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Combined effects of GSTM1 and GSTT1 polymorphisms on breast cancer risk

A MOOSE-compliant meta-analysis and false-positive report probabilities test

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Abstract

Many molecular epidemiology studies have reported an association between the combined effects of glutathione S-transferase M1 (*GSTM1*) and glutathione S-transferase T1 (*GSTT1*) polymorphisms on breast cancer risk. However, the results have been controversial.

A meta-analysis was performed to clarify this issue.

Meta-analysis of observational studies in epidemiology guidelines was used. Pooled the crude odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using a random-effects model or fixed-effects model. Several subgroup analyses were conducted by ethnicity, source of control, matching, and menopausal status. In addition, we also performed sensitivity analysis and publication bias. Moreover, a false-positive report probability (FPRP) test was applied to assess positive results.

A significantly increased breast cancer risk was observed in overall population (*GSTM1* null/*GSTT1* present [− +] vs *GSTM1* present/*GSTT1* present [+ +]: OR = 1.19, 95% CI: 1.03–1.36, *GSTM1* null/*GSTT1* null [− −] vs + +: OR = 1.63, 95% CI: 1.29–2.06, (− +) + *GSTM1* present/*GSTT1* null (+ −) vs + +: OR = 1.17, 95% CI: 1.05–1.31, (− +) + (+ −) + (− −) vs + +: OR = 1.27, 95% CI: 1.12–1.44, and − − vs (− +) + (+ −) + (+ +): OR = 1.39, 95% CI: 1.17–1.66) and several subgroup analyses, such as Caucasians, Indians, postmenopausal women, and so on. However, positive results were only considered noteworthy in overall population (− − vs + +: FPRP = 0.150 and (− +) + (+ −) + (− −) vs + +: FPRP = 0.162). Moreover, no significant association was observed when we used the trim and fill method to adjust the pooled data from all populations. Further, none of positive results of sensitivity analysis were considered noteworthy (FPRP >0.2).

These positive findings should be interpreted with caution and indicate that an increased breast cancer risk may most likely result from false-positive results, rather than from true associations or biological factors on the combined effects of *GSTM1* and *GSTT1*. Future studies should be based on sample sizes well-powered and attention needs to be paid to study design to further identify this issue.

Abbreviations: + + = *GSTM1* present/*GSTT1* present, + − = *GSTM1* present/*GSTT1* null, − + = *GSTM1* null/*GSTT1* present, − − = *GSTM1* null/*GSTT1* null, CIs = confidence intervals, CNKI = China National Knowledge Infrastructure, FPRP: false-positive report probability, *GSTM1* = glutathione S-transferase M1, *GSTs* = glutathione S-transferases, *GSTT1* = glutathione S-transferase T1, ORs = odds ratios, PAHs = polycyclic aromatic hydrocarbons, PB = population-based.

Keywords: breast cancer, FPRP, *GSTM1*, *GSTT1*, meta-analysis, polymorphism

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L-FM and X-YW contributed equally to this work.

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This is a meta-analysis, hence, ethical approval was waived or not necessary.

The study was designed by X-FH. L-FM, X-HY, and M-SC did the literature search, study quality assessment, and data extraction. X-FH and X-YW performed the statistical analysis and drafted the tables and figures. L-FM wrote the first draft of this analysis, and X-FH, X-HY, and X-YW helped to finish the final version. All authors approved the conclusions of our study.

The authors have no conflicts of interest to disclose.

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1. Introduction

Breast cancer is the most common malignant tumor and cause of cancer-related death among women, representing a major health problem worldwide.[1] In Portugal, it has the highest incidence and mortality rates among female diseases,[2] and is the second most common malignant tumor in Indian women[3] and the third most common malignant tumor in Korean women.[4] Some studies have indicated that alcohol consumption, tobacco, and particular food habits, especially high fat intake, are important risk factors for breast cancer.[5,6] In addition, previous studies indicated that cancer is related to the combined influences of genetic factors, environmental factors, and lifestyle. Hence, genetic polymorphism studies have become important in identifying the combined factors that may affect individual breast cancer susceptibility.[7,8]

Glutathione S-transferases (GSTs) are a family of multifunctional enzymes involved in the metabolism of a variety of xenobiotic compounds, including mammary carcinogens such as polycyclic aromatic hydrocarbons (PAHs).[9–11] GST_S have the capacity to detoxify the reactive product of metabolisms of PAHs, thereby preventing their interaction with DNA. According to their primary structure, the *GST* family is divided into 7 categories of genes in human.[12] In this meta-analysis, we studied glutathione S-transferase M1 (*GSTM1*) and glutathione S-transferase T1 (*GSTT1*) polymorphisms for breast cancer susceptibility. *GSTM1* and *GSTT1* genes are located on chromosome 1 (1p13.3) and chromosome 22 (22q11.2), respectively.[13] In humans, *GSTM1* is expressed in various tissues such as the liver, stomach, brain, and breast, while *GSTT1* is mainly expressed in the liver and erythrocytes.[14] Polymorphisms in both *GSTM1* and *GSTT1* result in gene deletions (null genotype), resulting in loss of expression and enzyme activity loss.[15,16] Lack of enzymatic activity may lead to the occurrence of cancer.

In 1998, the first study of the association between the combined effects of *GSTM1* and *GSTT1* polymorphisms on breast cancer risk was reported.[17] Subsequently, 34 articles[12–14,17–47] on this issue have been published. However, the results have been controversial and inconsistent. Some studies found no significant association; others reported an increased breast cancer risk. Several previously published meta-analyses did not assess the combined effects of *GSTM1* and *GSTT1* polymorphisms with breast cancer risk.[48–55] Hence, to address this association, a meta-analysis was performed to explore whether there was an association between the combined effects of *GSTM1* and *GSTT1* polymorphisms on breast cancer risk.

2. Materials and methods

2.1. Search strategy

PubMed, Embase, China National Knowledge Infrastructure (CNKI), and Wan Fang (WF) databases were searched (the last search was conducted on February 22, 2018). Two authors identified relevant studies using the following search strategy: breast and (glutathione S-transferase M1 OR *GSTM1*) and (glutathione S-transferase T1 or *GSTT1*) and (polymorph* or mutation* or variant* or genotype*). There were no restrictions on language in the meta-analysis. Additional studies were identified through a search of references of original studies or review articles on this topic and through personal contact with the authors if necessary.

2.2. Inclusion and exclusion criteria

The studies were included if they met the following criteria:

(1) case–control, cohort, or nested case–control study;
(2) the diagnosis of breast cancer cases was confirmed pathologically and controls were confirmed to be free of breast cancer;
(3) complete data was supplied to calculate ORs and the corresponding 95% confidence intervals (CIs).
(4) Studies were excluded if they met the following criteria:
(5) duplicate data or incomplete data,
(6) only case studies, and
(7) meta-analyses, letters, reviews, conference abstracts, and case reports.

2.3. Data extraction

Data were extracted independently by 2 authors. Any potential disagreement was adjudicated by a third investigator if required. The following data was collected from studies that met inclusion criteria: the surname of the first author, publication year, country, race, source of cases, source of controls, type of controls, matching, material used for assessment of genotype, sample size of case and control, and genotype frequencies of the combined effects of *GSTM1* present/null and *GSTT1* present/null polymorphisms.

