

REVIEW ARTICLE

MTHFR C677T polymorphism and breast cancer risk in the United States population: An updated meta-analysis

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Abstract

Breast cancer (BC) is a major global health concern, with genetic factors contributing significantly to individual risk. The *MTHFR* C677T (rs1801133) polymorphism has been extensively studied for its potential role in BC susceptibility; however, results have been inconsistent, particularly in the United States (US). Therefore, this study evaluates the association between the C677T variant and BC risk exclusively in populations from the US by pooling data from published case-control studies. A systematic literature search was conducted across multiple online resources to identify relevant studies published up to 2025. Eleven case-control studies comprising 9,137 BC cases and 12,427 controls were included. Genotypic and allelic data were extracted, and pooled odds ratios with 95% confidence intervals were calculated under different genetic models. Study quality was assessed using the Newcastle-Ottawa Scale, and heterogeneity and publication bias were examined. The pooled analysis showed no significant association between the C677T polymorphism and BC risk across different genetic models (allele model: odds ratio = 1.02, 95% confidence interval = 0.96–1.08, $p = 0.493$, adjusted p -value = 1) in the US population. Sensitivity analyses further confirmed the stability and robustness of these findings. Furthermore, heterogeneity across studies was low to moderate, and no evidence of significant publication bias was detected. In conclusion, this updated meta-analysis suggests that the *MTHFR* C677T variant is not significantly associated with BC susceptibility in US populations. Further research with larger sample sizes and considering gene-environment interactions is warranted to clarify the complex genetics of breast cancer risk.

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Keywords: *MTHFR*; C677T; rs1801133; Breast cancer; Genetic susceptibility

1. Introduction

Breast cancer (BC) is one of the most commonly diagnosed cancers among women worldwide. Its increasing global prevalence is driven by the complex interplay of environmental and genetic factors. While lifestyle and environmental exposures contribute significantly, a genetic predisposition also plays a crucial role in determining individual susceptibility.¹ The *MTHFR* gene (gene ID: 4524), located on chromosome 1p36.3 ([Figure 1](#)), is a notable genetic factor involved in BC risk.^{2,3} It encodes

methylenetetrahydrofolate reductase (MTHFR), an enzyme essential for converting 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-MTHF), critical for DNA synthesis and methylation.⁴

Given the central role of this enzyme in maintaining genomic stability, even subtle genetic variations in the *MTHFR* gene may have profound biological consequences. Specifically, a single nucleotide substitution can alter enzyme structure or stability, thereby disrupting cellular homeostasis and potentially contributing to carcinogenesis. In this context, the common functional polymorphism rs1801133 (C677T) has been extensively studied. This variant produces a thermolabile enzyme with approximately 50–70% reduced activity (Figure 1). Individuals carrying the TT genotype retain only about 30% of normal enzymatic activity compared with CC genotype carriers, mainly due to increased enzyme instability and degradation. This impaired enzymatic activity leads to reduced levels of 5-MTHF and elevated homocysteine, two metabolic disruptions that have been associated with increased BC risk.^{3,5-10} Despite numerous studies investigating the association between C677T and BC, the findings have remained inconsistent (Table 1), likely due to factors such as differences in sample sizes and genotyping methodologies. To resolve these discrepancies, this meta-analysis consolidates evidence from numerous studies conducted in the United States (US) to clarify

the role of the C677T variant in BC susceptibility in this population.

2. Method

2.1. Literature survey

We conducted a meta-analysis to investigate the association between the C677T polymorphism and BC risk, specifically among populations in the US. A systematic and comprehensive literature search was performed across multiple electronic databases, including PubMed, Google Scholar, and Semantic Scholar, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines to ensure methodological transparency and rigor (Tables S1 and S2).

A comprehensive set of keywords was utilized to capture all relevant studies, including terms such as “*MTHFR* C677T and BC risk,” “C677T polymorphism and BC susceptibility,” “methylenetetrahydrofolate reductase gene variant,” “*MTHFR* mutation and oncogenesis,” “single nucleotide polymorphism (SNP) C677T,” “genetic polymorphisms and breast neoplasms,” “BC genetic risk factors,” and “*MTHFR* gene polymorphism epidemiology.” Only studies conducted in the US were included to ensure population homogeneity and relevance. To enhance the reliability and minimize bias, unpublished, incomplete, or inaccessible studies, as well as sources such as book

