The C677T MTHFR Polymorphism as a Risk Factor for Breast Cancer

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
substitution in the N-terminal catalytic domain and results in an alloyme 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 ‘methylene tetrahydrofolate 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 I² metric (I²=(Q - df)/Q), which is independent of the number of studies in the meta-analysis. I² takes values of between 0 and 100%, with higher values denoting a greater degree of heterogeneity (Zintzaras and Hadjigeorgiou, 2004) (I²=0% to 25%: no heterogeneity; I²=25% to 50%: moderate heterogeneity; I²=50% to 75%: large heterogeneity; I²=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-Discount (Zamora et al., 2006), using two-sided P-value.

**Publication bias**

An estimate of potential publication bias was carried out by the funnel plot, Beggs’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
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Summary Statistics

In total thirty six studies, total cases were 8,040 with CC (3754), CT (3181) and TT (1105), and controls were 10,008 with CC (4869), CT (4069) and TT (1122). In controls genotype percentage of CC, CT and TT was 45.58%, 39.92% and 13.38% respectively. Frequencies of CC and CT genotype percentage of CC, CT and TT was 47.34%, 40.77% and 11.89% respectively. In total cases and controls were 10008 with CC (4869), CT (4069), and TT (1122).

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

| Study                  | Country | Case | Control |
|------------------------|---------|------|---------|
| 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. |
| Kart et al., 2007      | China   | 125  | 103     | Cancer Res Treat 34, 716-718. |
| Yu 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  | 642     | 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      | Mianjiang Med Coll. 30, 2-4. |
| Jin et al., 2009       | China   | 41   | 100     | Mol Med Rep 2009, 2, 283-289. |
| Alshatiwi 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     | Pakistan | 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. |

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 Laird, 1986) models.

Allele contrast meta-analysis:

Meta-analysis with allele contrast showed significant association with both fixed effect (OR_Tot=1.1, 95%CI: 1.05-1.14; p<0.0001; I^2=77.3; p_hetero=0.05) and random effect model (OR_Tot=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...
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 for TT vs CC =1.24; 95% CI=1.13-1.36; p=0.0001; I²=58.28%; P heterogeneity<0.0001; P=0.04) and random (Random-effects OR for TT vs CC=1.38; 95% CI: 1.16 -1.63; p=0.0003) effect models with high statistical heterogeneity between-study. Association of mutant heterozygous genotype (CT vs CC) with fixed (OR for CT vs CC=1.03; 95% CI=0.97-1.1; p=0.28; I²=33.73%; P heterogeneity=0.02; P=0.63) and random (OR for CT vs CC=1.05; 95% CI=0.96-1.14; p=0.29) effect models were observed insignificant. Similarly combined mutant genotypes (TT+CT vs CC) showed positive association with schizophrenia using both fixed (OR for TT+CT vs CC=1.08; 95% CI=1.02-1.19; p=0.002; I²=51.57%; P heterogeneity=0.0001; P=0.20) and random (OR for TT+CT vs CC=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 for TT vs CT+CC=1.22; 95% CI=1.12-1.33; p=0.0001; I²=50.35%; P heterogeneity=0.003; P=0.03) as well as random (OR for TT vs 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
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 (Baylin et 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 acknowledged (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.

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 I² Metric and Publication Bias p-value (Egger’s test).

| Genetic Models        | Fixed effect OR (95%CI), p | Random effect OR (95%CI), p | Heterogeneity p-value (Q test) | I² (%) | Publication Bias p-value (Egger’s test) |
|-----------------------|----------------------------|-----------------------------|-------------------------------|--------|----------------------------------------|
| Allele Contrast (T vs C) | 1.12(1.05-1.14),<0.0001 | 1.23(1.13-1.37),0.0000 | <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                          | 33.7   | 0.12                                   |
| Homozygote (TT vs CC)  | 1.24(1.13-1.36),<0.0001  | 1.38(1.16-1.63),0.0003     | <0.0001                      | 58.2   | 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                       | 51.7   | 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                        | 50.3   | 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)
References

Akram M, Malik FA, Kayani MA (2012). Mutational analysis of the MTHFR gene in breast cancer patients of Pakistani population. Asian Pac J Cancer Prev, 13, 1599-603.

