ORIGINAL RESEARCH

The clinicopathological significance and drug target potential of FHIT in breast cancer, a meta-analysis and literature review

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Abstract: *FHIT* is a bona fide tumor-suppressor gene and its loss contributes to tumorigenesis of epithelial cancers including breast cancer (BC). However, the association and clinicopathological significance between *FHIT* promoter hypermethylation and BC remains unclear. The purpose of this study is to conduct a meta-analysis and literature review to investigate the clinicopathological significance of *FHIT* methylation in BC. A detailed literature search was performed in PubMed, EMBASE, Web of Science, and Google Scholar databases. The data were extracted and assessed by two reviewers independently. Odds ratios with 95% corresponding confidence intervals were calculated. A total of seven relevant articles were available for meta-analysis, which included 985 patients. The frequency of *FHIT* hypermethylation was significantly increased in invasive ductal carcinoma compared to benign breast disease, the pooled odds ratio was 8.43, $P<0.00001$. The rate of *FHIT* hypermethylation was not significantly different between stage I/II and stage III/IV, odds ratio was 2.98, $P=0.06$. In addition, *FHIT* hypermethylation was not significantly associated with ER and PR status. *FHIT* hypermethylation was not significantly correlated with premenopausal and postmenopausal patients with invasive ductal carcinoma. In summary, our meta-analysis indicated that the frequency of *FHIT* hypermethylation was significantly increased in BC compared to benign breast disease. The rate of *FHIT* hypermethylation in advanced stages of BC was higher than in earlier stages; however, the difference was not statistically significant. Our data suggested that *FHIT* methylation could be a diagnostic biomarker of BC carcinogenesis. FHIT is a potential drug target for development of demethylation treatment for patients with BC.

Keywords: FHIT, methylation, tumor suppressor gene, meta-analysis, odds ratio, drug target

Introduction

Breast cancer (BC) is the most frequently diagnosed cancer in women and the major cause of cancer-related female mortality in the USA.[1] A series of epigenetic alterations and genetic abnormalities contribute to this process of BC onset and progression. Epigenetic alterations, which occur in transformed cells, involve changes in DNA methylation, including global hypomethylation, focal hypermethylation, histone modifications, and nucleosomal remodeling.[2] Specifically, abnormal promoter region methylation in tumor suppressor genes results in loss of gene function which contributes to tumorigenesis of epithelial cancers.[3] Therefore, it is critical to identify biomarkers for diagnosis and new molecular targets for development of personalized therapy. Common fragile sites are large chromosomal regions that are hot spots for alterations, especially in cancer cells. The three most frequently expressed common fragile site regions are FRA3B, FRA16D, and FRA6E which contain *FHIT*, *WWOX*, and *PARK2*.[4,5]

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The *FHIT* gene is a bona fide tumor-suppressor gene present on the short arm of chromosome 3 and its loss of function has been evaluated in different types of cancers including BC.[6,7] FHIT plays an important role in pro-apoptotic signaling, cell cycle control, and sensitivity to DNA damaging agents.[8–10] Viral-mediated *FHIT* gene transfer to FHIT-deficient mice not only prevents but reverses the carcinogen-induced tumor development in vivo and restoration of FHIT protein induces tumor suppression in 50% of tumor cell lines tested in vitro.[11] However, the association and clinical significance between *FHIT* promoter hypermethylation and BC remains under investigation. In this study, we systematically reviewed studies of *FHIT* promoter hypermethylation in this process of BC onset and progression, and quantified the association between *FHIT* promoter hypermethylation and BC by using meta-analysis methods. In addition, we summarize these findings and discuss the tumor suppressor function, as well as the clinical significance of FHIT in BC.

Materials and methods
Search strategy and selection criteria

We performed comprehensive literature searches in PubMed, EMBASE, Web of Science, and Google Scholar databases in May 2015 with no limit set for date and language of publication using the search terms: "breast cancer or breast carcinoma", "methylation", and "FHIT, or Fragile histidine triad". There were 103 articles identified from PubMed, 30 articles from EMBASE, 85 articles from Web of Science, and 16,600 articles from Google Scholar, first 400 of them were screened because the rest of them were not related to the present study. A total of 618 articles were screened by article titles and abstracts.

