Gene 533 (2014) 11–20

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Review

Folate: Metabolism, genes, polymorphisms and the associated diseases

Fakhira Hassan Nazki [a,1], Aga Syed Sameer [b,*,2], Bashir Ahmad Ganaie [a,3]

[a] Department of Biochemistry, University of Kashmir, Hazratbal, Srinagar, Kashmir 190006, India
[b] Department of Biochemistry, Sher-I-Kashmir Institute of Medical Sciences Associated Medical College, Bemina, Srinagar, Kashmir 190018, India

ARTICLE INFO

Article history:
Accepted 17 September 2013
Available online 1 October 2013

Keywords:
Folate
Polymorphisms
MTHFR
MTR
MTRR
TS

ABSTRACT

Folate being an important vitamin of B Complex group in our diet plays an important role not only in the synthesis of DNA but also in the maintenance of methylation reactions in the cells. Folate metabolism is influenced by several processes especially its dietary intake and the polymorphisms of the associated genes involved. Aberrant folate metabolism, therefore, affects both methylation as well as the DNA synthesis processes, both of which have been implicated in the development of various diseases. This paper reviews the current knowledge of the processes involved in folate metabolism and consequences of deviant folate metabolism, particular emphasis is given to the polymorphic genes which have been implicated in the development of various diseases in humans, like vascular diseases, Down's syndrome, neural tube defects, psychiatric disorders and cancers.

Contents

Abbreviations: THF, tetra-hydro folate; DHF, di-hydro folate; MTHFR, methylene tetra-hydro folate reductase; DHFR, di-hydro folate reductase; TS, thymidylate synthetase; MTR, methionine synthase; CBS, cystathionine β-synthase; CTH, cystathionine γ-lyase; dUMP, deoxyuridylate monophosphate; dTMP, deoxythymidylate monophosphate; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; DNMTs, DNA methyltransferases; NTD, neural tube defects; DS, Down's syndrome.

* Corresponding author. Tel.: +91 9906727948 (mobile).
E-mail addresses: faakhra.nazki@gmail.com (F.H. Nazki), mousvi786@gmail.com (A.S. Sameer), agsysa@gmail.com (B.A. Ganaie).

[1] Did the literature survey, analyzed the results of various studies and wrote the manuscript.
[2] Designed the review, did the literature survey and edited and structured the draft copy to make it in final form.
[3] Helped in the literature survey.

0378-1119/$ – see front matter.
http://dx.doi.org/10.1016/j.gene.2013.09.063

1. Introduction

The folates include the family of B-group vitamins composed of an aromatic pteridine ring attached through a methylene group to p-aminobenzoic acid and a glutamate residue (Shane, 1995). Folate metabolism plays a vital role in nucleic acid synthesis, methionine regeneration, shuttling and redox reactions of one carbon units required for normal metabolism and regulation (Bailey and Gregory, 1999). Folates mediate the transfer of one carbon units required in various biochemical reactions. It plays a critical role in the synthesis of S-adenosylmethionine (SAM) which serves as the methyl group donor in several methylation reactions; like DNA, RNA and protein methylation. DNA methylation in turn is an important epigenetic determinant in gene expression, DNA stability, DNA integrity and mutagenesis. Folate also plays an essential role in the de novo synthesis of purines and thymidylate, which is required in DNA replication and repair (Kim, 2000). Thus, deviant distribution of methyl groups due to abnormal folate metabolism affects both methylation and DNA synthesis-processes which play an essential role in the development of cancers (Hubner and Houlston, 2009). Abnormal folate status has also been implicated in the development of diseases like; cardiovascular diseases, neural tube defects, cleft lip and palate, late pregnancy