Alshatwi AA (2010). Breast cancer risk, dietary intake, and methylenetetrahydrofolate reductase (MTHFR) single nucleotide polymorphisms. Food Chem Toxicol, 48, 1881-5.

Antoniou AC, Pharaoh PD, McMullan G, et al (2001). Evidence for further breast cancer susceptibility genes in addition to BRCA1 and BRCA2 in a population-based study. Genet Epidemiol, 21, 1-18.

Bailey LB, Gregory JF (1999). Folate metabolism and requirements. J Nutr, 129, 779-82.

Bailey LB (2003). Folate, methyl-related nutrients, alcohol, and the MTHFR 677C>T polymorphism affect cancer risk: intake recommendations. J Nutr, 133, 3748-53.

Bax L, Yu LM, Beda N, et al (2006). Development and validation of MIX: comprehensive free software for meta-analysis of causal data. BMC Med Res Methodol, 6, 50.

Boccia S, Hung R, Ricciardi G, et al (2007). Meta- and Pooled Analyses of the Methylene tetrahydrofolate Reductase C677T and A1298C Polymorphisms and Gastric Cancer Risk: A Huge-GSEC Review. Am J Epidemiol, 167, 505-16.

Botto LD, Yang Q (2000). 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGe review. Am J Epidemiol, 151, 862-77.

Cam R, Eroglu A, Egin Y, et al (2009). Dihydrofolate reductase-Tase (DHFR) 19-bp intron-1 deletion and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms in breast cancer. Breast Cancer Res Treat, 115, 431-2.

Chen J, Gammon MD, Chan W, et al (2005). One-carbon metabolism, MTHFR polymorphisms, and risk of breast cancer. Cancer Res, 65, 1606-14.

Cheng CW, Yu JC, Huang CS, et al (2008). Polymorphism of cystosolic serine hydroxymethyltransferase, estrogen and breast cancer risk among Chinese women in Taiwan. Breast Cancer Res Treat, 111, 145-55.

Choi SW, Mason JB (2002). Folate status: effects on pathways of colorectal carcinogenesis. J Nutr, 132, 2413-4.

Chou YC, Wu MH, Yu JC, 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, 2295-300.

Colhoun HM, McKeigue PM, Davey Smith G (2003). Problems of reporting genetic associations with complex outcomes. Lancet, 361, 865-72.

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, 1047-59.

Deligezer U, Akisik EE, 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, 889-93.

DerSimonian R, Laird N (1986). Meta-analysis in clinical trials. Control Clin Trials, 7, 177-88.

Duthie SJ (1999). Folic acid deficiency and cancer: mechanisms of DNA instability. Br Med Bull, 55, 578-92.

Egger M, Davey Smith G, Schneider M, Minder C (1997). Bias in meta-analysis detected by a simple, graphical test. BMJ, 315, 629-34.

Ergul E, Sazei A, Utkan Z, Canturk NZ (2003). Polymorphisms in the MTHFR gene are associated with breast cancer. Tumour Biol, 24, 286-90.

Esteller M, Garcia A, Martinez-Palones JM, et al (1997). Germ line polymorphisms in cytochrome P450 1A1 (CYP1A1) and methylenetetrahydrofolate reductase (MTHFR) genes and endometrial cancer susceptibility. Carcinogenesis, 18, 2307-11.

Ferlay J, Bray F, Pisani P, Parkin DM (2000). Cancer incidence, mortality and prevalence worldwide. IARC Cancer Base No. 5 [CD-ROM]. Version 1.1. Lyon: IARC Press.

Friso S, Choi SW, Girelli D, et al (2002). A common mutation in the 5, 10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci USA, 99, 5606-11.

