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Review Article

MGMT promoter-enhancer single nucleotide polymorphism rs16906252 is associated with MGMT promoter methylation in cancer

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Abstract: The methylation of the O(6)-methylguanine DNA methyltransferase (*MGMT*) promoter is frequently observed in several cancer types. The potential correlation between the C>T allele at SNP rs16906252 and methylation in *MGMT* are being actively investigated and remain largely elusive. We studied the allelic pattern of *MGMT* methylation through meta-analysis. All eligible studies were identified by searching PubMed, Web of Science, Embase, and Chinese National Knowledge Infrastructure Database. Pooled odds ratio (OR) and 95% confidence interval (95% CI) were calculated using fixed- or random-effect model. Genotype and allelic frequency compared between cases and controls together with further subgroup analyses were carried out by cancer type. A total of 6 studies involving 1775 cancer cases were analyzed. Meta-analysis from this study indicated that the T allele of the rs16906252 SNP were strongly associated with increased risk for *MGMT* methylation in allelic model (*OR* = 9.70, 95% CI 4.12-22.86, $P_{heterogeneity}$ = 0.021), heterozygote comparison *OR* = 11.28, 95% CI 4.46-28.52, $P_{heterogeneity}$ = 0.024) and dominant model (*OR* = 6.61, 95% CI 3.23-13.54, $P_{heterogeneity}$ = 0.003). Stratified analysis by cancer type indicated that the association became more prominent among glioblastoma, colorectal cancer and other cancer. Our study provides strong evidence that the T allele of a *MGMT* promoter-enhancer SNP is a key determinant for *MGMT* methylation in cancer. Clarifying this association could advance our knowledge of the influence of *MGMT* promoter-enhancer single nucleotide polymorphism rs16906252 on *MGMT* promoter methylation in cancer.

Keywords: Cancer, meta-analysis, polymorphism, methylation, MGMT

Introduction

Transcriptional inactivation of DNA stability genes by aberrant methylation of the CpG island promoter predisposes to cancer development [1-3]. O^6-methylguanine-DNA methyltransferase (*MGMT*) protein removes mutagenic and cytotoxic adducts from the O^6 position of guanine and involved in the biology of cancer formation and behavior [4-6]. MGMT knockout mice display heightened sensitivity to the deleterious effects of alkylating agents [7]. Loss of expression of the MGMT protein contribute to tumor development and result in increased sensitivity to alkylating agents in many types of human malignancies [8, 9]. *MGMT* gene is located on the distal end of the long arm of chromosome 10 at 10q26 and transcribes messenger RNA under the regulation of a GC-rich promoter [10]. It is well known that the predominant mechanism for *MGMT* silencing is methylation of CpG island in the promoter region of MGMT that leads to transcriptional silencing in primary human neoplasia [11]. MGMT promoter methylation predicted significantly longer survival in glioblastoma patients who received alkylating chemotherapy with temozolomide [12-14]. Cancers with MGMT hypermethylation generate transition point mutations associated with epigenetic inactivation of the oncogene K-ras and the tumor suppressor gene p53 [5].

Single-nucleotide polymorphisms (SNPs) in the promoter/enhancer region of the MGMT gene, can affect MGMT transcription and its downstream protein expression [15]. The SNP rs16906252, within a 59-bp *cis*-acting enhancer

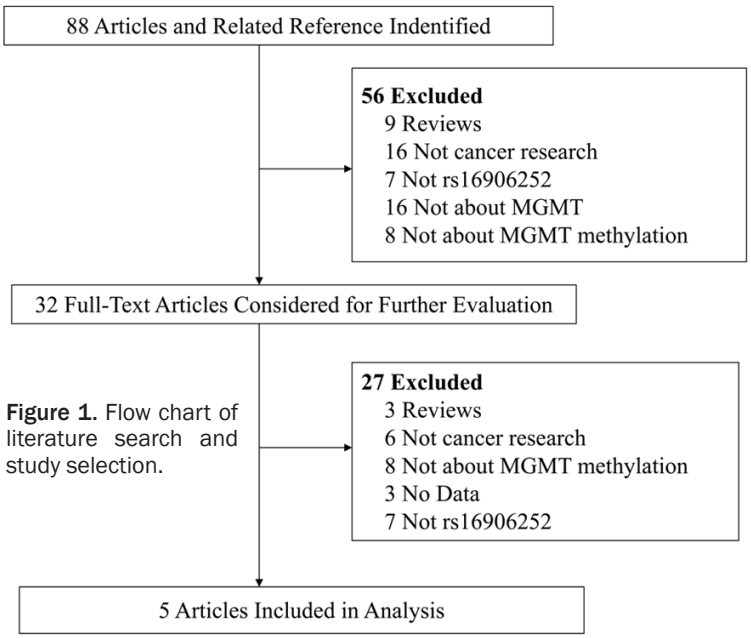

Figure 1. Flow chart of literature search and study selection.