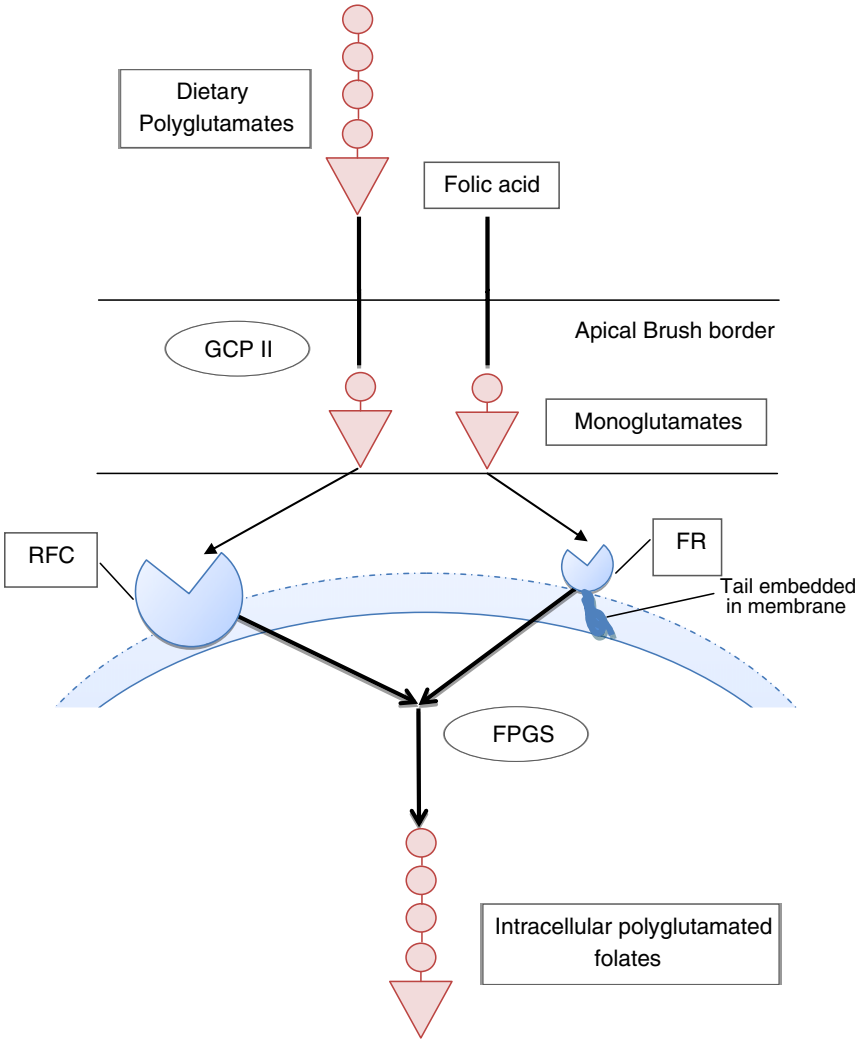

Fig. 1. Summary of folate transport. The polyglutamates are converted into monoglutamated folates by gamma-glutamylcarboxypeptidase activity in the intestinal juice. Folate is then absorbed into the blood through a pH dependent carrier mediated process in the jejunum brush border. The monoglutamates in the blood are then transported inside the cell via different processes involving membrane embedded folate receptors or reduced folate carrier. Folate is retained in tissues by polyglutamation. Abbreviations: FR, folate receptor; RFC, reduced folate carrier; FPGS, foly-poly-glutamate-synthase; GCP II, glutamate carboxypeptidase II.

complications, neurodegenerative and psychiatric disorders (Blom and Smulders, 2011).

2. Folate uptake and transport

The primary source of folates in mammals is diet (green vegetables, legumes, oranges and liver). Dietary folates primarily exist in polyglutamate forms and are unable to cross the cell membrane when the glutamate tail is longer than three residues (Shane, 1995). Therefore, in the small intestines of humans where folate is absorbed, the folates are first hydrolyzed into monoglutamates; this process is catalyzed by an exopeptidase glutamate carboxypeptidase II (GCPII) that is anchored to the intestinal apical brush border membrane (Chandler et al., 1986). After hydrolysis, this monoglutamyl folate is transported into the cells by three mechanisms (Fig. 1) (Antony, 1996; Kim, 2007; Sirotnak and Tolner, 1999).

1. Facilitative anion exchange: This mechanism involves the use of a facilitative anion exchanger, reduced folate carrier (RFC) which has a higher affinity for reduced folates (Km = 1–5 μM) than folic acid (Km = 100–200 μM).
2. Another process involves the use of folate receptors embedded in the cell membranes through a glycosyl-phosphatidylinositol moiety. These folate receptors transport folates via an endocytic process and have a very high affinity for folic acid (Km < 1 nM).
3. The third process involving passive diffusion has only been documented as a pharmacological effect (Antony, 1996; Sirotnak and Tolner, 1999).

Although monoglutamates are the only circulating forms of folate in blood and the only form of folate that is transported across the cell membrane, once inside the cell, they exist predominantly as polyglutamates (Fig. 1). This conversion into polyglutamates is brought about by folylpolyglutamate synthase (FPGS). This polyglutamylation of cellular folates is a form of metabolic trapping and allows the retention of folates that would otherwise be lost to efflux from cells. Moreover, polyglutamylated folates are better substrates than monoglutamates for intracellular folate dependent enzymes (Kim, 2007).

