Association between *MGMT* Promoter Methylation and Non-Small Cell Lung Cancer: A Meta-Analysis

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Abstract

Background: O^6-methylguanine-DNA methyltransferase (MGMT) is one of most important DNA repair enzyme against common carcinogens such as alkylate and tobacco. Aberrant promoter methylation of the gene is frequently observed in non-small cell lung cancer (NSCLC). However, the importance of epigenetic inactivation of the gene in NSCLC published in the literature showed inconsistence. We quantified the association between *MGMT* promoter methylation and NSCLC using a meta-analysis method.

Methods: We systematically reviewed studies of *MGMT* promoter methylation and NSCLC in PubMed, EMBASE, Ovid, ISI Web of Science, Elsevier and CNKI databases and quantified the association between *MGMT* promoter methylation and NSCLC using meta-analysis method. Odds ratio (OR) and corresponding 95% confidence interval (CI) were calculated to evaluate the strength of association. Potential sources of heterogeneity were assessed by subgroup analysis and meta-regression.

Results: A total of 18 studies from 2001 to 2011, with 1, 160 tumor tissues and 970 controls, were involved in the meta-analysis. The frequencies of *MGMT* promote methylation ranged from 1.5% to 70.0% (median, 26.1%) in NSCLC tissue and 0.0% to 55.0% (median, 2.4%) in non-cancerous control, respectively. The summary of OR was 4.43 (95% CI: 2.85, 6.89) in the random-effects model. With stratification by potential source of heterogeneity, the OR was 20.45 (95% CI: 5.83, 71.73) in heterogeneous control subgroup, while it was 4.16 (95% CI: 3.02, 5.72) in the autologous control subgroup. The OR was 5.31 (95% CI: 3.00, 9.41) in MSP subgroup and 3.06 (95% CI: 1.75, 5.33) in Q-MSP subgroup.

Conclusion: This meta-analysis identified a strong association between methylation of *MGMT* gene and NSCLC. Prospective studies should be required to confirm the results in the future.

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Introduction

Lung cancer is the leading cause of global cancer deaths in recent decades [1]. Human lung cancer contains two histological types, small-cell lung cancer (SCLC) and non-small lung cancer (NSCLC). The latter comprises the majority of lung cancer and has an increasing incidence and mortality in the last two decades worldwide. DNA methylation is an epigenetic modification of the genome and methylation associated with silencing can affect genes expression in cellular pathways [2]. The epigenetic alterations are early and frequent events occurred in carcinogenesis. O^6-methylguanine-DNA methyltransferase (MGMT) is a DNA damage reversal protein against DNA adduct formation of carcinogens [3,4]. It can protect cells from the carcinogenic effects of alkylating agents by removing adducts from the O^6 position of guanine [5]. Therefore, the repair capacity of the MGMT protein helps decrease the probability so that the damaged guanine becomes a mutagenic site. Methylation of *MGMT* gene promoter has been associated

with loss or decrease of *MGMT* expression in tumor tissues of various cancers, including lung tumors [6-8].

Taken together, methylation of *MGMT* gene has been considered as potentially useful candidate biomarker for early detection of lung cancer. Many studies had also shown that methylation of the gene can be found in clinical samples, such as tissues, serum and bronchoalveolar lavage fluid (BALF) of NSCLC [9-11].

The purpose of this study was to understand the difference of the prevalence of aberrant promoter methylation of *MGMT* in NSCLC tissue from control. We conducted a meta-analysis using available data in the literature on the basis of *MGMT* promoter methylation and NSCLC to better identify the association between *MGMT* promoter methylation and NSCLC.

Materials and Methods

Selection criteria and study search

We searched the electronic databases online including Pubmed, EMBASE, Ovid, ISI Web of Science, Elsevier and CNKI database, using the search terms "*MGMT*", "NSCLC" and "methylation". The following search strategy was performed in Pubmed "NSCLC" (MESH), "methylation" and "*MGMT* or O^6-methylguanine-DNA methyl-transferase" to collect eligible articles. The search was limited to articles published in English and Chinese. Similar searches were performed in other databases. The search was updated until June 1, 2013.

