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Meta Analysis

Effects of methylenetetrahydrofolate reductase single-nucleotide polymorphisms on breast, cervical, ovarian, and endometrial cancer susceptibilities

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Abstract

Background: Recent studies identifying *methylenetetrahydrofolate reductase* (*MTHFR*) polymorphisms associated with breast cancer (BC), ovarian cancer (OC), cervical cancer, and endometrial cancer (EC) have reported conflicting results and been underpowered. To clarify the correlation between *MTHFR* mutations and these common female malignancies, we conducted a comprehensive meta-analysis incorporating all eligible publications.

Methods: Relevant reports published before January 20, 2020, were retrieved from PubMed, Embase, the Cochrane Library, and the China National Knowledge Infrastructure databases. The odds ratio and 95% confidence interval summaries for the *MTHFR* 677C/T and 1298A/C polymorphisms in BC, OC, cervical cancer, and EC were estimated.

Results: A total of 171 studies comprising 56,675 cancer cases and 67,559 controls were included. The results showed a markedly elevated risk of cancer susceptibility related to *MTHFR* 677C/T based on all genetic models. Similarly, we identified a significant correlation between 1298A/C mutation and cancer risk based on overall comparisons among all models, except the heterozygous model. Moreover, subgroup analysis by cancer type revealed a significantly increased risk of BC associated with 677C/T in the five models and of cervical cancer associated with 1298A/C in some models. Based on ethnicity, significant associations were observed between Asian, African, and mixed populations for 677C/T and the Asian population for 1298A/C. With regard to the sample type used for analysis, we detected a positive association between using blood as the DNA source and cancer risk for 677C/T in all genetic models and for 1298A/C in some genetic models. Further stratification of the results revealed that a notably increased risk was associated with the use of polymerase chain reaction-restriction fragment-length polymorphism or TaqMan as the genotyping method, as well as with the use of population-or hospital-based groups as the controls for 677C/T and 1298A/C, respectively.

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Conclusion: This meta-analysis suggests that *MTHFR* 677C/T and 1298A/C polymorphisms correlate with the risk of common gynecological cancers, with these findings potentially applicable for overall comparisons of related data.

Keywords: Methylenetetrahydrofolate reductase; Breast cancer; Female genital neoplasms; Polymorphism; Meta-analysis

Introduction

Breast cancer (BC) and female reproductive-system-associated cancers, including ovarian cancer (OC), cervical cancer, and endometrial cancer (EC), significantly impact the health of women and present high associated mortality rates.[1,2] Nearly 2.7 million BC cases were newly diagnosed in 2018, accounting for approximately one in four cancer cases among women.[3] Among these cases, cervical cancer is globally the most common, accounting for a significant proportion of all gynecologic cancer deaths. Moreover, 14 new EC cases are reported annually per 100,000 women, and about 300,000 new OC cases are reported worldwide.[3] Although the specific factors associated with the pathogenesis of these common tumors have not been characterized, it is believed that their development involves multifactorial processes, including genetic factors, menstrual status, and environmental factors, among several others.[4] Indeed, studies show that cancer occurrence and outcomes vary among racial and ethnic groups.[5] Additionally, a previous report suggested that BC often occurs post-menopause when the ovaries have stopped producing estrogen.[6] It is widely believed that cancer is caused partly by genetic mutations, which subsequently lead to abnormal proliferation and/or inhibited apoptosis of breast, cervix, uterus, and ovary cells and result in corresponding cancers.[7,8] Additionally, increasing evidence suggests that single-nucleotide polymorphisms (SNPs) in specific oncogenes might be associated with the pathogenesis of these cancers.[9]

Recent studies suggest a correlation between the occurrence of common female malignancies and mutations in the methylenetetrahydrofolate reductase (*MTHFR*) gene.[10,11] *MTHFR* is located on chromosome 1p36.3[12] and encodes an enzyme that catalyzes transformation of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, which regulates methylation and folate metabolism and is related to DNA synthesis and methylation during normal development and growth.[13,14] Thus, *MTHFR* polymorphisms can cause gene instability and increase the risk of developing certain cancers by affecting DNA synthesis or methylation. Indeed, 677C/T (rs1801133) and 1298A/C (rs1801131), the two most common *MTHFR* variants, are reportedly highly associated with altered enzymatic activity.[15,16] Specifically, the 677C/T SNP is a missense mutation in exon 4 that results in replacement of a cytosine (the most frequent allele) with athymine, corresponding to conversion of an alanine into avaline codon. The 1298A/C SNP corresponds to an adenosine (the most frequent allele)-to-cytosine transversion in exon 7 that leads to a glutamate-to-alanine substitution.[17] Although studies have investigated the relationships between these polymorphisms and various cancer types, the results have been inconsistent, with some groups reporting a significant correlation between the *MTHFR*677TT genotype and an increased risk of BC or OC,[18,19] whereas others state that no such correlation exists.[20,21] Similarly, Liu et al[22] and Wang et al[23] report that the 1298A/C polymorphism is associated with significantly elevated risk of BC and OC, whereas no association was reported by other studies.[19,24]

The conflicting results regarding the relevance of these polymorphisms to common female malignancies, as well as the small associated sample sizes of the studies, make it difficult to draw definitive conclusions. Interestingly, previous studies indicate that the allele and genotype frequencies of the *MTHFR* 677C/T or *MTHFR* 1298A/C polymorphisms are similar between BC, OC, and cervical and uterine cancers.[25,26] For example, the allele frequencies of the *MTHFR* 677T and 1298C polymorphisms were 32.80% and 33.33%, respectively. Additionally, a previous study reported genotype frequencies of *MTHFR* CC, CT, and TT as 44.50%, 45.41%, and 10.09%, respectively, and the frequencies of *MTHFR* AA, AC, and CC as 46.58%, 40.18%, and 13.24% in EC patients, respectively.[27] These data were similar to findings reported for three other cancer types.