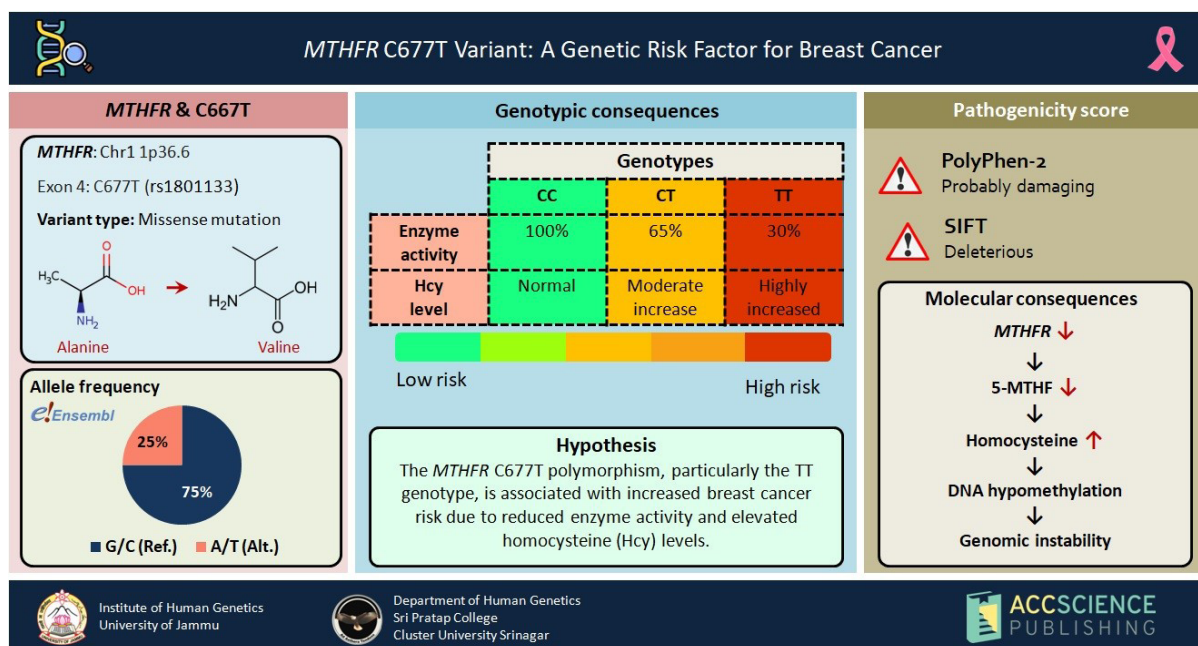


Figure 1. Genetic and molecular insight into the *MTHFR* C677T variant

Abbreviations: MTHFR: Methylenetetrahydrofolate reductase; 5-MTHF: 5-methyltetrahydrofolate; SIFT: Sorting Intolerant from Tolerant.

chapters, conference proceedings, and preprints (e.g., Research Square), were excluded from the analysis.

2.2. Inclusion and exclusion criteria, data extraction, and quality assessment

Stringent inclusion and exclusion criteria were applied to ensure the accuracy, reliability, and validity of the meta-analysis by selecting high-quality studies with relevant and consistent data. This approach minimized bias and heterogeneity, focusing on studies with clinically confirmed BC cases, well-characterized genetic polymorphisms, and sufficient statistical information to enable robust effect size estimation. By excluding studies with poor methodological quality, incomplete data, or inconsistent case definitions, the meta-analysis yields more precise and generalizable conclusions regarding the association between genetic variants and BC risk. Each study was evaluated for design, population characteristics, and methodology (Table 1), and relevant numerical data were extracted to facilitate analysis. Missing or incomplete data were addressed through review of previous meta-analyses, additional sources, or direct author contact when necessary. Data extraction was performed independently by two authors (AS and MY) and validated by the corresponding author (PK) to maintain quality control.

Study quality was assessed using the Newcastle–Ottawa Scale (NOS; Ottawa Hospital Research Institute), which evaluates selection, comparability, and outcome/exposure assessment, with scores ranging up to nine stars. Studies scoring below five stars were excluded, while those with five or more stars were included. Discrepancies in study selection or quality assessment were resolved by the corresponding author.

2.3. Statistical analysis

Genotypic and allelic data were extracted from each included study. Hardy–Weinberg equilibrium (HWE) in control groups was assessed using the chi-square test, with p -values adjusted by the Benjamini–Hochberg false discovery rate method. Genotypic and allelic frequencies were calculated using GeneRiskCalc software (version 1, India).¹¹ Publication bias was evaluated using Begg's and Egger's tests, and heterogeneity was measured by Cochran's Q and I^2 statistics ($p < 0.10$ indicating significant heterogeneity). Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to assess the association, with $p < 0.05$ considered significant. A random-effects model was applied when I^2 exceeded 30%; otherwise, a fixed-effects model was used. Sensitivity analyses were performed by excluding studies sequentially. The meta-analysis was conducted using the Meta-Genyo platform (V1, Bioinformatics Unit, Centre for Genomics and

Oncological Research [GENYO], Spain), a specialized tool for genetic association studies.¹²

Notably, the Bonferroni correction was applied to adjust the p -values for multiple comparisons across seven genetic models. By dividing the significance threshold (0.05) by the number of tests ($n = 7$), the adjusted p -value threshold was set to approximately 0.0071. Both the original and adjusted p -values were reported to ensure that the associations between the C677T polymorphism and BC risk are statistically robust and to minimize the likelihood of false positives.

2.4. Trial sequential analysis

To reduce random errors, trial sequential analysis (TSA) was conducted using software from the Copenhagen Trial Unit (software version 0.9.5.10 Beta, Copenhagen Trial Unit, Copenhagen, Denmark). The required information size (RIS) was calculated with a 5% type I error, 80% power, and a 20% relative risk reduction. If the cumulative Z -curve crosses the RIS, the evidence is considered sufficient; otherwise, additional studies are warranted.¹³

3. Result

3.1. Characteristics of studies

A total of 11 eligible case–control studies conducted in the US and published between 2003 and 2019 were included in this meta-analysis after systematic and critical filtering of the database (Figure 2). All studies employed a case–control design, comprising an overall sample size of 21,564 (BC cases = 9,137, controls = 12,427; Table 1). Quality assessment using the NOS indicated high methodological quality, with scores ranging from 7 to 8 (Table 1). Regarding study characteristics, most studies (9/11) used population-based controls, while two studies relied on hospital-based controls. The genotyping methods varied, with polymerase chain reaction–restriction fragment length polymorphism and TaqMan assays being the most frequently used, followed by quantitative polymerase chain reaction and allele-specific primer extension (Table 1).