Studies selected had to meet the following criteria: (a) The study was about *MGMT* methylation and NSCLC. (b) The authors offered a measure of the association either as an effect estimate with 95% CI and OR, or sufficient data in the original article to calculate it. (c) *MGTM* methylation status was examined using methylation-specific PCR (MSP) or real-time quantitative MSP (Q-MSP). (d) Specimens of NSCLC were surgically respected primary tumor sample and the styles of control were composed of plasma or non-cancerous lung tissues (NLT) including autologous control and heterogeneous control.

Studies were excluded according the following criteria: (a) The study did not provide control information. (b) The articles were repetitively reported, (c) The articles researched chromate lung cancer, brain metastases of lung cancers and malignant pleural mesothelioma [7,12,13]. Firstly, we evaluated whether a study met the inclusion criteria by title and abstract of initial searching articles. Then all the potentially relevant articles were evaluated by accessing full-text paper. The selection procedure of studies was illustrated in statement flow chart (Figure 1).

Abstraction of Data and Quality Assessment

All the data were independently abstracted by three authors (Changmei Gu, Cheng Lu, and Hao Shi) with the use of standardized data-extraction forms. For each study, the following characteristics were extracted: first author's name, year of publication, the study country, ethnicity, age, sample size, type of control, the method for methylating detection, and status of *MGMT* methylation were collected using standard data extraction forms. If there were disagreements, we discussed with Meixia Lu and Jiachun Lu repeatedly, until a consensus. To ensure the transparent and complete reporting of this meta-analysis, we designed and checked selected studies according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [14] statement.

Statistical Methods

The ORs and 95% CIs were abstracted or calculated to evaluate the strength of the association between *MGMT* promoter methylation and NSCLC risk. Summary of OR was acquired from all studies or calculated with the data in selected studies.

We, then, investigated between-study heterogeneity by using Cochran's Q test with a significance level of *P* value less than 0.1 and heterogeneity was also examined using I^2 statistic [15], which is a quantitative measure of inconsistency across studies. If $I^2 > 50\%$ or $P < 0.1$ is considered as a measure of severe heterogeneity, then the random-effects model was used to calculate summary OR according to the Der-Simonian Laird method; otherwise, the fixed-effects model (Matel–Haenszel method) was applied[16]. τ^2 was used to determine how much heterogeneity was explained by subgroup differences. The meta-regression was performed to explore the source of heterogeneity based on ethnicity (Asian and Caucasian), publication year, style of control (autogenous or heterogeneous), method (MSP or Q-MSP), and sample size. Subgroup analyses were performed according to ethnicity (Asian and Caucasian), control style (autogenous or heterogeneous) and method of methylation detection (MSP or Q-MSP) in consideration of the source of heterogeneity. Sensitivity analyses were also performed to assess the contributions of each study on the overall result by omitting one study at a time. The funnel plot asymmetry was used to evaluate the evidence of publication bias. Peter's test was applied to quantitatively evaluate the evidence for publication bias [17]. Fail-safe number (N_{fs}) adopted by Rosenthal [28] is considered to be a useful indicator of leaning [18]. The meta-trim method was used to re-estimate the effect size when there was possible bias. All *P* values are two-tailed with a significant level at 0.05. In forest plot displayed in Figure 2, the size of the box for each study was inversely proportional to the variance of the log relative risk, and the horizontal lines represent 95% CI.

All statistical analyses were conducted by using the Meta package (version 2.2-1) in R (version 3.0, http://www.r-project.org/).

Results

Study Characteristics

The electronic search found that 145 potentially relevant studies were initially identified. These studies were further screened based on inclusion and exclusion criteria. A total of 18 studies (2001-2011) were included in the analysis (Figure 1). In these studies, 6 studies were conducted in Asia (1 in Japan and 5 in China) and the remaining 11 were in the USA and 1 in the Netherlands.

