Review

Association of Intake Folate and Related Gene Polymorphisms with Breast Cancer

Xiang CHEN^{1,2}, Hadji AHAMADA³, Ting ZHANG⁴, Zhonghu BAI^{1,2,*} and ChunXin WANG^{5,**}

¹School of Biotechnology, Jiangnan University, Wuxi, 214122, China

²National Engineering Laboratory for Cereal Fermentation Technology, Jiang Nan University, China

³Hematology and Clinical Biochemistry Department, Hospital EL-Maarouf, Comoros

⁴The Affiliated Wuxi Maternity and Child Health Care Hospital of Nanjing Medical University, China

⁵Medical Laboratory, The Affiliated Wuxi People's Hospital of Nanjing Medical University, Wuxi, 214023, China

(Received February 14, 2019)

Summary Breast cancer is one of the most common malignancies in women worldwide and is associated with a variety of risk factors. Folate and vitamin B₁₂ are key elements of the one-carbon metabolism pathway where methylenetetrahydrofolate reductase (MTHFR) plays a significant role. Though many molecular and epidemiological studies have been performed to explore the relationship between intake folate, vitamin B₁₂, MTHFR gene polymorphism and breast cancer risk, there is no consensus to date. By reviewing the relevant literatures and summarizing the potential effect of dietary folate intake on MTHFR genes polymorphism and breast cancer risk, we conclude that MTHFR C677T gene polymorphism is associated with breast cancer risk among Asian, but not Caucasians, and the MTHFR A1298C gene polymorphism is not a susceptibility factor of breast cancers. Concomitant low activity of MTHFR enzyme resulted from C677T gene polymorphism and low dietary folate intake is associated with increased breast cancer risk.

Key Words vitamin B₉, MTHFR genes polymorphism, one-carbon metabolism pathway, DNA methylation, malignancies

Background

The interrelationship among genetics, metabolic needs and dietary adequacy is a topic of intense interest in the area of folate nutrition. Incidence of breast cancer is increasing around the world and it is still the leading cause of cancer mortality in women. About 235,303 new cases of breast cancer and approximately 40,430 deaths per year have been reported in the United States (1). The incidence of breast cancer is increasing in developing countries with a rate of 3% to 4% (2). The etiology of breast cancer is still not fully understood. Population studies suggest that genetic factors, including gene polymorphisms and the presence of mutations might be strong risk factors that influence the individual differences in breast cancer susceptibility (3). Epidemiological studies have demonstrated an association between folate deficiency and an increased risk of a variety of cancers (4). The factors associated with the increased breast cancer risk include not only genetic mutations and lifestyle, but also nutritional status. However, folate is plentiful in vegetables and fruits while vitamin B₁₂ is mainly stored in animal food. All these vitamins have been confirmed with reduced risk of several cancers. The crucial role of folate as the donor

of one-carbon groups in both DNA methylation and DNA synthesis may explain some of these observations (5). Methylenetetrahydrofolate reductase (MTHFR) is a type of folate-related enzymes (6). Biological functions of folate and vitamin B₁₂ within one-carbon metabolism are to facilitate deoxynucleoside triphosphate synthesis and to provide methyl groups required for intracellular methylation reactions. To date, many molecular, epidemiological studies have been performed to evaluate the association between MTHFR gene polymorphism and different types of cancer risk in diverse populations. Methionine synthase (MTR) is another key enzyme controlling folate metabolism. It catalyzes the remethylation of homocysteine to form methionine with the methyl donated by 5-methyltetrahydrofolate (7). One-carbon metabolism pathway plays a key role in genome integrity, DNA methylation, and gene expression. Any aberration in this pathway might be associated with the risk of breast cancer and its phenotype (8). Mutations in its coding gene can lead to a decrease in the efficiency of purine nucleotide and thymidylate synthesis, as well as to modifications in DNA methylation profiles. However, variants of MTHFR C677T and A1298C are good candidates to study the role of genetic variants of folate metabolizing enzymes in breast cancer risk. The present review summarizes the association of MTHFR C677T and MTHFR A1298C variants with folate and vitamin B₁₂ intake in breast cancer susceptibility to various

***To whom correspondence should be addressed.

*E-mail: baizhonghu@jiangnan.edu.cn

**E-mail: Wangcx_wxph@163.com

Fig. 1. Simplified scheme of folate metabolism involving DNA synthesis and methylation. DHF, dihydrofolate; THF, tetrahydrofolate; DHFR, dihydrofolate reductase; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine.

populations.

Evidence Acquisition

Relevant studies published before 31 November 2017 was identified by searching PubMed and EMBASE. Searching terms were "intake folate," "B vitamins" and "breast cancer," in combination with "methylene-tetrahydrofolate reductase" or "MTHFR" or "polymorphism." We also hand-checked the reference lists of all the included studies to make sure no study was missed.

Role of folate in human metabolic processes

Folate, also known as vitamin B₉, is a water-soluble vitamin and its name is derived from the Latin word folium, which means leaf. Folate is found naturally in green leafy vegetables, cereals, legumes and fruits, while folic acid is the synthetic form of the vitamin. Folate accepts one-carbon units from donor molecules and passes them on via various biosynthetic reactions (9). In their reduced form cellular folates function conjugated to a polyglutamate chain. These folates are a mixture of unsubstituted polyglutamyl tetrahydrofolates and various substituted one-carbon forms of tetrahydrofolate (e.g., 10-formyl, 5,10-methylene, and 5-methyl) (Fig. 1). The reduced forms of the vitamin, particularly the unsubstituted dihydro and tetrahydro forms, are unstable chemically. They are easily split between the C-9 and N-10 bond to yield a substituted pteridine and *p*-aminobenzoylglutamate, which have no biologic activity (10). Substituting a carbon group at N-5 or N-10 decreases the tendency of the molecule to split; however, the substituted forms are also susceptible to oxidative chemical rearrangements and, consequently, loss of activity. The

folate found in food consists of a mixture of reduced folate polyglutamates. However, folic acid is reduced in cells by the enzyme dihydrofolate reductase to the di- and tetrahydro forms. This takes place within the intestinal mucosal cells, and 5-methyltetrahydrofolate is released into the plasma. Functional folates have one-carbon groups derived from several metabolic precursors (e.g., serine, *N*-formino-L-glutamate, folate, etc.). With 10-formyltetrahydrofolate the formyl group is incorporated sequentially into C-2 and C-8 of the purine ring during its biosynthesis. Likewise the conversion of deoxyuridylate (a precursor to RNA) into thymidylate (a precursor to DNA) is catalyzed by thymidylate synthase, which requires 5,10-methylenetetrahydrofolate. Thus, folate in its reduced and polyglutamylated forms is essential for the DNA biosynthesis cycle shown in Fig. 1. The DNA and methylation cycles both regenerate tetrahydrofolate. However, there is a considerable amount of catabolism of folate and a small loss of folate via excretion from the urine, skin, and bile (11). There is a need to replenish the body's folate content by uptake from the diet. If there is inadequate dietary folate, the activity of both the DNA and the methylation cycles will be reduced. A decrease in the former will reduce DNA biosynthesis and thereby reduce cell division.

