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Original Article

Predictive value of MGMT promoter methylation status in Asian and Caucasian patients with malignant gliomas: a meta-analysis

Chao Chen, Feng Wang, Yuan Cheng, Yin Cheng, Xueying Ren, Haiqing Huai

Department of Medical Oncology of PLA Cancer Center, 81 Hospital of PLA, Nanjing 210002, China

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Abstract: Background: The prognostic significance of O6-methylguanine-DNA methyltransferase (MGMT) promoter status for survival of patients with malignant gliomas remains controversial. Thus, the meta-analysis was performed in order to identify the impact of MGMT expression on prognosis of malignant gliomas. Method: An extensive literature search for relevant studies was conducted on PubMed, Embase, Web of Science, Cochrane Library, and CBM databases. Version 12.0 STATA software was used for the current meta-analysis. Hazard ratios (HRs) with their corresponding 95% confidence interval (95% CI) were also calculated to clarify the correlation between MGMT expression and the prognosis of malignant gliomas. Results: Final analysis of 2,377 malignant gliomas patients from 32 clinical studies was performed. The meta-analysis results show that MGMT promoter group and unmethylated MGMT group has a significant difference (all $P < 0.01$). Combined HR of MGMT suggests that the methylated MGMT group has a longer overall survival than the unmethylated MGMT group ($P < 0.01$), but the Asians don't present a difference between the two groups. Conclusion: The meta-analysis study shows that the elevated MGMT promoter group may have a better prognosis in malignant gliomas patients, but the Asians don't have a better prognosis.

Keywords: Malignant gliomas, MGMT, temozolomide, prognosis, meta-analysis, O6-methylguanine-DNA methyltransferase

Introduction

Malignant gliomas, the most common type of brain malignancy, has increased over the past decades and is expected to keep rising in the aging population of developed countries despite the use of multiple treatment modalities, including surgical resection, radiotherapy, and chemotherapy [1-3]. Possible reasons include poor curative effect, poor survival, and the fear of treatment-related toxicity [4-7]. The current standard treatment for unresectable glioblastoma is concomitant radiotherapy with temozolomide followed by up to six cycles of adjuvant temozolomide, based on the results of the 2005 European Organization for Research and Treatment of Cancer (EORTC)-National Cancer Institute of Canada (NCIC) randomized phase III trial [8]. Promoter methylation of the O6-methylguanine-DNA methyltransferase gene (MGMT), which encodes a DNA repair protein associated with alkylator resistance, has been known to predict a favorable response to temozolamide [5, 9-11]. However, controversial issues remain regarding its efficacy. The aim of our study is to evaluate the impact of MGMT promoter status on prognosis of glioblastoma patients.

Methods

Search strategy an extensive literature search for relevant studies was conducted on PubMed, Embase, Cochrane Library, Web of Science, and CBM databases until OCT 20, 2014. We used the following keywords and MeSH terms: ["glioma" or "glioblastoma" or "malignant glioma"] and ["O-6-methylguanine-DNA methyltransferase" or "MGMT"] and ["temozolomide" or "TMZ"]. Reference lists from related articles were also reviewed. There were no language restrictions. Manual search of reference lists from potentially relevant articles was also performed to identify other potential studies.

