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# Prognostic role of methylated *GSTP1*, *p16*, *ESR1* and *PITX2* in patients with breast cancer

## A systematic meta-analysis under the guideline of PRISMA

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**Abstract**

**Background:** *BRCA1* and *RASSF1A* promoter methylation has been reported to be correlated with a worse survival in patients with breast cancer. However, the prognostic values of *GSTP1*, *p16*, *ESR1*, and *PITX2* promoter methylation in breast cancer remain to be determined. Here, we performed this study to evaluate the prognostic significance of *GSTP1*, *p16*, *ESR1,* and *PITX2* promoter methylation in breast cancer.

**Methods:** A range of online databases was systematically searched to identify available studies based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guideline. The pooled hazard ratios (HRs) with their 95% confidence intervals (95% CIs) were applied to estimate the prognostic effect of *GSTP1*, *p16*, *ESR1,* and *PITX2* promoter methylation in breast cancer for multivariate regression analysis.

**Results:** 13 eligible articles involving 3915 patients with breast cancer were analyzed in this meta-analysis. In a large patient population, *GSTP1* showed a trend toward a worse prognosis in overall survival (OS) (HR = 1.64, 95% CI = 0.93–2.87, $P = .085$). *PITX2* promoter methylation was significantly correlated with a worse prognosis in OS (HR = 1.57, 95% CI = 1.15–2.14, $P = .004$), but no association between *p16* promoter methylation and OS (HR = 0.92, 95% CI = 0.31–2.71, $P = .884$). *PITX2* promoter methylation was significantly correlated with an unfavorable prognosis of patients with breast cancer in metastasis-free survival (MFS) (HR = 1.73, 95% CI = 1.33–2.26, $P < .001$). The result from 3 studies with 227 cases showed that *ESR1* promoter methylation was linked to a worse prognosis in OS (HR = 1.55, 95% CI = 1.06–2.28, $P = .025$).

**Conclusions:** Our findings suggest *ESR1* and *PITX2* promoter methylation may be correlated with a worse survival of patients with breast cancer (*ESR1*: OS, *PITX2*: OS and MFS). The clinical utility of aberrantly methylated *ESR1* and *PITX2* could be a promising factor for the prognosis of breast cancer.

**Abbreviations:** 95% CI = 95% confidence interval, *BRCA1* = breast cancer susceptibility gene 1, DFS = disease-free survival, *ESR1* = estrogen receptor-α, *GSTP1* = glutathione S-transferase P 1, HER2 = human epidermal growth factor receptor-2, HR = hazard ratio, MFS = metastasis-free survival, OS = overall survival, *p16* = cyclin-dependent kinase inhibitor 2A, *PITX2* = paired-like homeodomain transcription factor 2, *RARbeta2* = retinoic acid receptor beta 2, *RASSF1A* = Ras association domain family 1 isoform, RFS = relapse-free survival, TSG = tumor suppressor gene.

**Keywords:** breast cancer, metastasis-free survival, multivariate analysis, overall survival, promoter methylation

## 1. Introduction

Breast cancer is the most common malignant tumor and the leading cause of cancer-related deaths among women with human cancers.[1] Based on global cancer estimates, approximately 1,676,600 new cases were diagnosed with breast carcinoma, leading to an estimate of approximately 521,900 deaths around the world in 2012.[1] Despite improvements in the early detection and treatment of breast cancer, patients with distant stage breast cancer remain to have an unfavorable 5-year survival rate of 26%.[2] In routine clinical practice, several clinicopathological features are applied as strong prognostic factors in the assessment of patients with breast cancer, such as lymph node metastasis, histological grade, tumor size, so on.[3,4] However, the basic molecular mechanism of this disease has not been fully understood. Thus, more noninvasive factors should be investigated to better predict prognosis.

Numerous studies suggest that epigenetic alterations are found to be an early and common event in cancer.[5–8] DNA methylation, a reversible epigenetic change, plays a crucial role in the carcinogenesis, progression, and prognosis of various

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human malignant tumors.[9–12] Tumor suppressor genes (TSGs) have been indicated to be frequently methylated in the promoter regions in breast cancer.[13–15] Gene with aberrant promoter methylation is identified to be closely correlated with breast cancer development and progression.[16–18] Located on human chromosome 11q13, the glutathione S-transferase P 1 (*GSTP1*) gene, a tumor suppressor gene, involves in the prevention of development of malignant tumors upon exposure to various carcinogens or electrophilic compounds.[19,20] The human cyclin-dependent kinase inhibitor 2A (*p16*) gene is mapped to human chromosome 9p21 and is a key cyclin-dependent kinase inhibitor that plays an important role in the regulation of cell cycle.[21,22] Estrogen receptor-α (*ESR1*) mediates the biological action of estrogen and dysregulation of its expression is found to be strongly implicated in breast cancer development and progression.[23] The paired-like homeodomain transcription factor 2 (*PITX2*), a bicoid-related homeobox transcription factor, has been suggested to be associated with the regulation of pituitary-specific gene and normal embryonic development.[24–26] Promoter methylation of the *GSTP1*, *p16*, *ESR1,* and *PITX2* genes has been frequently reported in breast cancer.[27–30]

