Meta Gene 6 (2015) 72–84

Contents lists available at ScienceDirect

Meta Gene

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

Pradeep Kumar, Upendra Yadav, Vandana Rai *

Human Molecular Genetics Laboratory, Department of Biotechnology, VBS Purvanchal University, Jaunpur 222 003, UP, India

ARTICLE INFO

Article history:
Received 9 May 2015
Revised 21 August 2015
Accepted 31 August 2015
Available online 1 October 2015

Keywords:
MTHFR
Folic acid
Breast cancer
Meta-analysis
Polymorphism

ABSTRACT

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 = 1.01–1.08, 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).

Contents

* Corresponding author at: Human Molecular Genetics Laboratory, Department of Biotechnology, VBS Purvanchal University, Jaunpur 222 003, India.
E-mail address: raivandana@rediffmail.com (V. Rai).

http://dx.doi.org/10.1016/j.mgene.2015.08.008

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 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-methylenetetrahydrofolate (10-MTHF) to 5-methylenetetrahydrofolate (5-MTHF), which is a cofactor for the remethylation of homocysteine to convert it to S-adenosyl methionine (SAM). SAM is 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; Gilbody 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 enzyme (Ala222Val) (Frosst et al., 1995). This change leads to reduced enzyme activity, and individuals heterozygous (677CT) or homozygous (677TT) for this variant had 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., 1988). The genotype frequencies of the polymorphism are CC, 0.583; CT, 0.35; TT, 0.067 in Europeans and CC, 0.267; CT, 0.444; TT, 0.289 in Asians (www.hapmap.org).

MTHFR gene T allele has been widely studied as a possible low-penetrance susceptibility allele for a variety of cancers, and in particular, BC. Several studies reported significant association between C677T polymorphism and BC risk (Kakkoura et al., 2015; Lu et al., 2015; He et al., 2014; Weiwei 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 reevaluate 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 with I^2. I^2 ranges from 0% to 100 (Huedo-Medina et al., 2006; Higgins and Thompson, 2002). When low heterogeneity ($I^2 < 50\%$) was observed, then overall OR was estimated under the fixed-effects model (Mantel and Haenszel, 1959), otherwise ($I^2 \geq 50\%$) under the random-effects model (DerSimonian and Laird, 1986).

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 x^2 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 was defined as low quality, and studies with score ≥ 7 was 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; Suzuki 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; Kalemi 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; Sharp et al., 2002). One author (eLe Marchand et al., 2004) investigated fiv 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.

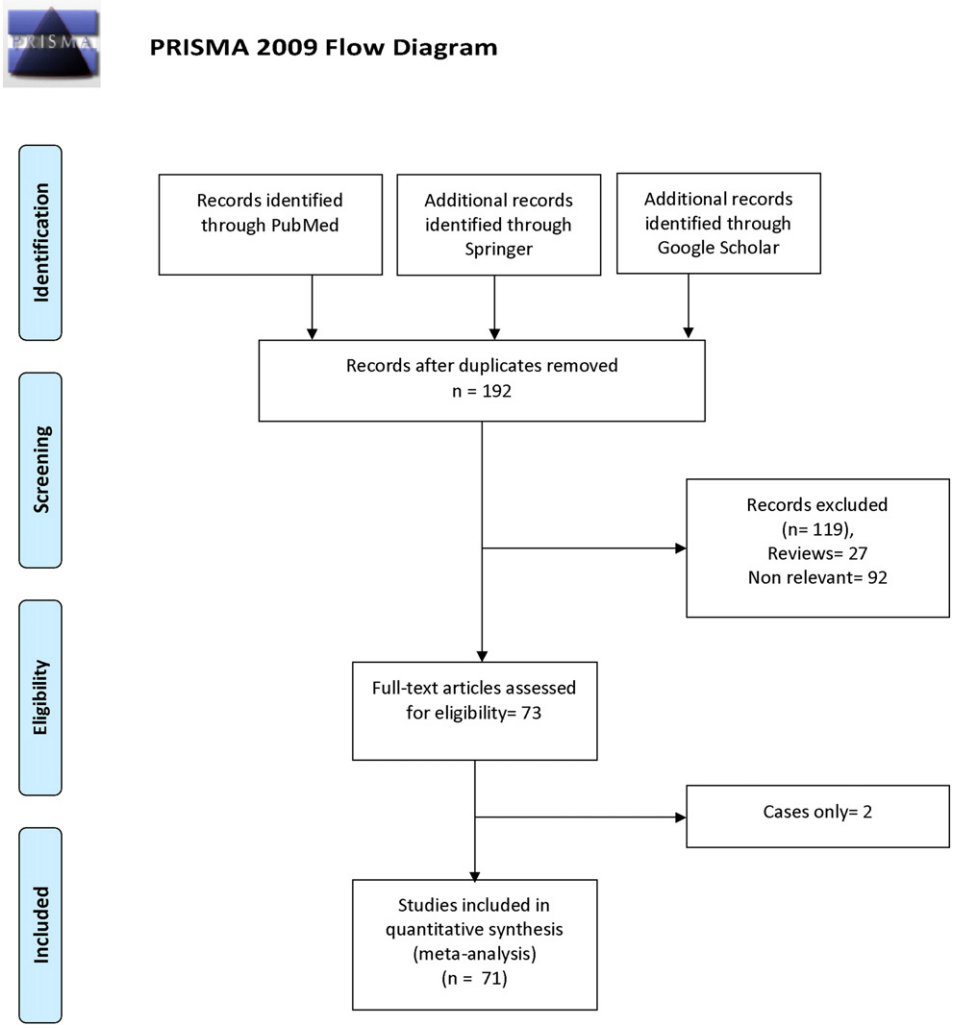

Fig. 1. Flow diagram of study search and selection process.

