Association of BRCA1 promoter methylation with sporadic breast cancers: Evidence from 40 studies

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Breast cancer susceptibility gene 1 (BRCA1) located at chromosome 17q12-21 is a classic tumor suppressor gene, and has been considered as a significant role in hereditary breast cancers. Moreover, numerous studies demonstrated the methylation status of CpG islands in the promoter regions of BRCA1 gene was aberrant in patients with sporadic breast tumors compared with healthy females or patients with benign diseases. However, these conclusions were not always consistent. Hence, a meta-analysis was performed to get a more precise estimate for these associations. Crude odds ratio with 95% confidence interval were used to assess the association of BRCA1 promoter methylation and the risk or clinicopathologic characteristics of breast cancers under fixed or random effect model. A total of 40 studies were eligible for this present study. We observed the frequency of BRCA1 promoter methylation was statistically significant higher in breast cancers than non-cancer controls. Furthermore, BRCA1 methylation was statistically associated with lymph node metastasis, histological grade 3, ER(-), PR(-), triple-negative phenotype, and decreased or lack levels of BRCA1 protein expression. In conclusion, this study indicated that BRCA1 promoter methylation appeared to be a useful predictive or prognostic biomarker for breast cancers in clinical assessment.

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always consistent. Therefore, we performed a meta-analysis to evaluate whether BRCA1 gene promoter methylation is a risk factor for sporadic breast cancers, and elucidated the association of BRCA1 promoter methylation with clinicopathological characteristics in patients with breast cancer.

Materials and Methods

Literature search strategy. We performed a comprehensive literature search from PubMed and EMBASE database (last search conducted in August 2015) without language restrictions. The following search terms were used, (“BRCA1” or “Breast cancer susceptibility gene 1”) and (“methylation” or “DNA methylation” or “promoter methylation”) and (“breast cancer” or “breast carcinoma” or “breast tumor” “breast carcinogenesis”). In addition, we carried out a manual search for other relevant articles via the reference lists of eligible studies.

Selection criteria. Eligible studies had to meet the following predefined criteria, (1) case-control studies evaluating the association between the prevalence of BRCA1 promoter methylation and sporadic breast cancer risk; or clinical cohort studies evaluating the associations of BRCA1 promoter methylation with clinicopathological features of sporadic breast cancer; (2) sufficient published data for calculating an odds ratio (OR) and 95% confidence interval (CI); (3) studies were confined to human female groups. It’s noteworthy that if the same study population was included in several different studies, we would only bring the most recent or comprehensive study into the meta-analysis.

Data extraction. A standard protocol was applied to extract data. For every eligible study, the following data were extracted: the first author's name, publication year, original country, methods for detecting methylation, the frequency of BRCA1 promoter methylation in case and control groups, control characteristics, sample materials and so on.

Statistical methods. The strength of the association between the BRCA1 promoter methylation and sporadic breast cancer risk or clinicopathological features was assessed by OR with corresponding 95%CI. A chi-square-based Q test was applied to test heterogeneity among studies. The p value of the Q test was >0.1, which suggested a lack of statistically significant heterogeneity, and we used the fixed-effect model (Mantel-Haenszel method)20 to calculate pooled ORs. Otherwise, heterogeneity was present and the random-effect model (DerSimonian-Laird method)21 was more appropriate. Additionally, the degree of heterogeneity was also quantitatively assessed by I²-test, which the value of I² ranged from 0 to 100% and was generally considered mild heterogeneity for I² < 25%, moderate heterogeneity for 25%–50%, large heterogeneity for 50%–75%, and extreme heterogeneity for I² > 75%22. Moreover, stratified-analyses were conducted based on ethnicity, methods for detecting methylation and sample materials to explore the potential source of heterogeneity. Sensitivity analyses, by which each study was omitted in each turn to confirm the influence of individual data set to the pooled OR, were implied to evaluate the robustness of the results. Furthermore, we estimated potential publication bias with funnel plot and Egger’s linear regression test. The funnel plot was visual symmetrical and the P-value of Egger's test was greater than 0.05, which indicated that there were no statistically significant publication bias. All statistical tests in the meta-analysis were two-tailed and P-value ≤ 0.05 was considered statistically significant unless otherwise noted. Statistical analyses were performed with Review Manager 5.2 software recommended by Cochrane Collaboration and STATA software version 12.0.

