

Is the prognostic significance of O6-methylguanine-DNA methyltransferase promoter methylation equally important in glioblastomas of patients from different continents? A systematic review with meta-analysis

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Background: O6-methylguanine-DNA methyltransferase (MGMT) is an independent predictor of therapeutic response and potential prognosis in patients with glioblastoma multiforme (GBM). However, its significance of clinical prognosis in different continents still needs to be explored.

Patients and methods: To explore the effects of MGMT promoter methylation on both progression-free survival (PFS) and overall survival (OS) among GBM patients from different continents, a systematic review of published studies was conducted.

Results: A total of 5103 patients from 53 studies were involved in the systematic review and the total percentage of MGMT promoter methylation was 45.53%. Of these studies, 16 studies performed univariate analyses and 17 performed multivariate analyses of MGMT promoter methylation on PFS. The pooled hazard ratio (HR) estimated for PFS was 0.55 (95% CI 0.50, 0.60) by univariate analysis and 0.43 (95% CI 0.38, 0.48) by multivariate analysis. The effect of MGMT promoter methylation on OS was explored in 30 studies by univariate analysis and in 30 studies by multivariate analysis. The combined HR was 0.48 (95% CI 0.44, 0.52) and 0.42 (95% CI 0.38, 0.45), respectively.

Conclusion: In each subgroup divided by areas, the prognostic significance still remained highly significant. The proportion of methylation in each group was in inverse proportion to the corresponding HR in the univariate and multivariate analyses of PFS. However, from the perspective of OS, compared with data from Europe and the US, higher methylation rates in Asia did not bring better returns.

Keywords: O6-methylguanine-DNA methyltransferase, methylation, glioblastoma, prognosis, meta-analysis

Introduction

Glioblastoma multiforme (GBM, WHO grade 4) is the most common primary brain tumor in adults with an annual incidence of 3–4/100,000 and is associated with poor prognosis.¹ Although some clinical trials have demonstrated that the standard treatment improves overall survival (OS) and progression-free survival (PFS), only less than one-third of GBM patients seem to benefit from these therapies, mainly because of GBM resistance to alkylating drugs.

Transcriptionally active O6-methylguanine-DNA methyltransferase (*MGMT*) gene encodes a ubiquitously expressed suicide DNA repair enzyme that counteracts the normally lethal effects of alkylating agents by removing the alkyl adducts, preventing the formation of cross-links and thereby causing resistance to alkylating drugs.³ The loss of *MGMT* protein expression caused by methylation of the *MGMT* promoter reduces the DNA repair activity of glioma cells, preventing their resistance to alkylating agents.^{2,4-6} It is believed that patients with GBM who have a methylated *MGMT* promoter are more sensitive to the killing effects of alkylating drugs, because tumor cells with low *MGMT* expression were unable to repair such DNA lesions and, thus, were prone to apoptosis, whereas those that do not have a methylated *MGMT* promoter do not have this benefit.^{68,69}

Various studies have shown that the *MGMT* promoter methylation status is an independent prognostic factor to GBM and the assessment of *MGMT* promoter methylation is currently considered as mandatory for patient selection in clinical trials.^{7-10,68} However, many differences in high risk factors and postoperative chemoradiation stay in guidelines for the treatment of glioblastoma, among countries, indicating different attitudes to *MGMT* promoter methylation status. Is the prognostic significance of *MGMT* promoter methylation independent equally among glioblastomas from different areas? Further explorations are needed in the prognostic value of *MGMT* promoter methylation on GBM including therapeutic intervention.^{11,12,20,21}

From the perspective of geography, we conducted this meta-analysis to test the independence of prognostic value of *MGMT* promoter methylation in both PFS and OS among patients with GBM.

Patients and methods

Publication selection

Ethical approval and patient consent are not required as this is a systematic review and meta-analysis of previously published studies. This study was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.¹³

Two reviewers (Yangyang Jiang and Wei Meng) participated in the citations search, study selection and data extraction, independently. Divergences between reviewers were resolved through consulting with Professor Jie Ma.

Electronic databases, including PubMed, EMBASE, Web of Science, China Biomedical Literature Database (CBM), Chinese National Knowledge Infrastructure (CNKI), China Wan Fang database and the Cochrane library, were searched

for relevant clinical trials published on the association between *MGMT* promoter methylation and GBM between January 2000 and June 2017.

The search combined key words: (“O6-methylguanine-DNA methyltransferase methylation” OR *MGMT* methylation”) AND (“glioblastoma” OR “GBM”) AND (“survival analysis” OR “meta analysis”) AND (“MSP” OR “PSQ”) AND (“survival analysis” OR “meta analysis”) AND (“methylation-specific polymerase chain reaction and pyrosequencing”).

The meta-analysis gathered complete databases from published cohort studies dealing with the prognostic value of *MGMT* promoter methylation in patients with GBM no matter which therapy was given.

The language in which the papers were written was restricted to English and Chinese. Abstracts were excluded because of insufficient data for meta-analysis. In order to identify the relevant publications, the references cited in the research papers were also scanned. To avoid duplication of data, we carefully noted the author names and the different research centers involved. We evaluated the eligible studies if all the following conditions were met: 1) *MGMT* promoter methylation status was measured by using identified method such as methylation-specific polymerase chain reaction (MSP) and pyrosequencing (PSQ); (2) inclusion of sufficient data or survival curves to calculate hazard ratio (HR) and 95% CI; and 3) full or special parts of papers investigated the relationship between *MGMT* promoter methylation and PFS or OS.

Data extraction

Two authors (Yangyang Jiang and Wei Meng) independently reviewed and extracted the data needed. Disagreements were resolved through discussion with each other.

We used a predesigned data extraction sheet to obtain the following information: first author, year of publication, region, HR form and sample size and style of postoperative chemoradiation, if given. The formula recommended by Spruance et al was adopted to calculate the corresponding HR of the missing data.¹⁴ Kaplan–Meier curve was read by using Engauge Digitizer version 4.1 (available at: <http://sourceforge.net/>) except if the paper has supplied HR directly.¹⁵ (All the data are shown in Table 1.)