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	494/495	PB	PCR-RFLP	0.33	7
Ergul et al. (2003)	Turkey	Caucasian	118/193	HB	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	186/147	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)	Hawaiian	Caucasian	1189/2414	PB	TaqMan	0.75	8.5
Qi et al. (2004)	China	Asian	217/218	PB	PCR-RFLP	0.59	4.5
Chen et al. (2005)	USA	Caucasian	1063/1104	PB	PCR-RFLP	0.68	9.5
Kalemi 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	557/633	PB	MALDI-TOF	0.19	8
Chou et al. (2006)	China	Asian	142/285	HB	PCR-RFLP	0.47	7
Kalyankumar and Jamil (2006)	India	Asian	88/95	HB	PCR-RFLP	0.69	6.5
Xu et al. (2007)	USA	Caucasian	1063/1104	PB	PCR-RFLP	0.68	8.5
Hekim et al. (2007)	Turkey	Caucasian	40/68	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	46/80	PB	TaqMan	0.51	4
Reljic et al. (2007)	Croatia	Caucasian	93/65	PB	PCR-RFLP	0.11	6
Stevens et al. (2007)	USA	Others	494/494	PB	TaqMan	0.01	7
Yu et al. (2007)	Taiwan	Asian	119/420	PB	PCR-RFLP	0.33	7.5
Inoue et al. (2008)	Singapore	Asian	380/662	PB	TaqMan	0.17	9
Kotsopoulos et al. (2008)	Canada	Caucasian	944/680	HB	Mass-array system	0.08	7.5
Suzuki et al. (2008)	Japan	Asian	454/909	HB	TaqMan	0.52	9.5
Cheng et al. (2008)	Taiwan	Asian	349/530	HB	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	HB	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
Platek et al. (2009)	USA	Caucasian	994/1802	PB	TaqMan	0.39	9
Hennquez-Hernandez et al. (2009)	Spain	Caucasian	135/292	PB	PCR-RFLP	0.82	7
Cam et al. (2009)	Turkey	Caucasian	110/95	NR*	PCR-RFLP	0.39	4.5
Maruti et al. (2009)	USA	Caucasian	318/647	PB	ASPE	0.67	8.5
Ma et al. (2009)	Brazil	Others	458/458	HB	NR*	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
Alshatwi (2010)	Arab	Asian	100/100	HB	TaqMan	0.80	6.5
Sangrajrang et al. (2010)	Indian	Asian	563/487	HB	TaqMan	0.42	9
Weiner et al. (2010)	Russia	Asian	837/778	PB	PCR-RFLP	0.80	8
Wu et al. (2010)	China	Asian	80/80	HB	PCR-RFLP	0.51	5.5
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
Hosseini et al. (2011)	Iran	Asian	294/300	HB	PCR-RFLP	<0.0001	3
Hua et al. (2011)	China	Asian	95/90	PB	PCR-RFLP	0.02	6.5
Nausad et al. (2011)	India	Asian	244/244	HB	PCR-RFLP	0.17	7
Prasad et al. (2011)	India	Asian	130/125	PB	PCR-RFLP	0.06	6
Akram et al. (2012)	Pakistan	Asian	110/110	HB	PCR-RFLP	0.85	5
Barbosa et al. (2012)	Mixed, Caucasian	Caucasian	176/176	HB	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	4778/3350	PB	TaqMan	0.15	7
Lajin et al. (2012)	Syria	Asian	119/126	HB	PCR-RFLP	0.35	6.5
Wu et al. (2012)	China	Asian	32/37	NR*	PCR-RFLP	0.03	6
Liu et al. (2013)	China	Asian	435/435	HB	PCR-RFLP	0.57	6
Ozen et al. (2013)	Turkey	Caucasian	51/106	NR*	Strip-assay	0.08	4
He et al. (2014)	China	Asian	310/381	HB	Sequenom	<0.0001	6
Huang et al. (2014)	Taiwan	Asian	1232/1232	HB	PCR-RFLP	0.01	8
Jiang-hua et al. (2014)	China	Asian	535/673	HB	PCR-RFLP	<0.0001	7
Wang et al. (2014)	China	Asian	435/435	HB	Sequenom	0.22	6
Weiwei et al. (2014)	China	Asian	297/306	HB	Sequenom	0.00	8
Kakkoura et al. (2015)	Cyprus	Caucasian	1065/1157	PB	TaqMan	0.09	9.5
Lopez-Cortes et al. (2015)	Ecuador	Others	114/195	HB	PCR-RFLP	0.00	7
Lu et al. (2015)	China	Asian	560/560	HB	TaqMan	0.27	9
Singh et al. (2015)	India	Asian	588/508	HB	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 (TTvs.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 = 1.02–1.09; p = <0.001) and random (OR°=°1.08; 95%CI = 1.03–1.14; p = <0.001) effect models. Similarly the recessive genotypes model (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 I^2 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)	I^2 (%)
All (75 studies)	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 (1.02–1.09), <0.001	1.08 (1.03–1.14), 0.002	<0.001	48
	Homozygote (TT vs. CC)	1.10 (1.04–1.16), <0.001	1.17 (1.06–1.28), 0.001	<0.001	60
	Co-dominant (CT vs. CC)	1.05 (1.01–1.08), 0.005	1.05 (1.01–1.10), 0.01	0.01	29
	Recessive (CC + CT vs. TT)	1.07 (1.02–1.13), 0.002	1.12 (1.03–1.22), 0.005	<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), 0.009	1.10 (1.00–1.20), 0.04	<0.001	64
	Homozygote (TT vs. CC)	1.15 (1.06–1.25), <0.001	1.26 (1.06–1.51), 0.009	<0.001	71
	Co-dominant (CT vs. CC)	1.04 (0.99–1.10), 0.08	1.05 (0.97–1.14), 0.19	0.003	43
	Recessive (CC + CT vs. TT)	1.12 (1.04–1.21), 0.003	1.21 (1.04–1.40), 0.01	<0.001	65
Caucasian (31 studies)	Allele contrast (T vs. C)	1.03 (1.00–1.06), 0.02	1.04 (1.00–1.09), 0.04	0.04	32
	Dominant (TT + CT vs. CC)	1.04 (1.00–1.08), 0.05	1.04 (1.00–1.08), 0.05	0.56	0
	Homozygote (TT vs. CC)	1.06 (0.99–1.14), 0.05	1.08 (0.97–1.21), 0.12	0.007	43
	Co-dominant (CT vs. CC)	1.03 (0.99–1.08), 0.12	1.03 (0.99–1.08), 0.12	0.79	0
	Recessive (CC + CT vs. TT)	1.05 (0.99–1.12), 0.09	1.07 (0.96–1.19), 0.18	0.002	47
Others (7 studies)	Allele contrast (T vs. C)	1.10 (0.99–1.21), 0.05	1.12 (0.97–1.28), 0.09	0.11	41
	Dominant (TT + CT vs. CC)	1.17 (1.02–1.33), 0.01	1.23 (0.99–1.53), 0.05	0.03	57
	Homozygote (TT vs. CC)	1.14 (0.91–1.42), 0.23	1.14 (0.91–1.44), 0.22	0.40	2
	Co-dominant (CT vs. CC)	1.19 (1.03–1.36), 0.01	1.24 (1.00–1.55), 0.04	0.03	55
	Recessive (CC + CT vs. TT)	1.03 (0.84–1.26), 0.74	1.03 (0.84–1.27), 0.73	0.77	0
Study design					
Hospital based (34 studies)	Allele contrast (T vs. C)	1.07 (1.03–1.12), <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
	Homozygote (TT vs. CC)	1.15 (1.06–1.25), <0.001	1.27 (1.08–1.50), 0.003	<0.001	69
	Co-dominant (CT vs. CC)	1.06 (1.01–1.12), 0.02	1.10 (1.01–1.20), 0.02	<0.001	54
	Recessive (CC + CT vs. TT)	1.12 (1.04–1.21), 0.002	1.20 (1.05–1.39), 0.008	<0.001	63
Population based (32 studies)	Allele contrast (T vs. C)	1.03 (1.00–1.06), 0.04	1.03 (0.98–1.09), 0.15	0.001	48
	Dominant (TT + CT vs. CC)	1.04 (0.99–1.08), 0.05	1.04 (0.98–1.09), 0.12	0.13	22
	Homozygote (TT vs. CC)	1.06 (0.99–1.13), 0.07	1.08 (0.96–1.21), 0.16	<0.001	50
	Co-dominant (CT vs. CC)	1.03 (0.99–1.08), 0.10	1.03 (0.99–1.08), 0.10	0.61	0
	Recessive (CC + CT vs. TT)	1.04 (0.97–1.10), 0.20	1.05 (0.95–1.16), 0.27	0.003	45
Menopausal status					
Pre-menopausal (9 studies)	Allele contrast (T vs. C)	1.01 (0.90–1.12), 0.84	1.01 (0.98–1.12), 0.84	0.68	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
	Homozygote (TT vs. CC)	1.01 (0.80–1.28), 0.89	1.01 (0.80–1.28), 0.89	0.63	0
	Co-dominant (CT vs. CC)	1.00 (0.85–1.17), 0.99	1.01 (0.84–1.21), 0.88	0.25	20
	Recessive (CC + CT vs. TT)	1.02 (0.82–1.27), 0.83	1.02 (0.81–1.27), 0.84	0.50	0
Post-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)	1.08 (0.96–1.20), 0.16	1.09 (0.95–1.25), 0.20	0.22	24
	Homozygote (TT vs. CC)	1.01 (0.85–1.20), 0.87	1.06 (0.77–1.45), 0.71	0.01	57
	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 (0.82–1.13), 0.68	0.99 (0.75–1.30), 0.96	0.04	48