Previous studies have revealed that promoter methylation of *BRCA1* and *RASSF1A* is linked to a poor prognosis of breast cancer patients in OS and DFS.[31,32] There were some inconsistent and conflicting results on multivariate regression analysis of *GSTP1*, *p16*, *ESR1,* or *PITX2* promoter methylation for the prognosis of breast cancer. For example, *GSTP1* promoter methylation was not correlated with the prognosis of breast cancer in DFS.[33] A significant correlation was found between *GSTP1* promoter methylation and DFS in breast cancer.[34]*p16* promoter methylation was associated with the prognosis of breast cancer in OS,[35] whereas *p16* promoter methylation was not correlated with the prognosis of breast cancer in OS.[33]*PITX2* promoter methylation was correlated with a poor OS of breast cancer in tissue or blood samples.[36,37]*ESR1* promoter methylation showed a trend toward a poor prognosis of breast cancer in OS.[38] No significant correlation was reported between *ESR1* promoter methylation and OS in breast cancer.[33] Therefore, we performed this meta-analysis to summarize the prognostic significance of *GSTP1*, *p16*, *ESR1,* or *PITX2* promoter methylation in breast cancer for multivariate regression analysis.

## 2. Materials and methods

### 2.1. Publication search

A systematic search strategy of the relevant publications was conducted in the PubMed, Embase, EBSCO, and Cochrane Library databases up to January 19th, 2017. The following combinations of key words and search terms were applied: (breast OR mammary) AND (cancer OR tumor OR carcinoma OR neoplasm) AND (methylation OR epigenetic silencing OR epigenetic inactivation) AND (prognos* OR survival OR outcome). Additionally, we also carefully scanned the references of the included studies to get other additional eligible papers.

### 2.2. Selection criteria

The eligible papers were included in this meta-analysis if they satisfied the following selection criteria: (1) all patients were limited to breast cancer using the diagnostic criteria; (2) studies provided sufficient information regarding the clinical outcome of *GSTP1*, *p16*, *ESR1,* or *PITX2* promoter methylation in overall survival (OS), disease-free survival (DFS), relapse-free survival (RFS), or metastasis-free survival (MFS) for multivariate regression analysis; (3) hazard ratio (HR) with its 95% confidence interval (95% CI) was reported from the original paper. If the original data were not recorded, we calculated the presented data in the survival plots by the methods described by Tierney et al[39]; (4) articles published in English were included in this meta-analysis. Methylated genes were excluded as follows: (1) a meta-analysis published involving the survival analysis of gene methylation in breast cancer; (2) methylated genes with fewer than 3 studies in survival analysis.

### 2.3. Ethical review

The present study was not primary research involving human samples, but rather a secondary analysis of human subject data published in the public domain.

### 2.4. Data extraction

The following information was collected from the included studies: first author's surname, publication year, country, ethnic population, age, tumor stage, testing method of methylation, the frequency of promoter methylation, the number of patients, OS, DFS, RFS, and MFS for multivariate regression analysis.

### 2.5. Statistical analysis

The current meta-analysis was conducted with Stata 12.0 software (Stata Corporation, College Station, TX). The pooled HRs and their 95% CIs were calculated to evaluate the strength of association between methylated genes and the prognosis of patients with breast cancer in OS, DFS, RFS, and MFS using multivariate regression analysis. Heterogeneity of the eligible studies was estimated based on Cochran's Q test.[40] The random-effects model was used when there was obvious evidence of heterogeneity ($P \leq .1$); otherwise, the fix-effects model was determined in this meta-analysis ($P > .1$).[41,42]

## 3. Results

### 3.1. Characteristics of the eligible studies

Figure 1 shows the detailed procedure of the relevant literature. According to the above selection criteria, final 13 eligible articles