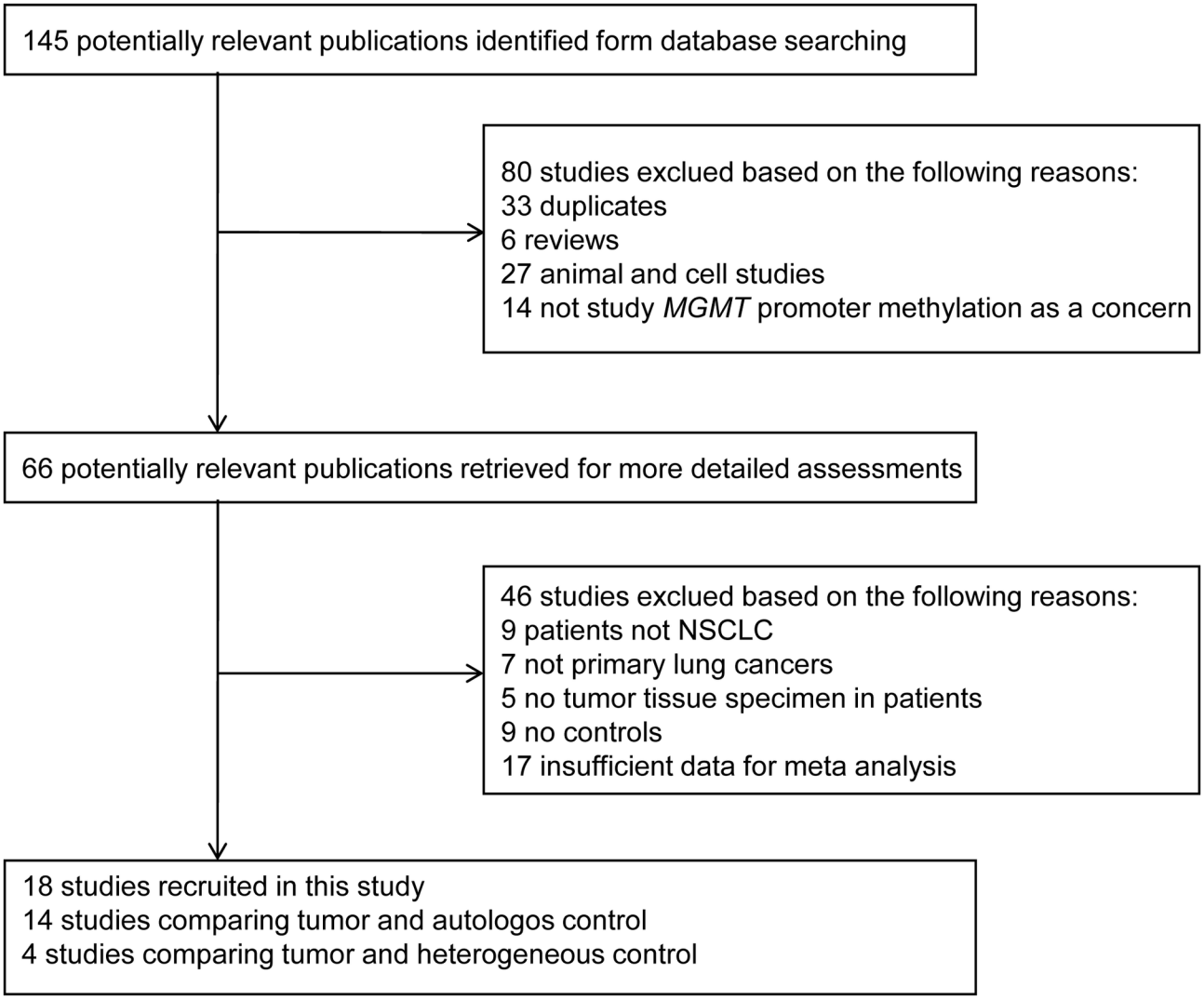

Figure 1. Flow diagram of the stepwise selection from associated studies.

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Among the 18 retrieved studies, 14 studies used methylation-specific polymerase chain reaction (MSP) and 4 studies used real-time quantitative MSP (Q-MSP) to explore *MGMT* methylation in NSCLC tissue and control. There were two control styles, including autogenous control (the tissues from the patients themselves) and heterogeneous control (i.e Plasma, tissue, and bronchoalveolar lavage fluid from other individuals). The main characteristics of these studies were presented in Table 1.

