Accepted Manuscript

Gene promoter methylation and cancer: An umbrella review

Emmanouil Bouras, Meropi Karakioulaki, Konstantinos I. Bougioukas, Michalis Aivaliotis, Giorgos Tzimagjorgis, Michael Chourdakis

PII: S0378-1119(19)30583-9
DOI: https://doi.org/10.1016/j.gene.2019.06.023
Reference: GENE 43933
To appear in: Gene
Received date: 28 February 2019
Revised date: 5 June 2019
Accepted date: 11 June 2019

Please cite this article as: E. Bouras, M. Karakioulaki, K.I. Bougioukas, et al., Gene promoter methylation and cancer: An umbrella review, Gene, https://doi.org/10.1016/j.gene.2019.06.023

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Gene promoter methylation and cancer: an umbrella review

Emmanouil Bouras\textsuperscript{a}, Meropi Karakioulaki\textsuperscript{a}, Konstantinos I. Bougioukas\textsuperscript{a}, Michalis Aivaliotis\textsuperscript{b}, Giorgos Tzimagiorgis\textsuperscript{b}, Michael Chourdakis\textsuperscript{a}

\textsuperscript{a} Laboratory of Hygiene, Social & Preventive Medicine and Medical Statistics, Department of Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Greece

\textsuperscript{b} Laboratory of Biochemistry, Department of Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Greece

**Corresponding author:** Michael Chourdakis, Department of Medicine, School of Health Sciences, Aristotle University, University Campus, 54124, Thessaloniki, Greece; Phone: +30 2310 999035, Fax: +30 2312 205270; Email: mhourd@gapps.auth.gr

* shared first authorship

**Running title:** Gene promoter methylation and Cancer: An umbrella Review
Abstract

Gene promoter methylation is a common epigenetic event, taking place in the early phase of tumorigenesis, which has a great potential as a diagnostic and prognostic cancer biomarker. In this umbrella review, we provide an overview on the association between gene-promoter methylation of protein-coding genes and cancer risk based on currently available meta-analyses data on gene promoter methylation. We searched MEDLINE via PubMed and the Cochrane Database of Systematic Reviews for meta-analyses that examine the association between gene-promoter methylation and cancer, published until January 2019 in English. We used AMSTAR to assess the quality of the included studies and applied a set of pre-specified criteria to evaluate the magnitude of each association. We provide a comprehensive overview of 80 unique combinations between 22 different genes and 18 cancer outcomes, all of which indicated a positive association between promoter hypermethylation and cancer. In total, the 70 meta-analyses produced significant results under a random-effects model with odds ratios that ranged from 1.94 to 26.60, with the summary effect being in favor of the unmethylated group in all cases. Three of the strong evidence associations involve RASSF1 methylation on bladder cancer risk (OR=18.46;95% CI:12.69-26.85;I²=0%), MGMT methylation on NSCLC (OR=4.25;95%CI:2.83-6.38; I²=22.4%) and RARB methylation on prostate cancer (OR=6.87;95%CI:4.68-10.08; I²=0%). Meta-analyses showed a moderate quality, AMSTAR score ranging from 4 to 9 (Mdn=8;IQR: 7.0to8.0). As primary studies and meta-analyses on the subject accumulate, more genetic loci may be found to be highly associated with specific cancer types and hence the biomarker sets will become wider.
Keywords: gene promoter; methylation; tumor suppressor genes; epigenetics; cancer; umbrella review

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
Background

DNA methylation is an epigenetic mechanism that modifies a cytosine base at the 5’-C-phosphate-G-3’ (CpG) nucleotide residues with the addition of a methyl group [1]. Vertebrate CpG islands are short interspersed DNA sequences that are rich in guanine and cytosine (GC) and are predominantly non-methylated [2,3]. They are a region with at least 200 bp, a GC percentage greater than 50% and an observed-to-expected CpG ratio greater than 60% [2,3]. Most of the CpG islands are sites of transcription initiation and are present in 70% of all mammalian promoters [2, 3]. Next Generation Sequencing (NGS) platforms have now provided genome-wide maps of CpG methylation and these maps have confirmed that 5-10% of normally unmethylated CpG promoter islands might become abnormally methylated in various cancer genomes [1]. Despite the fact, a global hypermethylation can be observed in malignant cells, the most extensively studied epigenetic alterations in cancer are the methylation changes that occur within CpG islands of the gene promoters [3, 4]. Gene promoter methylation is being catalyzed by specific enzymes, called DNA methyltransferases (DNMTs) and can be involved in the transcriptional silencing of various tumour suppressor genes, which is a common epigenetic event in the early phase of tumorigenesis [5].