After screening by titles and abstracts, individual studies were screened using the inclusion and exclusion criteria. We included studies that met the following criteria: 1) studies that evaluated *FHIT* hypermethylation in the primary BC tissues, 2) research that revealed the relationship between *FHIT* hypermethylation and BC clinicopathological parameters, 3) *FHIT* hypermethylation examined by polymerase chain reaction. The exclusion criteria included the following: 1) reviews, case reports, letters, editorials, expert opinion, conference abstracts and 2) all studies using cell lines serum, human xenografts, and in vitro/ex vivo studies were also excluded. The search process was conducted independently by two reviewers (XW and JL), discrepancies were discussed and resolved by the third reviewer (JX). Forward and backward citation chasing of each included article was conducted. The most complete study was chosen to avoid duplication if the same patient populations were reported in several publications. Seven articles were eligible for inclusion in this meta-analysis.

Data extraction and methodological assessment

Two authors (YS, XW) independently reviewed and extracted the following data: last name of the first author, year of publication, country(ies) where the study was conducted, number of BC cases, clinicopathological parameters, cancer tumor-node-metastasis stage, methylation detection method, methylation rate and/or expression. The detailed information of seven relevant articles is listed in Table 1.

Table I Basic characteristics of the included studies

Study	Country	Patients (n)	Methods	Primary aim	Methylation site	*FHIT* expression
Zaki et al[32]	Egypt	30	MSP	Determine the association between *FHIT* methylation and clinicopathological features	Promoter, CpG islands	–
Syeed et al[4]	India	130	PCR-SSCP	Study mutational and promoter hypermethylation status of *FHIT* in BC	Promoter	–
Raish et al[33]	India	379	MSP/immunochemistry, Western blot	Investigate the relationship of p16 and *FHIT* methylation with PR and ER in patients with BC	Promoter, CpG islands	+
Naqvi et al[34]	India	232	MSP/RT-PCR, Northern blot	Identify specific 5′CpG island methylation signatures of *FHIT* and p16 genes and their potential diagnostic relevance in BC	Promoter, CpG islands	+
Zhao et al[35]	People's Republic of China	50	MSP/RT-PCR, immunochemistry	Investigate whether abnormal *FHIT* methylation downregulates the expression of its mRNA and protein		+
Iliopoulos et al[36]	USA	28	MSP	Study fragile genes as biomarkers: epigenetic control of *WWOX* and *FHIT* in lung, breast, and bladder cancer	Intron	–
Yang et al[37]	Japan	46	MSP/immunochemistry	Study two-hit inactivation of *FHIT* by loss of heterozygosity and hypermethylation in BC		+

Abbreviations: BC, breast cancer; mRNA, messenger RNA; MSP, methylation-specific polymerase chain reaction; PCR-SSCP, polymerase chain reaction single-strand conformation polymorphism; RT-PCR, reverse transcription polymerase chain reaction; ER, estrogen receptor; PR, progesterone receptor.

Heterogeneity of investigation was evaluated to determine whether or not the data of various studies could be analyzed for a meta-analysis.

For the methodological evaluation of the studies, three investigators (JL, XW, and JX) read through each publication independently, and they assessed and scored them according to the Reporting Recommendations for Tumor Marker Prognostic Studies guidelines and European Lung Cancer Working Party quality scale.[12,13] Three readers provided the quality scores and compared them, and then reached a consensus value for each item.

Statistical analysis

Analysis was conducted using Review Manager 5.2 (Cochrane Collaboration, Oxford, UK). The pooled odds ratios (ORs) with its 95% confidence intervals (CIs) were calculated. Heterogeneity among studies was estimated using the Cochran's Q statistic and I^2 tests.[14] The I^2 statistics were used to examine the difference for between-study variability due to heterogeneity rather than chance, with a range from 0% to 100%. When heterogeneity (I^2) was less than 50%, a fixed effect model was used to calculate parameters. If there was substantial heterogeneity (I^2 values $\geq$50%), a random-effects model was used to pool data and attempt to identify potential sources of heterogeneity based on subgroup analyses. The analysis was performed to compare the frequency of *FHIT* methylation between BC and nonmalignant breast tissue. The frequency of *FHIT* hypermethylation was compared in different tumor characteristics. The pooled ORs were estimated for the correlation between *FHIT* hypermethylation and clinicopathological features. *P*-values tailed less than 0.05 were considered statistically significant. Publication bias is what occurs whenever the research in the published literature is systematically unrepresentative of population of completed studies. Funnel plots were used for detection of publication bias.

Results

Identification of relevant studies

The initial database search resulted in a total of 618 citations. After review, seven articles were included for meta-analysis, as shown in Figure 1.

