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REVIEW

The prognostic value of MGMT promoter methylation in Glioblastoma multiforme: a meta-analysis

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Abstract The prognostic significance of O^6-methylguanine-DNA methyltransferase (MGMT) promoter methylation on Glioblastoma multiforme (GBM) remains controversial. A meta-analysis of published studies investigating the effects of MGMT promoter methylation on both progression-free survival (PFS) and overall survival (OS) among GBM patients was performed. A total of 2,986 patients from 30 studies were included in the meta-analysis. In all, the frequency of MGMT promoter methylation was 44.27 %. Five studies undertook univariate analyses and nine undertook multivariate analyses of MGMT promoter methylation on PFS. The pooled hazard ratio (HR) estimate for PFS was 0.72 (95 % CI 0.55–0.95) by univariate analysis and 0.51 (95 % CI 0.38–0.69) by multivariate analysis. The effect of MGMT promoter methylation on OS was evaluated in 15 studies by univariate analysis and 14 studies by multivariate analysis. The combined HR was 0.67 (95 % CI 0.58–0.78) and 0.49 (95 % CI 0.38–0.64), respectively. For GBM patients treated with Alkylating agent, the meta-risk remained highly significant by both univariate (HR = 0.58; 95 % CI 0.42–0.79) and multivariate analysis (HR = 0.42; 95 % CI 0.29–0.60). This study showed that MGMT promoter methylation was associated with better PFS and OS in patients with GBM regardless of therapeutic intervention, and associated with longer OS in GBM patients treated with alkylating agents.

Keywords Glioblastoma multiforme · O^6-Methylguanine-DNA methyltransferase (MGMT) · Methylation · Prognostic factors · Meta-analysis

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Introduction

Glioblastoma multiforme (GBM, WHO grade 4) is one of the most frequently occurring brain tumors with an annual incidence of 3–4/100,000[1] in the primary central nervous system of adults and is highly malignant. The current WHO classification recognizes three variants, including conventional glioblastoma, giant cell glioblastoma, and gliosarcoma. GBM is a morphologically diverse and genetically instable neoplasm usually with rapidly fatal prognosis. After the trial by Stupp et al. [2, 3], the current standard of care for newly diagnosed GBM is surgical resection to the extent feasible followed by radiotherapy plus an

oral cytotoxic chemotherapy with the alkylating agent temozolomide (TMZ), given concomitantly with and after radiotherapy (RT). The median survival time is only 14 months from diagnosis, despite the use of aggressive treatment, surgery, postoperative radiotherapy, and adjuvant temozolomide (TMZ)-based chemotherapy [2, 4, 5]. The necessity of tumor markers that explain their biology is becoming increasingly important, mainly to recognize a potential molecular target of therapy.

The O^6-methylguanine-DNA methyltransferase (MGMT) gene encodes a ubiquitously expressed suicide DNA repair enzyme that counteracts the normally lethal effects of alkylating agents by removing alkyl adducts from the O^6-position of guanine [6]. O^6-Alkylated guanine causes base mispairing and double-strand breaks, thus inducing apoptosis and cell death [7]. The assessment of MGMT promoter methylation is currently considered as mandatory for patient selection in clinical trials [8]. The results of the European Organisation for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC) trial, in which methylation of the MGMT promoter was the strongest predictor for outcome and benefit from TMZ treatment [3, 8].

However, the prognostic significance of MGMT promoter methylation on GBM regardless of therapeutic intervention remains controversial [9, 10]. Based on the discordant results obtained by a large number of studies, we performed this meta-analysis with accumulated data from different studies to quantify the prognostic impacts of MGMT promoter methylation on both progression-free survival (PFS) and overall survival (OS) among patients with GBM.