The genotypic distribution of the *MTHFR* C677T variant (CC, CT, TT) was reported for both BC cases and controls across all included studies (Table 2). A pooled analysis revealed a total of 4,061 CC (44.45%), 3,901 CT (42.69%), and 1,175 TT (12.86%) genotypes among 9,137 BC cases, and 5,619 CC (45.22%), 5,300 CT (42.65%), and 1,508 TT (12.13%) genotypes among 12,427 controls. Among the cases, the allele frequencies were 65.79% for C and 34.21% for T, while in controls, the C and T allele frequencies were 66.54% and 33.46%, respectively. Before assessing the association, HWE was evaluated for all control groups. Most controls conformed to HWE, except for the

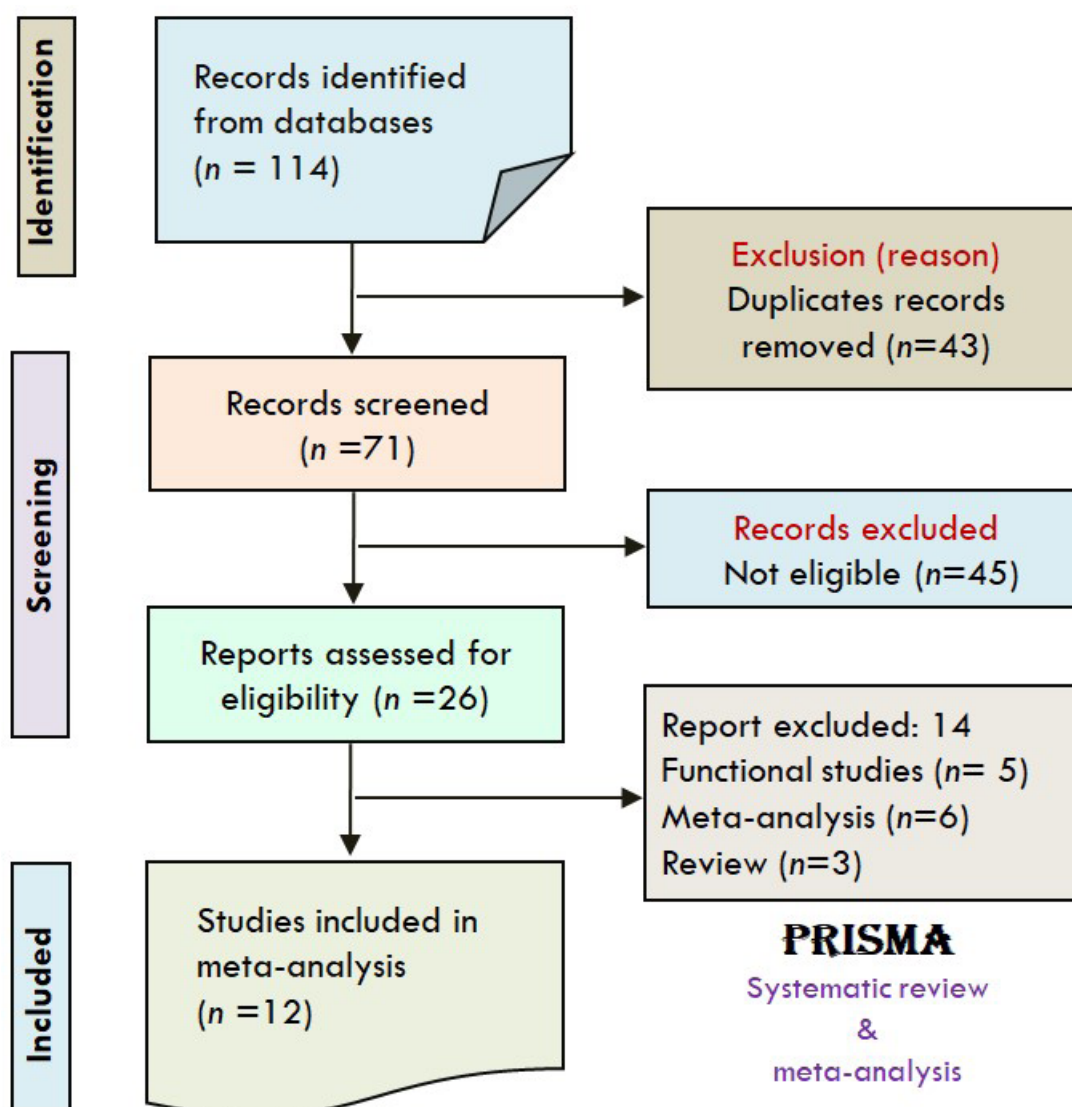


Figure 2. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram showing study selection for meta-analysis of *MTHFR* C677T polymorphism and breast cancer risk.

study by Le Marchand *et al.*,²² which showed a significant deviation ($p < 0.001$) and was therefore excluded from the final analysis (Table 1).