Study characteristics

Seven studies published from 2001 to 2011 were eligible for meta-analysis. A total of 985 BC patients from India, People's Republic of China, Japan, Egypt, and the USA were enrolled. Their basic characteristics are summarized in Table 1.
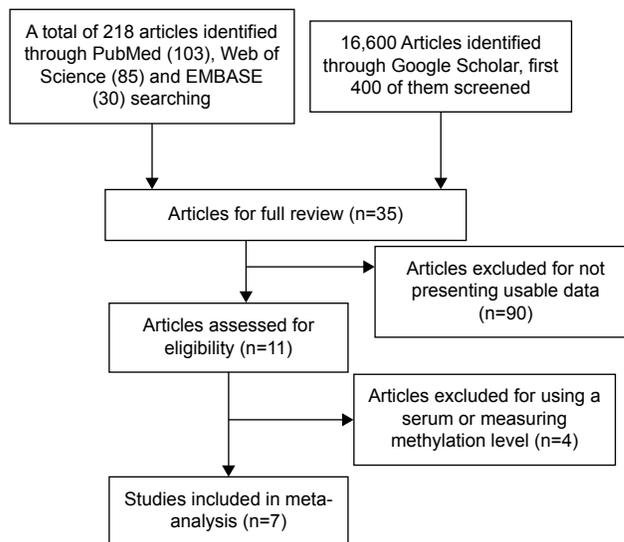

Figure 1 Schematic flow diagram for selection of included studies.

The correlation of FHIT hypermethylation with clinicopathological features

The frequency of *FHIT* hypermethylation was significantly increased in invasive ductal carcinoma (IDC) compared to benign breast disease, the pooled OR was 8.43 with 95% CI 4.31–16.50, z=6.22, P<0.00001 (Figure 2). *FHIT* hypermethylation rate was not significantly correlated with stage, although *FHIT* was more frequently methylated in advanced stages (stage III/IV) than earlier stages (I/II), closely approaching statistical significance, OR was 2.98 with 95% CI 0.94–9.40, z=1.86, P=0.06 (Figure 3). *FHIT* hypermethylation rate was not associated with ER status, the rate of *FHIT* hypermethylation was not higher in ER-positive IDC than that in ER-negative IDC, OR was 1.25 with 95% CI 0.37–4.23, z=0.36, P=0.72 (Figure 4). *FHIT* hypermethylation rate was not associated with PR status, the rate of *FHIT* hypermethylation was similar between PR-positive and PR-negative IDC, OR was 0.66 with 95% CI 0.12–3.57, z=0.49, P=0.63 (Figure 5). *FHIT* hypermethylation was not related to premenopausal and postmenopausal patients with IDC. The frequency of *FHIT* hypermethylation was similar between postmenopausal and premenopausal IDC patients, OR was 0.87 with 95% CI 0.43–1.79, z=0.37, P=0.71 (Figure 6).

Study quality, sensitivity analyses, and publication bias

The quality of each study was assessed with the Newcastle Ottawa Quality Assessment Scale. These scales were utilized to allocate a maximum of nine points for the quality of selection, comparability, exposure, and outcomes for study participants, and a score $\geq$7 indicates a good quality. Of the

Study or subgroup	IDC events	Total	Benign events	Total	Weight	Odds ratio M–H, random, 95% CI	Odds ratio M–H, random, 95% CI
Iliopoulos et al[36]	16	28	4	54	28.4%	16.67 (4.71, 58.99)	
Zaki et al[32]	21	30	5	20	27.8%	7.00 (1.95, 25.13)	
Zhao et al[35]	23	50	6	50	43.8%	6.25 (2.26, 17.29)	
Total (95% CI)		**108**		**124**	**100%**	**8.52 (4.34, 16.72)**	
Total events	60		15				

