RESEARCH ARTICLE

The Methylenetetrahydrofolate Reductase C677T Polymorphism and Breast Cancer Risk in Asian Populations

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Abstract

Background: Methylenetetrahydrofolate (MTHFR) is the key enzyme of the folate metabolic pathway and several studies have pointed to association between the MTHFR C677T polymorphism and breast cancer risk. Although significant association was observed in some studies, in others no clear link could be established. Objective: A meta-analysis of published Asian case control studies was therefor carried out to shed further light on any C677T breast cancer association. Materials and Methods: PubMed, Springer Link, Google Scholar and Elsevier databases were searched for case control studies of associations between MTHFR C677T polymorphism and breast cancer risk. Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated to assess the association. A total of 36 studies including 8,040 cases and 10,008 controls were included in the present meta-analysis. Results: Overall, a significantly elevated breast cancer risk was associated with the T allele and TT genotype in homozygote comparison and dominant genetic models when all studies were pooled into the meta-analysis (T *vs* C (allele contrast model): OR=1,23, 95%CI=1.13-1.37, p=0.000 ; TT *vs* CC(homozygote model): OR=1.38, 95%CI=1.16-1.63, p=0.0003; TT+CT *vs* CC (dominant model): OR=1.12, 95%CI=1.01-1.23, p=0.02). Conclusions: The present meta-analysis strongly suggested a significant association between the MTHFR C677T polymorphism and risk of breast cancer in Asian populations.

Keywords: Meta-analysis - breast cancer - polymorphism - MTHFR - C677T - polymorphism

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Introduction

Breast cancer is a primary cause of cancer death among women worldwide. Global breast cancer incidence has been increasing by more than one million new cases every year; the incidence is significantly higher in developed countries than in developing countries (Ferlay, 2000; Sturgeon et al., 2004; Liang et al., 2013). The cumulative lifetime risk for the development of the disease in the general population is estimated to be 10% (Yang and Lippman, 1999). The etiology of breast cancer is not fully understood. Besides age at menarche and menopause, diet, reproductive history, estrogen administration and genetic factors have been suggested as risk factors (Kelsey,1993;Hulka and Stark,1995; Collaborative Group on Hormonal Factors in Breast Cancer 1997; Langsenlehner et al., 2003). Approximately 25% of breast cancers are inherited by germ-line mutations in functional and/or oncogenes (Ozen et al., 2013). Only a small part of familial breast cancer cases can be explained by inherited mutations, the majority being most probably explained by a combination of common low-penetrance gene polymorphisms (Antoniou et al., 2001; Langsenlehner et al., 2003).

Dietary folate deficiency of an individual along with methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms leads to DNA hypomethylation (Friso et al., 2002). Methylation is genetically predetermined, either by imprinting or by inheritance of genes which influence methylation, such as MTHFR and other genes involved in the 1-carbon cycle. Methyl groups required for methylation are synthesized de novo or are supplied in the diet, primarily from folate. Thus, methylation may be modified by gene-exposure interactions occurring during development. Breast cancer is a manifestation of abnormal genetic and epigenetic changes. Interruption of folate metabolism may contribute to disease etiology as it facilitates cross-talk between genetic and epigenetic processes by effecting gene expression through DNA methylation and genome integrity through DNA synthesis and repair (Kim, 1999; Choi and Mason, 2002; Jakubowska et al., 2007).

Methylenetetrahydrofolate is an important enzyme in folate metabolism. It irreversibly converts 5,10-methylene tetrahydrofolate (THF) to 5-methyl THF which provides the methyl group for the de novo synthesis of methionine synthase and DNA methylation (Matthews et al., 1998). It also helps determine the folate levels available for DNA synthesis and repair (Bailey and Gregory,1999). The C677T polymorphism codes for an alanine to valine