Association of folate with breast cancer

Folate is involved in DNA synthesis, repair, and methylation. It has been hypothesized that high intake of folate may reduce the risk of human cancers, including breast cancer. In fact, breast cancer begins in any part of breast, caused by abnormal cells growth and division. Literature revealed that folate metabolism

imbalance may be involved in predisposition to cancer. Folate metabolism pathway regulates the intracellular folate pool for synthesis and methylation of DNA (12). The serum folate enters into tissue cells through folate receptors, and then it was turned into tetrahydrofolate via dihydrofolate reductase. Methylene-tetrahydrofolate reductase (MTHFR) then transformed the 5,10-methylene-tetrahydrofolate into 5-methyl-tetrahydrofolate, which supplied a methyl group for transformation of homocysteine to methionine in a reaction catalyzed by methionine synthase (MTR) (13). During the past several decades, many epidemiological studies have been conducted to evaluate the relationship between folate intake or blood folate levels and breast cancer risk (Table 1). A more pronounced inverse association between folate intake and breast cancer risk was observed among women who consumed high levels of folate cofactors (methionine, vitamin B₁₂, and vitamin B₆) than those whose intake levels of these nutrients were low (14). However, there is still no consensus on such a relationship. Several studies have suggested that high folate intake or high serum folate level may reduce the risk of breast cancer, especially for those with high alcohol consumption while some other studies found no such associations. Investigators used data from a population based case-control study of breast cancer conducted in urban Shanghai to evaluate the association of dietary folate intake and breast cancer risk, their studies found evidence of a decreased breast cancer risk associated with high folate consumption among women who do not regularly consume alcohol (14). This relationship was especially apparent among subjects who also consumed higher amounts of methionine, vitamin B₁₂, or vitamin B₆. One study found that folate intake was related to reduced breast cancer risk only among premenopausal women, whereas an inverse association was observed in postmenopausal women (15), particularly those who drank alcohol regularly. However, only a few studies have examined the association of dietary folate intake with breast cancer risk, and the results from these studies have been inconsistent (16). In addition, inadequate levels of serum folate, vitamin B₆, and vitamin B₁₂ have been associated with increased breast cancer risk (17, 18). These vitamins all participate as coenzymes in the synthesis of purines and thymidylate for DNA synthesis. Altered DNA methylation, disruption of DNA integrity, and interference with DNA repair are hypothesized mechanisms by which imbalances in folate and other B vitamins may influence nucleic acid metabolism and participate in carcinogenesis (19). The relation of folate intake with breast cancer risk has been investigated in five large prospective cohorts, most of which have not found an overall association of folate intake and breast cancer risk (15, 16, 20–22). However, in three of these studies, low folate intake seemed to increase breast cancer risk among women with high alcohol intake (15, 16, 22). This apparent contradiction may be explained by differences in folate intake between populations. Few epidemiologic studies have evaluated vitamin B₆ and vitamin B₁₂ and breast can-

cer risk. In addition, the association of micronutrient intake and breast cancer seems to differ by menopausal status and levels of other micronutrients (23, 24). It has been reported that among individuals with prevalence of homozygous TT genotype for the 677CT transition in the MTHFR gene with low folate intake has been associated with more substantial increased breast cancer risk than those with other genotypes (24).

Association of MTHFR gene polymorphisms with breast cancer

MTHFR gene polymorphisms. Breast cancer is a multifactorial disease, involving biological, endocrine factors, reproductive life, behavior and lifestyle. It is the leading cause of women death worldwide due to it metastasis to other organs. Development of human breast cancers is a multistage process, arising from genetic alterations that drive the transformation of normal mammary epithelial cells into highly malignant derivatives. Epidemiological evidence shows that folate deficiency is a breast cancer risk factor (22). MTHFR is a key enzyme in folate metabolism and regulates the intracellular folate pool for DNA synthesis and methylation (Fig. 1) (25). Two common allele variants of the MTHFR gene are C677T and A1298C, the point mutation of C to T and A to C may decrease the activity of enzyme (26). Heterozygous and homozygous carriers of the 677T allele variant reduced the activity of enzyme to 30–40% and 60–70%, respectively (27). The effect of the 1298C allele variant is less severe and homozygous carriers of this allele have a moderate 30–40% reduction of the enzyme activity, yet its function remains controversial (28). Furthermore, people who are heterozygous at both loci, C677T and A1298C, experience an intermediate activity loss of 40–50% (29). It has been shown that the 677T variant increases the plasma homocysteine concentration in humans and reduces DNA methylation in cancer patients, which indicates a reduced synthesis of methionine and a more limited availability of the methyl donor, S-adenosyl-methionine, in the presence of low activity T allele (30). Many studies investigated the association between the two genotypes and breast cancer incidence. Although significant associations were observed in some studies, a clear linkage between MTHFR gene polymorphisms and the risk to develop breast cancer has not been established (31). Recently, the two MTHFR genotypes were found to modulate the chemosensitivity of cancer cells to 5-fluorouracil and methotrexate. It was stated that two functional polymorphisms in the MTHFR gene affect the survival of ER-negative breast cancer patients (32). We did not observe significant interactions between the two SNPs and chemotherapy on breast cancer survival but observed interactions with race or ethnicity and alcohol consumption.

Prospective from meta-analysis and case-control studies. The gene encoding MTHFR is located in chromosome 1p36.3 and has several polymorphisms, the most common ones are MTHFR C677T and MTHFR A1298C. The point mutation of C to T at nucleotide 677 of the MTHFR gene (Ala222Val, rs1801133) causes an ala-

Table 1. A summary of different observational studies and meta-analysis that correlate folate intake, gene polymorphism and the risk of breast cancer.