Therefore, in the present study, we performed a comprehensive meta-analysis to explore the correlation between *MTHFR* mutations and these cancers within a larger pooled population.

Methods

Literature search strategy and inclusion criteria

PubMed, Embase, the Cochrane Library, and the China National Knowledge Infrastructure databases were searched for all available studies published up to January 1, 2020, and related to the correlation between *MTHFR* C677/T and A1298/C polymorphisms and susceptibility to the four common cancers among women. The search terms used were "MTHFR", "C677T", "A1298C", "rs1801133", "rs1801131", "polymorphism", "genital neoplasms", "BC", "cervical cancer", "OC", and "EC". We also searched for available articles in relevant meta-analyses and review references.

A total of 515 potential manuscripts were identified in the primary searches (Fig. 1). Eleven additional review studies and meta-analysis articles were also included, resulting in a total of 526 articles. The inclusion criteria for studies were as follows: (1) case-controlled studies; (2) studies where a correlation was investigated between *MTHFR*C677/T or A1298/C and one or more of the four common women-related cancers; (3) studies where control subjects met Hardy–Weinberg equilibrium (HWE) criteria; and (4) studies providing enough data to calculate a P-value, odds ratio (OR), and 95% confidence interval (CI).

Data extraction

Data were extracted from each publication independently by two authors, and the quality of the included studies was evaluated by at least two authors using the Newcastle–Ottawa Quality Assessment Scale (NOS). The NOS uses a "star" rating system to judge quality based on three aspects: comparability of

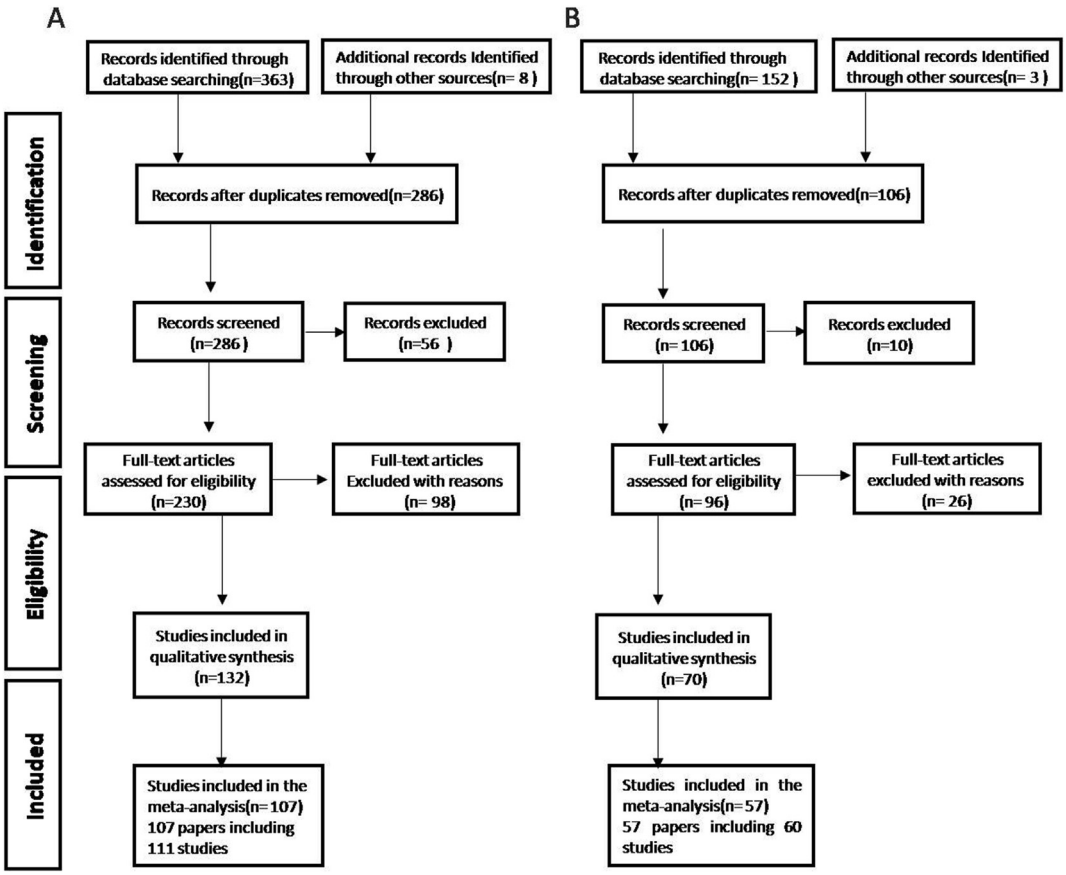

Fig. 1. Flowchart for included publications.

Table 1
Stratified analyses about MTHFR 677C/T polymorphism.