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substitution in the N-terminal catalytic domain and results in an allozyme with approximately 65 and 30% of the activity of the wild-type protein for heterozygotes and homozygotes, respectively (Frosst et al., 1995) Allele frequencies for the 677T variant range approximately from 0.24 to 0.44 in European and Caucasian populations, 0.06 in an African population, and 0.35 to 0.41 in Asian populations (Botto and Yang, 2000; Song et al., 2001; Rai et al., 2012). The frequency of homozygosity (TT) ranges from 1% in US African-American populations to more than 20% in US Latinos; 5% to 30% in White populations in Europe and North America; 32.2% in Mexico; 5.8% in White Canadians in Alberta to 14.3% in those in Quebec, Canada; 0.0% in Sub-Saharan Africa; 10.7% in Oceania; and 11.5% in Japanese and 16% in Chinese (Botto and Yang, 2000; Wilcken et al., 2003 Boccia et al., 2007). C677T variant has been associated with an increased risk for various cancers including endometrial cancer (Esteller et al., 1997), cervical intraepithelial neoplasia (Piyathilake et al., 2000), esophageal squamous cell carcinoma (Song et al., 2001), gastric cancer (Shen et al., 2001), bladder cancer (Lin et al., 2004) and squamous cell carcinoma of the head and neck (Neumann et al., 2005). Many studies investigated the association between the MTHFR C677T polymorphism and breast cancer incidence but results were controversial. Although significant association was observed in some studies (Liu et al., 2013; Ozen et al., 2013; Weiwei et al., 2013) whereas a clear association between MTHFR polymorphisms and the risk to develop breast cancer has not been established in other studies (Shrubsole et al., 2004; Chen et al., 2005; Le Marchand et al 2004;Wu et al., 2012).

Materials and Methods

Literature search

The literature included in the analysis was selected using PubMed, Elsevier, Google Scholar and Springer Link databases with keywords 'methylenetetrahydrofolate reductase' or 'MTHFR', 'C677T' and 'breast cancer'. All extracted articles read completely and carefully. The control group included individuals without any family history of breast cancer.

Inclusion and exclusion criteria

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

Data extraction

Relevant information's were extracted from all selected studies like- author family name, journal name, year of publication, country name and number of cases and controls for each C677T genotypes (CC,CT and TT genotypes). Allelic number for the cases and controls were calculated from corresponding genotypes.

Meta-analysis

Present meta-analysis examined the overall association for the allele contrast, the contrast of homozygotes, and the recessive, codominant and dominant models. Statistical analysis of MTHFR C677T polymorphism and BC risk was estimated by Odds ratio (ORs) with 95% confidence intervals (CIs).Cochran's Q statistic was used for heterogeneity test, and heterogeneity between studies was quantified with the I^2 metric (I^2=(Q - df)/Q), which is independent of the number of studies in the meta-analysis. I^2 takes values of between 0 and 100%, with higher values denoting a greater degree of heterogeneity (Zintzaras and Hadjigeorgiou, 2004) (I^2=0% to 25%: no heterogeneity; I^2=25% to 50%: moderate heterogeneity; I^2=50% to 75%: large heterogeneity; I^2=75% to 100%: extreme heterogeneity) (Higgins and Thompson, 2002; Zintzaras, 2007). The pooled OR was estimated using fixed effects (FE) (Mantel and Haenszel, 1959) and random effects (RE) (DerSimonian and Laird, 1986) models. Where large heterogeneity existed, the random effects model, which yields wider confidence intervals (CIs), should be adopted; otherwise both the fixed effects and random effects models should be deemed appropriate. All statistical analysis were performed using MIX version 1.7 (Bax et al., 2006) and Meta-Disc (Zamora et al., 2006), using two-sided P-value.

Publication bias

An estimate of potential publication bias was carried out by the funnel plot, Begg's and Egger's test. The significance of the intercept was determined by the t-test suggested by Egger ($p>0.05$ was considered representative of statistically significant publication bias) (Egger et al.,1997).