Results

Study Characteristics. Based on the above selection criteria, 20 case-control studies, involving 2747 cases and 2256 controls4–15,23–33 were included to analyze the association between BRCA1 promoter methylation and sporadic breast cancer risk. Among these studies, 12 studies4–15,17,23–28,32 confirmed the status of BRCA1 methylation in tissues derived from breast carcinoma, benign disease or normal breast epithelium. And the objects analyzed for the frequency of BRCA1 promoter methylation in breast cancers compared with non-cancer controls, and 2256 controls2–6,10,15–17,23–33, were included to analyze the association between BRCA1 promoter methylation with clinicopathological characteristics in patients with breast cancer. Due to the different type of sample materials, we considered this study as two case-control studies. Furthermore, 30 clinical studies3–5,6,12–14,16,18,27,29–49 with a total of 5058 breast cancer patients met our selection criteria for analyzing the association between BRCA1 promoter methylation and clinicopathological characteristics which included early age (<50 years) at diagnosis, premenopausal status, lymph node metastasis, histological grade 3, estrogen receptor (ER), progesterone receptor (PR), human epidermal receptor 2 (Her2), triple-negative phenotype and the expression of BRCA1 protein. In short, our meta-analysis included 40 eligible articles, among which 20 articles were analyzed for the frequency of BRCA1 promoter methylation in breast cancers compared with non-cancer controls, and 30 articles were analyzed for the association between BRCA1 promoter methylation and clinicopathological features. What was noteworthy was that 10 articles1,4,5,6,12,16,17,27,29–33 did not only studied the prevalence of BRCA1 promoter methylation, but also elaborated clinicopathological characteristics in breast cancer patients with BRCA1 promoter methylation versus BRCA1-unmethylated tumors. The flow diagram of study selection procedure was shown in Fig. 1. Every study characteristics were summarized in Tables 1 and 2.

Meta-analysis results. Association between BRCA1 promoter methylation and the risk of breast cancer. In general, our study indicated that the frequency of BRCA1 promoter methylation was statistically significant elevated in breast cancers compared with non-cancer controls (OR = 3.15, 95%CI 1.97–5.03, P < 0.001, Fig. 2). Because of large heterogeneity (P<0.001, I² = 74%), we explored the potential source of heterogeneity via stratified analysis based on sample materials, methods for detecting methylation and ethnicity. In the subgroup analysis about sample materials, the pooled OR for BRCA1 methylation in breast cancer tissues compared with normal or benign tissues was 4.75 (95%CI 2.37–9.54, P < 0.001), and was higher than the pooled OR (OR = 1.87, 95%CI 1.19–2.96, P = 0.007) in peripheral blood of breast cancers compared with non-cancer controls. Furthermore, the frequency
of BRCA1 methylation by MSP method (OR = 6.79, 95%CI 3.05–15.11, P < 0.001) was significantly higher than other methods (OR = 1.53, 95%CI 1.09–2.14, P = 0.01). Meanwhile, the prevalence of BRCA1 methylation in Asians (OR = 4.03, 95%CI 1.07–15.18, P = 0.04) was higher than that in Caucasians (OR = 3.16, 95%CI 1.78–5.62, P < 0.001) and in Australians (OR = 3.27, 95%CI 1.37–7.84, P = 0.008) in breast cancers compared with non-cancer controls. It’s worth mentioning that the degree of heterogeneity was apparently reduced in stratified analysis. The detailed results were summarized in Table 3.