Statistical analysis

In some studies, HR and 95% CI were directly obtained from published literature by using univariate or multivariate survival analysis. For studies in which the HR correspond-

Table 1 Main characteristics and results of eligible studies

References	Year	Country	M	U	PFS		OS	
					Univariate	Multivariate	Univariate	Multivariate
Kanemoto et al ⁴⁷	2014	Japan	36	17	Survival curve $p = 0.113$	N/A	Survival curve $p = 0.993$	N/A
Melguizo et al ⁴⁶	2012	Spain	34	42	Survival curve $p = 0.031$	N/A	Survival curve $p = 0.036$	N/A
Adeberg et al ²⁵	2015	Germany	14	18	HR, 95% CI $p = 0.02$	N/A	HR, 95% CI $p = 0.048$	N/A
Shen et al ⁴⁵	2014	USA	75	53	N/A	HR, 95%CI $p = 0.112$	N/A	HR, 95% CI $p = 0.029$
Gutenberg et al ²⁴	2013	Germany	46	35	HR, 95% CI $p = 0.942$	N/A	HR, 95% CI $p = 0.876$	N/A
Barault et al ⁴⁴	2015	Italy	N/A	N/A	Survival curve $p < 0.0001$	N/A	Survival curve $p = 0.0043$	N/A
Villani et al ²³	2015	Italy	25	26	HR, 95% CI $p = 0.18$	HR, 95%CI $p = 0.0045$	HR, 95% CI $p = 0.19$	HR, 95% CI $p = 0.0035$
Iaccarino C ⁴³	2015	Italy	17	15	N/A	N/A	Survival curve $p < 0.0001$	N/A
Cao et al ⁴²	2009	Korea	46	30	N/A	N/A	N/A	HR, 95% CI $p = 0.26$
Metellus et al ⁴¹	2009	France	6	15	N/A	HR, 95% CI $p = 0.0012$	N/A	HR, 95% CI $p = 0.019$
Gerstner et al ⁹⁴	2009	Arizona	12	11	N/A	N/A	Survival curve $p = 0.0009$	N/A
Brandes et al ⁵⁹	2009	Italy	16	21	Survival curve $p = 0.005$	N/A	Survival curve $p = 0.05$	N/A
Sonoda et al ⁴⁰	2009	Japan	4	12	N/A	N/A	N/A	HR, 95% CI $p = 0.02$
Park et al ³⁹	2009	Korea	26	22	N/A	N/A	HR, 95% CI $p = 0.518$	N/A
Zawlik et al ³⁸	2009	Switzerland	165	206	N/A	N/A	N/A	HR, 95% CI $p = 0.469$
Hegi et al ³⁷	2004	Switzerland	26	12	N/A	N/A	N/A	HR, 95% CI $p = 0.017$
Hegi et al ⁶⁸	2005	Switzerland	93	113	N/A	N/A	N/A	HR, 95% CI $p < 0.001$
Wemmert et al ³⁶	2009	Germany	15	12	N/A	N/A	HR, 95% CI $p = 0.490$	HR, 95% CI $p = 0.370$
Weller et al ³⁵	2009	Germany	111	137	N/A	HR, 95%CI $p < 0.0001$	N/A	HR, 95% CI $p < 0.0001$
Karayan-Tapon et al ⁵⁸	2010	France	55	26	N/A	N/A	Survival curve $p = 0.005$	N/A
Cheng et al ⁹⁵	2015	Korea	24	53	N/A	N/A	Survival curve $p = 0.04$	N/A
Thon et al ³⁴	2011	Germany	30	26	HR, 95% CI $p < 0.0001$	HR, 95%CI $p < 0.0001$	HR, 95% CI $p < 0.0001$	HR, 95% CI $p < 0.0001$
Minniti et al ³³	2011	Italy	42	41	N/A	N/A	N/A	HR, 95% CI $p = 0.0001$
Sonoda et al ³²	2010	Japan	35	27	N/A	HR, 95%CI $p = 0.011$	N/A	N/A
Rivera et al ⁵⁶	2010	USA	54	171	Survival curve $p = 0.009$	N/A	Survival curve $p = 0.019$	N/A
Morandi et al ⁵⁵	2010	Italy	70	89	N/A	N/A	Survival curve $p = 0.003$	N/A
Brandes et al ⁵⁴	2010	Italy	13	25	N/A	N/A	Survival curve $p = 0.04$	N/A
Costa et al ⁵³	2010	Portugal	38	42	N/A	N/A	Survival curve $p = 0.583$	N/A

(Continued)

Table 1 (Continued)

