Review Article

Methylenetetrahydrofolate Reductase A1298C Polymorphism and Breast Cancer Risk: A Meta-analysis of 33 Studies

Rai V

Department of Biotechnology, Human Molecular Genetics Laboratory, VBS Purvanchal University, Jaunpur, Uttar Pradesh, India

Address for correspondence:
Dr. Vandana Rai,
Department of Biotechnology,
VBS Purvanchal University,
Jaunpur - 222 001,
Uttar Pradesh, India.
E-mail: raivandana@rediffmail.com

Abstract

Methylenetetrahydrofolate reductase (MTHFR) enzyme is essential for DNA synthesis and DNA methylation, and its gene polymorphisms have been implicated as risk factors for birth defects, neurological disorders, and different types of cancers. Several studies have investigated the association between the MTHFR A1298C polymorphism and breast cancer (BC) risk, but the results were inconclusive. To assess the risk associated with MTHFR A1298C polymorphism, a comprehensive meta-analysis was performed. PubMed, Google Scholar, Elsevier and Springer Link databases were searched for case-control studies relating the association between MTHFR A1298C polymorphism and BC risk and estimated summary odds ratios (ORs) with confidence intervals (CIs) for assessment. Up to January 2014, 33 case-control studies involving 15,919 BC patients and 19,700 controls were included in the present meta-analysis. The results showed that the A1298C polymorphism was not associated with BC risk in all the five genetic models (C vs. A allele (allele contrast): OR = 0.99, 95% confidence interval (CI): 0.93–1.05; AC versus AA (heterozygote/codominant): OR = 0.97, 95% CI: 0.89–1.04; CC versus AA (homozygote): OR = 0.99, 95% CI: 0.91–1.06; CC + AC versus AA (dominant model): OR = 0.97, 95% CI: 0.90–1.05; and CC versus AC + AA (recessive model): OR = 0.99, 95% CI: 0.91–1.07). The present meta-analysis did not support any association between the MTHFR A1298C polymorphism and BC risk.

Keywords: A1298C, Breast cancer, Folate, Meta-analysis, Methylenetetrahydrofolate reductase, Polymorphism

Introduction

Breast cancer (BC) is a leading cause of morbidity and mortality in women in the developed world and its incidence in the developing world is on the rise. Worldwide, more than 1 million new cases of female BC are diagnosed each year.[1] The most rapid rises are seen in developing countries, where BC risk has historically been low-relative to industrialized countries. The cumulative lifetime risk for the development of the disease in the general population is estimated to be 10%.[2] However, 5-10% of all BC may represent hereditary cases. The most significant risk factor for breast or ovarian is the presence of the two cancer susceptibility genes, BRCA1 or BRCA2. Epigenetic alterations in cancer-related genes are recognized to play an important role in BC carcinogenesis. Epidemiological studies have consistently supported that cancer is related not only to mutations in functional genes, but also related to the aberrant epigenetic modifications of various genes.[3]

There is considerable interest in identifying other risk factors associated with BC that can be modified to reduce the risk of the disease. Accumulating evidence from epidemiologic studies suggests a protective role of folate and related B vitamins against BC. The folate metabolism pathway contributes to important metabolic processes such as DNA synthesis, methylation and repair.[4] Folate deficiency due to low-dietary or supplemental intake, or impaired absorption or metabolism, may result in increased numbers of DNA strand breaks, impaired DNA repair, enhanced mutagenesis and alterations in DNA methylation patterns and all of these events

Access this article online	
Quick Response Code:	Website: www.amhsr.org
	DOI: *****

have been implicated in carcinogenesis.[5,6] Epidemiologic studies have indicated that folate deficiency may be related to the development of several cancers, including BC.[7-9] It has been suggested that breast carcinogenesis could be associated with alteration of estrogen receptor gene methylation pattern and global DNA methylation.[10] It is biologically plausible that polymorphisms of folate pathway genes would have an impact on BC risk since functional polymorphisms contribute to the alteration of folate metabolism.[8]

There are several evidences that methylenetetrahydrofolate reductase (MTHFR) gene variants increase thymidylate synthase activity in cancer cells, because of increased supply of 5,10-methyleneTHF, the methyl donor for methylation of dUMP to dTMP.[11] MTHFR is a regulatory enzyme in folate metabolism that catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and directs the flux of intracellular folate toward the conversion of homocysteine to methionine at the expense of nucleotide synthesis.[12,13] MTHFR gene is located at 1p36.3.[9] Two SNP markers in the MTHFR gene (C677T and A1298C) have been associated with reduced enzyme activity, thereby making MTHFR polymorphisms a potential candidate cancer-predisposing factor due to genomic DNA hypomethylation, hyperhomocysteinemia and atherosclerosis.[3] The C677T polymorphism codes for an alanine to valine substitution in the N-terminal catalytic domain and results in an enzyme with ~65% and ~30% of the enzyme activity for heterozygotes and homozygotes, respectively.[12,14] The A→C polymorphism at nucleotide 1298 codes for glutamine to alanine substitution in the C-terminal regulatory domain.[13] Individuals homozygous for the A1298C have approximately the same enzyme activity as those heterozygous for C677T allele.[13,14] These variant genotypes are associated with a substantial decrease in enzymatic activity *in vitro*.[12,13] and may reduce the risk of colon cancer[15-17] and acute lymphocytic leukemia.[18] Conversely, the same variants have also been associated with an increased risk for various cancers including endometrial cancer,[19] cervical intraepithelial neoplasia,[20] esophageal squamous cell carcinoma,[21] gastric cancer,[22] bladder cancer,[23] and squamous cell carcinoma of the head and neck.[24] The role of folate in BC has been investigated in several studies, and most have shown folate consumption to be inversely related to BCs.[25]

A1298C allele frequency differs greatly in various ethnic groups of the world. The prevalence of the A1298C homozygote variant genotype ranges from 7% to 12% in White populations from North America and Europe. Lower frequencies have been reported in Hispanics (4-5%), Chinese (1-4%) and other Asian populations (1-4%).[26,27] Many studies investigated the association between the A1298C genotype and BC incidence. Although significant association was observed in some studies, a clear linkage between MTHFR polymorphisms and the risk to develop BC has not been established.[8,28-32] Hence in the present study a meta-analysis of all published case-control studies investigating A1298C polymorphism as a risk factor for BC was carried out to shed some lights on conclusive role of A1298C polymorphism in BC.

Materials and Methods

Articles included in the present meta-analysis were selected by PubMed, Elsevier, Google Scholar and Springer Link databases search with keywords MTHFR, 'A1298C' and 'BC' up to January, 2014. All extracted articles read completely and carefully. Relevant information's were extracted from all selected studies like-author family name, journal name, year of publication, country name and number of cases and controls for each A1298C genotypes (AA, AC and CC genotypes).

Eligible studies had to meet all of the following criteria: (1) They were published in a peer-reviewed journal, (2) they contained independent data, (3) they presented sufficient data to calculate the odds ratios (OR) with a CI and a *P* value, (4) they were case-control association studies, (5) they described the relevant genotyping protocols or provided reference to them, (6) they used healthy individuals as controls.