**Association of BRCA1 promoter methylation with clinicopathological features of breast cancer.** The results of our meta-analysis showed that the BRCA1 promoter methylation was statistically significant correlated with lymph node metastasis (OR = 1.25, 95%CI 1.06–1.48, P = 0.009, Fig. 3) and histological grade 3 (OR = 2.29, 95%CI 1.65–3.18, P < 0.001, Fig. 4), but had no correlation with early age (<50 years) at diagnosis (OR = 1.21, 95%CI 0.98–1.50, 2014 Poland 15 51 2 34 MS-HRM Blood Female without breast cancers

Bosviel. R^2^ 2012 France 425 477 454 516 QAMA Blood Female without breast cancers

Sturgeon. SR^3^ 2012 USA 192 112 147 87 Pyrosequencing Blood Benign breast disease controls

Al-moghrabi. N^{40} 2011 Saudi Arabic 2 5 8 65 MSP Blood Healthy female

Iwamoto. T^{39} 2011 Japan 43 157 27 173 QMSP Blood Female without breast cancers

Wong. EM^{11} 2011 Australia 28 227 6 163 MS-HRM Blood Female without breast cancers

Cho. YH^{17} 2010 USA 3 37 2 38 MethyLight Blood Ethnicty-matched healthy female

Cho. YH^{17} 2010 USA 7 33 2 25 MethyLight Tissue Paired adjacent normal breast tissue

Jing. F^{9} 2010 China 17 33 0 50 MSP Blood Female without breast cancers

Sharma. G^{29} 2010 India 25 75 0 30 MSP Blood Healthy female

Hasan. TN^{9} 2013 India 9 20 4 22 MSP Tissue Normal breast biopsies

Jung. El^{7} 2013 Korea 6 54 3 57 MS-MLPA Tissue Paired adjacent normal breast tissue

Ben Gacem. R^{32} 2012 Tunisia 71 46 5 60 MS-MLPA Tissue Paired adjacent normal breast tissue

Bhavani. V^{18} 2009 India 21 83 2 46 MS-MLPA Tissue Paired adjacent normal breast tissue

Buyru. N^{11} 2009 Turkey 4 73 0 77 MS-MLPA Tissue Paired adjacent normal breast tissue

Wei. M^{27} 2005 USA 39 92 0 3 MSP Tissue Paired adjacent normal breast tissue

Parrella. P^{26} 2004 Italy 9 45 2 8 MSP Tissue Benign breast disease

Chen. CM^{9} 2003 China 21 72 0 20 MSP Tissue Paired adjacent normal breast tissue

Jeronimo C^{25} 2003 Portugal 11 16 0 12 MSP Tissue Paired adjacent normal breast tissue

Esteller. M^{28} 2000 USA 11 73 0 84 MSP Tissue Paired adjacent normal breast tissue

Debrovic. A^{23} 1997 Australia 2 5 0 2 Southern blotting Tissue Normal breast biopsies