There are 1, 160 NSCLC tumor tissues and 970 controls. The frequencies of *MGMT* promote methylation ranged from 1.5% to 70.0% (median 26.1%) in NSCLC tissue and 0.0% to 55.0% (median 2.4%) in non-cancerous control, respectively, which indicated the methylation frequency in cancer tissue was much higher than that in the control group. Under the random-effects model, the pooled OR of *MGMT* methylation in NSCLC tissue was 4.43 (95% CI: 2.85, 6.89), in comparison with control group (Figure 2).

Meta-regression and subgroup analyses

Considering the existence of heterogeneity in the meta-analysis (I^2=32.0%, P= 0. 095), the meta-regression was performed for finding the source of heterogeneity. The restricted maximum likelihood modification was used to estimate between study variances. As the restriction to access raw data, we assumed the source of heterogeneity may appear from the year of publication, ethnicity, type of control, detection method and sample size. The results showed that the sources of the heterogeneity were control style (P = 0.073) and detection method (P = 0.094). Other factors such as sample size, year of publication, and ethnicity could not explain the heterogeneity (Table 2).

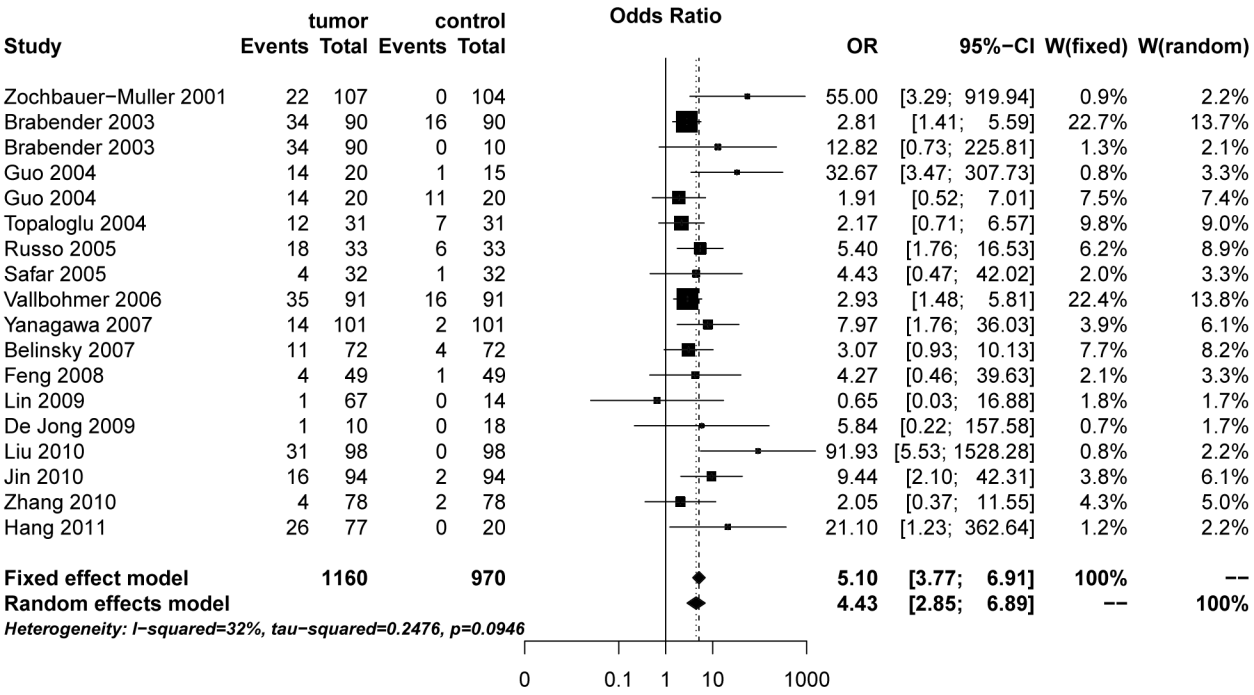

Figure 2. Forest plot of MGMT methylation in tumor tissue verse control group between *MGMT* promoter methylation and NSCLC.

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Table 1. Characteristics of studies included in this meta-analysis.