Materials and methods

Publication selection

We searched the PubMed and CNKI (China National Knowledge Infrastructure) databases for all articles within a range of published years from 2000 to 2012 on the association between MGMT promoter methylation and GBM (last search was update 20st June 2012). The following terms were used in this search: "MGMT promoter methylation" and "glioblastoma" and "survival analysis". The meta-analysis gathered complete databases from published cohort studies dealing with the prognostic value of MGMT promoter methylation in patients with GBM who underwent surgical resection of a tumor. The language in which the papers were written was not restricted. 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 centres involved. We evaluated the eligible studies if all the following conditions were met: (1) MGMT promoter methylation status were measured by using methylation-specific polymerase chain reaction (MSP); (2) surgically resected tumor tissue but not body fluids such as sputum, peritoneal fluid and serum were used; (3) inclusion of sufficient data to calculate HR and 95 % CI or inclusion of the HR and 95 % CI; and (4) full paper investigated the relationship between MGMT promoter methylation and PFS or OS.

Data extraction

Two authors (Kui Zhang and Bin Zhou) independently reviewed and extracted the data needed. Disagreements were resolved through discussion among the authors to achieve a consensus. Publications were read by Kui Zhang in order to check original data extraction. The following information was recorded for each study: first author, year of publication, region, HR form, and sample size (all of 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 corresponding to the 95 % CI were not given directly, published data and figures from original papers were used to calculate the HR according to the methods described by Parmar et al. [11]. 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 [12]. If there was no obvious heterogeneity, the fixed-effects model (the Mantel–Haenszel method) was used to estimate the pooled HR [13]; otherwise, the random-effects model (the DerSimonian and Laird method) was used [14]. The pejorative 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 1. Publication bias was evaluated with funnel plot and Begg's rank correlation method [15]. The statistical analyses were performed by STATA 12.0 software (Stata Corp., College Station, TX).

Results

Characteristics of studies

Out of a total of 109 articles were screened, 76 articles concerned topics not relevant to this study, 3 studies were

Table 1 Main characteristics and results of eligible studies

References	Years	Country	M	U	PFS		OS	
					Univariate	Multivariate	Univariate	Multivariate
Christians et al. [36]	2012	Germany	16	19	Survival curve ($p = 0.0011$)	N/A	N/A	N/A
Combs et al. [37]	2011	Germany	43	84	Survival curve ($p = 0.93$)	N/A	Survival curve ($p = 0.18$)	N/A
Kim et al. [19]	2012	Korea	43	35	N/A	HR, 95 % CI ($p = 0.008$)	N/A	HR, 95 % CI ($p = 0.002$)
Lechapt-Zalcman et al. [20]	2012	France	63	47	N/A	HR, 95 % CI ($p = 0.036$)	N/A	HR, 95 % CI ($p = 0.008$)
Yang et al. [38]	2012	Korea	10	12	N/A	N/A	Survival curve ($p = 0.156$)	N/A
Reifenberger et al. [21]	2011	Germany	134	99	N/A	HR, 95 % CI ($p = 0.646$)	N/A	HR, 95 % CI ($p = 0.352$)
Felsberg et al. [22]	2011	Germany	31	49	N/A	HR, 95 % CI ($p = 0.042$)	N/A	HR, 95 % CI ($p = 0.009$)
Balana et al. [23]	2011	Spain	27	42	N/A	HR, 95 % CI ($p = 0.018$)	N/A	HR, 95 % CI ($p = 0.028$)
Ellingson et al. [39]	2012	USA	141	238	N/A	N/A	Survival curve ($p < 0.0001$)	N/A
Lakomy et al. [24]	2011	Czech Republic	12	26	HR, 95 % CI ($p = 0.0201$)	N/A	HR, 95 % CI ($p = 0.0054$)	N/A
Park et al. [40]	2011	Korea	14	34	N/A	N/A	Survival curve ($p = 0.027$)	N/A
Costa et al. [41]	2010	Portugal	38	42	N/A	N/A	Survival curve ($p = 0.583$)	N/A
Brandes et al. [42]	2010	Italy	13	25	N/A	N/A	Survival curve ($p = 0.04$)	N/A
Morandi et al. [43]	2010	Italy	70	89	N/A	N/A	Survival curve ($p = 0.003$)	N/A
Rivera et al. [44]	2010	USA	54	171	Survival curve ($p = 0.009$)	N/A	Survival curve ($p = 0.019$)	N/A
Sonoda et al. [25]	2010	Japan	35	27	N/A	HR, 95 % CI ($p = 0.011$)	N/A	N/A
Minniti et al. [26]	2011	Italy	42	42	N/A	N/A	N/A	HR, 95 % CI ($p = 0.0001$)
Thon et al. [27]	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 ($p < 0.0001$)
Zunarelli et al. [45]	2011	Italy	24	53	N/A	N/A	Survival curve ($p < 0.04$)	N/A
Karayan-Tapon et al. [46]	2010	France	55	26	N/A	N/A	Survival curve ($p = 0.005$)	N/A
Weller et al [28]	2009	Germany	111	137	N/A	HR, 95 % CI ($p < 0.0001$)	N/A	HR, 95 % CI ($p < 0.0001$)
Wemmert et al. [29]	2009	Germany	15	12	N/A	N/A	HR, 95 % CI ($p = 0.490$)	HR, 95 % CI ($p = 0.370$)
Hegi et al. [30]	2004	Switzerland	26	12	N/A	N/A	N/A	HR, 95 % CI ($p = 0.017$)
Zawlik et al. [31]	2009	Switzerland	165	206	N/A	N/A	N/A	HR, 95 % CI ($p = 0.469$)
Park et al. [32]	2009	Korea	26	22	N/A	N/A	HR, 95 % CI ($p = 0.518$)	N/A
Sonoda et al. [33]	2009	Japan	4	12	N/A	N/A	N/A	HR, 95 % CI ($p = 0.02$)