3. Enzymes involved in folate metabolism

Folate metabolism involves reduction of the carbon atoms at the oxidation levels of formyl, methylene or methyl and covalently linked to nitrogen at position 5 or 10 (Fowler, 2001). Folate metabolism regulates these processes via a complex pathway involving at least 30 different enzymes (Lightfoot et al., 2005). A simplified version of the folate metabolism cycle that illustrates the key enzymes involved is shown in Fig. 2.

3.1. Dihydrofolate reductase (DHFR)

The enzyme DHFR catalyzes the reduction of dietary folic acid or dihydrofolate to THF, the predominant form of folate in plasma. The folate compounds are thereby reduced so as to synthesize the coenzymes which play a role in many metabolic pathways (Fowler, 2001; Shane, 1995).

3.2. C1-THF synthetase

C1-THF synthetase is a multi-enzyme complex which comprises of three enzymatic activities namely, 10-formyl-THF synthetase, 5,10-methenyl-THF cyclohydrolase, and 5,10-methylene-THF dehydrogenase catalyzing the interconversion of:

1) 10-Formyl-THF with THF and formate
2) 10-Formyl-THF with 5,10-methenyl-THF
3) 5,10-Methenyl-THFwith 5,10-methylene-THF (Fowler, 2001; Tan et al., 1977).

3.3. 5,10-Methylenetetrahydrofolate reductase (5,10-MTHFR)

5,10-MTHFR carries out a central reaction in folate metabolism. It irreversibly catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate — the primary circulating form of folate (Sharp and Little, 2004). The substrate of this reaction 5,10-MTHF is vital for DNA synthesis, it also acts as the entry point of the one carbon units from serine, through a reaction involving enzyme serine

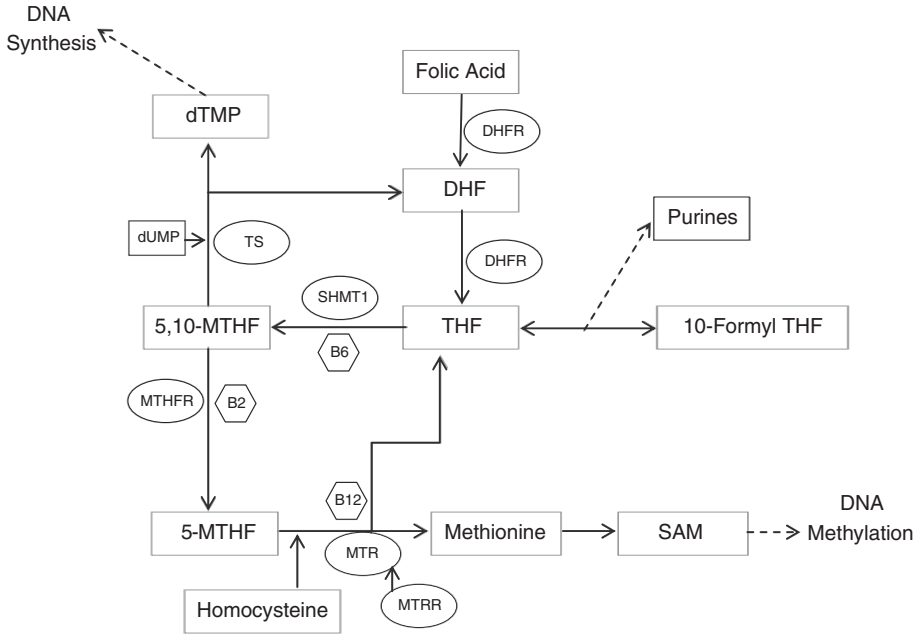

Fig. 2. Folate metabolism: DHFR, dihydrofolate reductase; SHMT1, serine hydroxymethyl transferase 1; B_6, vitamin B_6; MTHFR, methylenetetrahydrofolate reductase; B_2, vitamin B_2; TS, thymidylate synthase; MTR, methionine synthase; B_{12}, vitamin B_{12}; MTRR, methionine synthase reductase; DHF, dihydrofolate; THF, tetrahydrofolate; 5,10-MTHF, 5,10-methyltetrahydrofolate; 5-MTHF, 5-methyltetrahydrofolate; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidylate monophosphate; 10-Formyl THF, 10-formyl tetrahydrofolate; SAM, S-adenosylmethionine.