Cochran's Q statistic was used to test formally for heterogeneity, and the percentage variability of the pooled OR attributable to heterogeneity between studies was quantified with the I^2 metric (I^2= (Q – df)/Q), which is independent of the number of studies in the meta-analysis. I^2 takes values of between 0 and 100%, with higher values denoting a greater degree of heterogeneity[33] (I^2 = 0% to 25%: No heterogeneity; I^2 = 25% to 50%: Moderate heterogeneity; I^2 = 50% to 75%: Large heterogeneity; I^2 = 75% to 100%: Extreme heterogeneity).[34] The pooled OR was estimated using fixed effect (FE)[35] and random effect (RE)[36] models. Publication bias was investigated with the funnel plot. Funnel plot asymmetry was further assessed by the method of Egger's linear regression test.[37] All statistical analyses were undertaken using the program MIX version 1.7.[38] A $P < 0.05$ was considered as statistically significant, and all the *P* values were two-sided.

Results

Selection of included studies

Figure 1 presents a flow chart of the retrieved studies and the studies excluded, with specifying reasons and the information extracted from the studies included in the meta-analysis is provided in Tables 1 and 2. Totally 152 articles were retrieved using search strategies, but 98 articles did not meet the inclusion criteria after reviewing full paper. The excluded articles include seven case studies, two editorials, nine letter to the editor, 12 reviews and seven articles were not in English language, and 61 articles were irrelevant for the present meta-analysis. Out of remaining 54 articles, twenty-one articles were again excluded in which only C677T polymorphism were reported. Thirty-three studies were found suitable for the inclusion in the present meta-analysis.[3,8,9,28-31,39-64] The studies were carried out in Brazil,[54] Canada,[50] China,[28,41,44,53,57,60,62-64] Germany,[31]

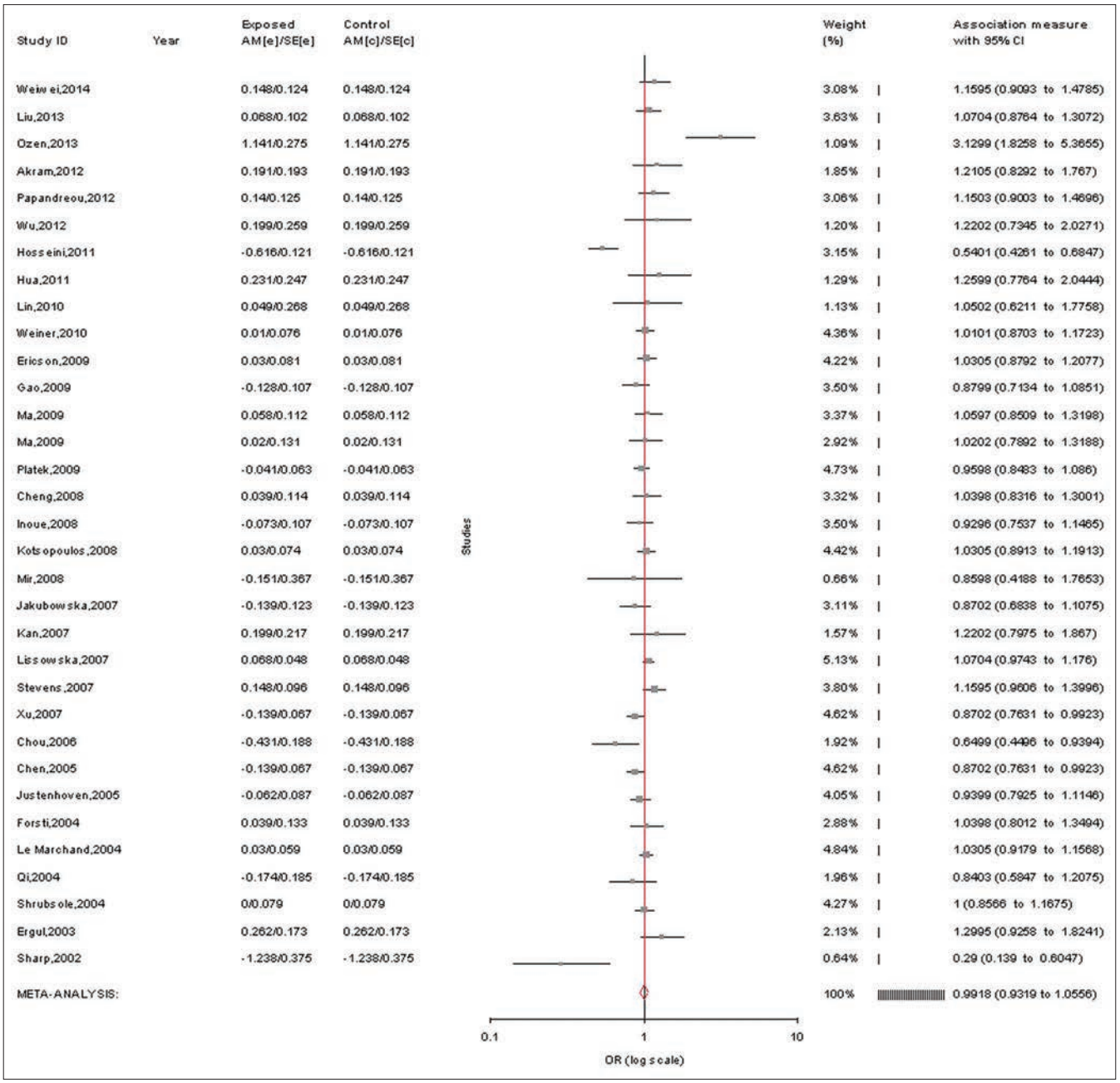

Figure 1: Forest plot for the association between MTHFR A1298C polymorphism and Breast Cancer for allele contrast model (C vs A) with random effect model. Results of individual and summary OR estimates, 95% CI, and weights of each study were shown

Greece,[9] India,[51] Iran,[59] Japan,[55] Finland,[40] Pakistan,[61] Poland,[45] Russia,[58] Singapore,[49] Taiwan,[42] Turkey,[3,39] UK,[8] and USA.[29,30,46,47,56] Among thirty-three included studies OR is above one in only 21 studies. Author has also assessed whether the frequencies of AA, AC and CC genotypes among controls in individual studies were consistent with the expected distribution (that is in Hardy-Weinberg equilibrium) by using the χ^2 test. Genotypes were in Hardy-Weinberg equilibrium in all controls. Thirty-three studies, reported the association of SNP A1298C polymorphism in the MTHFR gene with BC are summarized in Table 1.

Summary statistics

In total 33 studies, total cases were 15,919 with AA (8478), AC (6139) and CC (1302), and controls were 19,700 with AA (10479), AC (7622), and CC (1599). In controls genotypes percentage of AA, AC and CC were 53.19%, 38.69% and 8.12% respectively. In total cases genotype percentage of AA, AC, and CC was 53.26%, 38.56% and 8.18% respectively. Frequencies of AA and AC genotypes were highest in both cases and controls [Table 2]. Allelic number of A and C alleles were also calculated and presented in Table 2.