Results

Characteristics of included studies

Following these exclusions, 36 individual case-control studies with a total of 9,025 cases and 11,251 controls were included into this meta-analysis (Ergul et al., 2003; Lee et al., 2004; Le Marchand et al., 2004; Lin et al., 2004; Qi et al., 2004; Shrubsole et al., 2004; Deligezer et al., 2005; Chou et al., 2006; Kalyankumar et al., 2006; Hekim et al., 2007; Kan et al., 2007; Yu et al., 2007; Inoue et al., 2008; Suzuki et al., 2008; Cheng et al., 2008; Mir et al., 2008; Gao et al., 2009; Ma et al., 2009; Cam et al., 2009; Li et al., 2009; Yuan et al., 2009; Jin et al., 2009; Alshatwi et al., 2010; Sangrajrang et al., 2010; Wu et al., 2010; Hosseini et al., 2011; Hua et al., 2011; Mohammad et al., 2011; Nausad et al., 2011; Prasad et al., 2011; Akram et al., 2012; Lajin et al., 2012; Wu et al., 2012; Liu et al., 2013; Ozen et al., 2013; Weiwei et al., 2014). Twenty six studies were carried out in different countries/population like- Mixed Asian population (Le Marchand et al., 2004; Mohammad et al., 2011), Arab (Alshatwi et al., 2010), China (Qi et al., 2004; Shrubsole et al., 2004; Chou et al., 2006; Kan et al., 2007; Gao et al., 2009; Li et al., 2009; Yuan et al., 2009; Jin et al., 2009; Wu et al., 2010; Hua et al., 2011; Wu et al., 2012; Liu et al., 2013; Weiwei et al., 2014), India (Kalyankumar, et al.2006; Mir et al., 2008; Sangrajrang et al., 2010; Nausad et al., 2011; Prasad et al., 2011), Iran

(Hosseini et al., 2011), Japan (Suzuki et al., 2008; Ma et al., 2009), Korea (Lee et al., 2004), Pakistan (Akram et al., 2012), Singapore (Inoue et al., 2008), Syria (Lajin et al., 2012), Taiwan(Lin et al., 2004; Yu et al., 2007; Cheng et al., 2008),Turkey (Ergul et al., 2003; Deligezer et al., 2005; Hekim et al., 2007; Cam et al., 2009; Ozen et al., 2013). Genotypes were in Hardy-Weinberg equilibrium in all controls. Thirty six studies, reported the association of SNP C677T polymorphism in the MTHFR gene with breast cancer are summarized in Table 1.

Summary Statistics

In total thirty six studies, total cases were 8,040 with CC (3754), CT (3181) and TT (1105), and controls were 10008 with CC (4869), CT (4069), and TT (1122). In controls genotypes percentage of CC, CT and TT were 47.34%, 40.77% and 11.89% respectively. In total cases genotype percentage of CC, CT and TT was 45.58%, 39.92% and 13.38% respectively. Frequencies of CC and CT genotypes were highest in both cases and controls (Table 2). Number of C and T alleles were also calculated and presented in table 2. Eleven studies did not show any association (Le Merchand et al., 2004; Shrubsole et al., 2004; Chou et al., 2006; Inoue et al., 2008; Cheng et al., 2008; Mir et al., 2008; Ma et al., 2009; Hosseni et al., 2011; Hua et al., 2011; Prasad et al., 2011; Wu et al., 2012) and odds ratio was above one in other twenty five studies.

Meta-analysis

Table 3 summarizes the ORs with corresponding 95%CIs for association between C677T polymorphism and risk of breast cancer in allele contrast, homozygote, dominant, recessive and co-dominant models. The pooled Odd Ratios were estimated by both fixed effects (Mantel and Haenszel, 1959) and random effects (Der Simonian and Laired, 1986) models.

Allele contrast meta-analysis:

Meta-analysis with allele contrast showed significant association with both fixed effect (OR_{TvsC}=1.1 ;95%CI: 1.05-1.14; p<0.00011.05-1.17; P_{hetero}=<0.001; I^2=77.3; P_{Pb}=0.05) and random effect model (OR_{TvsC}=1.23; 95%CI: 1.13-1.37;p=,0.000). Breast cancer patients with showed a significantly increased frequency of the T allele (Table 3, Figure 1).

