Methylenetetrahydrofolate reductase gene C677T polymorphism and breast cancer risk: Evidence for genetic susceptibility

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There are several evidences supporting the role of 5–10 methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms in breast cancer (BC). Case control association studies on breast cancer have been repeatedly performed over the last two decades, but results are inconsistent. We performed a meta-analysis to confirm the association between MTHFR C677T polymorphism and BC risk.

The articles were retrieved by searching the PubMed, Google Scholar, and Springer Link databases. Crude odds ratios (OR) with 95% confidence intervals (CIs) was used to assess the strength of association between C677T polymorphism and BC. Publication bias was assessed by Egger's and Begg-Mazumdar tests. Meta-analysis was performed with Open Meta Analyst.

Total 75 studies with 31,315 cases and 35,608 controls were found suitable for the inclusion in the present meta-analysis. The results of meta-analysis suggested that there were moderate significant association between C677T polymorphism and BC risk using overall comparisons in five genetic models (T vs. C: OR = 1.08, 95% CI = 1.03–1.13, p = <0.001; TT + CT vs. CC: OR = 1.06, 95% CI = 1.02–1.09, p = <0.001; TT vs. CC: OR = 1.17, 95% CI = 1.06–1.28, p = 0.001; CT vs. CC OR = 1.05, 95% CI = 1.01–1.10, p = 0.005; TT vs. CT + CC: OR = 1.12, 95% CI = 1.03–1.22, p = 0.005). In conclusion, results of present meta-analysis showed modest association between MTHFR C677T polymorphism with breast cancer in total studies. However, sub-group analysis results based on ethnicity showed strong significant association between TT genotype and breast cancer (TT vs. CC; OR = 1.26; 95% CI: 1.06–1.51; p = 0.009) in Asian population but in Caucasian population such association was not observed (TT vs. CC; OR = 1.08; 95% CI: 0.99–1.14; p = 0.05).

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1. Introduction

Breast cancer (BC) is a leading cause of morbidity and mortality in women in the developed countries. Global BC incidence has been increasing by more than one million new cases every year, and it is significantly higher in developed countries than in developing countries (Liang et al., 2014; Sturgeon et al., 2004; Ferlay et al., 2000). The lifetime BC risk in the general population is estimated to be 10% (Yang and Lippman, 1999). Several risk factors for BC have been suggested like- age of menarche and menopause, diet, reproductive history, hormone administration and genetic factors (Langsenlehner et al., 2003; Collaborative Group on Hormonal Factors in Breast Cancer, 1997; Hulka and Stark, 1995; Kelsey, 1993). The etiology of breast cancer is not very well understood. However, it has been suggested that low-penetrance susceptibility genes combining with environmental factors may be important in the development of cancer (Zhang et al., 2010). In past decade, several common low-penetrant genes have been identified as potential breast cancer susceptibility genes, one of which is 5,10-methylenetetrahydrofolate reductase (MTHFR) gene (Zhang et al., 2010).

One carbon metabolism (OCM) and MTHFR enzyme play key roles in physiologic processes by regulating the one carbon units transfer between the DNA synthesis (nucleotide synthesis) and the DNA methylation cycle (Laanpere et al., 2010; Frankenburg, 2007). MTHFR reduces 10-methyltetrahydrofolate (10-MTHF) to S-methylenetetrahydrofolate (5-MTHF), which is a cofactor for the remethylation of homocysteine to convert it to S-adenosyl methionine (SAM). SAM is the sole methyl group donor for DNA, RNA and protein methylation. Dysfunction of the OCM cycle has been linked to congenital abnormalities (Rai et al., 2014; Zhang et al., 2013; van der Put et al., 2001), psychiatric disorders (Rai, 2011; Gibboly et al., 2007), and different types of cancers (Rai, 2014; Zhang et al., 2012; Kim, 1999).

C677T is the most common and functional polymorphism in the MTHFR gene, which involves a cytosine-to-thymine substitution at position 677, a consequence of transformation from an alanine to a valine in the gene, which involves a cytosine-to-thymine substitution at position 677, a consequence of transformation from an alanine to a valine in the gene. The 677 variant has enzyme activity reduced to approximately 60% and 30%, respectively, of that of the wild type (677CC) (Ueland et al., 2001) and elevate homocysteine levels (Holmes et al., 2011; Kang et al., 2014; Weiwei et al., 2014; Cheng et al., 2008), however some other studies have reported no association between BC and C677T polymorphism and BC risk (Kakkoura et al., 2015; Lu et al., 2015; He et al., 2014; Weiwai et al., 2014; Cheng et al., 2008), however some other studies have reported no association between BC and C677T polymorphism (Singh et al., 2015; Huang et al., 2014; Wu et al., 2012; Ma et al., 2009a, 2009b). The variation of these results might be induced by difference in ethnicities, sample size, study design and background of patients as well as random error (Wen et al., 2013). Hence we performed a meta-analysis of published case control studies to re-evaluate the association between C677T polymorphism and BC susceptibility. Meta-analysis is a technique that has proven useful in resolving discrepancies between association studies is meta-analysis (Sen et al., 2008; Lohmueller et al., 2003). Meta-analysis is a quantitative method of combining the results independent studies and synthesizing summaries and conclusions. This method increases power to distinguish between small effects and no effect.

2. Methods

2.1. Literature search and inclusion/exclusion criteria

The articles were retrieved by searching the PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Google Scholar (http://scholar.google.com), and Springer Link (http://link.springer.com) databases using the keywords “breast cancer”, “C677T”, “methylenetetrahydrofolate reductase” and “MTHFR” published up to March 31, 2015. In addition references of reviews and meta-analyses were examined to identify potential additional studies.

The inclusion criteria for the present meta-analysis were: (a) studies should investigated associations between MTHFR C677T polymorphism and BC; (b) studies should provide complete data on genotype number and frequencies of cases and controls for calculation of odd ratios (ORs) with 95% confidence intervals (CIs); (c) studies should be case–control studies. Exclusion criteria were as follows: (a) study design other than case–control (e.g., case reports, cohort study design without control group); (b) main outcome other than the risk of BC among genotypes (e.g., pharmacogenetic studies); and (c) reports were further excluded if they evaluated the role of MTHFR variants in other cancers. For duplicate publications, study with small sample size was excluded.