Table 1. Characteristics of seventeen studies included in the present meta-analysis

Study	Year	Country	Control	Case	Reference
Weiwei, 2014	2014	China	306	296	Pak J Med Sci, 30:106-110.
Liu, 2013	2013	China	435	435	Asian Pac J Cancer Prev, 14: 5189-5192
Ozen, 2013	2013	Turkey	106	51	Asian Pacific J Cancer Prev, 14 (5): 2903-2908.
Akram, 2012	2012	Pakistan	110	110	Asian pacific J Cancer Prev, 13:1599-1603.
Papandreou *et al.*	2012	Greece	283	300	DNA Cell Biology, 31:193-198.
Wu *et al.*	2012	China	75	75	Asian Pac J Cancer Prev, 13:2199-206.
Hosseini *et al.*	2011	Iran	300	294	Arch Med Sci, 7, 1: 134-137.
Hua *et al.*	2011	China	90	95	Mod Oncol ,19:428-31.
Lin *et al.*	2010	China	143	65	Prelim StudMod Hosp, 10:15-7.
Weiner *et al.*	2010	Russia	785	831	Mol Biol, 44 (5):720-727.
Ericson *et al.*	2009	Sweden	1072	541	Cancer Epidemiol Biomarkers Prev, 18:1101-1110.
Gao *et al.*	2009	China	682	669	J Hum Genet ,54:414-418.
Ma *et al.*	2009	Brazil	458	458	BMC Cancer, 9:122.
Ma *et al.*	2009	Japan	387	388	Nutr Cancer, 61:447-456.
Platek *et al.*	2009	USA	1781	928	Cancer Epidemiol Biomark Prev, 18:2453-2459.
Cheng *et al.*	2008	China	534	351	Breast Cancer Res Treat , 111:145-155.
Inoue *et al.*	2008	Singapore	662	380	Carcinogenesis, 29:1967-1972.
Kotsopoulos *et al.*	2008	Canada	780	941	Breast Cancer Res Treat , 112:585-593.
Mir *et al.*	2008	India	33	35	International Journal of Health Sciences, Qassim University, 2: pp. 3-14.
Jakubowska *et al.*	2007	Poland	290	319	Breast Cancer Res Treat, 115:431-432.
Kan *et al.*	2007	China	101	125	Cancer Res Prev Treat 34:716-718.
Lissowska *et al.*	2007	Poland	2278	1986	Int J Cancer 120: 2696-2703.
Stevens *et al.*	2007	USA	493	494	Cancer Epidemiol Biomarkers Prev 16:1140-1147.
Xu *et al.*	2007	USA	1103	1062	Carcinogenesis, 28:1504-1509.
Chou *et al.*	2006	Taiwan	285	142	Carcinogenesis, 27:2295-2300.
Chen *et al.*	2005	USA	1103	1062	Cancer Res, 65:1606-1614.
Justenhoven *et al.*	2005	Germany	634	582	Cancer Epidemiol Biomark Prev, 14:3015-3018.
Forsti *et al.*	2004	Finland	298	223	Oncol Rep, 11:917-922.
Le Marchand *et al.*	2004	USA	2414	1190	Cancer Epidemiol Biomarkers Prev 13:2071-2077.
Qi *et al.*	2004	China	218	217	Chin J Oncol, 26:287-289.
Shrubsole *et al.*	2004	China	1208	1121	Cancer Epidemiol Biomarkers Prev, 13:190-196.
Ergul *et al.*	2003	Turkey	193	118	Tumour Biol, 24:286-290.
Sharp *et al.*	2002	UK	60	35	Cancer Lett, 181:65-71.

Meta-analysis

Table 3 summarizes the ORs with corresponding 95% CIs for the association between A1298C polymorphism and risk of BC in allele contrast, homozygote, dominant, recessive and co-dominant models. The pooled ORs were estimated by both fixed effects (Mantel and Haenszel) and random effects (Der Simonian and Laired) models. Meta-analysis with allele contrast did not show any association with both fixed effect (OR_{CvsA} = 0.99; 95% CI: 0.95–1.02; P = 0.55) and random effect model (OR_{CvsA} = 0.99; 95% CI = 0.93–1.05; P = 0.79). The meta-analysis with fixed effects showed that there was 63.18% (P < 0.0001) heterogeneity between the 33 studies [Figure 2, Table 3].

Methylenetetrahydrofolate reductase A1298C polymorphism had no association with susceptibility to BC with genotype contrast meta-analysis using four genetic models (for CC + AC versus AA (dominant model): OR = 0.97; 95% CI = 0.90–1.05; P = 0.53; I^2 = 62.1%; $P_{heterogeneity}$ < 0.0001; for CC versus AA (homozygote model): OR = 0.99; 95% CI = 0.94–1.06; P = 0.74; I^2 = 41.82%; $P_{heterogeneity}$ = 0.006 [Figure 3]; for AC versus AA (heterozygote model): OR = 0.97; 95% CI = 0.89–1.04; P = 0.45; I^2 = 56.59%; $P_{heterogeneity}$ = 0.45; for CC vs. AC + AA (recessive model): OR = 0.99, 95% CI = 0.91–1.07, P = 0.85; I^2 = 28.16%; $P_{heterogeneity}$ = 0.069).

Publication bias

Funnel plots, Begg's and Egger's test were performed to estimate the risk of publication bias. The shape of funnel plots in all contrast models showed obvious evidence of symmetry [Figure 3]. In addition, all the P values of Egger›s test were more than 0.05, which provided statistical evidence for the symmetry of funnel plots in the meta-analysis (P = 0.89 for C vs. A; P = 0.21 for CC vs. AA; and P = 0.35 for AC vs. AA; P = 0.62 for CC + AC vs. AA; P = 0.06 for CC vs. AC + AA). Begg's test results also did not show publication bias (P = 0.78 for C vs. A; P = 0.28 for CC vs. AA; and P = 0.57 for AC vs. AA; P = 0.97 for CC + AC vs. AA; P = 0.06 for CC vs. AC + AA) [Table 3].

Table 2. The distributions of MTHFR A1298C genotypes and allele number for Breast cancer cases and controls

Study ID	Genotype						Alleles			
	AA		AC		CC		A		C	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
v	135	151	129	130	32	25	399	432	193	180
Liu, 2013	206	214	176	172	53	49	588	600	282	270
Ozen, 2013	17	71	29	35	5	0	63	177	39	35
Akram, 2012	35	30	55	75	20	5	125	135	95	85
Papandreou, 2012	129	136	135	116	36	31	393	388	207	178
Wu, 2012	37	42	32	28	6	5	106	112	44	38
Hosseini, 2011	162	105	96	135	36	60	420	345	168	255
Hua, 2011	50	55	42	32	3	3	142	142	48	38
Lin, 2010	45	98	14	35	6	10	104	231	26	55
Weiner, 2010	398	379	353	330	80	76	1149	1088	513	482
Ericson, 2009	242	487	242	480	57	105	726	1454	356	690
Gao, 2009	478	465	181	205	10	12	1137	1135	201	229
Ma, 2009	269	279	168	157	21	22	706	715	210	201
Ma, 2009	254	256	119	116	15	15	627	628	149	146
Platek, 2009	443	842	402	758	83	181	1288	2442	568	1120
Cheng, 2008	207	310	125	207	19	17	539	827	163	241
Inoue, 2008	225	387	139	234	16	41	589	1008	171	316
Kotsopoulos, 2008	466	398	390	309	85	73	1322	1105	560	455
Mir, 2008	15	11	19	22	1	0	49	44	21	22
Jakubowska, 2007	151	117	134	144	34	29	436	378	202	202
Kan, 2007	70	61	41	32	14	8	181	154	69	48
Lissowska, 2007	892	1086	874	941	220	251	2658	3113	1314	1443
Stevens, 2007	224	252	228	201	42	40	676	705	312	281
Xu, 2007	558	536	417	457	87	110	1533	1529	591	677
Chou, 2006	104	172	30	95	8	18	238	439	46	131
Chen, 2005	558	536	417	457	87	110	1533	1529	591	677
Justenhoven, 2005	273	295	256	266	53	73	802	856	362	412
Forsti, 2004	94	133	102	127	27	38	290	393	156	203
Le Marchand, 2004	741	1493	372	801	77	120	1854	3787	526	1041
Qi, 2004	155	144	58	71	4	3	368	359	66	77
Shrubsole, 2004	768	824	311	344	42	40	1847	1992	395	424
Ergul, 2003	50	90	48	85	20	18	148	265	88	121
Sharp, 2002	27	24	5	25	3	11	59	73	11	47