References	Year	Country	M	U	PFS		OS	
					Univariate	Multivariate	Univariate	Multivariate
Park et al ⁵²	2011	Korea	14	34	N/A	N/A	Survival curve $p = 0.027$	N/A
Lakomy et al ³¹	2011	Czech Republic	12	26	HR, 95% CI ($p = 0.0201$)	N/A	HR, 95% CI $p = 0.0054$	N/A
Ellingson et al ⁵¹	2012	USA	141	238	N/A	N/A	Survival curve $p < 0.0001$	N/A
Balana et al ³⁰	2011	Spain	27	42	N/A	HR, 95%CI $p = 0.018$	N/A	HR, 95% CI $p = 0.028$
Felsberg et al ²⁹	2011	Germany	31	49	N/A	HR, 95%CI $p = 0.042$	N/A	HR, 95% CI $p = 0.009$
Reifenberger et al ²⁸	2011	Germany	134	99	N/A	HR, 95%CI $p = 0.646$	N/A	HR, 95% CI $p = 0.352$
Yang et al ⁵⁰	2012	Korea	10	12	N/A	N/A	Survival curve ($p = 0.156$)	N/A
Lechapt-Zalcman et al ²⁷	2012	France	63	63	N/A	HR, 95%CI $p = 0.036$	N/A	HR, 95% CI $p = 0.008$
Kim et al ²⁶	2012	Korea	43	35	N/A	HR, 95%CI $p = 0.008$	N/A	HR, 95% CI $p = 0.002$
Combs et al ⁴⁹	2011	Germany	43	84	Survival curve($p = 0.93$)	N/A	Survival curve ($p = 0.18$)	N/A
Christians et al ⁴⁸	2012	Germany	16	19	Survival curve ($p = 0.0011$)	N/A	N/A	N/A
Dunn et al ⁷⁹	2009	England	58	51	Survival curve $p < 0.0001$	N/A	Survival curve $p < 0.0001$	N/A
Brell et al ¹²	2005	Spain	20	20	HR, 95%CI $p = 0.008$	N/A	HR, 95% CI $p = 0.850$	N/A
Glas et al ⁷⁷	2009	Switzerland	11	12	N/A	N/A	HR, 95% CI $p = 0.001$	N/A
Etcheverry et al ²²	2010	France	30	20	N/A	N/A	HR, 95% CI $p = 0.02$	N/A
Ellingson et al ⁵¹	2012	USA	128	225	N/A	N/A	Survival curve $p = 0.0001$	N/A
Stupp et al ⁸⁸	2009	Switzerland	106	100	N/A	N/A	HR, 95%CI $p = 0.035$	N/A
Murat et al ⁸⁰	2008	Switzerland	43	34	N/A	N/A	HR, 95% CI $p = 0.0001$	HR, 95% CI $p < 0.0001$
Schaich et al ⁸⁹	2008	Germany	37	63	N/A	N/A	N/A	HR, 95% CI $p < 0.0001$
Van den Bent et al ⁸⁷	2009	Lithuania	32	37	N/A	N/A	N/A	Survival curve $p = 0.005$
Carrillo et al ⁸⁴	2012	USA	24	36	N/A	N/A	N/A	HR, 95% CI $p < 0.001$
Ohka et al ⁸⁵	2011	Japan	62	49	N/A	HR, 95%CI $p = 0.075$	N/A	HR, 95% CI $p = 0.969$
Abhinav et al ⁸²	2013	UK	28	19	N/A	N/A	N/A	Survival curve $p = 0.005$
McDonald et al ⁷³	2013	Australia	27	49	HR, 95%CI $p = 0.011$	N/A	N/A	N/A
Thon et al ⁹³	2017	Germany	30	26	HR, 95% CI $p < 0.001$	HR, 95% CI $p < 0.001$	HR, 95% CI $p < 0.001$	HR, 95% CI $p < 0.001$

Abbreviations: HR, hazard ratio; MGMT, O6-methylguanine-DNA methyltransferase; PFS, progression-free survival; OS, overall survival; N/A, not available or not applicable; M/U, methylation/unmethylation case.

ing to the 95% CI was not given directly, published data and figures from original papers were used to calculate the HR according to the methods described by using Engauge Digitizer version 4.1.

The pooled HR corresponding to the 95% CI was used to assess the prognostic value of MGMT promoter methylation in patients with GBM. The statistical heterogeneity among studies was assessed with the Q -test and I_2 statistics.¹⁶

A random-effects model was used primarily regardless of heterogeneity. Level of heterogeneity (level of variance) across studies was evaluated using I_2 statistic. I_2 of 40, 70 and 100% was used to represent low, moderate and high variance, respectively.¹⁷ If obvious differences for clinical characteristics and methodology were not identified and $I_2 \leq 40\%$, a fixed-effects model was adopted. A random-effects model will be used if clinical characteristics and methodology were not identified to be great difference and $I_2 \leq 40\%$; in contrast, if the clinical characteristic and/or methodology across studies regardless of I_2 statistic was considered to be obviously different, qualitative analysis was adopted.¹⁸

The objective impact of MGMT promoter methylation on PFS and OS was considered to be statistically significant if the 95% CI for the HR did not overlap 0. Publication bias was evaluated with funnel plot and Begg's rank correlation method.¹⁹ The statistical analyses were performed by STATA/MP 13.0 software.

Results

Characteristics of studies

A total of 204 relevant citations were identified at the initial search stage; 151 articles concerned topics not relevant to this study, and finally 53 studies were included in the meta-analysis.

All the included studies were in English. The individual characteristics of the eligible studies are reported in Table 1. The total number of patients was 5103, and the total frequency of MGMT promoter methylation was 45.53%. Of the 53 publications eligible for systematic review, 31 studies reported the HR with corresponding to 95% CI directly, the other 22 studies reported the HR in the style of survival curve availability.

Meta-analysis

Sixteen studies (one in Asia, one in North America, one in Australia and 13 in Europe) reported the effect of MGMT promoter methylation on PFS using univariate analysis.^{12,23–25,31,44,46–49,56,59,68,73,79,93} As shown in Figure 1, the HR of the Asian group is 0.47, the HR of the American group is 0.88, the HR of the Australian group is 0.51 and the HR of the European group is 0.49; MGMT promoter methylation was significantly correlated with better PFS according to univariate analysis, with a combined HR of 0.55 (95% CI 0.50, 0.60). The random-effects model (the DerSimonian and Laird method) was used because significant heterogeneity was detected among these studies ($p = 0.000$, $I_2 = 88.3\%$).⁶¹

The effect of MGMT promoter methylation on PFS adjusted for other variables was evaluated in 17 studies (five in Asia, 11 in Europe and one in America)^{26–30,32,34,35,41,45,68,85,87,91,93–95} As shown in Figure 2, the HR of the Asian group is 0.49, the HR of the European group is 0.44 and the HR of the American group is 0.37; MGMT promoter methylation was significantly correlated with better PFS according to multivariate analysis, with a combined HR of 0.45 (95% CI 0.35, 0.54). The random-effects model (the DerSimonian and Laird method) was used because significant heterogeneity was detected among these studies ($p = 0.000$, $I_2 = 62.8\%$).⁶¹