| First author | Year | Country | Case | Control | Methods | Materials | Control characteristics |
|--------------|------|---------|------|---------|---------|-----------|-------------------------|
| Gupta. S^{33} | 2014 | Poland  | 15   | 51      | MS-HRM  | Blood     | Female without breast cancers |
| Bosviel. R^{2} | 2012 | France  | 425  | 477     | QAMA    | Blood     | Female without breast cancers |
| Sturgeon. SR^{3} | 2012 | USA     | 192  | 112     | Pyrosequencing | Blood | Benign breast disease controls |
| Al-moghrabi. N^{40} | 2011 | Saudi Arabic | 2  | 5       | MSP     | Blood     | Healthy female |
| Iwamoto. T^{39} | 2011 | Japan   | 43   | 157     | QMSP    | Blood     | Female without breast cancers |
| Wong. EM^{11} | 2011 | Australia | 28  | 227     | MS-HRM  | Blood     | Female without breast cancers |
| Cho. YH^{17} | 2010 | USA     | 3    | 37      | MethyLight | Blood | Ethnicty-matched healthy female |
| Cho. YH^{17} | 2010 | USA     | 7    | 33      | MethyLight | Tissue | Paired adjacent normal breast tissue |
| Jing. F^{9} | 2010 | China   | 17   | 33      | MSP     | Blood     | Female without breast cancers |
| Sharma. G^{29} | 2010 | India   | 25   | 75      | MSP     | Blood     | Healthy female |
| Hasan. TN^{9} | 2013 | India   | 9    | 20      | MSP     | Tissue    | Normal breast biopsies |
| Jung. El^{7} | 2013 | Korea   | 6    | 54      | MS-MLPA | Tissue    | Paired adjacent normal breast tissue |
| Ben Gacem. R^{32} | 2012 | Tunisia | 71   | 46      | MS-MLPA | Tissue    | Paired adjacent normal breast tissue |
| Bhavani. V^{18} | 2009 | India   | 21   | 83      | MS-MLPA | Tissue    | Paired adjacent normal breast tissue |
| Buyru. N^{11} | 2009 | Turkey  | 4    | 73      | MS-MLPA | Tissue    | Paired adjacent normal breast tissue |
| Wei. M^{27} | 2005 | USA     | 39   | 92      | MSP     | Tissue    | Paired adjacent normal breast tissue |
| Parrella. P^{26} | 2004 | Italy   | 9    | 45      | MSP     | Tissue    | Benign breast disease |
| Chen. CM^{9} | 2003 | China   | 21   | 72      | MSP     | Tissue    | Paired adjacent normal breast tissue |
| Jeronimo C^{25} | 2003 | Portugal | 11  | 16      | MSP     | Tissue    | Paired adjacent normal breast tissue |
| Esteller. M^{28} | 2000 | USA     | 11   | 73      | MSP     | Tissue    | Paired adjacent normal breast tissue |
| Debrovic. A^{23} | 1997 | Australia | 2  | 5       | Southern blotting | Tissue | Normal breast biopsies |

Table 1. Characteristics of studies included for the association between BRCA1 promoter methylation and breast cancer risk in the meta-analysis. M+: methylated; M−: unmethylated; MSP: methylation-specific PCR; MS-HRM: methylation-sensitive high-resolution melting; QMSP: methylation-specific quantitative PCR; QAMA: quantitative analysis of methylation alleles; MS-MLPA: methylation-specific multiplex ligation-dependent probe amplification.
P = 0.07, Fig. 5) or premenopausal status (OR = 1.21, 95%CI 0.98–1.50, P = 0.08, Fig. 6). As for hormone receptors, strong associations of \( \text{BRCA1} \) methylation were found with ER negative (OR = 2.36, 95%CI 1.67–3.33, \( P < 0.001 \), Fig. 7) and also with PR negative (OR = 2.14, 95%CI 1.49–3.07, \( P < 0.001 \), Fig. 8). In contrast, no association was found between \( \text{BRCA1} \) methylation and Her2 status (OR = 1.58, 95%CI 0.98–2.56, P = 0.06, Fig. 9). Interestingly, our study confirmed that the \( \text{BRCA1} \) promoter methylation was positively correlated with triple-negative phenotype (OR = 2.79, 95%CI 1.74–4.48, \( P < 0.001 \), Fig. 10). Furthermore, it’s worth mentioning that there was statistically significant correlation between \( \text{BRCA1} \) methylation and decreased or lack expression levels of \( \text{BRCA1} \) protein (OR = 4.44, 95%CI 2.56–7.70, \( P < 0.001 \), Fig. 11). The detailed results were summarized in Table 4.

**Sensitivity analyses and publication bias.** We performed sensitivity analyses to assess the robustness of the meta-analysis results by omitting each study in turn and no single study could essentially change the results. And the sensitivity analyses demonstrated that the results of our meta-analysis were statistically stable. Figure 12 showed the plot of sensitivity analysis for evaluating the association between \( \text{BRCA1} \) promoter methylation and breast cancer risk. The shapes of funnel plots and Egger’s linear regression test were used to evaluate the publication bias of the eligible literatures. In general, the funnel plots were not entirely symmetrical and the \( P \)-value of Egger’s test was not always greater than 0.05, indicating there was publication bias in our study. The funnel plot for evaluating the association of \( \text{BRCA1} \) promoter methylation with breast cancer risk was shown in Fig. 13 and the detailed results for \( P \)-value of Egger’s test were summarily in Table 3 and Table 4.