Heterogeneity: $\tau^2=0.00$; $\chi^2=1.53$, $df=2$ ($P=0.47$); $I^2=0\%$
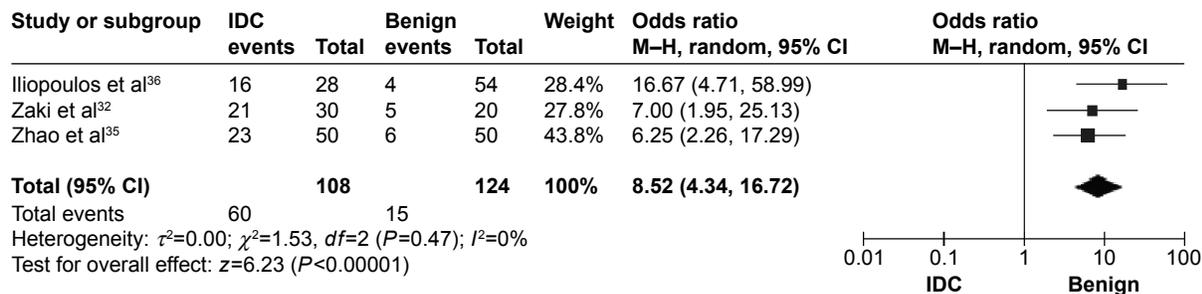Test for overall effect: $z=6.23$ ($P<0.00001$)

Figure 2 Forest plot for *FHIT* hypermethylation in IDC and benign breast disease.
Abbreviations: IDC, invasive ductal carcinoma; *df*, degrees of freedom; CI, confidence interval; M–H, Mantel–Haenszel.

Study or subgroup	Stage III/IV events	Total	Stage I/II events	Total	Weight	Odds ratio M–H, random, 95% CI	Odds ratio M–H, random, 95% CI
Naqvi et al[34]	114	126	58	106	29.2%	7.86 (3.88, 15.95)	
Raish et al[33]	63	170	66	181	31.3%	1.03 (0.66, 1.58)	
Syeed et al[4]	30	58	26	72	29.2%	1.90 (0.94, 3.83)	
Zaki et al[32]	10	10	11	20	10.3%	17.35 (0.90, 336.23)	
Total (95% CI)		**364**		**379**	**100%**	**2.98 (0.94, 9.40)**	
Total events	217		161				

Heterogeneity: $\tau^2=1.05$; $\chi^2=25.55$, $df=3$ ($P<0.0001$); $I^2=88\%$
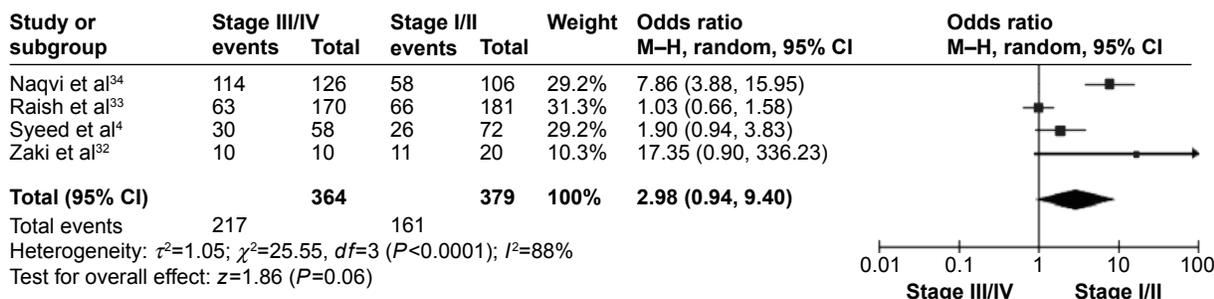Test for overall effect: $z=1.86$ ($P=0.06$)

Figure 3 Forest plot for *FHIT* hypermethylation in different stages of BC.
Abbreviations: BC, breast cancer; CI, confidence interval; *df*, degrees of freedom; M–H, Mantel–Haenszel.

Study or subgroup	ER (+) events	Total	ER (−) events	Total	Weight	Odds ratio M–H, random, 95% CI	Odds ratio M–H, random, 95% CI
Naqvi et al[34]	89	150	35	82	39.9%	1.96 (1.14, 3.38)	
Raish et al[33]	110	293	9	58	37.3%	3.27 (1.55, 6.92)	
Zaki et al[32]	4	10	17	20	22.8%	0.12 (0.02, 0.69)	
Total (95% CI)		**453**		**160**	**100%**	**1.25 (0.37, 4.23)**	
Total events	203		61				