The effect of MGMT promoter methylation on OS unadjusted for using univariate analysis was evaluated in 32 studies (four in Asia, six in North America, one in Australia and 21 in Europe).^{12,22–25,29,31,34,36,39,43,44,46,47,49–60,73,74,77–80,93} As shown in Figure 3, the HR of the American group is 0.49, the HR of the European group is 0.47, HR of the Asian group is 0.73 and the HR of the Australian group is 0.51; MGMT promoter methylation was significantly correlated with better OS according to univariate analysis, with a combined HR of 0.50 (95% CI 0.40, 0.59). The random-effects model (the DerSimonian and Laird method) was used as significant heterogeneity was detected among these studies ($p = 0.000$, $I_2 = 50.3\%$).⁶¹

Thirty-one studies (six in Asia, two in America and 23 in Europe) reported the effect of MGMT promoter methylation on OS using analyses adjusted for other factors.^{23,26–30,33–42,45,68,75,77,82–91,93} As shown in Figure 4, the HR of the Asian group is 0.56, the HR of the American group is 0.37 and the HR of the European group is 0.44; MGMT promoter methylation was significantly correlated with better OS according to multivariate analysis, with a combined HR of 0.44 (95% CI 0.38, 0.50). The random-effects model (the DerSimonian and Laird method) was used as significant heterogeneity was detected among these studies ($p = 0.000$, $I_2 = 50.3\%$).⁶¹

Publication bias statistics were determined; some publication bias (Begg's test, $p < 0.05$) was found. Sensitivity analysis was performed to investigate the influence of a single study on the overall meta-analysis by omitting one study at a time, and the omission of any study made no significant difference, indicating that our results were statistically reliable.

Discussion

The association between the MGMT promoter methylation and GBM has been reported in many studies. Evaluations of prognostic factors, such as patients age, gender, nationality, recurrence, tumor location and excision, MGMT testing method and the style of postoperative chemoradiation for tumors are all vital to improve research pursuing new thera-

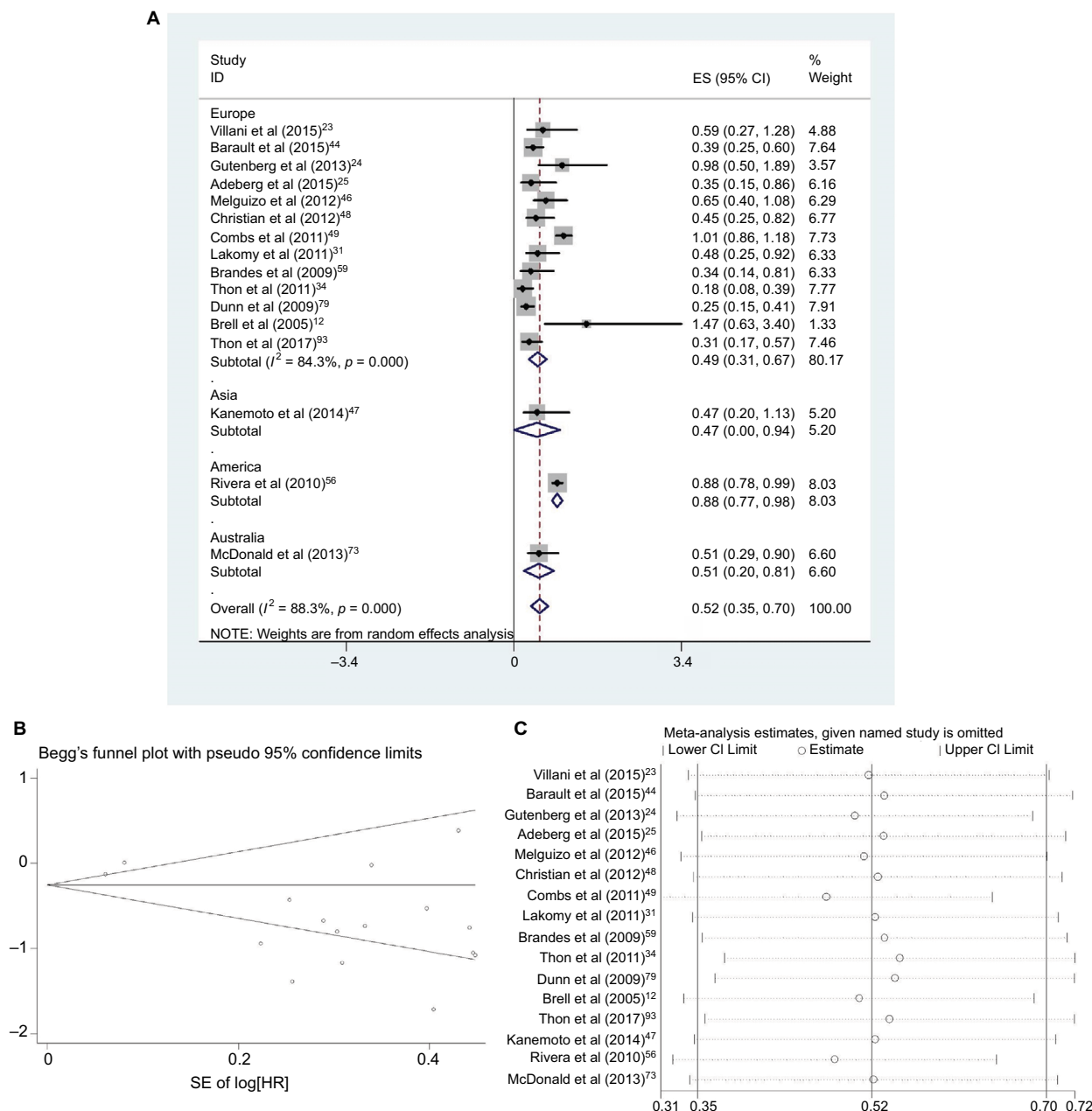


Figure 1 Data statistics on PFS using univariate analysis.

Notes: (A) Forest plot showing the combined relative HR from the random effect model for MGMT promoter methylation on PFS using univariate analysis with patients from different areas. The proportion of methylation in each group was in inverse proportion to the corresponding HR. (B) Begg's test on PFS using univariate analysis with different area. (C) Sensitivity analysis on PFS using univariate analysis with different area.