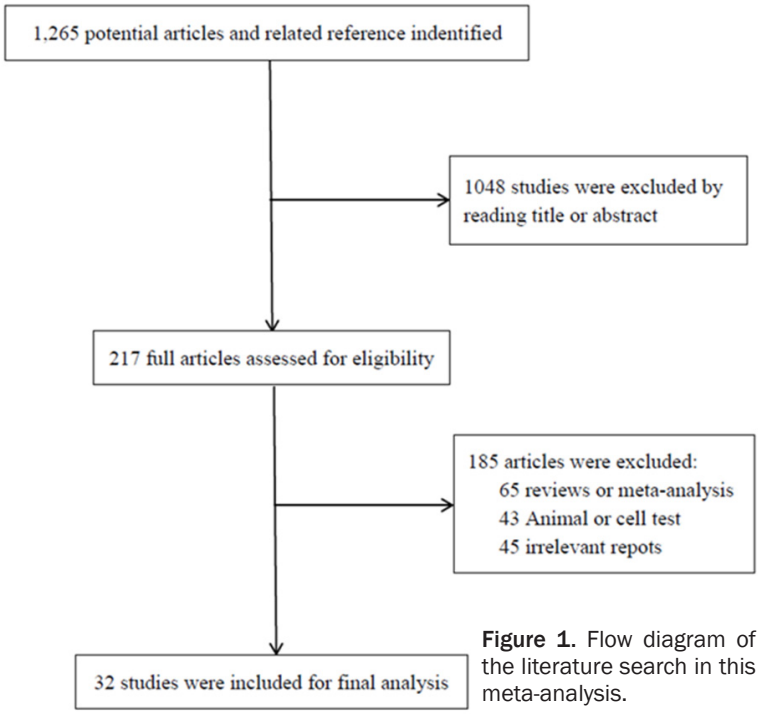


Figure 1. Flow diagram of the literature search in this meta-analysis.

Selection criteria The following criteria were used to determine the eligibility of included studies: (1) the study design must be a clinical cohort study; (2) the study must relate to the relationships of MGMT promoter methylation status and prognosis of the patients; (3) all patients met the diagnostic criteria for glioblastoma; (4) published data on Progression Free Survival (PFS) and Overall Survival (OS) estimates was sufficient. If a study could not meet the inclusion criteria, it was excluded. The most recent publication was included when the authors published other studies using the same subjects. The following information was extracted from eligible studies by two authors independently using a standardized form: surname of first author, year of publication, source of publication, country, language of publication, study design, total number of cases, method of MGMT promoter methylation status, statistical methods and survival data, etc. Any disagreement was resolved through discussions and subsequent consensus.

The time-to-event data from individual trials were summarized by the log hazard ratio (HR) and its variance. If the trials did not report this information directly, the time-to-event data were extracted from the survival curves. Kaplan-Meier curves were read by the Engauge Digitizer version 4.1 (free software downloaded from http://sourceforge.net). If the survival date can't be extracted from the article, the author will be contacted by e-mail. We estimated the degree of heterogeneity among studies using Cochran's Q-statistic, which is considered significant at $P < 0.05$. The I^2 test was also conducted to quantify the heterogeneity (ranges from 0 to 100%). The random-effect model was conducted when there existed a significant Q-test with $P < 0.05$ or $I^2 > 50\%$. When there was no statistical heterogeneity, the fixed-effects model was used.

The subgroup analyses will be taken according to patients' ethnicity. Funnel plots were constructed to investigate whether publication bias might have affected the validity of the estimates. Egger's linear regression test was used to evaluate the symmetry of the funnel plots. All tests were two-sided with a P value of < 0.05 being considered statistically significant. All analyses were calculated using the STATA software, version 12.0 (Stata Corp, College Station, TX, USA).

Results

Characteristics of studies: Our initial search yielded 1,265 potential publications, and finally 32 articles were included. The flow chart of the study selection process is shown in **Figure 1**. Publication years of the eligible studies ranged from 2004 to 2014. A total of 2,377 patients were involved in this meta-analysis, including 1,116 patients in the methylated MGMT promoter group and 1,261 patients in the unmethylated MGMT group. Overall, 3 studies including 253 patients were conducted in Asian populations [14, 16, 23], and the other 29 studies including 2,124 patients in Caucasia populations [15, 17-22, 24-32]. Detection methods include 2 studies of immunohistochemistry (IHC) [1, 28] and 30 studies of polymerase chain reaction (PCR) [2-27, 29-32]. The characteristics of the studies are summarized in **Table 1**.