Subgroup analysis

of 33 studies included in the present meta-analysis, 17 studies were carried out on Asian population, and 16 studies were carried out on Caucasian population. The subgroup analysis by ethnicity also revealed that the no significant association was found between MTHFR A1298C polymorphism and BC in Asian population (for C vs. A: OR = 1.0, 95% CI = 0.88–1.1, $P = 0.93$, $I^2 = 71.38\%$, $P_{heterogeneity} \leq 0.0001$; for AC vs. AA: OR = 0.93, 95% CI = 0.79–1.1, $P = 0.83$, $I^2 = 62.88\%$, $P_{heterogeneity} = 0.0003$; for CC vs. AA: OR = 1.1, 95% CI = 0.81–1.5, $P = 0.62$, $I^2 = 53.5\%$, $P_{heterogeneity} = 0.004$; for CC + AC vs. AA: OR = 0.96, 95% CI = 0.81–1.1, $P = 0.58$, $I^2 = 68.995$, $P_{heterogeneity} \leq 0.0001$; for CC vs AC + AA: OR = 1.1, 95% CI = 0.91–1.3, $P = 0.38$; $I^2 = 42.08\%$, $P_{heterogeneity} = 0.035$) [Table 4] and Caucasian population (for C vs. A: OR = 0.99, 95% CI = 0.93–1.0, $P = 0.73$, $I^2 = 50.3\%$, $P_{heterogeneity} \leq 0.01$; for AC vs. AA: OR = 0.83, 95% CI = 0.69–1.0, $P = 0.53$, $I^2 = 90.07\%$, $P_{heterogeneity} \leq 0.001$; for CC vs. AA: OR = 0.97, 95% CI = 0.88–1.0, $P = 0.47$, $I^2 = 15.59\%$, $P_{heterogeneity} = 0.26$; for CC + AC vs. AA: OR = 0.99, 95% CI = 0.92–1.1, $P = 0.92$, $I^2 = 54.1\%$, $P_{heterogeneity} = 0.006$; for CC vs. AC + AA: OR = 0.96, 95% CI = 0.88–1.0, $P = 0.6$, $I^2 = 0\%$, $P_{heterogeneity} = 0.60$) [Table 5].

Discussion

Breast cancer is a manifestation of abnormal genetic variants as well as epigenetic changes. Interruption of one-carbon metabolism may be important in BC etiology as it facilitates the cross-talk between genetic and epigenetic processes playing critical roles in both DNA methylation and DNA synthesis. Previous studies on the relationship between MTHFR A1298C polymorphism and BC risk were contradictory. These inconsistent results are possibly because of a small effect of the polymorphism on BC risk or the relatively low statistical power of the published studies. Hence, the meta-analysis was needed to provide a quantitative approach for combining the results of various studies with the same

Table: 3: Summary estimates for the odds ratio (OR) of MTHFR A1298C in various allele/genotype contrasts, the significance level (*P* value) of heterogeneity test (Q test), and the I^2 metric, and publication bias *P* value (Egger test)

Genetic models	OR (95% CI), *P*		Heterogeneity P value (Q test)	I^2 (%)	Publication Bias (p of Egger's test)
	Fixed effect	Random effect			
Allele contrast (C vs A)	0.99 (0.95-1.02),0.55	0.99 (0.93-1.05),0.79	<0.0001	63.18	0.89
Co-dominant (AC vs AA)	0.98 (0.94-1.02),0.47	0.97 (0.89-1.04),0.45	<0.0001	56.59	0.35
Homozygote (CC vs AA)	0.99 (0.91-1.06),0.74	0.99 (0.89-1.12),0.99	0.006	41.82	0.21
Dominant (CC+AC vs AA)	0.98 (0.94-1.02),0.46	0.97 (0.90-1.05),0.53	<0.0001	62.1	0.62
Recessive (AA+AC vs CC)	0.99 (0.91-1.07),0.85	1.00 (0.90-1.11),0.92	0.069	28.16	0.06

OR: Odds ratio, CI: Confidence interval, MTHFR: Methylenetetrahydrofolate reductase