Abbreviations: SE, standard error; ES, effect size; HR, hazard ratio; MGMT, O6-methylguanine-DNA methyltransferase; PFS, progression-free survival.

pies for GBM. In general, the population flows more and more frequently among the continents, and most of the prognostic factors are usually determined by circumstances and nationwide medical policies. Therefore, it is more reasonable to set subgroups by areas but not by races. Our meta-analysis was performed to define the prognostic and predictive value of MGMT promoter methylation in glioblastoma patients of different continents. The major strengths of this study include the deliberate distinction of area, the relatively

comprehensive sample size, the prospective data collection and the combination of the MSP and PSQ analysis to assess the MGMT promoter methylation status.

MGMT expression protects normal cells from carcinogens; however, it can also protect cancer cells from chemotherapeutic alkylating agents, which include mutations, sister chromatid exchanges, recombination and chromosomal aberrations.⁶² It has been shown that glial brain tumors are characterized by a low expression of MGMT, however, the

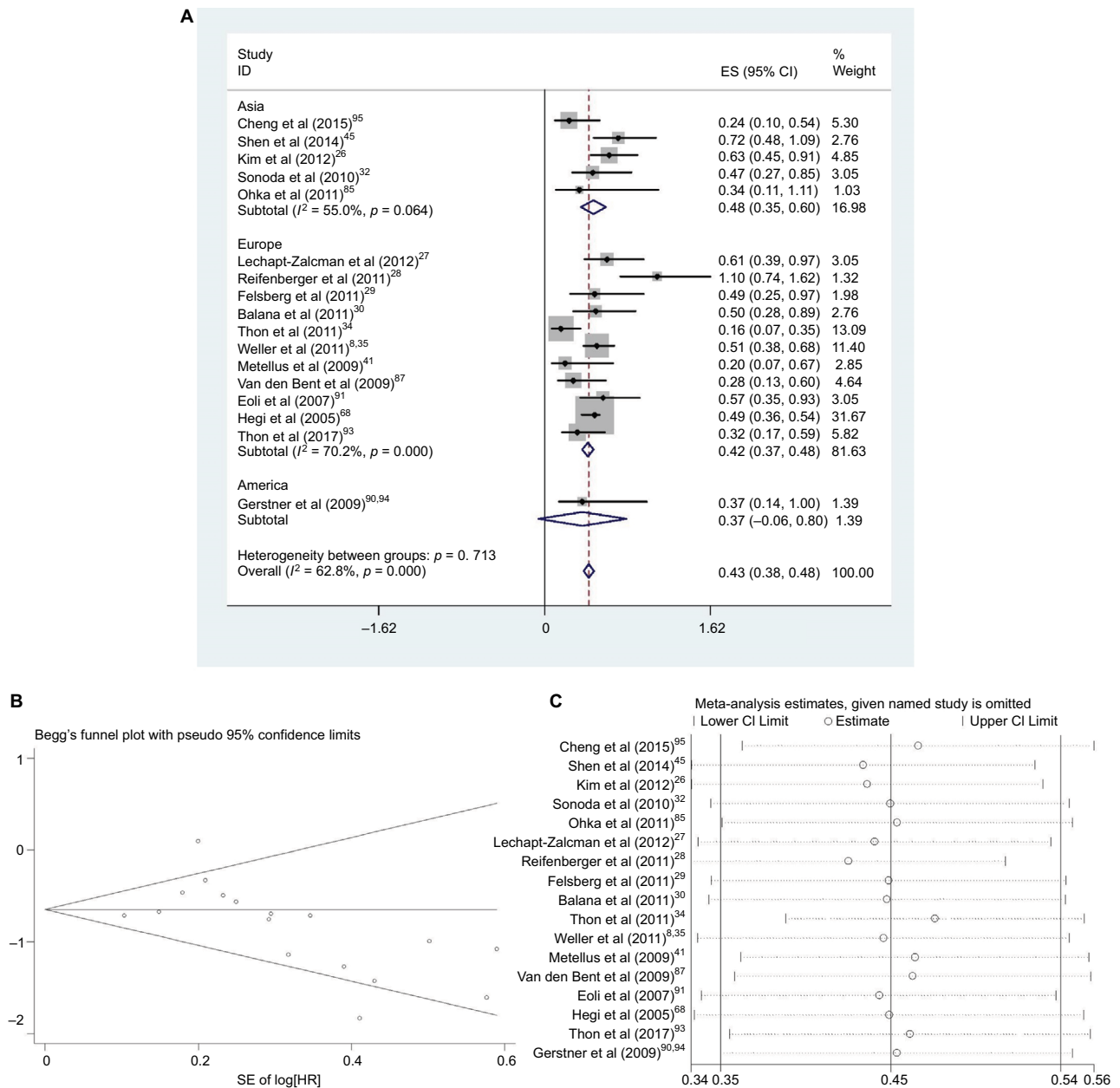


Figure 2 Data statistics on PFS using multivariate analysis. **Notes:** (A) Forest plot showing the combined relative HR from the random effect model for MGMT promoter methylation on PFS using multivariate analysis with patients from different areas. The proportion of methylation in each group was in inverse proportion to the corresponding HR. (B) Begg's test on PFS using multivariate analysis with different area. (C) Sensitivity analysis on PFS using multivariate analysis with different area. **Abbreviations:** SE, standard error; ES, effect size; HR, hazard ratio; MGMT, O6-methylguanine-DNA methyltransferase; PFS, progression-free survival.

activity of MGMT is commonly increased in relation to surrounding normal tissue.^{63,64}

The data of the Adeberg et al study show that delaying postoperative chemoradiation for GBM patients – carried out in order to determine MGMT promoter status – did not have a negative impact on survival time. Indeed, initiating radiation therapy sooner than 24 days after surgery has a negative impact on progression and survival.²⁵

In the older glioblastoma patient, MGMT promoter methylation status is still contentious on clinical decision making.

