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The association between the methylation frequency of BRCA1/2 gene promoter and occurrence and prognosis of breast carcinoma
A meta-analysis

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Abstract
Objectives: Breast cancer susceptibility gene 1/2 (BRCA1/2) is a promising tumor marker in many types of cancer. However, the methylation frequency of BRCA1/2 gene with occurrence risk and survival benefit of patients with breast carcinoma remains controversy. The aim of the present study was to assess the relationship between BRCA1/2 gene promoter methylation and the occurrence and prognosis in breast carcinoma based on a meta-analysis, meanwhile, this article explored the differential expression levels of BRCA1/2 gene promoter methylation in peripheral blood and tumor tissues of breast cancer patients.

Methods: Electronic databases (PubMed, Medline, Cochrane Library, and CNKI) were searched up to June 2019. The number of BRCA1/2 promoter methylation-positive and -negative patients in breast carcinoma patients were measured, and hazard ratio (HR) with 95% confidence interval (CI) for the association between BRCA1/2 gene promoter methylation and the prognosis of breast carcinoma patients. Primary end points were presence of breast cancer, overall survival (OS), disease-free survival (DFS). Statistical analysis was performed with STATA 12.0.

Results and conclusions: Fifty-eight articles including 19,084 individuals met full eligibility criteria. We observed that the frequency of BRCA1 gene promoter methylation was higher in breast cancer tissues compared with normal tissues, and the prognostic analysis suggested that BRCA1 gene promoter methylation was significantly associated with poor overall survival and poor disease-free survival. This study also verified that there was no statistically significant difference in the methylation frequency of BRCA1 gene promoter between peripheral blood and tumor tissues in breast cancer patients, which suggests that the detection of BRCA1 promoter methylation in peripheral blood may be a non-invasive and rapid way to monitor the occurrence breast cancer.

Abbreviations: BRCA1/2 = Breast cancer susceptibility gene 1/2, BRCA1-BARD1 = BRCA1 Associated Ring Domain-1, CI = Confidence interval, CpG = Cytosine phosphate guanine, DFS = Disease-free survival, HR = Hazard ratio, MRE11 = Meiotic Recombination-11, NBS1 = Nijmegen Breakage Syndrome, NOS = Newcastle-Ottawa Scale, OR = Odds ratio, OS = Overall survival, PLK1 = Polo-Like Kinase-1.

Keywords: BRCA1/2, breast carcinoma, methylation, peripheral blood, prognosis

1. Introduction

The latest national cancer statistics released by the China national cancer center showed that in 2014, the incidence rate of breast cancer among Chinese women ranked the first among all women with new cancer, reaching 17.10%, and the mortality rate also ranked the first among all female malignancy-related deaths.[1] Meanwhile, according to the American cancer society, published in 2018, the global cancer statistics show that breast cancer ranked the first among new tumors of women in the world, accounting for 24.2% and the highest mortality rate, accounting for 15.0%.[2] As breast cancer screening and treatment techniques have improved, the death rate from breast cancer has declined, but remains high.[3] There are many factors that cause breast cancer, genetic factors only account for less than 10%, and the other 90% of breast cancer occurrence mechanism is still unclear.[4]

BRCA-related breast cancer is characterized by more aggressive ability than sporadic breast cancer. Promoter methylation plays an important role in carcinogenesis of Mammary Cancer,

because methylation of cytosine phosphate guanine (CpG) sites in promoter regions may lead to downregulation of tumor suppressor genes.[5,6] Some literatures demonstrated that BRCA1/2 promoter methylation happen almost completely in the sporadic setting and it rarely occurs in patients with potential BRCA1/2 germline mutations.[7,8] This conclusion has potential clinical significance because promoter methylation tests can be used as screening tests when genetic properties are suspected, thus avoiding the need for germline mutation analysis in the patients with presence of promoter methylation.

Some researchers have identified BRCA1/2 as tumor suppressor genes that are primarily involved in maintenance of genome stability, specifically the homologous recombination pathway for double-strand DNA repair.[9,10] The promoter methylation of BRCA1/2 genes leaded to downregulated expression of BRCA1/2 proteins in mammary tissues, which may induce the occurrence of breast cancer. The effect of BRCA1/2 promoter methylation on the occurrence and survival of breast cancer patients has been reported extensively in recent years.[11–14] However, the role of BRCA1/2 gene promoter methylation in the occurrence and prognosis of breast cancer remains controversial. Based on the contradictory results from different studies, the aim of this meta-analysis was to assess the occurrence and prognostic value of BRCA1/2 promoter methylation in patients with breast cancer.

2. Materials and methods

2.1. Search strategy

We searched 4 major electronic databases (ie, PubMed, Medline, Cochrane Library, and CNKI) for this study. We used the following terms and their various combinations: "Breast Neoplasm*," or "Breast Tumor*," or "Breast Carcinoma*," or "Mammary Neoplasm*," or "Mammary Cancer*," or "Malignant Neoplasm of Breast," or "Breast Malignant Neoplasm*," or "Malignant Tumor of Breast" or "Breast Malignant Tumor*,"or "Cancer of Breast or Cancer of the Breast" and "BRCA1, or "BRCA2," or "BRCA" and "methylation". Last search updated in June 2019.

2.2. Eligibility criteria

All languages studies that meet the criteria are included. The selected studies for meta-analysis had to meet the following predefined criteria:

(1) The purpose and statistical methods of each study were similar, and data are complete.
(2) The studies evaluating the association between the prevalence of BRCA1/2 gene promoter methylation and occurrence risk of breast cancer or evaluating the role of BRCA1/2 gene promoter methylation in the prognosis of breast cancer.
(3) Adequate data about BRCA1/2 promoter methylation in tissues or peripheral blood were provided.
(4) All patients met the diagnostic criteria of breast cancer; and the nationality, race, and age of all patients were not limited.
(5) Publications including only the abstract were excluded because of incomplete information.
(6) Animal studies, Cell research, Reviews, Systematic reviews, and other non-original studies were excluded.

2.3. Screening and data extraction

Titles and abstracts were independently screened by 2 reviewers, and this constituted preliminary screening. According to the predefined inclusion criteria, we screened out the detected documents, removed duplicate documents, and read the full text of the literature that may meet the inclusion criteria (Fig. 1). Data were extracted from qualified literature. The extracted information was as follows: first author, year of publication, nationality of patients, age of patients, number of BRCA1/2 promoter methylation-positive and -negative patients, detection method of promoter methylation and sample type.

The quality of the eligible articles was independently evaluated by 2 reviewers using the Newcastle-Ottawa Scale (NOS). The quality of each study is graded with a maximum of 8 stars. Grading was as follows: <5 stars represented low quality and studies with a score of 6 or higher were classified as high-quality studies. The NOS for quality assessment is shown in Tables 1–3.

2.4. Statistical methods

STATA 12.0 (Stata Corporation, College Station, TX) software was used for statistical analysis. The effect measures of interest were the corresponding 95% confidence interval (CI). If the odds ratio (OR) was more than 1 and the 95% CI did not overlap with 1, BRCA1/2 gene promoter methylation could increase the risk of breast cancer. Meanwhile, if the OR was less than 1, BRCA1/2 gene methylation could not increase the risk of breast cancer.

HR and 95% CI were used to assess the relationships between BRCA1 promoter methylation and DFS (disease-free survival) and OS (overall survival). BRCA1 promoter methylation was a risk factor for poor prognosis in breast cancer, especially when the HRs for DFS and OS were >1 and the 95% CI did not overlap with 1.