2.2. Extraction of data

The characteristics of the included studies were independently extracted by two investigators (UY and VR) through a standardized protocol. They independently extracted the following data from each publication: author name; country of origin; selection and characteristics of cases and controls; source of control, demographic information; racial descent of the study population; numbers of eligible and genotyped cases and controls; and numbers of cases and controls for each MTHFR genotype. Number and frequency of genotypes and alleles in both case and control groups were extracted or calculated from published data to re-calculate crude ORs and their 95% confidence intervals (95% CIs). Results were compared and minor disagreements were resolved by discussion. If essential information was missing from the article, the authors of the respective papers were contacted and asked to provide additional data.

2.3. Statistical analysis

The strength of association between the MTHFR C677T polymorphism and BC was estimated using odds ratios (OR), with the corresponding 95% confidence intervals (95% CI). We estimated the risk of C677T polymorphism using all genetic models viz. allele contrast/additive model (T vs. C), homozygote model (TT vs. CC), co-dominant/heterozygote model (CT vs. CC), dominant model (TT + CT vs. CC) and recessive model (TT vs. CT + CC). We tested heterogeneity between studies using Cochran’s chi-square-based Q-statistic and estimated the degree of heterogeneity as follows: (a) study design other than case control (e.g., case reports, cohort study design without control group); (b) main outcome other than the risk of BC among genotypes (e.g., pharmacogenetic studies); and (c) reports were further excluded if they evaluated the role of MTHFR variants in other cancers. For duplicate publications, study with small sample size was excluded.

Two methods were used to detect possible publication bias in meta-analysis: graphical and statistical. The funnel plot is a commonly used graphical test and Egger’s (Egger et al., 1997) and Begg and Mazumdar (Begg and Mazumdar, 1994) are statistical methods. Pearson’s x² test was used to determine whether genotype of control population were in Hardy–Weinberg equilibrium (HWE) or not (P > 0.05). Sensitivity analyses were performed by excluding studies with a small number of cases (n = 100) and studies with control population violating HWE. Subgroup analyses based on ethnicity were also performed to investigate the cause of heterogeneity.

Meta-analysis was performed using Open Meta Analyst (Wallace et al., 2013) and publication bias analysis was performed using Mix version 1.7 (Bax et al., 2006). All P values are two-tailed with a significance level at 0.05.
2.4. Quality score assessment

Method of Guo et al. (2012) was adopted for quality score assessment. The quality scores ranged from 0 to 10 and studies with score < 5 were defined as low quality, and studies with score ≥ 7 were defined as high quality.

3. Results

3.1. Characteristics of included studies

A flow chart summarizing the process of study selection is shown in Fig. 1. Initially, the highly sensitive search strategy of Pubmed, Google Scholar, and Springer Link databases, 192 articles were retrieved. After screening the titles and abstracts of all retrieved articles, 119 articles were excluded. Then full texts were reviewed and 2 articles (only cases) were further excluded. Based on the inclusion and exclusion criteria, finally, seventy one studies were included in the present meta-analysis (Kakkoura et al., 2015; Lin et al., 2015; López-Cortés et al., 2015; Lu et al., 2015; Singh et al., 2015; He et al., 2014; Huang et al., 2014; Jiang-hua et al., 2014; Wang et al., 2014; Weiwei et al., 2014; Liu et al., 2013; Ozen et al., 2013; Akram et al., 2012; Barbosa Rde et al., 2012; Diakite et al., 2012; Jakubowska et al., 2012; Lajin et al., 2012; Wu et al., 2012; Batschauer et al., 2011; Cerne et al., 2011; Hosseini et al., 2011; Hua et al., 2011; Naushad et al., 2011; Prasad and Wilkhoo, 2011; Alshatwi, 2010; Bentley et al., 2010; Sangrajrang et al., 2010; Vainer et al., 2010; Wu et al., 2010; Cam et al., 2009; Ericson et al., 2009; Gao et al., 2009; Hennquez-Hernandez et al., 2009; Jin et al., 2009; Li and Chen, 2009; Ma et al., 2009a, 2009b; Maruti et al., 2009; Platek et al., 2009; Yuan et al., 2009; Cheng et al., 2008; Inoue et al., 2008; Kotsopoulos et al., 2008; Langsenlehner et al., 2008; Mir et al., 2008; Suzuiki et al., 2008; Hekim et al., 2007; Kan et al., 2007; Lissowska et al., 2007; Macis et al., 2007; Reljic et al., 2007; Stevens et al., 2007; Xu et al., 2007; Yu et al., 2007; Chou et al., 2006; Kalyankumar and Jamil, 2006; Chen et al., 2005; Deligezer et al., 2005; Justenhoven et al., 2005; Kalemci et al., 2005; Forsti et al., 2004; Grieu et al., 2004; Lee et al., 2004; Le Marchand et al., 2004; Lin et al., 2004; Qi et al., 2004; Shrubsole et al., 2004; Ergul et al., 2003; Langsenlehner et al., 2003; Semenza et al., 2003; Campbell et al., 2002; Sharpe et al., 2002). One author (eLe Marchand et al., 2004) investigated five different population. We included each population as separate article so total seventy five article were included in the present meta-analysis (Table 1).

In seventy five studies included in the present meta-analysis, the smallest case sample size was 32 (Wu et al., 2012) and highest sample size was 4778 (Jakubowska et al., 2012). In included studies, total cases were 31,315 with CC (13,960), CT (13,328) and TT (4027), and controls were 35,608 with CC (16,527), CT (14,868), and TT (4213). In controls genotype percentage of CC, CT and TT were 46.41%, 41.75% and 11.83% respectively. In cases genotype percentage of CC, CT and TT were 44.58%, 42.56% and 12.86% respectively. Frequencies of CC genotype and C allele were highest in both cases and controls.

