Research Article

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Correlation between *MTHFR* 677C > T polymorphism and response of pemetrexed-based chemotherapy in advanced NSCLC: A meta-analysis

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Abstract

Objective – The aim of this study was to investigate the correlation between *MTHFR* 677C > T polymorphism and response of pemetrexed-based chemotherapy in advanced non-small-cell lung cancer (NSCLC) by pooling the open published relevant studies.

Methods – Clinical studies associated with *MTHFR* 677C > T polymorphism and response of pemetrexed-based chemotherapy in advanced NSCLC were systematically searched in databases of Pubmed, Embase, Cochran Library, China national knowledge infrastructure (CNKI) and Wanfang. The correlation was expressed by odds ratio (OR) and corresponding 95% confidence interval (95% CI). The publication bias of the included studies was evaluated through Begg's funnel plot and Egger's line regression test.

Results – Ten prospective clinical studies relevant to *MTHFR* 677C > T polymorphism and response of pemetrexed-based chemotherapy in NSCLC were included in the present meta-analysis. The pooled results indicated that the partial response in NSCLC patients with TT or CT genotype was inferior to CC genotype in a dominant gene model (TT + CT vs CC) (OR = 0.16, 95% CI: 0.06–0.41, $P = 0.001$). NSCLC cases with T genotype were inferior to C genotype in the objective response rate treated with pemetrexed-based chemotherapy for dominant (OR = 0.28, 95% CI: 0.18–0.45, $P = 0.001$), recessive (OR = 0.43, 95% CI: 0.19–0.94, $P = 0.03$) and homozygous models (OR = 0.30, 95% CI: 0.13–0.67, $P = 0.003$). However, there was no statistical difference in disease control rate, progressive

disease between different genotypes of different gene models ($P_{all} > 0.05$).

Conclusion – The pemetrexed-based chemotherapy response was decreased in NSCLC cases with T genotype, which can be applied as a potential pemetrexed-based chemotherapy response marker.

Keywords: *MTHFR*, polymorphism, chemotherapy response, pemetrexed, NSCLC, meta-analysis

1 Introduction

Lung cancer, especially NSCLC, is known as the leading cause of tumor-associated mortality globally. It was estimated that 131,880 new lung cancer cases will be diagnosed in the year 2021, which accounts for almost one-quarter of all cancers diagnosed in the same year [1]. About 75–80% of NSCLC cases were at advanced stages and lost the opportunity of operation when was first diagnosed. Most of the patients who receive tumor resection also need adjuvant chemotherapy after operation except for stage Ia NSCLC cases. Therefore, chemotherapy is one of the major treatment methods for NSCLC. Pemetrexed-based chemotherapy is extensively applied clinically in NSCLC especially for lung adenocarcinoma [2–4]. Pemetrexed is a multitarget folate antagonist, which has been proved to be effective in the first-line treatment, second-line treatment and maintenance treatment of advanced NSCLC [5]. It plays an antitumor role by inhibiting the activities of several key enzymes in the folate metabolism pathway. *MTHFR* is a key enzyme in folate metabolism, which had been reported to be associated with the response of pemetrexed-based chemotherapy for NSCLC [6]. 677C > T polymorphism of *MTHFR* gene is a main single-nucleotide polymorphism, which is considered to be correlated with the pemetrexed-based chemotherapy response [7]. However, the correlation between *MTHFR*677C > T polymorphism and pemetrexed-based chemotherapy response is not conclusive. Therefore, we performed this meta-analysis by pooling the relevant open published studies to further investigate the correlation.

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2 Methods

2.1 Search of relevant studies in the electronic databases

Prospective clinical studies relevant to the correlation between *MTHFR* 677C > T polymorphism and response of pemetrexed-based chemotherapy in advanced NSCLC were systematically searched in the electronic databases of Pubmed, Embase, Cochran Library, CNKI and Wanfang. The prospective clinical studies on *MTHFR* 677C > T polymorphism and response of pemetrexed-based chemotherapy in advanced NSCLC published in English or Chinese were electronically searched through the following text words: pemetrexed, pemetrexed disodium, disodium, alimta, *MTHFR*, methylenetetrahydrofolate reductase, methylenetetrahydrofolate reductase (NADPH), methylene-THF reductase (NADPH), 5,10-methylenetetrahydrofolate reductase (NADPH), methylene tetrahydrofolate reductase, tetrahydrofolate reductase and methylene. The references of the included studies were also further screened to identify the

potentially suitable publications, which were not identified in the electronic databases.