The heterogeneity was evaluated using the χ^2 test and the I^2 test, if there was statistical heterogeneity ($P < .05$ and $I^2 > 50\%$), a random-effects model was used to combine effect quantities, or alternatively the fixed-effects model. Subgroup analysis was performed based on the patient's regional location, diagnostic method, sample type, and design type of clinical research to explore potential sources of heterogeneity. The sensitivity analysis was used to assess the stability of the results. If more than 10 articles were included, Begg funnel plot and Egger linear regression were used to assess potential publication bias.

2.5. Ethical review

The study is a meta-analysis, so ethical approval was not necessary.

3. Result

3.1. Study characteristics

Of the 288 potentially relevant articles, 230 were excluded, leaving 59 articles for analysis (Fig. 1). A total of 19,084 individuals were included in these studies, and they were divided into 2 groups based on the malignancy status. The study included 54 datasets of 48 literatures and 4 literatures that were used for assessing the association between BRCA1 and BRCA2 promoter methylation and occurrence of breast cancer, respectively (Tables 1 and 2). Six articles were used for comparing the relationship between BRCA1 promoter methylation in peripheral
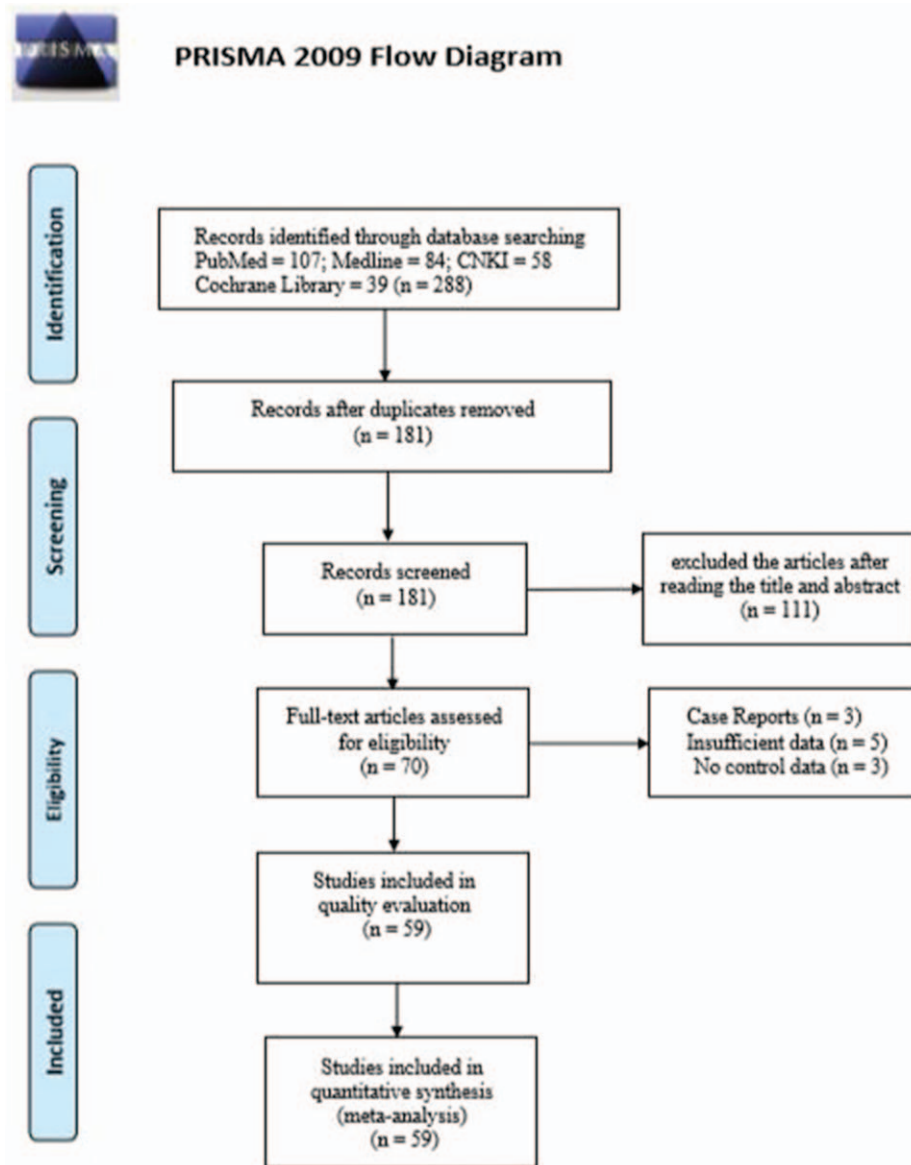
Figure 1. Flow diagram of study selection.

blood and matched tumor tissue samples (Table 3). Meanwhile, 13 datasets of 11 literatures and 9 datasets of 8 literatures were used for illustrating the association between BRCA1 promoter methylation and OS and DFS, respectively (Table 4). NOS evaluation showed that the overall methodological quality of the 58 studies included was high.

3.2. BRCA1 gene promoter methylation and breast carcinoma

The included 54 datasets of 48 literatures[11–13,15–59] reported on the relationship between BRCA1 promoter methylation and breast carcinoma. Given the heterogeneity found in the studies ($I^2 = 78.8\%$, $P < .001$), random-effects model was used for pooled analysis. The results showed that the patients with positive BRCA1 promoter methylation was 4.60 times higher risk of having breast carcinoma than that of the controls, and the

difference was significant (OR = 4.60, 95% CI: 3.21–6.59, $P < .001$) (Fig. 2).

To identify the sources of heterogeneity, further subgroup analysis based on sample materials, methods for detecting methylation, region for patients and design method of research was conducted. For the region for patients, the heterogeneity of the respectively pooled value of literatures of South Asia, West Asia and Europe and America research results was all smaller ($I^2 = 28.9\%$, $P = .218$; $I^2 = 0\%$, $P = .578$; $I^2 = 28.2\%$, $P = .141$, respectively). In particular, the prevalence of BRCA1 promoter methylation in East Asians (OR = 9.03, 95%CI: 5.16–15.80, $P < .001$) was higher than that in South Asians (OR = 6.22, 95% CI: 2.19–17.66, $P < .001$), West Asians (OR = 5.35, 95%CI: 1.90–15.07, $P = .002$), Europeans and Americans (OR = 1.41, 95%CI: 1.07–1.88, $P = .016$) and North Africans (OR = 5.00, 95%CI: 0.38–65.95, $P = .221$) in breast cancers compared with non-cancer controls. For the methods for detecting methylation,

Table 1

The main characteristics of eligible studies for the relationship between BRCA1 gene promoter methylation and the occurrence of breast cancer.