Table 1 continued

References	Years	Country	M	U	PFS		OS	
					Univariate	Multivariate	Univariate	Multivariate
Brandes et al. [47]	2009	Italy	16	21	Survival curve ($p = 0.005$)	N/A	Survival curve ($p = 0.05$)	N/A
Smith et al. [48]	2008	Arizona	12	11	N/A	N/A	Survival curve ($p = 0.0009$)	N/A
Metellus et al. [34]	2009	France	6	15	N/A	HR, 95 % CI ($p = 0.0012$)	N/A	HR, 95 % CI ($p = 0.019$)
Cao et al. [35]	2009	Korea	46	30	N/A	N/A	N/A	HR, 95 % CI ($p = 0.26$)

N/A no available or no applicable, *M/U* methylation/unmethylation cases

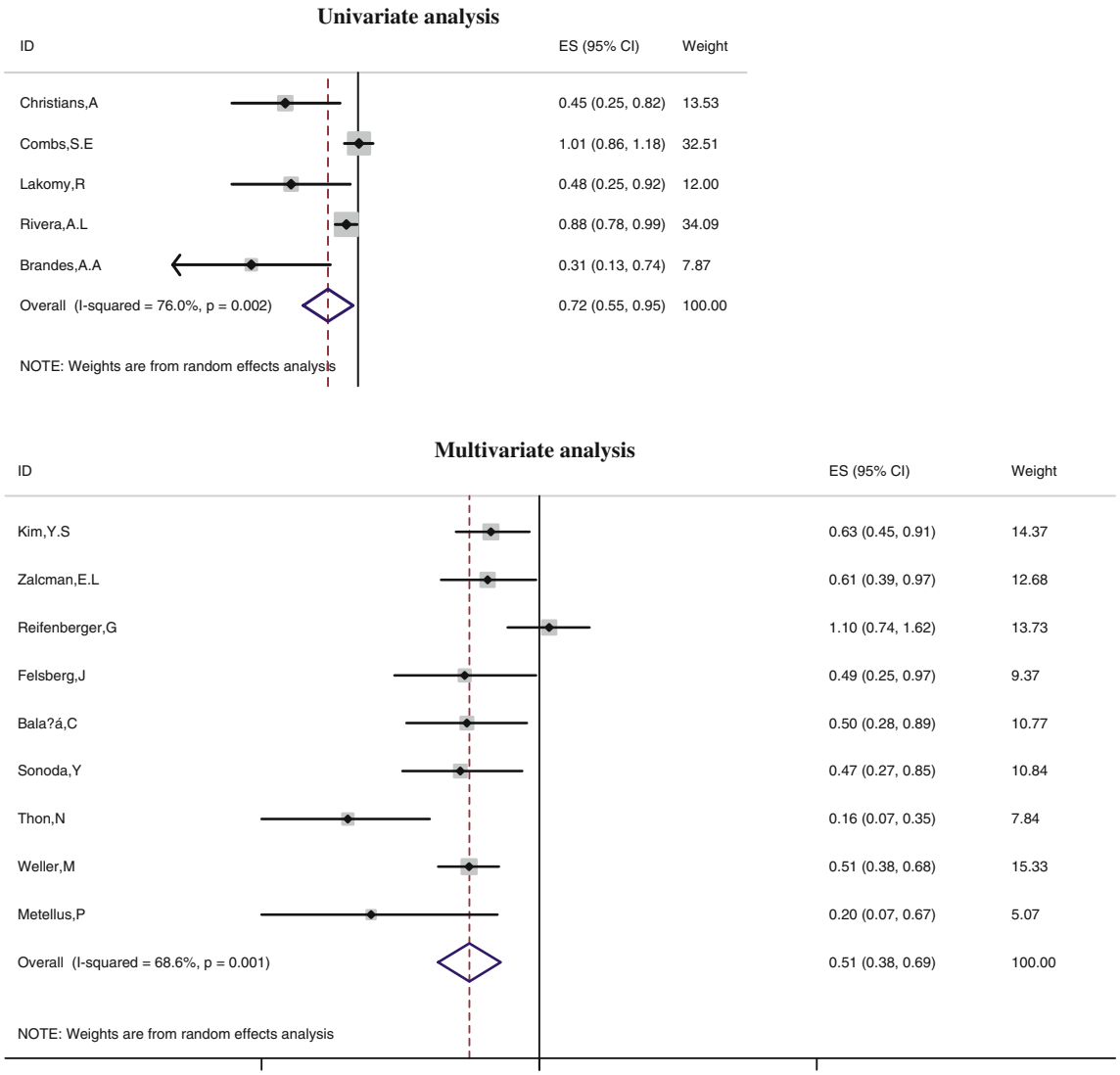

Fig. 1 Forest plot showing the combined relative HR from the random effect model for MGMT promoter methylation on PFS

excluded for determination of MGMT promoter methylation by Pyrosequencing [16–18], and finally 30 studies were available for this study. 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 2,986, and the frequency of MGMT promoter methylation was 44.27 %. Of the 30 publications eligible for systematic review, 17 studies reported the HR corresponding to 95 % CI directly [19–35], the other 13 studies only contain survival curve [36–48] available to calculate the HR.

Meta-analysis

Five studies [24, 36, 37, 44, 47], including 462 patients, reported the effect of MGMT promoter methylation on PFS using analyses unadjusted for other factors. As shown in Fig. 1, MGMT promoter methylation was significantly correlated with better PFS according to univariate analysis, with a combined HR of 0.72 (95 % CI 0.55–0.95). The random-effects model (the DerSimonian and Laird method) was used [14] because of