3.2. Pooled analysis

In the present pooled analysis comprising 11 case-control studies, no statistically significant association was observed between the *MTHFR* C677T variant and BC risk across any of the evaluated genetic models. The allele contrast model (Figure 3) (T vs. C) yielded an OR of 1.022 (95% CI = 0.96–1.08; $p = 0.49$; Table 3), suggesting a null association (adjusted $p = 1$). Similarly, the recessive model (TT vs. CT+CC) and the dominant model (TT+CT vs. CC) showed ORs of 1.042 and 1.016, respectively,

both with non-significant p -values ($p = 0.5749$ and $p = 0.690$, respectively). The over-dominant model (CT vs. TT+CC) also demonstrated a lack of association (OR = 0.99; 95% CI = 0.90–1.09; $p = 0.9632$). Further genotypic comparisons (homozygous recessive [HR] vs. homozygous wild-type [HW], HR vs. heterozygous [HT], and HT vs. HW) yielded similarly null results, with ORs close to unity and wide confidence intervals, reinforcing the absence of a detectable genetic effect (Table 3).

Heterogeneity was generally low to moderate, with I^2 values ranging from 39.4% to 56.54%, indicating relatively consistent findings across studies (Table 3). Egger's test revealed no evidence of publication bias across any model,

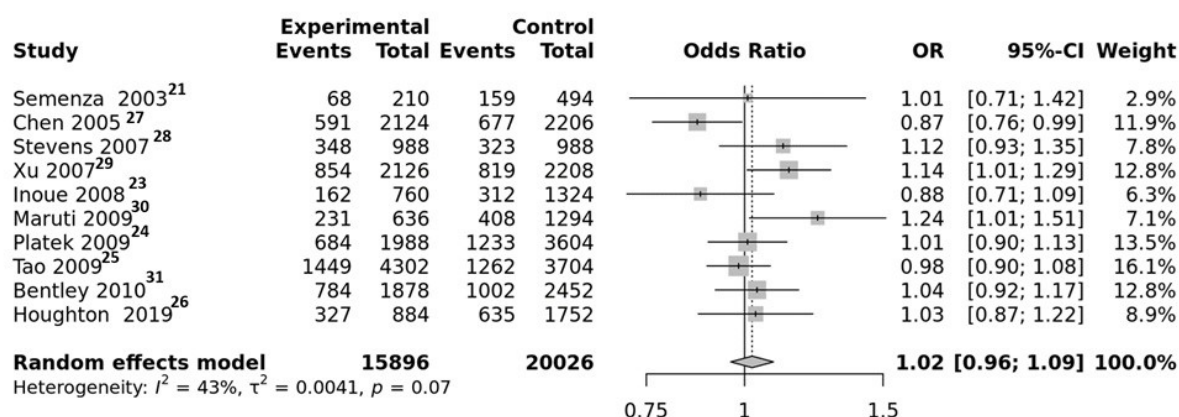


Figure 3. Pooled analysis of the association between the T allele of *MTHFR* C677T variant and breast cancer susceptibility using the allele contrast model (T vs. C).

Abbreviations: CI: Confidence interval; OR: Odds ratio.

Table 2. Genotypic and allelic distribution of *MTHFR* C667T polymorphism among breast cancer cases and controls in the United States population

Participants	Genotypic frequency			Allele frequency	
	CC (n [%])	CT (n [%])	TT (n [%])	C (n [%])	T (n [%])
Case (n = 9,137)	4,061 (44.45%)	3,901 (42.69%)	1,175 12.86%	0.6579	0.3421
Control (n = 12,427)	5,619 (45.22%)	5,300 (42.65%)	1,508 (12.13%)	0.6654	0.3346

Table 3. Meta-analysis of *MTHFR* C677T polymorphism and breast cancer risk in the United States population

Model	Test of association				Test of heterogeneity			Publication bias p-value (Egger's test)
	OR	95% CI	p-value	Adjusted p-value	Model	p-value	I ²	
Allele model	1.022	0.96–1.08	0.493058	1	Random	0.0695	0.4332	0.6665
Recessive mode	1.042	0.90–1.20	0.574916	1	Random	0.0218	0.5368	0.2365
Dominant model	1.016	0.93–1.10	0.690144	1	Random	0.0951	0.394	0.1463
Over-dominant	0.997	0.90–1.09	0.963269	1	Random	0.0191	0.5457	0.1016
HR vs. HW	1.048	0.91–1.20	0.511221	1	Random	0.0594	0.4505	0.531
HR vs. HT	1.033	0.88–1.21	0.679625	1	Random	0.014	0.5654	0.1416
HT vs. HW	1.008	0.92–1.10	0.852427	1	Random	0.061	0.4477	0.0936

Abbreviations: CI: Confidence interval; HR: Homozygous recessive; HT: Heterozygous; HW: Homozygous wild-type; OR: Odds ratio.

such as allele (Figure 4) (all $p > 0.05$), supporting the robustness of the results (Table 3). Additionally, sensitivity analyses confirmed the stability of the findings, as no single study significantly altered the pooled estimates when excluded (Figure 5).