2.4. Quality score assessment

The 2 authors assessed independently assessed the quality of the studies. The quality assessment criteria were modified from previous meta-analyses of molecular association studies.[56,57] Total scores ranging from 0 (worst) to 19 (best) were used to assess the quality of studies (Table 1). Low-quality studies were

Table 1

Scale for quality assessment of molecular association studies of breast cancer.

Criterion	Score
Source of case	
Selected from population or cancer registry	3
Selected from hospital	2
Selected from pathology archives, but without description	1
Not described	0
Source of control	
Population-based	3
Blood donors or volunteers	2
Hospital-based	1
Not described	0
Ascertainment of cancer	
Histological or pathological confirmation	2
Diagnosis of breast cancer by patient medical record	1
Not described	0
Ascertainment of control	
Controls were tested to screen out breast cancer	2
Controls were subjects who did not report breast cancer, no objective testing	1
Not described	0
Matching	
Controls matched with cases only by age	1
Not matched or not described	0
Genotyping examination	
Genotyping done blindly and quality control	2
Only genotyping done blindly or quality control	1
Unblinded and without quality control	0
Specimens used for determining genotypes	
Blood cells or normal tissues	1
Tumor tissues or exfoliated cells of tissue	0
Association assessment	
Assess association between genotypes and breast cancer with appropriate statistics and adjustment for confounders	2
Assess association between genotypes and breast cancer with appropriate statistics without adjustment for confounders	1
Inappropriate statistics used	0
Total sample size	
>1000	3
500–1000	2
200–500	1
<200	0

considered when scores were ≤11, while scores of >11 were considered to be of high quality. Inconsistent scores were adjudicated by a third author.

2.5. Statistical analysis

Pooled the crude odds ratios (ORs) and 95% CIs were calculated by Z-test and $P < .05$ was considered to be statistically significant. The combined genotypes of *GSTM1* and *GSTT1* were analyzed using the following 6 genetic models: *GSTM1* null/*GSTT1* null (− −) versus *GSTM1* present/*GSTT1* present (+ +), *GSTM1* present/*GSTT1* null (+ −) versus + +, *GSTM1* null/*GSTT1* present (− +) versus + +, (+ −) + (− +) versus + +, (− −) + (+ −) + (− +) versus + +, and − − versus (+ +) + (+ −) + (− +). − − represented *GSTM1* null/*GSTT1* null, + + represented *GSTM1* present/*GSTT1* present, + − represented *GSTM1* present/*GSTT1* null, and − + represented *GSTM1* null/*GSTT1* present. Heterogeneity among studies was assessed by Q test and I^2 value (significant heterogeneity was considered when $P < .10$ and $I^2 > 50\%$).[58] Pooled ORs were calculated using a fixed-effects model[59] when the heterogeneity was not significant, otherwise, a random-effects model was used.[60] However, the included studies cannot be pooled into together when I^2 value >75%. Subgroup analyses were performed by ethnicity, source of control, matching, and menopausal status. We carried out a sensitivity analysis to assess the stability by the following methods:

(1) a single study was excluded, 1 at a time,
(2) the studies of sample size <200 were excluded,
(3) low-quality studies were excluded, and
(4) we used a dataset that comprised only high-quality studies, matching studies, and genotyping performed blindly or with quality control.[61]

In addition, we applied a meta-regression analysis to explore the sources of heterogeneity. Moreover, publication bias was detected using the Begg funnel plot[62] and Egger regression asymmetry test (statistical significance was considered when $P < .05$).[63] If there was publication bias, a nonparametric "trim and fill" method was used to impute missing studies.[64] Last, a false-positive report probability (FPRP) test was applied to assess significant results. We preset a FPRP value of 0.2 for noteworthiness and set a prior probability of 0.001 to detect an OR of 1.50 for the combined genotypes with an increased risk. Noteworthy associations were considered when the FPRP values were less than 0.2.[65] All statistical analyses were calculated using STATA version 9.0 (STATA Corporation, College Station, TX).

3. Results

3.1. Characteristics of identified studies

A total of 144, 172, 12, and 15 studies were identified from PubMed, Embase, CNKI, and Wanfan databases (Fig. 1), respectively. In total, 309 records were removed when titles and abstracts were appraised for review articles, case reports, and meta-analyses. In addition, 5 studies[23,36,40,45,46] were also removed because their data had been included in another 3 studies.[18,22,34] Ultimately, 29 papers describing 30 case–control

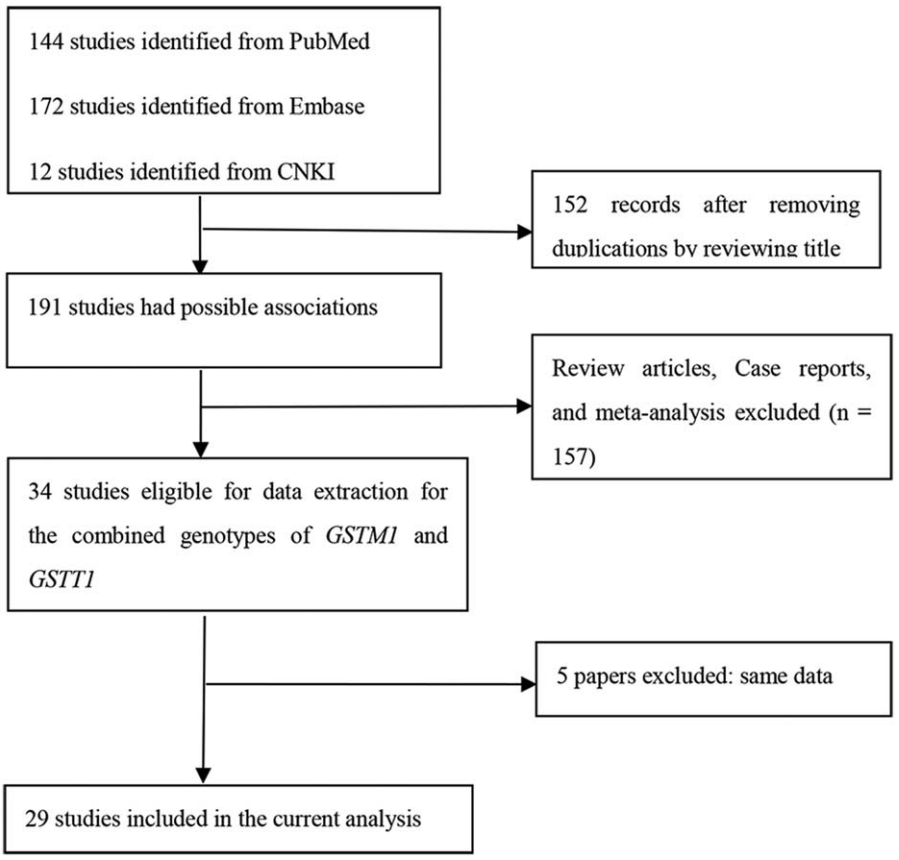

Figure 1. Flow diagram for identifying and including studies in the current meta-analysis.

Table 2

General characteristics of studies included in pooling gene effects.