In cumulative analysis using fixed and random effect models, the association of mutant 'T' allele with breast cancer turned statistically insignificant with the addition

Table 1. Characteristics of Thirty Six Studies Included in the Ppresent Meta-Analysis

Study	Country	Case	Control	Reference
Ergul et al., 2003	Turkey	118	193	Tumour Biol, 24, 286-290.
Shrubsole et al., 2004	China	1112	1160	Cancer Epidemiol Biomarkers Prev, 13, 190-196.
Lee et al., 2004	Korea	186	147	Exp Mol Med 36, 116-121.
Lin et al., 2004	Taiwan	88	342	Anticancer Res 2004, 24, 3863-3868.
Le Marchand et al., 2004	E.As.	318	410	Cancer Epidemiol Biomarkers Prev 13, 2071-2077
Qi et al., 2004	China	217	218	Chin J Oncol, 26, 287-289.
Deligezer et al., 2005	Turkey	189	223	In Vivo 2005, 19, 889-893.
Chou et al., 2006	China	142	285	Carcinogenesis, 27, 2295-2300.
Kalyankumar et al., 2006	India	88	95	Int J Cancer Res 2006, 2, 143-151.
Hekim et al., 2007	Turkey	40	68	Cell Biochem Funct 2007, 25, 115-117.
Kan et al., 2007	China	125	103	Cancer Res Prev Treat 34, 716-718.
Yu et al. et al., 2007	Taiwan	119	420	Anticancer Res 2007, 27, 1727-1732
Inoue et al., 2008	Singapore	380	662	Carcinogenesis, 29, 1967-1972.
Suzuki et al., 2008	Japan	454	909	Carcinogenesis 2008, 2, 356-362
Cheng et al. ,2008	Taiwan	349	530	Breast Cancer Res Treat , 111, 145-155.
Mir et al., 2008	India	35	33	International Journal of Health Sciences, Qassim University,2, pp. 3-14.
Gao et al., 2009	China	624	624	J Hum Genet ,54, 414-418.
Ma et al., 2009	Japan	388	387	Nutr Cancer,61, 447-456.
Cam et al., 2009	Turkey	110	95	Breast Cancer Res Treat 2009, 115, 431-432.
Li et al., 2009	China	65	143	Practical. J Med. 5, 2031-3.
Yuan et al., 2009	China	80	80	Mudanjiang Med Coll. 30, 2-4.
Jin et al., 2009	China	41	100	Mol Med Rep 2009, 2, 283-289.
Alshatwi et al., 2010	Arab	100	100	Food Chem Toxicol 2010, 48, 881-1885.
Sangrajrang et al., 2010	Indian	563	487	Breast Cancer Res Treat 2010, 123, 885-893.
Wu et al., 2010	China	80	80	Modern Oncology 2010;18, 2375-2378.
Hosseini et al., 2011	Iran	294	300	Arch Med Sci, 7, 1, 134-137.
Hua et al., 2011	China	95	90	Mod Oncol ,19, 428-31.
Mohammad et al., 2011	Asian	222	235	Mol Biol Rep 2011, 38, 4893-4901
Nausad et al., 2011	India	244	244	Cell Biochem Biophys, 61, 715-723
Prasad et al., 2011	India	130	125	Onkologie, 34, 422-426.
Akram et al., 2012	Pakisant	110	110	Asian Pac J Cancer Prev,13, 1599-1603
Lajin et al., 2012	Syria	119	126	Tumor Biol 2012, 33, 1133-1139.
Wu et al., 2012	China	32	37	Asian Pac J Cancer Prev, 13, 2199-206.
Liu et al., 2013	China	435	435	Asian Pac J Cancer Prev, 14, 5189-5192
Ozen et al., 2013	Turkey	51	106	Asian Pacific J Cancer Prev, 14 (5), 2903-2908.
Weiwei et al., 2014	China	297	306	Pak J Med Sci, 30, 106-110.