3.3. Ethnicity-based subgroup analysis

Subgroup analysis by ethnicity showed no significant association between the C677T polymorphism and BC risk in either Caucasian or multi-ethnic populations across all genetic models. For instance, in the allele model, the OR was 1.035 (95% CI = 0.9382–1.1429, $p = 0.4879$) in Caucasians and 1.0201 (95% CI = 0.9407–1.1062, $p = 0.6304$) in the multi-ethnic group. Similar null results were observed in the recessive, dominant, over-dominant, and genotype comparison models. Heterogeneity ranged from low to moderate, and no publication bias was detected (Egger's test $p > 0.1$). These findings suggest no ethnicity-specific risk contribution of this variant (Table 4).

Collectively, these findings suggest that the *MTHFR*

C677T variant is not significantly associated with BC susceptibility in the US population, and its contribution, if any, is likely minimal or confounded by other genetic or environmental factors.

3.4. Trial sequential analysis

The TSA indicates that the RIS = 2,839 has been achieved, suggesting that the cumulative sample size is sufficient to draw reliable conclusions. However, the cumulative Z-curve remains within the monitoring boundaries and fluctuates around the null effect line (Figure 6), indicating that the current evidence does not demonstrate a statistically significant association between the *MTHFR* C677T variant and BC risk. This pattern supports the interpretation of a true lack of association, rather than a result limited by insufficient power. In other words, despite reaching an adequate sample size, the absence of a definitive signal suggests that any potential genetic effect is likely minimal, weak, or context-dependent, such as due to gene–environment interactions or subpopulation differences. Therefore, further research focusing on well-

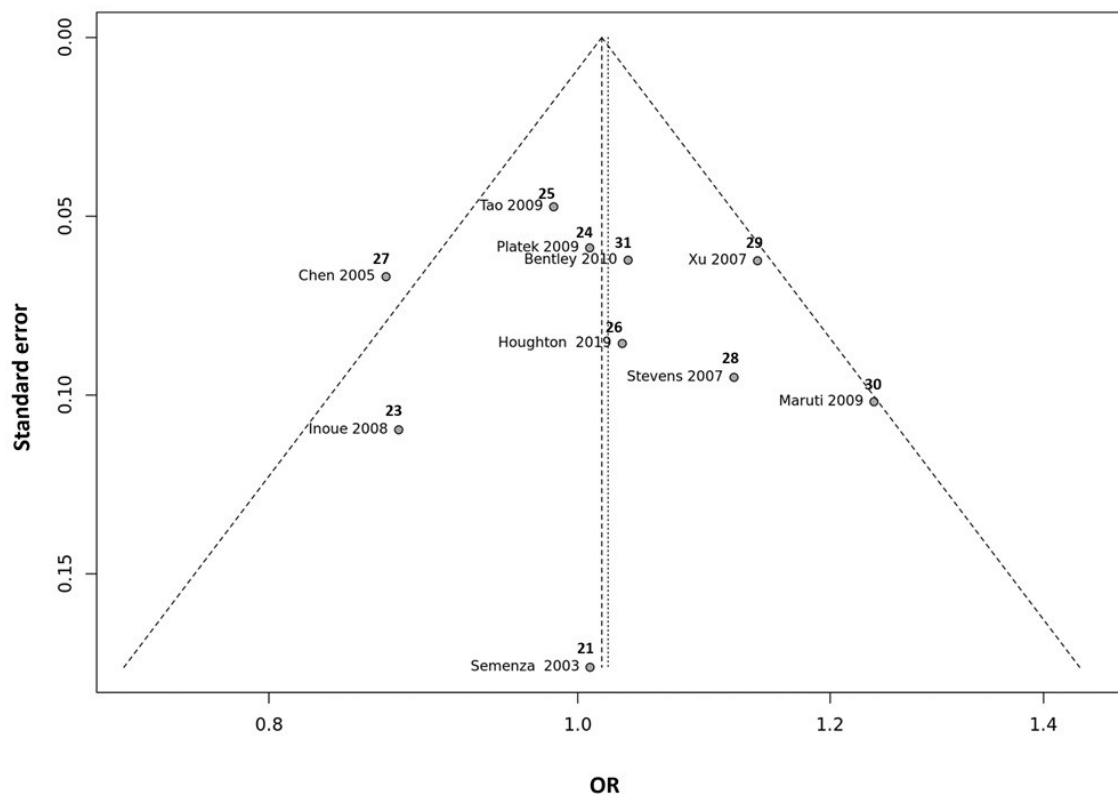


Figure 4. Funnel plot for publication bias assessment in the allelic model (T vs. C) of the *MTHFR* C677T variant