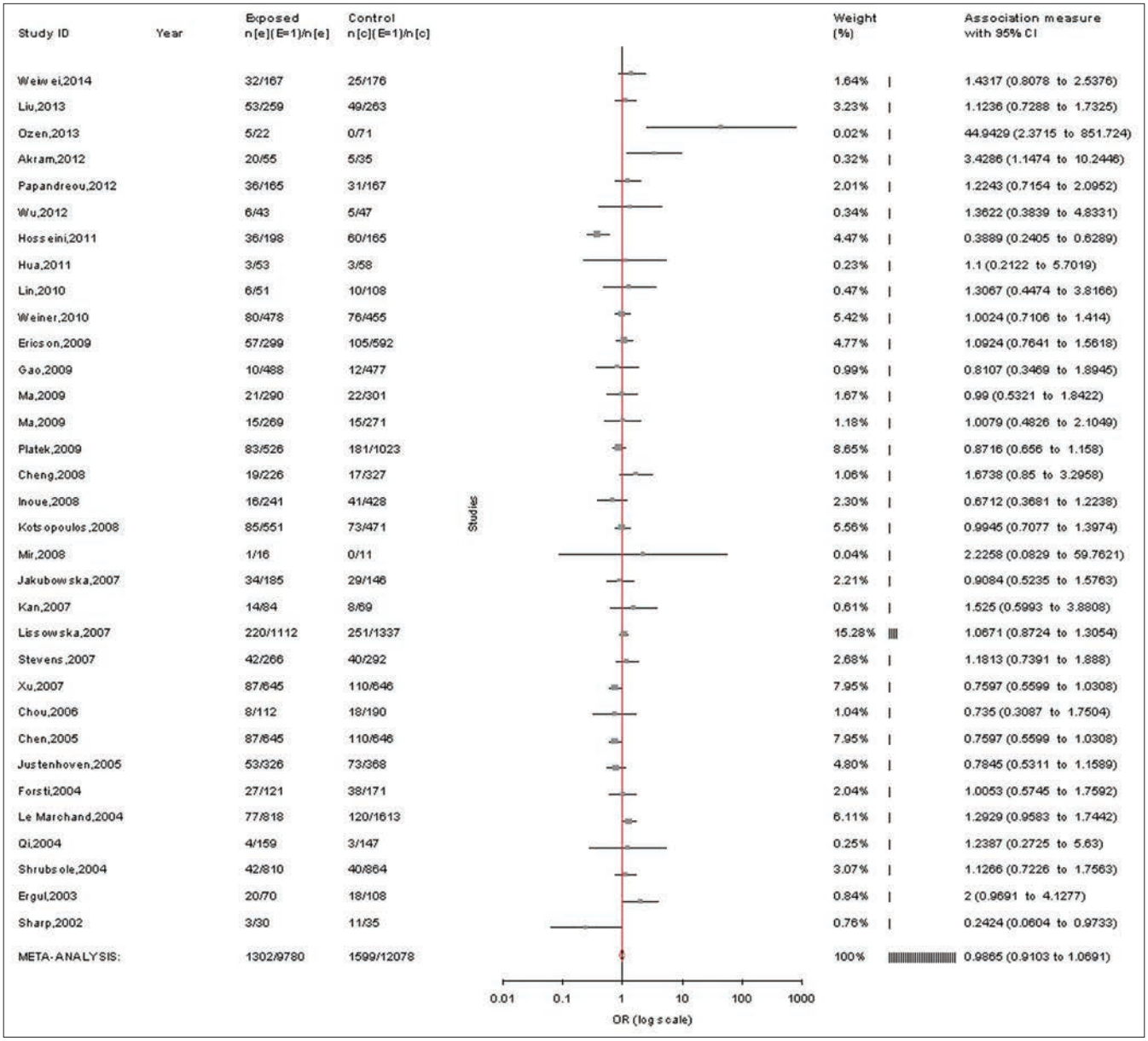

Figure 2: Forest plot for the association between MTHFR A1298C polymorphism and Breast cancer for homozygote model (CC vs AA) with fixed effect model. Results of individual and summary OR estimates, 95% CI, and weights of each study were shown

topic, and for estimating and explaining their diversity.[65] This meta-analysis examined the MTHFR A1298C polymorphism and its relationship to susceptibility for BC included 33 studies with 15,919 cases and 19,700 controls.

During the past decade several meta-analyses were published assessing MTHFR as a risk factor to various cancers like-esophageal cancer,[66,67] pancreatic cancer,[68,69] liver cancer,[70] ovary cancer,[68,71,72] lung cancer,[73-74] cervical

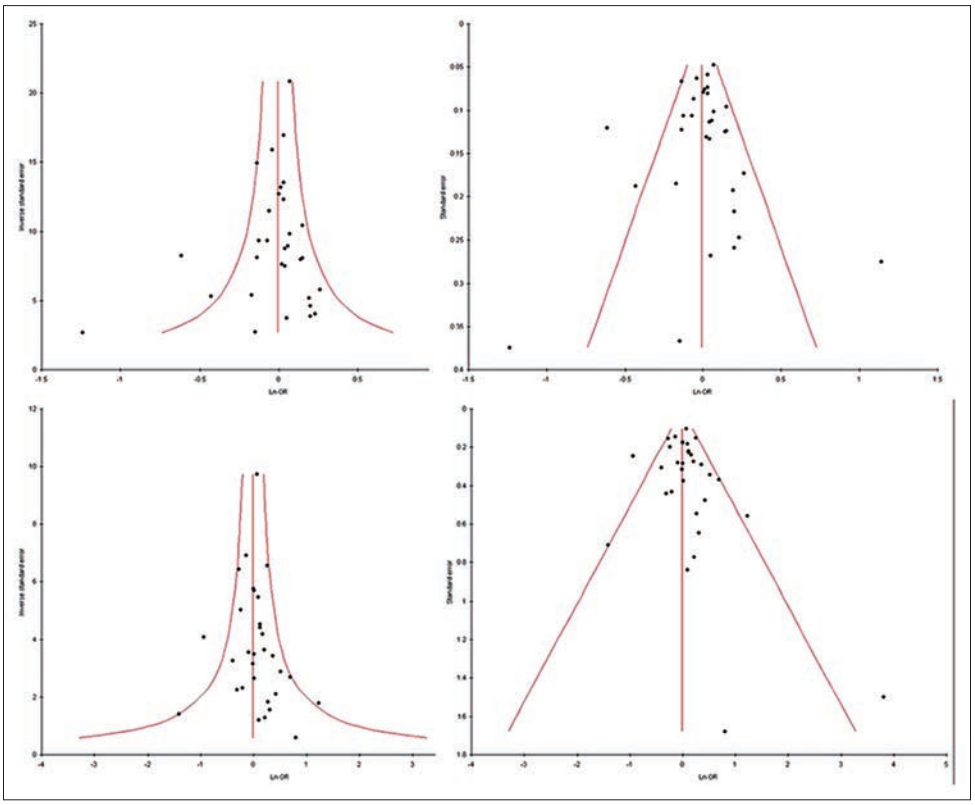

Figure 3: Funnel plots, A. precision versus OR for allele contrast model, B. standard error versus OR for allele contrast model (C vs A). C precision versus OR for homozygote model, D. Standard error versus OR for homozygote model

Table: 4: Summary estimates for the odds ratio (OR) of MTHFR A1298C in various allele/genotype contrasts, the significance level (*P* value) of heterogeneity test (Q test), and the I2 metric, and publication bias *P*-value (Egger and Begg tests) in Asian studies

Genetic Models	Fixed effect OR (95% CI), *P*	Random effect OR (95% CI), *P*	Heterogeneity *P*-value (Q test)	I2 (%)	Publication Bias (*P* of Egger's test)	Publication Bias (*P* of Begg's test)
Allele Contrast (C vs A)	0.97(0.91-1.03),0.38	1.00(0.88-1.1),0.93	<0.001	71.38	0.32	0.30
Heterozygote (AC vs AA)	0.92(0.85-1.0),0.07	0.93(0.79-1.1),0.83	0.0003	62.88	0.86	0.90
Homozygote (CC vs AA)	1.02(0.85-1.2),0.84	1.1(0.81-1.5), 0.62	0.004	53.5	0.08	0.13
Dominant (CC+AC vs AA)	0.94(0.86-1.0),0.12	0.96(0.81-1.1),0.58	<0.0001	68.95	0.62	0.64
Recessive (AA+AC vs CC)	1.1(0.91-1.3),0.38	1.13(0.87-1.4),0.34	0.035	42.08	0.06	0.17

Table: 5: Summary estimates for the odds ratio (OR) of MTHFR A1298C in various allele/genotype contrasts, the significance level (*P* value) of heterogeneity test (Q test), and the I2 metric, and publication bias *P*-value (Egger and Begg test) in Caucasian studies

Genetic Models	Fixed effect OR (95% CI), *P*	Random effect OR (95% CI), *P*	Heterogeneity *P*-value (Q test)	I2 (%)	Publication Bias (*P* of Egger's test)	Publication Bias (*P* of Begg's test)
Allele Contrast (C vs A)	0.99(0.95-1.0),0.73	0.99(0.93-1.0),0.72	0.01	50.3	0.1	0.48
Heterozygote (AC vs AA)	0.82(0.77-88),<0.001	0.83(0.69-1.0),0.53	<0.001	90.07	0.86	0.35
Homozygote (CC vs AA)	0.97(0.88-1.1),0.47	0.97(0.87-1.1),0.51	0.26	16.59	0.35	0.51
Dominant (CC+AC vs AA)	1.0(0.95-1.0),0.98	0.99(0.92-1.1),0.92	0.006	54.1	0.24	0.87
Recessive (AA+AC vs CC)	0.96(0.88-1.0),0.36	0.96(0.88-1.0),0.38	0.60	0	0.56	0.96

cancer,[76,77] gastric cancer,[34,78] prostate cancer[75] and head and neck cancer.[79] During the literature search seven meta-analysis on the same topic[45,65,80-84] were retrieved, out of which three meta-analysis investigated association between A1298C polymorphism and BC.[65,80,81] Zintzaras[79] reported insignificant [FE OR 0.97 (0.90–1.04)] association between A1298C polymorphism and BC. Qi *et al*.[82] and Yu *et al*.[65] demonstrated no significant association of A1298C polymorphism with BC risk. There are several published articles which were not included in the past meta-analyses,

so author conducted a comprehensive meta-analysis with the largest number of studies (33 studies) and largest sample size (35,619).