Study	Year	Country	Ethnicity	Gender M/F	Age (x)	Method	Patients M+/N	Control M+/N	Control style	Control source
Zochbauer[19]	2001	USA	Caucasian	76/31	28-81	MSP	22/107	0/104	A	tissue
Brabender[20]	2003	USA	Caucasian	68/22	63.3	Q-MSP	34/90	16/90	A	tissue
Brabender[20]	2003	USA	Caucasian	NA	NR	Q-MSP	34/90	0/10	H	tissue
Guo[9]	2004	USA	Caucasian	NA	42-83	MSP	14/20	1/15	A	tissue
Guo[9]	2004	USA	Caucasian	NA	42-83	MSP	14/20	11/20	A	BALF
Topaloglu[21]	2004	USA	Caucasian	NA	NA	Q-MSP	12/31	7/31	A	tissue
Russo[22]	2005	USA	Caucasian	NA	NA	MSP	18/33	6/33	A	serum
Safar[23]	2005	USA	Caucasian	NA	67	MSP	4/32	1/32	A	tissue
Vallbohmer[24]	2006	USA	Caucasian	69/22	63	MSP	35/91	16/91	A	tissue
Yanagawa[25]	2007	Japan	Asian	72/29	39-86	MSP	14/101	2/101	A	tissue
Belinsky[26]	2007	USA	Caucasian	49/23	37-80	MSP	11/72	4/72	A	serum
Feng[27]	2008	USA	Caucasian	24/23	64.3	MSP	4/49	1/49	A	tissue
Lin[28]	2009	China	Asian	NA	NA	MSP	1/67	0/14	H	tissue
De Jong[29]	2009	NE	Caucasian	NA	40-70	Q-MSP	1/10	0/18	H	tissue
Liu[30]	2010	China	Asian	58/40	32-68	MSP	31/98	0/98	H	tissue
Jin[31]	2010	China	Asian	83/11	32-75	MSP	16/94	2/94	A	tissue
Zhang[32]	2011	China	Asian	58/20	35-80	MSP	4/78	2/78	A	tissue
Hang[33]	2011	China	Asian	64/32	33-70	MSP	26/77	0/20	H	tissue

Abbreviation: A: Autologos control(the control from the NSCLC themselves); BALF: bronchoalveolar lavage fluid; H: Heterogeneous control(the control from other individuals, including serum, bronchoalveolar lavage or tissue); M+: The number of methylation; MSP: methylation-specific polymerase chain reaction; N: number of total; NA: not applicable; NE: Netherlands; Q-MSP: real-time quantitative methylation-specific polymerase chain reaction

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Table 3. Subgroup analysis of the association between *MGMT* promoter methylation and NSCLC.

	Tumor		Control		M-H pooled OR[b]	D+L pooled OR[a]	Heterogeneity		
Group	**M+**	**N**	**M+**	**N**	**OR (95%CI)**	**OR (95%CI)**	I^2 (%)	*P*	τ^2
Control group									
Auologous	202	818	69	810	4.16 (3.02, 5.72)	3.74 (2.54, 5.51)	16.6	0.276	0.080
Heterogeneous	93	342	0	160	20.45 (5.83, 71.73)	11.18 (2.14, 58.35)	34.0	0.194	1.208
Ethnicity									
Asian	92	515	6	405	10.96 (5.20, 23.14)	7.36 (2.33, 23.19)	45.1	0.105	0.8807
Caucasian	203	645	63	565	4.00 (2.85, 5.60)	3.49 (2.37, 5.14)	8.9	0.358	0.0418
Method									
MSP	214	939	46	821	6.17 (4.28, 8.91)	5.31 (3.00, 9.41)	40.2	0.060	0.4155
Q-MSP	81	221	23	149	3.06 (1.75, 5.33)	2.84 (1.62, 5.00)	0.0	0.674	<0.0001
Total	295	1160	69	970	5.10 (3.77, 6.91)	4.43 (2.85, 6.89)	32.0	0.095	0.2476

D+L pooled OR[a]: the result from random-effects model;M-H pooled OR[b]: the result from fixed-effects model.

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Table 2. Mixed-effects model of meta-regression analysis.