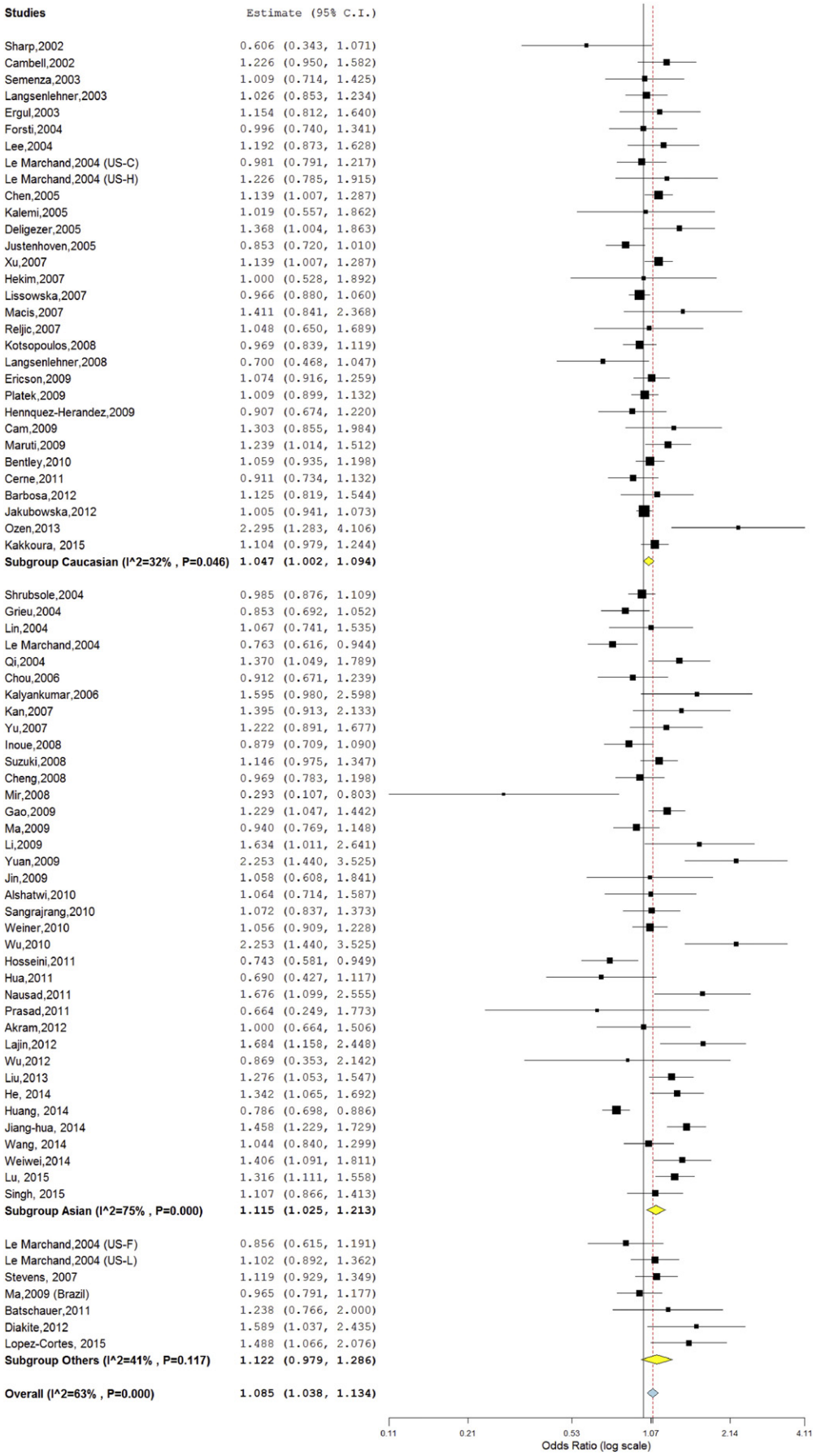

Fig. 2. Random effect Forest plot of allele contrast model (T vs. C) of MTHFR C677T polymorphism.

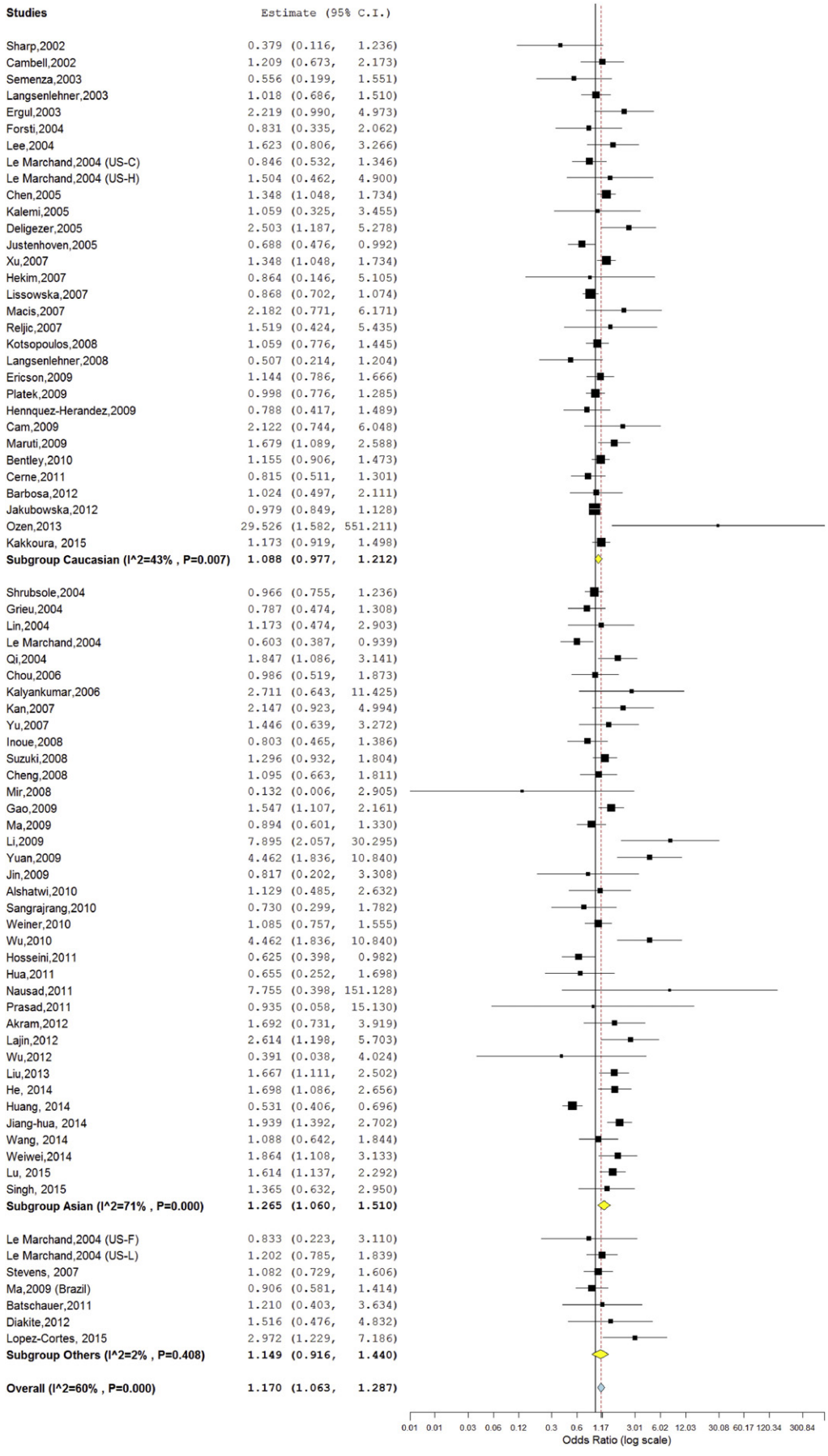

Fig. 3. Random effect Forest plot of homozygote model (TT vs. CC) of MTHFR C677T polymorphism.