Table 1
Important genes and associated enzymes of folate metabolism.

Candidate gene	Location in genome	Main functions	References
DHFR	5q11.2-13.2	It catalyzes tetrahydrofolate regeneration by reduction of dihydrofolate, using NADPH as a cofactor. Tetrahydrofolate is essential for de novo purine and thymidylate synthesis, hence DHFR has a critical role in cell growth and proliferation.	Shane (1995), Fowler (2001)
TS	18p11.31-p11.21	Thymidylate synthase catalyzes the methylation of deoxyuridylate to deoxythymidylate using 5,10-methylenetetrahydrofolate (methylene-THF) as a cofactor. This function maintains the dTMP (thymidine-5-prime monophosphate) pool critical for DNA replication and repair.	Blom and Smulders (2011)
MTHFR	18p11.32	The protein encoded by this gene catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine.	Fowler (2001), Sharp and Little (2004), Tan et al. (1977)
MTR	1q43	MTR encodes the enzyme 5-methyltetrahydrofolate-homocysteine methyltransferase. This enzyme, also known as cobalamin-dependent methionine synthase, catalyzes the final step in methionine biosynthesis.	Fowler (2001), Sharp and Little (2004);
MTRR	5p15.31	The protein encoded by this gene regenerates a functional methionine synthase via reductive methylation.	Wilson et al. (1999)
CBS	21q22.3	The protein encoded by this gene acts as a homotetramer to catalyze the conversion of homocysteine to cystathionine, the first step in the trans-sulfuration pathway.	Blom and Smulders (2011)
CTH	1p31.1	This gene encodes a cytoplasmic enzyme in the trans-sulfuration pathway that converts cystathionine derived from methionine into cysteine. Glutathione synthesis in the liver is dependent upon the availability of cysteine.	Blom and Smulders (2011)

hydroxymethyltransferase which catalyzes the interconversion of glycine and serine (Fowler, 2001). The product 5-MTHF in turn provides methyl groups for the formation of methionine, a reduced pool of which may alter DNA methylation (Tan et al., 1977).

3.4. Thymidylate synthase (TS)

TS catalyzes the conversion of dUMP to dTMP using 5,10-methylene-tetrahydrofolate as donor of methyl group. Dihydrofolate (DHF) is also generated in this reaction which is converted back to THF by the action of DHFR (Blom and Smulders, 2011).

3.5. Methionine synthase (MTR)

MTR catalyzes the remethylation of homocysteine to methionine through the transfer of a methyl group from 5-MTHF to homocysteine (Fowler, 2001). The reaction is vital as it ensures the provision of methionine for the production of S-adenosylmethionine (SAM), the universal donor of methyl groups (Sharp and Little, 2004). Cobalamin (Cbl) acts as a cofactor for this reaction, the resulting complex, Cbl(I) MTR, binds the methyl group from 5-methyl-THF to form methylcbl(III) MTR which transfers the methyl group to homocysteine leaving behind Cbl(I)MTR. Cob(I)alamin can also get oxidized to an inactive form, cob(II)alamin. Methionine synthase reductase (MTRR) reactivates the Cbl(II)MTR complex by reductive methylation, using AdoMet as a methyl donor (Leclerc et al., 1998).

3.6. Cystathionine β-synthase (CBS) and cystathionine γ-lyase (CTH)

Cystathionine β-synthase (CBS) and cystathionine γ-lyase (CTH) catalyze the irreversible degradation of homocysteine to cysteine. Vitamin B_6 is required for this reaction. CBS catalyzes the condensation of homocysteine and serine to cystathionine and CTH catalyzes the hydrolysis of cystathionine to cysteine and α-ketobutyrate. Cysteine not only is required in protein synthesis but also acts as a precursor of glutathione, a potent antioxidant and an important compound in detoxification of xenobiotics (Blom and Smulders, 2011).

4. Biochemical role of folate

The purpose of this complex biochemical network of one carbon transfer reactions is to transfer carbons from amino acids like serine, glycine and methionine as methyl groups for nucleotide synthesis and methylation reactions (Liu and Ward, 2010; Nijhout et al., 2008; Wagner, 1995).