Investigations	Study design	Findings	References
MTHFR polymorphisms and breast cancer risk	Population-based case-control study	Significant association between breast cancer, C677T & A1298C polymorphism exist	(51)
Dietary intake of folate, vitamin B ₂ , B ₆ , B ₁₂ , genetic polymorphism of related enzymes, and risk of breast cancer	A case-control study in Japan	No association between intake of folate, related B vitamins & genotypes of MTHFR/MTR & breast cancer	(70)
Dietary folate consumption and breast cancer risk	A case-control study in six Italian areas	No significant association between dietary folate consumption & breast cancer	(71)
Dietary folate consumption and breast cancer risk	A case-cohort analysis in Canada	No significant association between dietary folate consumption & breast cancer	(16)
A prospective study of folate intake and the risk of breast cancer	Prospective cohort study	Adequate folate intake may reduce breast cancer with excess alcohol consumption	(15)
Folate and other one-carbon metabolism-related nutrients and risk of postmenopausal breast cancer	Prospective cohort study	Dietary folate intake may be positively associated with postmenopausal breast cancer	(72)
A prospective study on folate, B ₁₂ , pyridoxal 5'-phosphate (B ₆) and breast cancer	Nested case-control study	Folate intake may be associated with postmenopausal breast cancer	(17)
Folate intake and risk of breast cancer by estrogen and progesterone receptor status	Swedish mammography cohort study	No association between dietary folate intake and risk of total breast cancer	(73)
Observational and genetic association studies of folate intakes and breast cancer risk	A meta-analysis	A lack of dietary folate intake is not associated with the risk of breast cancer	(74)
Folate intake and the risk of breast cancer	A systematic review & meta-analysis	The meta-analysis of total folate showed no statistically significant association with breast cancer	(75)
Dietary folate intake and breast cancer risk	A case-control study in urban Shanghai	Dietary intake of methionine, vitamin B ₁₂ , and vitamin B ₆ were not independently related to risk of breast cancer	(14)
Methionine synthase A2756G polymorphism and breast cancer risk	A meta-analysis	No significant association between A2756G and breast cancer risk	(76)
Association of MTHFR gene polymorphisms with breast cancer survival	A case-control study of African-American & Caucasian	MTHFR SNPs, C677T and A1298C, were associated with breast cancer survival	(32)
Association of MTHFR, MTRR and MTR polymorphisms with breast cancer risk	A case-control study in West China	MTHFR rs1801133 and MTRR rs1801394 polymorphisms are potential risk factors for the development of breast cancer	(77)

Table 1. (continued)

Investigations	Study design	Findings	References
Association between MTHFR C677T gene polymorphism with breast cancer	A case-control study in Iran	No genetic variation of MTHFR C677T polymorphism is involved in the breast cancer risk in a population of North Iranian patients	(78)
Association of MTHFR (C677T) gene polymorphism with breast cancer	A case-control study in North Indian	Association of the CT genotype and the T allele of the MTHFR (C667T) gene may increase the risk for breast cancer	(79)
Genetic polymorphisms of the methylenetetrahydrofolate reductase gene, plasma folate levels and breast cancer	A case-control study in Taiwan	The MTHFR 677T and 1298C variant alleles were associated with decreased risk for breast cancer	(80)
Methylenetetrahydrofolate reductase gene C677T polymorphism and breast cancer risk	A meta-analysis	Modest association between MTHFR C677T polymorphism with breast cancer exists	(81)
Methylenetetrahydrofolate reductase polymorphisms and breast cancer risk	A case-control study in China	MTHFR C677T polymorphism was significantly associated with breast cancer risk in the Chinese population	(82)
MTHFR and MTR polymorphisms and breast cancer	A case-control study in Brazil	677 C>T and 2756 A>G substitution does not appear to influence the risk of breast cancer	(83)
MTR and MTRR polymorphisms, dietary intake, and breast cancer risk	A case-control study in urban Shanghai	MTR and MTRR genotypes are not likely to play an important independent role in breast cancer etiology	(84)
Role of polymorphism of methylenetetrahydrofolatehomocysteine methyltransferase (MTR) A2756G and breast cancer risk	A case-control study in Iran	There was a significant association of breast cancer risk with MTR 2756 GG and AA polymorphism	(85)
Association of polymorphisms in one-carbon metabolism genes and postmenopausal breast cancer incidence	A case-control study	Polymorphisms (SNP) in three different genes were significantly associated with breast cancer	(86)
One carbon metabolism, MTHFR polymorphisms, and risk of breast cancer	A case-control study	The MTHFR 677T variant allele associated with increased risk of breast cancer	(36)
One carbon metabolism and breast cancer risk	A case-control study in Germany	A borderline inverse association was observed between dietary folate & breast cancer risk	(43)
Genetic polymorphisms in the one-carbon metabolism pathway and breast cancer risk	Case-control study and meta-analyses	No association was found between dietary folate intake and risk of breast cancer	(87)
Folate, vitamin B ₁₂ and postmenopausal breast cancer	Prospective study of French women	High intakes of folate & vitamin B ₁₂ were independently associated with decreased breast cancer risk	(88)
Folate and risk of breast cancer	A meta-analysis	No clear relationship between folate intake & breast cancer risk was found	(89)
Methylenetetrahydrofolate reductase polymorphism and susceptibility to breast cancer	A case-control study	The low activity C677T genotype of MTHFR may increase the risk of early onset breast cancer	(35)

Table 1. (continued)

Investigations	Study design	Findings	References
Vegetables, fruits, and related nutrients and risk of breast cancer	Case-control study in Uruguay	No evidence for an association between related nutrients, fruits, vegetables & breast cancer	(90)
Does dietary folate intake modify effect of alcohol consumption on breast cancer risk?	Prospective cohort study	No direct association was found between dietary folate intake and risk of breast cancer	(91)
Methylgroup dietary intake and risk of breast cancer among African-American women	A case-control study	High level of alcohol consumption seemed more likely to be related to tumors with un-methylated genes	(92)
MTHFR polymorphisms, diet, HRT, and breast cancer risk	Multiethnic cohort study	Folate intake exhibited no modifying effect on the genotype-breast cancer relationship	(93)
Folate, vitamin B ₆ , and vitamin B ₁₂ intake and the risk of breast cancer among Mexican women	A case-control study	High intake of folate & vitamin B ₁₂ were independently associated with decreased breast cancer risk	(94)
Plasma folate, vitamin B ₆ , vitamin B ₁₂ , homocysteine, and risk of breast cancer	A nested case-control study	Higher plasma levels of folate & vitamin B ₆ may reduce the risk of developing breast cancer	(18)
Association between MTHFR gene 1298A>C polymorphism and breast cancer susceptibility	A case-control study	No significant association between MTHFR gene 1298A>C polymorphism and breast cancer	(95)
Folate and breast cancer: the role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR)	A case-control study	No association between the MTHFR A1298C polymorphism and breast cancer risk	(31)
Methylenetetrahydrofolate reductase A1298C polymorphism and breast cancer risk	A case-control study	No association between the MTHFR A1298C polymorphism and breast cancer risk	(96)
Methylenetetrahydrofolate reductase gene and susceptibility to breast cancer	A case-control study	A significant association between MTHFR C677T polymorphism & risk of breast cancer exists in pre-menopausal women	(97)
Methylenetetrahydrofolate reductase polymorphism, diet, and breast cancer in Korean women	A case-control study	MTHFR polymorphism did not influence individual susceptibility to breast cancer	(98)
Polymorphisms of one-carbon metabolizing genes and risk of breast cancer	Population based study	no significant association between A2756G and breast cancer risk	(99)
One-carbon metabolism and breast cancer	An epidemiological perspective	No evidence of DNA methylation by one-carbon metabolism & risk of breast cancer	(100)
The methylenetetrahydrofolate reductase C677T polymorphism and breast cancer risk in Asian populations	A case-control study	A significant association between MTHFR C677T polymorphism & risk of breast cancer exists	(61)