First author/Yr	Country	Race	Source of case	Source of control	Type of control	Matching	Material used for assessment of genotype	Quality score
Kimi[19] 2016	India	Indian	HB	Healthy volunteers	Healthy women	Age	Whole blood	10
Chirilă[20] 2014	Romania	Caucasian	HB	ND	Healthy women	ND	Blood	4
Possuelo[44] 2013	Brazil	Mixed	HB	HB	Healthy women	Age	Peripheral blood	9
Hashemi[21] 2012	Iran	Caucasian	HB	PB	Healthy women	ND	Blood	12
Ramalhinho[22] 2012	Portugal	Caucasian	HB	Blood donors	Healthy women	ND	Blood	11
Kostrykina[24] 2009	Russia	Caucasian	HB	ND	Cancer-free women	ND	Blood	8
Saxena[25] 2009	India	Indian	HB	PB	Cancer-free women	ND	Blood	12
Unlu[26] 2008	Turkey	Caucasian	HB	ND	Healthy women	ND	Blood	6
Rajkumar[27] 2008	India	Indian	ND	ND	Healthy women	Age	Blood	9
Steck[28] 2007	USA	Mixed	HB	PB	Cancer-free women	Age	Blood	16
Spurdle[29] 2007	USA	Caucasian	CR	PB	Cancer-free women	Age	Blood	16
Cui[43] 2007	China	Asian	HB	ND	Cancer-free women	ND	Blood	8
Chang[30] 2006	China	Asian	HB	HB	Healthy women	Age	Peripheral blood	12
Vogl[14] 2004	Multiple	Mixed	PB+HB	PB+HB	ND	ND	Blood	13
Gago-Dominguez[32] 2004	Singapore	Asian	CR	PB	Cancer-free women	Age	Blood	16
Egan[33] 2004	China	Asian	PB	PB	Cancer-free women	Age	Blood	17
Park[34] 2004	Korea	Asian	HB	HB	Cancer-free women	Age	Blood	13
McCready[47] 2004	USA	Caucasian	HB	HB	Cancer-free patients	Age	Blood	9
Zheng T[18] 2003	USA	Mixed	HB	HB	Cancer-free patients	Age	Blood	14
Khedhaier[35] 2003	Tunisia	African	HB	Blood donors	Healthy women	ND	Peripheral blood leucocytes	12
da Fonte de Amorim[37] 2002	Brazil	Caucasian	HB	HB	Out-patients	Age	Blood	9
da Fonte de Amorim[37] 2002	Brazil	Mixed	HB	HB	Out-patients	Age	Blood	8
Zheng W[31] 2002	USA	Caucasian	PB	PB	Cancer-free women	ND	Blood	12
Gudmundsdottir[38] 2001	Iceland	Caucasian	ND	ND	Healthy women	ND	Blood and tumor (case), blood (control)	4
Dialyna[39] 2001	Greece	Caucasian	HB	HB	Healthy women	ND	Blood and tumor (case), blood (control)	7
Mitrunen[13] 2001	Finland	Caucasian	HB	PB	Health women	ND	Blood	14
Millikan[41] 2000	USA	Mixed	PB	PB	ND	Age	Peripheral blood	17
Curran[12] 2000	Australia	Caucasian	HB	Volunteers	Cancer-free women	Age	Blood	11
García-Closas[42] 1999	USA	Mixed	PB	PB	Cancer-free women	Age	Blood	16
Helzlsouer[17] 1998	USA	Mixed	PB	PB	ND	Age	Blood	13

HB = hospital-based, PB = population-based, CR = cancer registry, ND = not described.

studies were selected (including 10,406 breast cancer patients and 10,115 controls) in this meta-analysis (Tables 2 and 3). Among these studies, thirteen were conducted in Caucasian populations, 5 in Asian, 3 in Indian, 1 in an African population, with 8 in mixed populations. Furthermore, there were 16 high-quality studies and 14 low-quality studies as determined by quality assessment of molecular association studies (Table 1). Eight studies analyzed the combined effects of *GSTM1* and *GSTT1* polymorphisms among postmenopausal women, and 5 analyzed these associations among premenopausal women, as shown in Tables 4 and 5.

3.2. Quantitative synthesis

Significant heterogeneity was observed when all eligible studies were pooled in this meta-analysis. Hence, a random-effects model was used to pool the overall data. The pooled data yielded a statistically significant association between the combined effects of *GSTM1* and *GSTT1* polymorphisms and breast cancer risk (Table 6) in all races; respective OR was 1.19 (95% CI: 1.03–1.36, $P = .015$, $P_{het} < .001$, $I^2 = 60.7\%$) for − + versus + +, 1.63 (95% CI: 1.29–2.06, $P < .001$, $P_{het} < .001$, $I^2 = 74.5\%$) for − − versus + +, 1.17 (95% CI: 1.05–1.31, $P = .005$, $P_{het} < .001$, $I^2 = 57.9\%$) for (− +) + (+ −) versus + +, 1.27 (95% CI: 1.12–1.44, $P < .001$, $P_{het} < .001$, $I^2 = 69.2\%$) for (− +) + (+ −) + (− −) versus + +, and 1.39 (95% CI: 1.17–1.66, $P < .001$, $P_{het} < .001$, $I^2 = 66.0\%$) for − − versus (− +) + (+ −) + (+ +). Subgroup analyses were also performed by ethnicity, source of controls, matching, and menopausal status.

First of all, we analyzed subgroups by ethnicity (Table 6). Pooling data from Caucasians provided evidence of increased breast cancer risk; OR was 1.93 (95% CI: 1.31–2.83, $P = .001$, $P_{het} = .001$, $I^2 = 67.2\%$) for − − versus + +, 1.36 (95% CI: 1.10–1.68, $P = .005$, $P_{het} < .001$, $I^2 = 71.1\%$) for (− +) + (+ −) + (− −) versus + +, and 1.61 (95% CI: 1.22–2.12, $P = .001$, $P_{het=} .037$, $I^2 = 46.7\%$, Fig. 2) for − − versus (− +) + (+ −) + (+ +). Pooling data from Indian populations also showed a statistically significant elevated breast cancer risk; OR was 1.70 (95% CI: 1.09–2.64, $P = .019$, $P_{het=} .120$, $I^2 = 52.9\%$) for − + versus + +, 1.48 (95% CI: 1.19–1.84, $P < .005$, $P_{het} = .204$, $I^2 = 37.1\%$) for (− +) + (+ −) versus + +, and 1.54 (95% CI: 1.02–2.32, $P = .040$, $P_{het} = .082$, $I^2 = 60.0\%$) for (− +) + (+ −) + (− −) versus + +. No significant association was found between the combined effects of *GSTM1* and *GSTT1* polymorphisms and breast cancer risk in Asian populations.

Then, subgroups were analyzed by the source of controls (Table 6). A statistically significant association was also shown in the population-based (PB) studies (− − vs + +: OR = 1.40, 95% CI = 1.08–1.82, $P = .011$, $P_{het} = .003$, $I^2 = 65.5\%$, (− +) + (+ −) + (− −) vs + +: OR = 1.23, 95% CI = 1.04–1.45, $P = .015$, $P_{het} < .001$, $I^2 = 73.7\%$, − − vs (− +) + (+ −) + (+ +): OR = 1.22, 95% CI = 1.01–1.49, $P = .044$, $P_{het} = .021$, $I^2 = 54.0\%$) and no PB studies (− + vs + +: OR = 1.18, 95% CI = 1.01–1.38, $P = .038$, $P_{het} = .029$, $I^2 = 46.4\%$, (− +) + (+ −) + (− −) vs + +: OR = 1.32, 95% CI = 1.09–1.61, $P = .006$, $P_{het} < .001$, $I^2 = 67.9\%$, − − vs (− +) + (+ −) + (+ +): OR = 1.59, 95% CI = 1.20–2.11, $P = .001$, $P_{het} < .001$, $I^2 = 70.6\%$).