Table 1. Main characteristics of all eligible studies

First author	Years	Ethnicity	Country	Testing method	Number	Methylation MGMT	Unmethylation MGMT	Quality score*
Miyazaki M [14]	2014	Asian	JAPAN	IHC	117	55	62	6
Capdevila L [15]	2014	Caucasians	Spain.	PCR	22	10	12	7
Wang X [16]	2013	Asian	CHINA	PCR	68	25	43	5
Abhinav K [17]	2013	Caucasians	UK	PCR	45	28	17	6
Franceschi E [18]	2013	Caucasians	Italy	PCR	42	17	25	5
Fiorentino A [19]	2013	Caucasians	Italy	PCR	61	24	37	6
Malmstrom A [20]	2012	Caucasians	Sweden	PCR	203	91	112	7
Wick W [21]	2012	Caucasians	Germany	PCR	108	31	77	6
Reifenberger G [22]	2012	Caucasians	Germany	PCR	233	134	99	4
Motomura K [23]	2011	Asian	Japan	PCR	68	23	45	6
Gallego PL [24]	2011	Caucasians	France.	PCR	31	13	18	6
Minniti G [25]	2011	Caucasians	Italy	PCR	83	42	41	5
Lai A [26]	2011	Caucasians	USA	PCR	70	29	41	6
Metellus P [27]	2010	Caucasians	France	PCR	61	23	38	7
BRUNO MC [28]	2010	Caucasians	Portugal	PCR	80	38	42	6
Roger Stupp [29]	2010	Caucasians	Germany	PCR	45	23	22	5
Lucie KT [30]	2010	Caucasians	France	PCR	81	55	26	6
Brandes AA [31]	2009	Caucasians	Italy.	PCR	37	16	21	5
Gerstner ER [32]	2009	Caucasians	USA	PCR	64	37	27	6
Prados MD [33]	2009	Caucasians	USA	PCR	44	14	30	6
Dunn J [34]	2009	Caucasians	UK	PCR	109	51	58	7
Wemmert S [35]	2009	Caucasians	Germany	PCR	27	15	12	4
Kris A Smith [36]	2008	Caucasians	USA	PCR	23	12	11	4
Tosoni A [37]	2008	Caucasians	Italy	PCR	30	14	16	5
Brandes AA [38]	2008	Caucasians	Italy.	PCR	103	36	67	6
Sijben AE [39]	2008	Caucasians	Netherlands.	PCR	29	13	16	6
Preusser M [40]	2008	Caucasians	Austria	IHC	122	59	63	6
Eoli M [41]	2007	Caucasians	Italy.	PCR	86	41	45	5
Piccirilli [42]	2006	Caucasians	Italy	PCR	22	13	9	6
Herrlinger U [43]	2006	Caucasians	Germany	PCR	19	8	11	6
Hegi ME [44]	2005	Caucasians	Switzerland	PCR	206	114	92	7
Hegi ME [45]	2004	Caucasians	Switzerland	PCR	38	12	26	6

*The quality score of this paper was determinded by using the Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomised studies in meta-analyses. Abbreviative: Immunohistochemistry (IHC); polymerase chain reaction (PCR).

Meta-analysis results: All 32 studies reported OS data. Overall, the pooled hazard ratio for OS showed that there was a significant difference between the MGMT promoter group and the unmethylated MGMT group (HR, 0.525; 95% CI, 0.451-0. 61; $P = 0.00$, **Figure 2**).

A random effect model was used since heterogeneity across the trials was significant ($I^2 = 73.3\%$, $P = 0.000$). Results revealed a correlation of MGMT expression with temozolomide capsules in Caucasians (HR, 1.197; 95% CI, 0.55-2.606; $P = 0.00$, **Figure 3**). However, there was no benefit for the Asians (HR, 1.197; 95% CI, 0.55-2.606; $P = 0.00$, **Figure 3**).