Items	n	Case/controls	T allele vs C allele (allele model) OR (95% CI) *P* Ph	TT vs CC (homozygous model) OR (95% CI) *P* Ph	CT vs CC (heterozygous model) OR (95% CI) *P* Ph	CT + TT vs CC (dominant model) OR (95% CI) *P* Ph	TT vs CC + CT (recessive model) OR (95% CI) *P* Ph
Total	111	32,023/38,258	1.124(1.072 −1.178)0.000 0.000	1.257(1.149 −1.376)0.000 0.000	1.041(1.007 −1.076)0.019 0.000	1.109(1.047 −1.174)0.000 0.000	1.222(1.128 −1.322)0.000 0.000
Cancer type							
Breast			1.149(1.090 −1.211)0.000 0.000	1.285(1.164 −1.419)0.000 0.000	1.081(1.021 −1.145)0.008 0.000	1.139(1.070 −1.212)0.000 0.000	1.243(1.136 −1.360)0.000 0.000
Ovary			1.105 (0.974 −1.253)0.121 0.003	1.254 (0.921 −1.708)0.150 0.001	1.075 (0.956 −1.209)0.228 0.233	1.106 (0.962 −1.271)0.156 0.052	1.184 (0.906 −1.546)0.216 0.006
Cervical			0.967 (0.789 −1.186)0.747 0.000	1.057 (0.722 −1.548)0.774 0.000	0.859 (0.695 −1.062)0.160 0.003	0.898 (0.705 −1.146)0.388 0.000	1.140 (0.840 −1.547)0.402 0.001
Endometrial			1.123 (0.863 −1.461)0.389 0.034	1.098 (0.767 −1.572)0.610 0.223	1.207 (0.782 −1.865)0.396 0.012	1.220 (0.799 −1.865)0.357 0.009	1.057 (0.857 −1.305)0.604 0.716
Ethnicity							
Caucasian			1.008 (0.959 −1.060)0.755 0.010	1.011 (0.919 −1.113)0.818 0.121	1.018 (0.948 −1.094)0.620 0.009	1.016 (0.947 −1.089)0.665 0.005	1.012 (0.930 −1.102)0.780 0.210
Asian			1.196(1.101 −1.299)0.000 0.000	1.455(1.253 −1.690)0.000 0.000	1.077 (0.986 −1.177)0.100 0.000	1.165(1.055 −1.287)0.003 0.000	1.387(1.222 −1.574)0.000 0.000
African			1.361(1.055 −1.755)0.018 0.489	2.042(1.090 −3.827)0.026 0.801	1.187 (0.582 −2.419)0.638 0.036	1.328 (0.770 −2.291)0.308 0.115	1.917(1.045 −3.515)0.035 0.454
Mixed			1.179(1.085 −1.282)0.000 0.246	1.444(1.157 −1.802)0.001 0.064	1.062 (0.961 −1.175)0.239 0.881	1.145(1.041 −1.258)0.005 0.843	1.402(1.124 −1.749)0.003 0.027
Genotyping methods							
PCR-RFLP			1.211(1.124 −1.305)0.000 0.000	1.572(1.353 −1.827)0.000 0.000	1.111(1.017 −1.215)0.020 0.000	1.191(1.086 −1.306)0.000 0.000	1.480(1.298 −1.688)0.000 0.001
TaqMan			1.053 (0.962 −1.153)0.263 0.000	1.058 (0.929 −1.205)0.393 0.001	1.004 (0.910 −1.107)0.937 0.000	1.040 (0.929 −1.164)0.492 0.000	1.060 (0.953 −1.178)0.285 0.017
Others			1.059 (0.987 −1.136)0.110 0.002	1.100 (0.933 −1.298)0.257 0.001	1.022 (0.959 −1.089)0.497 0.446	1.054 (0.980 −1.133)0.155 0.162	1.087 (0.928 −1.274)0.301 0.000
Control source							
Population -based			1.104(1.036 −1.176)0.002 0.000	1.184(1.054 −1.330)0.005 0.000	1.030 (0.966 −1.098)0.365 0.001	1.083(1.006 −1.166)0.033 0.000	1.164(1.047 −1.294)0.005 0.000
Hospital -based			1.158(1.070 −1.252)0.000 0.000	1.355(1.161 −1.581)0.000 0.000	1.104(1.008 −1.210)0.034 0.000	1.155(1.047 −1.274)0.004 0.000	1.293(1.135 −1.473)0.000 0.000
DNA sample							
Blood			1.156(1.093 −1.223)0.000 0.000	1.323(1.190 −1.470)0.000 0.000	1.076(1.011 −1.146)0.022 0.000	1.141(1.066 −1.221)0.000 0.000	1.274(1.160 −1.399)0.000 0.000

(*continued on next page*)

Table 1 (*continued*)

Items	n	Case/controls	T allele vs C allele (allele model) OR (95% CI) *P* Ph	TT vs CC (homozygous model) OR (95% CI) *P* Ph	CT vs CC (heterozygous model) OR (95% CI) *P* Ph	CT + TT vs CC (dominant model) OR (95% CI) *P* Ph	TT vs CC + CT (recessive model) OR (95% CI) *P* Ph
Tissue			0.955 (0.805 −1.134)0.602 0.002	0.927 (0.659 −1.302)0.661 0.020	0.983 (0.792 −1.220)0.876 0.013	0.954 (0.757 −1.201)0.686 0.002	0.971 (0.757 −1.246)0.820 0.154
Blood + Tissue			1.044 (0.911 −1.198)0.535 0.010	1.111 (0.841 −1.467)0.458 0.042	0.977 (0.884 −1.079)0.643 0.487	0.999 (0.873 −1.143)0.986 0.162	1.134 (0.867 −1.482)0.358 0.033

research groups, selection of research groups, and determination of exposure of interest. Studies with a score of greater than or equal to seven stars are considered of high quality; however, a standard of five stars was required for inclusion in the present analysis. The extracted information was recorded as follows: author name, publication year, country, ethnicity, cancer type, genotyping data, sample size, source of control, case volume, and HWE score (Table 1).

