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...
have been implicated in carcinogenesis.\textsuperscript{[5,6]} Epidemiologic studies have indicated that folate deficiency may be related to the development of several cancers, including BC.\textsuperscript{[7–9]} It has been suggested that breast carcinogenesis could be associated with alteration of estrogen receptor gene methylation pattern and global DNA methylation.\textsuperscript{[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.\textsuperscript{[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-methylenetetrahydrofolate, the methyl donor for methylation of dUMP to dTMP.\textsuperscript{[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.\textsuperscript{[12,13]} MTHFR gene is located at 1p36.3. 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.\textsuperscript{[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 homoyzogotes, respectively.\textsuperscript{[12,14]} The A→C polymorphism at nucleotide 1298 codes for glutamine to alanine substitution in the C-terminal regulatory domain.\textsuperscript{[13]} Individuals homozygous for the A1298C have approximately the same enzyme activity as those heterozygous for C677T allele.\textsuperscript{[13,14]} These variant genotypes are associated with a substantial decrease in enzymatic activity in vitro\textsuperscript{[12,13]} and may reduce the risk of colon cancer\textsuperscript{[5,17–19]} and acute lymphocytic leukemia.\textsuperscript{[18]} Conversely, the same variants have also been associated with an increased risk for various cancers including endometrial cancer,\textsuperscript{[19]} cervical intraepithelial neoplasia,\textsuperscript{[20]} esophageal squamous cell carcinoma,\textsuperscript{[21]} gastric cancer,\textsuperscript{[22]} bladder cancer,\textsuperscript{[23]} and squamous cell carcinoma of the head and neck.\textsuperscript{[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.\textsuperscript{[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%).\textsuperscript{[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.\textsuperscript{[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\textsuperscript{2} metric (I\textsuperscript{2}=(Q−df)/Q), which is independent of the number of studies in the meta-analysis. I\textsuperscript{2} takes values of between 0 and 100%, with higher values denoting a greater degree of heterogeneity\textsuperscript{[33]} (I\textsuperscript{2} = 0% to 25%: No heterogeneity; I\textsuperscript{2} = 25% to 50%: Moderate heterogeneity; I\textsuperscript{2} = 50% to 75%: Large heterogeneity; I\textsuperscript{2} = 75% to 100%: Extreme heterogeneity).\textsuperscript{[34]} The pooled OR was estimated using fixed effect (FE)\textsuperscript{[35]} and random effect (RE)\textsuperscript{[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.\textsuperscript{[37]}

All statistical analyses were undertaken using the program MIX version 1.7.\textsuperscript{[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 included 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.\textsuperscript{[3,8,9,28–31,39,64]} The studies were carried out in Brazil,\textsuperscript{[54]} Canada,\textsuperscript{[50]} China,\textsuperscript{[28,41,44,53,57,60,62–64]} Germany,\textsuperscript{[31]...
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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 $\chi^2$ 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.
Meta-analysis

Table 1 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 Laird) models. Meta-analysis with allele contrast did not show any association with both fixed effect (OR<sub>CCvsA</sub> = 0.99; 95% CI: 0.95–1.02; P = 0.55) and random effect model (OR<sub>CCvsA</sub> = 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].