A random effect model was used since heterogeneity across the trials was significant ($I^2 = 86.4\%$, $P = 0.001$). Data for PFS were available from 11 studies. The pooled hazard ratio for PFS showed that there was a significant difference between the MGMT promoter group and

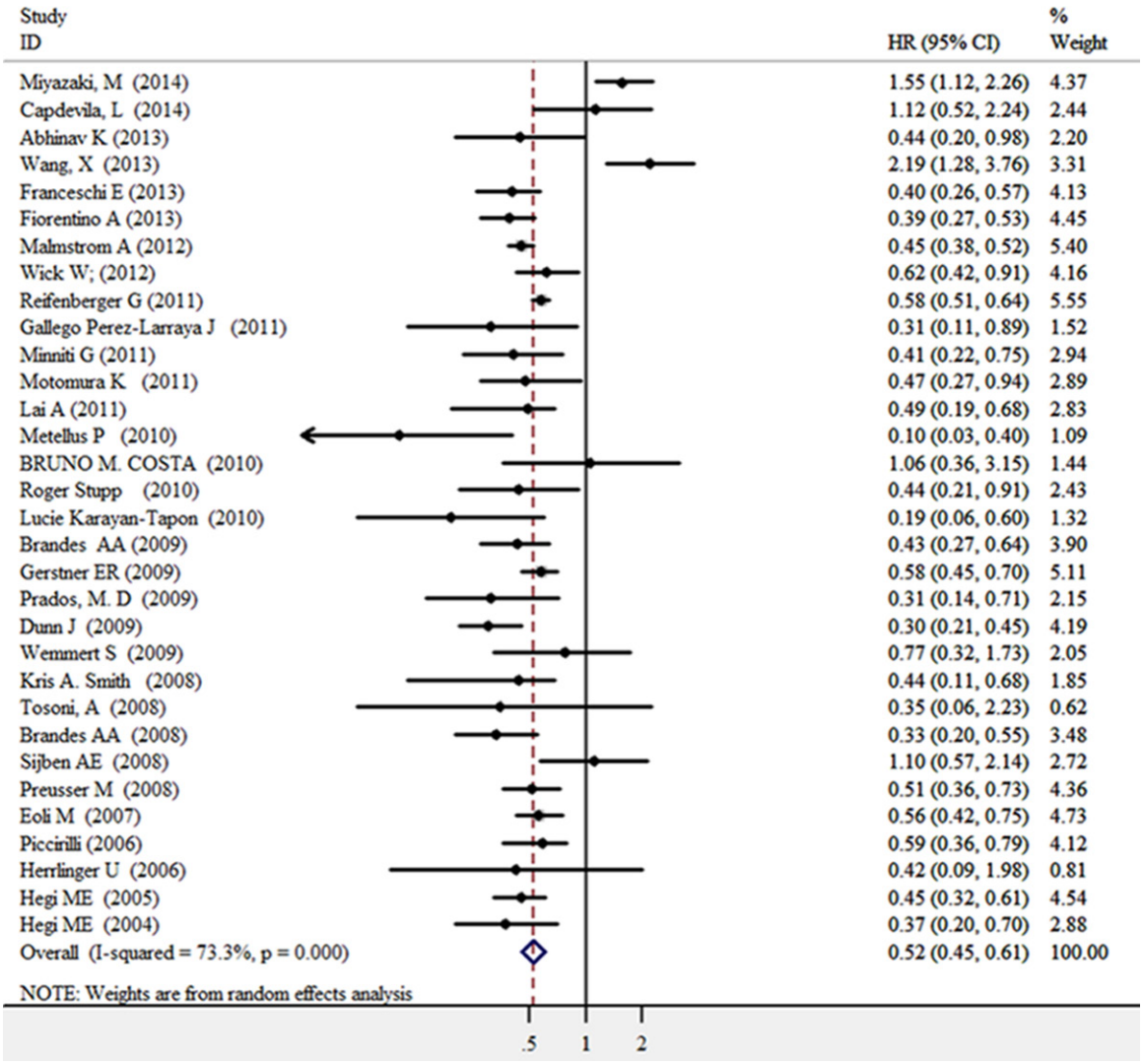


Figure 2. Forest Plots for the associations of MGMT promoter and overall survival treated with temozolomide (The squares and horizontal lines correspond to the study specific HR and 95% CI. The diamond represents the summary HR and 95% CI).

the unmethylated MGMT group (HR, 0.518; 95% CI, 0.319-0.84; *P* = 0.00, **Figure 4**). A random effect model was used since heterogeneity across the trials was significant (I^2 = 84.2%, *P* = 0.000).