Statistical analysis

Stata software (v.14.0; StataCorp, College Station, TX, USA) was used for summary statistics. Chi-squared tests of the control groups were conducted to assess the deviation of *MTHFR* 677C/T and 1298A/C mutations from HWE, with a deviation from HWE identified as a result of $P < 0.05$. The correlation between *MTHFR* 677C/T and 1298A/C mutations and susceptibility to the four cancers was assessed using ORs with 95% CIs. Five genetic models, including allelic (T vs C for 677C/T and C vs A for 1298A/C), homozygote (TT vs CC for 677C/T and CC vs AA for 1298A/C), heterozygote (CT vs CC for 677C/T and AC vs AA for 1298A/C), dominant (CT + TT vs CC for 677C/T and AC + CC vs AA for 1298A/C), and recessive (TT vs CC + CT for 677C/T and CC vs AA + AC for 1298A/C), were used in this study. The ORs and 95% CIs were used to quantify the subgroup analyses, which were based on cancer type, ethnicity, genotyping methods, control source, and DNA source. Genotyping methods other than PCR-RFLP and TaqMan were assigned to the "others" group. The Q-test and I^2statistics were used to evaluate statistical heterogeneity. A $P_{\text{heterogeneity (het)}}<0.1$ or $I^2>50\%$ was considered statistically significant for heterogeneity using the random-effects model[28]; otherwise, the Mantel–Haenszel method was used in a fixed-effect model.[29] The sources of heterogeneity were investigated by means of subgroup and meta-regression analyses. Furthermore, sensitivity analysis was performed by removing individual studies sequentially to evaluate the stability of our results. Begg's funnel plot and Egger's test were used to quantitatively evaluate publication bias.[30,31]

Results

Study characteristics

A total of 526 articles were initially retrieved from multiple databases, of which 362 articles were excluded based on their titles and abstracts. Specifically, 102 papers were reviews and meta-analyses; 36 were duplicates; 107 were unassociated with *MTHFR* 677C/T or1298A/C; 76 did not investigate BC, cervical cancer, OC, or EC; 30 lacked complete data; and 11 were inconsistent with HWE. Ultimately, 164 articles, including 171 case-control studies (111 for 677C/T and 60 for 1298A/C), covering 56,675 cancer cases and 67,559 controls were selected (Fig. 1).

In terms of genotyping methods for *MTHFR* 677C/T and 1298A/C, 63 and 27 articles cited the use of PCR-RFLP, 25 and 20 articles cited TaqMan, and 22 and 13 articles cited other methods, respectively. The genotype distribution for both polymorphisms among controls was consistent with HWE. The characteristics of each included study are shown in Table S1.

Quantitative synthesis

The results are summarized in Tables 1 and 2. In the overall comparison, a notable correlation between *MTHFR* 677C/T mutation and the four cancers was

Table 2
Stratified analyses about MTHFR 1298A/C polymorphism.

Items	n	Case/controls	C allele vs A allele (allele model) OR (95% CI) *P* Ph	CC vs AA (homozygous model) OR (95% CI) *P* Ph	AC vs AA (heterozygous model) OR (95% CI) *P* Ph	AC + CC vs AA (dominant model) OR (95% CI) *P* Ph	CC vs AA + AC (recessive model) OR (95% CI) *P* Ph
Total	60	24,857/29,620	1.083(1.024 −1.145)0.005 0.000	1.145(1.030 −1.273)0.012 0.000	1.025 (0.987 −1.064)0.195 0.001	1.070(1.007 −1.137)0.029 0.000	1.110(1.045 −1.179)0.001 0.000
Cancer type							
Breast			1.038 (0.992 −1.086)0.109 0.001	1.086 (0.993 −1.187)0.072 0.113	1.016 (0.972 −1.062)0.475 0.385	1.031 (0.981 −1.083)0.232 0.063	1.079 (0.991 −1.175)0.078 0.088
Ovary			1.014 (0.943 −1.091)0.702 0.412	1.079 (0.889 −1.308)0.441 0.319	0.971 (0.879 −1.071)0.553 0.726	0.991 (0.902 −1.088)0.844 0.932	1.102 (0.895 −1.357)0.361 0.227
Cervical			1.974(1.085 −3.592)0.026 0.000	3.723 (0.922 −15.034) 0.0650.000	1.732(1.056 −2.841)0.030 0.000	2.072(1.088 −3.946)0.027 0.000	2.894 (0.932 −8.984)0.066 0.000
Endometrial			1.049 (0.920 −1.196)0.477 0.677	1.099 (0.783 −1.543)0.585 0.993	1.035 (0.876 −1.223)0.687 0.389	1.046 (0.893 −1.227)0.576 0.491	1.112 (0.800 −1.544)0.528 0.839
Ethnicity							
Caucasian			0.997 (0.962 −1.034)0.888 0.551	1.001 (0.921 −1.086)0.990 0.664	0.993 (0.944 −1.044)0.773 0.587	0.994 (0.948 −1.042)0.799 0.549	1.006 (0.933 −1.084)0.878 0.719
Asian			1.203(1.075 −1.346)0.001 0.000	1.415(1.140 −1.758)0.002 0.000	1.100 (0.995 −1.216)0.063 0.000	1.173(1.042 −1.320)0.008 0.000	1.361(1.120 −1.653)0.002 0.000
African			0.992 (0.691 −1.426)0.967 -	0.933 (0.361 −2.411)0.885 -	1.019 (0.637 −1.629)0.938 -	1.007 (0.642 −1.578)0.977 -	0.925 (0.365 −2.345)0.870 -
Mixed			1.109 (0.956 −1.287)0.172 0.575	1.206 (0.809 −1.797)0.358 0.404	1.117 (0.922 −1.353)0.257 0.821	1.128 (0.939 −1.357)0.198 0.817	1.183 (0.773 −1.808)0.439 0.330
Genotyping methods							
PCR-RFLP			1.088 (0.995 −1.189)0.064 0.000	1.241(1.064 −1.448)0.006 0.145	0.996 (0.922 −1.076)0.926 0.270	1.036 (0.951 −1.129)0.420 0.061	1.273(1.077 −1.504)0.005 0.021
TaqMan			1.114(1.006 −1.234)0.039 0.000	1.144 (0.944 −1.386)0.171 0.000	1.098 (0.995 −1.211)0.062 0.000	1.129(1.005 −1.269)0.041 0.000	1.086 (0.932 −1.265)0.290 0.002
Others			1.029 (0.938 −1.130)0.543 0.003	1.028 (0.844 −1.251)0.784 0.017	1.015 (0.931 −1.105)0.740 0.263	1.031 (0.929 −1.144)0.567 0.039	1.013 (0.855 −1.200)0.883 0.058
Control source							
Population -based			1.033 (0.980 −1.089)0.226 0.001	1.071 (0.964 −1.190)0.200 0.047	0.999 (0.952 −1.049)0.977 0.377	1.014 (0.962 −1.070)0.605 0.124	1.078 (0.971 −1.196)0.160 0.013
Hospital -based			1.156(1.027 −1.302)0.017 0.000	1.270(1.015 −1.588)0.036 0.000	1.102 (0.987 −1.230)0.084 0.000	1.148(1.008 −1.307)0.038 0.000	1.235(1.013 −1.505)0.037 0.000
DNA sample							
Blood			1.053(1.004 −1.103)0.032 0.000	1.115(1.019 −1.219)0.017 0.148	1.023 (0.974 −1.076)0.361 0.155	1.042 (0.989 −1.098)0.123 0.032	1.110(1.019 −1.208)0.016 0.115
Tissue			1.707 (0.943 −3.091)0.078 0.000	2.407 (0.847 −6.842)0.099 0.000	1.507 (0.889 −2.556)0.128 0.000	1.783 (0.906 −3.511)0.094 0.000	1.981 (0.827 −4.742)0.125 0.000