element of the promoter that spans the first exon-intron boundary, resides 56 bp upstream of the MGMT translation start site and is required for efficient *MGMT* promoter function [16, 17]. Promoter reporter assays performed in both lung cancer and GBM cell lines have shown that the haplotype bearing the SNP rs16906252 T allele has reduced promoter activity compared with the wild-type sequence [18, 19]. In the recent years, a number of molecular epidemiological studies have investigated the impact of SNP rs16906252 on MGMT promoter methylation in cancer [16, 18, 20-22]. However reported results varied across studies and remain inconclusive. We implemented a meta-analysis that integrated all studies to address the association between MGMT rs16906252 within the *MGMT* promoter-enhancer region and methylation of *MGMT* in cancers in order to obtain an accurate assessment.

Methods

Literature search strategy

Relevant publications were identified by conducting a comprehensive literature search in PubMed, Web of Science, Embase, and Chinese National Knowledge Infrastructure Database up to Jan 28, 2016. We used different combinations of the following customized terms "*MGMT*", "rs16906252", "polymorphism", and "cancer", without restrictions on publication language. Search strings were adjusted accordingly for the other databases. Furthermore, reference lists of identified articles were also searched manually to identify additional relevant publications.

Selection criteria

Identified studies meeting all of the following criteria were satisfied for further meta-analysis: (a) articles which focused on the correlation of MGMT rs16906252 polymorphism with *MGMT* methylation; (b) providing sufficient data to calculate the odds ratio (OR) with a 95% confidence interval (CI).

Data extraction

After systematic search and selection, we reviewed all papers in accordance with the criteria for further analysis. Two authors extracted the following information independently from each publication with the inclusion criteria mentioned above: the first author's surname, publication year, country of origin, ethnicity, cancer type, total number of cases, numbers of cases and controls with the CC, CT, TT and CTTT genotypes. We conducted stratification analyses by cancer type according to the number of each investigation. Disagreements between the two investigators were resolved by discussing the results with a third investigator.

Statistical methods

Hardy-Weinberg equilibrium (HWE) for the genotype distribution in controls was tested by a chi-squared goodness-of-fit test ($P<0.05$ was considered significant). The strength of the association between MGMT rs16906252 polymorphism and *MGMT* methylation was calculated using odds ratios (ORs) with 95% confidence intervals (CIs). The pooled ORs were calculated in dominant model (TT+CT versus CC), heterozygote comparison (TC versus CC) and allelic model (T versus C). The significance of the pooled ORs was determined using a Z-test, and the level of statistical significance was

Table 1. Main characteristics of all studies included in the meta-analysis

First author	Year	Country	HWE	Cancer type	Cases	MGMT methylated				MGMT unmethylated			
						CC	CT	TT	CTTT	CC	CT	TT	CTTT
Ogino	2007	USA	0.06	Colorectal cancer	179	36	24	2	26	112	5	0	5
Hawkins	2009	Australia	0.25	Colorectal cancer	1039	195	91	2	93	691	60	0	60
Kristensen	2011	Denmark	0.64	Pleural mesothelioma	95	6	6	1	7	74	8	0	8
Leng	2011	USA	0.96	Lung cancer	169	36	23	0	23	109	1	0	1
Rapkins	2015	Australia (AGOG)	-	Glioblastoma	138	61	-	-	25	45	-	-	7
Rapkins	2015	America (UCLA/KPLA)	-	Glioblastoma	155	44	-	-	12	88	-	-	11

HWE: *P* values for Hardy-Weinberg equilibrium (HWE) for each study's control group.