Fig. 1. Flow diagram of study search and selection process.
## Table 1
Characteristics of the eligible studies considered in the meta-analysis.

| Study ID          | Country | Ethnicity | Case/control | Control source | Genotyping method | HWE | Study quality |
|-------------------|---------|-----------|--------------|----------------|-------------------|-----|---------------|
| Sharp et al. (2002)| UK      | Caucasian | 54/57        | PB             | PCR-RFLP          | 0.10 | 4             |
| Campbell et al. (2002) | Australia | Caucasian | 335/233      | HB             | PCR-RFLP          | 0.41 | 6.5           |
| Semenza et al. (2003) | USA     | Caucasian | 105/247      | HB             | PCR-RFLP          | 0.64 | 6             |
| Langsenlehner et al. (2003) | Austria | Caucasian | 94/495       | PB             | PCR-RFLP          | 0.33 | 5             |
| Ergul et al. (2003) | Turkey  | Caucasian | 118/193      | PB             | PCR-RFLP          | 0.16 | 6.5           |
| Shrubsole et al. (2004) | China   | Asian     | 1112/1160    | PB             | PCR-RFLP          | 0.44 | 8.5           |
| Forsti et al. (2004) | Poland  | Caucasian | 223/298      | NR             | PCR-RFLP          | 0.68 | 7             |
| Lee et al. (2004) | Australia | Caucasian | 106/1147     | HB             | PCR-RFLP          | 0.07 | 7.5           |
| Grieu et al. (2004) | Korea   | Asian     | 334/551      | PB             | PCR-RFLP          | 0.10 | 7             |
| Lin et al. (2004) | Taiwan  | Asian     | 88/342       | PB             | PCR-RFLP          | 0.38 | 7             |
| Le Marchand et al. (2004) | Hawaii | Caucasian | 1189/2414    | PB             | TaqMan            | 0.75 | 8.5           |
| Qi et al. (2004) | China   | Asian     | 1112/1160    | PB             | PCR-RFLP          | 0.44 | 8.5           |
| Chen et al. (2005) | Australia | Caucasian | 186/147      | HB             | PCR-RFLP          | 0.07 | 7.5           |
| Bastaschier et al. (2005) | Greece | Caucasian | 42/51        | NR             | PCR-RFLP          | 0.31 | 5             |
| Deligezer et al. (2005) | Turkey | Caucasian | 189/223      | NR             | PCR-RFLP          | 0.75 | 7             |
| Justenhoven et al. (2005) | Germany | Caucasian | 575/633      | PB             | MALDI-TOF         | 0.19 | 8             |
| Chou et al. (2005) | China   | Asian     | 142/285      | PB             | PCR-RFLP          | 0.47 | 7             |
| Kalyankumar and Jamil (2006) | India | Asian     | 88/95        | HB             | PCR-RFLP          | 0.69 | 6.5           |
| Xu et al. (2006) | USA      | Caucasian | 106/1104     | PB             | PCR-RFLP          | 0.68 | 8.5           |
| Hekimi et al. (2006) | Turkey  | Caucasian | 40/66        | NR             | PCR-RFLP          | 0.87 | 6             |
| Kan et al. (2007) | China   | Asian     | 125/103      | PB             | PCR-RFLP          | 0.04 | 7             |
| Lissowska et al. (2007) | Poland | Caucasian | 1974/2282    | PB             | TaqMan            | 0.01 | 8             |
| Macis et al. (2007) | Italy   | Caucasian | 45/900       | PB             | TaqMan            | 0.52 | 4.5           |
| Cheng et al. (2007) | Taiwan  | Asian     | 349/530      | PB             | PCR-RFLP          | 0.62 | 6.5           |
| Langsenlehner et al. (2008) | Austria | Caucasian | 105/105      | NR             | PCR-RFLP          | 0.68 | 6             |
| Mir et al. (2008) | India   | Asian     | 35/33        | PB             | PCR-RFLP          | 0.95 | 4             |
| Ericson et al. (2009) | Sweden | Caucasian | 540/1074     | PB             | MALDI-TOF         | 0.70 | 6             |
| Gao et al. (2009) | China   | Asian     | 624/624      | PB             | PCR-RFLP          | 0.59 | 9             |
| Ma et al. (2009) | Japan   | Asian     | 388/387      | HB             | TaqMan            | 0.66 | 6.5           |
| Plunkett et al. (2009) | USA     | Caucasian | 49/612       | PB             | TaqMan            | 0.51 | 4.5           |
| Hennquez-Hernandez et al. (2009) | USA | Others | 494/494      | PB             | TaqMan            | 0.11 | 7             |
| Cam et al. (2009) | Turkey  | Caucasian | 110/95       | NR             | PCR-RFLP          | 0.39 | 4.5           |
| Maruti et al. (2009) | USA     | Caucasian | 318/647      | HB             | ASPE              | 0.67 | 8.5           |
| Ma et al. (2009) | Brazil  | Others    | 458/458      | HB             | TaqMan            | 0.30 | 8.5           |
| Li et al. (2009) | China   | Asian     | 65/143       | PB             | PCR-RFLP          | 0.18 | 7             |
| Yuan et al. (2009) | China   | Asian     | 80/80        | HB             | PCR-RFLP          | 0.51 | 6.5           |
| Jin et al. (2009) | China   | Asian     | 41/100       | NR             | PCR-RFLP          | 0.74 | 7.5           |
| Bentley et al. (2010) | USA    | Caucasian | 939/1163     | HB             | PCR-RFLP          | 0.05 | 8             |
| Alishati (2010) | Arab    | Arab      | 100/100      | HB             | TaqMan            | 0.80 | 6.5           |
| Sangrajgar et al. (2010) | India | Asian     | 563/487      | HB             | TaqMan            | 0.42 | 7             |
| Weiner et al. (2010) | Austria | Caucasian | 83/778       | PB             | PCR-RFLP          | 0.80 | 8             |
| Batschauer et al. (2011) | Brazil | Others    | 68/85        | PB             | PCR-RFLP          | 0.59 | 4.5           |
| Cerne et al. (2011) | Caucasian | Caucasian | 522/269      | PB             | Sequencing        | 0.88 | 6             |
| Holen et al. (2011) | Iran    | Asian     | 294/300      | HB             | PCR-RFLP          | 0.001 | 3 |
| Hua et al. (2011) | China   | Asian     | 95/90        | PB             | PCR-RFLP          | 0.02 | 6.5           |
| Nausad et al. (2011) | India   | Asian     | 244/244      | PB             | PCR-RFLP          | 0.17 | 7             |
| Prasad et al. (2011) | India   | Asian     | 130/125      | PB             | PCR-RFLP          | 0.06 | 6             |
| Prasad et al. (2011) | Pakistan | Asian     | 110/110      | PB             | PCR-RFLP          | 0.85 | 5             |
| Barbosa et al. (2012) | Mixed, Caucasian | Caucasian | 176/176      | PB             | PCR-RFLP          | 0.38 | 5.5           |
| Diakite et al. (2012) | Morocco | Others    | 96/117       | HB             | PCR-RFLP          | 0.78 | 7             |
| Jakubowska et al. (2012) | Mixed, Caucasian | Caucasian | 478/3350     | PB             | TaqMan            | 0.15 | 7             |
| Lajin et al. (2012) | India   | Asian     | 254/254      | PB             | PCR-RFLP          | 0.17 | 7             |
| Costantini et al. (2012) | USA     | Caucasian | 310/310      | HB             | Sequenom          | 0.001 | 6 |
| Huang et al. (2012) | Taiwan  | Asian     | 1232/1232    | PB             | PCR-RFLP          | 0.01 | 8             |
| Jiang-hua et al. (2012) | China | Asian     | 535/673      | HB             | PCR-RFLP          | 0.001 | 7 |
| Weiwei et al. (2012) | China   | Asian     | 1065/1157    | PB             | TaqMan            | 0.09 | 9.5           |
| Kakkoura et al. (2015) | Cyprus  | Caucasian | 297/306      | PB             | Sequenom          | 0.00 | 8             |
| Singh et al. (2015) | India   | Asian     | 588/508      | PB             | PCR-RFLP          | 0.37 | 5.5           |