**Discussion**

As is well-known that breast carcinoma is a heterogeneous group of tumors. It’s essential to find a reliable biomarker for the early diagnosis and prognosis prediction of breast cancer. In the meta-analysis, the strength of relationships...
Figure 2. Forest plot for evaluating the association between BRCA1 promoter methylation and breast cancer risk. Random-effect model was used for the analysis.

| Study or Subgroup | breast cancer | OR 95%CI | P | Pm | I² | Pbias |
|-------------------|---------------|----------|---|----|----|-------|
| A-Moghaddam 2011  | 2             | 7        | 8 | 73 | 4.2% | 3.25 [0.54, 19.60] |
| Ben Gacem 2012    | 71            | 117      | 5 | 65 | 7.3% | 18.52 [0.92, 49.59] |
| Bhawari 2009      | 21            | 104      | 2 | 48 | 5.1% | 5.82 [1.31, 25.94] |
| Bosveld 2012      | 425           | 902      | 454 | 990 | 10.6% | 1.05 [0.88, 1.26] |
| Buyu 2009         | 4             | 77       | 0 | 77 | 2.1% | 9.49 [0.50, 179.35] |
| Chen 2003         | 21            | 93       | 0 | 20 | 2.2% | 12.18 [0.71, 209.44] |
| Choc 2010         | 7             | 40       | 2 | 40 | 4.0% | 1.54 [0.24, 9.75] |
| Choc 2010         | 7             | 40       | 2 | 27 | 4.6% | 2.65 [0.51, 13.88] |
| Dobrovič 1997     | 2             | 7        | 0 | 2 | 1.6% | 2.27 [0.08, 67.05] |
| Esteller 2000      | 11            | 84       | 0 | 84 | 2.2% | 26.44 [1, 456.50] |
| Gupta 2014        | 15            | 66       | 2 | 36 | 5.0% | 5.00 [1.07, 23.27] |
| Hasan 2013        | 9             | 39       | 4 | 26 | 5.8% | 2.46 [0.66, 9.31] |
| Iwamoto 2011      | 43            | 200      | 27 | 200 | 9.5% | 1.75 [1.04, 2.97] |
| Jeronimou 2003     | 11            | 27       | 0 | 12 | 2.1% | 17.42 [0.93, 324.77] |
| Jing 2010         | 17            | 50       | 0 | 50 | 2.2% | 52.78 [3.07, 907.44] |
| Jung 2013         | 6             | 60       | 3 | 60 | 5.3% | 2.11 [0.50, 8.87] |
| Parreiras 2004     | 9             | 54       | 2 | 10 | 4.4% | 0.80 [0.16, 4.41] |
| Sharma 2010       | 25            | 100      | 0 | 30 | 2.2% | 20.60 [1.22, 349.21] |
| Stangor 2012      | 192           | 304      | 147 | 234 | 10.1% | 1.01 [0.71, 1.44] |
| Wei 2005          | 3             | 39       | 0 | 3 | 2.0% | 2.99 [0.15, 59.24] |
| Wong 2011         | 28            | 255      | 6 | 169 | 7.7% | 3.35 [1.36, 8.28] |
| Total             | 2747          | 2258     | 100% | 3.15 [1.97, 5.03] |

Total events 961 664
Heterogeneity: Tau² = 0.53; Chi² = 76.38; df = 20 (P < 0.0001); I² = 74%
Test for overall effect: Z = 4.80 (P < 0.00001)

Table 3. Stratified analysis of the frequency of BRCA1 promoter methylation in breast cancers compared with non-cancer controls. N: the total number of eligible studies; Pm: the p-value of Q test for heterogeneity among studies. Pbias: the p-value of Egger linear regression test for evaluating publication bias.