Publication Bias and Sensitivity Analysis Begg's funnel plot and Egger's test were used to assess the publication bias in this present work. The results indicated that no evidence of publication bias was shown in OS (Begg's test *P* = 0. 773, Egger's test *P* = 0. 608) (**Figure 5**), and Egger's test showed a remarkable evidence of publication bias in PFS (Begg's test *P* = 0. 087, Egger's test *P* = 0.007) (**Figure 5**). The results showed that no evidence of publication bias was found in Asian and Caucasians' OS (Begg's test *P* = 0. 733, Egger's test *P* = 0. 608) (**Figure 6**).

Discussion

How to enhance effects of chemotherapy for malignant gliomas has always been a target in the field of medical oncology. At present, empirical chemotherapy is the main method used clinically, but a large quantity of patients show poor curative effect. Resistance of tumor cells to chemotherapy drugs is an important influence factor of curative effect [8, 11, 12]. Research indicates that the expression drug

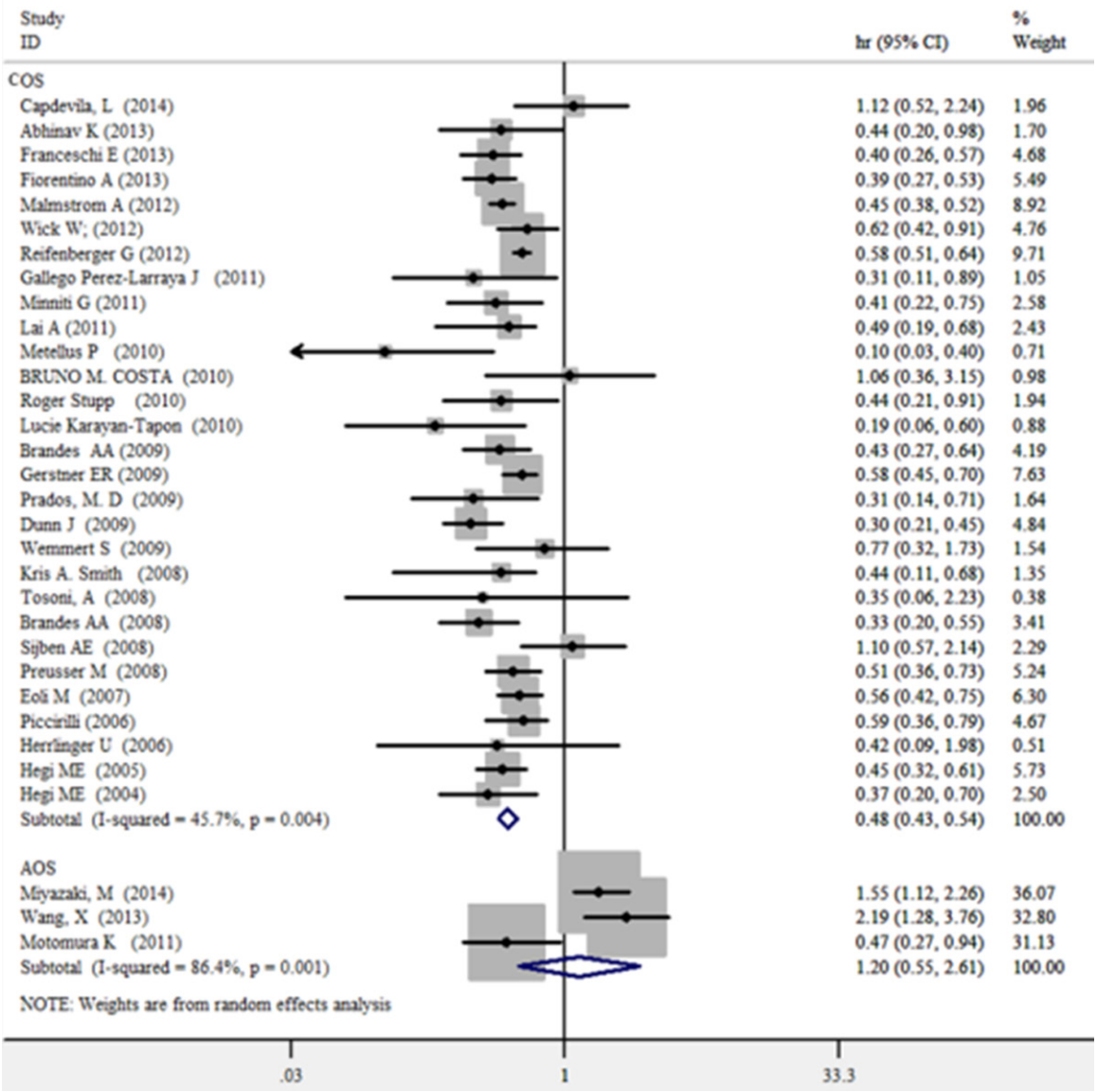


Figure 3. Forest plots for the associations of MGMT promoter with overall survival in Asian and Caucasians (The squares and horizontal lines correspond to the study specific HR and 95% CI. The diamond represents the summary HR and 95% CI Abbreviative: COS = Caucasians OS; AOS = Asian OS).

resistance gene is a significant mechanism in the resistance of tumor to medicines. Hence, testing of such expression can provide a further reference for selection of chemotherapy drug [11, 13]. The role of MGMT in killing alkylating agents is the molecular basis that determines the resistance of tumor cells to these agents. After entering people's bodies, temozolomide is converted into active products such as methyl hydrazine, which acts on each stage of tumor cell division and results in DNA alkylation damage. Among these active products, O6-ethylguanine brings the greatest hazard to cells, causes wrong base pairing, and leads to cell mutation and death. As a DNA repair enzyme, MGMT can transfer the alkylating group from the O6 site of O6-ethylguanine to the cysteine residue so that damage to guanine on the DNA chain is repaired while MGMT itself is irreversibly inactivated. MGMT is the only protein that is able to remove O6 guanine compound from DNA. Therefore, cells' ability of repairing DNA damage depends on the content and synthetic rate of MGMT in the cell. The expression level of MGMT in tumor cells varies greatly. Consequently, tumor cells with high