4.1. Nucleotide biosynthesis

The folates 5,10-methylene-THF, 5,10-methenyl-THF, and 10-formyl-THF are required for DNA synthesis. In the synthesis of purines, 10-formyl-THF donates one-carbon units to carbon atoms 2 and 8 of the purine ring (Liu and Ward, 2010). 5,10-Methylene-THF also plays a crucial role in the methylation of deoxyuridylate monophosphate (dUMP) to deoxythymidylate monophosphate (dTMP). This reaction is the sole de novo source of thymidine and the rate limiting step in mammalian DNA synthesis (Choi and Mason, 2002). Consequently, folate deficiency in tissues with rapidly replicating cells results in ineffective DNA synthesis (Kim, 2007). For example, impaired thymidylate synthesis can increase the misincorporation of dUTP into DNA thereby creating strand nicks and if two nicks appear transversely within 12 bases of each other, a strand breakage can occur, causing DNA instability and increased mutagenesis (Liu and Ward, 2010). Uracil misincorporation and double strand breaks have been observed in cultured tumor cells, and blood and tissues from rodents and humans with low folate status (Duthie and Hawdon, 1998; Duthie et al., 2000, 2002; Liu and Ward, 2010).

Similarly, impaired purine synthesis restricts the cell's capacity to synthesize and repair DNA. When cells were cultured in folate-deplete media supplemented with hypoxanthine (a purine precursor which bypasses the need for folate-dependent purine synthesis), chromosomal damage was significantly reduced compared to cells cultured in folate-deplete media alone (Libbus et al., 1990).

4.2. Methylation pathways

5-Methyl-THF donates methyl groups for the remethylation of homocysteine to methionine (Hubner and Houlston, 2009). Methionine in turn acts as a substrate for S-adenosylmethionine (SAM), a cofactor and methyl group donor for methylation reactions including the methylation of DNA, RNA, neurotransmitters, lipids and proteins like, histones (Crider et al., 2012). These methylation reactions are catalyzed by cellular methyltransferases, for example, DNA methyltransferases (DNMTs), methylate cytosine residues in CpG dinucleotides, forming 5-methylcytosine (Liu and Ward, 2010). After donating its methyl group, SAM is converted to S-adenosylhomocysteine (SAH), a competitive inhibitor of numerous methyltransferases (Ryan and Weir, 2001). SAM itself is a potent inhibitor of MTHFR. When SAM is present in high concentration, MTHFR is inhibited, which decreases the synthesis of 5-methyl-THF and hence remethylation of homocysteine. Conversely, when SAM concentrations are low, it favors the remethylation of homocysteine. 5-Methyl-THF formation can also be modified by common MTHFR gene polymorphisms (Crider et al., 2012).

Folate deficiency therefore can result in a reduction in the availability of one carbon groups required for methylation reactions, which in turn

causes accumulation of homocysteine. Homocysteinemia is associated with vascular disease, neural tube defects and Alzheimer's disease, although the precise mechanism and causal association have yet to be determined (Wilcken and Wilcken, 2001). Elevated homocysteine also leads to an accumulation of SAH, which actively inhibits SAM-dependent methyltransferases, including DNMTs (Yi et al., 2000). Both a deficiency of SAM and an accumulation of SAH may cause DNA hypomethylation (Liu and Ward, 2010) and affect malignancy.

4.3. Unsubstituted folate

Folate species DHF and THF do not play a direct role in metabolism, but receive one-carbon moieties and transfer them toward either methylation or DNA-synthesis pathways. Methotrexate is a competitive and irreversible inhibitor of the enzyme DHF reductase which converts folic acid and DHF to THF, and thus inhibits both nucleotide biosynthesis and remethylation reactions.

Therefore, given its metabolic importance, the folate cycle is tightly regulated via feedback regulation and end product inhibition to ensure the efficient utilization of dietary folate and maintain proper balance of intracellular folate pools (Liu and Ward, 2010).

5. Functional polymorphisms of folate metabolic genes

Folate status could potentially be perturbed by polymorphisms in the genes involved in its metabolism. In this section the polymorphisms in the various genes involved in folate metabolism and their association with the development of diseases is discussed (Table 1).

5.1. MTHFR

The MTHFR gene, *MTHFR*, is located at 1p36.3 (Goyette et al., 1994). Several polymorphisms in the *MTHFR* gene have been reported, and most studied polymorphisms are:

1) C677T: C → T at nucleotide 677, resulting in an alanine to valine conversion in the protein (Frosst et al., 1995)
2) A1298C: A → C at nucleotide 1298, leading to an alanine to glutamate conversion in the protein (29, 30). These polymorphisms are located 2.1 kb apart. The other reported polymorphisms are—T1059C, T1317C, and G1793A (Rady et al., 2002; Sharp and Little, 2004; Trembath et al., 1999; Weisberg et al., 1998).