significant heterogeneity was detected among these studies ($p = 0.002$, $I^2 = 76.0$ %). The effect of MGMT promoter methylation on PFS adjusted for other variables was evaluated in nine studies [19–23, 25, 27, 28, 34], including 957 patients. As shown in Fig. 1, MGMT promoter methylation was significantly correlated with better PFS according to multivariate analysis, with a combined HR of 0.51 (95 % CI 0.38–0.69). The random-effects model (the DerSimonian and Laird method) was used [14] because of significant heterogeneity was detected among these studies ($p = 0.001$, $I^2 = 68.6$ %).

The effect of MGMT promoter methylation on OS unadjusted for other variables was evaluated in 15 studies [24, 29, 32, 37–48], including 1,409 patients. As shown in Fig. 2, MGMT promoter methylation was significantly correlated with better OS according to univariate analysis, with a combined HR of 0.67 (95 % CI 0.58–0.78). The random-effects model (the DerSimonian and Laird method) was used [14] because of significant heterogeneity was detected among these studies ($p = 0.010$, $I^2 = 52.0$ %). 14 studies [19–23, 26–31, 33–35], including 1,507 patients, reported the effect of MGMT promoter methylation on OS using analyses adjusted for other factors. As shown in Fig. 2, MGMT promoter methylation was significantly correlated with better OS according to multivariate analysis, with a combined HR of 0.49 (95 % CI 0.38–0.64). The random-effects model (the DerSimonian and Laird method) was used [14] because of significant heterogeneity was detected among these studies ($p < 0.001$, $I^2 = 68.6$ %).