2.2 Inclusion and exclusion criteria

The inclusion criteria of the studies were as follows: (1) clinical studies relevant to *MTHFR* 677C > T polymorphism and response of pemetrexed-based chemotherapy; (2) the patients included in the original publication should be diagnosed with NSCLC; (3) genotype of CC, CT, and TT frequency of the included cases can be extracted or calculated from the original study; (4) the genotyping methods were PCR or Taqman; (5) studies were published in English or Chinese; (6) the treatment response of pemetrexed-based chemotherapy in NSCLC of CC, CT and TT genotypes can be extracted or calculated from the original study. The exclusion criteria of the studies were as follows: (1) case report or literature review studies; (2) studies on animals; (3) studies published in other languages not in English or Chinese; (4) studies

Figure 1: The publication searching flowchart with reasons for studies exclusion.

Table 1: The general characteristics of the included studies

Study	Region	Sample size	Age	Pathology	Chemotherapy regimen	Genotyping	HWE	NOS
Tiseo et al. 2012	Italy	208	60 (36–84)	Adenocarcinoma	Pemetrexed, Pemetrexed + Carboplatin	Taqman	Yes	7
Jung et al. 2013	South Korea	90	59 (34–85)	Adenocarcinoma	Pemetrexed	PCR	Yes	7
Li et al. 2013	China	45	63 (39–91)	NSCLC	Pemetrexed + Cisplatin	Taqman	Yes	6
Krawczyk et al. 2014	Poland	115	61 ± 8.5	Non-SCC	Pemetrexed + Cisplatin	PCR	Yes	7
Kucharczyk et al. 2016	Poland	72	61	Non-SCC	Pemetrexed	PCR	Yes	7
Lan et al. 2017	China	51	57	Non-SCC	Pemetrexed, Pemetrexed + Cisplatin	PCR	Yes	6
Dong et al. 2015	China	92	57 (40–67)	Adenocarcinoma	Pemetrexed + Carboplatin	PCR	No	6
Zhao et al. 2017	China	88	57 (40–74)	NSCLC	Pemetrexed + Cisplatin	Taqman	No	6
Bai et al. 2019	China	25	NA	Adenocarcinoma	Pemetrexed + Carboplatin + Cisplatin	PCR	Yes	6
Li et al. 2012	China	37	63 (39–79)	Adenocarcinoma	Pemetrexed + Cisplatin	Pemetrexed + Cisplatin	Yes	5

about small cell lung cancer; (5) genotype and/or treatment response cannot be extracted or calculated from the original study.

2.3 Data extraction from the original study

The studies were first screened by two reviewers (HF and XWG) independently. The publications were included according to the inclusion and exclusion criteria. When there was a controversy for inclusion or exclusion criteria of a certain study, the discussion was first adapted and then a third reviewer was consulted. The main characteristics of the included studies such as the author name, year of publication, region, genotyping methods and outcomes were extracted and shown in a summary table. The data of TT, CT and CC genotype frequency, treatment response such as complete response, partial response, objective response rate, disease control rate and progressive disease were extracted or calculated from each original included study and cross-checked by two reviewers.

2.4 Studies quality evaluation

The general quality of the 10 included studies was evaluated by Newcastle-Ottawa Scale (NOS). The highest score of NOS is 9 points. The high, moderate, and low quality of the original studies were considered as score ≥6 points, 3–5 points and <3 points [8].

2.5 Publication bias evaluation

Begg's funnel plot and Egger's line regression test were applied for publication bias evaluation for the correlation between *MTHFR* 677C > T polymorphism and response of pemetrexed-based chemotherapy in advanced NSCLC.