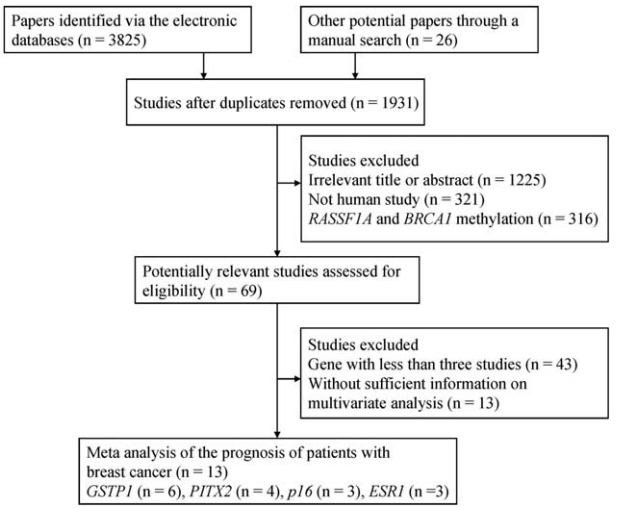


**Figure 1.** Flow chart of the study selection.

**Table 1**

**General characteristics of the eligible studies in this meta-analysis.**

| Gene | First author | Country | Ethnicity | Stage | Method | Sample | Breast cancer | | OS | DFS | RFS | MFS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | M | Total | MA-HR (95% CI) | MA-HR (95% CI) | MA-HR (95% CI) | MA-HR (95% CI) |
| *GSTP1* | | | | | | | | | | | | |
| | Arai 2006 | Japan | Asians | NA | MSP | Tissue | 13.79% | 174 | NA | NA | 2.711 (1.22–6.04) | NA |
| | Sharma 2009 | India | Caucasians | 1–3 | MSP | Tissue | 24.75% | 101 | 4.90 (1.10–21.88) | 1.76 (0.87–3.59) | NA | NA |
| | Sharma 2010 | India | Caucasians | 1–3 | MSP | Tissue | 25.00% | 100 | NA | 2.8 (1.1–7.1) | NA | NA |
| | Dejeux 2010 | France | Caucasians | 2–4 | PSQ | Tissue | NA | 163 | 7.52 (1.76–32.07) | NA | NA | NA |
| | Cho 2012 | USA | Mix | NA | MethyLight | Tissue | 27.84% | 765 | 1.43 (1.05–1.97) | NA | NA | NA |
| | Klajic 2013 | Norway | Caucasians | 1–4 | PSQ | Tissue | 17.18% | 206 | 0.935 (0.902–0.970) | NA | NA | NA |
| *PITX2* | | | | | | | | | | | | |
| | Nimmrich 2008 | Germany | Caucasians | NA | QMPCR | Tissue | 21.00% | 412 | 1.46 (1.05–2.01) | NA | NA | 1.74 (1.26–2.40) |
| | Harbeck 2008 | Germany | Caucasians | NA | QMPCR | Tissue | 47.87 | 399 | NA | NA | NA | 2.35 (1.20–4.60) |
| | Hartmann 2009 | Germany | Caucasians | NA | PCR | Tissue | NA | 241 | NA | NA | NA | 1.28 (1.03–3.83) |
| | Göbel 2011 | Austria | Caucasians | NA | Methylight | Blood | 13.90% | 428 | 3.4 (1.2–9.8) | NA | NA | NA |
| *p16* | | | | | | | | | | | | |
| | Sharma 2009 | India | Caucasians | 1–3 | MSP | Tissue | 50.50% | 101 | 0.66 (0.18–2.39) | 1.49 (0.73–3.05) | NA | NA |
| | Xu 2010 | USA | Mix | NA | MSP | Tissue | 3.63% | 800 | 2.09 (1.14–3.84) | NA | NA | NA |
| | Klajic 2013 | Norway | Caucasians | 1–4 | PSQ | Tissue | 4.68% | 206 | 0.432 (0.144–1.294) | NA | NA | NA |
| *ESR1* | | | | | | | | | | | | |
| | Widschwendter 2004 | Austria | Caucasians | 1–4 | MethyLight | Tissue | NA | 57 | 1.5 (1.0–2.4) | 1.5 (0.9–2.3) | NA | NA |
| | Sharma 2009 | India | Caucasians | 1–3 | MSP | Tissue | 64.36% | 101 | 0.68 (0.15–3.05) | 1.18 (0.55–2.56) | NA | NA |
| | Ramos 2010 | Brazil | Caucasians | 1–4 | MSP | Tissue | 40.58% | 69 | 2.575 (0.983–6.748) | NA | NA | 2.757 (1.020–7.449) |

95% CI = 95% confidence interval, DFS = disease-free survival, *ESR1* = estrogen receptor-α, *GSTP1* = glutathione S-transferase P 1, HR = hazard ratio, M = methylation, MA = multivariate regression analysis, MFS = metastasis-free survival, mix = mixed population, MSP = methylation-specific polymerase chain reaction, NA = not applicable, OS = overall survival, *p16* = cyclin-dependent kinase inhibitor 2A, PCR = polymerase chain reaction, *PITX2* = paired-like homeodomain transcription factor 2, PSQ = pyrosequencing, QMPCR = real-time polymerase chain reaction, RFS = relapse-free survival.

using multivariate regression analysis[30,33–38,43–48] were identified in the present meta-analysis, including 3915 patients with breast cancer. Of the included studies, 6 studies with 1509 breast cancer patients analyzed the prognostic role of *GSTP1* promoter methylation in OS, DFS, and RFS.[33,34,43–45,47] Four studies with 1480 breast cancer patients analyzed the prognostic value of *PITX2* promoter methylation in OS and MFS.[30,36,37,46] Three studies involving 1107 patients with breast cancer evaluated the correlation between *p16* promoter methylation and the prognosis in OS and DFS.[33,35,43] Three studies involving 227 patients with breast cancer assessed the association between *ESR1* promoter methylation and the prognosis in OS, DFS, and MFS.[33,38,48] The baseline characteristics of the eligible studies are listed in Table 1.