A true heterogeneity existed between studies for allele contrast ($P_{heterogeneity}$ = <0.001, Q = 203.99, I^2 = 63%, t^2 = 0.019, z = 3.73), homozygote ($P_{heterogeneity}$ = <0.001, Q = 186.33, I^2 = 60%, t^2 = 0.079, z = 3.24), dominant ($P_{heterogeneity}$ = <0.001, Q = 147.7, I^2 = 48%, t^2 = 0.019, z = 3.29) and recessive ($P_{heterogeneity}$ = <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; Kalemi 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%; $P_{heterogeneity}$ = <0.001; CT vs. CC: OR = 1.04; 95% CI = 0.99–1.10; p = 0.08; I^2 = 43%; $P_{heterogeneity}$ = 0.003; TT vs. CC: OR = 1.26; 95% CI = 1.06–1.51; p = 0.009; I^2 = 71%; $P_{heterogeneity}$ = <0.001; TT + CT vs. CC: OR = 1.10; 95% CI = 1.00–1.20; p = 0.04; I^2 = 64; $P_{heterogeneity}$ = <0.001; TT vs. CT + CC: OR = 1.21; 95% CI = 1.04–1.40; p = 0.01; I^2 = 65%; $P_{heterogeneity}$ = <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%; $P_{heterogeneity}$ = 0.04; CT vs. CC: OR = 1.03; 95% CI = 0.99–1.12; p = 0.09; I^2 = 0%; $P_{heterogeneity}$ = 0.79; TT vs. CC: OR = 1.06; 95% CI = 0.99–1.14; p = 0.05; I^2 = 43%; $P_{heterogeneity}$ = 0.007; TT + CT vs. CC: OR = 1.04; 95% CI = 1.00–1.08; p = 0.05; I^2 = 0; $P_{heterogeneity}$ = 0.56; TT vs. CT + CC: OR = 1.05; 95% CI = 0.99–1.12; p = 0.09; I^2 = 47%; $P_{heterogeneity}$ = 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%; $P_{heterogeneity}$ = 0.11; CT vs. CC: OR = 1.24; 95% CI = 1.0–1.55; p = 0.04; I^2 = 55%; $P_{heterogeneity}$ = 0.03; TT vs. CC: OR = 1.14; 95% CI = 0.91–1.42; p = 0.23; I^2 = 2%; $P_{heterogeneity}$ = 0.40; TT + CT vs. CC: OR = 1.23; 95% CI = 0.99–1.53; p = 0.05; I^2 = 57%; $P_{heterogeneity}$ = 0.03; TT vs. CT + CC: OR = 1.03; 95% CI = 0.84–1.26; p = 0.74; I^2 = 0%; $P_{heterogeneity}$ = 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

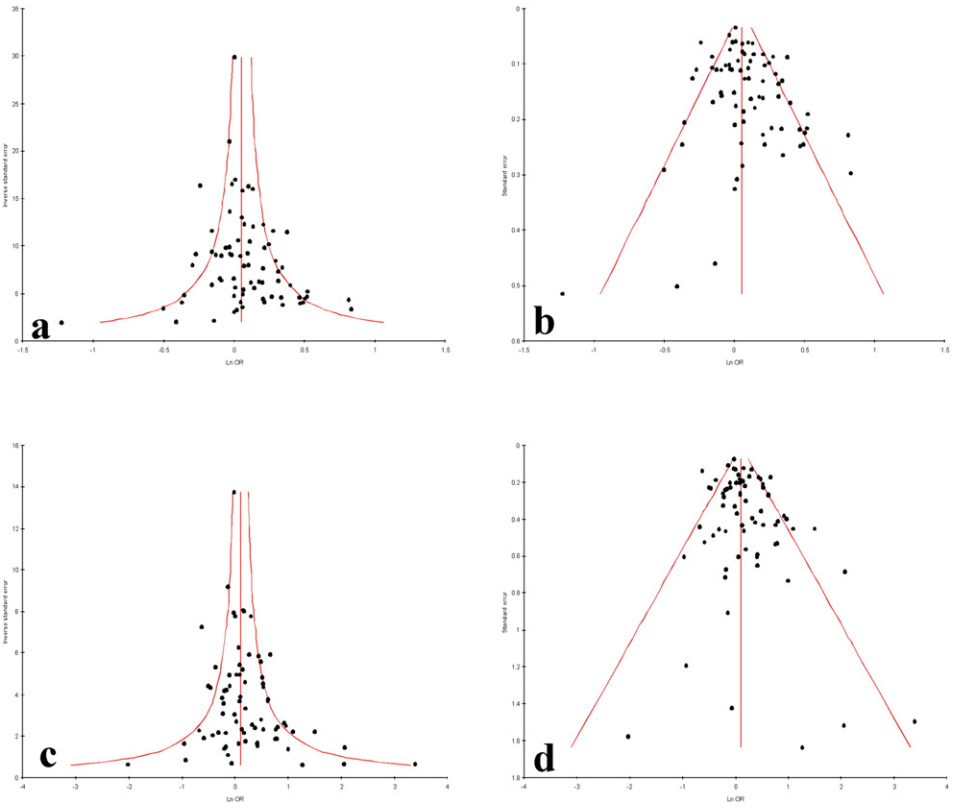

Fig. 4. Funnel plots of a. precision by OR; b. standard error by OR of MTHFR C677T allele contrast model (T vs. C); c. precision by OR; d. standard error by OR of MTHFR C677T homozygote model (TT vs. CC).

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_{TvsC} = 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_{TvsC} = 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: $P_{Begg's\ test}$ = 0.03, $P_{Egger's\ test}$ = 0.03; CT vs. CC: $P_{Begg's\ test}$ = 0.41, $P_{Egger's\ test}$ = 0.29; TT vs. CC: $P_{Begg's\ test}$ = 0.10, $P_{Egger's\ test}$ = 0.03; Dominant model TT + CT vs. CC:, $P_{Begg's\ test}$ = 0.27, $P_{Egger's\ test}$ = 0.06; Recessive model TT vs. CT + CC: $P_{Begg's\ test}$ = 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 but did not find such association in Caucasian 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 *MTHFR* 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 *MTHR* C677T polymorphism and breast cancer.

SN	Author	No. of studies	Sample size			OR	95% confidence interval	Model	I^2
			Case	Control	Total				
1	Zintzaras (2006)	18	5467	7336	12,803	1.03	0.97–1.08	T vs. C	34
						1.07	0.95–1.20	TT vs. CC	36
						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	10,825	19,155	1.01	0.95–1.08	CT vs. CC	NA
						0.99	0.86–1.15	TT vs. CC	NA
3	Macis et al. (2007)	18				1.01	0.87–1.18	TT vs. CT + CC	NA
						1.04	0.97–1.11	TT + CT vs. CC	NA
4	Qi et al. (2011)	41	16,480	22,388	38,868	1.04	1.00–1.07	T vs. C	NA
						1.13	1.01–1.25	TT vs. CC	NA
						1.03	0.99–1.07	TT + CT vs. CC	NA
						1.11	1.01–1.23	TT vs. CT + CC	NA
5	Zhang et al. (2010)	37	15,260	20,411	35,671	1.04	0.99–1.08	CT vs. CC	NA
						1.11	1.01–1.23	TT vs. CC	NA
						1.04	1.00–1.09	TT + CT vs. CC	NA
						1.09	0.99–1.20	TT vs. CT + CC	NA
6	Yu and Chen (2012)	51	20,907	23,905	44,812	0.93	0.88–0.98	T vs. C	NA
						0.96	0.92–1.01	CT vs. CC	NA
						0.87	0.78–0.95	TT vs. CC	NA
						0.89	0.82–0.97	TT vs. CT	NA
						0.88	0.80–0.96	TT + CT vs. CC	NA
						0.94	0.89–0.99	TT vs. CT + CC	NA
7	Liang et al. (2014)	13	3273	4419	7692	1.12	1.02–1.23	T vs. C	NA
						1.35	1.10–1.67	TT vs. CC	NA
						1.37	1.11–1.70	TT vs. CT + CC	NA
8	Li et al. (2014)	57	25,877	29,781	55,658	0.94	0.89–0.98	T vs. C	NA
						0.98	0.96–1.00	CT vs. CC	NA
						0.98	0.96–0.99	TT vs. CC	NA
						0.98	0.96–1.00	TT vs. CT	NA
						0.95	0.92–0.99	TT + CT vs. CC	NA
						0.99	0.98–0.99	TT vs. CT + CC	NA
9	Rai (2014)	36	8040	10,008	18,048	1.23	1.13–1.37	T vs. C	77.3
						1.03	0.97–1.10	CT vs. CC	33.7
						1.38	1.16–1.63	TT vs. CC	58.2
						1.12	1.01–1.23	TT + CT vs. CC	51.5
						1.33	1.15–1.43	TT vs. CT + CC	50.3
10	Singh et al. (2015)	61	28,031	31,880	59,911	0.97	0.93–1.00	TT + CT vs. CC	29.5
						1.05		TT vs. CT + CC	29.5
11	Present study, 2015	75	31,315	35,608	66,923	1.08	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 deoxyuridylate monophosphate to deoxythymidylate monophosphate, and low levels of 5,10-methylenetetrahydrofolate would lead to an increased deoxyuridylate monophosphate/deoxythymidylate monophosphate ratio. In this situation, increased incorporation of uracil into DNA in place of thymine 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 ($p = 0.08$, $I^2 = 34\%$) and non-significant association (OR = 1.02; 95% confidence interval (0.95–1.10) 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, 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; $p = 0.05$). 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.