2.6 Statistical analysis

Stata/SE 11.0 (StataCorp LP, <http://www.stata.com>) statistical software was used for the data analysis. The correlation between *MTHFR* 677C > T polymorphism and

response of pemetrexed-based chemotherapy in advanced NSCLC was demonstrated by the odds ratio (OR) and its 95% CI. The statistical heterogeneity across the 10 included publications was evaluated by I^2 test. Two-tailed $P < 0.05$ was considered as statistically significant.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

3.1 Main characteristics of the included original studies

Ten prospective studies [6,7,9–16] relevant to *MTHFR* 677C > T polymorphism and response of pemetrexed-based

Table 2: The genotyping distribution for the original included studies

Study	Region	Sample size	CC	CT	TT
Tiseo et al. 2012	Italy	208	95	92	21
ORR			28	7	0
PFS			3.4 (2.7–4.1)	5.4 (3.6–7.2)	NA
OS			8.5 (6.8–10.2)	16.4 (7.7–25.0)	NA
Jung et al. 2013	South Korea	90	24	47	19
ORR			5	9	0
DCR			17	28	11
Li et al. 2013	China	45	17	21	7
PFS			5.6 (3.7–7.5)	3.8 (1.6–6.0)	5.8 (0.0–12.5)
OS			10.3 (7.6–132.0)	10.6 (4.6–16.6)	8.1 (4.5–11.7)
Krawczyk et al. 2014	Poland	115	53	49	13
PD			9	1	10
SD/PR			44	48	3
PFS			6	7.5	7
OS			25	13	12
Kucharczyk et al. 2016	Poland	72	32	29	11
PD			10	12	2
SD/PR			22	17	9
PFS			5.5	4	5
OS			11	13.5	17.5
Lan et al. 2017	China	51	21	20	10
ORR			6	4	
DCR			15	20	
Dong et al. 2015	China	92	56	23	13
PR			20	0	2
SD			19	13	7
PD			17	10	4
ORR			20	0	2
DCR			39	13	9
Zhao et al. 2017	China	88	54	22	12
PR			18	0	2
SD			19	13	6
PD			17	9	4
ORR			18	0	2
DCR			37	13	8
Bai et al. 2019	China	25	10	15	
CR			0	0	
PR			1	2	
SD			6	9	
PD			3	4	
DCR			7	11	
ORR			1	2	
Li et al. 2012	China	37	15	16	6
ORR			4	5	3
PFS			4.7	6.9

Figure 2: The funnel plot of the correlation between *MTHFR* 677C > T polymorphism and pemetrexed-based chemotherapy partial response in different gene models.

chemotherapy in advanced NSCLC were included in the present meta-analysis (Figure 1). Of the 10 included studies, six were from China, two were from Poland, one was from South Korea and one was from Italy. The sample size ranges from 25 to 208. The general quality of the included studies was relatively high. The NOS score ranges from 5 to 7 with the median NOS score of 6.0. The main characteristics including the study region, age of the subject, the sample size, chemotherapy regimen, the outcome and others were presented in Table 1. The number of responses in each of the included studies is presented in Table 2.

3.2 Correlation between *MTHFR* 677C > T polymorphism and partial response

The statistical heterogeneity was not significant in dominant, recessive and homozygous models ($P_{all} > 0.05$). Therefore, the data were pooled in a fixed effect model. The pooled results indicated that the partial response in NSCLC patients with TT or CT genotype was inferior to the CC genotype in a dominant gene model (TT + CT vs CC;

OR = 0.16, 95% CI: 0.06–0.41, $P = 0.001$). However, the difference was not statistically different in recessive (TT vs CT + CC; OR = 0.59, 95% CI: 0.19–1.81, $P = 0.351$) and homozygous gene models (TT vs CC; OR = 0.36, 95% CI: 0.12–0.52, $P = 0.08$), Figure 2.