Table 2. Stratified analyses of the MGMT rs16906252 polymorphism on MGMT promoter methylation in cancer

Variables	n[a]	Cases	T versus C			CT versus CC			Dominant model		
			OR (95% CI)	P^b	I^2 (%)	OR (95% CI)	P^b	I^2 (%)	OR (95% CI)	P^b	I^2 (%)
Total	6		9.70 (4.12-22.86)[c]	0.021	69.3	11.28 (4.46-28.52)[c]	0.024	68.2	6.61 (3.23-13.54)[c]	0.003	72.2
Cancer type											
Colorectal cancer	2		7.17 (2.64-19.48)[c]	0.049	74.2	6.16 (4.40-8.65)	0.067	70.3	6.37 (4.55-8.92)	0.051	73.7
Glioblastoma	2								2.41 (1.27-4.57)	0.773	0.00
Other cancer	2		20.67 (7.96-53.65)	0.071	69.3	27.03 (9.26-78.84)	0.071	69.4	28.24 (9.71-82.13)	0.093	64.5

[a]Number of studies. [b]*P* value of Q-test for heterogeneity test. [c]Random-effects model was used when *P* value for heterogeneity test <0.05; otherwise, fix-effects model was used.

established as P<0.05. Between-study heterogeneity was calculated using the Cochran's *Q* test and quantified by the I^2. When a significant Q test (*P*>0.05) indicated homogeneity across studies, a fixed-effect model based on the Mantel-Haenszel method were hired to calculate the pooled OR and 95% CI [23]. Otherwise, the DerSimonian and Laird method-based random effects model was adopted [24]. Analysis of sensitivity was performed to evaluate the stability of the results by omitting each study in turn. Finally, potential publication bias was evaluated using Begg's funnel plot and Egger's regression test [25, 26]. All of the statistical analyses were performed using a software program, STATA version 11.0 (Stata, College Station, TX, USA).

Results

Study selection and description

The flow chart of the detailed steps of study selection is shown in **Figure 1**. A total of 6 studies with 1775 cancer cases were included in the current meta-analysis [16, 18, 20-22]. The main characteristics of the eligible studies are listed in **Table 1**. ALL of the 6 studies involved Caucasian populations. These studies included 2 colorectal studies, 2 glioblastoma studies, 1 pleural mesothelioma studies and 1 lung cancer studies. Among the 6 studies, 3 studies were performed in America, 2 in Australia and 1 in Denmark. The distribution of the genotypes in the control subjects was in agreement with HWE (**Table 1**).

Quantitative synthesis

We studied the effect of SNP rs16906252 in the promoter-enhancer region on MGMT promoter methylation through meta-analysis. *The main results of this meta-analysis and the heterogeneity test were shown in* **Table 2** (**Figure 2**). *We firstly analyzed the association in the overall population. The results declared that* MGMT rs16906252 *polymorphism might be significantly associated with* MGMT promoter methylation in cancer *(allele model: OR* = 9.70, 95% CI 4.12-22.86, $P_{heterogeneity}$ = 0.021*;* heterozygote comparison*: OR* = 11.28, 95% CI 4.46-28.52, $P_{heterogeneity}$ = 0.024*;* dominant model*: OR* = 6.61, 95% CI 3.23-13.54, $P_{heterogeneity}$ = 0.003*; respectively).*

Then in order to obtain the exact consequence of the relationship between MGMT rs16906252 *polymorphism and* MGMT promoter methylation in cancer*, stratified analyses by cancer type were performed* (**Figure 3**). The results of