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 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 pacif J Cancer Prev, 13:1599-1603.      |
| Papandreou et al., 2012 | 2012 | Greece  | 283     | 300    | DNA Cell Biology, 31:193-198.                 |
| Wu et al., 2012       | 2012 | China   | 75      | 75     | Asian Pac J Cancer Prev, 13:2199-206.         |
| Hosseini et al., 2011 | 2011 | Iran    | 300     | 294    | Arch Med Sci, 7, 1: 134-137.                  |
| Hua et al., 2011      | 2011 | China   | 90      | 95     | Mod Oncol., 19:428-31.                        |
| Lin et al., 2010      | 2010 | China   | 143     | 65     | Prelim StudMod Hosp, 10:15-7.                 |
| Weiner et al., 2010   | 2010 | Russia  | 785     | 831    | Mol Biol, 44 (5):720-727.                     |
| Ericson et al., 2009  | 2009 | Sweden  | 1072    | 541    | Cancer Epidemiol Biomarkers Prev, 18:1101-1110.|
| Gao et al., 2009      | 2009 | China   | 682     | 669    | J Hum Genet, 54:414-418.                     |
| Ma et al., 2009       | 2009 | Japan   | 458     | 458    | BMC Cancer, 9:122.                            |
| Ma et al., 2009       | 2009 | Brazil  | 387     | 388    | Nutr Cancer, 61:447-456.                     |
| Platek et al., 2009   | 2009 | USA     | 1781    | 928    | Cancer Epidemiol Biomark, 18:2453-2459.       |
| Cheng et al., 2008    | 2008 | China   | 534     | 351    | Breast Cancer Res Treat , 111:145-155.        |
| Inoue et al., 2008    | 2008 | Singapore| 662    | 380    | Carcinogenesis, 29:1967-1972.                 |
| Kotsopoulos et al., 2008 | 2008 | Canada  | 780     | 941    | Breast Cancer Res Treat , 112:585-593.         |
| Mir et al., 2008      | 2008 | India   | 33      | 35     | International Journal of Health Sciences, Qassim University, 2: pp. 3-14.|
| Jakubowska et al., 2007 | 2007 | Poland  | 290     | 319    | Breast Cancer Res Treat, 115:431-432.          |
| Kan et al., 2007      | 2007 | China   | 101     | 125    | Cancer Res Prev Treat 34:716-718.             |
| Lissowska et al., 2007 | 2007 | Poland  | 2278    | 1986   | Int J Cancer 120: 2696-2703.                  |
| Stevens et al., 2007  | 2007 | USA     | 493     | 494    | Cancer Epidemiol Biomarkers Prev 16:1140-1147.|
| Xu et al., 2007       | 2007 | USA     | 1103    | 1062   | Carcinogenesis, 28:1504-1509.                 |
| Chou et al., 2006     | 2006 | Taiwan  | 285     | 142    | Carcinogenesis, 27:2295-2300.                 |
| Chen et al., 2005     | 2005 | USA     | 1103    | 1062   | Cancer Res, 65:1606-1614.                     |
| Justenhoven et al., 2005 | 2005 | Germany | 634     | 582    | Cancer Epidemiol Biomark, 14:3015-3018.        |
| Forstl et al., 2004   | 2004 | Finland | 298     | 223    | Oncol Rep, 11:917-922.                        |
| Le Marchand et al., 2004 | 2004 | USA     | 2414    | 1190   | Cancer Epidemiol Biomarkers Prev 13:2071-2077.|
| Qi et al., 2004       | 2004 | China   | 218     | 217    | Chin J Oncol, 26:287-289.                     |
| Shrubsole et al., 2004 | 2004 | China   | 1208    | 1121   | Cancer Epidemiol Biomarkers Prev, 13:190-196.  |
| Ergul et al., 2003    | 2003 | Turkey  | 193     | 118    | Tumour Biol, 24:286-290.                      |
| Sharp et al., 2002    | 2002 | UK      | 60      | 35     | Cancer Lett, 181:65-71.                       |
Table 2. The distributions of MTHFR A1298C genotypes and allele number for Breast cancer cases and controls