Table 3

Genotype frequencies of the combined effects of *GSTM1* present/null and *GSTT1* present/null between breast cancer and control groups.

		+ +		+ −		− +		(+ −) + (− +)		(+ −) + (− +) + (+ +)		− −		All risk genotypes	
First author/Yr	Case/control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Kimi[19] 2016	22/10	2	2	2	3	6	4	8	7	10	9	12	1	20	8
Chiril⊠[20] 2014	59/39	10	18	NA	NA	NA	NA	41	19	51	37	8	2	49	21
Possuelo[44] 2013	49/49	5	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	44	47
Hashemi[21]] 2012	134/152	48	81	0	0	71	59	71	59	119	140	15	12	86	71
Ramalhinho[22] 2012	101/121	20	61	15	15	34	36	49	51	69	112	32	9	81	60
Kostrykina[24] 2009	695/263	306	112	61	28	257	107	318	135	624	247	71	16	389	151
Saxena[25] 2009	399/396	141	202	45	61	162	106	207	167	348	369	51	27	258	194
Unlu[26] 2008	65/108	21	40	11	33	17	24	28	57	49	97	16	11	44	68
Rajkumar[27] 2008	250/500	152	324	33	66	55	91	88	157	240	481	10	19	98	176
Steck[28] 2007	971/998	394	400	107	144	368	378	475	522	869	922	102	76	577	598
Spurdle[29] 2007	1235/659	480	267	83	63	541	283	624	346	1104	613	131	46	755	392
Cui[43] 2007	105/100	33	56	20	19	23	22	43	41	76	97	29	3	72	44
Chang[30] 2006	189/417	35	82	47	109	43	126	90	235	125	317	64	100	154	335
Vogl[14] 2004	1186/849	460	327	NA	NA	NA	NA	607	412	1067	739	119	110	726	522
Gago-Dominguez[32] 2004	180/466	NA	NA	NA	NA	NA	NA	NA	NA	146	370	34	96	NA	NA
Egan[33] 2004	1132/1193	245	263	252	253	332	340	584	593	829	856	303	337	887	930
Park[34] 2004	202/299	33	70	NA	NA	NA	NA	117	165	150	235	50	54	167	219
McCready[47] 2004	70/69	NA	NA	NA	NA	NA	NA	NA	NA	57	60	8	5	NA	NA
Zheng T[18] 2003	312/319	100	115	47	31	119	133	166	164	266	279	46	40	212	204
Khedhaier[35] 2003	309/242	NA	NA	NA	NA	NA	NA	NA	NA	254	206	55	36	NA	NA
da Fonte de Amorim[37] 2002	79/123	NA	NA	NA	NA	NA	NA	NA	NA	74	107	5	16	NA	NA
da Fonte de Amorim[37] 2002	49/133	NA	NA	NA	NA	NA	NA	NA	NA	45	128	4	5	NA	NA
Zheng W[31] 2002	152/325	47	131	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	105	194
Gudmundsdottir[38] 2001	500/395	179	143	48	38	222	171	270	209	449	452	51	43	321	252
Dialyna[39] 2001	207/171	85	76	14	6	92	78	106	84	191	160	16	11	122	95
Mitrunen[13] 2001	481/478	219	236	NA	NA	NA	NA	233	221	452	457	29	21	262	242
Millikan[41] 2000	570/555	278	265	60	53	194	196	254	249	532	514	38	41	292	290
Curran[12] 2000	128/128	45	48	11	8	56	60	67	68	112	116	16	12	83	80
García-Closas[42] 1999	465/464	198	192	35	45	197	192	232	237	430	429	35	35	267	272
Helzlsouer[17] 1998	110/112	26	47	13	13	54	41	67	54	93	101	17	11	84	65

+ − = *GSTM1* present/*GSTT1* null, − + = *GSTM1* null/*GSTT1* present, − − = *GSTM1* null/*GSTT1* null, + + = *GSTM1* present/*GSTT1* present, NA = not available.

Table 4

Genotype frequencies of the combined effects of *GSTM1* present/null and *GSTT1* present/null between post-menopausal breast cancer and control groups.

		+ +		+ −		− +		(+ −) + (− +)		(+ −) + (− +) + (+ +)		− −		All risk genotypes	
First author/Yr	Case/control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Gago-Dominguez[32] 2004	180/466	NA	NA	NA	NA	NA	NA	NA	NA	146	370	34	96	NA	NA
Khedhaier[35] 2003	112/242	NA	NA	NA	NA	NA	NA	NA	NA	95	206	17	36	NA	NA
Steck[28] 2007	641/614	262	247	64	94	247	231	311	325	573	572	68	42	379	367
Zheng W[31] 2002	152/325	47	131	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	105	194
Mitrunen[13] 2001	317/277	142	147	NA	NA	NA	NA	159	118	301	265	16	12	175	130
García-Closas[42] 1999	357/346	148	142	31	35	152	144	183	179	331	321	26	25	209	204
Park[34] 2004	80/122	16	34	NA	NA	NA	NA	47	65	63	99	17	23	64	88
Zheng T[46] 2002	229/201	73	74	31	21	87	89	118	110	191	184	38	17	156	127

+ − = *GSTM1* present/*GSTT1* null, − + = *GSTM1* null/*GSTT1* present, − − = *GSTM1* null/*GSTT1* null, + + = *GSTM1* present/*GSTT1* present, NA = not available.

Table 5

Genotype frequencies of the combined effects of *GSTM1* present/null and *GSTT1* present/null between pre-menopausal breast cancer and control groups.

		+ +		+ −		− +		(+ −) + (− +)		(+ −) + (− +) + (+ +)		− −		All risk genotypes	
First author/Year	Case/control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Khedhaier[35] 2003	194/242	NA	NA	NA	NA	NA	NA	NA	NA	159	206	38	36	NA	NA
Steck[28] 2007	310/339	125	136	38	45	117	126	155	171	280	307	30	32	185	203
Mitrunen[13] 2001	164/201	77	89	NA	NA	NA	NA	74	103	150	192	13	9	87	112
García-Closas[42] 1999	108/118	50	50	4	10	45	48	49	58	99	108	9	10	58	68
Park[34] 2004	120/167	17	36	NA	NA	NA	NA	70	100	87	136	33	31	103	131
Zheng T[46] 2002	83/118	27	41	16	10	32	44	48	54	75	95	8	23	56	77

+ − = *GSTM1* present/*GSTT1* null, − + = *GSTM1* null/*GSTT1* present, − − = *GSTM1* null/*GSTT1* null, + + = *GSTM1* present/*GSTT1* present, NA = not available.

Table 6

Pooled results of the combined effects of *GSTM1* present/null and *GSTT1* present/null on breast cancer risk and FPRP test.