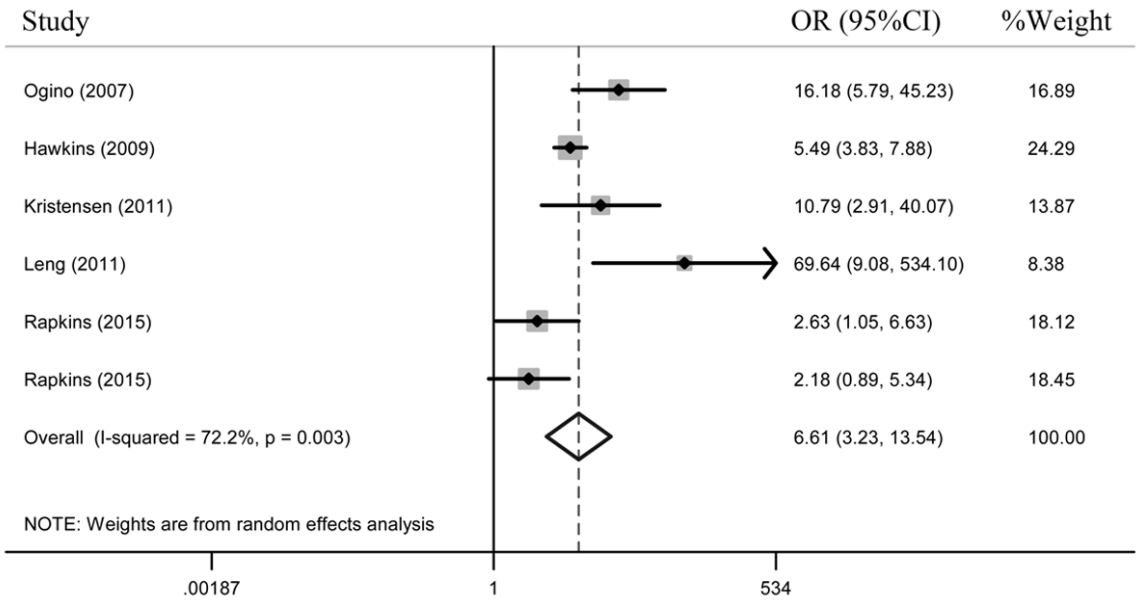

Figure 2. Forest plot for pooled OR of association between the MGMT rs16906252 polymorphism and *MGMT* methylation in cancer under dominant model (CT+TT vs. CC).

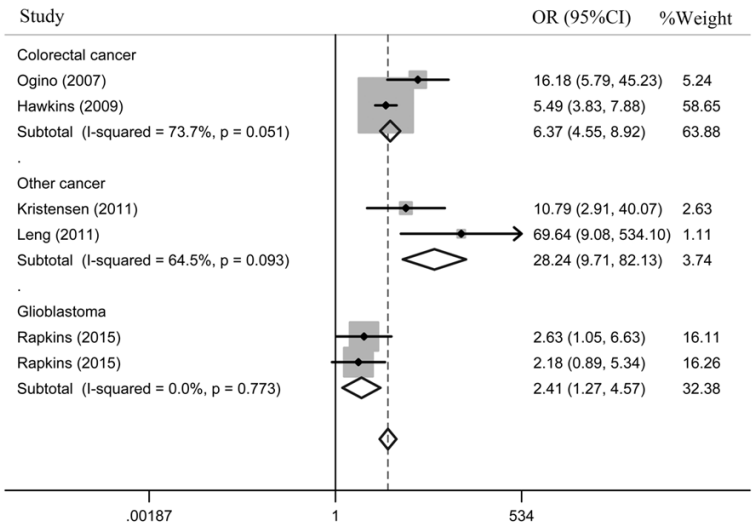

Figure 3. Forest plots of the subgroup analysis (*cancer type*) for the association between MGMT rs16906252 polymorphism and *MGMT* methylation in cancer under dominant model (CT+TT vs. CC).

our study suggested that there was a positive correlation between the MGMT rs16906252 *polymorphism and* MGMT promoter methylation in glioblastoma (dominant model*: OR* = 2.41, 95% CI 1.27-4.57, $P_{heterogeneity}$ = 0.773), colorectal cancer (*allele model: OR* = 7.17, 95% CI 2.64-19.48, $P_{heterogeneity}$ = 0.049*;* heterozygote comparison*: OR* = 6.16, 95% CI 4.40-8.65, $P_{heterogeneity}$ = 0.067*;* dominant model*: OR* = 6.37, 95% CI 4.55-8.92, $P_{heterogeneity}$ = 0.051) and other cancer (*allele model: OR* = 20.67, 95% CI 7.96-53.65, $P_{heterogeneity}$ = 0.071*;* heterozygote comparison*: OR* = 27.0, 95% CI 9.26-78.84, $P_{heterogeneity}$ = 0.071*;* dominant model*: OR* = 28.24, 95% CI 9.71-82.13, $P_{heterogeneity}$ = 0.093).

Heterogeneity and sensitivity analyses

The heterogeneities that originated within the collection of selected studies and within each subgroup of studies are shown in **Table 2**. Significant heterogeneity existed in all genetic models. However, stratification based on *cancer type* reduced the heterogeneity in glioblastoma subgroup (Dominant model: $P_{heterogeneity}$ = 0.773), colorectal cancer subgroup (CT versus CC: $P_{heterogeneity}$ = 0.067; Dominant model: $P_{heterogeneity}$ = 0.051) and other cancer (T versus C: $P_{heterogeneity}$ = 0.071; CT versus CC: $P_{heterogeneity}$ = 0.071; Dominant model: $P_{heterogeneity}$ = 0.093).