* NR = not reported.
Out of 75 studies, only twenty studies reported OR above one and significant association between C677T polymorphism and BC (López-Cortés et al., 2015; Lu et al., 2015; He et al., 2014; Jiang-hua et al., 2014; Weiwei et al., 2014; Liu et al., 2013; Ozen et al., 2013; Lajin et al., 2012; Naushad et al., 2011; Wu et al., 2010; Gao et al., 2009; Maruti et al., 2009; Li and Chen, 2009; Yuan et al., 2009; Xu et al., 2007; Chen et al., 2005; Deligezer et al., 2005; Qi et al., 2004). Control population of eleven studies (López-Cortés et al., 2015; He et al., 2014; Jiang-hua et al., 2014; Wang et al., 2014; Weiwei et al., 2014; Wu et al., 2012; Hosseini et al., 2011; Hua et al., 2011; Lissowska et al., 2007; Stevens et al., 2007) was not in Hardy–Weinberg equilibrium (Table 1).

### 3.2. Meta-analysis

The meta-analysis was carried out using all five genetic models—allele contrast (T vs. C), co-dominant (CT vs. CC), homozygote (TT vs. CC), dominant (TT + CT vs. CC), and recessive (TT vs. CT + CC) models. Meta-analysis with allele contrast (T vs. C) showed moderate significant association with both fixed effect (OR = 1.05; 95% CI = 1.02–1.07; p = <0.001) and random effect model (OR = 1.08; 95% CI = 1.03–1.13; p = <0.001). Subjects with T allele showed a slightly increased risk of BC (Table 2; Fig. 2).

An increased significant association was found between BC and mutant genotype (TTvss.CC; homozygote model) with both fixed (OR = 1.10; 95% CI = 1.04–1.16; p = <0.001) and random (OR = 1.17; 95% CI = 1.06–1.28; p = 0.001) effect models (Table 2, Fig. 3). Association of mutant heterozygous genotype (CT vs.CC; co-dominant model) was observed significant with BC using fixed (OR = 1.05; 95% CI = 1.01–1.08; p = 0.005) and random (OR = 1.05; 95% CI = 1.01–1.10; p = 0.01) effect models (Table 2). Combined mutant genotypes (TT + CT vs. CC; dominant model) showed positive association with BC using both fixed (OR = 1.06; 95% CI = 1.02–1.10; p = <0.001) and random (OR = 1.08; 95% CI = 1.03–1.14; p = <0.001) effect models. Similarly the recessive genotypes models (TT vs. CT + CC) also showed positive association fixed (OR = 1.07; 95% CI = 1.02–1.13; p = 0.002) and random (OR = 1.12; 95% CI = 1.03–1.22; p = 0.005) effect models (Table 2). In allele contrast cumulative meta-analysis, after addition of Bentley et al. (2010) study, the pooled turned statistically significant and remained significant after addition of subsequent studies (details not given).

### Table 2

Summary estimates for the odds ratio (OR) of MTHFR C677T in various allele/genotype contrasts, the significance level (p-value) of heterogeneity test (Q test), and the I² metric: overall analysis, and subgroup analyses.