Variable	n	Cases/ Controls	Test of association		Test of heterogeneity			FPRP test	
			OR (95% CI)	*P*	P_h	I^2 (%)	Model	Power	0.001
+ − vs + +									
Overall	19	3692/3764	1.04 (0.89, 1.22)	.619	.052	38.0	R	—	—
Race									
Asian	3	632/782	1.10 (0.89, 1.37)	.370	.425	0.0	F	—	—
Indian	3	375/658	1.05 (0.77, 1.45)	.744	.943	0.0	F	—	—
Caucasian	7	1427/1019	1.06 (0.74, 1.53)	.744	.039	54.7	R	—	—
Source of controls									
PB	8	2405/2349	0.92 (0.80, 1.05)	.230	.200	29.9	F	—	—
NPB	11	1287/1415	1.18 (0.97, 1.43)	.093	.145	31.8	F	—	—
Matching									
Yes	11	2645/2793	0.97 (0.85, 1.10)	.610	.144	31.9	F	—	—
No	8	1047/971	1.19 (0.84, 1.67)	.329	.070	48.5	R	—	—
Menopausal status									
Post-menopausal	3	609/613	0.88 (0.55, 1.41)	.596	.077	61.0	R	—	—
Pre-menopausal	3	260/292	1.02 (0.44, 2.35)	.965	.056	65.4	R	—	—
− + vs + +									
Overall	19	5631/5223	**1.19 (1.03, 1.36)**	.015	<.001	60.7	R	1.000	0.914
Race									
Asian	3	711/889	1.05 (0.86, 1.28)	.642	.219	34.2	F	—	—
Indian	3	518/729	**1.70 (1.09, 2.64)**	.019	.120	52.9	R	0.289	0.984
Caucasian	8	2474/1646	1.19 (0.96, 1.48)	.119	.025	56.2	R	—	—
Source of controls									
PB	8	3729/3312	—	—	<.001	77.9	—	—	—
NPB	11	1902/1911	1.08 (0.95, 1.24)	.241	.177	28.1	F	—	—
Matching									
Yes	11	3920/3849	1.04 (0.95, 1.14)	.416	.451	0.0	F	—	—
No	8	1711/1374	**1.47 (1.07, 2.01)**	.018	<.001	74.8	R	0.550	0.966
Menopausal status									
Post-menopausal	3	969/927	1.01 (0.84, 1.21)	.943	.997	0.0	F	—	—
Pre-menopausal	3	396/445	1.01 (0.77, 1.32)	.955	.934	0.0	F	—	—
− − vs + +									
Overall	23	4771/4464	**1.63 (1.29, 2.06)**	<.001	<.001	74.5	R	**0.243**	**0.150**
Race									
Asian	4	792/965	—	—	<.001	87.3	—	—	—
Indian	3	368/575	2.12 (0.91, 4.97)	.082	.091	58.3	R	—	—
Caucasian	10	1798/1265	**1.93 (1.31, 2.83)**	.001	.001	67.2	R	0.098	0.885
Source of controls									
PB	9	2750/2559	**1.40 (1.08, 1.82)**	.011	.003	65.5	R	0.697	0.945
NPB	14	2021/1905	—	—	<.001	79.3	—	—	—
Matching									
Yes	12	2812/2847	**1.30 (1.07, 1.56)**	.007	.076	39.7	R	0.938	0.836
No	11	1959/1617	—	—	<.001	85.0	—	—	—
Menopausal status									
Post-menopausal	5	806/763	**1.49 (1.14, 1.94)**	.004	.498	0.0	F	0.520	0.855
Pre-menopausal	5	389/457	1.16 (0.83, 1.63)	.389	.150	40.6	F	—	—
(+ −) + (− +) vs + +									
Overall	23	8255/7679	**1.17 (1.05, 1.31)**	.005	<.001	57.9	R	1.000	0.866
Race									
Asian	4	1180/1505	1.13 (0.95, 1.34)	.162	.181	38.5	F	—	—
Indian	3	598/859	**1.48 (1.19, 1.84)**	<.001	.204	37.1	F	0.548	0.432
Caucasian	10	3220/2331	1.23 (0.99, 1.51)	.057	.002	64.9	R	—	—
Source of controls									
PB	9	4776/4401	1.17 (0.99, 1.38)	.066	.001	71.2	R	—	—
NPB	14	3479/3278	**1.18 (1.01, 1.38)**	.038	.029	46.4	R	0.999	0.975
Matching									
Yes	12	4760/4872	1.03 (0.95, 1.13)	.434	.323	12.5	F	—	—
No	11	3495/2807	**1.35 (1.09, 1.67)**	.006	<.001	71.2	R	0.834	0.872
Menopausal status									
Post-menopausal	5	1459/1441	1.05 (0.91, 1.22)	.501	.216	30.9	F	—	—
Pre-menopausal	5	692/838	1.01 (0.81, 1.23)	.993	.495	0.0	F	—	—
(+ −) + (− +) + (− −) vs. + +									
Overall	25	9717/9090	**1.27 (1.12, 1.44)**	<.001	<.001	69.2	R	**0.995**	**0.162**
Race									
Asian	4	1626/1999	1.42 (0.94, 2.12)	.092	.006	76.2	R	—	—
Indian	3	671/906	**1.54 (1.02, 2.32)**	.040	.082	60.0	R	0.450	0.989

(*continued*)

Table 6

(continued).

Variable	n	Cases/ Controls	Test of association		Test of heterogeneity		Model	FPRP test	
			OR (95% CI)	P	P_h	I^2 (%)		Power	0.001
Caucasian	11	3757/2839	**1.36 (1.10, 1.68)**	.005	<.001	71.1	R	0.818	0.841
Source of controls									
PB	10	5649/5332	**1.23 (1.04, 1.45)**	.015	<.001	73.7	R	0.991	0.932
NPB	15	4068/3758	**1.32 (1.09, 1.61)**	.006	<.001	67.9	R	0.896	0.873
Matching									
Yes	13	5633/5693	1.06 (0.98, 1.15)	.141	.203	23.8	F	—	—
No	12	4084/3397	—	—	<.001	80.0	R	—	—
Menopausal status									
Post-menopausal	6	1776/1885	1.14 (0.99, 1.31)	.052	.216	29.2	F	—	—
Pre-menopausal	5	785/943	1.01 (0.83, 1.24)	.896	.523	0.0	F	—	—
− − vs (+ −) + (− +) + (+ +)									
Overall	28	10,198/9,845	**1.39 (1.17, 1.66)**	<.001	<.001	66.0	R	0.800	0.257
Race									
Asian	5	1806/2465	—	—	<.001	83.9	R	—	—
Indian	3	671/906	1.85 (0.85, 4.01)	.118	.106	55.4	R	—	—
Caucasian	12	3749/2802	**1.61 (1.22, 2.12)**	.001	.037	46.7	R	0.307	0.693
Source of controls									
PB	10	5677/5473	**1.22 (1.01, 1.49)**	.044	.021	54.0	R	0.979	0.981
NPB	18	4521/4372	**1.59 (1.20, 2.11)**	.001	<.001	70.6	R	0.343	0.793
Matching									
Yes	16	5957/6431	**1.21 (1.03, 1.44)**	.024	.031	44.0	R	0.992	0.970
No	12	4241/3414	—	—	<.001	78.4	R	—	—
Menopausal status									
Post-menopausal	7	1916/2268	**1.25 (1.02, 1.53)**	.030	.247	23.9	F	0.961	0.969
Pre-menopausal	6	981/1185	1.18 (0.91, 1.53)	.201	.143	39.4	F	—	—

NPB = no population-based, PB = population-based, + − = *GSTM1* present/*GSTT1* null, − + = *GSTM1* null/*GSTT1* present, − − = *GSTM1* null/*GSTT1* null, + + = *GSTM1* present/*GSTT1* present, R = random-effects model, F = fixed-effects model.