Table 2. Related methylene tetrahydrofolate reductase C677T polymorphism and breast cancer in various populations.

Population	Findings	Reference
Chinese, Iranian, American, European, Mexican, Jordanian, Turkish, Kazakh, Indian & East Asian	Association with breast cancer risk	(40, 41, 48, 51, 52, 55–60, 62, 101)
Turkish & British	Association with pre-menopausal breast cancer risk	(35, 46, 47)
Swedish, Japanese, Brazilian & East Asian	Association with post-menopausal breast cancer	(37, 39, 49)
Caucasians, Canadian, Spanish, German & Russian	Lack of association with the risk of breast cancer	(38, 42–45, 59, 60)

nine to valine change at position 222 of the polypeptide that result in a thermolabile enzyme with reduced catalytic activity (33). Each copy of the 677T allele results in a 35% decreased MTHFR activity. In homozygous genotype of MTHFR 677TT, the MTHFR enzyme has 30% full activity. However, in the presence of heterozygous genotype of MTHFR 677CT, the activity of enzyme is 65% (34). There are controversial reports related to the role of MTHFR C677T variants in susceptibility to breast cancer in various populations (Table 1 and Table 2). Studies found an association between the increased risk of breast cancer with MTHFR C677T polymorphism in population of England, Mexican, south-eastern European women, and in population of Swedish and Brazilian post-menopausal women (35–41). In contrast, in a large sample of Canadian, Spanish, German and West Siberian Region of Russia women, the MTHFR C677T was not a risk factor for breast cancer (42–45). There are several studies from Asian populations reporting the influence of MTHFR C677T variants in breast cancer susceptibility, but also with inconsistency. It seems that the MTHFR 677TT genotype was associated with breast cancer in Chinese, Iranians, Jordanese, Turkish, Indian, Kazakhstan, and in population of Japanese post-menopausal women while unassociated with Thai, Pakistani and Syrian women (46–58). The higher risk of breast cancer in post-menopausal women than the premenopausal women could be due to different expression of estrogen receptor in the first group and the possible estrogen receptor gene misregulation in the presence of MTHFR 677TT genotype (49). In two meta-analyses, strong association was found between this polymorphism and the risk of breast cancer in East Asian population. However, the meta-analysis did not find such association in Caucasian population (59, 60). The latter meta-analysis suggested the risk of breast cancer was significantly associated with postmenopausal status (60). Also, two recent meta-analyses suggested a significant relation between MTHFR 677TT genotype and breast cancer risk in Asian populations (61, 62). The association of MTHFR C677T polymorphism with hypomethylation of DNA might suggest a role for this polymorphism in the development of cancer. According

to the literature, it seems that MTHFR C677T is associated with breast cancer risk among Asian populations, especially East Asians, but not in Caucasian populations. The inconsistent findings of association between the MTHFR C677T in various populations may underlie differences in ethnicity, lifestyle, and disease prevalence as well as possible limitations due to the relatively small sample size. There are a wide variation in the T allele frequencies of control resources in Asians (0.396), Indians (0.132), Caucasians (0.326), Middle Eastern countries (0.201), and Africans (0.196) that might account for the discrepancy in association between the MTHFR C677T polymorphism and cancer risk in different ethnic groups (62).

Association of DNA methylation with breast cancer

Diets deficient in methyl group donors (choline, folate, methionine, and vitamin B₁₂) are associated with spontaneous and chemically induced development of hepatocellular carcinoma in rats (63). Folate, in the form of 5-methyltetrahydrofolate, is involved in remethylation of homocysteine to methionine, which is a precursor of S-adenosylmethionine (SAM), the primary methyl group donor for most biological methylations including that of DNA (Fig. 1). After transferring the methyl group, SAM is converted to S-adenosylhomocysteine (SAH), a potent inhibitor of most SAM-dependent methyltransferases (64). Cravo et al. firstly proposed that a mechanism by which folate deficiency enhances carcinogenesis might be through an induction of genomic DNA hypomethylation based on the biochemical function of folate in mediating one-carbon transfer and on evidence from animal experiments that demonstrated methyl group donor deficiency-induced DNA hypomethylation (65). Thus, several human studies have investigated the correlations between DNA methylation and folate status. In human subjects with normal folate status, no significant correlations between genomic lymphocyte DNA methylation and RBC folate and plasma homocysteine concentrations were observed (66). Therefore, DNA hypomethylation was proposed as one of the possible mechanisms for the development of hepatocellular carcinoma associated with methyl-deficient diets (67). Furthermore, there is evidence that folate status influ-

ences DNA methylation through an interaction with the MTHFR C677T polymorphism (5). MTHFR is a critical enzyme in folate metabolism that catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, thereby playing an important role in DNA synthesis, maintenance of nucleotide pool balance, and DNA methylation (Fig. 1). Christensen et al. examined a cohort of women with breast cancer and the DNA methylation level at 1413 sites in 733 genes (68). They found that dietary folate intake was associated with DNA methylation class membership in primary breast tumors. Genomic DNA methylation in peripheral blood mononuclear cells was recently shown to directly correlate with folate status and inversely correlate with plasma homocysteine levels. MTHFR TT genotypes had a diminished level of genomic DNA methylation compared with those with the CC wild-type (69). When analyzed according to folate status, however, only the TT subjects with low levels of folate accounted for the diminished genomic DNA methylation.