			95%CI		
Heterogeneity sources	**Coefficients**	***Z***	**Lower**	**Upper**	***P***
Year	-0.1964	-1.4009	-0.4712	0.0784	0.161
Control	1.4501	1.7915	-0.1364	3.0365	0.073
Ethnicity	0.9485	1.3741	-0.4044	2.3015	0.169
Method	-0.8260	-1.6752	-1.7925	0.1404	0.094
Sample size	0.0014	0.4581	-0.0046	0.0075	0.065

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With these observations, we performed subgroup analyses based on control style and detection method. The OR in the heterogeneous control subgroup was 20.45 (95% CI: 5.83, 71.73; fixed-effects model), while it was 4.16 (95% CI: 3.02,5.72; fixed-effects model) in the autologous control subgroup. I^2 changed to 16.6% and 34.0% in those subgroups, respectively, compared with 32% of the total. Stratification by control style showed that the OR in the heterogeneous control subgroup was higher than that in the autologous control subgroup. After stratification by detection method, the OR was 5.31 (95% CI: 3.00, 9.41; random-effects model) in MSP subgroup and 3.06 (95% CI: 1.75, 5.33; fixed-effects model) in Q-MSP subgroup (Table 3).

Sensitivity analysis

To determine the effects of omitting a single study at a time on the overall effect, the sensitivity analysis was performed. Omission of a single study changed the overall OR from 3.85 (95% CI: 2.66, 5.56) to 4.87 (95% CI: 2.96, 8.02) using the random-effects model, which demonstrated that no single sensitivity analysis existed (Figure 3).

Publication bias

A funnel plot of methylation status of tumor versus control showed that there were two studies exceed the 95% confidence limits as shown in Figure 4. However, no publication bias was detected by using Peter's test (P = 0.624). We also used a fail-safe number (N_{fs}) to assess the efficacy of meta-analysis (Z = 45.63, $N_{fs0.\ 05}$ = 756.12, $N_{fs0.\ 01}$ = 365.52), which indicated a slight publication bias in the meta-analysis.

Discussion

Aberrant methylation of promoter regions in DNA repair genes is a key event in the formation and progression of cancer. It was concluded that genes methylation was potentially a new generation of cancer biomarker [34]. Epigenetic change of CpG islands in the gene promoter region is an important reason for gene dysfunction [35], which can lead to the transcription of the gene down or stops [36].

MGMT promoter methylation is very common in the primary NSCLC, which has been reported in some studies. However, the results about the association between the status of *MGMT* methylation and NSCLC were inconsistent. The OR values fluctuated from 0.65 [28] to 114.98 [24], so we performed this meta-analysis to identify the association between *MGMT* promoter methylation and NSCLC.

A total of 18 studies including 1, 160 tumor tissues and 970 controls were involved in the meta-analysis. The frequencies of *MGMT* promote methylation ranged from 1.5% to 70.0% (median; 26.1%) in NSCLC tissue and 0.0% to 55.0% (median; 2.4%) in non-cancerous control, respectively. The findings indicated that the methylation frequency in cancer tissue was much higher than that in the control group. *MGMT* methylation had an increased risk in tumor tissue (OR = 4.43; 95% CI: 2.85, 6.89) in comparison with non-cancerous samples including plasma, tissue, and bronchoalveolar lavage fluid. This finding was consistent with other studies [10,25]. Furthermore, subgroup analysis of control style showed that an OR was 20.45 (95% CI: 5.83, 71.73; fixed-effects model) in the heterogeneous control subgroup verse 4.16 (95% CI: 3.02, 5.72; random-effects model) in the autologous tissues subgroup, indicating that the OR of methylation in heterogeneous control group was higher than that in the autologous control group. It suggested that *MGMT* gene