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Table 2 (*continued*)

Items	n	Case/controls	C allele vs A allele (allele model) OR (95% CI) *P* Ph	CC vs AA (homozygous model) OR (95% CI) *P* Ph	AC vs AA (heterozygous model) OR (95% CI) *P* Ph	AC + CC vs AA (dominant model) OR (95% CI) *P* Ph	CC vs AA + AC (recessive model) OR (95% CI) *P* Ph
Blood + Tissue			1.007 (0.918–1.104)0.888 0.315	0.934 (0.760–1.149)0.519 0.467	1.039 (0.935–1.154)0.479 0.583	1.024 (0.926–1.133)0.644 0.442	0.926 (0.757–1.131)0.451 0.516

found in all genetic models (T vs C: OR = 1.124, 95% CI = 1.072–1.178, P_{het} = 0.000; TT vs CC: OR = 1.257, 95% CI = 1.149–1.376, P_{het} = 0.000; CT vs CC: OR = 1.041, 95% CI = 1.007–1.076, P_{het} = 0.000; CT + TT vs CC: OR = 1.109, 95% CI = 1.047–1.174, P_{het} = 0.000; and TT vs CC + CT: OR = 1.222, 95% CI = 1.128–1.322, P_{het} = 0.000).

Similarly, we observed an obvious association between *MTHFR* 1298A/C and susceptibility to the four cancers in most genetic models, except the heterozygous model (C vs A: OR = 1.083, 95% CI = 1.024–1.145, P_{het} = 0.000; CC vs AA: OR = 1.145, 95% CI = 1.030–1.273, P_{het} = 0.000; AC + CC vs AA: OR = 1.070, 95% CI = 1.007–1.137, P_{het} = 0.000; and CC vs AA + AC: OR = 1.110, 95% CI = 1.045–1.179, P_{het} = 0.000).

Furthermore, we performed subgroup analysis to evaluate the effect of certain factors on the pooled results, including cancer type, genotyping method, ethnicity, and DNA source (Tables 1 and 2; Supplementary Fig. 1). Subgroup analysis based on cancer type showed that the *MTHFR* 677C/T variant was highly associated with increased risk of BC (T vs C: OR = 1.149, 95% CI = 1.090–1.211, P_{het} = 0.000; TT vs CC: OR = 1.285, 95% CI = 1.164–1.419, P_{het} = 0.000; CT vs CC: OR = 1.081, 95% CI = 1.021–1.145, P_{het} = 0.000; CT + TT vs CC: OR = 1.139, 95% CI = 1.070–1.212, P_{het} = 0.000; and TT vs CC + CT: OR = 1.243, 95% CI = 1.136–1.360, P_{het} = 0.000), whereas the *MTHFR* 1298A/C mutation was associated with increased susceptibility to cervical cancer (C vs A: OR = 1.974, 95% CI = 1.085–3.592, P_{het} = 0.000; AC vs AA: OR = 1.732, 95% CI = 1.056–2.841, P_{het} = 0.000; and AC + CC vs AA: OR = 2.072, 95% CI = 1.088–3.946, P_{het} = 0.000).

When stratified by ethnicity, we observed an obvious correlation between *MTHFR* 677C/T and the four cancer types among Asian, African, and mixed populations in most of the genetic models. Notably, a similar result was obtained for *MTHFR* 1298A/C in the Asian population (C vs A: OR = 1.203, 95% CI = 1.075–1.346, P_{het} = 0.000; CC vs AA: OR = 1.415, 95% CI = 1.140–1.758, P_{het} = 0.000; AC + CC vs. AA: OR = 1.173, 95% CI = 1.042–1.320, P_{het} = 0.000; and CC vs AA + AC: OR = 1.361, 95% CI = 1.120–1.653, P_{het} = 0.000).