In case of C → T polymorphisms it has been found that compared with the normal homozygous variants (677CC) the heterozygous (677CT) variants have 65% of their enzyme activity levels in vitro whereas homozygotes for the variant (677TT) have 30% activity (Rozen, 1997). The microbiological assay studies have shown that compared with CC homozygotes, heterozygotes have 10% lower and TT homozygotes have 18% lower red cell folate levels (Molloy et al., 1997). Individuals with the TT variant have also been found to have lowered plasma folate and vitamin B_{12} levels and raised homocysteine levels (Ma et al., 1997, 1999). With respect to DNA methylation, one small study involving MTHFR polymorphisms have found that DNA from subjects homozygous for (677TT) variant had a significantly higher methyl group acceptance capacity than DNA from subjects homozygous for the (677CC) variant (Stern et al., 2000) however, this finding was not confirmed in a larger study (Narayanan, 2001). In a study involving 292 subjects (66% of whom had coronary atherosclerosis) selected by MTHFR genotype (187CC, 105TT), DNA methylation status was found to be affected by genotype among only those with lower plasma folate levels; subjects with the TT variant who had lower plasma folate concentrations had lower methylation levels than all other groups of subjects (Friso et al., 2002). A few in vitro and in vivo studies investigating MTHFR and uracil misincorporation, DNA strand breaks, or genetic instability have shown inconclusive results (Andreassi et al., 2003; Crott et al., 2001; Narayanan, 2001; Zijno et al., 2003).

Similarly for A1298C, compared with the normal homozygotes (AA) enzyme activity in vitro has been shown to be lowered in homozygote variants (CC) and, to a lesser extent, in heterozygotes (AC) (van der Put et al., 1998). Studies of A1298C and plasma folate and homocysteine are inconsistent (Chen et al., 2002; Friedman et al., 1999; Lievers et al., 2001; Weisberg et al., 1998). Enzyme activity in vitro for compound heterozygotes (i.e., heterozygotes for C677T and for A1298C) is unclear (Lievers et al., 2001).

5.2. MTR

The MTR gene, *MTR* is located on 1q43 (Leclerc et al., 1996). The A → G polymorphism at position 2756 in the protein binding region of *MTR* which replaces aspartic acid with glycine has been identified (van der Put et al., 1997). Most studies have shown that plasma homocysteine level is lower in individuals with the, G, than the common, A, allele (Anwar et al., 2001; Chen et al., 2001; Dekou et al., 2001; Ma et al., 1999; Silaste et al., 2001). In one study however, the individuals with the GG variant were shown to have significantly higher folate levels that in those with the common AA variant (49), but this finding was not observed in another study (Ma et al., 1999).

5.3. MTRR

MTRR gene, *MTRR*, is located at 5p15.31 and the A66G polymorphism in the *MTRR* gene causes the substitution of isoleucine with methionine at codon 22 (Wilson et al., 1999). Studies have shown that subjects homozygous for the common allele (AA) had elevated homocysteine levels compared with those who had other genotypes (Gaughan et al., 2001; Geisel et al., 2001); however one study has shown that, genotype was not a significant predictor of homocysteine level (O'Leary et al., 2002).

5.4. TS

Thymidylate synthase gene (*TS*) is located on chromosome 18p11.32 (Sharp and Little, 2004). Several polymorphisms in the *TS* have been reported, which may influence TS mRNA transcription, message stability or protein expression, which have been described recently (Zhang et al., 2004). One of them is a unique tandem repeat sequence in the TS 5′UTR immediately upstream of the ATG codon initiation site that contains two, three or more 28-bp repeats (Kaneda et al., 1987). The presence of the triple (3R) versus double (2R) 28-bp repeat sequence has been shown to enhance mRNA transcription and protein expression in vitro and in vivo studies (Horie et al., 1995; Kawakami et al., 1999). In vitro studies have shown that compared with the double repeat, the triple repeat is associated with 2.6 fold greater thymidylate synthase expression. (Horie et al., 1995). Also studies have shown that the folate levels were significantly lower and homocysteine levels non-significantly higher in 3 rpt/3 rpt subjects than in other genotypes (Trinh et al., 2002). A

Table 2
Folate gene polymorphisms and diseases.