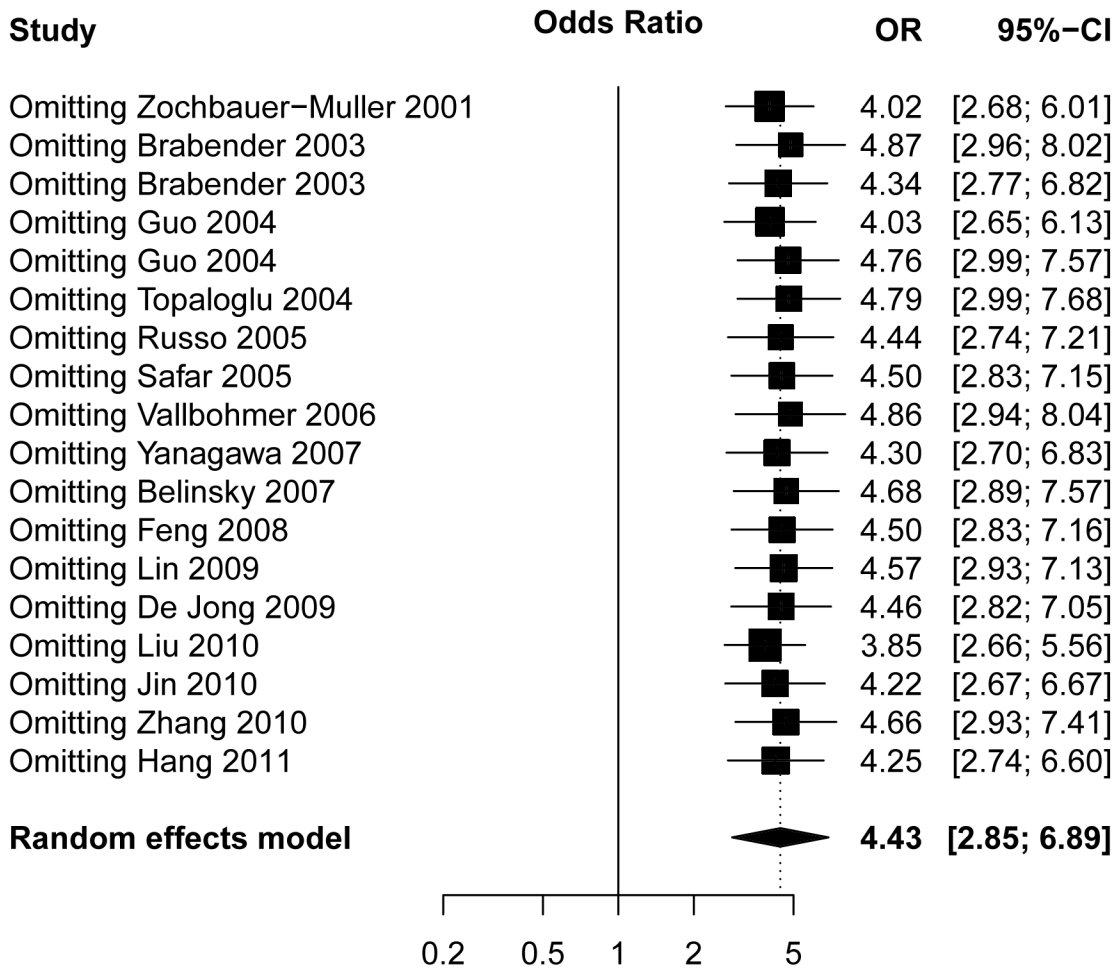

Figure 3. Sensitivity analysis by omitting a single study at a time on the overall effect.

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promoter methylation was a frequent event in NSCLC, but it rarely happened in the non-tumor group. Stratification analysis by different methods of methylation detection showed that the OR for the MSP subgroup was higher than that for Q-MSP subgroup.

Sensitivity analysis was performed to determine the effects when omitting a single study at a time on the overall effect. The analytic results demonstrated that no single study could affect the summarized OR. In addition, the shape of the funnel plot did not show any evidence of funnel plot asymmetry and no publication bias was detected.

The current meta-analysis has some limitations. Firstly, selection bias is inevitable due to the strategy restricted to articles published in English or Chinese. Secondly, although the total sample sizes in the meta-analysis were not more than 1, 000, the results maybe have still no vigorous power. Thirdly, we did not study the methylation status in histological subtypes, smoking and different clinical stages because of limitation of insufficient raw materials.