Acknowledgments

The authors are highly grateful to Leon Bax (Chief Scientific Officer at BiostatXL, UMC Utrecht) for his valuable suggestions, which help us in statistical analysis. We also thank all authors of the included studies for their cooperation.

References

Akram, M., Malik, F.A., Kayani, M.A., 2012. Mutational analysis of the MTHFR gene in breast cancer patients of Pakistani population. Asian Pac. J. Cancer Prev. 13, 1599–1603.

Alshatwi, A.A., 2010. Breast cancer risk, dietary intake, and methylenetetrahydrofolate reductase (MTHFR) single nucleotide polymorphisms. Food Chem. Toxicol. 48, 1881–1885.

Barbosa Rde, C., Menezes, D.C., Freire, T.F., Sales, D.C., Alencar, V.H., 2012. Associations of polymorphisms of folate cycle enzymes and risk of breast cancer in a Brazilian population are age dependent. Mol. Biol. Rep. 39, 4899–4907.

Batschauer, A.P., Cruz, N.G., Oliveira, V.C., Coelho, F.F., Santos, I.R., 2011. HFE, MTHFR, and FGFR4 genes polymorphisms and breast cancer in Brazilian women. Mol. Cell. Biochem. 357, 247–253.

Bax, L., Yu, L.M., Ikeda, N., Tsuruta, H., Moons, K.G., 2006. Development and validation of MIX: comprehensive free software for meta-analysis of causal research data. BMC Med. Res. Methodol. 6, 50.
Begg, C.B., Mazumdar, M., 1994. Operating characteristics of a rank correlation test for publication bias. Biometrics 50, 1088–1101.
Bentley, A.R., Raiszadeh, F., Stover, P.J., Hunter, D.J., Hankinson, S.E., 2010. No association between cSHMT genotypes and the risk of breast cancer in the Nurses' Health Study. Eur. J. Clin. Nutr. 64, 108–110.
Blount, B.C., Mack, M.M., Wehr, C.M., MacGregor, J.T., Hiatt, R.A., Wang, G., et al., 1997. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc. Natl. Acad. Sci. U. S. A. 94, 3290–3295.
Boccia, S., Boffetta, P., Brennan, P., Ricciardi, G., Gianfagna, F., Matsuo, K., et al., 2009. Meta-analyses of the methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and risk of head and neck and lung cancer. Cancer Lett. 273, 55–61.
Boccia, S., Hung, R., Ricciardi, G., Gianfagn, F., Ebert, M.P.A., Fang, J.Y., et al., 2008. Meta- and pooled analyses of the methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and gastric cancer risk: a huge-GSEC review. Am. J. Epidemiol. 167 (5), 505–516.
Cam, R., Eroglu, A., Egin, Y., Akar, N., 2009. Dihydrofolate reductase (DHRF) 19-bp intron-1 deletion and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms in breast cancer. Breast Cancer Res. Treat. 115, 431–432.
Campbell, I.G., Baxter, S.W., Eccles, D.M., Choong, D.Y., 2002. Methylenetetrahydrofolate reductase polymorphism and susceptibility to breast cancer. Breast Cancer Res. 4, R14.
Cerne, J.Z., Pohar-Perme, M., Cerkovnik, P., Gersak, K., Novakovic, S., 2011. Age at menarche and menopause is not associated with two common genetic variants in the methylenetetrahydrofolate reductase (MTHFR) gene. Eur. J. Contracept. Reprod. Health Care 16 (4), 241–247.
Chen, J., Gammon, M.D., Chan, W., Palomeque, C., Wetmur, J.G., Kabat, G.C., et al., 2005. One-carbon metabolism, MTHFR polymorphisms, and risk of breast cancer. Cancer Res. 65, 1606–1614.
Cheng, C.W., Yu, J.C., Huang, C.S., Shieh, J.C., Fu, Y.P., Wang, H.W., et al., 2008. Polymorphism of cytosolic serine hydroxymethyltransferase, estrogen and breast cancer risk among Chinese women in Taiwan. Breast Cancer Res. Treat. 111, 145–155.
Chou, Y.C., Wu, M.H., Yu, J.C., Lee, M.S., Yang, T., Shih, H.L., et al., 2006. Genetic polymorphisms of the methylenetetrahydrofolate reductase gene, plasma folate levels, and breast cancer susceptibility: a case–control study in Taiwan. Carcinogenesis 27, 2295–2300.
Collaborative Group on Hormonal Factors in Breast Cancer, 1997. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52 705 women with breast cancer and 108 411 women without breast cancer. Lancet 350, 1047–1059.
Deligezer, U., Akisik, E.E., Dalay, N., 2005. Homozygosity at the C677T of the MTHFR gene is associated with increased breast cancer risk in the Turkish population. In Vivo 19, 889–893.
DerSimonian, R., Laird, N., 1986. Meta-analysis in clinical trials. Control. Clin. Trials 7, 177–188.
Diakite, B., Tazzite, A., Hamzi, K., Jouhadi, H., Nadifi, S., 2012. Methylenetetrahydrofolate reductase C677T polymorphism and breast cancer risk in Moroccan women. Afr. Health Sci. 12 (2), 204–209.
Ding, X.P., Feng, L., Ma, L., 2012. MTHFR C677T polymorphism and ovarian cancer risk: a meta-analysis. Asian Pac. J. Cancer Prev. 13, 3937–3942.
Duthie, S.J., 1999. Folic acid deficiency and cancer: mechanisms of DNA instability. Br. Med. Bull. 55, 578–592.
Egger, M., Davey, S., Schneider, G., Minder, M.C., 1997. Bias in meta-analysis detected by a simple, graphical test. BMJ 315, 629–634.
Ergul, E., Sazci, A., Utkan, Z., Canturk, N.Z., 2003. Polymorphisms in the MTHFR gene are associated with breast cancer. Tumor Biol. 24, 286–290.
Ericson, U., Sonestedt, E., Ivarsson, M.I., Gullberg, B., Carlson, J., Olsson, H., et al., 2009. Folate intake, methylenetetrahydrofolate reductase polymorphisms, and breast cancer risk in women from the Malmö Diet and Cancer cohort. Cancer Epidemiol. Biomark. Prev. 18, 1101–1110.
Ferlay, J., Bray, F., Pisani, P., Parkin, D.M., 2000. Cancer incidence, mortality and prevalence worldwide. IARC Cancer Base No. 5 [CD-ROM]. Version 1.1. IARC Press, Lyon.
Forsti, A., Angelini, S., Festa, F., Sanyal, S., Zhang, Z., Grzybowska, E., et al., 2004. Single nucleotide polymorphisms in breast cancer. Oncol. Rep. 11, 917–922.
Frankenburg, F.R., 2007. The role of one-carbon metabolism in schizophrenia and depression. Harv. Rev. Psychiatry 15 (4), 146–160.
Frosst, P., Bloom, H.J., Milos, R., Goyette, P., Sheppard, C.A., Matthews, R.G., et al., 1995. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat. Genet. 10, 111–113.
Gao, C.M., Tang, J.H., Cao, H.X., Ding, J.H., Wu, J.Z., Wang, J., et al., 2009. MTHFR polymorphisms, dietary folate intake and breast cancer risk in Chinese women. J. Hum. Genet. 5, 414–418.
Gilbody, S., Lewis, S., Lightfoot, T., 2007. Methylenetetrahydrofolate reductase (MTHFR) genetic polymorphisms and psychiatric disorders: a HuGE review. Am. J. Epidemiol. 165 (1), 1–13.
Goodman, J.E., Lavigne, J.A., Wu, K., Helzlsouer, K.J., Strickland, P.T., Selhub, J., et al., 2001. COMT genotype, micronutrients in the folate metabolic pathway and breast cancer risk. Carcinogenesis 22 (10), 1661–1665.