Table 2. The Distributions of MTHFR C677T Genotypes and Allele Number for Breast Cancer Cases and Ccontrols

Study ID	Genotype						Alleles			
	CC		CT		TT		C		T	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Ergul et al., 2003	60	94	41	87	17	12	161	275	75	11
Shrubsole et al., 2004	374	387	555	577	183	196	1303	1351	921	969
Lee et al., 2004	58	50	96	80	32	17	212	180	160	114
Lin et al., 2004	43	173	38	145	7	24	124	491	52	193
Le Marchand et al., 2004	135	140	140	196	43	74	410	476	226	344
Qi et al., 2004	42	59	104	105	71	54	188	223	246	213
Deligezer et al., 2005	98	128	68	83	23	12	264	339	114	107
Chou et al., 2006	73	132	51	120	18	33	197	384	87	186
Kalyankumar et al., 2006	45	61	37	31	6	3	127	153	49	37
Hekim et al., 2007	22	38	16	26	2	4	60	102	20	30
Kan et al., 2007	74	65	29	29	22	9	177	159	73	47
Yu et al. et al., 2007	56	225	54	170	9	25	166	620	72	220
Inoue et al., 2008	239	393	120	226	21	43	598	1012	162	312
Suzuki et al., 2008	150	338	220	425	84	146	520	1101	388	717
Cheng et al. ,2008	185	268	133	221	31	41	503	757	195	303
Mir et al., 2008	29	19	6	12	0	2	64	50	6	16
Gao et al., 2009	202	235	305	301	117	88	709	771	539	477
Ma et al., 2009	124	115	183	188	81	84	431	418	345	356
Cam et al., 2009	48	47	49	42	13	6	145	136	75	54
Li et al., 2009	38	90	17	50	10	3	93	230	37	56
Yuan et al., 2009	16	32	35	35	29	13	67	99	93	61
Jin et al., 2009	18	49	20	41	3	10	56	139	26	61
Alshatwi et al., 2010	34	36	50	49	16	15	118	121	82	79
Sangrajrang et al., 2010	410	366	144	110	9	11	964	1098	162	132
Wu et al., 2010	16	32	35	35	29	13	67	99	93	61
Hosseini et al., 2011	168	150	84	90	42	60	420	390	168	210
Hua et al., 2011	65	52	21	27	9	11	151	131	39	49
Mohammad et al., 2011	168	198	53	37	1	0	389	433	55	37
Nausad et al., 2011	185	205	56	39	3	0	426	449	62	39
Prasad et al., 2011	124	116	5	8	1	1	253	240	7	10
Akram et al., 2012	65	55	25	45	20	10	155	155	65	55
Lajin et al., 2012	44	65	52	48	23	13	140	178	98	74
Wu et al., 2012	23	27	8	7	1	3	54	61	10	13
Liu et al., 2013	139	168	216	209	80	58	494	545	376	325
Ozen et al., 2013	28	76	18	30	5	0	74	182	28	30
Weiwei et al., 2014	156	185	97	93	44	28	409	463	185	149

of study of Lee et al (2004) and became significant after addition of Kalyankumar et al. (2006) and remained significant till the addition of Ma et al. (2009). After addition of study of Yuan et al. (2009) association turned significant and stayed significant thereafter (result no shown).