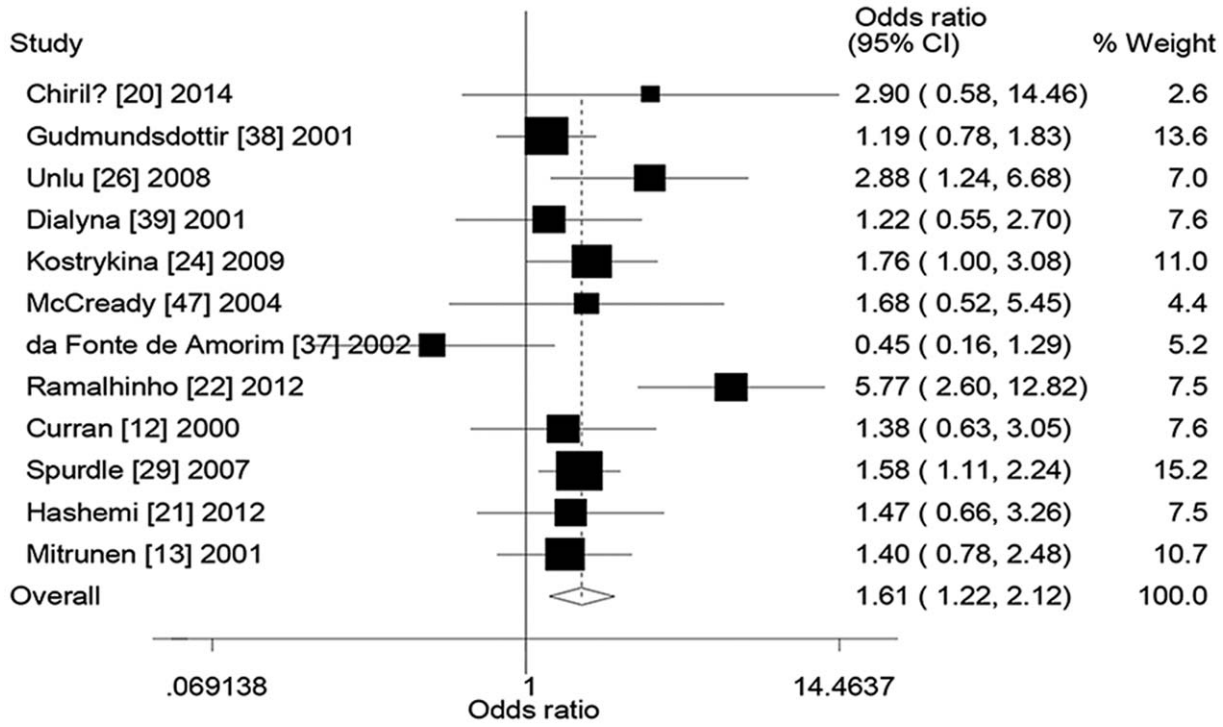

Figure 2. Forest plot for the association between the combined effects of GSTM1 and GSTT1 polymorphisms and breast cancer risk in Caucasians (− − vs (− +) + (+ −) + (+ +)). + − = *GSTM1* present/*GSTT1* null, − + = *GSTM1* null/*GSTT1* present, − − = *GSTM1* null/*GSTT1* null, + + = *GSTM1* present/*GSTT1* present, GSTM1 = glutathione S-transferase M1, GSTT1 = glutathione S-transferase T1.

Table 7

The results of sensitivity analysis and FPRP test in this meta-analysis.

Variable	n	Cases/controls	Test of association		Test of heterogeneity		Model	FPRP test	
			OR (95% CI)	*P*	P_h	I^2 (%)		Power	0.001
+ − vs + +									
Sample size≥200	11	3326/3,256	0.95 (0.85, 1.07)	.400	.236	21.8	F	—	—
Quality score >11	10	2634/2,686	0.98 (0.82, 1.18)	.834	.084	42.5	R	—	—
Only studies with high quality studies, matching, and genotyping examination done bindly or quality control									
Yes	7	2318/2,151	0.98 (0.78, 1.23)	.849	.034	56.1	R	—	—
− + vs + +									
Sample size≥200	14	5395/4,890	1.10 (0.97, 1.25)	.143	.004	57.3	R	—	—
Quality score >11	11	4427/4,082	1.19 (0.99, 1.43)	.070	<.001	73.1	R	—	—
Only studies with high quality studies, matching, and genotyping examination done bindly or quality control									
Yes	7	3526/3,112	1.04 (0.94, 1.14)	.492	.269	21.1	F	—	—
− − vs + +									
Sample size≥200	14	4320/3,963	**1.26 (1.04, 1.52)**	.017	.002	60.6	R	0.966	0.942
Quality score >11	13	3657/3,457	**1.35 (1.09, 1.68)**	.006	<.001	66.9	R	0.827	0.896
Only studies with high quality studies, matching, and genotyping examination done bindly or quality control									
Yes	8	2476/2,259	**1.27 (1.02, 1.59)**	.032	.038	53.0	R	0.927	0.976
(+ −) + (− +) vs + +									
Sample size≥200	17	7907/7,226	1.09 (0.99, 1.19)	.076	.037	41.6	R	—	—
Quality score >11	13	6384/5,971	1.16 (0.99, 1.36)	.054	.016	51.7	R	—	—
Only studies with high quality studies, matching, and genotyping examination done bindly or quality control									
Yes	8	4273/3,949	1.10 (0.97, 1.25)	.146	.106	44.9	F	—	—
(+ −) + (− +) + (− −) vs + +									
Sample size≥ 200	21	9552/8,884	**1.25 (1.10, 1.41)**	<.001	<.001	70.6	R	0.998	0.220
Quality score >11	14	7536/7,206	**1.20 (1.05, 1.36)**	.006	<.001	66.3	R	1.000	0.811
Only studies with high quality studies, matching, and genotyping examination done bindly or quality control									
Yes	8	4,995/4,589	1.08 (0.95, 1.23)	.210	.069	46.6	R	—	—
− − vs (+ −) + (− +) + (+ +)									
Sample size≥200	23	9938/9,490	**1.32 (1.11, 1.58)**	.002	<.001	68.1	R	0.918	0.729
Quality score >11	15	7873/7,589	**1.21 (1.03, 1.42)**	.021	.003	57.8	R	0.996	0.952
Only studies with high quality studies, matching, and genotyping examination done bindly or quality control									
Yes	9	5175/5055	1.16 (0.98, 1.38)	.094	.067	45.2	R	—	—

+ − = *GSTM1* present/*GSTT1* null, − + = *GSTM1* null/*GSTT1* present, − − = *GSTM1* null/*GSTT1* null, + + = *GSTM1* present/*GSTT1* present, R = Random-effect model, F = Fixed-effect model.