Among 13 studies, which reported the prognostic value of MGMT promoter methylation on OS in GBM patients treated with alkylating agent, nine studies, including 653 patients, reported the effect of MGMT promoter methylation on OS using analyses unadjusted for other factors. Six studies, including 371 patients, reported the effect on OS using analyses adjusted for other factors. As shown in Fig. 3, MGMT promoter methylation were significantly correlated with better OS according to both univariate analysis and multivariate analysis, with combined HR of 0.58 (95 % CI 0.42–0.79) for univariate analysis, and combined HR of 0.42 (95 % CI 0.29–0.60) for multivariate analysis. The random-effects model (the DerSimonian and Laird method) was used [14] because of significant heterogeneity was detected among these studies ($p < 0.001$, $I^2 = 75.2$ % for univariate analysis and $p = 0.125$, $I^2 = 42.1$ % for multivariate analysis).

Publication bias statistics were determined, no 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 prognostic role of a specific molecular marker is more powerful when used to help make therapeutic decisions. Despite progress in our understanding of the genetic alterations in GBM, clinically useful molecular markers predictive of the therapeutic response and prognosis are still rare. Usually, meta-analysis is used to evaluate pooled results from different randomized controlled trials.

Our meta-analysis focuses on MGMT promoter methylation in patients with GBM. Although the findings of two studies [21, 37] which reported the status of MGMT promoter methylation on PFS, and the results of nine studies [21, 29, 31, 32, 35, 37, 38, 40, 41] which reported the status of MGMT promoter methylation on OS were in the opposite direction to those observed in the meta-analysis, our meta-analysis with accumulated data suggested MGMT promoter methylation was associated with longer PFS and OS according to both univariate analyses and multivariate analyses.

MGMT protects cells against the potentially deleterious effects of alkylating agents, which include mutations, sister chromatid exchanges, recombination, and chromosomal aberrations [49, 50]. It has been shown that glial brain tumors are characterized by a low expression of MGMT, however, the activity of MGMT is commonly increased in relation to surrounding normal tissue [51, 52]. MGMT activity is partly mediated through methylation of the MGMT promoter region; this epigenetic mechanism contributes to a loss of