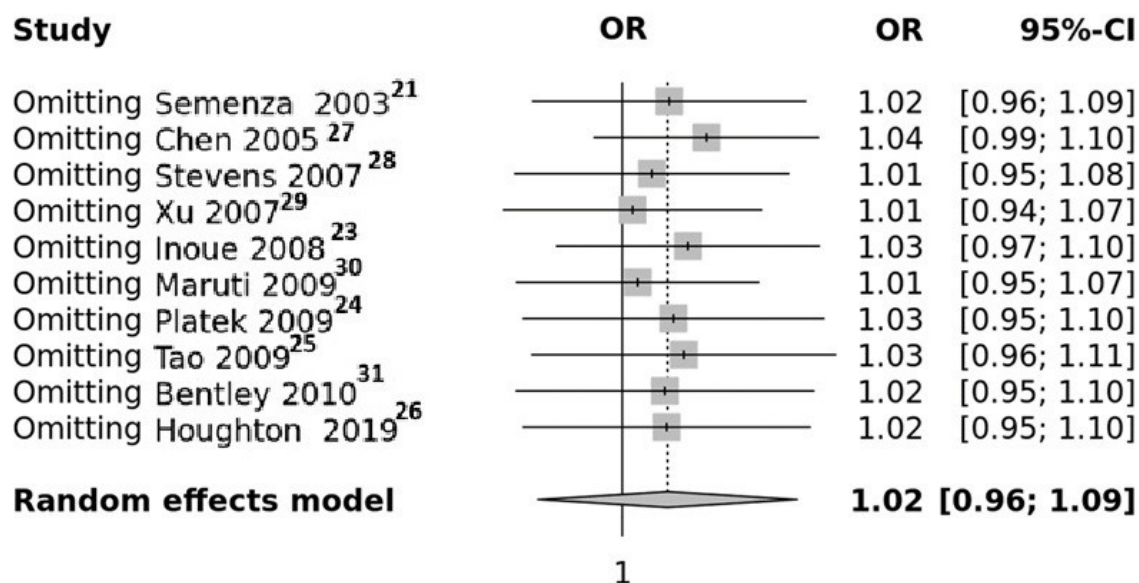


Figure 5. Leave-one-out forest plot for the *MTHFR* C677T variant
Abbreviations: CI: Confidence interval; OR: Odds ratio.

Table 4. Ethnicity-based subgroup analysis of *MTHFR* C677T polymorphism and breast cancer risk

Model	Ethnicity	Number of studies	Test of association				Test of heterogeneity			Publication bias <i>p</i> -value (Egger's test)
			OR	95% CI	<i>p</i> -value	Adjusted <i>p</i> -value	Model	<i>p</i> -value	<i>I</i> ²	
Allele	Caucasian	2	1.0355	0.93–1.14	0.487936	1	Fixed	0.9683	0	NA
	Multi-ethnic	8	1.0201	0.94–1.10	0.63037	1	Random	0.0278	0.5548	0.7072
Recessive	Caucasian	2	1.0509	0.69–1.593	0.815043	1	Random	0.0425	0.757	NA
	Multi-ethnic	8	1.0323	0.87–1.21	0.70747	1	Random	0.0378	0.5291	0.4281
Dominant	Caucasian	2	1.0024	0.87–1.15	0.973937	1	Fixed	0.127	0.5707	NA
	Multi-ethnic	8	1.0193	0.92–1.12	0.69647	1	Random	0.0846	0.441	0.2455
Over-dominant	Caucasian	2	0.9947	0.64–1.53	0.980955	1	Random	0.0023	0.8925	NA
	Multi-ethnic	8	0.9851	0.92–1.05	0.661252	1	Fixed	0.1805	0.31	0.1189
HR vs. HW	Caucasian	2	1.0836	0.88–1.32	0.440054	1	Fixed	0.341	0	NA
	Multi-ethnic	8	1.0414	0.87–1.24	0.656166	1	Random	0.0314	0.545	0.6824
HR vs. HT	Caucasian	2	1.0364	0.57–1.85	0.904215	1	Random	0.0073	0.8612	NA
	Multi-ethnic	8	1.0272	0.87–1.20	0.745479	1	Random	0.0774	0.4528	0.2781
HT vs. HW	Caucasian	2	1.0087	0.69–1.46	0.963251	1	Random	0.0171	0.8242	NA
	Multi-ethnic	8	0.9958	0.92–1.06	0.907902	1	Fixed	0.1615	0.3339	0.1275

Abbreviations: CI: Confidence interval; HR: Homozygous recessive; HT: Heterozygous; HW: Homozygous wild-type; NA: Not available; OR: Odds ratio.

defined subgroups, environmental modifiers, or gene–gene interactions may be necessary to fully elucidate any subtle or conditional effects of this polymorphism on disease risk.

4. Discussion

Folate, or vitamin B9, is a hydrophilic, anionic molecule essential for human health. Unlike plants and some bacteria, humans cannot synthesize folate and must obtain it from dietary sources such as okra, beans, sunflower seeds, broccoli, asparagus, and spinach.^{14,15} Folate is transported into cells via three main carriers: reduced folate carrier, proton-coupled folate transporter, and folate receptors. Inside the cell, folate is crucial for nucleotide synthesis and one-carbon metabolism.¹⁶ The enzyme MTHFR converts 5,10-methylenetetrahydrofolate to 5-MTHF, which is vital for remethylating homocysteine to methionine. Methionine then forms S-adenosylmethionine, a key methyl donor for DNA methylation. The common *MTHFR* C677T variant reduces enzyme activity, lowering 5-MTHF and folate availability, impairing homocysteine remethylation and S-adenosylmethionine production.¹⁷ This can disrupt DNA methylation, an epigenetic process often linked to cancer development.^{18–20}

Therefore, the present pooled analysis brought together data from 11 different case–control studies (Table 1) across the US to investigate whether the *MTHFR* C677T variant increases the risk of BC. However, when we analyzed the

data across multiple genetic models, including comparisons of different genotypes and alleles, we consistently found no statistically significant association between the *MTHFR* C677T variant and BC risk (Table 3). We also examined the consistency of the results across the studies and found that heterogeneity ranged from low to moderate, indicating that the findings were generally in agreement. Even in models with slightly higher variability, such as the recessive model or the HR vs. HT comparison, the overall direction and strength of the association remained unchanged (Table 3).