In addition, we also performed subgroup analysis by matching (Table 6). A statistically significant increased breast cancer risk was yielded in the studies of matching (− − vs + +: OR = 1.30, 95% CI = 1.07–1.56, $P = .007$, $P_{het} = .076$, $I^2 = 39.7\%$, − − vs (− +) + (+ −) + (+ +): OR = 1.21, 95% CI = 1.03–1.44, $P = .024$, $P_{het} = .031$, $I^2 = 44.0\%$) and no matching (− + vs + +: OR = 1.47, 95% CI = 1.07–2.01, $P = .018$, $P_{het} < .001$, $I^2 = 74.8\%$, (− +) + (+ −) vs + +: OR = 1.35, 95% CI = 1.09–1.67, $P = .006$, $P_{het} < .001$, $I^2 = 71.2\%$).

Last, analysis of subgroups on the basis of menopausal status (Table 6) showed that the increased breast cancer risk was found in postmenopausal women (− − vs + +: OR = 1.49, 95% CI = 1.14–1.94, $P = .004$, $P_{het} = .498$, $I^2 = 0.0\%$, − − vs (− +) + (+ −) + (+ +): OR = 1.25, 95% CI = 1.02–1.53, $P = .030$, $P_{het} = .247$, $I^2 = 23.9\%$).

3.3. Heterogeneity and sensitivity analyses

Significant heterogeneity was detected in this meta-analysis (Table 6). Source of heterogeneity was assessed on the basis of ethnicity, source of controls, matching, sample size, and quality score using a meta-regression analysis. The results demonstrated that sample size (+ − vs + +: $P = .023$, − + vs + +: $P = .006$, − − vs + +: $P = .004$, (− +) + (+ −) vs + +: $P = .001$) and matching (− + vs + +: $P = .023$) were sources of heterogeneity in several genetic models.

Sensitivity analysis was carried out to assess the robustness of results in this meta-analysis. Table 7 lists the results of sensitivity analysis. The results are stable when a single study was removed each time (Fig. 3). However, the results changed in overall population when the studies of sample size <200 were excluded (− + vs + +: OR = 1.10, 95% CI = 0.97–1.25, (− +) + (+ −) vs + +: OR = 1.09, 95% CI = 0.99–1.19). The results also changed in overall population when the studies of low-quality were excluded (− + vs + +: OR = 1.19, 95% CI = 0.99–1.43, (− +) + (+ −) vs + +: OR = 1.16, 95% CI = 0.99–1.36). Last, significantly increased breast cancer risk was found when the studies only included with high-quality, matching, and genotyping examination performed bindly or with quality control (− − vs + +: OR = 1.27, 95% CI = 1.02–1.59, $P = .032$, $P_{het} = .038$, $I^2 = 53.0\%$).

3.4. Publication bias

Publication bias was detected using the Begg funnel plot and Egger regression asymmetry test. The shapes of Begg funnel plots (figure not shown) and the results of Egger regression asymmetry test (− + vs + +: $P = .049$, − − vs + +: $P < .001$, (+ −) + (− +) vs (+ +): $P = .004$, (− +) + (+ −) + (− −) vs + +: $P = .002$, − − vs (− +) + (+ −) + (+ +): $P = .001$) suggested that evidence of publication bias was observed in this meta-analysis. The funnel plots of the nonparametric "trim and fill" method are listed in Figure 4. The results were changed using the nonparametric trim and fill method in the following 4 genetic models (− + vs + + : OR = 1.02, 95% CI = 0.86–1.20, − − vs + +: OR = 1.12, 95% CI = 0.88–1.44, (+ −) + (− +) vs (+ +): OR = 1.12, 95% CI = 0.88–1.44, − −

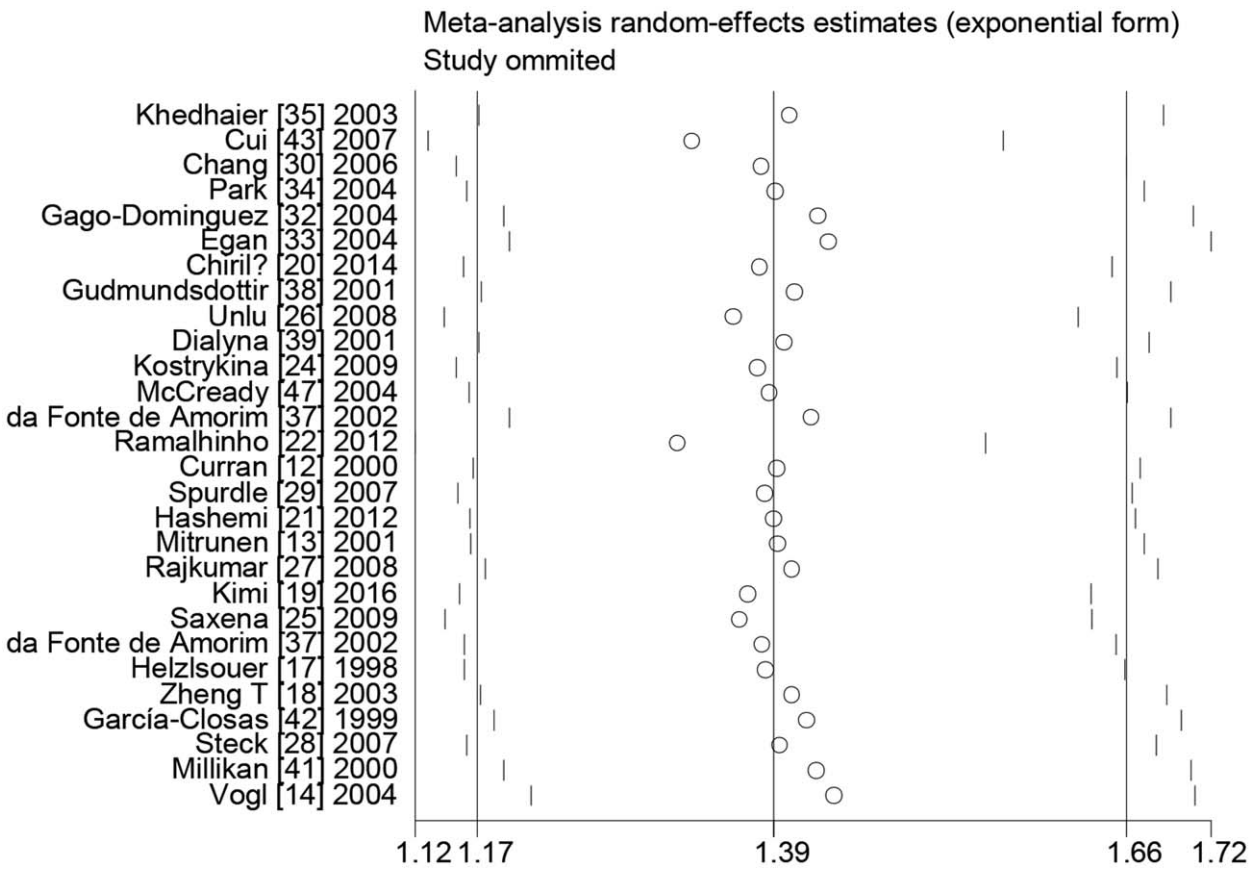

Figure 3. Sensitive analysis between the combined effects of GSTM1 and GSTT1 polymorphisms and breast cancer risk in overall population ((− +) + (+ −) + (− −) vs + +). + − = *GSTM1* present/*GSTT1* null, − + = *GSTM1* null/*GSTT1* present, − − = *GSTM1* null/*GSTT1* null, + + = *GSTM1* present/*GSTT1* present, GSTM1 = glutathione S-transferase M1, GSTT1 = glutathione S-transferase T1.