Recently, the use of specific gene promoter methylation status as biomarker to detect cancer has attracted much attention, as it offers advantages over biopsy-related immunohistochemistry techniques. A plethora of meta-analyses studies have been published that examine the association between gene promoter methylation and cancer outcomes with diverse methodological quality and reporting completeness. The aim of this umbrella review is to examine the breadth and the validity of the currently available meta-analyses on the association between gene promoter
methylation and risk of cancer and provide a classification of each unique association in terms of magnitude and absence of bias [6, 7].

Materials and methods

Eligibility criteria

Eligible for inclusion were meta-analyses (MA) that examined the association between gene promoter methylation status and malignancies in human subjects. No restrictions were made regarding population characteristics, such as age, gender, ethnicity or setting. Studies that examined survival or recurrence, pharmacogenetic studies, narrative reviews and also mixed type of studies were excluded. Studies that pooled classification function measures, such as sensitivity and specificity were also excluded.

Information sources and search strategy

Two reviewers independently searched the Cochrane Database of Systematic Reviews (CDSR) (via Cochrane Library) and MEDLINE via PubMed for eligible meta-analyses published in English from inception to January 5th 2019 using free text terms and controlled vocabulary specific for methylation and cancer (Supplementary Table S1).

Selection process

Title, abstract and full text screening was performed by two reviewers independently (EB and MK) by use of Endnote v. X7. Discrepancies were resolved by a third reviewer (MC). In case more than one meta-analysis was identified on a specific combination of gene promoter and cancer outcome, the most recent or most
complete study (the one including the most overview-related information) was selected for the analyses.

Data collection process and data items

Data were extracted by one reviewer (MK) using a predefined data extraction form and verified by another reviewer (EB). Each unique meta-analysis item that was extracted included: methylation status among cases and controls in each primary study, number of primary studies, total number of cases and controls, ethnicity, age and gender of the participants. Furthermore, search-specific information that authors used, such as the date of last search, databases, language restriction posed were extracted along with information regarding quality assessment of the primary studies and presence of matching.

Statistical analysis

For each unique gene-cancer combination we estimated the odds ratios (OR) along with 95% confidence intervals (CI) and prediction intervals (PI) under a random effects (RE) model. We used inconsistency index ($I^2$) to quantify heterogeneity. We tested for small study effect using a rank correlation asymmetry test [8]. Summary statistics were presented as median (Mdn) and interquartile range (IQR). All statistical analyses were performed in R v 3.5.3.

Quality assessment

We further classified each unique association as Class I (strong evidence), Class II (highly suggestive evidence), Class III (suggestive evidence), Class IV (nominal associations) and Class V (non-significant), with regard to potential hints of bias, as shown in Table 1. Lastly, all studies that were included in our analysis were independently evaluated by two reviewers (EB and MK) using AMSTAR [9].
Results

From the literature search, 571 unique records were identified and after title and abstract screening, 274 studies were evaluated in full text. In total, 147 meta-analyses were deemed eligible for inclusion, of which we selected the most recent or complete meta-analyses for each unique combination of gene promoter and cancer outcome (Figure 1). Finally, 80 publications including a total of 1,323 primary studies involving approximately 85,337 cases and 58,084 controls that examined the association between the promoter methylation status of 22 different genes and cancer on 18 major sites (i.e. Bladder; Breast; Cervical; Colorectal; Endometrial; Esophageal; Gastric; Hepatocellular; HNSCC; Lung; Melanoma; Multiple myeloma; Ovarian; Pancreatic; Prostate; Renal; Testicular; Thyroid). From the total number of meta-analyses, 10 gene-cancer studies did not provide sufficient data to perform our meta-analytical evaluation. Hence these outcomes were not included in the quantitative analysis but only presented in a descriptive manner (Supplementary Table S2).

