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Review Article

# Potential of *RASSF1A* promoter methylation as biomarker for endometrial cancer: A systematic review and meta-analysis

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## HIGHLIGHTS

- Association between *RASSF1A* methylation and endometrial cancer risk was significant.
- Subgroup outcomes by ethnicity confirmed the overall pooled effects.
- Heterogeneity was erased with outlier treatment.
- Endometrial cancer risk was highlighted by consistency, significance and robustness.
- We conclude that *RASSF1A* has good biomarker potential for endometrial cancer.

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## ABSTRACT

*Background.* An epigenetic approach to explaining endometrial carcinogenesis necessitates good understanding of *Ras association domain family 1 isoform A* (*RASSF1A*) promoter methylation data from primary studies.

*Aims.* Differential magnitude of reported associations between *RASSF1A* promoter methylation and endometrial cancer (EC) prompted a meta-analysis to obtain more precise estimates.

*Methods.* Literature search yielded eight included articles. We calculated pooled odds ratios (OR) and 95% confidence intervals and subgrouped the data by race. Sources of heterogeneity were investigated with outlier analysis.

*Results.* The pooled ORs indicated increased risk, mostly significant. The overall effect (OR 11.46) was reflected in the European outcome (OR 15.07). However, both findings were heterogeneous ($I^2$ = 57-70%) which when subjected to outlier treatment, erased heterogeneity ($I^2$ = 0%) and retained significance (OR 9.85-12.66). Significance of these pre- and post-outlier outcomes were pegged at $P \leq 0.0001$. Only the Asian pre-outlier (OR 6.85) and heterogeneous ($I^2$ = 82%) outcome was not significant ($P = 0.12$) but when subjected to outlier treatment, erased heterogeneity ($I^2$ = 0%) and generated significance (OR 23.74, $P \leq 0.0001$).

*Conclusions.* Consistent increased risk associations underpinned by significance and robustness render *RASSF1A* with good biomarker potential for EC.



**Contents**



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## 1. Introduction

Endometrial cancer (EC) is the seventh most common cancer in women worldwide and increasing incidence [1] warrants better understanding of the contribution of molecular events in its carcinogenesis. As a gynecological malignancy, EC has multifactorial features driven by abnormal genetic and epigenetic alterations, as well as environmental factors [2]. Because, variations in gene expression and mutation or deletion of cancer-related genes may not fully explain carcinogenesis of the endometrium, epigenetic changes in gene expression through effects on chromatin without DNA mutation are drawing attention [3]. An epigenetic change is aberrant promoter methylation, which has been found to be an early and widespread alteration in endometrial tumorigenesis [4]. *RASSF1A* (*Ras association domain family 1 isoform A*) is one of the tumor suppressor genes whose promoter is frequently found to be inactivated by methylation in EC [4,5]. This epigenetic silencing of *RASSF1A* gene suggests that it might be implicated in the pathogenesis of EC. The human *RASSF1A* gene, which is located on chromosome 3p21.3, contains eight exons and generates seven transcripts, designated as *RASSF1A* to *G* [6]. Aberrant methylation in the *RASSF1A* promoter has been identified as the main cause of the inactivation of *RASSF1A* expression, thereby contributing to malignant transformation from early benign tumor to later invasive carcinoma [7]. A multi-gene hypermethylation study [8] showed that *RASSF1A* was a significant indicator of EC with high sensitivity (100%) and specificity (97.2%). Therefore, *RASSF1A* promoter methylation might be correlated with the development and progression of EC [4,9]. DNA methylation associated with particular genes is one of the earliest detectable changes and may even precede tumor formation with the potential to predict the condition [6,10]. Given the screening potential of *RASSF1A* promoter methylation, it is an attractive biomarker for early cancer detection which, for most cancers, results in improved clinical outcome [11]. We undertake this study for a number of reasons; (i) while *RASSF1A* inactivation by promoter methylation is known to perform important functions in tumorigenesis, its specific action in EC has neither been thoroughly investigated nor reviewed. (ii) primary study outcomes of *RASSF1A* promoter methylation on EC have differed in magnitude making this feature suitable for meta-analysis; (iii) primary studies have also been methodologically inconsistent, warranting a meta-analysis to obtain more precise estimates.

## 2. Materials and methods

### 2.1. Literature search and article selection

Using the terms,"*RASSF1A*", "methylation", "uterine" and "endometrial cancer" without language restriction, we searched MEDLINE using PubMed, ScienceDirect and Google Scholar for publications as of March 25, 2017. References cited in the retrieved publications were screened manually to identify additional eligible articles. Inclusion criteria included articles that presented *RASSF1A* data indicating methylation status.

### 2.2. Data extraction

Two investigators (NP and AK) independently extracted data and reached consensus on all the items. For each eligible study, the following information was extracted: the first author's name, publication year, country, race, method of methylation detection, source of control samples, number of methylation in tumor and controls. For control values that were unavailable, we contacted the corresponding authors of the primary studies for more detailed information. Nonmalignant endometrial tissues were defined as controls, which included normal tissues from healthy subjects with benign conditions or adjacent normal tissues from the same cancer patients which were histologically confirmed as cancer-negative endometrial samples.

### 2.3. Quality assessment of the studies

The Newcastle–Ottawa Scale (NOS) assessment [12] was used to assess methodological quality of the included studies. These studies were judged based on three broad perspectives: selection, comparability, and exposure. The star rating system has scores ranging from zero (worst) to 9 (best). Scores of 5–6 and ≥7 stars indicate moderate and high quality, respectively.

### 2.4. Meta-analysis protocol

Using Review Manager 5.3 (Copenhagen: Nordic Cochrane Centre, Cochrane Collaboration, 2014), pooled odds ratios (OR) and 95% confidence interval (CI) were calculated to evaluate the association between *RASSF1A* promoter methylation and EC risk. Where methylation values were zero, we applied the Laplace correction by adding a pseudocount of one to all values of the data set [13] prior to generating the forest plots. From methylation data in tumors compared to those in normal tissues, OR estimates were interpreted from the fulcrum of 1 (null association) where less and more than this number indicate reduced and increased associations, respectively. Pooled estimates were obtained using either the fixed [14] (absence of heterogeneity) or random [15] (in its presence) effects models. Heterogeneity between studies was estimated using the $\chi^2$-based Q test [16]. Recognizing the low power of this test [17], significance threshold was set at $P = 0.10$. Sources of heterogeneity were identified with outlier analysis using the Galbraith plot method [18]. Heterogeneity was quantified with the $I^2$ statistic which measures the degree of inconsistency among studies [19]. Pooled estimates were subjected to sensitivity analysis which involved omitting one study at a time followed by recalculation to test for robustness of the summary effects. Subgroup analysis was based on ethnicity where we examined East Asians and Europeans. Publication bias was not investigated because of the low sensitivity of the qualitative and quantitative tests when the number of studies is lower than ten [20]. All *P* values were two-sided with significance set at ≤0.05 except in heterogeneity estimation.

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