Grieu, F., Powell, B., Beilby, J., Iacopetta, B., 2004. Methylenetetrahydrofolate reductase and thymidylate synthase polymorphisms are not associated with breast cancer risk or phenotype. Anticancer Res. 24, 3215–3219.
Guo, J., Jin, M., Zhang, M., Chen, L.A., 2012. Genetic variant in miR-196a2 increased digestive system cancer risks: a meat-analysis of 15 case–control studies. PLoS ONE 7 (1), e30585.
He, J.M., Pu, Y.D., Wu, Y.J., Qin, R., Zhang, Q.J., Sun, Y.S., et al., 2014. Association between dietary intake of folate and MTHFR and MTR genotype with risk of breast cancer. Genet. Mol. Res. 13 (4), 8925–8931.
Hekim, N., Ergen, A., Yaylim, I., Yilmaz, H., Zeybek, U., Oztürk, O., et al., 2007. No association between methylenetetrahydrofolate reductase C677T polymorphism and breast cancer. Cell Biochem. Funct. 25, 115–117.
Hennquez-Hernandez, L.A., Murias-Rosales, A., Hernandez Gon-zalez, A., Cabrera De Leon, A., Dıaz-Chico, B.N., Mori De Santiago, M., et al., 2009. Gene polymorphisms in TYMS, MTHFR, p53 and MDR1 as risk factors for breast cancer: a case–control study. Oncol. Rep. 22, 1425–1433.
Higgins, J.P., Thompson, S.G., 2002. Quantifying heterogeneity in a meta-analysis. Stat. Med. 21, 1539–1558.
Holmes, M.V., Newcombe, P., Hubacek, J.A., Sofat, R., Ricketts, S.L., Cooper, J., et al., 2011. Effect modification by population dietary folate on the association between MTHFR genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomized trials. Lancet 378, 584–594.
Hosseini, M., Houshmand, M., Ebrahimi, A., 2011. MTHFR polymorphisms and breast cancer risk. Arch. Med. Sci. 7, 134–137.
Hua, Z., Wang, Y., Ni, J., Ge, F., Zou, T., 2011. Serum folate, vitamin B12 concentration and MTHFR, MS gene polymorphism associated with risk of breast cancer research. Mod. Oncol. 19, L428–L431.
Huang, C.Y., Chang, W.S., Shui, H.A., Hsieh, Y.H., Loh, C.H., Wang, H.C., et al., 2014. Evaluation of the contribution of methylenetetrahydrofolate reductase genotypes to Taiwan breast cancer. Anticancer Res. 34, 4109–4115.
Huedo-Medina, T.B., Sanchez-Meca, J., Marin-Martinez, F., Botella, J., 2006. Assessing heterogeneity in meta-analysis: Q statistic or I^2 index? Psychol. Methods 11, 193–206.
Hulka, B.S., Stark, A.T., 1995. Breast cancer: cause and prevention. Lancet 346, 883–887.
Inoue, M., Robien, K., Wang, R., Van Den Berg, D.J., Koh, W.P., Yu, M.C., 2008. Green tea intake, MTHFR/TYMS genotype and breast cancer risk: the Singapore Chinese health study. Carcinogenesis 29, 1967–1972.
Jakubowska, A., Rozkrut, D., Antoniou, A., Hamann, U., Scott, R.J., McGuffog, L., et al., 2012. Association of PHB 1630 C>T and MTHFR 677 C>T polymorphisms with breast and ovarian cancer risk in BRCA1/2 mutation carriers: results from a multicenter study. Br. J. Cancer 106, 2016–2024.
Jiang-Hua, Q., De-Chuang, J., Zhen-Duo, L., Shu-de, C., Zhenzhen, L., 2014. Association of methylenetetrahydrofolate reductase and methionine synthase polymorphisms with breast cancer risk and interaction with folate, vitamin B6, and vitamin B 12 intakes. Tumor Biol. 35 (12), 11895–11901.
Jin, Z.Z., Lu, Q., Ge, D.H., Zong, M., Zhu, Q.H., 2009. Effect of the methylenetetrahydrofolate reductase gene C677T polymorphism on C-erbB-2 methylation status and its association with cancer. Mol. Med. Rep. 2, 283–289.
Justenhoven, C., Hamann, U., Pierl, C.B., Rabstein, S., Pesch, B., 2005. One-carbon metabolism and breast cancer risk: no association of MTHFR, MTR, and TYMS polymorphisms in the GENICA study from Germany. Cancer Epidemiol. Biomark. Prev. 14, 3015–3018.
Kakkoura, M.G., Demetriou, C.A., Loizidou, M.A., Loucaides, G., Neophytou, I., Marcou, Y., Hadjisavvas, A., et al., 2015. Single-nucleotide polymorphisms in one-carbon metabolism genes, Mediterranean diet and breast cancer risk: a case–control study in the Greek-Cypriot female population. Genes Nutr. 10 (2), 453.
Kalemi, T.G., Lambropoulos, A.F., Gueorguiev, M., Chrisafi, S., Papazisis, K.T., 2005. The association of p53 mutations and p53 codon 72, Her 2 codon 655 and MTHFR C677T polymorphisms with breast cancer in Northern Greece. Cancer Lett. 222, 57–65.
Kalyankumar, C., Jamil, K., 2006. Methylene tetrahydofolate reductase (MTHFR) C677T and A1298C polymorphisms and breast cancer in South Indian population. Int. J. Cancer Res. 2, 143–151.
Kan, X., Zou, T., Wu, X., Wang, X., 2007. Yunnan methylenetetrahydrofolate reductase gene polymorphism associated with breast cancer susceptibility. Cancer Res. 34, 716–718.
Kang, S.S., Zhou, J., Wong, P., Kowalisyn, J., Strokosch, G., 1988. Intermediate homocysteinemia: a thermolabile variant of methylenetetrahydrofolate reductase. Am. J. Hum. Genet. 43, 414.
Kelsey, J.L., 1993. Breast cancer epidemiology: summary and future directions. Epidemiol. Rev. 15, 256–263.
Kim, Y.I., 1999. Folate and carcinogenesis: evidence, mechanisms, and implications. J. Nutr. Biochem. 10, 66–88.
Kotsopoulos, J., Zhang, W.W., Zhang, S., McCready, D., Trudeau, M., Zhang, P., et al., 2008. Polymorphisms in folate metabolizing enzymes and transport proteins and the risk of breast cancer. Breast Cancer Res. Treat. 8, 9895–9896.
Laanpere, M., Altmäe, S., Stavreus-Evers, A., Nilsson, T.K., Yngve, A., Salumets, A., 2010. Folate-mediated one-carbon metabolism and its effect on female fertility and pregnancy viability. Nutr. Rev. 68 (2), 99–113.
Lajin, B., Sakur, A.A., Ghabreau, L., Alachkar, A., 2012. Association of polymorphisms in one-carbon metabolizing genes with breast cancer risk in Syrian women. Tumor Biol. 33, 1133–1139.
Langsenlehner, U., Krippl, P., Renner, W., Yazdani-Biuki, B., Wolf, G., Wascher, T.C., et al., 2003. The common 677C>T gene polymorphism of methylenetetrahydrofolate reductase gene is not associated with breast cancer risk. Breast Cancer Res. Treat. 81, 169–172.
Langsenlehner, T., Renner, W., Yazdani-Biuki, B., Langsenlehner, U., 2008. Methylenetetrahydrofolatereductase (MTHFR) and breast cancer risk: a nested-case–control study and a pooled meta-analysis. Breast Cancer Res. Treat. 107, 459–460.
Le Marchand, L., Haiman, C.A., Wilkens, L.R., Kolonel, L.N., Henderson, B.E., 2004. MTHFR polymorphisms, diet, HRT, and breast cancer risk: the multiethnic cohort study. Cancer Epidemiol. Biomark. Prev. 13, 2071–2077.