Sensitivity analysis was performed to explore the influence of individual data set on the pooled OR by repeating the meta-analysis while

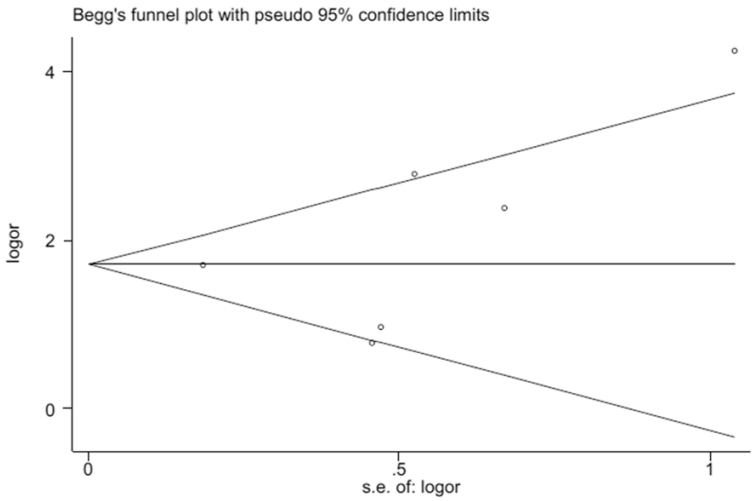

Figure 4. Funnel plot assessing evidence of publication bias from the eligible studies.

omitting each study one at a time. The corresponding pooled ORs were not materially altered, indicating our results are reliable and robust.

Publication bias

Begg's funnel plot and Egger's linear regression test were performed to assess the publication biases of included studies. The funnel plots failed to reveal any obvious asymmetry under the dominant model, which was further proven by Egger's linear regression test (P = 0.488) (**Figure 4**).

Discussion

To we knowledge, this is the first study showing the *MGMT* SNP modulate interindividual susceptibility to *MGMT* methylation in cancer. Taken together all analyzed data, our study provides strong evidence that the T allele of a *MGMT* promoter-enhancer SNP (rs16906252) underlies the predisposition to methylate in the tumors of patients.

O^6-methylguanine-DNA methyltransferase (MGMT), a conserved protein in all organisms, is a candidate for tumor suppression that repairs adducts at O^6 of guanine in DNA [27, 28]. *MGMT* promoter methylation was demonstrated to result in decreased MGMT expression and correlates with a survival benefit in glioblastoma patients treated with therapeutic alkylating agents [29]. Hypermethylation of the MGMT CpG island is the cause of *MGMT* transcriptional silencing in the tumor suppressor p53 and the oncogene K-ras. Interindividual variability in MGMT promoter methylation can significantly influence individual response to environmental and alkylating chemotherapeutics such as temozolomide [30].

The mechanism by which the T allele of the rs16906252 *MGMT* promoter SNP influences MGMT promoter methylation is currently unknown. The SNP rs16906252 may decrease the affinity of the DNA for certain transcription factors, which was significantly associated with lower transcription levels [31]. Functional studies have suggested that MGMT promoter rs16906252 SNP genotyping may contribute to increased CpG island methylation by recruiting DNA methyltransferases [16, 32]. We cannot exclude a possibility that rs16906252 is in perfect LD with other polymorphisms in this area which may cause *MGMT* promoter methylation. Studies have identified that deletion of the 59-bp enhancer element, within which this SNP is located, reduces MGMT transcriptional activity by 95% [17]. Promoter reporter assays performed in lung cancer cell lines have shown the haplotype carrying the T allele has a 20-41% reduction in promoter activity correlated with lower *MGMT* expression [18]. With the wild-type haplotype as the reference, a 30% reduction in normalized promoter reporter activity was observed in MGMT methylated glioblastoma cell lines transfected with the haplotype carrying the T allele of rs16906252 [19].

Stratified analysis by cancer type indicated that the association became more prominent among glioblastoma, colorectal cancer and other cancer. It cannot be excluded that the effect of the T-allele on MGMT methylation propensity is cell-type specific.

Some limitations of the present meta-analysis should be taken into consideration when interpreting the results. The first limitation was that

the small number of studies was short of sufficient statistical power to assess the correlations between MGMT rs16906252 *polymorphism and* MGMT promoter methylation in cancer. Further large scale multicenter studies were needed to gain a more reliable. Second, our meta-analysis did not consider gene-gene and gene-environment interactions due to the lack of sufficient data. Third, there are only one ethnicity groups (Caucasian), which may limit the general application of our results.

In summary, our results suggest that the germline C>T genotype represents a strong determinant of *MGMT* methylation in cancer. The mechanism of this effect of a promoter SNP on CpG island methylation needs to be elucidated by additional studies. More larger and well-designed studies with more ethnic groups are needed to validate the risk identified in our meta-analysis.

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Disclosure of conflict of interest

None.

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