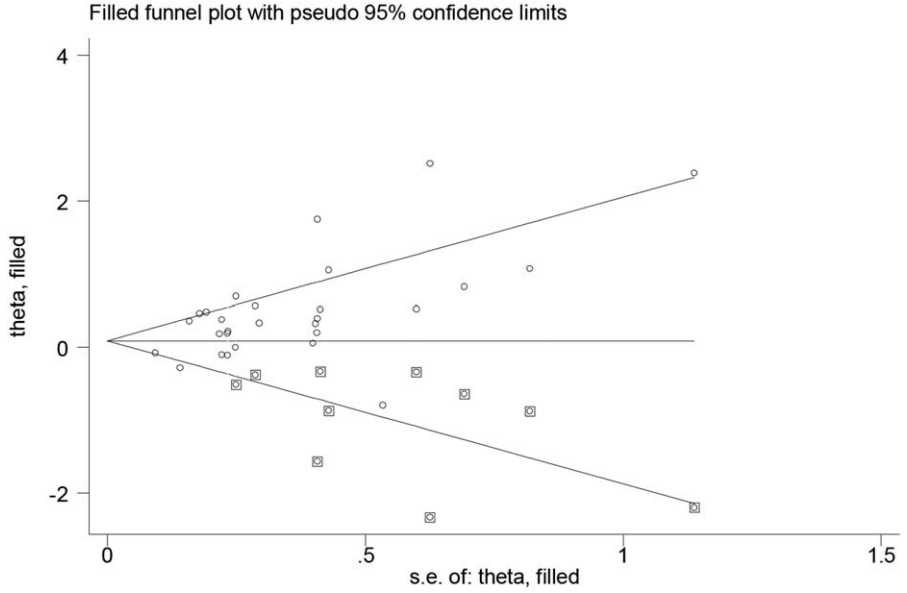

Figure 4. "Trim and fill" plots for the publication bias evaluation between the combined effects of GSTM1 and GSTT1 polymorphisms and breast cancer risk (− − vs (− +) + (+ −) + (+ +)). + − = *GSTM1* present/*GSTT1* null, − + = *GSTM1* null/*GSTT1* present, − − = *GSTM1* null/*GSTT1* null, + + = *GSTM1* present/*GSTT1* present, GSTM1 = glutathione S-transferase M1, GSTT1 = glutathione S-transferase T1.

vs (− +) + (+ −) + (+ +): OR = 1.13, 95% CI = 0.93–1.35) in the overall meta-analysis.

3.5. FPRP test results

Statistically significant associations were further investigated on the basis of an FPRP test (Tables 6 and 7). For a pre-specified prior probability of 0.001, the results were only considered noteworthy in overall pooled analysis (FPRP = 0.150 for − − vs + + and FPRP = 0.162 for (− +) + (+ −) + (− −) vs + +, Table 6). However, none of the results were considered noteworthy, especially in the results of sensitivity analysis (Table 7).

4. Discussion

We performed a meta-analysis to assess the association between the combined effects of *GSTM1* and *GSTT1* polymorphisms on breast cancer risk, including 10,406 breast cancer patients and 10,115 controls. To our knowledge, this is the first meta-analysis to explore whether there was an association on this issue.

The pooled data from all eligible studies yielded an association between the combined effects of *GSTM1* and *GSTT1* polymorphisms and breast cancer risk. In addition, statistically significant increased breast cancer risk was also found in several subgroups, such as Caucasians, Indians, postmenopausal women, and so on, as shown in Table 6. The pooled data were analyzed using 6 different genetic models in this study. Under the circumstances, the *P* value must be adjusted to explain the multiple comparisons.[66] However, when *P* values were adjusted according to the FPRP method, none of the results in this meta-analysis were considered noteworthy, except the overall pooled analysis on the basis of a pre-specified prior probability of 0.001. Further, there were only 12 studies in which genotyping examination was performed blindly or with quality control. There were 18 studies that were age-matched in cases and controls, but bias may exist in the non-matched studies. Hence, we further performed a sensitivity analysis restricted to studies that only included high-quality articles, matching, and genotyping examination performed blindly or with quality control. The pooled results were not still considered noteworthy by FPRP methods. This was an attempt to avoid random errors and confounding bias that sometimes distorted the results of molecular epidemiological studies.[67–69] Overall, the results of the present meta-analysis are more close to real value. Based on biochemical properties described for *GSTM1* and *GSTT1* polymorphisms, we expected that the combined effects of the 2 genes were associated with risk of breast cancer risk in all races. However, a significantly increased breast cancer risk may most likely be from false-positive results. Therefore, future studies should be based on sample sizes well-powered and attention needs to be paid to study design to further identify our findings.

There was significant heterogeneity in this meta-analysis. A meta-regression analysis was performed to explore the source of heterogeneity. We found sample size have contributed to the heterogeneity. In addition, evidence of publication bias was observed in this work (Fig. 4 indicates that bias is from small-size studies). therefore, the potential source of type I error (elevation of false-positive results) may be based on publication bias in this study.[70] Moreover, some small sample studies may be easier to accept if there was a positive report as they tend to yield false-positive results because they may be not rigorous and are often of low-quality. Furthermore, the results were also changed in overall analysis when we used the nonparametric trim and fill method. Random error and bias were common in these studies with small sample sizes, and the results were unreliable, especially in molecular epidemiological studies.[71] In addition, research indicated that the absence of SNPs is a frequent occurrence in tumor cells.[72] Hence, data from studies of genetic polymorphisms should be more reliable when DNA was isolated from blood cell rather than tumor cells.

There are some limitations in this meta-analysis. First, only published articles were selected in this study. Second, we did not uniformly define the controls. There were controls of 12 studies from healthy women, 11 studies from cancer-free women, 4 studies from cancer-free patients, and 3 studies with undefined controls. Hence, non-differential misclassification bias was possible exist. Third, we did not consider whether the genotype distribution in the controls was in Hardy–Weinberg equilibrium (HWE). Under normal circumstances, the HWE in the meta-analysis of genetic polymorphisms must be calculated to assess the quality, genotyping errors, and selection bias in the study.[73,74] However, we cannot calculate or extract the relevant data in the original studies. Fourth, no data were extracted on other risk factors, such as hormonal readiness, obesity, smoking, and so on. This study has also several strengths. First, a meta-analysis can increase the statistical power more than any single study. Second, we used the FPRP value to explore the false-positive results. Third, we performed an important sensitivity analysis, a dataset was used that the studies with high-quality, matching, and genotyping examination performed bindly or with quality control were only included.

After more than 10 years of extensive research on this issue, our findings should be interpreted with caution and indicate that an increased breast cancer risk may most likely result from false-positive results, rather than from true associations or biological factors on the combined effects of *GSTM1* and *GSTT1*. Future studies should be based on sample sizes well-powered and attention needs to be paid to study design to further identify this issue.

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