Heterogeneity is a very important part of a meta-analysis, and finding the possible sources for the high heterogeneity is very important and can greatly affect the results of a meta-analysis.[76] To explore the possible sources for the high heterogeneity in the present meta-analysis, subgroup analysis was performed (results not shown). By subgroup analysis author found that the ethnicity was the major source of the high heterogeneity in the present meta-analysis, which could be explained by the race-specific effect of MTHFR A1298C polymorphism on susceptibility to BC. However, ethnicity didn't explain all heterogeneity in this meta-analysis. Present meta-analysis had several strengths like-publication bias was not detected, which indicated that the pooled results were unbiased. Further substantial studies were pooled which increased the power of the study. Some limitation of the present meta-analysis should also be acknowledged like (i) unadjusted OR was used, (ii) sample size in some studies was low, (iii) controls in some studies were not well defined and were hospital based noncancerous patients, (iv) meta-analysis was restricted on only single polymorphism, other polymorphism of folate pathway genes should also be included in future meta-analysis and (v) except genetic polymorphism, other important factors such as age, ethnicity, folate intake, and smoking status were not considered.

In conclusion, the present meta-analysis suggests that A1298C polymorphism in MTHFR gene independent of other factors, such as folate levels etc., may not play a significant role in the development of BC.

Acknowledgment

The author is highly grateful to Leon Bax (Chief Scientific Officer at BiostatXL, UMC Utrecht) for his valuable suggestions, which help us in statistical analysis.

References

1. Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2000: Cancer Incidence, Mortality and Prevalence Worldwide. IARC Cancer Base No. 5 [CD-ROM]. Version 1.1. Lyon: IARC Press; 2000.
2. Yang X, Lippman ME. BRCA1 and BRCA2 in breast cancer. Breast Cancer Res Treat 1999;54:1-10.
3. Ozen F, Erdis E, Sik E, Silan F, Uludag A, Ozdemir O. Germ-line MTHFR C677T, FV H1299R and PAI-1 5G/4G variations in breast carcinoma. Asian Pac J Cancer Prev 2013;14:2903-8.
4. Ulrich CM. Nutrigenetics in cancer research – folate metabolism and colorectal cancer. J Nutr 2005;135:2698-702.
5. Duthie SJ. Folate and cancer: How DNA damage, repair and methylation impact on colon carcinogenesis. J Inherit Metab Dis 2011;34:101-9.
6. Kim YI. Does a high folate intake increase the risk of breast cancer? Nutr Rev 2006;64:468-75.
7. Kim YI. Folate and carcinogenesis: Evidence, mechanisms, and implications. J Nutr Biochem 1999;10:66-88.
8. Sharp L, Little J, Schofield AC, Pavlidou E, Cotton SC, Miedzybrodzka Z, *et al*. Folate and breast cancer: The role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR). Cancer Lett 2002;181:65-71.
9. Papandreou CN, Doxani C, Zdoukopoulos N, Vlachostergios PJ, Hatzidaki E, Bakalos G, *et al*. Evidence of association between methylenetetrahydrofolate reductase gene and susceptibility to breast cancer: A candidate-gene association study in a South-eastern European population. DNA Cell Biol 2012;31:193-8.
10. Suzuki T, Matsuo K, Hirose K, Hiraki A, Kawase T, Watanabe M, *et al*. One-carbon metabolism-related gene polymorphisms and risk of breast cancer. Carcinogenesis 2008;29:356-62.
11. Sohn KJ, Croxford R, Yates Z, Lucock M, Kim YI. Effect of the methylenetetrahydrofolate reductase C677T polymorphism on chemosensitivity of colon and breast cancer cells to 5-fluorouracil and methotrexate. J Natl Cancer Inst 2004;96:134-44.
12. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, *et al*. A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111-3.
13. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 1998;64:169-72.
14. Weisberg IS, Jacques PF, Selhub J, Bostom AG, Chen Z, Curtis Ellison R, *et al*. The 1298A--> and gt; C polymorphism in methylenetetrahydrofolate reductase (MTHFR): *In vitro* expression and association with homocysteine. Atherosclerosis 2001;156:409-15.
15. Ma J, Stampfer MJ, Giovannucci F, Artigas C, Hunter DJ, Fuchs C, *et al*. Methylenetetrahydrofolate reductase polymorphism, dietry interactions and risk of colorectal cancer. Cancer Res 1997;57:1098-102.
16. Chen J, Giovannucci EL, Hunter DJ. MTHFR polymorphism, methyl-replete diets and the risk of colorectal carcinoma and adenoma among U.S. men and women: An example of gene-environment interactions in colorectal tumorigenesis. J Nutr 1999;129:560S-64.
17. Slattery ML, Potter JD, Samowitz W, Schaffer D, Leppert M. Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 1999;8:513-8.
18. Skibola CF, Smith MT, Kane E, Roman E, Rollinson S, Cartwright RA, *et al*. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc Natl Acad Sci U S A 1999;96:12810-5.
19. Esteller M, Garcia A, Martinez-Palones JM, Xercavins J, Reventos J. Germ line polymorphisms in cytochrome-P450 1A1 (C4887 CYP1A1) and methylenetetrahydrofolate reductase (MTHFR) genes and endometrial cancer susceptibility. Carcinogenesis 1997;18:2307-11.
20. Piyathilake CJ, Macaluso M, Johanning GL, Whiteside M, Heimburger DC, Giuliano A. Methylenetetrahydrofolate reductase (MTHFR) polymorphism increases the risk

of cervical intraepithelial neoplasia. Anticancer Res 2000;20:1751-7.