Candidate gene	Known polymorphism	Diseases implicated in
MTHFR	C677T A1298C	Breast cancer, colon cancer, gastric cancer, head & neck cancer, acute lymphoblastic leukemia, coronary artery disease, neural tube defect, depression, chromosomal abnormalities
TS	28-bp repeat sequence	Breast cancer, lung cancer
MTR	A2756G	Down's syndrome
MTRR	A66G	Breast cancer, head & neck cancer, acute lymphoblastic leukemia, Down's syndrome

novel G → C single nucleotide polymorphism (SNP) in the second repeat of the 3R alleles has been identified recently. The 3R sequence with guanine (3G) shows three to four times greater efficiency of translation than the 3R with cytidine (3C) and the 2R sequence (Kawakami and Watanabe, 2003; Mandola et al., 2003). Another TS polymorphism, a 6-bp deletion in the 3′UTR, was discovered by searching the expressed sequence tag database (Ulrich et al., 2000). It has been reported recently that the allele with a 6-bp deletion (6 bp) is associated with decreased mRNA stability in vitro and lower intra-tumoral TS expression in vivo (Mandola et al., 2004).

6. Association of folate gene polymorphisms with various diseases

6.1. Cancer

Altered folate metabolism due to variation in the distribution of methyl groups affects DNA biosynthesis and DNA methylation, both of which are very crucial in relation to carcinogenesis (Choi and Mason, 2002). The first mechanism through which altered folate metabolism can affect the DNA integrity and stability and contribute to neoplastic transformation is through altered methylation. Methylation of the cytosine residues of cytosine–guanine dinucleotide pairs is an important epigenetic determinant of gene expression and also has a role in maintaining DNA stability. Hypermethylation of gene promoter regions results in loss of tumor suppressor gene function, whereas reduced 'global' methylation results in chromosomal instability and an increase in mutational events (Eden et al., 2003). Long-term dietary folate deficiency in humans results in global DNA hypomethylation in lymphocytes, which is reversible on repletion of folate status (Jacob et al., 1998). The underlying process is thought to be the reduction in the availability of methyl groups which affects the methylation of homocysteine to methionine, leading to a rise in homocysteine levels which in turn results in raised levels of S-adenosylhomocysteine (SAH) — an inhibitor of methylation reactions (Table 2).

The second mechanism through which folate deficiency can disrupt DNA integrity and promote carcinogenesis is the reduced thymidylate synthesis leading to uracil misincorporation. Removal of a uracil base from the newly synthesized DNA results in the creation of a single-strand DNA break, and where two adjacent uracil bases lie on opposite strands, a double-strand DNA break will occur which is difficult to repair and is associated with an increased cancer risk (McKinnon and Caldecott, 2007). Increased DNA uracil content and chromosomal breaks occur in folate-deficient humans, and both can be reversed by restoration of adequate folate status (Blount et al., 1997).

The fact that folate metabolism can affect both DNA synthesis and methylation has made gene-environmental variants that impact on this pathway attractive candidates as cancer susceptibility factors. These include dietary intakes of folate and folic acid, and functional polymorphisms in the genes coding for folate metabolism enzymes. The role of these variants in some cancers is discussed below.

6.1.1. Breast cancer

MTHFR polymorphisms have been extensively studied in breast cancer but the results are inconsistent (Lewis et al., 2006). Relationships between folate status and *MTHFR* genotype have been examined in respect to breast cancer risk in Chinese women (Shrubsole et al., 2004). Although there was no difference in the distribution of *MTHFR C677T* genotype among cases and controls, there was a significant inverse association of breast cancer risk with dietary folate intake for each of the genotypes that appeared to be stronger for those carrying the TT version of the gene. Many studies reported the interactive effect between folate intake and the one-carbon metabolism-related gene on breast cancer (Chen et al., 2005; Ericson et al., 2009; Hosseini et al., 2011; Le Marchand et al., 2004; Lissowska et al., 2007; Stevens et al., 2007; Suzuki et al., 2008; Xu et al., 2007). The gene–diet interactions between folate, vitamins B_2, B_6, B_{12}, and methionine as dietary factor, and *MTHFR*, *MTR*, *MTRR*, *SHMT1*, *MTHFD1*, *TS*, *FTHFD*, *CBS*, as the one-carbon metabolism related gene have also been studied. Among these gene–diet combinations, there was an interactive effect between *MTHFR* and folate, *MTRR* and folate, and *MTR* and vitamin B_2 on the breast cancer risk (Lissowska et al., 2007; Shrubsole et al., 2006; Stevens et al., 2007; Suzuki et al., 2008).