The majority of the meta-analyses were on lung cancer (16/80; 20%) [10-25] followed by breast cancer (12/80; 15%) [26-37] and gastric cancer (8/80; 10%) [38-45]. Seven meta-analyses were on colorectal cancer [46-52], five on hepatocellular cancer [53-57], four meta-analyses were on bladder [58-61], ovarian [62-65], prostate [66-69] and esophageal [70-73], three on cervical [74-76], two on endometrial [77, 78], renal [79, 80], thyroid [81, 82], multiple myeloma [83, 84] and head and neck squamous cell carcinoma (HNSCC) [85, 86] and one on melanoma [87], pancreatic cancer [88] and testicular [89]. The genes that were studied the most were the Ras association domain family member 1 coding gene (RASSF1, old gene name:
RASSF1A) and the cyclin-dependent kinase inhibitor 2A coding gene (CDKN2A), analyzed in 13/80 (16%) of meta-analyses, followed by the Cadherin (CDH) coding gene analyzed, in 11/80 (14%) meta-analyses. Other genes commonly analyzed were: O-6-Methylguanine-DNA methyltransferase (MGMT), runt related transcription factor 3 (RUNX3), death-associated protein kinase gene (DAPK), human mutL homolog 1 (hMLH1), phosphatase and tensin homolog (PTEN) and glutathione S-transferase Pi 1 (GSTPi), Fragile histidine triad protein (FHIT), Secreted frizzled-related protein 1 (SFRP1), vimentin (VIM), Metalloproteinase inhibitor 3 (TIMP3), E3 ubiquitin-protein ligase (CHFR), Retinoic acid receptor beta (RARβ) and von Hippel–Lindau tumor suppressor (VHL).

Study characteristics are shown in supplementary Table S3. Among the 80 meta-analyses, the number of primary studies ranged from 4 to 91 (Mdn=13; IQR:10.75 to 17.25), the number of cases ranged from 112 to 4654 (Mdn=771; IQR:556 to 1252.25) and the number of controls ranged from 72 to 5175 (Mdn=518.5; IQR: 291 to 814.25). More than half of the meta-analyses (44/80; 55%) presented information regarding the gender distribution of the participants and a quarter of the meta-analyses included information regarding the age (25/80; 25%). Quality assessment of the included primary studies was reported in the minority of the meta-analyses (16/80; 20%), most of which (14/20) used the Newcastle-Ottawa Scale (NOS) for that purpose. In most meta-analyses (56/80; 70%) the reviewers reported that at least four databases had been included in their literature search strategy, and 49/80 (61%) were not restricted to English as a sole publication language, while Medline was the most commonly reported database (77/80; 96%) (Supplementary Table S4).
Quantitative analysis

The 70 meta-analyses included in the quantitative analysis produced significant results under a random effects model with odds ratios that ranged from 1.94 (CDKN2A hypermethylation on ovarian cancer risk) to 26.60 (RARB hypermethylation on prostate cancer risk). In all cases the summary effect size was larger than one indicating that methylation was associated with higher risk of cancer. However, when 95% prediction intervals were calculated, only 44/70 (63%) of the MA retained a significant effect (the null value was not included in the 95% PI). With regard to the small study effects, asymmetry (p < 0.1) was evident in 12 meta-analysis as shown in Figure 2. Heterogeneity ranged from 0, which was found in 11 meta-analyses, to 88.5% (Mdn: 51.8; IQR: 19.8 to 69.9).

Grading the evidence

Three of the associations claimed strong evidence (Figure 2). In particular RASSF1 methylation on bladder cancer risk (OR=18.46; 95% CI:12.69-26.85; I²=0%), MGMT methylation on NSCLC (OR=4.25; 95%CI: 2.83-6.38; I²=22.4%) and RARB methylation on prostate cancer (OR=6.87; 95% CI: 4.68-10.08; I²=0%). Highly suggestive evidence (Class II) was found on two meta-analyses: CDH13 on bladder cancer risk (OR=15.77; 95% CI:7.00-35.57; I²=0%) and RASSF1 on ovarian cancer risk (OR=8.42; 95% CI:5.19-13.65; I²=13.8%). Suggestive evidence (Class III) was found for 27 meta-analyses and the remaining 38 were characterized as weak, according to the predefined criteria that we posed.