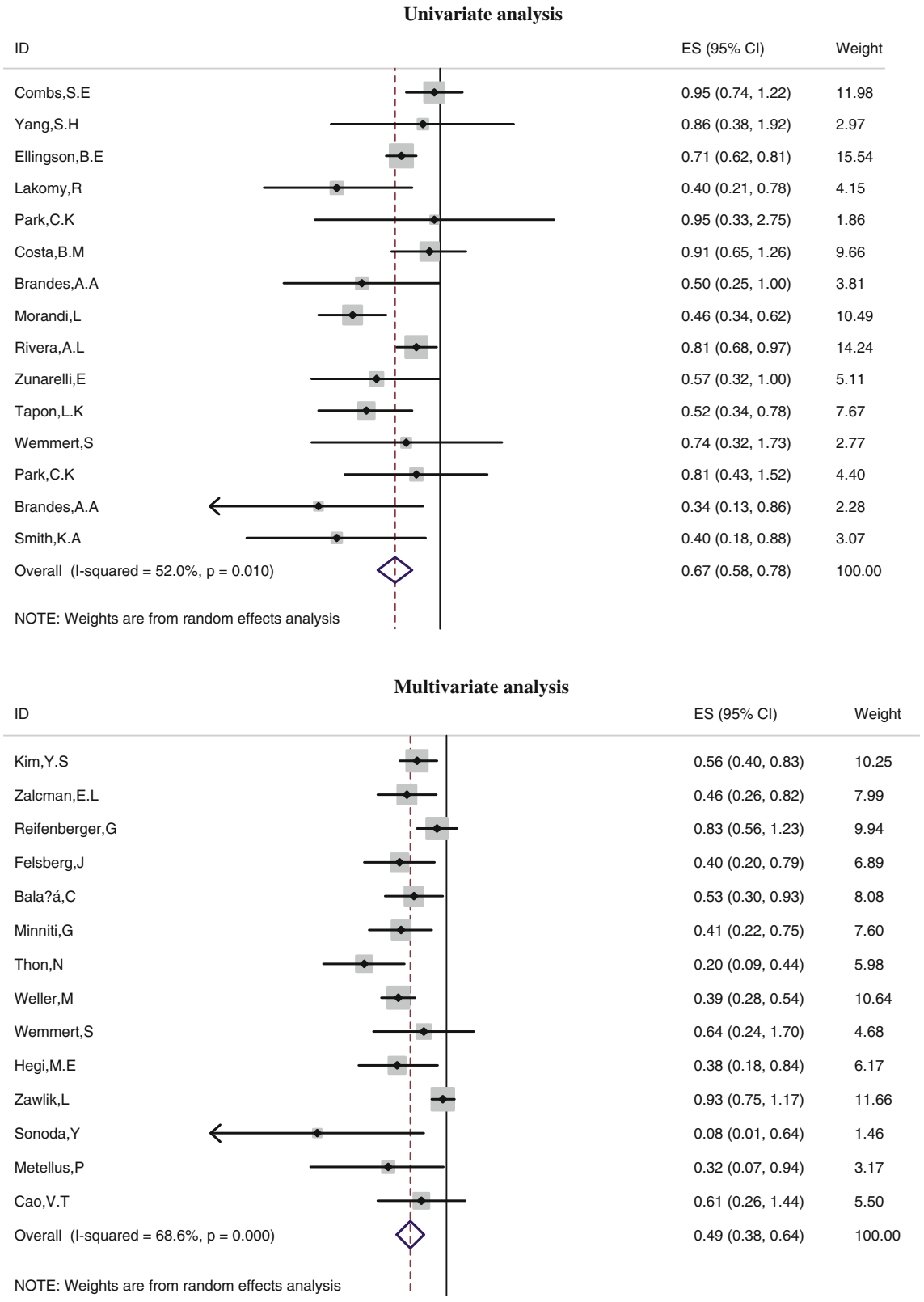

Fig. 2 Forest plot showing the combined relative HR from the random effect model for MGMT promoter methylation on OS