Author	Year	Country	Age	Experimental group		Control group		Method	DM	ESM	CSM	NOS
				M+	M-	M+	M-					
Cao (TT)	2010	China	–	35	67	0	102	MSP	CCS	TT	PT	6
Cao (PB)	2010	China	–	32	70	0	102	MSP	CCS	TB	NB	6
Fen (TT)	2009	China	29–77	22	41	0	63	MSP	CCS	TT	PT	5
Fen (PB)	2009	China	29–77	20	43	0	29	MSP	CCS	TB	NB	5
Fen	2005.11	China	–	17	41	0	58	MSP	CCS	TT	PT	6
Fen (TT)	2005.05	China	–	27	66	0	93	MSP	CCS	TT	PT	6
Fen (PB)	2005.05	China	–	23	70	0	93	MSP	CCS	TB	NB	6
Fu	2014	China	26–75	26	80	8	98	MSP	CCS	TT	PT	6
Guo	2016	China	–	21	69	6	94	MSP	CCS	TT	PT	5
Li	2018	China	35–59	261	89	7	43	MSP	CS	TB	NB	6
Lu	2011	China	31–78	70	45	7	58	MSP	CS	TB	NB	6
Ma	2011	China	–	9	22	1	30	MSP	CS	TT	PT	5
Ni	2014	China	–	15	38	0	8	MSP	CS	TT	PT	5
Ren	2007	China	36–72	7	44	0	10	MSP	CCS	TT	BBT	5
Wei	2014	China	29–64	21	29	0	50	MSP	CCS	TT	PT	5
Wei	2015	China	31–76	19	41	0	60	BSP	CCS	TT	PT	6
Wei	2006	China	–	7	44	0	10	MSP	CCS	TT	BBT	5
Wu	2011	China	39–72	13	17	5	25	MSP	CCS	TT	BBT	5
Yang	2008	China	32–65	15	8	11	12	BSP	CCS	TT	PT	5
Liu	2015	China	–	4	32	1	29	BSP	CCS	TT	BBT	6
Jing	2010	China	23.1–75.9	17	33	0	50	MSP	CCS	TB	NB	6
Chen	2003	Taiwan, China	–	21	72	1	19	MTA	CCS	TT	PT	7
Iwamoto	2011	Japan	30–69	43	157	27	173	MSP	CCS	TB	NB	7
Otani	2014	Japan	–	15	15	9	21	MSP	CS	TT	PT	7
Jung	2013	Korea	29–77	6	54	3	57	MS-MLPA	CCS	TT	PT	6
Bae	2004	South Korea	29–90	45	55	0	8	MSP	CCS	TT	BBT	6
Vu	2018	Vietnam	–	46	33	41	38	MSP	CCS	TT	PT	6
Sharma (TT)	2010	India	30–81	27	73	0	100	MSP	CS	TT	PT	7
Sharma (PB)	2010	India	30–82	25	75	0	30	MSP	CS	TB	NB	7
Mirza (TT)	2007	India	30–81	13	37	0	5	MSP	CS	TT	PT	6
Mirza (PB)	2007	India	30–82	11	39	0	5	MSP	CS	TB	NB	6
Hasan	2013	India	–	9	20	4	22	MSP	CS	TT	PT	5
Bhavani	2009	India	–	21	83	2	46	MethyLight assay	CCS	TT	PT	5
Paydar	2019	Iran	49.28±7.25	12	15	3	33	MSP	CCS	TT	PT	5
Shakeri	2016	Iran	28–68	7	68	0	10	MS-MLPA	CCS	TT	BBT	5
Al-moghrabi	2011	SaudiArabic	22–80	2	5	8	65	MSP	CCS	TB	NB	5
Ben Gacem	2012	Tunisia	31–87	71	46	5	60	MSP	CCS	TT	PT	8
Hosny	2016	Egypt	–	17	23	14	26	MSP	CS	TT	PT	6
Cho (TT)	2010	Turkey	50.8±10.8	7	33	2	25	MethyLight assay	CCS	TT	PT	6
Cho (PB)	2010	Turkey	50.8±10.9	3	37	2	38	MethyLight assay	CCS	TB	NB	6
Buyru	2009	Turkey	–	4	73	0	77	MS-MLPA	CCS	TT	PT	7
Kontorovich	2009	Israel	–	6	94	8	122	Real-time PCR	CCS	TT	PT	7
Sturgeon	2012	USA	Mean:67	192	112	147	87	Pyrosequencing	CCS	TB	NB	8
Wei	2005	USA	–	39	92	0	3	MSP	CS	TT	PT	7
Cho	2015	USA	–	18	1003	13	1023	MSP	CS	TB	NB	8
Esteller	2000	USA	–	11	73	0	84	MSP	CS	TT	PT	8
Dobrovic	1997	Australia	–	2	5	0	2	Southern blotting	CCS	TT	PT	5
Snell	2008	Australia	–	3	4	1	6	MS-HRM	CS	TT	PT	5
Wong	2011	Australia	–	28	227	6	163	MS-HRM	CCS	TB	NB	8
Gupta	2014	Poland	Mean:61	15	51	2	34	MS-HRM	CS	TB	NB	7
Bosviel	2012	France	26–89	425	477	454	536	QAMA	CS	TB	NB	8
Jerónimo	2003	Portugal	18–92	30	46	8	17	MSP	CS	TT	PT	6
Parrella	2004	Italy	–	9	45	2	8	MSP	CS	TT	BBT	6
Vos	2017	Netherlands	21–86	68	84	3	6	MS-MLPA	CCS	TT	BBT	7

BBT = benign breast tissue, BSP = bisulfite sequencing PCR, CCS = case-control study, CS = cohort study, CSM = control sample organization, DM = design method of research, ESM = experimental sample organization, M+ = methylated, M- = unmethylated, MS-HRM = methylation-sensitive high-resolution melting, MS-MLPA = methylation-specific multiplex ligation-dependent probe amplification, MSP = methylation-specific PCR, MTA = methylation target array, NB = nontumor blood, NOS = Newcastle-Ottawa scale, PB = peripheral blood, PT = paracancerous tissue, QAMA = quantitative analysis of methylated alleles, TB = tumor blood, TT = tumor tissue, TT = tumor tissues, "-" = not mentioned.

Table 2

The main characteristics of eligible studies for the relationship between BRCA2 gene promoter methylation and the occurrence of breast cancer.

Author	Year	Country	Age	Experimental group		Control group		Method	DM	ESM	CSM	NOS
				M+	M−	M+	M−					
Ni	2014	China	–	4	49	1	37	MSP	CS	TT	PT	5
Jung	2013	Korea	29–77	1	59	0	60	MS-MLPA	CCS	TT	PT	6
Vos	2017	Netherlands	21–86	60	92	5	4	MS-MLPA	CCS	TT	BBT	7
Ben Gacem	2012	Tunisia	31–87	81	36	3	62	MSP	CCS	TT	PT	8

BBT = benign breast tissue, CS = cohort study, CSM = control sample organization, DM = design method of research, CCS = case-control study, ESM = experimental sample organization, M+ = methylated, M− = unmethylated, MS-MLPA = methylation-specific multiplex ligation-dependent probe amplification, MSP = methylation-specific PCR, NOS = Newcastle-Ottawa scale, PT = paracancerous tissue, TT = tumor tissues, "-" = not mentioned.

Table 3

The main characteristics of eligible studies for the comparison of BRCA1 gene promoter methylation in peripheral blood and matched tumor tissue samples.

Author	Year	Country	Age	PB		TT		method	DM	NOS
				M+	M−	M+	M−			
Cao	2010	China	–	32	70	35	67	MSP	CCS	6
Fen	2009	China	29–77	20	43	22	41	MSP	CCS	6
Fen	2005.05	China	–	23	70	27	66	MSP	CCS	6
Sharma	2010	India	30–81	25	75	27	73	MSP	CS	7
Mirza	2007	India	30–81	11	39	13	37	MSP	CS	6
Cho	2010	Turkey	50.8 ± 10.8	3	37	7	33	MethyLight assay	CCS	6

CCS = case-control study, CS = cohort study, DM = design method of research, M+ = methylated, M− = unmethylated, MSP = methylation-specific PCR, NOS = Newcastle-Ottawa scale, PB = peripheral blood, TT = tumor tissue, "-" = not mentioned.

Table 4

The main characteristics of eligible studies for the relationship between BRCA1 gene promoter methylation and prognosis of breast cancer.