| Study or Subgroup | methylated | unmethylated | OR 95%CI | P | Pm | I² | Pbias |
|-------------------|------------|--------------|----------|---|----|----|-------|
| A-Moghaddam 2011  | 9           | 3            | 17       | 0.7% | 1.33 [0.18, 9.91] |
| Ben Gacem 2012    | 11          | 18           | 34       | 1.6% | 1.07 [0.27, 4.17] |
| Bhawari 2009      | 9            | 78           | 14       | 61 | 5.7% | 0.44 [0.18, 1.05] |
| Bosveld 2012      | 25           | 86           | 10       | 46 | 3.6% | 1.48 [0.64, 3.42] |
| Buyu 2009         | 7            | 31           | 35       | 131 | 4.2% | 0.80 [0.32, 2.02] |
| Chen 2003         | 5            | 44           | 137      | 3.0% | 0.61 [0.27, 2.42] |
| Choc 2010         | 19           | 33           | 34       | 69 | 3.0% | 1.40 [0.61, 3.22] |
| Choc 2010         | 3            | 6            | 26       | 54 | 11% | 1.08 [0.20, 5.82] |
| Dobrovič 1997     | 1            | 15           | 2        | 15 | 0.8% | 0.48 [0.04, 5.75] |
| Esteller 2000      | 18           | 192          | 63       | 112 | 7.8% | 1.62 [0.93, 2.85] |
| Gupta 2014        | 21           | 36           | 43       | 86 | 4.3% | 1.40 [0.64, 3.07] |
| Hasan 2013        | 30           | 202          | 19       | 125 | 6.7% | 2.36 [1.33, 4.19] |
| Iwamoto 2011      | 131          | 292          | 340      | 869 | 38.4% | 1.27 [0.97, 1.65] |
| Jeronimou 2003     | 6            | 11           | 16       | 58 | 9.9% | 3.15 [0.84, 11.78] |
| Jing 2010         | 192          | 304          | 147      | 234 | 10.1% | 1.01 [0.71, 1.44] |
| Jung 2013         | 131          | 292          | 340      | 869 | 38.4% | 1.27 [0.97, 1.65] |
| Parreiras 2004     | 9            | 54           | 2        | 10 | 4.4% | 0.80 [0.16, 4.41] |
| Sharma 2010       | 25           | 100          | 0        | 30 | 2.2% | 20.60 [1.22, 349.21] |
| Stangor 2012      | 192          | 304          | 147      | 234 | 10.1% | 1.01 [0.71, 1.44] |
| Wei 2005          | 3            | 39           | 0        | 3 | 2.0% | 2.99 [0.15, 59.24] |
| Wong 2011         | 28            | 255          | 6        | 169 | 7.7% | 3.35 [1.36, 8.28] |
| Total             | 1223         | 1970         | 100% | 1.25 [1.06, 1.48] |

Total events 537 757
Heterogeneity: Chi² = 17.27; df = 16 (P = 0.31); I² = 7%
Test for overall effect: Z = 2.62 (P = 0.009)

Figure 3. Forest plot for evaluating the association between BRCA1 promoter methylation and lymph node metastasis. Fix-effect model was used for the analysis.
of BRCA1 promoter methylation with breast cancer risk and its clinicopathological features were systematically investigated. The results of our study confirmed that BRCA1 promoter methylation was significantly correlated with the increased risk of breast cancer and associated with lymph node metastasis, histological grade 3, ER(-), PR(-), triple-negative phenotype and BRCA1 protein expression, which indicated that BRCA1 promoter methylation may be utilized as an effective biomarker in the management of breast tumors.