In conclusion, *MGMT* gene is an important DNA repair gene on maintaining the integrity of the genome. Aberrant *MGMT* promoter methylation may be associated with the occurrence and development of NSCLC. This meta-analysis identified a strong association between *MGMT* promoter methylation and NSCLC. Prospective studies should be required to confirm the results in the future.

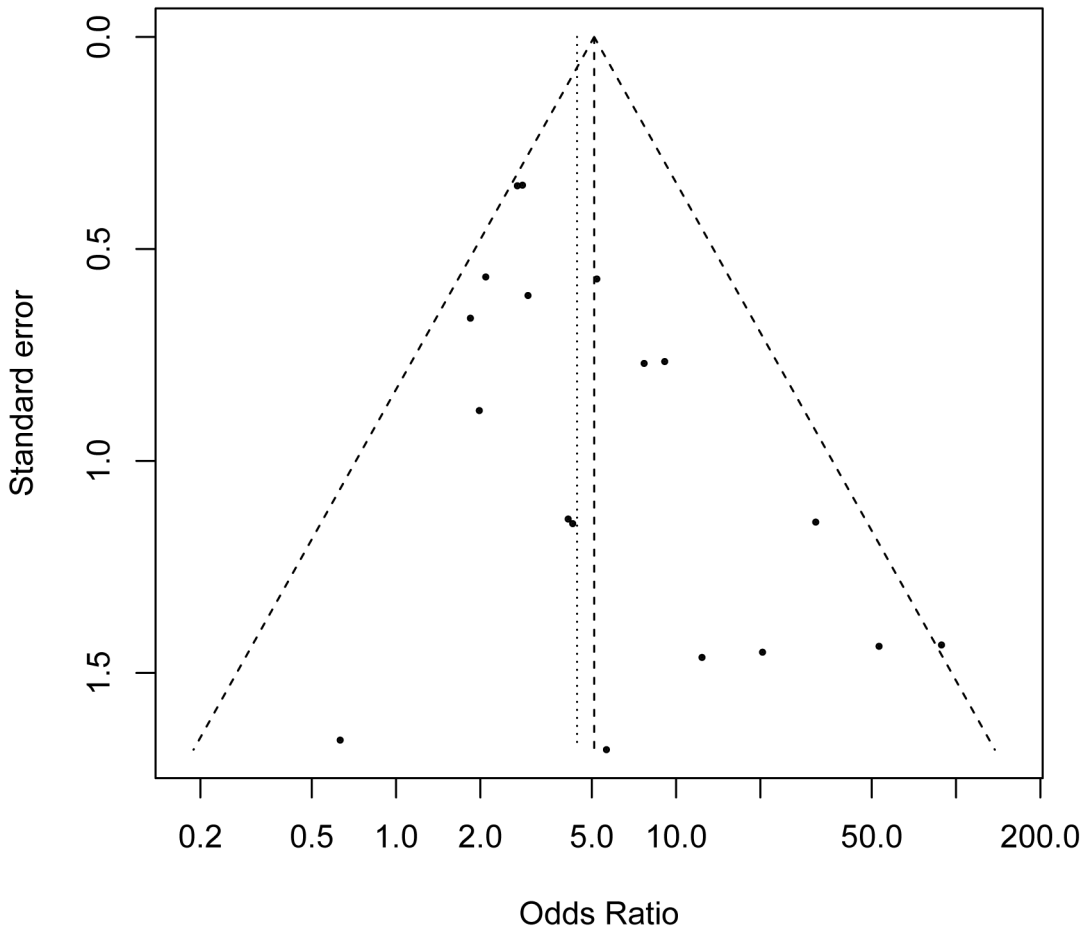

Figure 4. Funnel plot for assessment of publication bias. Each hollow point represents a separate study for the indicated association. The area of the hollow point reflects the weight (inverse of the variance). Horizontal line stands for the mean magnitude of the effect.

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Supporting Information

Checklist S1. PRISMA Checklist.
(DOCX)

Author Contributions

Conceived and designed the experiments: M-XL. Performed the experiments: J-CL T-PC HS W-MX. Analyzed the data: C-MG CL X-LY X-BY Y-XH. Contributed reagents/materials/analysis tools: CL X-BY Y-XH. Wrote the manuscript: C-MG M-XL Y-XH.

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