### 3.2. GSTP1 promoter methylation and the prognosis of patients with breast cancer

As depicted in Fig. 2, the result from 4 studies with 1235 breast cancer patients demonstrated that *GSTP1* promoter methylation had a trend toward a poor prognosis in OS (HR = 1.64, 95%

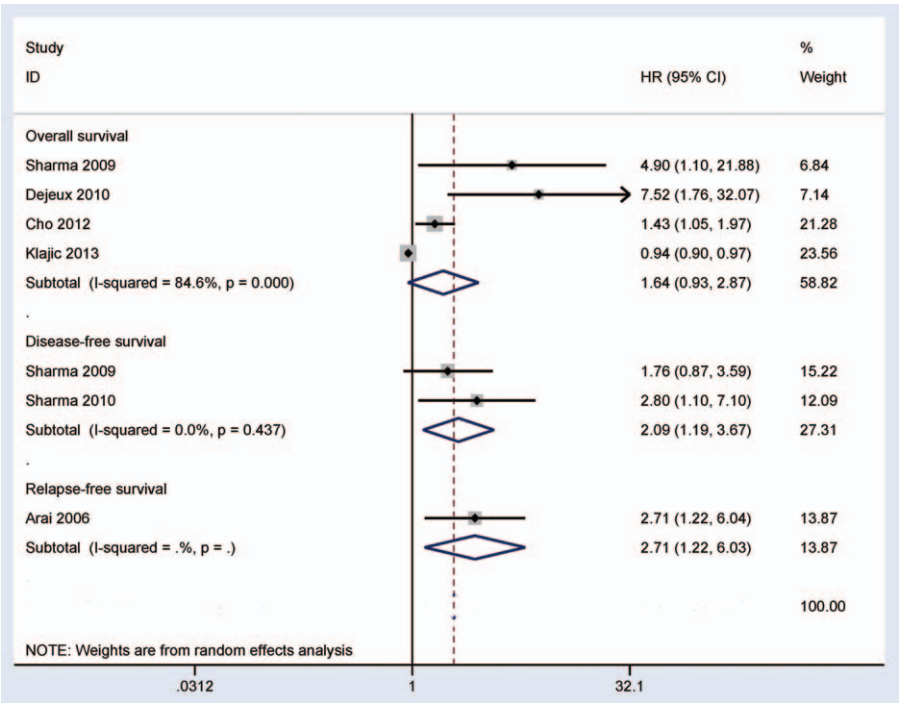


**Figure 2.** Forest plot of the association between *GSTP1* promoter methylation and the prognosis of patients with breast cancer in OS, DFS, and RFS for multivariate regression analysis. DFS = disease-free survival, *GSTP1* = glutathione S-transferase P 1, OS = overall survival, RFS = relapse-free survival.

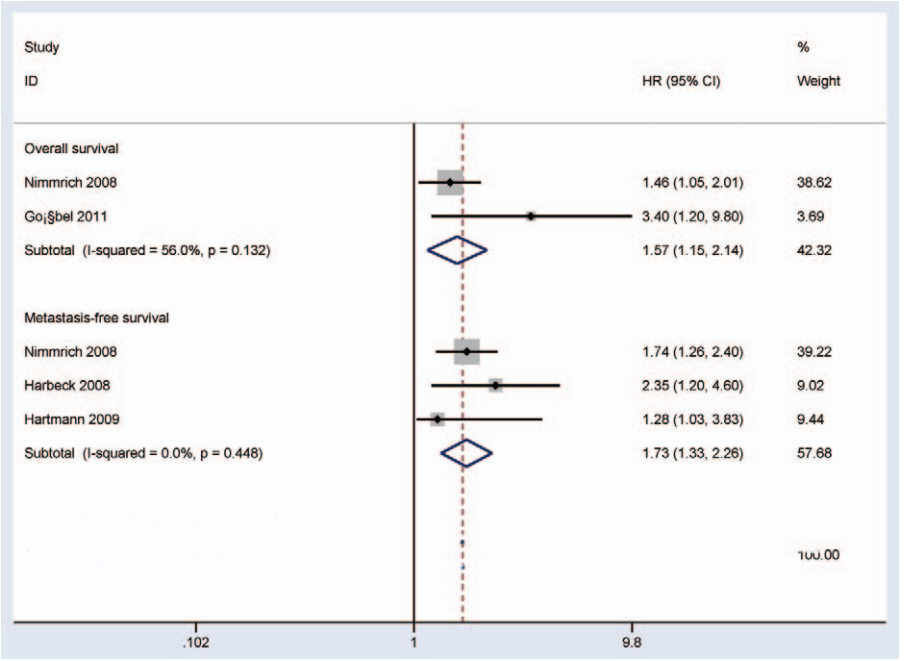


**Figure 3.** Forest plot of the association between *PITX2* promoter methylation and the prognosis of patients with breast cancer in MFS and OS for multivariate regression analysis. MFS = metastasis-free survival, OS = overall survival, *PITX2* = paired-like homeodomain transcription factor 2.

CI = 0.93–2.87, $P = .085$). Significant correlation was found between *GSTP1* promoter methylation and DFS, and RFS (HR = 2.09, 95% CI = 1.19–3.67, $P = .011$; HR = 2.71, 95% CI = 1.22–6.03, $P = .015$; respectively), including 2 studies with 201 breast cancer patients and 1 study with 174 patients with breast cancer, respectively. *GSTP1* promoter methylation was not notably correlated with a poor prognosis in breast cancer in OS.