Frosst P, Bloom HJ, Milos R, et al (1995). A Candidate Genetic Risk Factor for Vascular Disease: a Common Mutation in Methylenetetrahydrofolate Reductase. Nat Genet, 10, 111-3.

Gao CM, Tang JH, Cao RX, et al (2009). MTHFR polymorphisms, dietary folate intake and breast cancer risk in Chinese women. J Hum Genet, 5, 414-8.

Hekim N, Ergen A, Yaylim I, et al (2007). No association between methylenetetrahydrofolate reductase C677T polymorphism and breast cancer. Cell Biochem Funct, 25, 115-17.

Higgins JP, and Thompson SG (2002). Quantifying heterogeneity in a meta-analysis. Stat Med, 21, 1539-58.

Hosseini M, Houshmard M, Ebrahimi A (2011). MTHFR polymorphisms and breast cancer risk. Arch Med Sci, 7, 134-7.

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, 428-31.

Hulka BS, Stark AT (1995). Breast cancer: cause and prevention. Lancet, 346, 883-7.

Inoue M, Robien K, Wang R, et al (2008). Green tea intake, MTHFR/TYMS genotype and breast cancer risk: the Singapore Chinese Health Study. Carcinogenesis, 29, 1967-72.

Jakubowska A, Gronwald J, Menkiszak J, et al (2007). Methylenetetrahydrofolate reductase polymorphisms modify BRCA1-associated breast and ovarian cancer risks. Breast Cancer Res Treat, 104, 299-308.

Jin ZZ, Lu Q, Ge DH, Zong M, Zhu QH (2009). Effect of the methylenetetrahydrofolate reductase gene C677T polymorphism on C-erbB-2 methylation status and its association with cancer. Mol Med Rep, 2, 283-9.

Jones PA, Laird PW (1999). Cancer epigenetics comes of age. Nat Genet, 21, 163-7.

Kalyankumar C, Jamil K (2006). Methylene tetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms and breast cancer in South Indian population. Breast Cancer Res Treat, 97, 428-31.

Kelsey JL (1993). Breast cancer epidemiology: summary and implications. J Nutr Biochem, 4, 66-88.

Kim YI (1999). Folate and carcinogenesis: evidence, mechanisms, and implications. J Nutr Biochem, 10, 66-88.