Our meta-analysis demonstrated that the prevalence of BRCA1 promoter methylation in the breast cancer group was statistically significant elevated in comparison with the control group. This suggested a possibility that...
aberrant methylation of BRCA1 promoter was correlated with an increased risk of breast cancer. And this almost was in line with the report by Wong et al. which confirmed that BRCA1 methylation in peripheral blood DNA was correlated with 3.5-fold evaluated risk of having early-onset breast tumors. In the stratification analysis based on sample materials, the summary OR was 4.75 in tissues and 1.87 in peripheral bloods, indicating that the association of BRCA1 methylation with breast cancer risk in tissues was stronger than in peripheral bloods. Because there was only one study in which the tissue of the control group was derived from patients with benign breast diseases, we omitted the study to evaluate the frequency of BRCA1 promoter methylation in breast cancer tissues compared with normal breast tissues, and the pooled OR was 7.23 (95% CI 4.35–12.01, P < 0.00001), which showed that the
frequency of BRCA1 methylation in breast carcinoma tissues was 7.23-fold higher than that in normal breast tissues. The result demonstrated that the difference for the frequency of BRCA1 methylation between breast tumors and non-cancerous breast tissues group was smaller than that between cancers and normal breast tissues group. Therefore, it suggested that normal breast tissues had a lower prevalence of BRCA1 methylation than benign and malignant breast tissues, which also implied that the methylation of BRCA1 gene promoter may play a certain role in the initiation of breast carcinoma. Similarly, a recent research confirmed that the BRCA1 promoter methylation of histological normal breast epithelial cells may result in BRCA1-methylated breast tumors\(^47\). Additionally, our study demonstrated that the methylation of BRCA1 promoter in peripheral blood DNA was correlated with a 1.87-fold increased risk of breast cancer, which was accordance with the result of a previous study\(^16\).
of extracting DNA from peripheral blood is simple and barely invasive for detecting the methylation of BRCA1 promoter. Therefore, the finding could open a new avenue for screening the risk of breast cancer.

In the subgroup analysis based on ethnicity, there were 15 studies in Caucasians, 4 in Asians and 2 in Australians for the association of BRCA1 methylation with breast cancer risk. Meanwhile, significant difference in the level of BRCA1 methylation was found in Caucasians (OR = 3.16), Asians (OR = 4.03) and Australians (OR = 3.27) in the cancer group compared with the control group, which suggested that different ethnicity and environment had certain impact on the prevalence of BRCA1 methylation. Additionally, the results of stratified analysis based on methods showed that the different methods had different efficiency for detecting methylation. It’s essential to find an appropriate method based on feasibility of clinical practice in order to apply BRCA1 methylation as a biomarker in clinical management.

In our meta-analysis, 18 articles discussed the association of BRCA1 methylation with age at diagnosis in breast cancers. However, it’s meaningless to extract data from these articles to calculate the pooled OR and 95%CI on account of the difference in the definition of early age among these studies. Nevertheless, due to 10 articles considering early age as less than 50 years, we then investigated the correlation between BRCA1 methylation and early age (<50 years) at diagnosis. However, there was no statistically significant association. In addition, no association between BRCA1 methylation and premenopausal status was observed in our study. This was inconsistent with a previous report which showed that BRCA1-methylated breast cancers tended to occur at an early age (<50 years) and were more frequently observed in premenopausal or perimenopausal women than postmenopausal women. Furthermore, our results showed that BRCA1 promoter methylation was strongly related to breast cancer patients with high histological grade and lymph node metastasis, revealing that aberrant methylation of BRCA1 promoter may be implicated in the invasion and metastasis of breast cancer. In this sense, a report investigated the methylation profiles of 12 genes in the matched axillary lymph nodes compared with primary tumor tissues and the adjacent normal tissues from the same breast cancer patients, and demonstrated that the proportion of
BRCA1 methylation was higher in the matched axillary lymph nodes metastasis than normal tissue. Thus, a hypothesis may be proposed that the methylation status of BRCA1 promoter may be served as a biomarker for screening metastasis in breast tumors.

As expected from previous studies, we demonstrated that there was a remarkable correlation between BRCA1 promoter hypermethylation and breast tumors with lack of ER and PR expression. However, no inverse relationship was found between BRCA1 methylation and Her2 status. Interestingly, it's noteworthy that the triple negative phenotype was more common in BRCA1-methylated breast cancers than unmethylated tumors, which also was in agreement with numerous reports. Altogether, these indicate that the patients with BRCA1-methylated breast tumors are likely to have little benefit from traditional hormone or targeted therapies. Additionally, a considerable amount of researches investigated that hypermethylation of BRCA1 promoter resulted in the down-regulation of BRCA1 expression. Likewise, a statistically significant association of BRCA1 methylation with lack or decreased expression of BRCA1 protein was confirmed in our study. It's interesting to note that there were positive and negative methylation-expression relationships in diverse gene regions, which differently affected genes expression and prognosis in breast cancer subtypes. Therefore, it is reasonably predicted that methylation of BRCA1 different promoter region and distinct region of BRCA1 gene play different role in the BRCA1 expression and prognosis in breast tumors, and this needs further study.