21. Song C, Xing D, Tan W, Wei Q, Lin D. Methylenetetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population. Cancer Res 2001;61:3272-5.
22. Shen H, Xu Y, Zheng Y, Qian Y, Yu R, Qin Y, *et al.* Polymorphisms of 5,10-methylenetetrahydrofolate reductase and risk of gastric cancer in a Chinese population: A case-control study. Int J Cancer 2001;95:332-6.
23. Lin J, Spitz MR, Wang Y, Schabath MB, Gorlov IP, Hernandez LM, *et al.* Polymorphisms of folate metabolic genes and susceptibility to bladder cancer: A case-control study. Carcinogenesis 2004;25:1639-47.
24. Neumann AS, Lyons HJ, Shen H, Liu Z, Shi Q, Sturgis EM, *et al.* Methylenetetrahydrofolate reductase polymorphisms and risk of squamous cell carcinoma of the head and neck: A case-control analysis. Int J Cancer 2005;115:131-6.
25. Zhang H, Somasundaram K, Peng Y, Tian H, Zhang H, Bi D, *et al.* BRCA1 physically associates with p53 and stimulates its transcriptional activity. Oncogene 1998;16:1713-21.
26. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: A HuGE review. Am J Epidemiol 2000;151:862-77.
27. Robien K, Ulrich CM. 5,10-Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: A HuGE minireview. Am J Epidemiol 2003;157:571-82.
28. Shrubsole MJ, Gao YT, Cai Q, Shu XO, Dai Q, Hébert JR, *et al.* MTHFR polymorphisms, dietary folate intake, and breast cancer risk: Results from the Shanghai Breast Cancer Study. Cancer Epidemiol Biomarkers Prev 2004;13:190-6.
29. Chen J, Gammon MD, Chan W, Palomeque C, Wetmur JG, Kabat GC, *et al.* One-carbon metabolism, MTHFR polymorphisms, and risk of breast cancer. Cancer Res 2005;65:1606-14.
30. Le Marchand L, Haiman CA, Wilkens LR, Kolonel LN, Henderson BE. MTHFR polymorphisms, diet, HRT, and breast cancer risk: The multiethnic cohort study. Cancer Epidemiol Biomarkers Prev 2004;13:2071-7.
31. Justenhoven C, Hamann U, Pierl CB, Rabstein S, Pesch B, Harth V, *et al.* One-carbon metabolism and breast cancer risk: No association of MTHFR, MTR, and TYMS polymorphisms in the GENICA study from Germany. Cancer Epidemiol Biomarkers Prev 2005;14:3015-8.
32. Zhang SM, Hankinson SE, Hunter DJ, Giovannucci EL, Colditz GA, Willett WC. Folate intake and risk of breast cancer characterized by hormone receptor status. Cancer Epidemiol Biomarkers Prev 2005;14:2004-8.
33. Zintzaras E, Hadjigeorgiou GM. The role of G196A polymorphism in the brain-derived neurotrophic factor gene in the cause of Parkinson's disease: A meta-analysis. J Hum Genet 2005;50:560-6.
34. Zintzaras E. Maternal gene polymorphisms involved in folate metabolism and risk of Down syndrome offspring: A meta-analysis. J Hum Genet 2007;52:943-53.
35. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959;22:719-48.
36. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177-88.
37. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-34.
38. Bax L, Yu LM, Ikeda N, Tsuruta H, Moons KG. Development and validation of MIX: Comprehensive free software for meta-analysis of causal research data. BMC Med Res Methodol 2006;6:50.
39. Ergul E, Sazci A, Utkan Z, Canturk NZ. Polymorphisms in the MTHFR gene are associated with breast cancer. Tumour Biol 2003;24:286-90.
40. Försti A, Angelini S, Festa F, Sanyal S, Zhang Z, Grzybowska E, *et al.* Single nucleotide polymorphisms in breast cancer. Oncol Rep 2004;11:917-22.
41. Qi J, Miao XP, Tan W, Yu CY, Liang G, Lü WF, *et al.* Association between genetic polymorphisms in methylenetetrahydrofolate reductase and risk of breast cancer. Zhonghua Zhong Liu Za Zhi 2004;26:287-9.
42. Chou YC, Wu MH, Yu JC, Lee MS, Yang T, Shih HL, *et al.* Genetic polymorphisms of the methylenetetrahydrofolate reductase gene, plasma folate levels and breast cancer susceptibility: A case-control study in Taiwan. Carcinogenesis 2006;27:2295-300.
43. Jakubowska A, Gronwald J, Menkiszak J, Górski B, Huzarski T, Byrski T, *et al.* Methylenetetrahydrofolate reductase polymorphisms modify BRCA1-associated breast and ovarian cancer risks. Breast Cancer Res Treat 2007;104:299-308.
44. Kan X, Zou T, Wu X, Wang X. Yunnan methylenetetrahydrofolate reductase gene polymorphism associated with breast cancer susceptibility. Cancer Res 2007;34:716-8.
45. Lissowska J, Gaudet MM, Brinton LA, Chanock SJ, Peplonska B, Welch R, *et al.* Genetic polymorphisms in the one-carbon metabolism pathway and breast cancer risk: A population-based case-control study and meta-analyses. Int J Cancer 2007;120:2696-703.
46. Stevens VL, McCullough ML, Pavluck AL, Talbot JT, Feigelson HS, Thun MJ, *et al.* Association of polymorphisms in one-carbon metabolism genes and postmenopausal breast cancer incidence. Cancer Epidemiol Biomarkers Prev 2007;16:1140-7.
47. Xu X, Gammon MD, Wetmur JG, Bradshaw PT, Teitelbaum SL, Neugut AI, *et al.* B-vitamin intake, one-carbon metabolism, and survival in a population-based study of women with breast cancer. Cancer Epidemiol Biomarkers Prev 2008;17:2109-16.
48. Cheng CW, Yu JC, Huang CS, Shieh JC, Fu YP, Wang HW, *et al.* Polymorphism of cytosolic serine hydroxymethyltransferase, estrogen and breast cancer risk among Chinese women in Taiwan. Breast Cancer Res Treat 2008;111:145-55.
49. Inoue M, Robien K, Wang R, Van Den Berg DJ, Koh WP, Yu MC. Green tea intake, MTHFR/TYMS genotype and breast cancer risk: The Singapore Chinese Health Study. Carcinogenesis 2008;29:1967-72.
50. Kotsopoulos J, Zhang WW, Zhang S, McCready D, Trudeau M, Zhang P, *et al.* Polymorphisms in folate metabolizing enzymes and transport proteins and the risk of breast cancer. Breast Cancer Res Treat 2008;112:585-93.
51. Mir MM, Dar JA, Dar NA, Dar MS, Salam I, Lone MM, *et al.* Combined impact of polymorphism of folate metabolism genes; glutamate carboxypeptidase, methylene tetrahydrofolate reductase and methionine synthase reductase on breast cancer

susceptibility in Kashmiri women. Int J Health Sci (Qassim) 2008;2:3-14.