| Genetic contrast | Fixed effect OR (95% CI), p | Random effect OR (95% CI), p | Heterogeneity p-value (Q test) | I² (%) |
|------------------|-----------------------------|-----------------------------|-------------------------------|-------|
| **Allele contrast (T vs. C)** | 1.05 (1.02–1.07), <0.001 | 1.08 (1.03–1.13), <0.001 | <0.001 | 63 |
| Dominant (TT + CT vs. CC) | 1.06 (1.02–1.10), <0.001 | 1.10 (1.03–1.18), <0.001 | <0.001 | 75 |
| Recessive (CC + CT vs. TT) | 1.00 (1.03–1.09), 0.005 | 1.05 (1.01–1.10), 0.01 | 0.001 | 55 |
| **Ethnicity** | | | | |
| **Asian (37 studies)** | | | | |
| Allele contrast (T vs. C) | 1.06 (1.02–1.11), <0.001 | 1.11 (1.02–1.21), 0.01 | <0.001 | 75 |
| Dominant (TT + CT vs. CC) | 1.07 (1.01–1.12), 0.000 | 1.10 (1.00–1.20), 0.04 | <0.001 | 64 |
| **Caucasian (31 studies)** | | | | |
| Allele contrast (T vs. C) | 1.00 (1.00–1.02), 0.05 | 1.04 (1.00–1.08), 0.05 | 0.56 | 0 |
| Dominant (TT + CT vs. CC) | 1.00 (1.00–1.02), 0.05 | 1.04 (1.00–1.08), 0.05 | 0.56 | 0 |
| **Others (7 studies)** | | | | |
| Allele contrast (T vs. C) | 1.10 (1.09–1.12), 0.01 | 1.12 (0.99–1.14), 0.19 | 0.003 | 43 |
| Dominant (TT + CT vs. CC) | 1.10 (1.09–1.12), 0.01 | 1.12 (0.99–1.14), 0.19 | 0.003 | 43 |
| **Hospital based (34 studies)** | | | | |
| Allele contrast (T vs. C) | 1.07 (1.03–1.14), <0.001 | 1.14 (1.05–1.23), <0.001 | <0.001 | 73 |
| Dominant (TT + CT vs. CC) | 1.08 (1.03–1.14), 0.001 | 1.14 (1.04–1.26), 0.004 | <0.001 | 65 |
| Co-dominant (CT vs. CC) | 1.15 (1.06–1.25), <0.001 | 1.27 (1.08–1.50), 0.003 | <0.001 | 69 |
| Recessive (CC + CT vs. TT) | 1.06 (1.01–1.12), 0.02 | 1.10 (1.01–1.20), 0.02 | <0.001 | 54 |
| Allele contrast (T vs. C) | 1.12 (1.04–1.21), 0.003 | 1.21 (1.04–1.40), 0.01 | <0.001 | 65 |
| Dominant (TT + CT vs. CC) | 1.12 (1.04–1.21), 0.003 | 1.21 (1.04–1.40), 0.01 | <0.001 | 65 |
| **Population based (32 studies)** | | | | |
| Allele contrast (T vs. C) | 1.04 (1.09–1.19), 0.01 | 1.07 (0.96–1.19), 0.18 | 0.002 | 47 |
| Dominant (TT + CT vs. CC) | 1.04 (1.09–1.19), 0.01 | 1.07 (0.96–1.19), 0.18 | 0.002 | 47 |
| **Menopausal status** | | | | |
| Allele contrast (T vs. C) | 1.01 (0.90–1.12), 0.84 | 1.01 (0.98–1.12), 0.84 | 0.08 | 0 |
| Dominant (TT + CT vs. CC) | 1.00 (0.87–1.17), 0.90 | 1.00 (0.86–1.16), 0.92 | 0.45 | 0 |
| Co-dominant (CT vs. CC) | 1.00 (0.88–1.28), 0.89 | 1.01 (0.88–1.28), 0.89 | 0.63 | 0 |
| Recessive (CC + CT vs. TT) | 1.02 (0.82–1.27), 0.83 | 1.02 (0.81–1.27), 0.84 | 0.50 | 0 |
| **Pre-menopausal (9 studies)** | | | | |
| Allele contrast (T vs. C) | 1.03 (0.95–1.12), 0.40 | 1.05 (0.92–1.20), 0.39 | 0.03 | 51 |
| Dominant (TT + CT vs. CC) | 1.05 (0.95–1.12), 0.40 | 1.05 (0.92–1.20), 0.39 | 0.03 | 51 |
| Co-dominant (CT vs. CC) | 1.10 (0.98–1.23), 0.10 | 1.10 (0.98–1.23), 0.10 | 0.62 | 0 |
| Recessive (CC + CT vs. TT) | 0.96 (0.83–1.13), 0.68 | 0.99 (0.75–1.30), 0.96 | 0.04 | 48 |
Fig. 2. Random effect Forest plot of allele contrast model (T vs. C) of MTHFR C677T polymorphism.
Fig. 3. Random effect Forest plot of homozygote model (TT vs. CC) of MTHFR C677T polymorphism.
A true heterogeneity existed between studies for allele contrast ($P_{\text{heterogeneity}} = 0.001, Q = 203.99, I^2 = 63\%, t^2 = 0.019, z = 3.73$), homozygote ($P_{\text{heterogeneity}} = 0.001, Q = 186.33, I^2 = 60\%, t^2 = 0.079, z = 3.24$), dominant ($P_{\text{heterogeneity}} = 0.001, Q = 147.7, I^2 = 48\%, t^2 = 0.019, z = 3.29$) and recessive ($P_{\text{heterogeneity}} = 0.001, Q = 163.7, I^2 = 55\%, t^2 = 0.054, z = 2.83$) comparisons.

3.3. Sensitivity analysis

Sensitivity analysis was performed by eliminating studies with small sample size ($<100$) and control population deviating from HWE. Control population of eleven studies was not in HWE (López-Cortés et al., 2015; He et al., 2014; Huang et al., 2014; Jiang-hua et al., 2014; Weiwei et al., 2014; Wu et al., 2012; Hosseini et al., 2011; Hua et al., 2011; Kan et al., 2007; Lissowska et al., 2007; Stevens et al., 2007) and heterogeneity was decreased after exclusion of these studies ($I^2 = 52\%; p = 0.001$). Sample size of seventeen studies was less than 100 (Ozen et al., 2013; Diakite et al., 2012; Wu et al., 2012; Batschauer et al., 2011; Hua et al., 2011; Wu et al., 2010; Jin et al., 2009; Li and Chen, 2009; Yuan et al., 2009; Mir et al., 2008; Hekim et al., 2007; Macis et al., 2007; Reljic et al., 2007; Kalyankumar and Jamil, 2006; Kalemí et al., 2005; Le Marchand et al., 2004; Sharp et al., 2002) and after exclusion of these studies heterogeneity was slightly decreased ($I^2 = 61\%; p = 0.002$).