Author	Year	Country	Age	HR	ll	ul	Total (n)	Follow-up (month)	Method	DM	ESM	NOS
OS												
Zhu	2015	China	25–83	2.32	1.12	4.81	239	120	MSP	CCS	TT	7
Xu	2013	China	25–87	1.29	0.96	1.73	1163	108	MSP	CCS	TT	8
Hsu	2013	Taiwan, China	24–71	4.23	1.08	16.52	139	–	MSP	CCS	TT	6
Chen	2009	China	25–86	1.56	1.02	2.37	536	96	MSP	CCS	TT	7
Feng	2008	China	–	6.4	2	20.5	102	–	MSP	CCS	TT	7
Mori	2016	Japan	30–87	3.1	1.1	13.2	262	54	MLPA	CCS	TT	7
Sharma	2009	India	–	5.06	1.58	16.22	101	33.6	MSP	CS	TT	7
Sharma (TT)	2010	India	30–81	5.32	1.17	24.06	100	14	MSP	CS	TT	7
Sharma (PB)	2010	India	30–81	5.27	1.12	24.79	100	14	MSP	CS	TB	7
Callahan-1	2016	USA	35–79	0.42	0.18	1.01	170	89	BSP	CCS	TT	8
Callahan-2	2016	USA	35–79	0.71	0.32	1.54	363	89	BSP	CCS	TT	8
Xu	2009	USA	mean:59	1.45	0.99	2.11	851	67.2	MSP	CCS	TT	8
Krasteva	2012	Bulgaria	54.11 ± 11.5	0.47	0.14	1.54	135	60	MSP	CCS	TT	7
DFS												
Zhu	2015	China	25–83	2.36	1.13	4.9	239	120	MSP	CCS	TT	7
Xu	2013	China	25–87	1.34	1.06	1.71	1163	108	MSP	CCS	TT	8
Hsu	2013	Taiwan, China	24–71	2.43	1.11	5.31	139	-	MSP	CCS	TT	6
Chen	2009	China	25–86	1.45	1.01	2.09	536	96	MSP	CCS	TT	7
Mori	2016	Japan	30–87	2.6	1.1	7.8	262	54	MLPA	CCS	TT	7
Sharma	2009	India	–	3.88	2.05	7.34	101	36.05	MSP	CS	TT	7
Sharma (TT)	2010	India	30–81	9	3.4	23.8	100	14	MSP	CS	TT	7
Sharma (PB)	2010	India	30–81	8.11	2.44	26.94	100	14	MSP	CS	TB	7
Karray-Chouayekh	2009	Tunisia	28–73	3.67	1.2	11.18	78	60	MSP	CCS	TT	6

BSP = bisulfite sequencing PCR, CCS = case-control study, CS = cohort study, DFS = disease-free survival, DM = design method of research, ESM = experimental sample organization, HR = hazard ratio, ll = lower limit, M+ = methylated, M− = unmethylated, MLPA = multiplex ligation-dependent probe amplification, MSP = methylation-specific PCR, NOS = Newcastle-Ottawa Scale, OS = overall survival, TB = tumor blood, TT = tumor tissues, TT = tumor tissues, ul = upper limit, "-" = not mentioned.
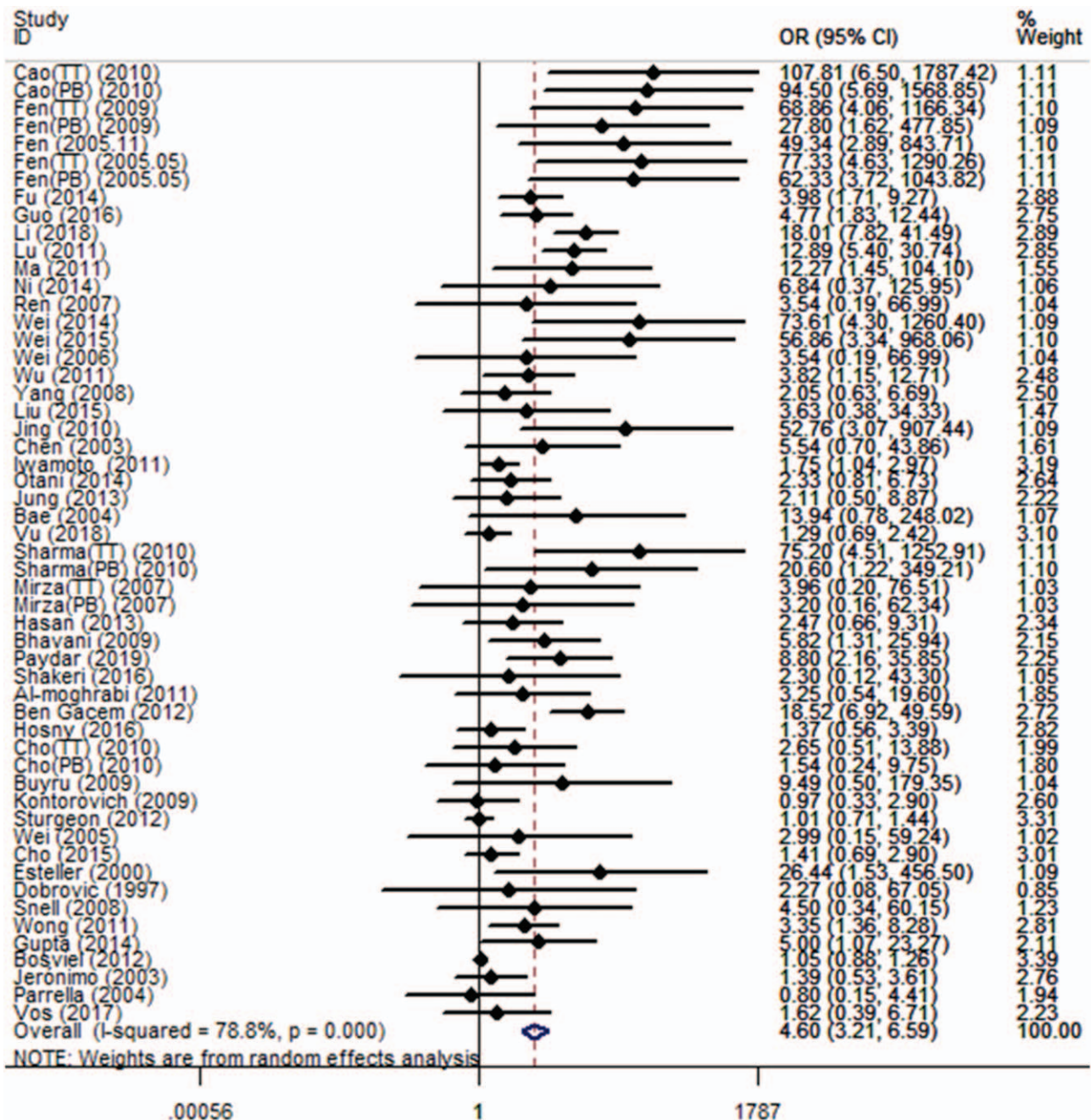
Figure 2. Forest plot for evaluating the relationship between BRCA1 promoter methylation and occurrence risk of breast cancer.

the heterogeneity of literatures of MS-MLPA (Methylation-specific multiplex ligation-dependent probe amplification, MS-MLPA), MethyLight assay and MS-HRM (methylation-sensitive high-resolution melting, MS-HRM) was all Significantly reduced ($I^2 = 0\%$, $P = .761$; $I^2 = 0\%$, $P = 0.521$; $I^2 = 0\%$, $P = .899$, respectively). Meanwhile, heterogeneity was not related to sample materials and design method of research, because there was no significant change in heterogeneity after stratification. It is worth mentioning that the degree of heterogeneity was apparently reduced in stratified analysis. The detailed results were summarized in Table 5.