Quality of the included studies
Methodological quality assessment of the studies included in our sample (n=80) with AMSTAR (Figure 3) showed that an overall moderate quality, ranging from 4 to 9 (Mdn=8; IQR: 7.0 to 8.0) and more than half of the studies (46/80; 57.5%) met the criteria for at least 8/11 domains. Nearly no study (79/80) provided information of a registered protocol nor included grey literature in their search strategy. The majority of the studies 64/80 (80%) did not assess the scientific quality of the included studies.

Discussion

In this umbrella review we provide a comprehensive overview of 80 unique combinations between 22 different protein-coding genes and 18 cancer outcomes all of which showed a positive association between promoter hypermethylation and cancer. Using a defined set of criteria we classified each unique association in terms of magnitude and absence of common bias. Furthermore, having assessed the quality of the included studies we describe key points of methodological frailty.

Aberrantly methylated genes can be used as molecular targets for the detection of neoplastic cells in body fluids and small quantities of tissue providing a minimally invasive early diagnosis of cancer [5]. Hypermethylation of individual genetic loci was found to be closely associated with more than one type of cancers, indicating that no single hypermethylated gene can be used as a cancer-specific biomarker. For instance, RASSF1 promoter hypermethylation was associated with several cancer outcomes such as bladder, endometrial, esophageal squamous cell carcinoma, gastric, HNSCC, lung, melanoma, ovarian, prostate, renal, testicular and thyroid. However, in a literature analysis, Strmsek and Kunej collected data from 150 studies and demonstrated that in 36 cancer types, 180 miRNA genes were regulated via DNA
methylation and 54.4% of them were found to be specific for a certain type of cancer [90]. That is to say, miRNA gene methylation appears to represent a more cancer-specific biomarker potential than protein-coding gene methylation.

Additionally, in our study, hypermethylation of multiple genes was found to be associated with a specific type of cancer, indicating that a number of particular genes may be used to provide diagnosis for a certain cancer type. For instance, bladder cancer patients were presented with a hypermethylated state of RASSF1 and CDH1, which were supported with highly suggestive or strong evidence (Class I or II) and also DAPK and CDKN2A. In a similar fashion, breast cancer patients were presented with a hypermethylated state of RARB, PTEN, BRCA and APC while NSCLC patients were presented with a hypermethylated state of MGMT, FHIT and hMLH1. Limited evidence supports that hypermethylation of defined sets of genes is associated with a specific type of cancer [91-93]. For instance, it was found that CDKN2A, RUNX3, MGMT, and DAPK may play an important role in the pathogenesis of gastric precancerous lesions, the latter three of which were supported by our analyses’ results [94]. As primary studies and meta-analyses on the subject accumulate, more genetic loci may be found to be highly associated with specific cancer types and hence the number of the biomarker sets will be wider.

Three associations claimed strong evidence namely RASSF1 methylation on bladder cancer, MGMT methylation on NSCLC and RARB methylation on prostate cancer risk. RASSF1 has been characterized as a tumor suppressor gene as it has shown the potential to inhibit cell proliferation, control cell cycle and promote cell apoptosis. Its inactivation due to methylation of the promoter region, has been associated with an increased risk of a number of different types of cancer, such as small cell lung cancer, breast cancer, gastric, prostate cancer, renal cell carcinoma,
and nasopharyngeal carcinoma [61]. The MGMT protein, has demonstrated DNA repair capacity as it can protect against DNA adduct formation of carcinogens. Methylation of *MGMT* gene promoter has been associated with loss or decrease of *MGMT* expression in tumor tissues of various cancers, including lung, gastric and esophageal tumors [10, 44, 95]. The *RAR* has been shown to function as a tumor suppressor gene and inactivation through its promoter hypermethylation has been implicated in lung, breast, and prostate cancer [26, 66].

The majority of the meta-analyses results were based on populations with a mixed ethnic background, indicating that they can be possibly extrapolated to the general population, although it should be noted that we did not investigate for potentially differential estimates in specific ethnic subgroups. Additionally, most meta-analyses were based on primary studies that reported using samples for the biochemical analyses of both blood and various tissues, limiting our ability to support the use of hypermethylation status as a blood-derived biomarker.