Lee, S.A., Kang, D., Nishio, H., Lee, M.J., Kim, D.H., Han, W., et al., 2004. Methylenetetrahydrofolate reductase polymorphism, diet, and breast cancer in Korean women. Exp. Mol. Med. 36, 116–121.
Li, W.D., Chen, S.Q., 2009. Association of methylenetetrahydrofolate reductase C677T polymorphism and breast cancer risk. J. Prac. Med. 25, 2031–2033.
Li, K., Li, W., Dong, X., 2014. Association of 677 C>T (rs1801133) and 1298A>C (rs1801131) polymorphisms in the MTHFR gene and breast cancer susceptibility: a meta-analysis based on 57 individual studies. PLoS ONE 9, e71290.
Liang, H., Yan, Y., Li, T., Li, R., Li, M., Li, S., et al., 2014. Methylenetetrahydrofolate reductase polymorphisms and breast cancer risk in Chinese population: a meta-analysis of 22 case–control studies. Tumor Biol. 35, 1695–1701.
Lin, W., Cen, Y.L., Lin, Y., Su, F.X., Wu, B.H., Tang, L.Y., et al., 2015. Joint effects between urinary selenium and polymorphisms in methylation related genes on breast cancer risk. Neoplasma (Epub ahead of print: DOI:10.4149/neo_2015_059).
Lin, W.Y., Chou, Y.C., Wu, M.H., Huang, H.B., Jeng, Y.L., Wu, C.C., et al., 2004. The MTHFR C677T polymorphism, estrogen exposure and breast cancer risk: a nested case–control study in Taiwan. Anticancer Res. 24, 3863–3868.
Lissowska, J., Gaudet, M.M., Brinton, L.A., Chanock, S.J., Peplonska, B., 2007. Genetic polymorphisms in the one carbon metabolism pathway and breast cancer risk: a population-based case–control study and metaanalyses. Int. J. Cancer 120, 2696–2703.
Liu, Y., Zhou, L.S., Xu, X.M., Deng, L.Q., Xiao, Q.K., 2013. Association of dietary intake of folate, vitamin B6 and B12 and MTHFR genotype with breast cancer risk. Asian Pac. J. Cancer Prev. 14, 5189–5192.
Lohmueller, K.E., Pearce, C.L., Pike, M., Lander, E.S., Hirschhorn, J.N., 2003. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat. Genet. 33 (2), 177–182.
López-Cortés, A., Echeverría, C., Oña-Cisneros, F., Sánchez, M.E., Herrera, C., Cabrera-Andrade, A., et al., 2015. Breast cancer risk associated with gene expression and genotype polymorphisms of the folate-metabolizing MTHFR gene: a case–control study in a high altitude Ecuadorian mestizo population. Tumor Biol. (Epub ahead of print: DOI 10.1007/s13277-015-3335-0).
Lu, Q., Jiang, K., Li, Q., Ji, Y.J., Chen, W.L., Xue, X.H., 2015. Polymorphisms in the MTHFR gene are associated with breast cancer risk and prognosis in a Chinese population. Tumor Biol. (Epub ahead of print: DOI 10.1007/s13277-014-3016-4).
Ma, E., Iwasaki, M., Junko, I., Hamada, G.S., Nishimoto, I.N., 2009a. 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 9, 122.
Ma, E., Iwasaki, M., Kobayashi, M., Kasuga, Y., Yokoyama, S., Onuma, H., et al., 2009b. 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 61, 447–456.
Macis, D., Maisonneuve, P., Johansson, H., Bonanni, B., Botteri, E., Iodice, S., et al., 2007. Methylenetetrahydrofolate reductase (MTHFR) and breast cancer risk: a nested-case–control study and a pooled meta-analysis. Breast Cancer Res. Treat. 106, 263–271.
Mantel, N., Haenszel, W., 1959. Statistical aspects of the analysis of data from retrospective studies of disease. J. Natl. Cancer Inst. 22, 719–748.
Maruti, S.S., Ulrich, C.M., Jupe, E.R., White, E., 2009. MTHFR C677T and postmenopausal breast cancer risk by intakes of one-carbon metabolism nutrients: a nested case–control study. Breast Cancer Res. 11, R9.
Mei, Q., Zhou, D., Gao, J., Shen, S., Wu, J., Guo, L., et al., 2012. The association between MTHFR 677C > T polymorphism and cervical cancer: evidence from a meta-analysis. BMC Cancer 12, 467–476.
Mir, M.M., Dar, J.A., Dar, N.A., Dar, M.S., Salam, I., Lone, M.M., et al., 2008. Combined impact of polymorphism of folate metabolism genes; glutamate carboxypeptidase, methylene tetrahydrofolate reductase and methionine synthase reductase on breast cancer susceptibility in Kashmiri women. In. J. Health Sciences. 2, 3–14.
Naushad, S.M., Reddy, C.A., Rupasree, Y., Pavani, A., Digumarti, R.R., Gottumukkala, S.R., et al., 2011. Cross-talk between one-carbon metabolism and xenobiotic metabolism: implications on oxidative DNA damage and susceptibility to breast cancer. Cell Biochem. Biophys. 61, 715–723.
Ozen, F., Erdis, E., Sik, E., Silan, F., Uludag, A., Ozdemir, O., 2013. Germ-line MTHFR C677T, FV H1299R and PAI-1 5G/4G variations in breast carcinoma. Asian Pac. J. Cancer Prev. 14, 2903–2908.
Platek, M.E., Shields, P.G., Marian, C., McCann, S.E., Bonner, M.R., Nie, J., et al., 2009. Alcohol consumption and genetic variation in methylenetetrahydrofolate reductase and 5-methyltetrahydrofolate-homocysteine methyltransferase in relation to breast cancer risk. Cancer Epidemiol. Biomarkers Prev. 18 (2459), 2453–2459.
Prasad, V.V., Wilkhoo, H., 2011. Association of the functional polymorphism C677T in the methylenetetrahydrofolate reductase gene with colorectal, thyroid, breast, ovarian, and cervical cancers. Onkologie 34, 422–426.
van der Put, N., van Straaten, M., Trijbels, H.W., Blom, F.J., H.J., 2001. Folate, homocysteine and neural tube defects: an overview. Exp. Biol. Med. (Maywood) 226 (4), 243–270.
Qi, X., Ma, X., Yang, X., Fan, L., Zhang, Y., 2011. 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. 123, 499–506.
Qi, J., Miao, X.P., Tan, W., Yu, C.Y., Liang, G., Lü, W.F., et al., 2004. Association between genetic polymorphisms in methylenetetrahydrofolate reductase and risk of breast cancer. Zhonghua Zhong Liu Za Zhi 26, 287–289.
Rai, V., 2011. Evaluation of methylenetetrahydrofolate reductase gene variant (C677T) as risk factor for bipolar disorder. Cell. Mol. Biol. (Noisy-le-grand). 57, Suppl:OL1558-OL1566.
Rai, V., 2014. The methylenetetrahydrofolate reductase C677T polymorphism and breast cancer risk in Asian populations. Asian Pac. J. Cancer Prev. 15, 5853–5860.
Rai, V., Yadav, U., Kumar, P., Yadav, S.K., Mishra, O.P., 2014. Maternal methylenetetrahydrofolate reductase C677T polymorphism and down syndrome risk: a meta-analysis from 34 studies. PLoS ONE 9, e108552.
Reljic, A., Simundic, A.M., Topic, E., Nikolac, N., Justinic, D., 2007. The methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and cancer risk: the Croatian case–control study. Clin. Biochem. 40, 981–985.
Robien, K., Ulrich, C.M., 2003. 5,10-Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: a HuGE minireview. Am. J. Epidemiol. 157 (7), 571–582.
Rohan, T.E., Jain, M.G., Howe, G.R., Miller, A.B., 2000. Dietary folate consumption and breast cancer risk. J. Natl. Cancer Inst. 92, 266–269.
Rosenberg, N., Murata, M., Ikeda, Y., Opare-Sem, O., Zivelin, A., Geffen, E., et al., 2002. The frequent 5,10-methylenetetrahydrofolate reductase C677T polymorphism is associated with a common haplotype in whites, Japanese, and Africans. Am. J. Hum. Genet. 70, 758–762.
Sangrajrang, S., Sato, Y., Sakamoto, H., Ohnami, S., Khuhaprema, T., Yoshida, T., 2010. Genetic polymorphisms in folate and alcohol metabolism and breast cancer risk: a case–control study in Thai women. Breast Cancer Res. Treat. 123, 885–893.
Semenza, J.C., Delfino, R.J., Ziogas, A., Anton-Culver, H., 2003. Breast cancer risk and methylenetetrahydrofolate reductase polymorphism. Breast Cancer Res. Treat. 77, 217–223.
Sen, S., Duman, R., Sanacora, G., 2008. Serum BDNF, depression and anti-depressant medications: meta-analyses and implications. Biol. Psychiatry 64 (6), 527–532.
Sharp, L., Little, J., 2004. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. Am. J. Epidemiol. 159 (5), 423–443.
Sharp, L., Little, J., Schofield, A.C., Pavlidou, E., Cotton, S.C., Meidzybrodzka, Z., et al., 2002. Folate and breast cancer: the role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR). Cancer Lett. 181, 65–71.
Shrubsole, M.J., Gao, Y.T., Cai, Q., Shu, X.O., Dai, Q., Hébert, J.R., et al., 2004. MTHFR polymorphisms, dietary folate intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. Cancer Epidemiol. Biomark. Prev. 13, 190–196.
Singh, P., Carlus, J., Sekhar, D., Francis, A., Gupta, N., Konwar, R., et al., 2015. MTHFR 677C>T polymorphism and the risk of breast cancer: evidence from an original study and pooled data for 28031 cases and 31880 controls. PLoS ONE 10 (3), e0120654.
Sohn, K.J., Jang, H., Campan, M., Weisenberger, D.J., Dickhout, J., Wang, Y.C., et al., 2009. The methylenetetrahydrofolate reductase C677T mutation induces cell-specific changes in genomic DNA methylation and uracil misincorporation: a possible molecular basis for the site-specific cancer risk modification. Int. J. Cancer 124, 1999–2005.
Stern, L.L., Mason, J.B., Selhub, J., Choi, S.W., 2000. Genomic DNA hypomethylation, a characteristic of most cancers, is present in peripheral leukocytes of individuals who are homozygous for the C677T polymorphism in the methylenetetrahydrofolate reductase gene. Cancer Epidemiol. Biomark. Prev. 9, 849–853.
Stevens, V.L., McCullough, M.L., Pavluck, A.L., Talbot, J.T., Feigelson, H.S., Thun, M.J., et al., 2007. Association of polymorphisms in one-carbon metabolism genes and postmenopausal breast cancer incidence. Cancer Epidemiol. Biomark. Prev. 16, 1140–1147.
Sturgeon, S.R., Schairer, C., Grauman, D., El Ghormli, L., Devesa, S., 2004. Trends in breast cancer mortality rates by region of the United States, 1950–1999. Cancer Causes Control 15, 987–995.
Suzuki, T., Matsuo, K., Hirose, K., Hiraki, A., Kawase, T., Watanabe, M., et al., 2008. One-carbon metabolism-related gene polymorphisms and risk of breast cancer. Carcinogenesis 2, 356–362.
Tu, Y.L., Wang, S.B., Tan, X.L., 2012. MTHFR gene polymorphisms are not involved in pancreatic cancer risk: a meta-analysis. Asian Pac. J. Cancer Prev. 13, 4627–4630.
Ueland, P.M., Hustad, S., Schneede, J., Refsum, H., Vollset, S.E., 2001. Biological and clinical implications of the MTHFR C677T polymorphism. Trends Pharmacol. Sci. 22, 195–201.
Vainer, A.S., Boiarskikh, U.A., Voronina, E.N., Selezneva, I.A., Sinkina, T.V., 2010. 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) 44, 816–823.
Wallace, B.C., Dahabreh, I.J., Trikalinos, T.A., Lau, J., Trow, P., Schmid, C.H., 2013. Closing the gap between methodologists and end-users: R as a computational back-end. J. Stat. Softw. 49, 1–15.
Wang, Z.G., Cui, W., Yang, L.F., Zhu, Y.Q., Wei, W.H., 2014. Association of dietary intake of folate and MTHFR genotype with breast cancer risk. Genet. Mol. Res. 13, 5446–5451.
Weiwei, Z., Liping, C., Dequan, L., 2014. Association between dietary intake of folate, vitamin B6, B12 & MTHFR, MTR genotype and breast cancer risk. Pak. J. Med. Sci. 30, 106–110.
Wen, Y.Y., Yang, S.J., Zhang, J.X., Chen, X.Y., 2013. Methylenetetrahydrofolate reductase genetic polymorphisms and esophageal squamous cell carcinoma susceptibility: a meta-analysis of case–control studies. Asian Pac. J. Cancer Prev. 14, 21–25.
Wilcken, B., Bamforth, F., Li, Z., Zhu, H., Ritvanen, A., Renlund, M., et al., 2003. Geographical and ethnic variation of the 677C>T allele of 5,10 methylenetetrahydrofolate reductase (MTHFR): findings from over 7000 newborns from 16 areas worldwide. J. Med. Genet. 40 (8), 619–625.
Wu, X.Y., Ni, J., Xu, W.J., Zhou, T., Wang, X., 2012. Interactions between MTHFR C677T-A1298C variants and folic acid deficiency affect breast cancer risk in a Chinese population. Asian Pac. J. Cancer Prev. 13, 2199–2206.
Wu, Y., Yuan, X., Zheng, H., et al., 2010. Northeast methylenetetrahydrofolate reductase gene c677t single nucleotide polymorphisms and susceptibility to breast cancer research. Mod. Oncol. 18, 2375–2378.
Xu, X., Gammon, M.D., Zhang, H., Wetmur, J.G., Rao, M., 2007. Polymorphisms of one-carbon-metabolizing genes and risk of breast cancer in a population-based study. Carcinogenesis 28, 1504–1509.