Heterogeneity: $\tau^2=0.89$; $\chi^2=11.58$, $df=2$ ($P=0.003$); $I^2=83\%$
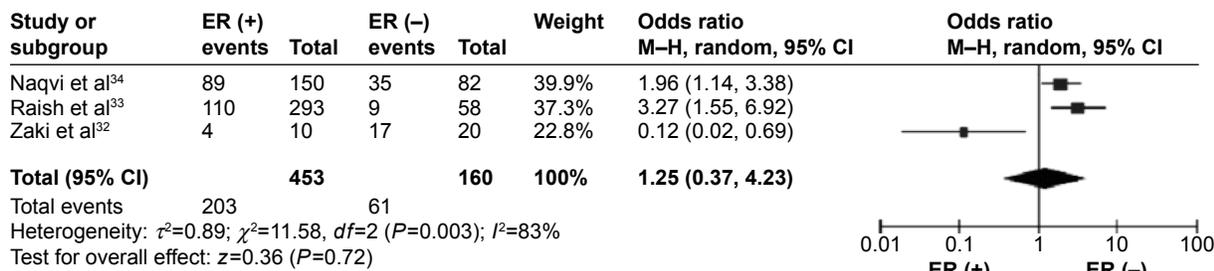Test for overall effect: $z=0.36$ ($P=0.72$)

Figure 4 Forest plot for *FHIT* hypermethylation in ER-positive and -negative BC.
Abbreviations: BC, breast cancer; CI, confidence interval; *df*, degrees of freedom; M–H, Mantel–Haenszel; ER, estrogen receptor.
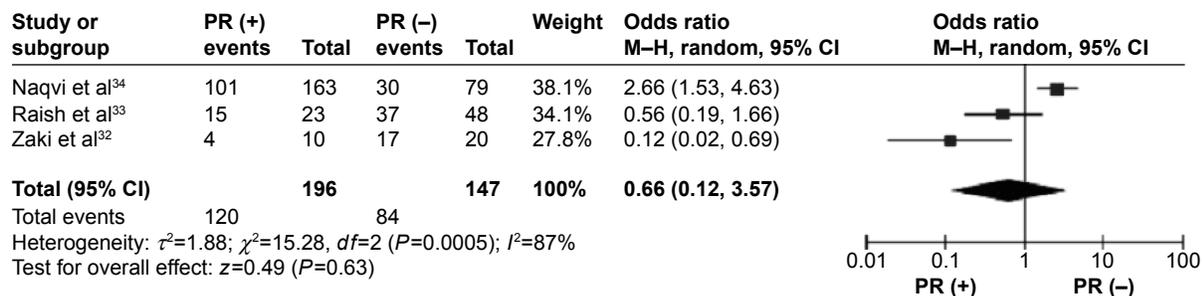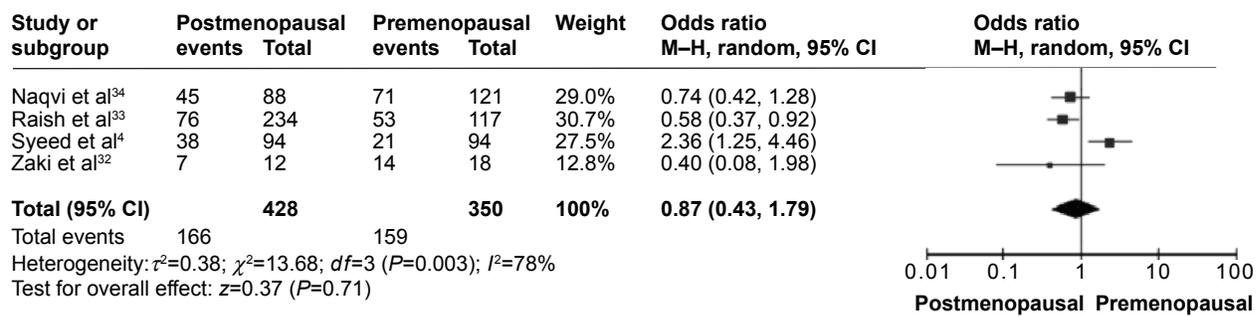
Study or subgroup	PR (+) events	Total	PR (−) events	Total	Weight	Odds ratio M–H, random, 95% CI	Odds ratio M–H, random, 95% CI
Naqvi et al[34]	101	163	30	79	38.1%	2.66 (1.53, 4.63)	
Raish et al[33]	15	23	37	48	34.1%	0.56 (0.19, 1.66)	
Zaki et al[32]	4	10	17	20	27.8%	0.12 (0.02, 0.69)	
Total (95% CI)		**196**		**147**	**100%**	**0.66 (0.12, 3.57)**	
Total events	120		84				

Heterogeneity: $\tau^2=1.88$; $\chi^2=15.28$, $df=2$ ($P=0.0005$); $I^2=87\%$
Test for overall effect: $z=0.49$ ($P=0.63$)

Figure 5 Forest plot for *FHIT* hypermethylation in PR-positive and -negative BC.
Abbreviations: BC, breast cancer; CI, confidence interval; *df*, degrees of freedom; M–H, Mantel–Haenszel; PR, progesterone receptor.