3.3 Correlation between *MTHFR* 677C > T polymorphism and objective response rate

Statistical heterogeneity was found in a dominant gene model ($P = 0.038$), but not in recessive and homozygous gene models ($P > 0.05$). The data were pooled by random and fixed-effect models in dominant and recessive models and homozygous gene model, respectively. The combined results showed that NSCLC cases with T genotype were inferior to C genotype in the objective response rate when treated with pemetrexed-based chemotherapy for dominant (OR = 0.28, 95% CI: 0.18–0.45, $P = 0.001$), recessive (OR = 0.43, 95% CI: 0.19–0.94, $P = 0.03$) and homozygous gene models (OR = 0.30, 95% CI: 0.13–0.67, $P = 0.003$), Figure 3.

Figure 3: The funnel plot of the correlation between *MTHFR* 677C > T polymorphism and pemetrexed-based chemotherapy objective response rate in different gene models.

3.4 Correlation between *MTHFR* 677C > T polymorphism and disease control rate

The data were pooled in the fixed-effect model in the disease control rate due to nonsignificant statistical heterogeneity ($P > 0.05$). The pooled results indicated that the disease control rates were not statistically different in different genotypes in dominant, recessive and homozygous gene models ($P_{\text{all}} > 0.05$), Figure 4.

3.5 Correlation between *MTHFR* 677C > T polymorphism and progressive disease

The data were pooled by the fixed-effect model for the dominant gene model and the random-effect model for recessive and homozygous gene models. The combined

results showed that progressive disease was not statistically different in different genotypes (CC, CT or TT) in dominant, recessive and homozygous gene models ($P_{\text{all}} > 0.05$). However, NSCLC cases with T genotype had a trend of an increased risk for disease progression, Figure 5.

3.6 Progression-free survival and overall survival analysis

The progression-free survival and overall survival time for CC, CT and TT allele were presented in Table 3. There was no statistical difference in progression-free survival and overall survival time for NSCLC patients with CC, CT or TT alleles treated with pemetrexed-based chemotherapy ($P_{\text{all}} > 0.05$), Figure 6.

Figure 4: The funnel plot of the correlation between *MTHFR* 677C > T polymorphism and pemetrexed-based chemotherapy disease control rate in different gene models.

3.7 Publication bias evaluation

Begg's funnel plots of objective response rate and disease control rate were in general left and right symmetrical, which indicated no obviously publications bias (Figure 7). Egger's line regression test also showed that the publication bias was not significant ($P > 0.05$).

4 Discussion

Ten prospective clinical studies relevant to *MTHFR* 677C > T polymorphism and response of pemetrexed-based chemotherapy in advanced NSCLC were included in the present meta-analysis. The general quality of the 10 publications was relatively high with the median NOS score of 6.0. The original data of the ten included studies were pooled and indicated that the partial response in NSCLC patients with T genotype was inferior to C genotype in the

dominant gene model (TT + CT vs CC; OR = 0.16, 95% CI: 0.06–0.41, $P = 0.001$). The objective response rate in cases with T genotype was inferior to C genotype in dominant (OR = 0.28, 95% CI: 0.18–0.45, $P = 0.001$), recessive (OR = 0.43, 95% CI: 0.19–0.94, $P = 0.03$) and homozygous models (OR = 0.30, 95% CI: 0.13–0.67, $P = 0.003$). However, the progression-free survival and overall survival were not statistically different in NSCLC patients with CC, CT or TT alleles treated with pemetrexed-based chemotherapy. The results indicated that NSCLC with T allele only had 0.16 odds of partial response in pemetrexed-based chemotherapy response compared to C allele in the dominant gene model. For the objective response rate, the chemotherapy odds ranges from 0.28 to 0.43 for T allele compared with C allele in NSCLC treated with pemetrexed-based chemotherapy. Therefore, NSCLC cases with T genotype of *MTHFR* gene may have decreased response when treated with pemetrexed-based chemotherapy, which indicated that T allele of *MTHFR* 677 SNP was a contraindication for pemetrexed-based chemotherapy regimen in NSCLC.

Figure 5: The funnel plot of the correlation between *MTHFR* 677C > T polymorphism and pemetrexed-based chemotherapy progressive disease in different gene models.