### 3.3. PITX2 promoter methylation and the prognosis of patients with breast cancer

The data involving *PITX2* promoter methylation included 3 studies with 1052 breast cancer patients in MFS and 2 studies with 840 breast cancer patients in OS (Fig. 3). The results showed that *PITX2* promoter methylation was significantly associated with the prognosis in MFS and OS (HR = 1.73, 95% CI = 1.33–2.26, $P < .001$; HR = 1.57, 95% CI = 1.15–2.14, $P = .004$, respectively). Thus, *PITX2* promoter methylation was significantly correlated with a poor prognosis of breast cancer patients in MFS and OS.

### 3.4. p16 promoter methylation and the prognosis of patients with breast cancer

No significant relationship was observed between *p16* promoter methylation and the prognosis in OS and DFS (HR = 0.92, 95% CI = 0.31–2.71, $P = .884$; HR = 1.49, 95% CI = 0.73–3.05, $P = .274$; respectively) (Fig. 4), including 3 studies with 1107 breast cancer patients in OS and 1 study with 101 breast cancer patients. The analysis revealed that *p16* promoter methylation was not linked to the prognosis of breast cancer patients in OS.

### 3.5. ESR1 promoter methylation and the prognosis of patients with breast cancer

The results showed that *ESR1* promoter methylation was significantly correlated with the prognosis in OS and MFS (HR = 1.55, 95% CI = 1.06–2.28, $P = .025$; HR = 2.76, 95% CI = 1.02–7.45, $P = .046$, respectively) (Fig. 5), including 3 studies with 227 cases in OS and 1 study with 69 cases in MFS. The result from 2 studies involving 158 breast cancer cases revealed that *ESR1* promoter methylation had a trend toward an unfavorable prognosis in DFS (HR = 1.41, 95% CI = 0.94–2.10, $P = .096$) (Fig. 5). Promoter methylation of the *ESR1* gene may be significantly linked to a poor prognosis of patients with breast cancer in OS.

## 4. Discussion

The silencing of tumor suppressor genes (TSGs) via promoter methylation may prompt the carcinogenesis and progression of breast cancer.[49] Dedeurwaerder and Fuks et al[50] reported that high expression of some T-cell marker genes was correlated with a better clinical outcome in breast cancer. The Chi-square (and Fisher's exact) test had a notably ($P < .05$) higher percentage of promoter methylation of the *ESR1* gene in breast cancer patients with triple negative and HER2 phenotypes with poorer prognosis by Martinez-Galan et al.[51] Some methylated genes within the promoter (i.e., *RASSF1A*, *RARbeta2*, *BRCA1*, and *GSTP1*) have been identified to be associated with poor prognosis of breast cancer patients,[31–33,44] suggesting that aberrantly methylated genes may become potential prognostic factors. Therefore, aberrant promoter methylation of a gene may provide more independent prognostic information as a prognostic indicator in the treatment and management of breast cancer.

In the present study, 4 genes consisted of the *GSTP1*, *p16*, *ESR1*, and *PITX2* genes. However, the prognostic values of *GSTP1*, *p16*, *ESR1*, and *PITX2* promoter methylation in breast cancer remain to be elucidated. Thus, we first determined whether these 4 cancer-related genes with promoter methylation were correlated with the prognosis of patients with breast cancer in multivariate regression analysis.

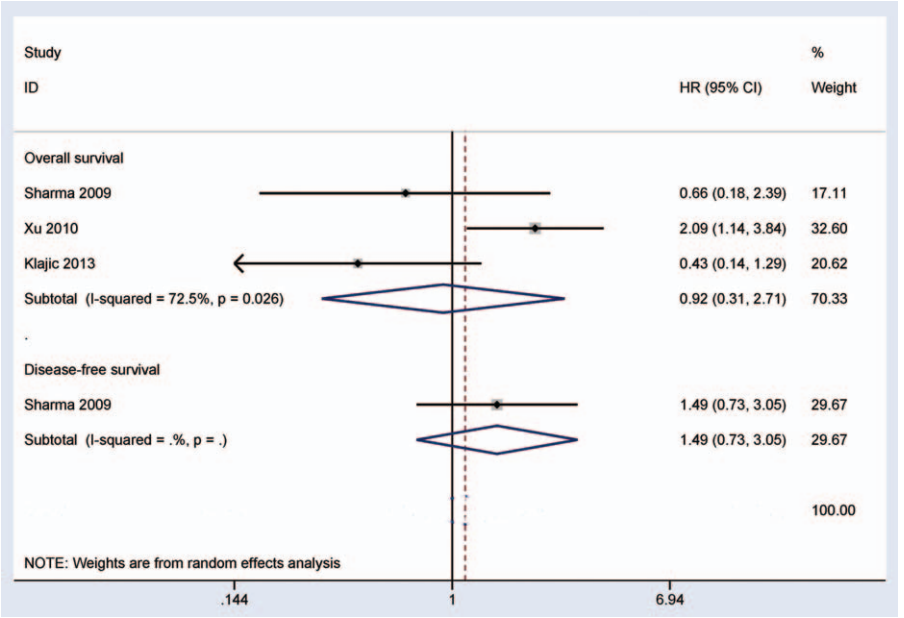


**Figure 4.** Forest plot of the correlation between *p16* promoter methylation and the prognosis of patients with breast cancer in OS and DFS for multivariate regression analysis. DFS = disease-free survival, OS = overall survival, *p16* = cyclin-dependent kinase inhibitor 2A.