Genotype contrast meta-analysis

Table 3 summarizes the ORs with corresponding 95%CIs for association between C677T polymorphism and risk of breast cancer in dominant, recessive, homozygote and co-dominant models. Thirty six studies allowed author to make all planned comparisons of genotypes. With primary analysis, there was an increased risk of breast cancer among mutant homozygote variants (TT), with both fixed (OR_{TTvCC}=1.24; 95%CI=1.13-1.36;p<0.0001; I^2=58.28%; $P_{heterogeneity}$ <0.0001; P_{Pb}=0.04) and random (Random-effects OR_{TTvCC}=1.38; 95%CI: 1.16- 1.63; p=0.0003) effect models with high statistical heterogeneity between-study. Association of mutant heterozygous genotype (CTvsCC) with fixed (OR_{CTvCC}=1.03; 95%CI=0.97-1.1;p=0.28; I^2=33.73%; Pheterogeneity=0.02; P_{Pb}=0.63) and random (OR_{CTvCC}=1.05; 95%CI=0.96-1.14;p=0.29) effect models were observed insignificant. Similarly combined mutant genotypes (TT+CTvsCC) showed positive association with schizophrenia using both fixed ($OR_{TT+CTvCC}$=1.08; 95%CI=1.02-1.159 p=0.002; I^2=51.57%; $P_{heterogeneity}$=0.0001; P_{Pb}=0.20) and random ($OR_{TT+CTvsCC}$=1.12; 95%CI: 1.01-1.23; p=0.02) effect models. Association between recessive genetic model (TT vs CT+CC) were also found significant with both fixed ($OR_{TTvsCT+CC}$=1.22;95%CI=1.12-1.33;p<0.0001; I^2=50.35%; $P_{heterogeneity}$=0.003; P_{Pb}=0.03) as well as random ($OR_{TTvs\ CT+CC}$=1.33; 95%CI: 1.15-1.43; p=0.0001) effects model.(Table 3).

Publication bias

Funnel plots, Begg's and Egger's test were performed to estimate the risk of publication bias. The shape of funnel plots in all contrast models showed obvious evidence of symmetry (Figure 2). In addition, all the P values of Egger's test were more than 0.05, which provided

Table 3. 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 and Publication Bias p-value (Egger Test).

Genetic Models	Fixed effect OR (95%CI), p	Random effect OR (95%CI), p	Heterogeneity p-value (Q test)	I^2 (%)	Publication Bias (p of Egger's test)
Allele Contrast (T *vs* C)	1.1(1.05-1.14),<0.0001	1.23(1.13-1.37),0.000	<0.0001	77.3	0.05
Co-dominant (CT *vs* CC)	1.03(0.97-1.1),0.28	1.05(0.96-1.14),0.29	0.02	33.73	0.63
Homozygoote (TT *vs* CC)	1.24(1.13-1.36),<0.001	1.38(1.16-1.63),0.0003	<0.0001	58.28	0.04
Dominant (TT+CT *vs* CC)	1.08(1.02-1.15), 0.009	1.12(1.01-1.23),0.02	0.0001	51.57	0.20
Recessive (TT *vs* CT+CC)	1.22(1.12-1.33),<0.0001	1.33(1.15-1.43),0.0001	0.003	50.35	0.03

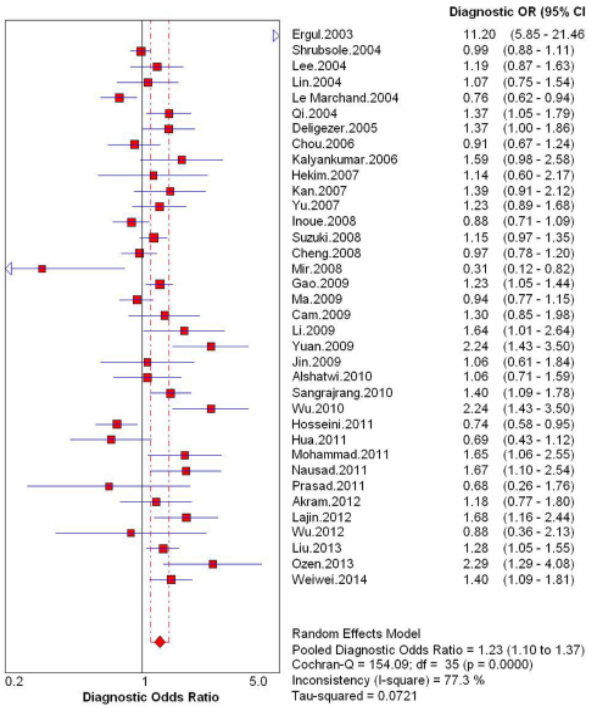

Figure 1. Forest Plots for the Association between MTHFR C677T Polymorphism and BC for Allele Contrast (T vs. C) with Random Effect Model