For the elderly with malignant glioma, two recently published Phase III trials have evaluated the place of dose-dense/conventional temozolomide (TMZ) regimes alone as compared with conventional/hypofractionated radiotherapy.^{65–67} OS in methylated patients was better if TMZ treatment was applied, whereas in unmethylated patients radiotherapy alone was more effective. However, in contrast, Gutenberg et al study showed no significant differences in OS for concomitant plus adjuvant administration of TMZ, as the current standard treatment specifies, to sequentially administered TMZ.²⁴

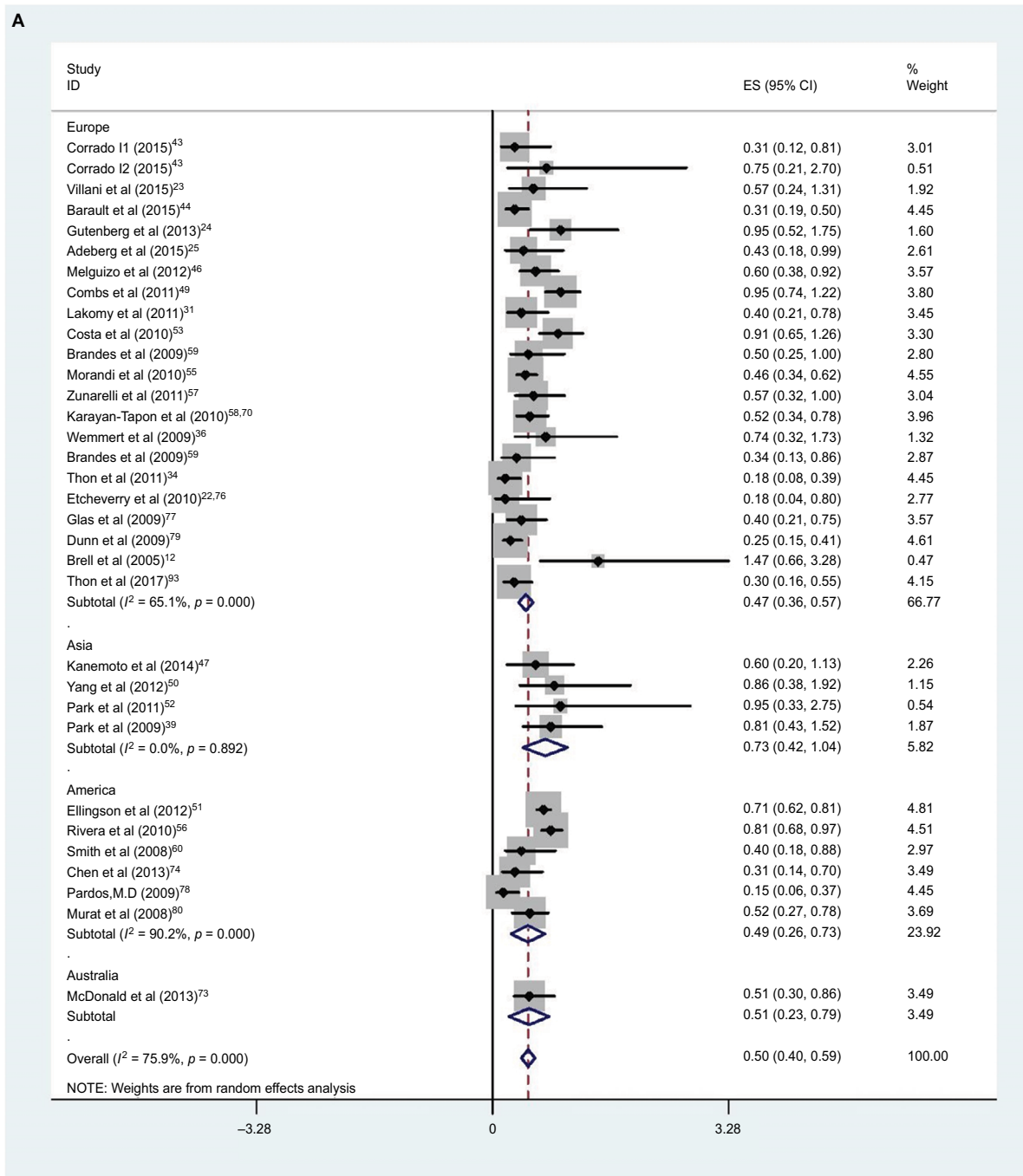


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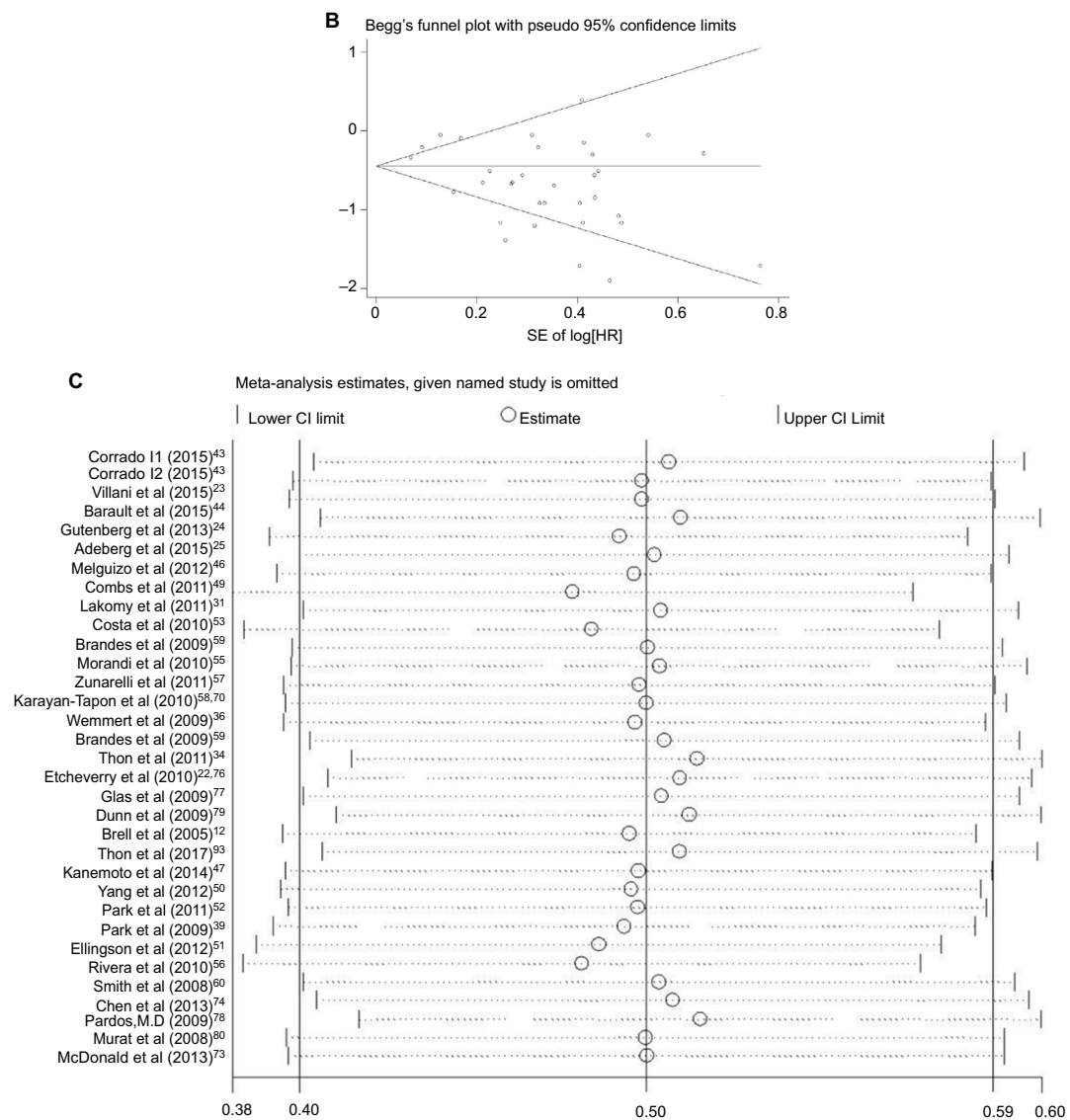


Figure 3 Data statistics on OS using univariate analysis.

Notes: (A) Forest plot showing the combined relative HR from the random effect model for MGMT promoter methylation on OS using univariate analysis with patients from different areas. The proportion of methylation in European and American groups was in inverse proportion to the corresponding HR except for the Asian group. (B) Begg's test on OS using univariate analysis with different area. (C) Sensitivity analysis on OS using univariate analysis with different area.

Abbreviations: SE, standard error, ES, effect size; HR, hazard ratio; MGMT, O₆-methylguanine-DNA methyltransferase; OS, overall survival.

Concerning age, the findings of Gutenberg et al suggest that patients over 65 years of age showed significantly longer PFS and a trend toward longer OS when receiving concomitant plus adjuvant TMZ as compared to the sequential TMZ regimen. Thus, MGMT promoter methylation is an important biomarker for personalized treatment strategies in the elderly subpopulation.

It was found that GBM patients with MGMT promoter methylation had better OS and PFS than those without methylated status by univariate or multivariate analysis regardless of therapeutic intervention and area.^{72,74–78} MGMT gene promoter methylation levels can be used as a sensitive biomarker of using alkylating agents in GBM

patients.^{86,88,89,92} The results suggested that MGMT promoter methylation indicated better clinical prognosis of GBM, and played an independent role with GBM development.^{80,82–84} Yang et al once have explored the connection between MGMT promoter methylation in glioblastoma and different race, conducting a primary conclusion, GBM patients with MGMT promoter methylation only had longer OS by multivariate analysis in Asian, but with no further exploration of subgroup.⁸¹ Also, because of population flow, it is more reasonable and accurate to set subgroup by continent but not by race. Therefore, is the prognostic significance of MGMT promoter methylation independent equally in glioblastomas of different areas?