The results in a large patient population showed that promoter methylation of the *GSTP1* gene just showed a trend toward a poor prognosis in OS (HR = 1.64, 95% CI = 0.93–2.87, *P* = .085). *PITX2* promoter methylation was significantly correlated with an unfavorable prognosis of breast cancer patients in OS, but no significant correlation was found between *p16* promoter methylation and OS. In addition, *PITX2* promoter methylation was also found to be significantly associated with a poor prognosis of patients with breast cancer in MFS. Based on small sample sizes, we found that *GSTP1* and *ESR1* promoter methylation in 2 studies was correlated with an unfavorable prognosis of patients in DFS, but no association was observed between *p16* promoter methylation and DFS in 1 study.[33] A significant correlation was observed between *ESR1* promoter methylation and a worse prognosis of patients with breast cancer in 3 studies with a small patient population in OS. Only 1 study

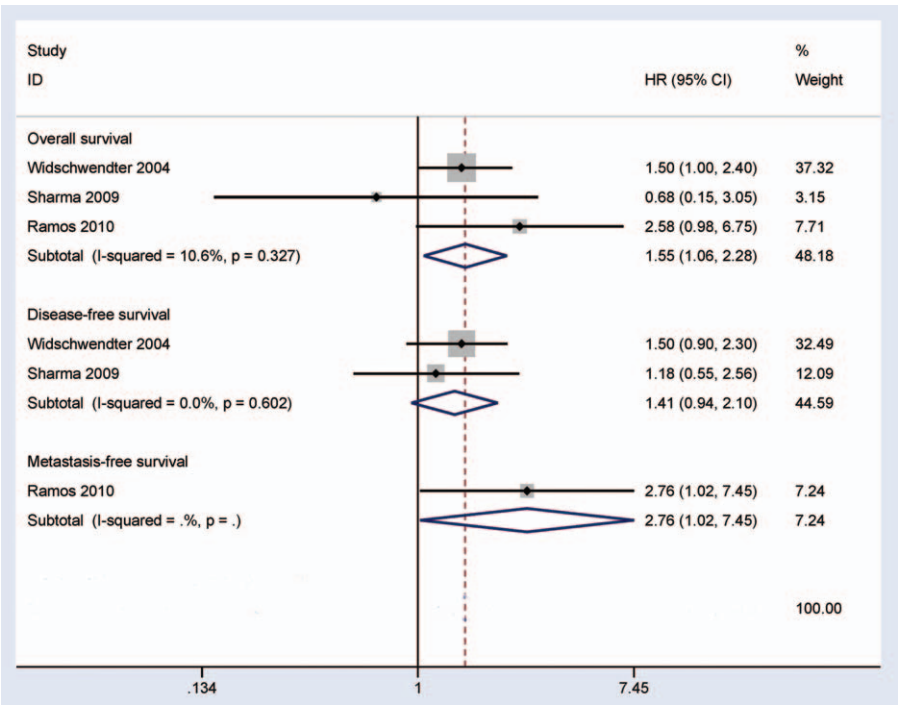


**Figure 5.** Forest plot of the correlation between *ESR1* promoter methylation and the prognosis of patients with breast cancer in OS, DFS, and MFS for multivariate regression analysis. DFS = disease-free survival, *ESR1* = estrogen receptor-α, MFS = metastasis-free survival, OS = overall survival.

involving 174 breast cancer patients reported that *GSTP1* promoter methylation was linked to a worse prognosis in RFS.[47] *ESR1* promoter methylation was reported to be associated with a worse prognosis in 1 study with 69 breast cancer patients in MFS.[38] For the results with small sample sizes, more studies with large sample sizes should be necessary to further validate the prognostic values in OS, DFS, RFS, and MFS.

Some limitations should be addressed in this meta-analysis. First, only publications written in English were identified in our study, which can lead to a bias in literature selection. Articles with positive results were more easily accepted than articles with negative results. Second, the main population included Caucasians in the current study, and other ethnic populations, such as Africans and Asians, were insufficient. In the future, additional studies are still needed to confirm the prognostic role of *GSTP1*, *p16*, *ESR1,* or *PITX2* promoter methylation in the African and Asian populations with breast cancer. Third, studies of the blood with large sample sizes should be done to confirm whether *GSTP1*, *p16*, *ESR1,* or *PITX2* promoter methylation could be a noninvasive prognostic factor based on blood samples.

In conclusion, our findings show that *ESR1* and *PITX2* may be notably associated with a worse prognosis of patients with breast cancer in OS, but no significant relationship was found between *p16* or *GSTP1* promoter methylation and OS. Moreover, *PITX2* promoter methylation was found to be significantly correlated with an unfavorable prognosis of patients with breast cancer in MFS. *ESR1* or *PITX2* promoter methylation could serve as a potential drug target in the treatment of breast cancer.