52. Ericson U, Sonestedt E, Ivarsson MI, Gullberg B, Carlson J, Olsson H, *et al*. Folate intake, methylenetetrahydrofolate reductase polymorphisms, and breast cancer risk in women from the Malmö Diet and Cancer cohort. Cancer Epidemiol Biomarkers Prev 2009;18:1101-10.
53. Gao CM, Tang JH, Cao HX, Ding JH, Wu JZ, Wang J, *et al*. MTHFR polymorphisms, dietary folate intake and breast cancer risk in Chinese women. J Hum Genet 2009;54:414-8.
54. Ma E, Iwasaki M, Junko I, Hamada GS, Nishimoto IN, Carvalho SM, *et al*. Dietary intake of folate, vitamin B6, and vitamin B12, genetic polymorphism of related enzymes, and risk of breast cancer: A case-control study in Brazilian women. BMC Cancer 2009;9:122.
55. Ma E, Iwasaki M, Kobayashi M, Kasuga Y, Yokoyama S, Onuma H, *et al*. Dietary intake of folate, vitamin B2, vitamin B6, vitamin B12, genetic polymorphism of related enzymes, and risk of breast cancer: A case-control study in Japan. Nutr Cancer 2009;61:447-56.
56. Platek ME, Shields PG, Marian C, McCann SE, Bonner MR, Nie J, *et al*. Alcohol consumption and genetic variation in methylenetetrahydrofolate reductase and 5-methyltetrahydrofolate-homocysteine methyltransferase in relation to breast cancer risk. Cancer Epidemiol Biomarkers Prev 2009;18:2453-9.
57. Lin J, Chen S, Li W. Mthfr C677T and A1298C gene polymorphisms associated with breast cancer. Prelim Stud Mod Hosp 2010;10:15-7.
58. Vainer AS, Boiarskikh UA, Voronina EN, Selezneva IA, Sinkina TV, Lazarev AF, *et al*. Polymorphic variants of folate metabolizing genes (C677T and A1298C MTHFR, C1420T SHMT1 and G1958A MTHFD) are not associated with the risk of breast cancer in West Siberian Region of Russia. Mol Biol (Mosk) 2010;44:816-23.
59. Hosseini M, Houshmand M, Ebrahimi A. MTHFR polymorphisms and breast cancer risk. Arch Med Sci 2011;7:134-7.
60. Hua Z, Wang Y, Ni J, Ge F, Zou T. Serum folate, vitamin b12 concentration and MTHFR, MS gene polymorphism associated with risk of breast cancer research. Mod Oncol 2011;19:428-31.
61. Akram M, Malik FA, Kayani MA. Mutational analysis of the MTHFR gene in breast cancer patients of Pakistani population. Asian Pac J Cancer Prev 2012;13:1599-603.
62. Wu XY, Ni J, Xu WJ, Zhou T, Wang X. Interactions between MTHFR C677T-A1298C variants and folic acid deficiency affect breast cancer risk in a Chinese population. Asian Pac J Cancer Prev 2012;13:2199-206.
63. Liu Y, Zhou LS, Xu XM, Deng LQ, Xiao QK. Association of dietary intake of folate, vitamin B6 and B12 and MTHFR genotype with breast cancer risk. Asian Pac J Cancer Prev 2013;14:5189-92.
64. Weiwei Z, Liping C, Dequan L. Association between dietary intake of folate, vitamin B6, B12 and amp; MTHFR, MTR Genotype and breast cancer risk. Pak J Med Sci 2014;30:106-10.
65. Yu L, Chen J. Association of MTHFR Ala222Val (rs1801133) polymorphism and breast cancer susceptibility: An update meta-analysis based on 51 research studies. Diagn Pathol 2012;7:171.
66. Liu YX, Wang B, Wan MH, Tang WF, Huang FK, Li C. Meta-analysis of the relationship between the metholenetetrahydrofolate reductase C677T genetic polymorphism, folate intake and esophageal cancer. Asian Pac J Cancer Prev 2011;12:247-52.
67. Wen YY, Yang SJ, Zhang JX, Chen XY. Methylenetetrahydrofolate reductase genetic polymorphisms and esophageal squamous cell carcinoma susceptibility: A meta-analysis of case-control studies. Asian Pac J Cancer Prev 2013;14:21-5.
68. Liu L, Liao SG, Wang YJ. MTHFR polymorphisms and ovarian cancer risk: A meta-analysis. Mol Biol Rep 2012;39:9863-8.
69. Tu YL, Wang SB, Tan XL. MTHFR gene polymorphisms are not involved in pancreatic cancer risk: A meta-analysis. Asian Pac J Cancer Prev 2012;13:4627-30.
70. Sun H, Han B, Zhai H, Cheng X, Ma K. Significant association between MTHFR C677T polymorphism and hepatocellular carcinoma risk: A meta-analysis. Tumour Biol 2014;35:189-93.
71. Li C, Chen P, Hu P, Li M, Li X, Guo H, *et al*. Folate intake and MTHFR polymorphism C677T is not associated with ovarian cancer risk: Evidence from the meta-analysis. Mol Biol Rep 2013;40:6547-60.
72. Ma C, Liu Y, Zhang W, Liu P. The association between MTHFR C677T polymorphism and ovarian cancer risk: A meta-analysis of 18,628 individuals. Mol Biol Rep 2013;40:2061-8.
73. Boccia S, Hung R, Ricciardi G, Gianfagna F, Ebert MP, Fang JY, *et al*. Meta-and pooled analyses of the methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and gastric cancer risk: A huge-GSEC review. Am J Epidemiol 2008;167:505-16.
74. Hou XH, Huang YM, Mi YY. Methylenetetrahydrofolate reductase gene C677T polymorphism and lung cancer: An updated meta-analysis. Asian Pac J Cancer Prev 2012;13:2025-9.
75. Zhang WB, Zhang JH, Pan ZQ, Yang QS, Liu B. The MTHFR C677T polymorphism and prostate cancer risk: New findings from a meta-analysis of 7306 cases and 8062 controls. Asian Pac J Cancer Prev 2012;13:2597-604.
76. Guo LN. Methylenetetrahydrofolate reductase C677T polymorphism and cervical cancer risk: A meta-analysis. Asian Pac J Cancer Prev 2012;13:2193-7.
77. Mei Q, Zhou D, Gao J, Shen S, Wu J, Guo L, *et al*. The association between MTHFR 677C > T polymorphism and cervical cancer: Evidence from a meta-analysis. BMC Cancer 2012;12:467.
78. Larsson SC, Giovannucci E, Wolk A. Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: A meta-analysis. Gastroenterology 2006;131:1271-83.
79. Niu YM, Shen M, Li H, Ni XB, Zhou J, Zeng XT, *et al*. No association between MTHFR A1298C gene polymorphism and head and neck cancer risk: A meta-analysis based on 9,952 subjects. Asian Pac J Cancer Prev 2012;13:3943-7.
80. Zintzaras E. Methylenetetrahydrofolate reductase gene and susceptibility to breast cancer: A meta-analysis. Clin Genet 2006;69:327-36.
81. Macis D, Maisonneuve P, Johansson H, Bonanni B, Botteri E, Iodice S, *et al*. Methylenetetrahydrofolate reductase (MTHFR) and breast cancer risk: A nested-case-control study and a pooled meta-analysis. Breast Cancer Res Treat 2007;106:263-71.
82. Qi X, Ma X, Yang X, Fan L, Zhang Y, Zhang F, *et al*.

Methylenetetrahydrofolate reductase polymorphisms and breast cancer risk: A meta-analysis from 41 studies with 16,480 cases and 22,388 controls. Breast Cancer Res Treat 2010;123:499-506.

83. Zhang J, Qiu LX, Wang ZH, Wu XH, Liu XJ, Wang BY, *et al.* MTHFR C677T polymorphism associated with breast cancer susceptibility: A meta-analysis involving 15,260 cases and 20,411 controls. Breast Cancer Res Treat 2010;123:549-55.
84. Liang H, Yan Y, Li T, Li R, Li M, Li S, *et al.* Methylenetetrahydrofolate reductase polymorphisms and breast cancer risk in Chinese population: A meta-analysis of 22 case-control studies. Tumour Biol 2014;35:1695-701.

How to cite this article: ????

Source of Support: Nil. **Conflict of Interest:** None declared.