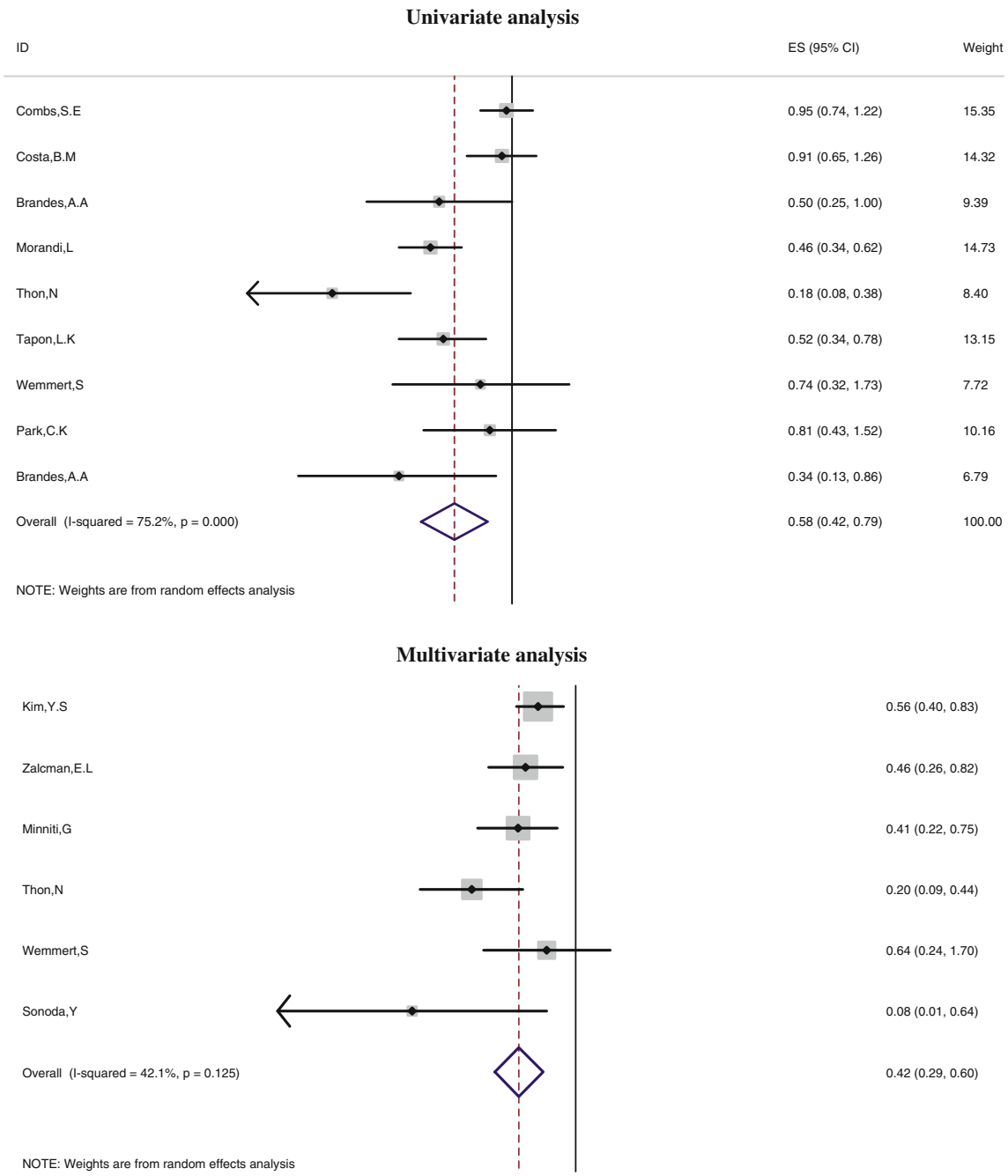

Fig. 3 Forest plot showing the combined relative HR from the random effect model for MGMT promoter methylation on OS in patients treated with Alkylating agent

MGMT expression [49]. Epigenetic MGMT gene silencing by promoter methylation associated with loss of MGMT expression may contribute to diminished DNA repair [53], which may be the potential mechanism that results in longer PFS and OS.

Esteller et al. [9] first determined that MGMT promoter methylation is related to the responsiveness of gliomas to carmustine (BCNU). Alkylating agents are the currently leading chemotherapeutic agents for GBM patients [2, 54]. Evaluation of prognostic factors is vital to improve research pursuing new therapies for GBM. Although 4 previous studies [29, 32, 37, 41] failed to find significant association of MGMT promoter methylation on OS in GBM patients treated with alkylating agents, our meta-analysis with pooled data suggested that MGMT promoter methylation was associated with prolonged OS in GBM

patients treated with alkylating agents according to both univariate analysis and multivariate analysis. Indeed, it was demonstrated that MGMT-hypermethylated tumors were 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 [55].

The various methods of measurement of MGMT promoter methylation sometimes show discrepant results. It is generally accepted that a methylation-specific polymerase chain reaction (MSP) evaluating 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 outcome [56]. The vast majority of previous studies of MGMT promoter methylation in GBM have used MSP, which is a qualitative method. Our meta-analysis was performed under the bases of the same methods of measurement of MGMT promoter methylation, which eliminated the disparity from the method differences.

Publication bias statistics were determined, no publication bias (Begg's test, $p > 0.05$) was found. Sensitivity analysis was also 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.

In conclusion, MGMT promoter methylation was associated with better PFS and OS in patients with GBM regardless of therapeutic intervention, and associated with longer OS in GBM patients treated with alkylating agents. Our results suggested that MGMT promoter methylation was an independent indicator of better prognosis for GBM. The presence of a methylated MGMT promoter may be a marker for response to therapy with alkylating agents.

Conflict of interest The authors declare no conflict of interest.

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