## References


[1] Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87–108.

[2] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:7–30.

[3] Rakha EA, Reis-Filho JS, Baehner F, et al. Breast cancer prognostic classification in the molecular era: the role of histological grade. Breast Cancer Res 2010;12:207.

[4] Rakha EA, El-Sayed ME, Lee AH, et al. Prognostic significance of Nottingham histologic grade in invasive breast carcinoma. J Clin Oncol 2008;26:3153–8.

[5] Mehrmohamadi M, Mentch LK, Clark AG, et al. Integrative modelling of tumour DNA methylation quantifies the contribution of metabolism. Nat Commun 2016;7:13666.

[6] Khan SA, Reddy D, Gupta S. Global histone post-translational modifications and cancer: biomarkers for diagnosis, prognosis and treatment? World J Biol Chem 2015;6:333–45.

[7] Nawaz I, Hu LF, Du ZM, et al. Integrin alpha9 gene promoter is hypermethylated and downregulated in nasopharyngeal carcinoma. Oncotarget 2015;6:31493–507.

[8] Ye M, Huang T, Ni C, et al. Diagnostic capacity of RASSF1A promoter methylation as a biomarker in tissue, brushing, and blood samples of nasopharyngeal carcinoma. EBioMedicine 2017;18:32–40.

[9] Molina-Pinelo S, Salinas A, Moreno-Mata N, et al. Impact of DLK1-DIO3 imprinted cluster hypomethylation in smoker patients with lung cancer. Oncotarget 2016.

[10] Sakaguchi H, Muramatsu H, Okuno Y, et al. Aberrant DNA methylation is associated with a poor outcome in juvenile myelomonocytic leukemia. PloS One 2015;10:e0145394.

[11] Shukla HD, Mahmood J, Vujaskovic Z. Integrated proteo-genomic approach for early diagnosis and prognosis of cancer. Cancer Lett 2015;369:28–36.

[12] Huang T, Chen X, Hong Q, et al. Meta-analyses of gene methylation and smoking behavior in non-small cell lung cancer patients. Sci Rep 2015;5:8897.

[13] Ye M, Huang T, Ying Y, et al. Detection of 14-3-3 sigma (() promoter methylation as a noninvasive biomarker using blood samples for breast cancer diagnosis. Oncotarget 2017;8:9230–42.

[14] Sayar N, Karahan G, Konu O, et al. Transgelin gene is frequently downregulated by promoter DNA hypermethylation in breast cancer. Clin Epigenetics 2015;7:104.

[15] Rodriguez-Miguel C, Moral R, Escrich R, et al. The role of dietary extra virgin olive oil and corn oil on the alteration of epigenetic patterns in the rat DMBA-induced breast cancer model. PloS One 2015;10:e0138980.

[16] Ma Z, Song J, Liu S, et al. Decreased expression of the CHD5 gene and its clinicopathological significance in breast cancer: correlation with aberrant DNA methylation. Oncol Lett 2016;12:4021–6.

[17] Moelans CB, Vlug EJ, Ercan C, et al. Methylation biomarkers for pleomorphic lobular breast cancer—a short report. Cell Oncol 2015;38: 397–405.

[18] Conway K, Edmiston SN, May R, et al. DNA methylation profiling in the Carolina Breast Cancer Study defines cancer subclasses differing in clinicopathologic characteristics and survival. Breast Cancer Res 2014;16:450.

[19] Masood N, Kayani MA. Expression patterns of carcinogen detoxifying genes (CYP1A1, GSTP1 & GSTT1) in HNC patients. Pathol Oncol Res 2013;19:89–94.

[20] Strange RC, Spiteri MA, Ramachandran S, et al. Glutathione-S-transferase family of enzymes. Mutat Res 2001;482:21–6.

[21] Piepkorn M. Melanoma genetics: an update with focus on the CDKN2A (p16)/ARF tumor suppressors. J Am Acad Dermatol 2000;42(5 pt 1):705–22. quiz 723–706.

[22] Lukas J, Parry D, Aagaard L, et al. Retinoblastoma-protein-dependent cell-cycle inhibition by the tumour suppressor p16. Nature 1995;375: 503–6.

[23] Anderson E. The role of oestrogen and progesterone receptors in human mammary development and tumorigenesis. Breast Cancer Res 2002;4: 197–201.

[24] Martin DM, Skidmore JM, Philips ST, et al. PITX2 is required for normal development of neurons in the mouse subthalamic nucleus and midbrain. Dev Biol 2004;267:93–108.

[25] Kioussi C, Briata P, Baek SH, et al. Identification of a Wnt/Dvl/beta-Catenin –> Pitx2 pathway mediating cell-type-specific proliferation during development. Cell 2002;111:673–85.

[26] Kitamura K, Miura H, Miyagawa-Tomita S, et al. Mouse Pitx2 deficiency leads to anomalies of the ventral body wall, heart, extra- and periocular mesoderm and right pulmonary isomerism. Development 1999;126:5749–58.