3.3. BRCA2 gene promoter methylation and breast carcinoma

The 4 included studies[13,24,36,44] reported about the relationship between BRCA2 gene promoter methylation and breast carcinoma. A random-effects model was used to perform the meta-analysis due to significant heterogeneity ($I^2 = 87.7\%$, $P < .001$). The pooled OR of BRCA2 gene promoter methylation was 4.01 (95%CI: 0.31–50.97, $P = .285$), which showed no statistically significant association between BRCA2 gene promoter methylation and breast carcinoma (Fig. 3).

Table 5

Stratified analysis of the relationship between the frequency of BRCA1 promoter methylation and occurrence of breast cancer.

Study	N	Z	OR	95%CI	P	I^2	P_H
Total	54	8.37	4.60	3.21–6.59	<.001	78.8%	<.001
Region for patients							
East Asia	26	7.76	9.03	5.16–15.80	<.001	67.0%	<.001
Southeast Asia	1	0.80	1.29	0.69–2.42	.424	/	/
South Asia	6	3.43	6.22	2.19–17.66	.001	28.9%	.218
west Asia	3	3.17	5.35	1.90–15.07	.002	0.0%	.578
North Africa	2	1.22	5.00	0.38–65.95	.221	93.3%	<.001
European and America	16	2.40	1.41	1.07–1.88	.016	28.2%	.141
Methods for detecting methylation							
MSP	36	7.74	6.65	4.12–10.75	<.001	73.6%	<.001
BSP	3	1.71	5.65	0.77–41.26	.088	65.3%	.056
MTA	1	1.62	5.54	0.70–43.86	.105	/	/
MS-MLPA	4	1.71	2.20	0.89–5.47	.088	0%	.761
MethyLight assay	3	2.37	3.16	1.22–8.17	.018	0%	.521
Real-time PCR	1	0.05	0.97	0.33–2.90	.961	/	/
Pyrosequencing	1	0.08	1.01	0.71–1.44	.936	/	/
Southern blotting	1	0.48	2.27	0.08–67.05	.634	/	/
MS-HRM	3	3.49	3.77	1.79–7.96	<.001	0%	.899
QAMA	1	0.55	1.05	0.88–1.26	.583	/	/
Design method of research							
Case-control study	35	7.01	5.27	3.29–8.44	<.001	73.6%	<.001
Cohort study	19	4.17	3.88	2.05–7.34	<.001	82.4%	<.001
Sample materials							
Tumor tissue	38	7.14	4.54	2.98–6.91	<.001	58.2%	<.001
Peripheral blood	16	4.80	4.41	2.41–8.09	<.001	87.5%	<.001

BSP = bisulfite sequencing PCR, MS-HRM = methylation-sensitive high-resolution melting, MS-MLPA = methylation-specific multiplex ligation-dependent probe amplification, MSP = methylation-specific PCR, MTA = methylation target array, N = Number of articles, P_H = P of heterogeneity, QAMA = quantitative analysis of methylated alleles, "/" = There was only one study, and the heterogeneity could not be calculated.
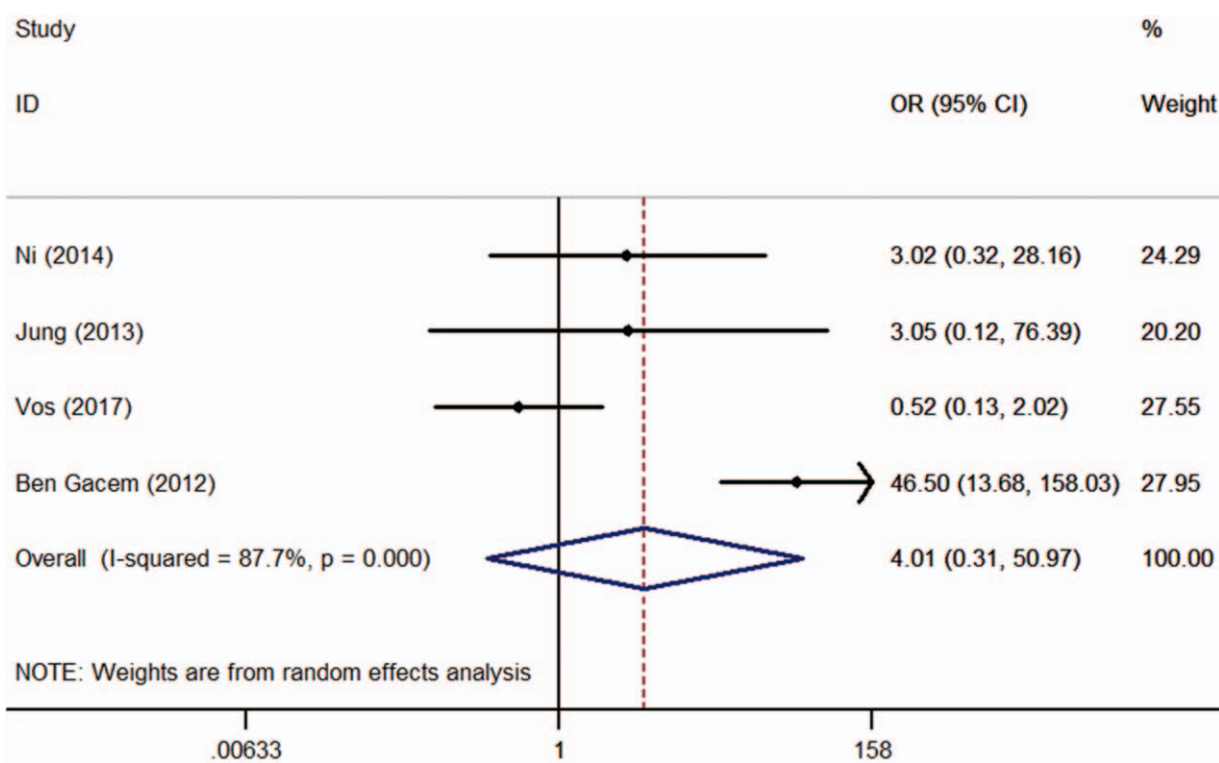

Figure 3. Forest plot for evaluating the relationship between BRCA2 promoter methylation and occurrence risk of breast cancer.
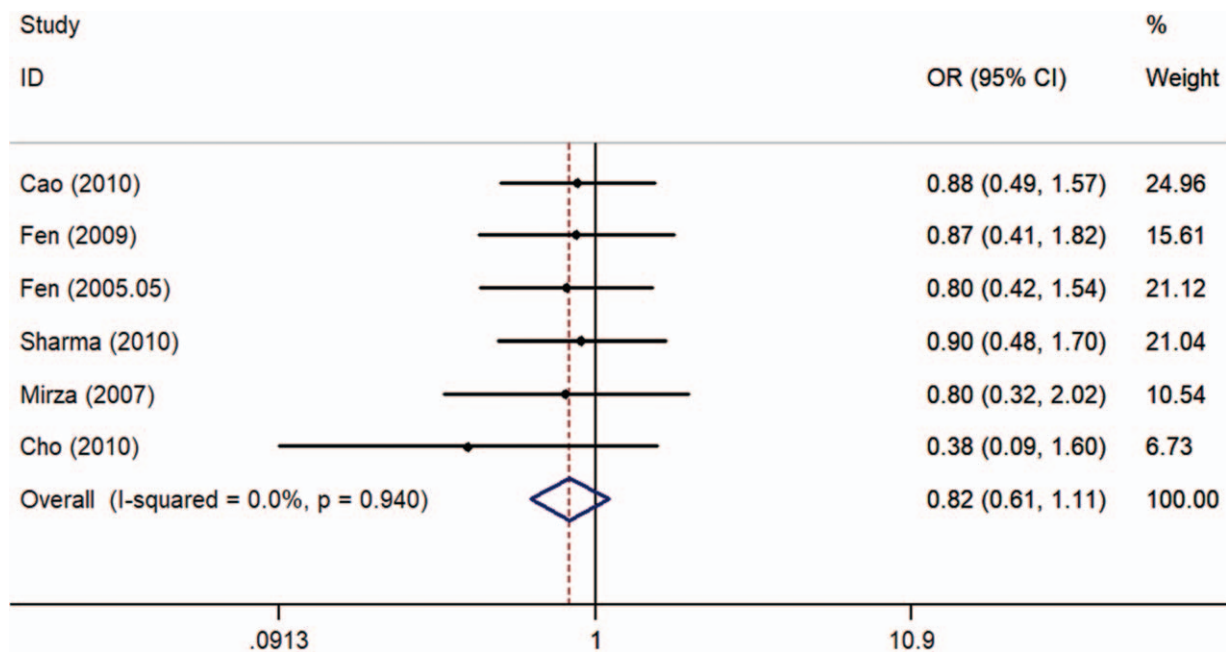
Figure 4. Forest plot that the comparison of BRCA1 gene promoter methylation in peripheral blood and tumor tissues of the same group of tumor patients.