Yadav, U., Kumar, P., Rai, V., 2014. Global prevalence of MTHFR C677T gene polymorphism: a meta-analysis of population based studies. Indian J. Clin. Biochem. 29 (1), 123–124.
Yadav, U., Kumar, P., Yadav, S.K., Mishra, O.P., Rai, V., 2015. Polymorphisms in folate metabolism genes as maternal risk factor for neural tube defects: an updated meta-analysis. Metab. Brain Dis. 30, 7–14.
Yang, X., Lippman, M.E., 1999. BRCA1 and BRCA2 in breast cancer. Breast Cancer Res. Treat. 54, 1–10.
Yu, L., Chen, J., 2012. Association of MHTFR Ala222Val (rs1801133) polymorphism and breast cancer susceptibility: an update meta-analysis based on 51 research studies. Diagn. Pathol. 7, 171.
Yu, C.P., Wu, M.H., Chou, Y.C., Yang, T., You, S.L., Chen, C.J., et al., 2007. Breast cancer risk associated with multigenotypic polymorphisms in folate metabolizing genes: a nested case–control study in Taiwan. Anticancer Res. 27, 1727–1732.
Yuan, H., Xu, X.Y., Wang, Z.L., 2009. The relation between polymorphisms of methylenetetrahydrofolate reductase C677T and the risk of breast cancer. J. MuDan Jiang Med. Univ. 30, 2–4.
Zhang, S., Hunter, D.J., Hankinson, S.E., Giovannucci, E.L., Rosner, B.A., et al., 1999. A prospective study of folate intake and the risk of breast cancer. J. Am. Med. Assoc. 281, 1632–1637.
Zhang, T., Lou, J., Zhong, R., Wu, J., Zou, L., Sun, Y., et al., 2013. Genetic variants in the folate pathway and the risk of neural tube defects: a meta-analysis of the published literature. PLoS ONE 8, e59570.
Zhang, J., Qiu, L.X., Wang, Z.H., Wu, X.H., Liu, X.J., 2010. MTHFR C677T polymorphism associated with breast cancer susceptibility: a meta-analysis involving 15,260 cases and 20,411 controls. Breast Cancer Res. Treat. 123, 549–555.
Zhang, W.B., Zhang, J.H., Pan, Z.Q., Yang, Q.S., Liu, B., 2012. 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. 13, 2597–2604.
Zintzaras, E., 2006. Methylenetetrahydrofolate reductase gene and susceptibility to breast cancer: a meta-analysis. Clin. Genet. 69, 327–336.