| Study ID | Genotype | Alleles |
|----------|----------|---------|
|          | AA       | AC      | CC       | Case | Control |
| v        | 135      | 151     | 129      | 130  |         |
| Liu, 2013| 206      | 214     | 176      | 172  |         |
| Ozen, 2013| 17     | 71      | 29       | 35   |         |
| Akram, 2012| 35  | 30      | 55       | 75   |         |
| Papandreou, 2012| 129 | 136  | 135      | 116  |         |
| Wu, 2012  | 37       | 42      | 32       | 28   |         |
| Hosseini, 2011 | 162 | 105  | 96       | 135  |         |
| Hua, 2011 | 50       | 55      | 42       | 32   |         |
| Lin, 2010 | 45       | 98      | 14       | 35   |         |
| Weiner, 2010| 398    | 379     | 353      | 330  |         |
| Ericson, 2009| 242 | 487    | 242      | 480  |         |
| Gao, 2009 | 478      | 465     | 181      | 205  |         |
| Ma, 2009  | 269      | 279     | 168      | 157  |         |
| Ma, 2009  | 254      | 256     | 119      | 116  |         |
| Platek, 2009| 443  | 842     | 402      | 758  |         |
| Cheng, 2008| 207    | 310     | 125      | 207  |         |
| Inoue, 2008| 225    | 387     | 139      | 234  |         |
| Kotsopoulos, 2008| 466 | 398    | 390      | 309  |         |
| Mir, 2008 | 15       | 11      | 19       | 22   |         |
| Jakubowska, 2007| 151  | 117     | 134      | 144  |         |
| Kan, 2007 | 70       | 61      | 41       | 32   |         |
| Lissowska, 2007| 892 | 1086   | 874      | 941  |         |
| Stevens, 2007| 224    | 252     | 228      | 201  |         |
| Xu, 2007  | 558      | 536     | 417      | 457  |         |
| Chou, 2006| 104      | 172     | 30       | 95   |         |
| Chen, 2005| 558      | 536     | 417      | 457  |         |
| Justenhoven, 2005| 273 | 295    | 256      | 266  |         |
| Forst, 2004| 94      | 133     | 102      | 127  |         |
| Le Marchand, 2004| 741 | 1493   | 372      | 801  |         |
| Qi, 2004  | 155      | 144     | 58       | 71   |         |
| Shrubsole, 2004| 768 | 824    | 311      | 344  |         |
| Ergul, 2003| 50       | 90      | 48       | 85   |         |
| Sharp, 2002| 27      | 24      | 5        | 25   |         |

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 = 0.98–1.1, P = 0.93, I² = 71.38%, P heterogeneity ≤ 0.0001; for AC vs. AA: OR = 0.93, 95% CI = 0.79–1.1, P = 0.83, I² = 62.88%, P heterogeneity = 0.0003; for CC vs. AA: OR = 1.1, 95% CI = 0.81–1.5, P = 0.62, I² = 53.5%, P heterogeneity = 0.004; for CC + AC vs. AA: OR = 0.96, 95% CI = 0.81–1.1, P = 0.58, I² = 68.99%, P heterogeneity ≤ 0.0001; for CC vs. AC + AA: OR = 1.1, 95% CI = 0.91–1.3, P = 0.38, I² = 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, I² = 50.3%, P heterogeneity ≤ 0.01; for AC vs. AA: OR = 0.83, 95% CI = 0.69–1.0, P = 0.53, I² = 90.07%, P heterogeneity ≤ 0.001; for CC vs. AA: OR = 0.97, 95% CI = 0.88–1.0, P = 0.47, I² = 15.59%, P heterogeneity = 0.26; for CC + AC vs. AA: OR = 0.99, 95% CI = 0.92–1.1, P = 0.92, I² = 54.1%, P heterogeneity = 0.006; for CC vs. AC + AA: OR = 0.96, 95% CI = 0.88–1.0, P = 0.6, I² = 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
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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, pancreatic cancer, liver cancer, ovarian cancer, lung cancer, cervical cancer.

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 I² metric, and publication bias P value (Egger test)

| Genetic models       | OR (95% CI), P   | Heterogeneity P value (Q test) | I² (%) | Publication Bias P value (p of Egger's test) |
|----------------------|------------------|-------------------------------|-------|------------------------------------------|
| 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 (0.91-1.10),0.64 | 0.99 (0.89-1.12),0.66 | 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 | 62.10 | 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 ratios, 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. 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.
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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.

**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(0.77-0.88),<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\(^{[79]}\)
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.

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