3.4. The comparison of BRCA1 gene promoter methylation in peripheral blood and matched tumor tissue samples

In order to compare the methylation status between BRCA1 promoter in peripheral blood and matched tumor tissue samples, the 6 articles were included in this study,[15,16,18,38,39,46] which met the requirements. Given no heterogeneity found in the studies ($I^2 = 0\%$, $P = .940$), fixed-effects model was used for pooled analysis. The results suggested that there was no statistically significant difference in the level of BRCA1 promoter methylation between peripheral blood and matched tumor tissue samples (OR = 0.82, 95% CI: 0.61–1.11, $P = .199$) (Fig. 4).

3.5. BRCA1 gene promoter methylation and prognosis of breast cancer

The study included 13 datasets of 11 literatures[14,38,60–68] and 9 datasets of 8 literatures[14,38,60–63,65,69] that reported the HR and 95%CI for OS and DFS in breast cancer patients with BRCA1 gene promoter methylation, respectively. Given the heterogeneity found in the studies (OS: $I^2 = 67.4\%$, $P < .001$; DFS: $I^2 = 75.4\%$; $P < .001$, separately), random-effects model was used for pooled analysis. The pooled HR confirmed statistically significant association between BRCA1 gene promoter methylation and poor prognosis of breast cancer (OS: HR = 1.72, 95%CI: 1.19–2.49, $P = 0.004$; DFS: HR = 2.75, 95%CI: 1.80–4.20, $P < .001$, respectively) (Fig. 5).

Further subgroup analysis was conducted because of higher heterogeneity. For OS, the pooled HR of BRCA1 gene promotor methylation in South Asians (HR = 5.19, 95%CI: 2.35–11.45, $P < .001$) was higher than it in East Asians (HR = 2.07, 95%CI: 1.36–3.17, $P = .001$) and Europeans and Americans (HR = 0.74, 95%CI: 0.38–1.47, $P = .394$), and heterogeneity of the group of South Asians ($I^2 = 0\%$, $P = .998$) was significantly reduced. For DFS, the pooled HR of BRCA1 gene promoter methylation in

South Asians (HR = 5.68, 95%CI: 3.20–10.08, $P < .001$) was higher than it in East Asians (HR = 1.58, 95%CI: 1.24–2.00, $P < .001$) and North Africans (HR = 3.67, 95%CI: 1.20–11.20, $P = .022$), and heterogeneity of the group of South Asians ($I^2 = 21.0\%$, $P = .282$) and East Asians ($I^2 = 20.4\%$, $P = .285$) were significantly reduced. The detailed results were summarized in Table 6.

3.6. Sensitivity analysis

Sensitivity analysis for the relationship between BRCA1 gene promoter methylation and occurrence of breast carcinoma and prognosis of breast cancer by alternately omitting one study showed that no individual study influenced the pooled risk estimates.

3.7. Publication bias assessment

We used the Egger linear regression model and Begg funnel plot to test the publication bias. The significant publication bias was found of the association between BRCA1 gene promoter methylation and occurrence of breast carcinoma (Begg test: $P = .202$ and Egger test: $P < .001$, respectively). Trim-and-fill method was used to adjust this bias and calculate the number of unpublished studies that could lead to asymmetry, and 27 articles were added. The publication bias was evaluated using the 81 articles, the change of OR value was small (before and after the filling process: OR = 4.60, 95% CI: 3.21–6.59, $P < .001$ vs OR = 4.42; 95% CI: 2.84–8.33, $P = .029$), suggesting that the results of meta-analysis was reliable and robust (Fig. 6). At the same time, for DFS that the relationship between BRCA1 gene promoter methylation and prognosis of breast cancer, the results of Begg test ($P = .076$) and Egger test ($P = .001$) indicated there were publication bias, but the changes in the estimated HR before and after Trim-and-fill method was small (HR = 2.75, 95%CI: 1.80–4.20, $P < .001$ and HR = 2.75; 95% CI: 1.80–4.20, $P < .001$),
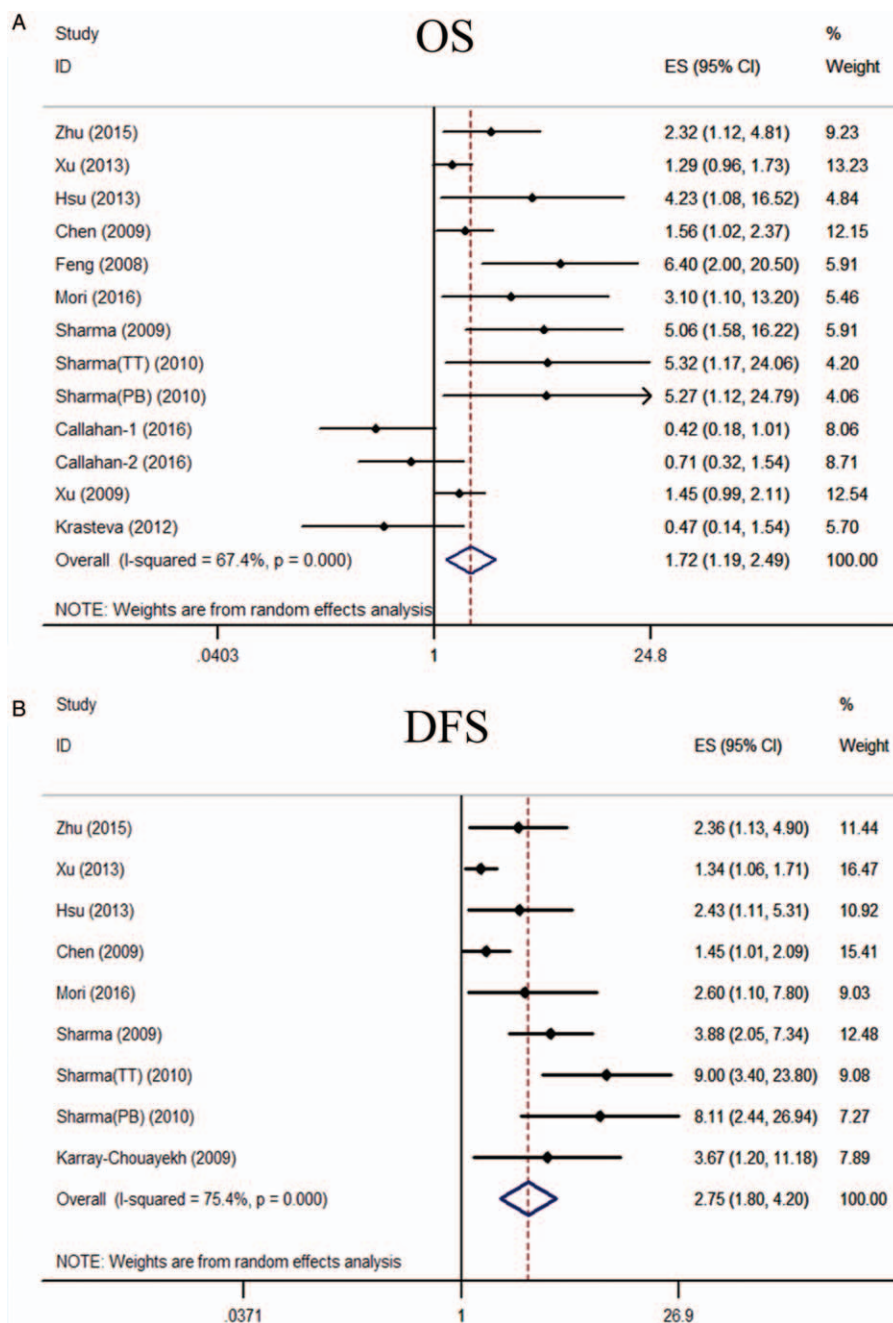
Figure 5. Forest plot for evaluating the relationship between BRCA1 gene promoter methylation and prognosis of breast cancer. A: OS; B: DFS.