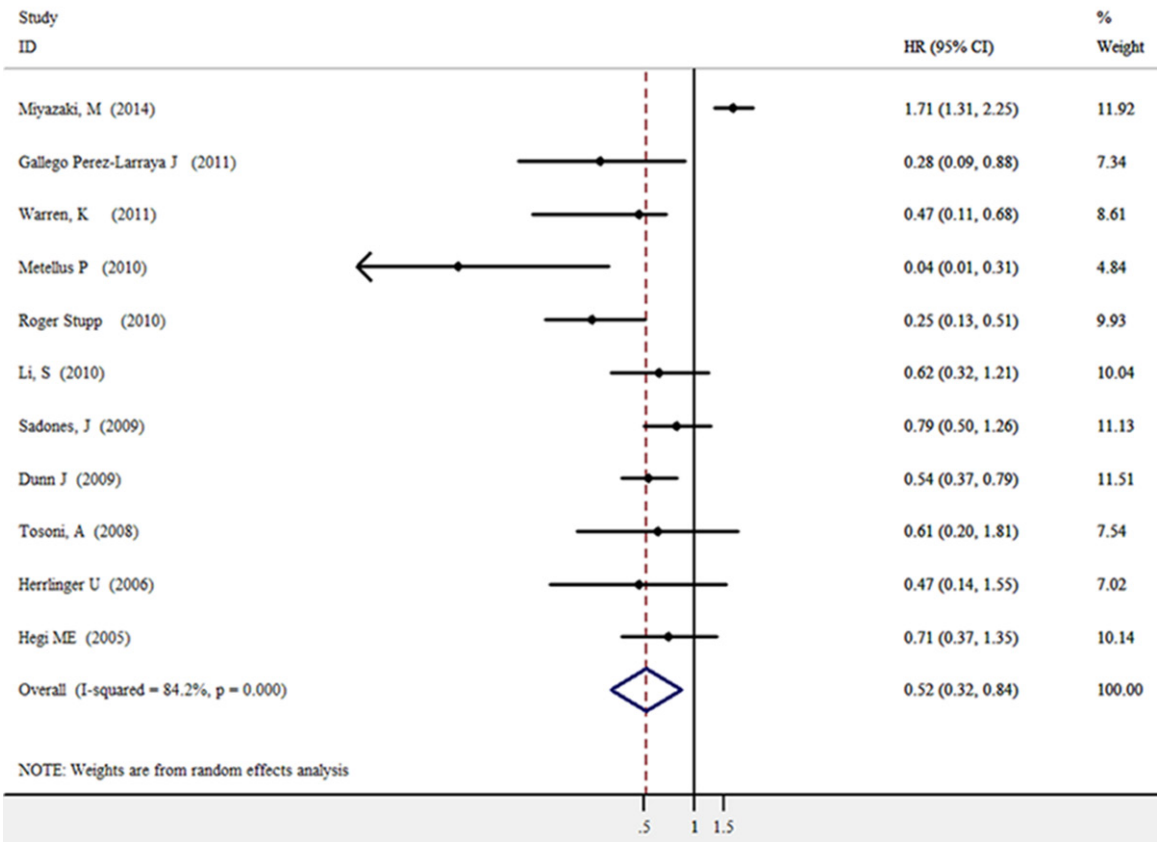


Figure 4. Forest plots for the associations of MGMT promoter and progression free survival Treated with temozolomide (The squares and horizontal lines correspond to the study specific HR and 95% CI. The diamond represents the summary HR and 95% CI).

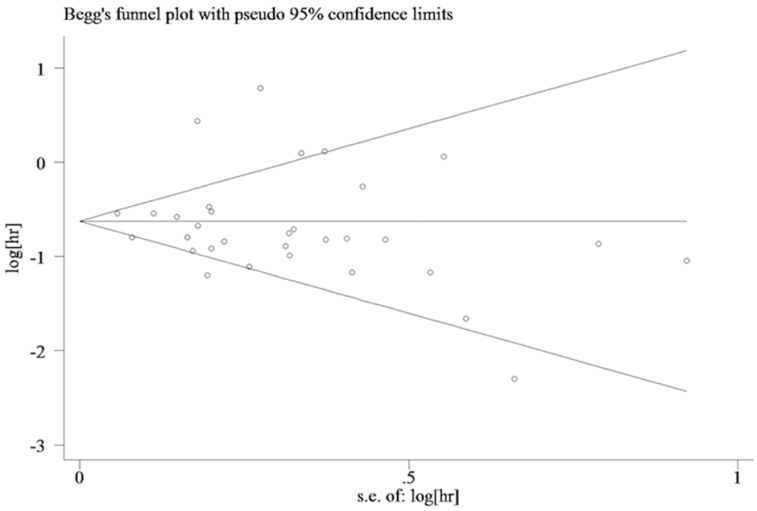


Figure 5. Funnel plot of the associations of MGMT promoter with overall survival and progression free survival.

endogenous MGMT activity can reduce the killing effect of alkylating agents and cause drug resistance. This is possibly the fundamental cause for failure of temozolomide treatment in some patients with malignant gliomas [5, 11, 12].

With regard to META analysis, this paper mainly investigated the correlation between MGMT expression and temozolomide chemotherapy sensitivity. A total of 2,377 patients from 32 studies were included. HR was calculated as 0.525 (95% CI, 0.451-0.61; *P* = 0.00, **Figure 1**). These results demonstrated that patients subject to MGMT methylation get more benefits from treatment with temozolomide capsules.