Stratified analysis by genotyping method revealed an obvious increased risk in the PCR-RFLP group for 677C/T (T vs C: OR = 1.211, 95% CI = 1.124–1.305, P_{het} = 0.000; TT vs CC: OR = 1.572, 95% CI = 1.353–1.827, P_{het} = 0.000; CT vs CC: OR = 1.111, 95% CI = 1.017–1.215, P_{het} = 0.000; CT + TT vs CC: OR = 1.191, 95% CI = 1.086–1.306, P_{het} = 0.000; and TT vs CC + CT: OR = 1.480, 95% CI = 1.298–1.688, P_{het} = 0.001). Similarly, we observed an obvious correlation between *MTHFR* 1298A/C and PCR-RFLP (CC vs AA: OR = 1.241, 95% CI = 1.064–1.448, P_{het} = 0.145; and CC vs AA + AC: OR = 1.273, 95% CI = 1.077–1.504, P_{het} = 0.021) and TaqMan (C vs A: OR = 1.114, 95% CI = 1.006–1.234, P_{het} = 0.000; and AC + CC vs AA: OR = 1.129, 95% CI = 1.005–1.269, P_{het} = 0.000).

Stratification based on the source of controls indicated an obvious correlation between *MTHFR* 677C/T and the population-based group (T vs C: OR = 1.104, 95% CI = 1.036–1.176, P_{het} = 0.000; TT vs CC: OR = 1.184, 95% CI = 1.054–1.330, P_{het} = 0.000; CT + TT vs CC: OR = 1.083, 95% CI = 1.006–1.166, P_{het} = 0.000; and TT vs CC + CT: OR = 1.164, 95% CI = 1.047–1.294, P_{het} = 0.000) and the hospital-based group (T vs C: OR = 1.158, 95% CI = 1.070–1.252, P_{het} = 0.000; TT vs CC: OR = 1.355, 95% CI = 1.161–1.581, P_{het} = 0.000; CT vs CC: OR = 1.104, 95% CI = 1.008–1.210, P_{het} = 0.000; CT + TT vs CC: OR = 1.155, 95% CI = 1.047–1.274, P_{het} = 0.000; and TT vs CC + CT: OR = 1.293, 95% CI = 1.135–1.473, P_{het} = 0.000). Similarly, we observed a significant correlation between *MTHFR* 1298A/C and the hospital-based group (C vs A: OR = 1.156, 95% CI = 1.027–1.302, P_{het} = 0.000; CC vs AA: OR = 1.270, 95% CI = 1.015–1.588, P_{het} = 0.000; AC + CC vs AA: OR = 1.148, 95% CI = 1.008–1.307, P_{het} = 0.000; and CC vs AA + AC: OR = 1.235, 95% CI = 1.013–1.505, P_{het} = 0.000).

Further stratified analysis based on DNA source revealed a significantly elevated risk of the four cancers in the blood group for *MTHFR* 677C/T (T vs C: OR = 1.156, 95% CI = 1.093–1.223, P_{het} = 0.000; TT vs CC: OR = 1.323, 95% CI = 1.190–1.470, P_{het} = 0.000; CT vs CC: OR = 1.076, 95% CI = 1.011–1.146, P_{het} = 0.000; CT + TT vs CC: OR = 1.141, 95% CI = 1.066–1.221, P_{het} = 0.000; and TT vs CC + CT: OR = 1.274, 95% CI = 1.160–1.399, P_{het} = 0.000) and *MTHFR* 1298A/C(C vs A: OR = 1.053, 95% CI = 1.004–1.103, P_{het} = 0.000; and CC vs AA: OR = 1.115, 95% CI = 1.019–1.219, P_{het} = 0.148). However, we identified no significant association between the tissue or blood + tissue groups and *MTHFR* 677C/T or 1298A/C.

Heterogeneity testing

Heterogeneity was detected in the pooled and stratified analyses of *MTHFR* 677C/T and 1298A/C studies. To further explore the source of heterogeneity, we performed meta-regression analysis using cancer type, publication year, ethnicity, genotyping method, source of control, and DNA source as co-variables. We found that the control source (TT vs CC + CT, P = 0.018) might have accounted for up to 5.2% of the heterogeneity associated with *MTHFR* 677C/T, whereas the publication year (AC + CC vs AA, P = 0.035) might have accounted for up to 44.9% of the heterogeneity associated with *MTHFR* 1298A/C.

Sensitivity analysis

Sensitivity analyses were performed to evaluate the effects of each study on the pooled results by omitting one individual study at a time. However, we identified no significant effects following the removal of any study, indicating that the results were statistically robust (Supplementary Fig. S2).

Publication bias

We used the Begg's funnel plot and Egger's test to assess possible publication bias. Fig. 2 shows the funnel plots suggesting relationships between *MTHFR* 677C/T and 1298A/C mutations and cancer risk. The results demonstrated obvious publication bias for *MTHFR* 677C/T (T vs C, P = 0.020; TT vs CC, P = 0.019; and TT vs CC + CT, P = 0.021) and *MTHFR* 1298A/C (C vs A. P = 0.007; CC vs AA, P = 0.023; and AC + CC vs AA, P = 0.010) in certain genetic models, whereas no bias was noted in the other comparisons.

Discussion

In this study, we comprehensively evaluated the correlation between *MTHFR* 677C/T and 1298A/C mutations and the susceptibility to BC, OC, cervical cancer, and EC. The results indicated that both *MTHFR* 677C/T and 1298A/C were associated with increased cancer susceptibility in the pooled analysis.

