and lung cancer risk were pooled. We determined T allele frequency in lung cancer patients was higher when compared to healthy controls in all gene expression models, indicating that populations with T alleles had an increased risk of developing lung cancer in all genetic models which were in accordance

Figure 5: Funnel plot of *MTHFR* gene rs1801133 C>T polymorphisms and lung cancer susceptibility in the recessive genetic model (TT vs CT+CC).

Figure 6: Forrest plot of *MTHFR* gene rs1801133 C>T polymorphisms and lung cancer susceptibility in the homologous model (TT vs CC).

Figure 7: Funnel plot of *MTHFR* gene rs1801133 C>T polymorphisms and lung cancer susceptibility in the homologous model (TT vs CC)

with Hou et al., results. However, compared to Hou et al., we discussed the correlation between *MTHFR* gene C677T polymorphisms and lung cancer in dominant, recessive and homologous genetic models and also added 4 newly published studies which were not included in Hou et al. Although the results of the present meta-analysis were similar to Hou et al., our study provided further evidence for the potential correlation between *MTHFR* gene C677T polymorphisms and lung cancer susceptibility. Our work concluded that T allele increased the lung cancer risk compared to C. However, there are several limitations for the present study. Firstly, the statistical heterogeneity was observed in all genetic models, and the data were pooled by a random effect model. The statistical power is weaker than the fixed effect model. Secondly, significant publication bias was found in the meta-analysis which indicating studies with negative results may not be published. The heterogeneity across the included studies was potentially from two major aspects. One is from the clinical heterogeneity such as the subjects included in each study were varied in gender and ethnicity. The second clinical heterogeneity may come from the control types (hospital-based or population-based) and the third source of clinical heterogeneity may be due to the different methods of genotyping detection. The other major aspect of heterogeneity was statistical heterogeneity. The statistical heterogeneity may have arisen in describing the properties of a dataset, or several datasets. They relate to

the validity of the often-convenient assumption that the statistical properties of any one part of an overall dataset are the same as any other part. In a meta-analysis which combines the data from several studies, homogeneity measures the differences or similarities between the several studies.

Methylenetetrahydrofolate reductase is a key enzyme in the folate metabolism pathway coded by the *MTHFR* gene which plays a key role in folate metabolism, catalyzing the irreversible conversion of 5,10-MTHF to 5-MTHF. *MTHFR* rs1801133 C>T can cause alanine to be replaced with valine, which reduces enzyme activity. Reduced *MTHFR* activity generally leads to increased 5,10-MTHF for DNA synthesis and decreased 5-MTHF for DNA methylation and finally increased the cancer risk [6, 20].

Conclusion

Based on the present data, lung cancer susceptibility may be increased in subjects with TT or CT alleles, compared to CC alleles. However, due to the statistical heterogeneity and publication bias limitations, conclusions should be drawn with caution. We recommend more well-designed original case-control studies, cohort studies or high-quality meta-analysis relevant to (*MTHFR*) gene rs1801133 C>T polymorphisms and lung cancer susceptibility in order to further elucidate its correlation.

Conflict of interest: Authors state no conflict of interest

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