3.4. Subgroup analysis

Out of 75 studies included in the present meta-analysis, 37 studies were carried out on Asian population, and 31 studies were carried out on Caucasian population and other studies were carried on other ethnic group and we grouped those studies in mixed population subgroup (7 studies). The subgroup analysis by ethnicity revealed significant association between $MTHFR$ C677T polymorphism and BC in Asian population (T vs. C: OR = 1.11; 95% CI = 1.02–1.21; p = 0.01; $I^2 = 75\%$; $P_{\text{heterogeneity}} = 0.001$; CT vs. CC: OR = 1.04; 95% CI = 0.99–1.10; p = 0.08; $I^2 = 43\%$; $P_{\text{heterogeneity}} = 0.003$; TT vs. CC: OR = 1.26; 95% CI = 1.06–1.51; p = 0.009; $I^2 = 71\%$; $P_{\text{heterogeneity}} = 0.001$; TT + CT vs. CC: OR = 1.10; 95% CI = 1.00–1.20; p = 0.04; $I^2 = 64\%$; $P_{\text{heterogeneity}} = 0.001$; TT vs. CT + CC: OR = 1.21; 95% CI = 1.04–1.40; p = 0.01; $I^2 = 65\%$; $P_{\text{heterogeneity}} = 0.001$) (Table 2). In Caucasian subgroup analysis, heterogeneity was low and except allele contrast model, significant association was not found between C677T polymorphism and BC risk (T vs. C: OR = 1.03; 95% CI = 1.00–1.06; p = 0.02; $I^2 = 32\%$; $P_{\text{heterogeneity}} = 0.04$; CT vs. CC: OR = 1.03; 95% CI = 0.99–1.12; p = 0.09; $I^2 = 0\%$; $P_{\text{heterogeneity}} = 0.007$; TT vs. CC: OR = 1.06; 95% CI = 0.99–1.14; p = 0.05; $I^2 = 43\%$; $P_{\text{heterogeneity}} = 0.007$; TT + CT vs. CC: OR = 1.04; 95% CI = 1.00–1.08; p = 0.05; $I^2 = 0\%$; $P_{\text{heterogeneity}} = 0.56$; TT vs. CT + CC: OR = 1.05; 95% CI = 0.99–1.12; p = 0.09; $I^2 = 47\%$; $P_{\text{heterogeneity}} = 0.002$). In mixed subgroup analysis, significant association was found in allele contrast, co-dominant and dominant models (T vs. C: OR = 1.10; 95% CI = 0.99–1.21; p = 0.05; $I^2 = 41\%$; $P_{\text{heterogeneity}} = 0.11$; CT vs. CC: OR = 1.24; 95% CI = 1.0–1.55; p = 0.04; $I^2 = 55\%$; $P_{\text{heterogeneity}} = 0.03$; TT vs. CC: OR = 1.14; 95% CI = 0.91–1.42; p = 0.23; $I^2 = 2\%$; $P_{\text{heterogeneity}} = 0.40$; TT + CT vs. CC: OR = 1.23; 95% CI = 0.99–1.53; p = 0.05; $I^2 = 57\%$; $P_{\text{heterogeneity}} = 0.03$; TT vs. CT + CC: OR = 1.03; 95% CI = 0.84–1.26; p = 0.74; $I^2 = 0\%$; $P_{\text{heterogeneity}} = 0.77$) (Table 2; Figs. 2, 3).

Sub-group analysis based on menstrual status i.e. premenopausal and postmenopausal was performed. Out of 75 included studies, in 9 studies BC cases was from premenopausal group and in other 9 studies BC cases was from postmenopausal group. In remaining 57 studies menstrual status was not mentioned. In both the group, pre and postmenopausal groups no significant association was observed using all five genetic models.

Sub-group analysis based on source of control population i.e. hospital based or population based was also performed. Out of 75 included studies.
studies, control population in 34 studies was hospital based and in 32 studies control population was from population and in 9 studies source of controls was not mentioned. In hospital based control group studies, (number of studies = 34; 12,515/13,560 cases/controls), allele contrast meta-analysis showed significant association (OR_{T>C} = 1.14; 95% CI = 1.05–1.23; p < 0.001). In population based control group studies, (number of studies = 32; 2916/4300 cases/controls), allele contrast meta-analysis did not show significant association (OR_{T>C} = 1.03; 95% CI = 0.98–1.09; p = 0.15).

3.5. Publication bias

Funnel plots and Egger's test were performed to estimate the risk of publication bias. Except allele contrast and homozygote model, publication bias was absent (T vs. C: PBegg's test = 0.03, P_Egger's test = 0.03; C vs. CC: PBegg's test = 0.41, P_Egger's test = 0.29; TT vs. CC: PBegg's test = 0.10, P_Egger's test = 0.03; Dominant model TT + CT vs. CC: PBegg's test = 0.27, P_Egger's test = 0.06; Recessive model TT vs. CT + CC: PBegg's test = 0.18, P_Egger's test = 0.05) (Fig. 4).

4. Discussion

Present meta-analysis investigated association of the MTHFR C677T polymorphism with BC risk (31,315 patients and 35,608 controls from 75 case–control studies). Results of meta-analysis suggested moderate significant genetic association between the MTHFR C677T polymorphism and BC. This result is in line with that of eight other previously published meta-analyses that had included fewer case control studies of the MTHFR C677T polymorphism and BC (Li et al., 2014; Liang et al., 2014; Rai, 2014; Yu and Chen, 2012; Qi et al., 2011; Zhang et al., 2010; Macis et al., 2007; Zintzaras, 2006). This is the largest meta-analysis carried out so far to investigate the association between MTHFR and BC.