Moreover, stratified analyses demonstrated that the correlation between *MTHFR* 677C/T and risk of the four common female malignancies was more predominant among Asian, African, and mixed populations when PCR-RFLP was used for genotyping (with population- and hospital-based groups used as controls) and blood as the DNA source (i.e., T-allele carriers and TT genotypes showed increased susceptibility to the four common cancers under these conditions). Similarly, subgroup analysis of *MTHFR* 1298A/C indicated that C-allele carriers and CC genotypes correlated significantly with an increased risk of cervical cancer in Asian populations (when hospital-based groups were used as the controls, and blood was used

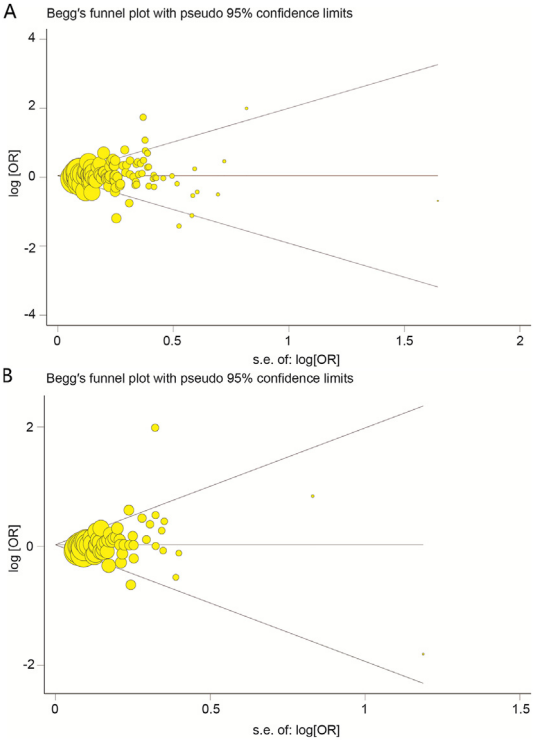

Fig. 2. Determination of publication bias using Begg's funnel plot and Egger's test. Each point represents an individual study. *MTHFR* 677C/T (A) and *MTHFR* 1298A/C (B) in respective heterozygous models.

as the DNA source). Moreover, CC genotypes and C-allele carriers correlated with significantly elevated risks in the PCR-RFLP and TaqMan genotyping groups, respectively.

The results obtained for *MTHFR* 677C/T agree with those reported by other studies,[20,32] in that a significantly increased cancer risk was observed according to the overall analysis. However, our overall results were also inconsistent with other studies that reported no significant correlation of the *MTHFR* 677C/T polymorphism with an elevated risk of OC or cervical cancer in the five genetic models.[33] Additionally, although our pooled results for *MTHFR* 1298A/C were similar to those from a previous study,[34] they completely contradicted another study[22] when stratified by certain factors.

We observed various unique characteristics in our subgroup analyses. For example, analysis of *MTHFR* 677C/T based on cancer type revealed a significant association with BC for T-allele carriers and TT genotypes, whereas we observed no correlation in the other models. These results are consistent with those of Yu et al[35] and Naushad et al[36] both of whom found significantly increased risks of BC among T-allele carriers. By contrast, Lewis et al[37] found no association with BC in their genetic models. Moreover, Zintzaras et al[38] reported no overall associations with BC but rather only detected a positive correlation between BC and pre-menopausal women. In the present study, we identified no significant correlations between *MTHFR* 677C/T and OC, cervical cancer, or EC susceptibility in any models, which agrees with the findings reported by Xu et al,[39] Liu et al[26] and Wang et al;[40] however, the present results are inconsistent with the findings reported by Chen et al[41] and Pu et al[19] who observed that TT genotypes or the T allele elevated the risk of cervical cancer or OC. However, when the *MTHFR* 1298A/C results were stratified by cancer type, C-allele carriers and CC genotypes were found to remarkably increase the risk of cervical cancer as compared with that of non-cervical cancers. This outcome agrees with the findings of Yi et al[11] who also observed a significantly increased risk of cervical cancer. Nevertheless, conflicting observations have been previously reported. For example, Long et al[42] reported no correlation between *MTHFR* 1298A/C variation and susceptibility to cervical cancer, whereas Liu et al[22] found a significantly increased risk for BC and OC among carriers of the C allele and the CC genotype and no correlations with cervical cancers. These results indicate that the T allele of *MTHFR* 677C/T and C allele of *MTHFR* 1298A/C can increase susceptibility to BC and cervical cancer, respectively; however, the precise mechanisms underlying these processes remain unclear.

There have been several investigations on the biochemical functions of MTHFR and the corresponding effects of SNPs. Specifically, the *MTHFR* 677C/T and 1298A/C variants reportedly correlate with MTHFR production and affect enzyme activity, which plays an important role in folate metabolism and results in multifarious pathological outcomes, such as BC and OC.[43–46] Therefore, the T allele, C allele, TT genotype, and CC genotype might be involved in tumor development.