The associations that claimed strong evidence (*MGMT* methylation on NSCLC; *RASSF1* on bladder cancer and *RAR* on prostate cancer risk) were based on studies that presented with moderate methodological quality according to the evaluation with AMSTAR, a fact which in part limits the credibility of the findings, indicating that they should be interpreted with caution. A moderate quality is reflected in the majority of the included in our analysis studies, which poses a limitation to our umbrella review. Furthermore, a qualitative assessment of component studies was only reported in the minority of the original meta-analyses (20%).

Another limitation is the moderate to high heterogeneity (≥50%) that was observed for approximately half of the meta-analyses. Differences in population characteristics, such as racial composition, gender proportion and age distribution
along with methodological variability in test methods, and primers used may have introduced a considerable amount of heterogeneity in meta-analytical results. It was common to report multiple different methods to quantify methylation in the primary studies with variant performance, or diverse sources of genetic material, within a single meta-analysis [96]. Additionally, although it has been shown that methylation status can be affected by external factors, such as diet or other exposures, in the vast majority of the studies such parameters were not taken into account [97]. Furthermore, in many occasions within the same meta-analysis, cases had been considered at various stages in terms of cancer progression, which may also have introduced heterogeneity in the summary effects. We did not perform subgroup analysis involving only primary studies that used high quality methodological standards as it would limit the number of participants and hence the power of our analysis.

Using evidence that comes from the highest in hierarchy level, we provide a wide panel of methylation markers related to cancer outcomes, analyzed through the prism of rigor and quality assessment. Through our analysis, suggestive evidence supports the use of promoter methylation status for different sets of genes in cancer diagnosis. Observed patterns should be validated in the clinical setting and their diagnostic performance should be tested before conclusion can be drawn.

**Statement of authorship**

EB and MC conceived and designed the study. MK and KB were involved in the study selection process. Quality assessment of the studies was performed by MK and KB. EB and MK wrote the manuscript, while KB and EB performed the statistical analyses. GT and MA contributed in the interpretation of the data and the final pre-
submission revisions of the manuscript. MC supervised all procedures. All authors participated in the interpretation of data, revision of the manuscript for important intellectual content and agreed to be accountable for all aspects of the work. The manuscript has been read and its submission has been approved by all co–authors.

Conflict of interest

The authors declare that they have no conflicts of interest.

Funding sources

The authors received no funding for this work.

Acknowledgements

We would like to express our gratitude to Maria Grammatikopoulou for her valuable assistance in editing the manuscript tables and figures.
References


97. Singh, S.M., R.L. Murphy B Fau - O'Reilly, and R.L. O'Reilly, Involvement of gene-diet/drug interaction in DNA methylation and its contribution to complex diseases: from cancer to schizophrenia. (0009-9163 (Print)).
**Figure 1.** Flow chart of study selection. The diagram includes article identification, screening, eligibility, and final inclusion in the analysis.
Figure 2. Classification of the evidence on gene promoter methylation and cancer. Each line in the forest plot corresponds to a unique Meta-Analysis (a unique association) of Gene-Cancer. Odds ratio values above "one" indicate that there is an increased cancer risk when the promoter is hypermethylated. For each MA the total number of cases in the component studies, the amount of heterogeneity ($I^2$), the 95% Prediction Intervals (PI), the small study effects (p-value) are presented in the forest plot. Each association was evaluated according to the aforementioned criteria and the classification is shown at the far right of the plot.

**Abbreviations:** RE: Random effects model; OR: Odds ratio; CI: Confidence intervals; PI: Prediction intervals; MA: Meta-Analysis; Class: Classification; NSCLC: Non-small-cell lung cancer; ESCC: Esophageal squamous cell carcinoma; HNSCC: Head and neck squamous cell carcinoma; APC: Adenomatous polyposis coli; BRCA1: BRCA1 DNA repair associated; CDH: Major cadherins; CDH1: cadherin 1; CDH13: cadherin 13; CDKN2A: cyclin-dependent kinase Inhibitor 2A; CDKN2B: cyclin-
dependent kinase Inhibitor 2B; DAPK: Death-associated protein kinases; DAPK1: Death-associated protein kinase 1; FHit: fragile histidine triad diadenosine triphosphatase; GSTP1: glutathione S-transferase pi 1; MGMT: methylguanine-DNA-methyltransferase; MLH1: mutL homolog 1; PTEN: Phosphatase and tensin homolog; RARb: Retinoic acid receptor beta; RASSF1: Ras association domain family member 1; RUNX3: RUNX family transcription factor 3; SOCS1: suppressor of cytokine signaling 1; VHL: von Hippel-Lindau tumor suppressor; WIF1: WNT inhibitory factor 1
Figure 3. Quality assessment of the included studies with A.M.S.T.A.R. The graph is showing a summary (n=80 studies) of the evaluation on each of the A.M.S.T.A.R domains. The part of each domain that acquired a positive answer is highlighted and the pertinent frequency is presented inside the bars.
Table 1. Criteria used for the classification of the evidence.