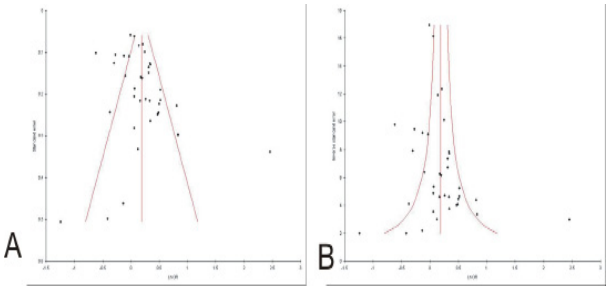

Figure 2. Funnel Plots A) Precision Versus OR (T vs. C); B) Standard Error Versus OR (T vs. C)

statistical evidence for the symmetry of funnel plots in the meta-analysis (p=0.05 for T *vs* C; p=0.04 for TT vs CC; and p=0.63 for CT *vs* CC; p=0.20 for TT+CT *vs* CC; p=0.03 for TT vs CT+CC) (Table 3).

Discussion

Deficiency of nutrients, such as vitamins and microelements, were observed to be correlated with breast cancer (Norat et al., 2014; Weiwei et al., 2014). Folate is as an important nutritional factor which may have a role as a cancer-preventing agent (Kim, 1999; Kotsopoulos et al., 2008). It plays an integral role in DNA synthesis and methylation, and as an epigenetic regulator of gene expression, DNA integrity and stability (Wagner, 1995; Kim,1999; Kotsopoulos et al., 2008). Folate deficiency may result in increased numbers of DNA strand breaks, impaired DNA repair, enhanced mutagenesis and alterations in DNA methylation patterns. All of these events have been implicated in neoplastic transformation (Baylinet al., 1999; Duthie, 1999; Jones and Laird, 1999; Kim, 2004; Kotsopoulos et al., 2008). This link between folate, folate metabolism, and DNA methylation therefore provides a plausible biologic mechanism for the observed association between MTHFR and breast cancer.

MTHFR 677TT polymorphism has been associated with risk for many different types of cancer, including esophageal, colorectal, gastric, pancreatic, prostate, cervical, lung, and leukemia (Boccia et al., 2007; Tu et al., 2012; Mei et al., 2012; Zhang et al., 2012; Wen et al., 2013). Impaired MTHFR activity might influence cancer risk is determined by the level of S-adenosyl-L-methionine, the common donor of methyl that is necessary for maintenance of the methylation patterns in DNA. Changes in methylation modify DNA conformation and gene expression. A less active form of MTHFR leads to lower S-adenosyl-L-methionine levels and consequently to hypomethylation; this phenomenon would be expected to increase the risk of some cancers (Stern et al., 2000; Boccia et al., 2007). Similarly,low folate intake may modify cancer risk by inducing uracil misincorporation during DNA synthesis, leading to chromosomal damage, DNA strand breaks and impaired DNA repair, and DNA hypomethylation (Duthie, 1999; Boccia et al., 2007).

Limitations of the meta-analysis should also be acknowledge like (is)crude odds ratio was used, (ii)studies with small sample size were included and (iii)publication bias was observed. Publication bias was observed in the present meta-analysis. Publication bias is an important problem particularly in relation to meta-analyses of genetic association studies. Negative results, especially smaller ones, may not be submitted for publication, let alone accepted, rendering any systematic review of published results misleading (Colhoun et al., 2003; Lewis et al., 2005). Despite of several limitations, present meta-analysis provided evidence of the association between the MTHFR C677T polymorphisms and breast cancer risk in Asian population, supporting the hypothesis that MTHFR C677T polymorphism is contributed to overall BC risk

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