Kim YI (2004). Folate, colorectal carcinogenesis, and DNA methylation: lessons from animal studies. Environ Mol...
Matagen, 44, 10-25.
Kotsopoulos J, Zhang WW, Zhang S et al (2008). Polymorphisms in folate metabolizing enzymes and transport proteins and the risk of breast cancer. Breast Cancer Res Treat, 8, 9895-6.
Lajin B, Sakur AA, Ghlibreul AL, Alachkar A (2012). Association of polymorphisms in one-carbon metabolizing genes with breast cancer risk in Syrian women. Tumor Biol, 33, 1133-9.
Langsenlehner U, Kripp P, Renner W, 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, 169-72.
Le Marchand L, Haiman CA, Wilkens LR et al (2004). Association of polymorphism of folate metabolizing enzymes, carboxypeptidase, methylene tetrahydrofolate reductase and candidate genes with breast cancer risk in a multiethnic cohort study. Cancer Epidemiol Biomarkers Prev, 13, 2071-7.
Lee SA, Kang D, Nishio H, et al (2004). Methylenetetrahydrofolate reductase polymorphism, diet, and breast cancer in Korean women. Exp Mol Med, 36, 116-21.
Lewis SJ, Zammit S, Gunnell D, Smith GD (2005). A Meta-Analysis of the MTHFR C677T Polymorphism and Schizophrenia Risk. Am J Med Genet Part B, 135, 2-4.
Li WD, Chen SQ (2009). Association of methylenetetrahydrofolate reductase C677T polymorphism and breast cancer risk. J Pract Med, 25, 2031-3.
Lin J, Spitz MR, Wang Y, et al (2004). Polymorphisms of folate metabolic genes and susceptibility to bladder cancer: a case-control study. Carcinogenesis, 25, 1639-47.
Lin WY, Zhou YC, Wu MH, et al (2004). The MTHFR C677T polymorphism, estrogen exposure and breast cancer risk: a nested case-control study in Taiwan. Anticancer Res, 24, 3863-8.
Li W, Chen SH (2009). Association of methylenetetrahydrofolate reductase polymorphism and breast cancer risk in Chinese population: a meta-analysis of 22 case-control studies. Tumor Biol, 35, 1695-701.
Liu Y, Zhou LS, Xu XM, et al (2013). Association of dietary intake of folate, vitamin B6 and B12 and MTHFR genotype with breast cancer risk. Asian Pac J Cancer Prev, 14, 5189-92.
Ma E, Iwasaki M, Kobayashi M, Kasuga Y, et al (2009). 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, 447-56.
Mantel N, Haenszel W (1959). Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst, 22, 719-48.
Matthews RG, Sheppard C, Goulding C (1998). Methylenetetrahydrofolate reductase and methionine synthase: biochemistry and molecular biology. Eur J Pediatr, 157, 54-9.
Meng Q, Zhou D, Gao J, et al (2012). The association between MTHFR 677C>T polymorphism and cervical cancer: evidence from a meta-analysis. BMC Cancer, 12, 467-76.
Mir MM, Dar JD, Dar NA, 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, 4-13.
Muhammad A, Malik FA, Mahmood Akhtar K (2012). Mutational analysis of the MTHFR gene in breast cancer patients of Pakistani population. Asian Pac J Cancer Prev, 13, 1599-603.
Naushad SM, Reddy CA, Rupasree Y, 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, 715-23.
Neumann AS, Lyons HJ, Shen H, et al (2005). Methylenetetrahydrofolate reductase polymorphisms and risk of squamous cell carcinoma of the head and neck: a case-control analysis. Int J Cancer, 115, 131-6.
Norton T, Aune D, Chan D, Romaguera D (2014). Fruits and Vegetables: Updating the Epidemiologic Evidence for the WCRF/AICR Lifestyle Recommendations for Cancer Prevention. Cancer Treat Res, 159, 35-50.
Ozen F, Erdis E, Sik E, et al (2004). Germ-line MTHFR C677T, FV H1299R and PAI-1 5G/4G Variations in Breast Carcinoma. Asian Pac J Cancer Prev, 14, 2903-8.
Piyathilake CJ, Macakuso M, Johanning GL, et al (2000). Methylenetetrahydrofolate reductase (MTHFR) polymorphism increases the risk of cervical intraepithelial neoplasia. Anticancer Res, 20, 1751-7.
Prasad VV, Wilkho H (2011). Association of the functional polymorphism C677T in the methylenetetrahydrofolate reductase gene with colorectal, thyroid, breast, ovarian, and cervical cancers. Onkologie, 34, 422-6.
Qi J, Miao XP, Tan W, et al (2004). Association between genetic polymorphisms in methylenetetrahydrofolate reductase and risk of breast cancer. Zhonghua Zhong Liu Za Zhi, 26, 287-9.
Rai V, Yadav U, Kumar P (2012). Prevalence of methylenetetrahydrofolate reductase C677T polymorphism in eastern Uttar Pradesh. Indian J Human Genetics, 18, 43-6.
Sangrajrang S, Sato Y, Sakamoto H, et al (2010). Genetic polymorphisms in folate and alcohol metabolism and breast cancer risk: a case-control study in Thai women. Breast Cancer Res Treat, 123, 885-93.
Shen HB, Xu YC, Zheng YX, et al (2001). Polymorphisms of 5,10- Methylenetetrahydrofolate reductase and risk of gastric cancer in a Chinese population a case-control study. Int J Cancer, 95, 332-6.
Shrubsole MJ, Gao YT, Cai Q, et al (2004). MTHFR polymorphisms, dietary folate intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. Cancer Epidemiol Biomarkers Prev, 13, 190-6.
Song C, Xing D, Tan W, et al (2001). Methylenetetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in Chinese population. Cancer Res, 61, 3272-5.
Song C, Xing D, Tan W, Wei Q, Lin D (2001). Methylenetetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population. Cancer Res, 61, 3272-5.
Stern LL, Mason JB, Sellhub J, et al (2000). Genomic DNA hypomethylation, a characteristic of most cancers, is present in peripheral leucocytes of individuals who are homozygous for the C677T polymorphism in the methylenetetrahydrofolate reductase gene. Cancer Epidemiol Biomarkers Prev, 9, 849-53.
Sturgeon SR, Schairer C, Grauman D, et al (2004). Trends in breast cancer mortality rates by region of the United States, 1950-1999. Cancer Causes Control, 15, 987-95.
Suzuki T, Matsuo K, Hirose K, et al (2008). One-carbon metabolism-related gene polymorphisms and risk of breast cancer. Carcinogenesis, 2, 356-62.
Tu YL, Wang SB, Tan XL (2012). MTHFR gene polymorphisms are not involved in pancreatic cancer risk: a meta-analysis. Asian Pac J Cancer Prev, 13, 4627-30.
Wagner C (1995). Biochemical role of folate in cellular metabolism. In: Bailey LB (ed) Folate in health and disease. Marcel Dekker Inc, New York, pp 23-42.
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, 106-10.
Wen YY, Yang SJ, Zhang JX, Chen XY (2013).
Methylenetetrahydrofolate reductase genetic polymorphisms and esophageal squamous cell carcinoma susceptibility: a meta-analysis of case-control studies. Asian Pac J Cancer Prev, 14, 21-5.