In the present study, we also used ethnicity as a stratified factor, observing a significantly increased risk among Asian, African, and mixed groups for *MTHFR* 677C/T. Moreover, we observed a significant increase in *MTHFR* 1298A/C risk in the Asian population, whereas no association was found in other races. These results agree with those of previous studies. Mo et al[18] found that T-allele carriers and TT-genotype individuals among Asians have a higher BC risk, whereas Zhu et al[34] found that carriers of the C allele carriers and CC genotype in the Asian population have a significantly increased risk of cervical cancer. Conversely, Zintzaras et al[38] and Zhu et al[47] found no associations between Asian populations and the risk of BC or cervical cancer. These findings indicate that the *MTHFR* 677C/T and 1298A/C variants might be linked to ethnicity for susceptibility to BC, OC, cervical cancer, and EC. Although the causes of these discrepancies are unclear, the frequency of specific gene mutations and polymorphisms might differ among ethnicities due to different environmental effects (gene–environment interactions), compliance with natural-selection principles, different lifestyles, or disease prevalence, such that *MTHFR* 677C/T carriers in the Asian, African, and mixed groups or *MTHFR* 1298A/C carriers in Asian populations are likely to be eliminated relative to other individuals. Furthermore, we identified variations in the T-allele frequencies of control resources in Asians (0.307), Caucasians (0.339), Africans (0.268) and mixed populations (0.330), which might account for the association between the C677T polymorphism and cancer risk in different ethnicities. These results are consistent with previous studies.[32] Furthermore, this phenomenon might explain why certain polymorphisms can increase the risk of cancers in certain races but not in others. Additionally, other studies[3,48] report that BC, OC, cervical cancer, and EC are more prevalent among Asian and African populations as compared with other ethnicities, which agrees with our observed correlations between both polymorphisms and BC or cervical cancer.

The source of the control was another factor investigated in this meta-analysis. Specifically, we detected an obvious increase in cancer risk in both population- and hospital-based groups for *MTHFR* 677C/T, whereas for *MTHFR* 1298A/C, we found significantly elevated risks for cancer among carriers of the C allele and CC genotypes in the hospital-based group. This is similar to findings reported by Zhang et al[49] but is inconsistent with those of Yi et al[11] Considering that the hospital-based groups are not representative of the whole population, we were cautious with regard to how we interpreted our final conclusions.

When stratified based on genotyping method, we found a notably increased risk of cancer for *MTHFR* 677C/T in the PCR-RFLP genotyping group and positive correlations for *MTHFR*1298A/C in the PCR-RFLP and TaqMan groups. This subgroup factor was rarely discussed in other reports.

When stratified based on the DNA source, we found a strong association between cancer susceptibility and the blood-sample group for *MTHFR* 677C/T and 1298A/C, which might have resulted from differences in the sample-collection procedures and interference factors associated with the DNA-extraction methods. Generally, DNA-extraction protocols for blood and tissue are similar, with the exception of the tissue-management steps.[50,51] In the present study, the analysis included paraffin-embedded tissue, frozen tissue, and tissue-exfoliated cells.[52–54] Considering that formaldehyde, the main component of formalin-fixation solution, increases the brittleness of DNA strands while fixing the tissue, and that DNA is readily degraded during the paraffin-embedding and dewaxing processes, the quality and purity of the DNA extraction might have been compromised.[55] Furthermore, for frozen tissue, the grinding process and the degree of fineness can impact the quality of extracted DNA.[53] Accordingly, we believe that blood collection represents a more appropriate strategy for DNA extraction.

To achieve accurate results, it is crucial to identify probable sources of heterogeneity. Therefore, we assessed heterogeneity using the Q-test and I^2 forms. Overall, along with subgroup analyses, evaluation of *MTHFR* 677C/T and 1298A/C revealed significant heterogeneity. Additionally, meta-regression analysis determined that the source of controls was associated with heterogeneity for *MTHFR* 677C/T, whereas the publication year might have partially contributed to the observed heterogeneity for *MTHFR* 1298A/C. However, considering that these factors did not account for all of the heterogeneity in the present study, additional aspects must be investigated.

We also noted publication bias among certain models of *MTHFR* 677C/T and 1298A/C using Begg's and Egger's tests. For example, we observed publication bias for *MTHFR* 677C/T (T vs C, P = 0.020; TT vs CC, P = 0.019; and TT vs CC + CT, $P = 0.021$) and *MTHFR* 1298A/C (C vs A, $P = 0.007$; CC vs AA, $P = 0.023$; and AC + CC vs AA, $P = 0.010$) in certain genetic models. Additionally, sensitivity analyses showed that the present findings were stable, as the overall results were unaltered after individual studies were eliminated.

The study has several limitations that might have impacted the results. First, in the subgroup analyses, data were not stratified by age, folate intake, or other suspected aspects due to insufficient data from the publications. Therefore, it is assumed that a more comprehensive study would have been conducted had adequate data been available. Second, only published articles meeting the inclusion criteria were selected for analysis; therefore, the possibility of publication bias cannot be eliminated. Third, due to the lack of data, we did not include gene–gene or gene–environment interactions in the present study. Moreover, the present study was based on unadjusted estimates, given that some individual data were unavailable and that might have allowed adjustment for other co-variants, including environmental factors and other lifestyles. Fourth, we detected heterogeneity in several models, which might be attributed to the publication year, cancer type, and control source. Additionally, although most controls were population-based, this was not clearly stated in selected studies. Furthermore, some controls were hospital-based but not adequately representative. Accordingly, certain factors must be considered in future studies, such as well-matched control groups, unbiased methods, and gene–gene and gene–environment interactions.

In conclusion, this study using a large sample size showed that the *MTHFR* 677C/T mutation likely increases susceptibility to BC, OC, EC, and cervical cancer. Specifically, the findings suggested BC susceptibility in non-Caucasian individuals who have their blood samples genotyped by PCR-RFLP. Additionally, we found that the *MTHFR* 1298A/C mutation serves as a significant genetic factor in the pathogenesis of BC, OC, EC, and cervical cancer and particularly for cervical cancer among Asian populations when blood is used as the DNA source. Interestingly, we observed significantly increased susceptibility for these cancers among the hospital-based group. These findings offer a baseline for the comparison of factors influencing the risk of

developing BC, OC, EC, and/or cervical cancer in clinical settings.

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Data availability

All data used to support the study are included in this article.

Conflicts of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cdtm.2021.06.003.

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