To confirm the robustness of our findings, we first evaluated publication bias, which was not detected (Table 3). Next, sensitivity analyses showed consistent, stable results, indicating no single study unduly influenced the outcome. Additionally, TSA demonstrated that while our total sample size surpassed the required threshold, the cumulative evidence (Z-curve) did not reach statistical significance (Figure 6), indicating that the data are reliable and suggesting there is likely no strong association between the *MTHFR* C677T variant and BC risk. However, these findings cannot be interpreted with complete certainty, and further research is warranted to confirm them.

Thus, our analysis corroborates earlier individual studies that reported no significant association between the C677T polymorphism and BC risk.^{21–26} However, it stands in contrast to other findings that observed a significant association,^{27–31} emphasizing the continued inconsistencies

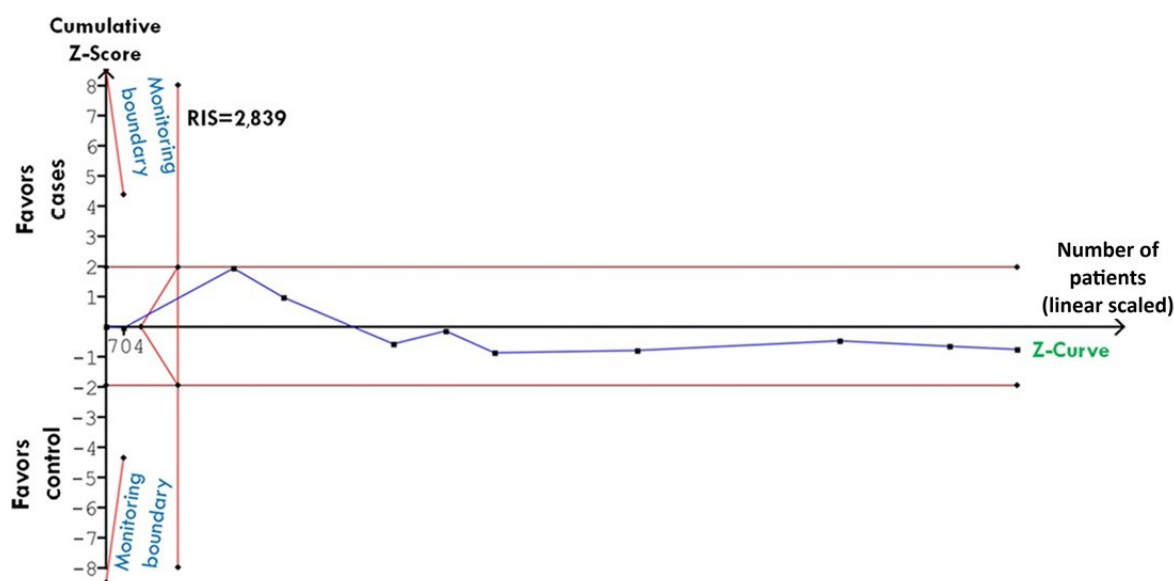


Figure 6. Trial sequential analysis plot for the association between C677T polymorphism and breast cancer risk in the United States under the allelic model
Abbreviation: RIS: Required information size.

Table 1. Detailed characteristics of studies examining the association between the *MTHFR* C677T polymorphism and breast cancer risk

No.	Study	Publication year	Ethnicity	Type of study	Source of control	Genotyping	Sample size	Case			Control			Significant association	NOS	HWE (p-value)
								CC	CT	TT	CC	CT	TT			
1	Semenza <i>et al.</i> ²¹	2003	Multi-ethnic	Case-control	HB	PCR-RFLP	105/247	42	58	5	112	111	24	No	7	0.7575
2	Le Marchand <i>et al.</i> ²²	2004	Multi-ethnic	Case-control	PB	TaqMan	1,189/2,414	573	479	137	1,211	920	283	No	7	p < 0.001
3	Chen <i>et al.</i> ²⁷	2005	Multi-ethnic	Case-control	PB	PCR-RFLP	1,062/1,103	558	417	87	536	457	110	Yes	7	0.7467
4	Stevens <i>et al.</i> ²⁸	2007	Multi-ethnic	Case-control	PB	TaqMan	494/494	208	224	62	236	193	65	Yes	8	0.0693
5	Xu <i>et al.</i> ²⁹	2007	Multi-ethnic	Case-control	PB	PCR-RFLP	1,063/1,104	398	476	189	440	509	155	Yes	8	0.7575
6	Inoue <i>et al.</i> ²³	2008	Multi-ethnic	Case-control	PB	TaqMan	380/662	239	120	21	393	226	43	No	8	0.6534
7	Maruti <i>et al.</i> ³⁰	2009	Multi-ethnic	Case-control	PB	ASPE	318/647	133	139	46	301	284	62	Yes	8	0.7575
8	Platek <i>et al.</i> ²⁴	2009	Multi-ethnic	Case-control	PB	TaqMan	274/531	429	446	119	788	795	219	No	8	0.7467
9	Tao <i>et al.</i> ²⁵	2009	Multi-ethnic	Case-control	PB	qPCR	2,151/1,852	969	915	267	813	816	223	No	7	0.7467
10	Bentley <i>et al.</i> ³¹	2010	Caucasian	Case-control	HB	PCR-RFLP	939/1,226	346	402	191	429	592	205	Yes	7	0.9747
11	Houghton <i>et al.</i> ²⁶	2019	Caucasian	Case-control	PB	TaqMan	442/876	166	225	51	360	397	119	No	8	0.7575
12	Meta-analysis (present study)	2025	Multi-ethnic	Systematic review	Mixed	Mixed	9,137/12,427	4,061	3,901	1,175	5,619	5,300	1,508	No	-	-