Conclusion

Folate and MTHFR gene are key elements of the one-carbon metabolism pathway which are significantly associated with breast cancers. The association between folate and breast cancer risk largely rely on MTHFR gene polymorphism. MTHFR C677T is associated with breast cancer risk among Asians but not Caucasians, and MTHFR A1298C is not a susceptibility factor of breast cancer. Lower activity of MTHFR enzyme in the presence of C677T allele and low dietary folate intake may result in uracil disincorporation in DNA and breast cancer development. As vitamin absorption and utilization efficiency vary considerably in different populations. Further studies on relationship between serum folate and MTHFR gene polymorphism with breast cancers should be conducted.

Disclosure of state of COI

No potential conflicts of interest were disclosed.

Grant support

This work was supported by Jiangsu key R & D program (BE2018621), the national first-class discipline program of Light Industry Technology and Engineering (LITE2018-24), the Collaborative Innovation Center of Jiangsu Modern Industrial Fermentation, the 111 Project (111-2-06), the Priority Academic Program Development of Jiangsu Higher Education Institutions.

REFERENCES

- Sanjari Moghaddam A, Nazarzadeh M, Noroozi R, Darvish H, Mosavi Jarrahi A. 2016. XRCC1 and OGG1 gene polymorphisms and breast cancer: a systematic review of literature. *Iran J Cancer Prev* **9**(1): e3467.
- Yari K, Rahimi Z, Moradi MT, Rahimi Z. 2014. The MMP-2-735 C allele is a risk factor for susceptibility to breast cancer. *Asian Pac J Cancer Prev* **15**(15): 6199–6203.
- Yari K, Rahimi Z, Payandeh M, Rahimi Z. 2015. MMP-7 A-181G polymorphism in breast cancer patients from Western Iran. *Breast Care (Basel)* **10**(6): 398–402.
- Choi SW, Mason JB. 2000. Folate and carcinogenesis: an integrated scheme. *J Nutr* **130**(2): 129–132.
- Kim YI. 1999. Folate and carcinogenesis: evidence, mechanisms, and implications. *J Nutr Biochem* **10**(2): 66–88.
- Friedman G, Goldschmidt N, Friedlander Y, Ben-Yehuda A, Selhub J, Babaey S, Mendel M, Kidron M, Bar-On H. 1999. A common mutation A1298C in human methylenetetrahydrofolate reductase gene: association with plasma total homocysteine and folate concentrations. *J Nutr* **129**(9): 1656–1661.
- Fodinger M, Horl WH, Sunder-Plassmann G. 2000. Molecular biology of 5,10-methylenetetrahydrofolate reductase. *J Nephrol* **13**(1): 20–33.
- Naushad SM, Pavani A, Rupasree Y, Divyia S, Deepti S, Digumarti RR, Gottumukkala SR, Prayaga A, Kutala VK. 2012. Association of aberrations in one-carbon metabolism with molecular phenotype and grade of breast cancer. *Mol Carcinog* **51** (Suppl 1): E32–41.
- Scott J, Weir D. 1994. Folate/vitamin B12 inter-relationships. *Essays Biochem* **28**: 63–72.
- Blakley RL. 1969. Biochemistry of Folic Acid and Related Pteridines. North-Holland Publishing, Amsterdam.
- McPartlin J, Halligan A, Scott JM, Darling M, Weir DG. 1993. Accelerated folate breakdown in pregnancy. *Lancet* **341**(8838): 148–149.
- Das PM, Singal R. 2004. DNA methylation and cancer. *J Clin Oncol* **22**(22): 4632–4642.
- Biselli JM, Goloni-Bertollo EM, Haddad R, Eberlin MN, Pavarino-Bertelli EC. 2008. The MTR A2756G polymorphism is associated with an increase of plasma homocysteine concentration in Brazilian individuals with Down syndrome. *Braz J Med Biol Res* **41**(1): 34–40.
- Shrubsole MJ, Jin F, Dai Q, Shu XO, Potter JD, Hebert JR, Gao YT, Zheng W. 2001. Dietary folate intake and breast cancer risk: results from the Shanghai Breast Cancer Study. *Cancer Res* **61**(19): 7136–7141.
- Zhang S, Hunter DJ, Hankinson SE, Giovannucci EL, Rosner BA, Colditz GA, Speizer FE, Willett WC. 1999. A prospective study of folate intake and the risk of breast cancer. *JAMA* **281**(17): 1632–1637.
- Rohan TE, Jain MG, Howe GR, Miller AB. 2000. Dietary folate consumption and breast cancer risk. *J Natl Cancer Inst* **92**(3): 266–269.
- Wu K, Helzlsouer KJ, Comstock GW, Hoffman SC, Nadeau MR, Selhub J. 1999. A prospective study on folate, B12, and pyridoxal 5'-phosphate (B6) and breast cancer. *Cancer Epidemiol Biomarkers Prev* **8**(3): 209–217.
- Zhang SM, Willett WC, Selhub J, Hunter DJ, Giovannucci EL, Holmes MD, Colditz GA, Hankinson SE. 2003. Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. *J Natl Cancer Inst* **95**(5): 373–380.
- Mason JB. 2003. Biomarkers of nutrient exposure and status in one-carbon (methyl) metabolism. *J Nutr* **133** (Suppl 3(3)): 941s–947s.
- Cho E, Spiegelman D, Hunter DJ, Chen WY, Stampfer MJ, Colditz GA, Willett WC. 2003. Premenopausal fat intake and risk of breast cancer. *J Natl Cancer Inst* **95**(14): 1079–1085.
- Feigelson HS, Jonas CR, Robertson AS, McCullough ML, Thun MJ, Calle EE. 2003. Alcohol, folate, methionine, and risk of incident breast cancer in the American Cancer Society Cancer Prevention Study II Nutrition Cohort. *Cancer Epidemiol Biomarkers Prev* **12**(2): 161–164.