Wilcken B, Bamforth F, Li Z, 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, 619-25.

Wu Y, Yuan X, Zheng H, et al (2010). Methylenetetrahydrofolate reductase gene c677t single nucleotide polymorphisms and susceptibility to breast cancer research. Modern Oncol, 18, 2375-8.

Wu XY, Ni J, Xu WJ, et al (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, 2199-06.

Yang X, Lippman ME (1999). BRCA1 and BRCA2 in breast cancer. Breast Cancer Res Treat, 54, 1-10.

Yu CP, Wu MH, Chou YC, Yang T, You SL, Chen CJ, Sun CA: Breast cancer risk associated with multigenotypic poly-Morphisms in folate metabolizing genes: a nested case-control study in Taiwan. Anticancer Res, 27, 1727-32.

Yuan H, Xu XY, Wang ZL (2009). The relation between polymorphisms of methylenetetrahydrofolate reductase C677T and the risk of breast cancer. J MuDan Jiang Med Univ, 30, 2-4.

Zamora J, Abraira V, Muriel A, et al (2006). Meta-DiSc: a software for meta-analysis of test accuracy data. BMC Medical Research Method, 6, 31.

Zhang SM, Hankinson SE, Hunter DJ, et al (2005). Folate intake and risk of breast cancer characterized by hormone receptor statuss. Cancer Epidemiol Biomarkers Prev, 14, 2004-8.

Zhang WB, Zhang JH, Pan QZ, et al (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, 2597-604.

Zintzaras E (2006). Association of methylenetetrahydrofolate reductase (MTHFR) polymorphisms with genetic susceptibility to gastric cancer: a meta-analysis. J Hum Genet, 51, 618-24.

Zintzaras E, Hadjigeorgiou GM (2004). The role of G196A polymorphism in the brain-derived neurotrophic factor gene in the cause of Parkinson’s disease: a meta-analysis. J Hum Genet, 50, 560-6

Zintzaras E (2007). Maternal gene polymorphisms involved in folate metabolism and risk of Down syndrome offspring: a meta-analysis. J Hum Genet, 52, 943-53.