suggesting that the meta-analysis was reliable (Fig. 7A). For OS that the relationship between BRCA1 gene promoter methylation and prognosis of breast cancer, we did not find publication bias (Begg test: $P = .161$ and Egger test: $P = .191$, respectively) (Fig. 7B).

4. Discussion

Malignant tumor has become one of the major public health problems that seriously threaten human health, it ranks high among all the causes of death of residents.[9] The incidence and mortality of malignant tumors have been increasing in recent

decades. Breast cancer is the common type of cancer and leading cause of malignant cancer death in women all over the world.[1] Among a large number of genes that have been identified as methylated genes in breast cancer, BRCA1/2 were extensively studied because their multifunctional roles in numerous cellular pathways.

BRCA1 is a nuclear phosphoprotein, which interacts with Rad51, a human homolog of RecA, and the Rad50-MRE11 (Meiotic Recombination-11)-NBS1 (Nijmegen Breakage Syndrome) complex, either in response to DNA damage or during S-Phase of the Cell Cycle. BRCA1, BRCA2 and Rad51 are important in maintaining genomic stability by promoting

Table 6

Stratified analysis of the relationship between the frequency of BRCA1 promoter methylation and prognosis of breast cancer.

Study	N	Z	HR	95%CI	P	I^2	P_H
Total for OS	13	2.90	1.72	1.19–2.49	.004	67.4%	<.001
Region for patients							
East Asia	6	3.36	2.07	1.36–3.17	.001	56.7%	.041
South Asia	3	4.07	5.19	2.35–11.45	<.001	0%	.998
European and America	4	0.85	0.74	0.38–1.47	.394	69.4%	.020
Methods for detecting methylation							
MSP	10	3.75	2.03	1.40–2.94	<.001	61.2%	.006
BSP	1	1.78	0.56	0.31–1.00	.050	0%	.378
MLPA	2	1.96	3.10	0.89–10.74	.074	/	/
Design method of research							
Case-control study	10	1.91	1.43	0.99–2.05	.056	66.2%	.002
Cohort study	3	4.07	5.19	2.35–11.45	<.001	0%	.998
Total for DFS	9	2.90	2.75	1.80–4.20	<.001	75.4%	<.001
Region for patients							
East Asia	5	3.77	1.58	1.24–2.00	<.001	20.4%	.285
South Asia	3	5.94	5.68	3.20–10.08	<.001	21.0%	.282
North Africa	1	2.28	3.67	1.20–11.20	.022	/	/
Design method of research							
Case-control study	6	3.85	1.71	1.30–2.24	<.001	32.7%	.190
cohort study	3	5.94	5.68	3.20–10.08	<.001	21.0%	.282

BSP = bisulfite sequencing PCR, DFS = disease-free survival, MS-HRM = methylation-sensitive high-resolution melting, MS-MLPA = methylation-specific multiplex ligation-dependent probe amplification, MSP = methylation-specific PCR, MTA = methylation target array, N = Number of articles, OS = overall survival, P_H = P of heterogeneity, QAMA = quantitative analysis of methylated alleles, "/" = There was only one study, and the heterogeneity could not be calculated.

homologous recombination repair.[70] BRCA1-BARD1 (BRCA1 Associated Ring Domain-1) co-localizes with DNA replication and repair factors in response to DNA damage.[71] The ring finger of BRCA1 confers ubiquitin ligase activity that is markedly enhanced when complexed with another ring-containing protein, BARD1, and is required for the function of this tumor suppressor protein in protecting genomic integrity.[71] BRCA1 induces GADD45, a p53-regulated and stress-inducible gene that plays an important role in cellular response to DNA damage.[72] BRCA1 acts in concert with STAT1 to differentially activate transcription of a subset of IFN-Gamma target genes and mediates growth inhibition by this cytokine.[73] BRCA1 participating in double strand DNA break repair, genome surveillance, transcription-coupled DNA repair, transcriptional regulation, chromatin
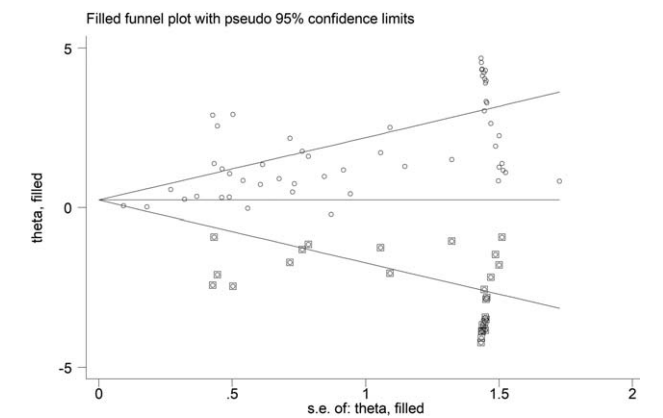

Figure 6. Publication bias test for evaluating the relationship between BRCA1 gene promoter methylation and the occurrence risk of breast cancer using trim-and-fill method.
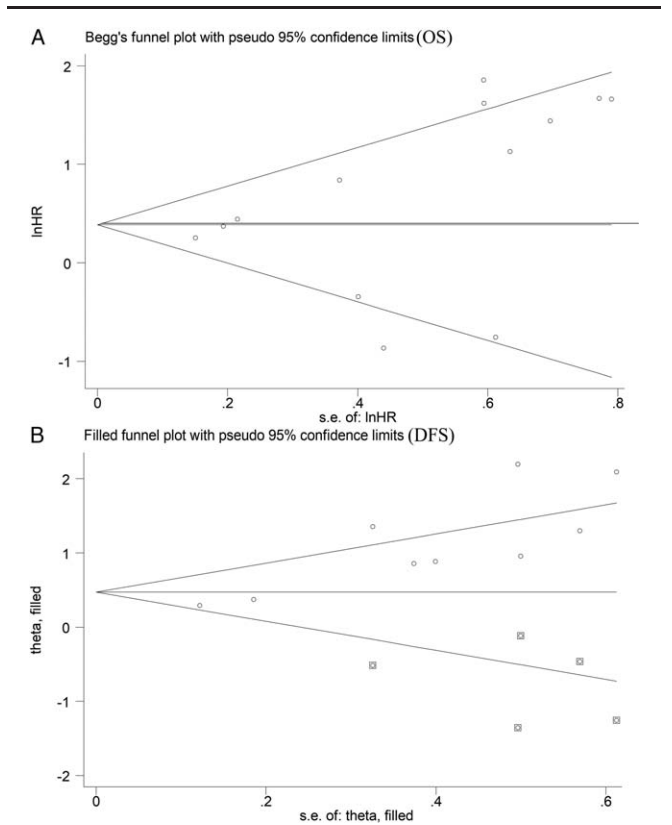