- 22) Sellers TA, Kushi LH, Cerhan JR, Vierkant RA, Gaps-tur SM, Vachon CM, Olson JE, Therneau TM, Folsom AR. 2001. Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women. *Epidemiology* **12**(4): 420–428.
- 23) Gaudet MM, Britton JA, Kabat GC, Steck-Scott S, Eng SM, Teitelbaum SL, Terry MB, Neugut AI, Gammon MD. 2004. Fruits, vegetables, and micronutrients in relation to breast cancer modified by menopause and hormone receptor status. *Cancer Epidemiol Biomarkers Prev* **13**(9): 1485–1494.
- 24) Shrubsole MJ, Gao YT, Cai Q, Shu XO, Dai Q, Hébert JR, Jin F, Zheng W. 2004. MTHFR polymorphisms, dietary folate intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. *Cancer Epidemiol Biomarkers Prev* **13**(2): 190–196.
- 25) Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE. 2001. Biological and clinical implications of the MTHFR C677T polymorphism. *Trends Pharmacol Sci* **22**(4): 195–201.
- 26) Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP. 1995. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* **10**(1): 111–113.
- 27) Sohn KJ, Croxford R, Yates Z, Lucock M, Kim YI. 2004. Effect of the methylenetetrahydrofolate reductase C677T polymorphism on chemosensitivity of colon and breast cancer cells to 5-fluorouracil and methotrexate. *J Natl Cancer Inst* **96**(2): 134–144.
- 28) Yamada K, Chen Z, Rozen R, Matthews RG. 2001. Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. *Proc Natl Acad Sci USA* **98**(26): 14853–14858.
- 29) Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. 1998. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab* **64**(3): 169–172.
- 30) Paz MF, Avila S, Fraga MF, Pollan M, Capella G, Peinado MA, Sanchez-Cespedes M, Herman JG, Esteller M. 2002. Germ-line variants in methyl-group metabolism genes and susceptibility to DNA methylation in normal tissues and human primary tumors. *Cancer Res* **62**(15): 4519–4524.
- 31) Sharp L, Little J, Schofield AC, Pavlidou E, Cotton SC, Miedzybrodzka Z, Baird JO, Haites NE, Heys SD, Grubb DA. 2002. Folate and breast cancer: the role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR). *Cancer Lett* **181**(1): 65–71.
- 32) Martin DN, Boersma BJ, Howe TM, Goodman JE, Mechanic LE, Chanock SJ, Ambs S. 2006. Association of MTHFR gene polymorphisms with breast cancer survival. *BMC Cancer* **6**: 257.
- 33) Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG, Rozen R. 1994. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. *Nat Genet* **7**(2): 195–200.
- 34) Lopez-Cortes A, Jaramillo-Koupermann G, Muñoz MJ, Cabrera A, Echeverría C, Rosales F, Vivar N, Paz-y-Miño C. 2013. Genetic polymorphisms in MTHFR (C677T, A1298C), MTR (A2756G) and MTRR (A66G) genes associated with pathological characteristics of prostate cancer in the Ecuadorian population. *Am J Med Sci* **346**(6): 447–454.
- 35) Campbell IG, Baxter SW, Eccles DM, Choong DY. 2002. Methylenetetrahydrofolate reductase polymorphism and susceptibility to breast cancer. *Breast Cancer Res* **4**(6): R14.
- 36) Chen J, Gammon MD, Chan W, Palomeque C, Wetmur JG, Kabat GC, Teitelbaum SL, Britton JA, Terry MB, Neugut AI, Santella RM. 2005. One-carbon metabolism, MTHFR polymorphisms, and risk of breast cancer. *Cancer Res* **65**(4): 1606–1614.
- 37) Ericson U, Sonestedt E, Ivarsson MI, Gullberg B, Carlsson J, Olsson H, Wirfält E. 2009. Folate intake, methylenetetrahydrofolate reductase polymorphisms, and breast cancer risk in women from the Malmo Diet and Cancer cohort. *Cancer Epidemiol Biomarkers Prev* **18**(4): 1101–1110.
- 38) Platek ME, Shields PG, Marian C, McCann SE, Bonner MR, Nie J, Ambrosone CB, Millen AE, Ochs-Balcom HM, Quick SK, Trevisan M, Russell M, Nochajski TH, Edge SB, Freudenheim JL. 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**(9): 2453–2459.
- 39) de Cassia Carvalho Barbosa R, da Costa DM, Cordeiro DE, Vieira AP, Rabenhorst SH. 2012. Interaction of MTHFR C677T and A1298C, and MTR A2756G gene polymorphisms in breast cancer risk in a population in Northeast Brazil. *Anticancer Res* **32**(11): 4805–4811.
- 40) Papandreou CN, Doxani C, Zdoukopoulos N, Vlachostergios PJ, Hatzidaki E, Bakalos G, Ziogas DC, Koufakis T, Zintzaras E. 2012. Evidence of association between methylenetetrahydrofolate reductase gene and susceptibility to breast cancer: a candidate-gene association study in a South-eastern European population. *DNA Cell Biol* **31**(2): 193–198.
- 41) Ramos-Silva A, Figueroa LE, Soto-Quintana OM, Puebla-Pérez AM, Ramírez-Patiño R, Gutiérrez-Hurtado I, Carrillo-Moreno DI, Zúñiga-González GM, Dávalos-Rodríguez IP, Gallegos-Arreola MP. 2015. Association of the C677T polymorphism in the methylenetetrahydrofolate reductase gene with breast cancer in a Mexican population. *Genet Mol Res* **14**(2): 4015–4026.
- 42) Kotsopoulos J, Zhang WW, Zhang S, McCready D, Trudeau M, Zhang P, Sun P, Narod SA. 2008. Polymorphisms in folate metabolizing enzymes and transport proteins and the risk of breast cancer. *Breast Cancer Res Treat* **112**(3): 585–593.
- 43) Justenhoven C, Hamann U, Pierl CB, Rabstein S, Pesch B, Harth V, Baisch C, Vollmert C, Illig T, Brüning T, Ko Y, Brauch H. 2005. One-carbon metabolism and breast cancer risk: no association of MTHFR, MTR, and TYMS polymorphisms in the GENICA study from Germany. *Cancer Epidemiol Biomarkers Prev* **14**(12): 3015–3018.
- 44) Henriquez-Hernandez LA, Murias-Rosales A, Hernández González A, Cabrera De León A, Díaz-Chico BN, Mori De Santiago M, Fernández Pérez L. 2009. Gene polymorphisms in TYMS, MTHFR, p53 and MDR1 as risk factors for breast cancer: a case-control study. *Oncol Rep* **22**(6): 1425–1433.
- 45) Vainer AS, Boiarskikh UA, Voronina EN, Selezneva IA, Sinkina TV, Lazarev AE, Petrova VD, Filipenko ML. 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**(5): 816–823.