Study or subgroup	Postmenopausal events	Total	Premenopausal events	Total	Weight	Odds ratio M–H, random, 95% CI
Naqvi et al[34]	45	88	71	121	29.0%	0.74 (0.42, 1.28)
Raish et al[33]	76	234	53	117	30.7%	0.58 (0.37, 0.92)
Syeed et al[4]	38	94	21	94	27.5%	2.36 (1.25, 4.46)
Zaki et al[32]	7	12	14	18	12.8%	0.40 (0.08, 1.98)
Total (95% CI)		**428**		**350**	**100%**	**0.87 (0.43, 1.79)**
Total events	166		159			

Heterogeneity: τ^2=0.38; χ^2=13.68; df=3 (P=0.003); I^2=78%
Test for overall effect: z=0.37 (P=0.71)

Figure 6 Forest plot for *FHIT* hypermethylation in postmenopausal and premenopausal BC.
Abbreviations: BC, breast cancer; CI, confidence interval; *df*, degrees of freedom; M–H, Mantel–Haenszel.

studies, four scored eight points, two scored seven points, and one scored six points. Hence, the studies were of a relatively high quality (data not shown). A sensitivity analysis was conducted by removing one study from the meta-analysis at a time; the overall results were not significantly affected. The pooled ORs were not significantly changed, indicating the stability of our analyses. The funnel plots were largely symmetric (Figure 7A–E), suggesting there were no publication biases in the meta-analysis of *FHIT* hypermethylation and clinicopathological features.

Discussion

Aberrant DNA methylation has been recognized to contribute to breast carcinogenesis, and promoter hypermethylation of several tumor suppressor genes including *APC*, *GSTP1*, *P14 (ARF)*, *P16 (CDKN2A)*, *P21 (CDKN1A)*, *PTEN*, and *TIMP3* has been correlated with decreased gene expression.[5,15–17] *FHIT* gene, encompassing the FRA3B fragile site at chromosome 3p14.2, is a putative tumor suppressor gene involved in the pathogenesis of different tumor types including BC.[18,19] FHIT messenger RNA (mRNA) is expressed in most normal human tissue, with highest level in epithelial cells and tissues.[20] The *FHIT* gene is inactivated by genetic and epigenetic changes, ie, loss of heterozygosity or promoter hypermethylation, in numerous cancers.[21] Originally, the absence of FHIT protein in tumors was examined as a loss of heterozygosity, mainly due to biallelic deletions of the gene. Recent evidence indicates that inactivation of *FHIT*
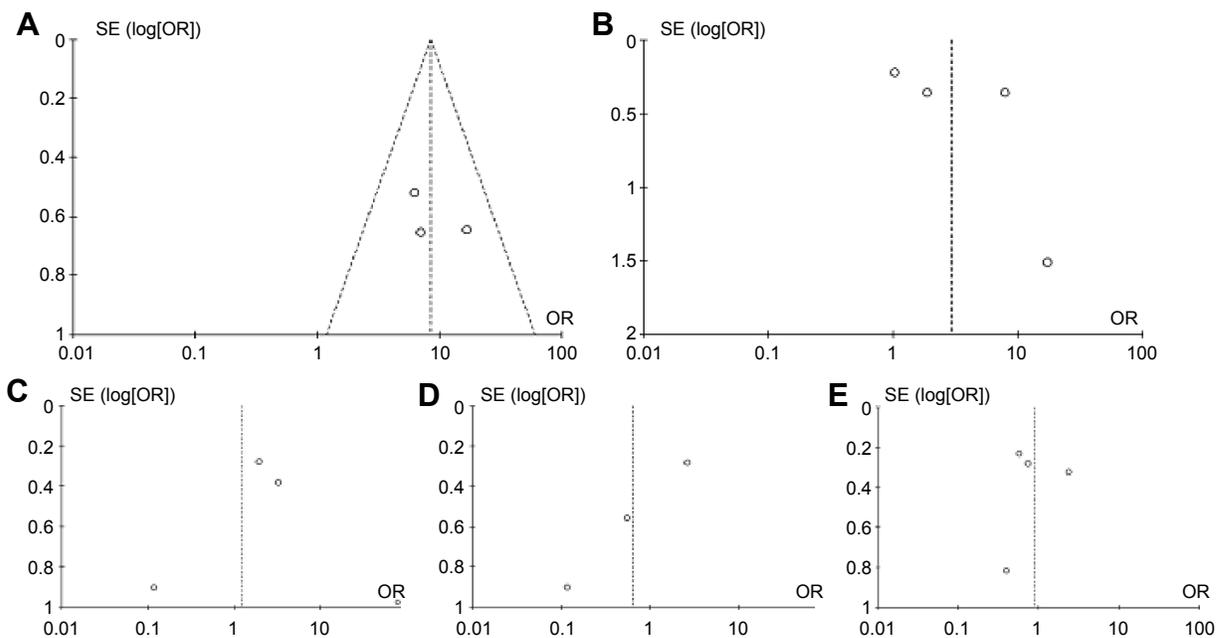