A subgroup analysis of MGMT test methods and gliomas patients was done in the present study so as to further examine and evaluate

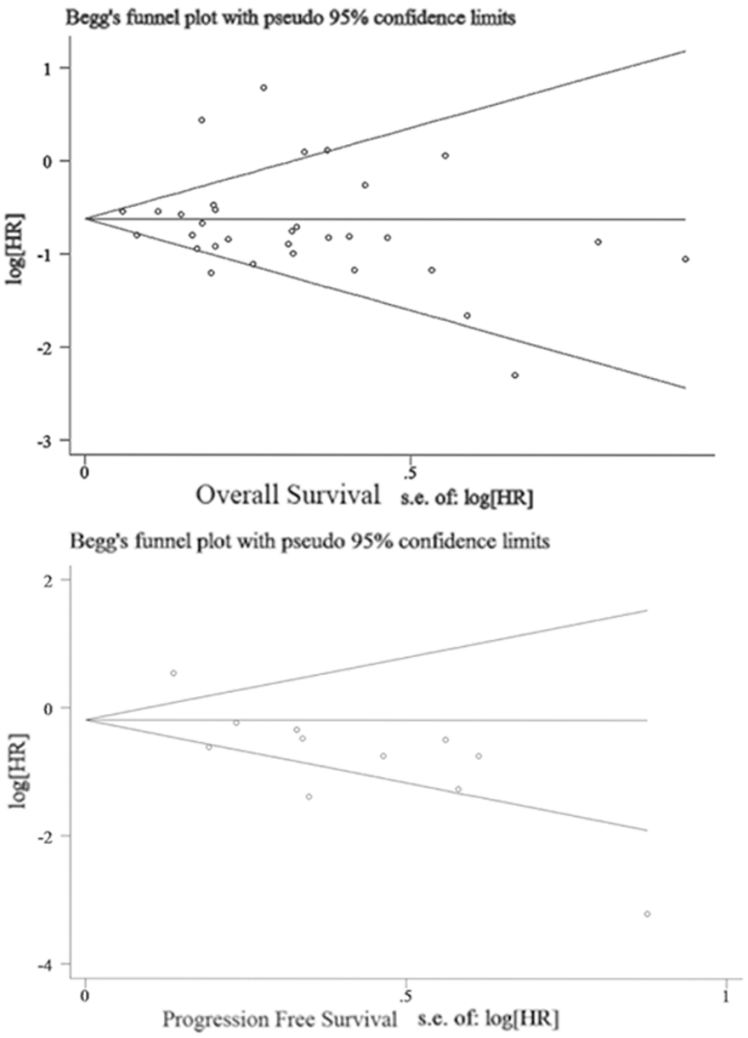


Figure 6. Funnel plot of the associations of MGMT promoter with overall survival in Asian and Caucasians.

clinical agreement. Results showed the association of MGMT expression with temozolomide capsules in Caucasians (HR, 0.481; 95% CI, 0.429-0.538; *P* = 0.00, **Figure 4**). However, there was no benefit for the Asians (HR, 1.197; 95% CI, 0.55-2.606; *P* = 0.00, **Figure 4**).

Funnel plots of PFS analysis and results of Egger's test presented in this paper suggest that the present study has publication bias. Meta-analysis is a quantitative analysis method based on results of previous research and greatly influenced by quality of previous research data. In this work, literatures with poor quality, repeated report, and insufficient information have been eliminated. However, due to limitation of Meta-analysis itself, various biases may still exist in the analysis process. The most common is publication bias. Compared with negative results or results against mainstream theories, positive results or research results in agreement with mainstream theories are easier to publish and provide more detailed information. The other is language bias. In this study, language used for literature search is limited to English, which affects data comprehensiveness. In addition, the subgroup analysis shows MGMT methylation doesn't cause a difference in temozolomide capsule efficacy in the Asians. This is because effects of medicines may vary among different races. Only three related dependent studies are available, so such results shall be verified by further studies.

In conclusion, analysis of this paper indicates patients subject to MGMT methylation are more sensitive to treatment by temozolomide capsules. Non-MGMT-methylation patients show a high probability of being resistant to chemotherapy by temozolomide capsules, especially the Caucasian population. Testing of MGMT expression can be used to predict effects of temozolomide capsules on gliomas.

Disclosure of conflict of interest

None.

Address correspondence to: Haiqing Huai, Department of Medical Oncology of PLA Cancer Center, 81 Hospital of PLA, Nanjing 210002, China. E-mail: 149764146@qq.com

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