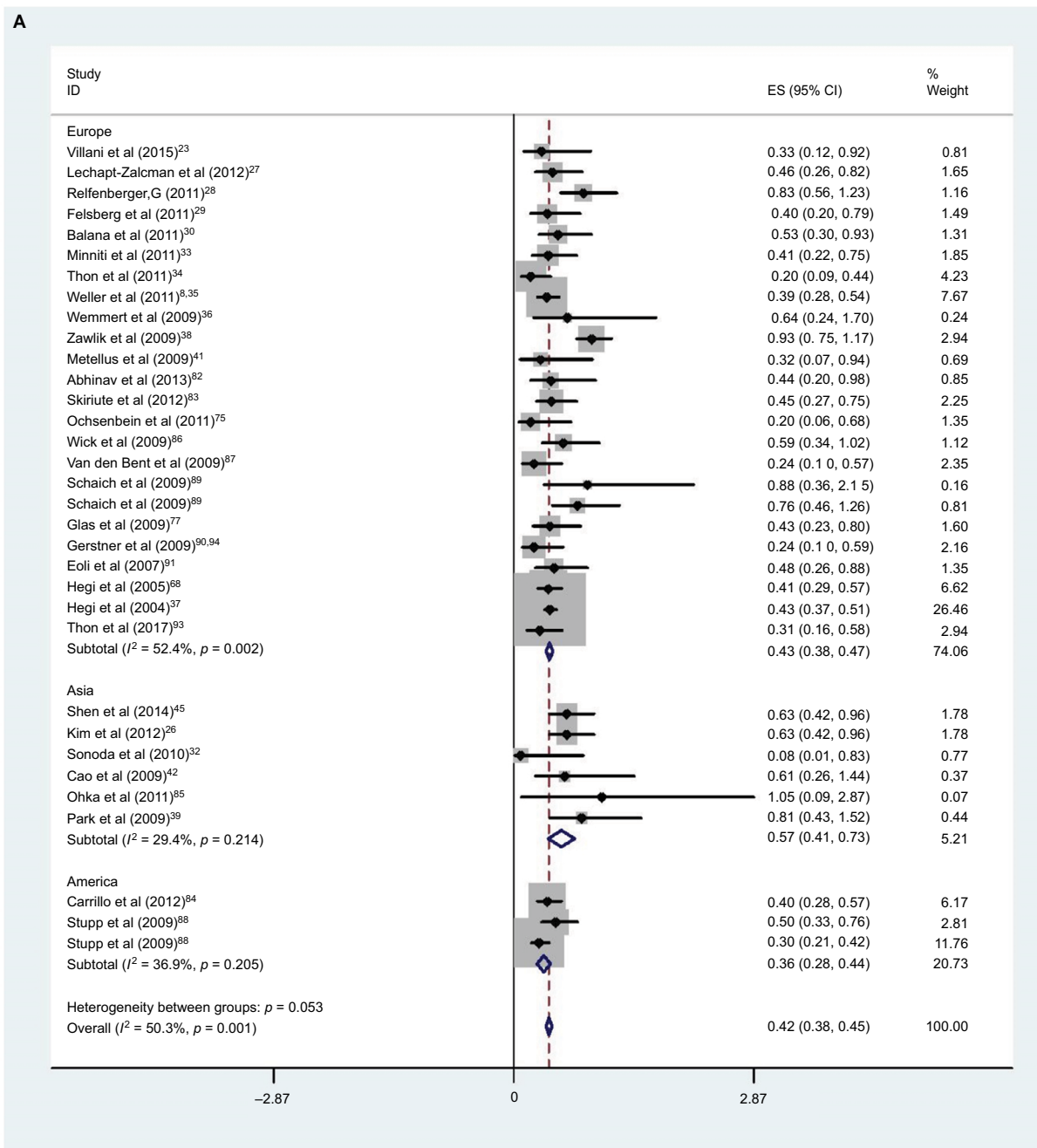


Figure 4 (Continued)

of OS, MGMT promoter methylation ratio of Asian groups is 0.53, the European is 0.53 and the American is 0.72. The HR of Asian groups is 0.56, the European is 0.43 and the American is 0.36. The proportion of methylation in the European and American group was in inverse proportion to the corresponding HR but Asian group doesn't follow the inverse relation.

Our meta-analysis with pooled data suggested that MGMT promoter methylation was associated with prolonged PFS in GBM patients according to both univariate analysis and multivariate analysis. From the perspective of PFS, the prognostic significance of MGMT promoter methylation is independent and basically equal in glioblastomas of different areas. Prolonged OS in GBM patients was also accompanied by MGMT promoter methylation through univariate analysis and multivariate analysis. However, from the perspective of OS, the prognostic significance of MGMT promoter methylation in the Asian group was not so important as in the European and American groups.

There are still two public questions. First, what is the most appropriate method for the assessment of methylation? The various technologies of measurement of MGMT promoter methylation sometimes show discrepant or even opposite results. It is originally regarded that MSP which evaluates the methylation status of the MGMT promoter is the best way to predict the MGMT expression of the tumor in a manner that also correlates with clinical prognosis.⁵⁶ In the last 5 years, more and more studies have reported that a series of more accurate values have been obtained by PSQ compared to MSP.⁷⁰ Studies with PSQ showed that this technique, having a higher reproducibility and sensitivity than MSP, is also a qualitative method. Therefore, besides MSP, our meta-analysis also absorbed measurement of MGMT promoter methylation from PSQ, which make our results more persuasive.

Second, what is the best threshold indicating methylated or unmethylated status? The definition of a prognostically relevant threshold for the percentage of MGMT methylation remains one of the most critical issues in the use of PSQ analysis. In 2015, the Receiver Operating Characteristics analysis from Villani et al showed that the best possible criteria for PSQ-detected percentage of MGMT methylation that predicted PFS and OS were 19% and 13%, respectively.²³

This meta-analysis has several potential limitations that may be taken into account. First, only English and Chinese language literature studies were scanned for publication. If the search had included literature studies published in other languages, it is possible that more additional relevant trials may have been considered. Second, some ongoing studies,

most of which being of high quality, were ineligible for inclusion. Therefore, limitations in quality cannot be excluded, and the pooled results of this meta-analysis may have been affected, more or less. Moreover, subgroup analysis still needs a larger number of trials to make results convincing. Additionally, we are unable to assess the effects of other clinically meaningful end points on PFS or OS, such as quality of life, patient and physician satisfaction with surgical resection and cytotoxic chemotherapy with the alkylating agent TMZ or concomitant radiotherapy, because of sparse and inconsistent reporting across studies. Finally, because all of the Asian studies included in the meta-analysis were carried out in Japan and South Korea, clinicians and pharmacists should carefully and judiciously assess the feasibility of applying the results in the clinical setting in China.

Conclusion

MGMT promoter methylation was an independent indicator of better prognosis for GBM and epigenetic *MGMT* gene silencing by promoter methylation associated with loss of MGMT expression may contribute to diminished DNA repair, which may be the potential mechanism that results in longer PFS and OS.⁷¹ From the perspective of PFS, the prognostic significance of MGMT promoter methylation is independent and basically equal in glioblastomas of different areas. However, from the perspective of OS, the proportion of methylation in the Asian group was not in basically inverse proportion to the corresponding HR as in European and American groups, in the univariate and multivariate analyses. The different prognosis might result from the intervention of age, percentage of MGMT methylation and the style of postoperative chemoradiation. More exploration is needed to investigate the clinical chemotherapy effect on MGMT promoter of the glioblastoma, screen a more sensitive alkylating agent combination for glioblastoma and apparent genetic targets for potential therapeutic value.

Acknowledgments

The authors thank the Intensive Care Unit of Shanghai Deji Hospital, the Ninth Clinical Medical College of Qingdao University for their help on this article. Our paper has no funding.

Author contributions

Yangyang Jiang and Wei Meng independently reviewed and extracted the data needed. Disagreements were resolved through discussion with Professor Jie Ma. All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interests in this work.

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