- 46) Ergul E, Sazci A, Utkan Z, Canturk NZ. 2003. Polymorphisms in the MTHFR gene are associated with breast cancer. *Tumour Biol* **24**(6): 286–290.
 - 47) Semenza JC, Delfino RJ, Ziogas A, Anton-Culver H. 2003. Breast cancer risk and methylenetetrahydrofolate reductase polymorphism. *Breast Cancer Res Treat* **77**(3): 217–223.
 - 48) Cheng CW, Yu JC, Huang CS, Shieh JC, Fu YP, Wang HW, Wu PE, Shen CY. 2008. Polymorphism of cytosolic serine hydroxymethyltransferase, estrogen and breast cancer risk among Chinese women in Taiwan. *Breast Cancer Res Treat* **111**(1): 145–155.
 - 49) Suzuki T, Matsuo K, Hirose K, Hiraki A, Kawase T, Watanabe M, Yamashita T, Iwata H, Tajima K. 2008. One-carbon metabolism-related gene polymorphisms and risk of breast cancer. *Carcinogenesis* **29**(2): 356–362.
 - 50) Sangrajrang S, Sato Y, Sakamoto H, Ohnami S, Khuha-prema 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**(3): 885–893.
 - 51) Hosseini M, Houshmand M, Ebrahimi A. 2011. MTHFR polymorphisms and breast cancer risk. *Arch Med Sci* **7**(1): 134–137.
 - 52) Mohammad NS, Yedluri R, Addepalli P, Gottumukkala SR, Digumarti RR, Kutala VK. 2011. Aberrations in one-carbon metabolism induce oxidative DNA damage in sporadic breast cancer. *Mol Cell Biochem* **349**(1–2): 159–167.
 - 53) 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**(4): 1599–1603.
 - 54) Lajin B, Alhaj Sakur A, Ghabreau L, Alachkar A. 2012. Association of polymorphisms in one-carbon metabolizing genes with breast cancer risk in Syrian women. *Tumour Biol* **33**(4): 1133–1139.
 - 55) Akilzhanova A, Nurkina Z, Momynaliev K, Ramanulov E, Zhumadilov Z, Rakhypbekov T, Hayashida N, Nakashima M, Takamura N. 2013. Genetic profile and determinants of homocysteine levels in Kazakhstan patients with breast cancer. *Anticancer Res* **33**(9): 4049–4059.
 - 56) He JM, Pu YD, Wu YJ, Qin R, Zhang QJ, Sun YS, Zheng WW, Chen LP. 2014. Association between dietary intake of folate and MTHFR and MTR genotype with risk of breast cancer. *Genet Mol Res* **13**(4): 8925–8931.
 - 57) 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**(1): 106–110.
 - 58) Awwad N, Yousef AM, Abuhaliema A, Abdalla I, Yousef M. 2015. Relationship between genetic polymorphisms in MTHFR (C677T, A1298C and their haplotypes) and the incidence of breast cancer among Jordanian females—case-control study. *Asian Pac J Cancer Prev* **16**(12): 5007–5011.
 - 59) Qi X, Ma X, Yang X, Fan L, Zhang Y, Zhang F, Chen L, Zhou Y, Jiang J. 2010. 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**(2): 499–506.
 - 60) Zhong S, Chen Z, Yu X, Li W, Tang J, Zhao J. 2014. A meta-analysis of genotypes and haplotypes of methylenetetrahydrofolate reductase gene polymorphisms in breast cancer. *Mol Biol Rep* **41**(9): 5775–5785.
 - 61) Rai V. 2014. The methylenetetrahydrofolate reductase C677T polymorphism and breast cancer risk in Asian populations. *Asian Pac J Cancer Prev* **15**(14): 5853–5860.
 - 62) Xie SZ, Liu ZZ, Yu JH, Liu L, Wang W, Xie DL, Qin JB. 2015. Association between the MTHFR C677T polymorphism and risk of cancer: evidence from 446 case-control studies. *Tumour Biol* **36**(11): 8953–8972.
 - 63) Newberne PM, Rogers AE. 1986. Labile methyl groups and the promotion of cancer. *Amu Rev Nutr* **6**: 407–432.
 - 64) Selhub J, Miller JW. 1992. The pathogenesis of homocystinemia: interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. *Am J Clin Nutr* **55**(1): 131–138.
 - 65) Cravo ML, Mason JB, Dayal Y, Hutchinson M, Smith D, Selhub J, Rosenberg IH. 1992. Folate deficiency enhances the development of colonic neoplasia in dimethylhydrazine-treated rats. *Cancer Res* **52**(18): 5002–5006.
 - 66) Fenech M, Aitken C, Rinaldi J. 1998. Folate, vitamin B12, homocysteine status and DNA damage in young Australian adults. *Carcinogenesis* **19**(7): 1163–1171.
 - 67) Zapisek WF, Cronin GM, Lyn-Cook BD, Poirier LA. 1992. The onset of oncogene hypomethylation in the livers of rats fed methyl-deficient, amino acid-defined diets. *Carcinogenesis* **13**(10): 1869–1872.
 - 68) Christensen BC, Kelsey KT, Zheng S, Houseman EA, Marsit CJ, Wrensch MR, Wiemels JL, Nelson HH, Karagas MR, Kushi LH, Kwan ML, Wiencke JK. 2010. Breast cancer DNA methylation profiles are associated with tumor size and alcohol and folate intake. *PLoS Genet* **6**(7): e1001043.
 - 69) Friso S. 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**(8): 5606–5611.
 - 70) Ma E, Iwasaki M, Kobayashi M, Kasuga Y, Yokoyama S, Onuma H, Nishimura H, Kusama R, Tsugane S. 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**(4): 447–456.
 - 71) Negri E, La Vecchia C, Franceschi S. 2000. Re: dietary folate consumption and breast cancer risk. *J Natl Cancer Inst* **92**(15): 1270–1271.
 - 72) Stevens VL, McCullough ML, Sun J, Gapstur SM. 2010. Folate and other one-carbon metabolism-related nutrients and risk of postmenopausal breast cancer in the Cancer Prevention Study II Nutrition Cohort. *Am J Clin Nutr* **91**(6): 1708–1715.
 - 73) Larsson SC, Bergkvist L, Wolk A. 2008. Folate intake and risk of breast cancer by estrogen and progesterone receptor status in a Swedish cohort. *Cancer Epidemiol Biomarkers Prev* **17**(12): 3444–3449.
 - 74) Lewis SJ, Harbord RM, Harris R, Smith GD. 2006. Meta-analyses of observational and genetic association studies of folate intakes or levels and breast cancer risk. *J Natl Cancer Inst* **98**(22): 1607–1622.
 - 75) Tio M, Andrici J, Eslick GD. 2014. Folate intake and the risk of breast cancer: a systematic review and meta-analysis. *Breast Cancer Res Treat* **145**(2): 513–524.
 - 76) Zhong S, Xu J, Li W, Chen Z, Ma T, Zhao J. 2013. Methionine synthase A2756G polymorphism and breast can-