Figure 7 Funnel plot for publication bias.
Notes: (**A**) *FHIT* hypermethylation in IDC and benign breast disease; (**B**) *FHIT* methylation in different stages of BC; (**C**) *FHIT* hypermethylation in ER-positive and -negative BC; (**D**) *FHIT* hypermethylation in PR-positive and -negative BC; (**E**) *FHIT* hypermethylation in postmenopausal and premenopausal BC.
Abbreviations: BC, breast cancer; IDC, invasive ductal carcinoma; SE, standard error; OR, odds ratio; ER, estrogen receptor; PR, progesterone receptor.

by promoter hypermethylation plays an important role in tumorigenesis in several types of tumors including BC.[22–27] However, the reported correlation between *FHIT* methylation and clinicopathological features in BC was inconsistent. We conducted the meta-analysis to determine the association between *FHIT* hypermethylation and clinicopathological characteristics in BC.

Our data revealed that the frequency of *FHIT* hypermethylation in BC was 8.43 times higher than that in benign breast tissues. Haroun et al observed that the methylation frequencies of the genes tested in non-small cell lung carcinoma specimens were 53.6% for *FHIT*.[28] Jeong et al evaluated the methylation of *FHIT* in 60 BC samples; *FHIT* methylation was detected in 96.7% and the positive expression rate of *FHIT* in 87.3% of the patients.[27] Our data and previous studies indicate that *FHIT* promoter hypermethylation is a useful diagnostic biomarker of BC. *FHIT* promoter hypermethylation is reversible, drug treatment through demethylation may be useful to delay tumorigenesis and progression, as well as improving prognosis. Inhibitors of DNA methylation such as 5-Aza-CdR and FdCyd were applied to human BC cells and induced apoptosis,[16,28] and FdCyd is now in clinical trials for the treatment of BC and other solid tumors.[29–31] More specifically, Stewart et al observed that there was a statistically significant increase in expression of FHIT as well as two other tumor suppressor genes, *FUS1* and *WWOX*, in patients with tumor regression following decitabine, which is a demethylating agent.[19] These preclinical studies and early clinical trials showed the potential to restore the activity of epigenetically silenced *FHIT* through epigenetic modulating agents and promise of reexpressed tumor suppressors as markers and effectors of responses. This approach provides a promising drug target for development of personalized therapy of BC in the future.

Our data showed the nonsignificant association of *FHIT* hypermethylation with ER, PR, and menopausal status of BC patients. Those data were different from previous studies which reported significant associations between *FHIT* hypermethylation and ER-negative as well as in PR-negative cases. Since the power of our analysis is small, further evaluation needs to be carried out in a larger sample study. Sensitivity analysis showed that no individual study significantly biased the pooled result and no evidence of publication bias was found. There are several potential limitations: 1) the search strategy was restricted to articles published in English and Chinese, some studies published in other languages were not included in current study; 2) the possibility of information and selection biases as well as

unidentified confounders could not be completely excluded because all of the included studies were observational. Therefore, caution should be exercised when our findings are interpreted among the general population.

In conclusion, our meta-analysis indicated that the frequency of *FHIT* hypermethylation was significantly increased in BC compared to benign breast disease. Our data suggested FHIT could be a diagnostic biomarker. In addition, FHIT is a potential drug target for development of demethylation treatment for patients with BC.

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Disclosure

The authors declare that they have no competing interests, and have no financial disclosures relevant to this work.

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