[27] Chattopadhyay S, Deo SV, Shukla NK, et al. Association of promoter methylation of ERalpha and ERbeta with sporadic breast cancer–a study from North India. Tumour Biol 2014;35:7911–9.

[28] Yamamoto N, Nakayama T, Kajita M, et al. Detection of aberrant promoter methylation of GSTP1, RASSF1A, and RARbeta2 in serum DNA of patients with breast cancer by a newly established one-step methylation-specific PCR assay. Breast Cancer Res Treat 2012;132:165–73.

[29] Zhu W, Qin W, Hewett JE, et al. Quantitative evaluation of DNA hypermethylation in malignant and benign breast tissue and fluids. Int J Cancer 2010;126:474–82.

[30] 2008;Harbeck N, Nimmrich I, Hartmann A, et al. Multicenter study using paraffin-embedded tumor tissue testing PITX2 DNA methylation as a marker for outcome prediction in tamoxifen-treated, node-negative breast cancer patients, Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 26:5036–42.

[31] Wu L, Wang F, Xu R, et al. Promoter methylation of BRCA1 in the prognosis of breast cancer: a meta-analysis. Breast Cancer Res Treat 2013;142:619–27.

[32] Jiang Y, Cui L, Chen WD, et al. The prognostic role of RASSF1A promoter methylation in breast cancer: a meta-analysis of published data. PloS One 2012;7:e36780.

[33] Sharma G, Mirza S, Yang YH, et al. Prognostic relevance of promoter hypermethylation of multiple genes in breast cancer patients. Cell Oncol 2009;31:487–500.

[34] Sharma G, Mirza S, Parshad R, et al. Clinical significance of promoter hypermethylation of DNA repair genes in tumor and serum DNA in invasive ductal breast carcinoma patients. Life Sci 2010;87:83–91.

[35] Xu X, Gammon MD, Zhang Y, et al. Gene promoter methylation is associated with increased mortality among women with breast cancer. Breast Cancer Res Treat 2010;121:685–92.

[36] Nimmrich I, Sieuwerts AM, Meijer-van Gelder ME, et al. DNA hypermethylation of PITX2 is a marker of poor prognosis in untreated lymph node-negative hormone receptor-positive breast cancer patients. Breast Cancer Res Treat 2008;111:429–37.

[37] Gobel G, Auer D, Gaugg I, et al. Prognostic significance of methylated RASSF1A and PITX2 genes in blood- and bone marrow plasma of breast cancer patients. Breast Cancer Res Treat 2011;130:109–17.

[38] Ramos EA, Camargo AA, Braun K, et al. Simultaneous CXCL12 and ESR1 CpG island hypermethylation correlates with poor prognosis in sporadic breast cancer. BMC Cancer 2010;10:23.
[39] Tierney JF, Stewart LA, Ghersi D, et al. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007;8:16.
[40] Zintzaras E, Ioannidis JP. HEGESMA: genome search meta-analysis and heterogeneity testing. Bioinformatics 2005;21:3672–3.
[41] Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. BMJ 2003;327:557–60.
[42] DerSimonian R. Meta-analysis in the design and monitoring of clinical trials. Statistics in medicine 1996;15:1237–48. discussion 1249–1252.
[43] Klajic J, Fleischer T, Dejeux E, et al. Quantitative DNA methylation analyses reveal stage dependent DNA methylation and association to clinico-pathological factors in breast tumors. BMC Cancer 2013;13: 456.
[44] Cho YH, Shen J, Gammon MD, et al. Prognostic significance of gene-specific promoter hypermethylation in breast cancer patients. Breast Cancer Res Treat 2012;131:197–205.
[45] Dejeux E, Ronneberg JA, Solvang H, et al. DNA methylation profiling in doxorubicin treated primary locally advanced breast tumours identifies novel genes associated with survival and treatment response. Mol Cancer 2010;9:68.
[46] Hartmann O, Spyratos F, Harbeck N, et al. DNA methylation markers predict outcome in node-positive, estrogen receptor-positive breast cancer with adjuvant anthracycline-based chemotherapy. Clin Cancer Res 2009;15:315–23.
[47] Arai T, Miyoshi Y, Kim SJ, et al. Association of GSTP1 CpG islands hypermethylation with poor prognosis in human breast cancers. Breast Cancer Res Treat 2006;100:169–76.
[48] Widschwendter M, Siegmund KD, Muller HM, et al. Association of breast cancer DNA methylation profiles with hormone receptor status and response to tamoxifen. Cancer Res 2004;64:3807–13.
[49] Maziveyi M, Alahari SK. Breast cancer tumor suppressors: a special emphasis on novel protein nischarin. Cancer Res 2015;75:4252–9.
[50] Dedeurwaerder S, Fuks F. DNA methylation markers for breast cancer prognosis: unmasking the immune component. Oncoimmunology 2012;1:962–4.
[51] Martinez-Galan J, Torres-Torres B, Nunez MI, et al. ESR1 gene promoter region methylation in free circulating DNA and its correlation with estrogen receptor protein expression in tumor tissue in breast cancer patients. BMC Cancer 2014;14:59.