- cer risk: an up-to-date meta-analysis. *Gene* **527**(2): 510–515.
- 77) Niu Z, Zhao H, Hou X. 2017. Association of MTHFR, MTRR and MTR polymorphisms with breast cancer risk: A study in Chinese females. *Int J Clin Exp Pathol* **10**(6): 7059–7066.
 - 78) Hedayatzadeh-Omran A, Alizadeh-Navaei R, Toghiani-Hulari F, Amjadi O. 2017. Association between MTHFR (C677T) gene polymorphism with breast cancer in Northern Iran. *WCRJ* **4**(2): e876.
 - 79) Waseem M, Hussain SR, Kumar S, Serajuddin M, Mahdi F, Sonkar SK, Bansal C, Ahmad MK. 2016. Association of MTHFR (C677T) Gene polymorphism with breast cancer in North India. *Biomark Cancer* **8**: 111–117.
 - 80) Chou YC, Wu MH, Yu JC, Lee MS, Yang T, Shih HL, Wu TY, Sun CA. 2006. Genetic polymorphisms of the methylenetetrahydrofolate reductase gene, plasma folate levels and breast cancer susceptibility: a case-control study in Taiwan. *Carcinogenesis* **27**(11): 2295–2300.
 - 81) Kumar P, Yadav U, Rai V. 2015. Methylenetetrahydrofolate reductase gene C677T polymorphism and breast cancer risk: Evidence for genetic susceptibility. *Meta Gene* **6**: 72–84.
 - 82) Liang H, Yan Y, Li T, Li R, Li M, Li S, Qin X. 2014. Methylenetetrahydrofolate reductase polymorphisms and breast cancer risk in Chinese population: a meta-analysis of 22 case-control studies. *Tumour Biol* **35**(2): 1695–1701.
 - 83) Da Conceicao LL, Pessoa MC, Hermsdorff HHM, De Freitas RN, Do Carmo Gouveia Peluzio M. 2016. MTHFR and MTR polymorphisms and breast cancer in Brazilian women. *World J Res Rev* **2**(6): 29–32.
 - 84) Shrubsole MJ, Gao YT, Cai Q, Shu XO, Dai Q, Jin F, Zheng W. 2006. MTR and MTRR polymorphisms, dietary intake, and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* **15**(3): 586–588.
 - 85) Hosseini M. 2013. Role of polymorphism of methyltetrahydrofolate-homocysteine methyltransferase (MTR) A2756G and breast cancer risk. *Pol J Pathol* **3**: 191–195.
 - 86) Stevens VL, McCullough ML, Pavluck AL, Talbot JT, Feigelson HS, Thun MJ, Calle EE. 2007. Association of polymorphisms in one-carbon metabolism genes and postmenopausal breast cancer incidence. *Cancer Epidemiol Biomarkers Prev* **16**(6): 1140–1147.
 - 87) Lissowska J, Gaudet MM, Brinton LA, Chanock SJ, Peplonska B, Welch R, Zatonski W, Szeszenia-Dabrowska N, Park S, Sherman M, Garcia-Closas M. 2007. Genetic polymorphisms in the one-carbon metabolism pathway and breast cancer risk: a population-based case-control study and meta-analyses. *Int J Cancer* **120**(12): 2696–2703.
 - 88) Lajous M, Romieu I, Sabia S, Boutron-Ruault MC, Clavel-Chapelon F. 2006. Folate, vitamin B12 and postmenopausal breast cancer in a prospective study of French women. *Cancer Causes Control* **17**(9): 1209–1213.
 - 89) Larsson SC, Giovannucci E, Wolk A. 2007. Folate and risk of breast cancer: a meta-analysis. *J Natl Cancer Inst* **99**(1): 64–76.
 - 90) Ronco A, De Stefani E, Boffetta P, Deneo-Pellegrini H, Mendilaharsu M, Leborgne F. 1999. Vegetables, fruits, and related nutrients and risk of breast cancer: a case-control study in Uruguay. *Nutr Cancer* **35**(2): 111–119.
 - 91) Baglietto L, English DR, Gertig DM, Hopper JL, Giles GG. 2005. Does dietary folate intake modify effect of alcohol consumption on breast cancer risk? Prospective cohort study. *BMJ* **331**(7520): 807.
 - 92) Zhu K, Davidson NE, Hunter S, Yang X, Payne-Wilks K, Roland CL, Phillips D, Bentley C, Dai M, Williams SM. 2003. Methyl-group dietary intake and risk of breast cancer among African-American women: a case-control study by methylation status of the estrogen receptor alpha genes. *Cancer Causes Control* **14**(9): 827–836.
 - 93) Le Marchand L, Haiman CA, Wilkens LR, Kolonel LN, Henderson BE. 2004. MTHFR polymorphisms, diet, HRT, and breast cancer risk: the multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev* **13**(12): 2071–2077.
 - 94) Lajous M, Lazcano-Ponce E, Hernandez-Avila M, Willett W, Romieu I. 2006. Folate, vitamin B(6), and vitamin B(12) intake and the risk of breast cancer among Mexican women. *Cancer Epidemiol Biomarkers Prev* **15**(3): 443–448.
 - 95) Zhang J, Zhang L, Li G. 2016. Association between MTHFR gene 1298A>C polymorphism and breast cancer susceptibility: a meta-analysis based on 38 case-control studies with 40,985 subjects. *World J Surg Oncol* **14**(1): 230.
 - 96) Rai V. 2014. Methylenetetrahydrofolate reductase A1298C polymorphism and breast cancer risk: A meta-analysis of 33 studies. *Ann Med Health Sci Res* **4**(6): 841–851.
 - 97) Zintzaras E. 2006. Methylenetetrahydrofolate reductase gene and susceptibility to breast cancer: a meta-analysis. *Clin Genet* **69**(4): 327–336.
 - 98) Lee SA, Kang D, Nishio H, Lee MJ, Kim DH, Han W, Yoo KY, Ahn SH, Choe KJ, Hirvonen A, Noh DY. 2004. Methylenetetrahydrofolate reductase polymorphism, diet, and breast cancer in Korean women. *Exp Mol Med* **36**(2): 116–121.
 - 99) Xu X, Gammon MD, Zhang H, Wetmur JG, Rao M, Teitelbaum SL, Britton JA, Neugut AI, Santella RM, Chen J. 2007. Polymorphisms of one-carbon-metabolizing genes and risk of breast cancer in a population-based study. *Carcinogenesis* **28**(7): 1504–1509.
 - 100) Xu X, Chen J. 2009. One-carbon metabolism and breast cancer: an epidemiological perspective. *J Genet Genomics* **36**(4): 203–214.
 - 101) Ozen F, Erdi S, 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**(5): 2903–2908.