Taken together, the clinicopathological features in sporadic BRCA1-methylated breast cancers compared with BRCA1-unmethylated tumors in our meta-analysis showed that sporadic breast carcinomas with BRCA1 promoter methylation had molecular and clinicopathologic phenotype similar to those of hereditary BRCA1-mutated breast cancers, which was in line with several reports. Furthermore, several lines of evidences confirmed that the expression pattern of sporadic BRCA1-methylated breast cancers was the same as that of inherited BRCA1 mutations. Herein, numerous preclinical researches investigated the antitumor activity of DNA-damaging agents in BRCA1-mutated breast cancers had a similar activity in BRCA1-methylated tumors, and the results demonstrated that the BRCA1 hypermethylation conferred the same extent of sensitivity to poly adeno-sine diphosphate-ribose polymerase-1 (PARP1) inhibitors and platinum-derived drugs as did the BRCA1 mutation. Moreover, Xu et al. reported that BRCA1-methylated triple-negative breast tumor patients were sensitive to adjuvant chemotherapy and had a significantly better 10-year disease-free survival (DFS) and disease-specific survival (DSS) than patients with BRCA1-unmethylated triple-negative tumors. Importantly, a recent meta-analysis including 9 studies with 3205 breast cancer patients indicated that there was significant association of BRCA1 methylation with poor overall survival and DFS of breast tumors. Hence, BRCA1 promoter methylation may be a potential biomarker for targeted therapy and prognostic assessment.

Despite the advantage of a considerable number of included literatures, our meta-analysis had some limitations that should be thought over. First and most importantly, there was large heterogeneity in the outcome of the association between BRCA1 promoter methylation and breast cancer risk. Nevertheless, stratification analyses based on sample materials, methods for detecting methylation or ethnicities showed to reduce the degree of heterogeneity among studies, which demonstrated that the three factors may be contributed to the heterogeneity. Additionally, there was large heterogeneity among studies for the correlation of BRCA1 methylation with ER status, PR status and triple negative phenotype. The expression of ER and PR protein were almost assayed by immunohistochemical staining, but different antibody source and dilution ratio or even cut-off value for result evaluation should be taken into account for the source of heterogeneity. On the other hand, our included studies did not illustrate specific promoter regions of BRCA1 gene for methylation detection, and whether this may cause heterogeneity need to be further studied. Second, it's noteworthy that there were no related articles for the prevalence of BRCA1 promoter methylation in breast cancers in the African population among our eligible literatures. Therefore, further research is needed to evaluate whether this section of our results may be consistent with studies for the African ethnicity. Third, the control groups included healthy females and patients with benign breast diseases. No uniform definition of control groups may, to some extent, partly affected our research results. Finally, there was apparent publication bias in our study, which may be generated by defective design method of small sample studies or absent publication of small trials with negative results. In addition, language bias may be present on the basis of the fact that only English articles were included for our meta-analysis.

In conclusion, this meta-analysis indicated that BRCA1 promoter methylation was associated with an increased risk of breast cancer. The prevalence of BRCA1 methylation was, especially in mammary tissue, was high in patients with breast cancers compared with healthy females or patients with benign breast diseases. Furthermore, there were significant associations between BRCA1 promoter methylation and clinicopathological characteristics in breast tumors, such as lymph node metastasis, high histological grade, ER-negative, PR-negative, triple-negative phenotype and reduced or lack expression of BRCA1 protein. It's necessary to need large-scale researches which use uniform criterion of control groups, detection methods for methylation and sample materials, before BRCA1 promoter methylation can be a useful predictive or diagnostic biomarker for patients with breast cancer and applied to novel targeted therapeutic strategies in the future.

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