<table>
<thead>
<tr>
<th>Class</th>
<th>Criteria</th>
</tr>
</thead>
</table>
| I (strong evidence) | More than 1,000 cases  
MA P-value < $10^{-9}$  
Non-significant small study effect (P-value > 0.1)  
Non-significant heterogeneity ($I^2 < 40\%$)  
95% PI not including the null. |
| II (highly suggestive evidence) | More than 750 cases  
MA P-value < $10^{-6}$  
Non-significant small study effect (P-value > 0.1)  
Non-significant heterogeneity ($I^2 < 40\%$) |
| III (suggestive evidence) | More than 500 cases  
MA P-value < $10^{-3}$,  
Non-significant small study effect (P-value > 0.1) |
| IV (weak evidence) | MA P-value < 0.05 |
| V (non-significant) | MA P-value > 0.05 |

MA: Meta-Analysis; PI: Prediction Intervals
Abbreviations

FE: Fixed effects model; RE: Random effects model; OR: Odds ratio; Mdn: Median;
IQR: Interquartile range; CI: Confidence intervals; PI: Prediction intervals;
BRISQ: Biospecimen Reporting for Improved Study Quality; CASP: Critical
Appraisal Skills Program; CBM: Chinese Biomedical Database; CDC: Centers for
Disease Control; CINAHL: Cumulative Index to Nursing and Allied Health
Literature; CN: Chinese; CNKI: China National Knowledge Infrastructure; EBSCO:
Elton B. Stephens Co.; ELCWP: European Lung Cancer Working Party; EN: English;
NOS: Newcastle-Ottawa Scale; n/r: not reported; REMARK: REporting
recommendations for tumour MARKer prognostic studies; SCI: Science Citation
Index; WoS: Web of Science; ER: Estrogen receptor; GC: Gastric cancer; ESCC:
esophageal squamous-cell carcinomas; HNSCC: Head and Neck squamous cell
carcinoma; NSCLC: Non-Small Cell Lung Cancer; n/r: not reported; PR: Progesteron
Receptor; APC: Adenomatous polyposis coli; BRCA1: BRCA1 DNA repair
associated; CDH: Major cadherins; CDH1: cadherin 1; CDH13: cadherin 13;
CDKN2A: cyclin-dependent kinase Inhibitor 2A; CDKN2B: cyclin-dependent kinase
Inhibitor 2B; CIN: Cervical intraepithelial neoplasia; CHFR: checkpoint with
forkhead and ring finger domains; DAPK: Death-associated protein kinases; DAPK1:
Death-associated protein kinase 1; FHIT: fragile histidine triad diadenosine
triphosphatase; GSTP1: glutathione S-transferase pi 1; SCGB3A1: Secretoglobin
family 3A member 1; MLHI: mutL homolog 1; MGMT: methylguanine-DNA-
methyltransferase; PTEN: Phosphatase and tensin homolog; RARB: Retinoic acid
receptor beta; RASSF1: Ras association domain family member 1; RUNX3: RUNX
family transcription factor 3; SRFPI: Secreted Frizzled Related Protein 1; VIM:
Vimentin. *VHL*: von Hippel-Lindau tumor suppressor; *WIF1*: WNT inhibitory factor 1; *TIMP3*: TIMP Metalloproteinase inhibitor 3; *SOCS1*: Suppressor of cytokine signaling 1
Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Highlights

- Overview of 80 unique combinations between 22 genes and 18 cancer outcomes.
- All showed a positive association between promoter hypermethylation and cancer.
- Strong evidence linked methylation of *MGMT, RASSF1* and *RARβ* to various cancers.
- Quality of studies was moderate, AMSTAR ranged from 4 to 9 (Mdn=8; IQR: 7 to 8)