In subgroup analysis based of ethnicity, we find significant association between C677T polymorphism and BC risk in Asian population. These discrepancies in the results could be arise because of the multitude of the factors such as the differences in the allele frequencies due to ethnic variations, nutritional status especially folate intake and sample size studied etc. Frequency of C677T polymorphism varies in different ethnic populations. Recently, Yadav et al. (2014) reported that T allele

### Table 3

A comparative analysis of details of odds ratio, 95% CI, genetic models reported in total 11 (including present) meta-analysis published so far analyzing case–control studies of MTHFR C677T polymorphism and breast cancer.

| SN | Author               | No. of studies | Sample size       | OR  | 95% confidence interval | Model       | I²  |
|----|----------------------|----------------|-------------------|-----|-------------------------|-------------|-----|
| 1  | Zintzaras (2006)     | 18             | 5467              | 1.03| 0.97–1.08               | T vs. C     | 34  |
|    |                      |                | 7336              | 1.07| 0.95–1.20               | TT vs. CC   | 36  |
|    |                      |                | 12,803            | 1.06| 0.95–1.19               | TT vs. CT + CC| 33  |
|    |                      |                |                   | 1.02| 0.95–1.10               | TT + CT vs. CC| 14  |
| 2  | Lissowska et al. (2007) | 22            | 8330              | 1.01| 0.95–1.08               | CT vs. CC   | NA  |
|    |                      |                | 10,825            | 0.99| 0.86–1.15               | TT vs. CC   | NA  |
|    |                      |                | 19,155            | 1.01| 0.87–1.18               | TT vs. CT + CC| NA  |
|    |                      |                |                   | 1.04| 0.97–1.11               | TT + CT vs. CC| NA  |
| 3  | Macis et al. (2007)  | 18             | 15,260            | 1.04| 1.00–1.07               | T vs. C     | NA  |
|    |                      |                | 20,411            | 1.13| 1.01–1.25               | TT vs. CC   | NA  |
|    |                      |                | 35,671            | 1.03| 0.99–1.07               | TT + CT vs. CC| NA  |
|    |                      |                |                   | 1.11| 1.01–1.23               | TT vs. CT + CC| NA  |
| 4  | Qi et al. (2011)     | 41             | 16,480            | 0.93| 0.88–0.98               | T vs. C     | NA  |
|    |                      |                | 22,388            | 0.96| 0.92–1.01               | CT vs. CC   | NA  |
|    |                      |                | 38,868            | 0.87| 0.78–0.95               | TT vs. CC   | NA  |
|    |                      |                |                   | 0.89| 0.82–0.97               | TT + CT vs. CC| NA  |
|    |                      |                |                   | 0.88| 0.80–0.96               | TT + CT vs. CC| NA  |
|    |                      |                |                   | 0.94| 0.89–0.99               | TT vs. CT + CC| NA  |
| 5  | Zhang et al. (2010)  | 37             | 15,260            | 1.12| 1.02–1.23               | T vs. C     | NA  |
|    |                      |                | 20,411            | 1.04| 1.00–1.09               | TT vs. CC   | NA  |
|    |                      |                | 35,671            | 1.09| 0.99–1.20               | TT + CT vs. CC| NA  |
|    |                      |                |                   | 1.04| 1.00–1.09               | TT vs. CT + CC| NA  |
| 6  | Yu and Chen (2012)   | 51             | 20,907            | 0.93| 0.88–0.98               | T vs. C     | NA  |
|    |                      |                | 23,905            | 0.96| 0.92–1.01               | CT vs. CC   | NA  |
|    |                      |                | 44,812            | 0.87| 0.78–0.95               | TT vs. CC   | NA  |
|    |                      |                |                   | 0.89| 0.82–0.97               | TT + CT vs. CC| NA  |
|    |                      |                |                   | 0.88| 0.80–0.96               | TT + CT vs. CC| NA  |
|    |                      |                |                   | 0.94| 0.89–0.99               | TT vs. CT + CC| NA  |
|    |                      |                |                   | 1.12| 1.11–1.70               | TT + CT + CC| NA  |
|    |                      |                |                   | 1.37| 1.11–1.70               | TT vs. CC   | NA  |
|    |                      |                |                   | 1.35| 1.10–1.67               | TT vs. CT + CC| NA  |
| 7  | Liang et al. (2014)  | 13             | 3273              | 0.94| 0.89–0.98               | T vs. C     | NA  |
|    |                      |                | 4419              | 0.98| 0.96–1.00               | CT vs. CC   | NA  |
|    |                      |                | 7692              | 0.98| 0.96–0.99               | TT vs. CC   | NA  |
|    |                      |                |                   | 0.98| 0.96–1.00               | TT + CT vs. CC| NA  |
|    |                      |                |                   | 0.95| 0.92–0.99               | TT + CT + CC| NA  |
|    |                      |                |                   | 0.99| 0.98–0.99               | TT vs. CT + CC| NA  |
| 8  | Li et al. (2014)     | 57             | 25,877            | 1.23| 1.13–1.37               | T vs. C     | 77.3|
|    |                      |                | 27,981            | 1.03| 0.97–1.10               | TT vs. CC   | 33.7|
|    |                      |                | 55,658            | 1.03| 0.97–1.10               | TT vs. CC   | 58.2|
|    |                      |                |                   | 1.38| 1.16–1.63               | TT vs. CC   | 51.5|
|    |                      |                |                   | 1.12| 1.01–1.23               | TT + CT vs. CC| 50.3|
|    |                      |                |                   | 1.33| 1.15–1.43               | TT vs. CT + CC| 29.5|
|    |                      |                |                   | 1.05| 1.03–1.13               | T vs. C     | 63  |
|    |                      |                |                   | 1.05| 1.01–1.08               | CT vs. CC   | 29  |
|    |                      |                |                   | 1.17| 1.06–1.28               | TT vs. CC   | 60  |
|    |                      |                |                   | 1.06| 1.02–1.09               | TT + CT vs. CC| 48  |
|    |                      |                |                   | 1.12| 1.03–1.22               | TT vs. CT + CC| 55  |

NA = not given in paper.
and TT genotype frequencies in Asian population (37.2% and 16.9%) are higher in comparison to Caucasian populations (33.6% of T allele and 12.1% of TT genotype).