Figure 7. Publication bias test for evaluating the relationship between BRCA1 gene promoter methylation and prognosis of breast cancer using trim-and-fill method. A: OS; B: DFS of trim-and-fill method.

remodeling, ubiquitin ligation and cell cycle checkpoint arrests through several mechanisms described above.[73] BRCA1 is a tumor suppressor gene implicated in the predisposition to breast and ovarian cancer. BRCA1 promoter methylation could silence BRCA1, and associated with reduced mRNA in tumor, thus it further leading to the occurrence of tumors.[42]

Several studies on the relationship between BRCA1 promoter methylation and breast cancer have been conducted. Paydar et al[12] found that the methylation levels of BRCA1 and MGMT in breast cancer tissues were significantly higher than those in non-tumor tissues, BRCA1 promoter was methylated in 44.4% of malignant specimens compared with 9.7% in benign specimens ($P = .005$). Shakeri et al[42] found that the methylation rate of BRCA1 was 9.33% and APC was 30.67% in breast tumor tissues, while BRCA1 and APC promoter were unmethylated in the normal samples ($P < .05$). Vu et al[11] confirmed that the methylation frequencies of BRCA1 detected from 79 breast tumor samples were 58.23%, while those detected from 79 matched normal adjacent tissue samples were 51.90%, and the difference concerning BRCA1 promoter methylation frequency between these 2 tissues was not statistically significant ($P = .424$). Meanwhile, Parrella et al[59] demonstrated that BRCA1 promoter methylation frequency in breast cancer tissues was lower than those in benign breast tissue (16.7% vs 20%). Kontorovich et al[48] also verified that BRCA1 promoter methylation level in breast cancer tissues was slightly lower than those in normal breast tissues (6% vs 6.2%). These findings suggested that the role of BRCA1 promoter methylation in breast cancer remains controversial. So, in present meta-analysis, we explored the potential relationship between BRCA1 promoter methylation and breast carcinoma. We found that individuals with BRCA1 promoter methylation may had higher risk of breast carcinoma, and the frequency of BRCA1 promoter methylation was significantly higher in breast carcinoma tissues (OR = 4.60, 95% CI: 3.21–6.59, $P < .001$) (Fig. 2). At the same time, the methylation frequency of BRCA2 promoter in breast cancer tissues was higher than those in the non-tumor tissues, but which showed no statistically significant difference (OR = 4.01, 95% CI: 0.31–50.97, $P = .285$) (Fig. 3). Therefore, BRCA1/2 promoter methylation may serve as a biological predictor of breast cancer.

The literature on new circulating tumor biomarkers for breast cancer has increased in recent years. BRCA1 mRNA was released from tumors into the circulation. Compared to tissue biopsies, use of BRCA1 mRNA in peripheral blood allows minimally invasive access and ease of serial monitoring.[57] In present meta-analysis, we included 6 literatures with both BRCA1 promoter methylation frequency in peripheral blood and tumor tissue, the results showed that there was no statistically significant difference in the methylation frequency of BRCA1 promoter between peripheral blood and matched tumor tissue samples (OR = 0.82, 95% CI: 0.61–1.11, $P = .199$) (Fig. 4). The conclusion might provide a new idea for the rapid screening of breast cancer. Some other potential clinical utilities were existent for BRCA1 gene promoter methylation in peripheral blood in patients with breast cancer. For example, monitoring of BRCA1 promoter methylation in peripheral blood, it can preclinically signal the emergence of early recurrence in asymptomatic patients who have undergone curative surgery for primary breast cancer, identify mechanisms of acquired resistance to ongoing treatments, and monitor response to therapy in patients.[74–76]

BRCA1 interacts with Chk1 and PLK1 (Polo-Like Kinase-1) to regulate the G2/M and G1/S checkpoints, possibly via GADD45; thereby linking BRCA1 to the regulation of apoptosis.[77] Methylation of the BRCA1 gene promoter may result in the loss of this function of BRCA1, which in turn affects the prognosis of patients. Hsu et al[62] found that the mutation of BRCA1 gene does not play a significant role in the occurrence of breast carcinoma in Taiwanese population, but BRCA1 gene promoter methylation play a pivotal role in occurrence and prognosis of breast carcinoma, and BRCA1 gene promoter methylation was detected in 78 (56%) of the 139 tumors. BRCA1 promoter methylation was significantly associated with poor overall survival (HR = 4.23, $P = .026$) and poor disease-free survival (HR = 2.43, $P = .001$). Sharma et al[38] detected frequency of BRCA1 gene promoter methylation in patients with breast cancer, and found that the negative correlation between methylation frequency of BRCA1 gene promoter and OS and DFS. However, the study of Callahan et al[66] showed that BRCA1 gene promoter methylation could decrease risk of breast cancer related mortality. Meanwhile, Krasteva et al[68] also confirmed that the positive correlation between the methylation status of BRCA1 gene promoter and mortality risk of patients of breast cancer. This controversy might affect the treatment of breast cancer patients, meta-analysis was used to pool all relevant studies. The pooled HR suggested statistically significant association between BRCA1 gen promoter methylation and poor prognosis of breast cancer (OS: HR = 1.72, 95%CI: 1.19–2.49, $P = .004$; DFS:HR = 2.75, 95%CI: 1.80–4.20, $P < .001$, respectively) (Fig. 5). Therefore, BRCA1 gene promoter methylation status has been considered as a potential biomarker for prognosis of breast cancer.

A meta-analysis can aggregate different findings to improve statistical value and provide more accurate and reliable estimates of risk. However, there were still some limitations in this meta-analysis. On the one hand, despite the fact that all studies met the inclusion criteria, there was large heterogeneity in the outcome of the association between BRCA1 gene promoter methylation and risk of breast carcinoma. Nevertheless, stratification analyses based on sample materials, methods for detecting methylation, region for patients and design method of research showed to reduce the degree of heterogeneity among studies, and sensitivity analysis showed that heterogeneity did not affect the stability of meta-analysis results. On the other hand, some of the original studies included did not provide data on tumor stage, age, and chemotherapy regimens, therefore, we failed to conduct a comprehensive subgroup analysis to explore the sources of heterogeneity.

In conclusion, the results of this meta-analysis indicated that BRCA1 gene promoter methylation is statistically significant association with the occurrence of breast cancer. Meanwhile, the prognostic analysis showed that BRCA1 gene promoter methylation was significantly associated with poor overall survival and poor disease-free survival, suggesting that BRCA1 gene promoter methylation could leads to shorter overall survival and disease-free survival. Finally, our study verified that there was no statistically significant difference in the methylation frequency of BRCA1 gene promoter between peripheral blood and matched tumor tissue samples that suggests detection of BRCA1 promoter methylation in peripheral blood may be a non-invasive and rapid way to monitor the occurrence, recurrence, treatment effect and prognosis of breast cancer. So, the methylation frequency of BRCA1 gene promoter may be a

potential biomarker for the diagnosis and prognosis of breast cancer. Our findings may provide a new theoretical basis for the diagnosis and targeted therapy of breast cancer.

Author contributions

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Formal analysis: Shu Li.
Methodology: Shu Li.
Software: Shu Li, Qiuhong Li.
Writing – original draft: Shu Li, Qiuhong Li.
Writing – review & editing: Shu Li.

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