Pemetrexed is a multitarget folate antagonist. Its main anticancer mechanism is inhibiting cell proliferation by destroying the key folate-dependent metabolic process of cell replication [17–19]. *MTHFR* is a key enzyme in folate metabolism, which can irreversibly catalyze the reduction of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, which is the main form of folate in plasma [20,21]. Therefore, the activity of *MTHFR* can affect the metabolism of folic acid and further affect the response of pemetrexed-based chemotherapy. Currently, the study on *MTHFR* activity mainly focuses on

its SNP, and C677T is the most-studied SNP site. However, the relationship between *MTHFR* 677C > T polymorphism and the response of pemetrexed-based chemotherapy in patients with advanced NSCLC is not consistent according to the previous publications [6,7]. Zhao et al. [10] found that patients with CC allele of *MTHFR* gene had better chemotherapy response and longer progression-free survival than patients with CT + TT genotype. However, Tiseo et al. [6] found that patients with TT genotype of *MTHFR* gene had longer progression-free survival and overall survival time compared to CC genotype. Therefore, whether *MTHFR* 677C > T polymorphism is correlated with the response of pemetrexed-based chemotherapy in advanced NSCLC is not conclusive. Our meta-analysis results showed pemetrexed-based chemotherapy response was decreased in NSCLC cases with T genotype. The reason of the inferior pemetrexed-based chemotherapy response in NSCLC cases with T genotype may be due to the decreased *MTHFR* activity and folic acid metabolism of the T genotypes. In addition, it has also been

Table 3: The PFS and OS between *MTHFR* 677C > T polymorphism and Pemetrexed-based chemotherapy progressive disease

Prognosis	CC	CT	TT	F	P-value
PFS	5.04 ± 1.03	5.18 ± 1.71	5.93 ± 1.01	0.48	0.63
OS	13.70	13.38	12.53	0.04	0.96

Figure 6: Scatter plot of progression-free survival and overall survival between *MTHFR* 677C > T polymorphism and pemetrexed-based chemotherapy progression disease. (a) Progression-free survival in CC, CT and TT allele. (b) Overall survival in CC, CT and TT allele.

Figure 7: The Begg's funnel plots showed no significant publication bias in evaluation of *MTHFR* C677T polymorphism and response of pemetrexed-based chemotherapy in advanced NSCLC. (a) Funnel plot of object response rate. (b) Disease control rate.

pointed out that the increase of folic acid in the serum of NSCLC cases can increase the pemetrexed chemotherapy response, which validates the aforementioned point of view [22].

Our results showed that pemetrexed-based chemotherapy response was decreased in NSCLC cases with T genotype, which can be applied as a potential pemetrexed-based chemotherapy response marker. However, the meta-analysis also had its own limitations: First, only 10 studies were included in the meta-analysis with a small sample size. Therefore, the statistical power is limited. Second, the studies screened and included in the meta-analysis were limited to English or Chinese publications. Third, the long-term survival such as overall survival and progression-free survival were not pooled due to inadequate data. Fourth, clinical heterogeneity such as mutations in EGFR or the presence of the ROS1 or EML4-ALK fusion oncogenes might affect the treatment response and outcome.

However, the information relevant to the aforementioned molecular characteristics was not mentioned in the original study. Therefore, how much the molecular characteristics affects the results of the meta-analysis was not clear.

5 Conclusion

The pemetrexed-based chemotherapy response was decreased in NSCLC cases with T genotype, which can be applied as a potential pemetrexed-based chemotherapy response marker. However, due to the aforementioned limitations, large-scale well-designed prospective clinical trials relevant to this topic are still necessary to further investigate the correlation between *MTHFR* 677C > T polymorphism and response of pemetrexed-based chemotherapy in advanced NSCLC to provide more reliable evidence.

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Data availability statement: The datasets analyzed during the current study are available in the PubMed repository, <https://pubmed.ncbi.nlm.nih.gov>; Embase repository, <https://www.embase.com>; Cochrane Library repository, <https://www.cochranelibrary.com>; CNKI repository, <https://oversea.cnki.net/index/> and Wanfang repository, <http://www.wanfangdata.com/>

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