Abbreviations: ASPE: Allele-specific primer extension; HB: Hospital-based; HWE: Hardy-Weinberg equilibrium; NOS: Newcastle-Ottawa Scale; PB: Population-based; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; qPCR: Quantitative polymerase chain reaction.

within the existing literature. To our knowledge, no prior pooled analysis has been specifically conducted in this population.

It is important to note that although the US population is predominantly Caucasian, the samples included in our analysis consisted of both multi-ethnic and Caucasian groups, with only a limited number of studies exclusively involving Caucasian participants ($n = 2$) (Table 1). We assessed the influence of ethnicity on the association; however, no statistically significant ethnic-specific association was observed (Table 4). Upon further exploration of the literature, evidence from ethnic subgroup analyses suggests potential variability in genetic susceptibility to BC related to the C677T polymorphism. Notably, previous studies reported no significant association in Caucasian populations,^{32,33} while a consistent and significant association was observed among Asian populations.^{32,33} This ethnicity-specific pattern is further supported by a meta-analysis conducted by Liang *et al.*,³⁴ which focused on the Chinese population, a representative subgroup of Asians, and identified a significant association between the C677T variant and BC risk. Collectively, these findings suggest that the C677T polymorphism may contribute to increased BC risk in certain ethnic groups, particularly among Asians. This underscores the critical importance of incorporating ethnic background into the design and interpretation of genetic association studies.

In summary, it is biologically plausible that the C677T variant reduces the activity of the MTHFR enzyme, potentially affecting DNA methylation, a process known to play a role in cancer development.³⁵ However, in the US population, the effect does not appear to be strong enough on its own to significantly influence BC risk (Table 3). This is likely because BC is a multifactorial disease, influenced by a combination of genetic, hormonal, environmental, and lifestyle-related factors.³⁶ A single gene variant like C677T may have only a modest impact (low penetrant variant), especially when factors such as dietary folate intake, interacting genes, and environmental exposures are not accounted for. The data were sufficient to make a robust assessment, with findings that were consistent and reliable. The results suggest that if the C677T variant does influence BC risk, its effect is likely to be small or conditional on other modifying factors such as diet, lifestyle, or additional genetic variations. Future research should aim to investigate these complex gene-environment and gene-gene interactions more comprehensively.

4.1. Strengths, limitations, and future perspectives

This meta-analysis benefits from a rigorous and systematic approach, including a comprehensive literature search

restricted to the US population, ensuring both population homogeneity and relevance. The high methodological quality of the included studies, assessed using the NOS, enhances the reliability of the findings. The use of multiple genetic models, Bonferroni correction for multiple testing, and TSA further strengthens the robustness and validity of the results.

While this meta-analysis offers valuable insights, several limitations should be acknowledged. First, the possibility of residual confounding from unmeasured environmental or lifestyle factors cannot be ruled out. Differences in genotyping techniques across studies may have introduced methodological variability, and moderate heterogeneity in some models may suggest underlying inter-study differences. Although statistical tests revealed no evidence of publication bias, the exclusion of unpublished and non-English studies could still pose a subtle risk. In addition, we acknowledge that the absence of recent studies after 2019 is a limitation that may affect the applicability of our findings to current populations. Lastly, the predominance of US Caucasian participants limits the broader applicability of the findings to ethnically diverse populations.

Future research should focus on large-scale, multi-ethnic cohort studies integrating gene-environment interactions to better elucidate the complex role of *MTHFR* gene polymorphisms in BC risk. Advanced genomic techniques and functional studies are also needed to explore mechanistic pathways and potential interactions with other susceptibility genes.

5. Conclusion

This meta-analysis of 11 case-control studies from the US found no significant association between the C677T polymorphism and BC risk. Pooled analyses across multiple genetic models showed ORs close to 1, with non-significant p -values. TSA confirmed an adequate sample size, but it did not support a true effect. Overall, the findings suggest that the *MTHFR* C677T variant is not a major risk factor for BC in the US population. Further research is needed to explore gene-environment interactions and other potential genetic contributors.

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Conflict of interest

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Author contributions

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Writing—original draft: Amrit Sudershan

Writing—review & editing: Mohd Younis, Parvinder Kumar

Availability of data

All data generated and/or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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