MTHFR enzyme function may influence cancer risk in two ways. The substrate of MTHFR enzyme, 5,10-methylenetetrahydrofolate, is involved in the conversion of deoxyuridyldate monophosphate to deoxothymidylate monophosphate, and low levels of 5,10-methylenetetrahydrofolate would lead to an increased deoxyuridyldate monophosphate/deoxothymidylate monophosphate ratio. In this situation, increased incorporation of uracil into DNA in place of thymin may follow, resulting in an increased chance of point mutations and DNA/chromosome breakage (Sohn et al., 2009; Boccia et al., 2008; Blount et al., 1997). The second way by which dysfunctional MTHFR increases risk of cancer is determined by the level of SAM, which is necessary for maintenance of the methylation patterns in DNA. Altered methylation pattern may modify DNA conformation and gene expression. A less active form of MTHFR leads to lower SAM levels and consequently to hypomethylation and increase the risk of cancers (Boccia et al., 2008; Stern et al., 2000; Duthie, 1999).

The role of folate in breast cancer has been investigated in several dietary studies and most have shown folate consumption to be inversely related to breast cancer risk (Zhang et al., 1999; Rohan et al., 2000; Goodman et al., 2001; Xu et al., 2007) and adequate folate intake has been associated with a substantially decreased risk of cancer. Cancer risk modification conferred by C677T polymorphism is further modified by the status of folate and nutrients involved in one-carbon and folate metabolism (Ueland et al., 2001; Robien and Ulrich, 2003; Sharp and Little, 2004). We did not done sub group analysis on the basis of folate concentrations. In total 75 included studies, folate intake information was reported only in 12 studies, out of which few authors reported folate uptake dose and others reported blood level of folate. With increased folic acid fortification in the Caucasian population, the general intake of folate may be higher than that from the Asian population, whose folate intake is primarily obtained from unfortified diets. Further, in Asian population malnutrition, low folate intake and impaired folate absorption due to infectious diseases were already reported (Rosenberg et al., 2002; Wilcken et al., 2003). Folate supplementation would outweigh the negative effects of C677T polymorphism. Hence the effect of MTHFR on breast cancer risk in a particular population may depend on the intake level of folate food in that population.

Meta-analysis is a powerful tool for analyzing cumulative data of studies where the individual sample sizes are small and the statistical power low (Yadav et al., 2015; Rai et al., 2014). Several meta-analyses were published to assess the role of MTHFR polymorphism in cancer development like: lung cancer (Boccia et al., 2009), pancreatic cancer (Tu et al., 2012), prostate cancer (Zhang et al., 2012), esophageal cancer (Wen et al., 2013), ovarian cancer (Ding et al., 2012) and cervical cancer (Mei et al., 2012).

We identified ten meta-analyses (Singh et al., 2015; Liang et al., 2014; Li et al., 2014; Rai, 2014; Yu and Chen, 2012; Qi et al., 2011; Zhang et al., 2010; Lissowska et al., 2007; Macis et al., 2007; Zintzaras, 2006) identified concerning similar topic as we did during the literature search. A comparative details of all the meta-analysis published so far (including present) were presented in Table 3. Zintzaras (2006) carried out first meta-analysis of MTHFR C677T genotype of 18 studies and reported significant heterogeneity (p = 0.08, I² = 34%) and non-significant association (OR = 1.02; 95% confidence interval (0.95–1.01) in allele contrast model. Lissowska et al. (2007) carried out meta-analysis of 22 studies and showed no association between TT (mutant homozygote) vs. CC genotypes and breast cancer risk (OR = 0.99; 95% CI = 0.86–1.15), based on 8330 cases and 10,825 controls. Macis et al. (2007) performed a meta-analysis of 18 studies examining the association between polymorphisms C677T and BC risk and found positive association between the TT genotype BC risk. A meta-analysis of 41 retrospective studies (16,480 cases and 22,388 controls) was carried out by Qi et al. (2011) and reported significant elevated breast cancer risk using all five genetic model (TT vs. CC: OR = 1.13, 95% CI = 1.01–1.25). Zhang et al. (2010) reported significant association between 677T polymorphism with BC (TT vs. CC: OR = 1.11, 95% CI = 1.01–1.23 and suggested MTHFR T allele as a low-penetrant risk factor for developing breast cancer. Yu and Chen (2012) carried out meta-analysis of 51 studies including 20,907 cases and 23,905 controls and reported significant associations between MTHFR C677T polymorphism and BC risk. Liang et al. (2014), Li et al. (2014); Rai (2014) and Singh et al. (2015) conducted meta-analyses on 37 studies (15,260 cases and 20,411 controls), 57 studies (25,877 breast cancer cases and 29,781 controls), 36 studies (8040 cases and 10,008 controls) and 41 studies (16,480 cases and 22,388 controls), and 61 studies (28,031 Cases and 31,880 Controls), respectively, and except Singh et al. (2015), all were reported significant association between C677T polymorphism and BC risk. Compared with present meta-analysis, most of these meta-analyses included less number of studies and smaller total sample was analyzed.

Presence of higher heterogeneity showed that there were significant differences between individual studies. Hence, sensitivity and subgroup analyses were performed to explore the causes of heterogeneity. Sensitivity analysis showed that even after excluding studies with a small number of cases (n < 100), or having controls violating the HWE, the heterogeneity decreased slightly. However, the larger sample size does not mean the study is without limitations. The current meta-analysis has few limitations also like - (i) only published studies were included, thus possibility of publication bias cannot be excluded, (ii) single gene polymorphism of folate metabolic pathway was considered, and (iii) finally, due to lack of data, gene–gene and gene–environment interactions could not be included.

We hope that this meta-analysis of the most comprehensive literature addressing the association is yielded convincing evidence to determine the role of MTHFR C677T polymorphism in BC risk. In summary, results of present meta-analysis showed modest association between MTHFR C677T polymorphism with breast cancer in total studies. However, subgroup analysis results based on ethnicity showed strong significant association between TT genotype and breast cancer (TT vs. CC; OR* = 1.26; 95% CI: 1.06–1.51; p = 0.009) in Asian population but in Caucasian population such association was not observed (TT vs. CC; OR* = 1.08; 95% CI: 0.99–1.14; p = 0.05). However, presence of publication bias and higher between study heterogeneity suggested that results should be interpreted cautiously and also indicated that the observed association may differ in strength between populations, or may not exist at all in some populations.

Conflict of interest

None.

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