6.1.2. Colorectal cancer

The C677T variant of the *MTHFR* gene has been extensively investigated for an association with CRC and CRA risk and interactions with intakes of folate, other B vitamins, and alcohol. Studies where genotype was considered in isolation, 677TT individuals showed a modest reduction in CRC risk compared to those with wild-type 677CC genotype (Hubner and Houlston, 2007), whereas CRA risk did not appear to be significantly influenced by *MTHFR C677T* genotype (Sharp and Little, 2004). Studies in which both genotype and methyl status were stratified, however, have indicated a more complex relationship with 677TT individuals being at reduced CRC risk if they also have high methyl status (low alcohol, high folate), but at increased risk if their methyl status is low (high alcohol, low folate) (Chen et al., 1996; Ma et al., 1999). These interactions are not universally reported in small case–control series, although a recent case–control study also indicated interactions between *MTHFR C677T* genotype and folate status or alcohol intake in determining CRC risk (Le Marchand et al., 2005).

Similarly, Sameer et al. (2011), in a study of *MTHFR* gene polymorphism in CRC cases in Kashmir, found a varied difference in the genotype frequency of C677T *MTHFR* between CRC cases and the matched controls. The incidence of the *MTHFR TT* allele was increased in patients with colorectal cancer compared to healthy controls. The frequency of CT genotype in CRC cases was 20.9% and that of TT was 10.4%, as compared to healthy controls, where it was 16.9% and 7.5%, respectively. The overall hazard ratio of the *MTHFR* T allele in patients with CRC was 1.41 (95% CI = 0.79–2.54). Overall, both the heterozygous CT genotype and the homozygous variant TT genotype were associated with a modestly elevated risk for colorectal cancer {OR = 1.36; 95% CI = 0.70–2.67 and OR = 1.53; 95% CI = 0.61–3.85, respectively}.

The complex relationship between folate intake and CRC risk may be further modulated by other functional polymorphisms impacting on folate metabolism. These include the *MTHFR A1298C* variant which also confers a reduced MTHFR enzyme function, the thymidylate synthase promoter (*TSER*) and the 3′ untranslated region (TS 1494del6) variant (Sharp and Little, 2004; Ulrich et al., 2005). Furthermore, recent data indicates folate may have differential influences on the development of CRC caused by mismatch repair deficiency and chromosomal instability (Hubner and Houlston, 2007). To date, studies investigating the relationships between the multiple genetic and dietary factors that impact on folate metabolism have had sample sizes at least one order of magnitude too small to convincingly confirm or refute potential gene–gene or gene–nutrient interactions in determining colorectal neoplasia risk (Hubner and Houlston, 2009).

6.1.3. Head and neck cancer

Many studies have assessed the association of *MTHFR C667T* polymorphism with head and neck cancer (Kruszyna et al., 2010; Kureshi et al., 2004; Neumann et al., 2005; Reljic et al., 2007; Solomon et al., 2008; Suzuki et al., 2007; Vairaktaris et al., 2006; Weinstein et al., 2002). Of these, only three studies have confirmed an association of the *MTHFR C677T* polymorphism with a risk of head and neck cancer (Reljic et al., 2007; Solomon et al., 2008; Vairaktaris et al., 2006).

Vairaktaris et al. (2006) studied 110 subjects with mouth cancer and 102 cancer-free individuals among Germans and Greeks and found that the 677CT genotype was associated with an increased risk of this cancer. Reljic et al. (2007) conducted a case–control study of 81 patients with head and neck cancer and 102 subjects without a history of cancer among a Croatian population and found that the 677TT genotype decreases the risk of this disease. On the other hand, Solomon et al.

(2008) assessed 126 individuals who were alcohol abusers (33 chronic and significant consumers of alcohol, 56 moderate consumers of alcohol, and 37 social drinkers) and who had mouth cancer and found that the 677TT genotype was associated with the group of chronic and significant alcohol abusers and the group of moderate consumers of alcohol (to a lesser degree).

A1289C variant has also been associated with the risk of head and neck cancer however data on the risk of head and neck cancer in relation to the *MTHFR* A1298C polymorphism is contradictory. Studies by Suzuki et al. (2007) and Kruszyna et al. (2010) have found no association between this polymorphism and the risk of head and neck carcinoma. A study by Neumann et al. (2005) involving 537 patients with head and neck cancer and 545 control subjects in Texas showed that individuals with the 1298AC or 1298CC genotypes had a 35% lower risk of head and neck cancer.

Similarly, few studies have investigated an association of the *MTRR* A66G polymorphism and the risk of head and neck cancer. Suzuki et al. (2007) showed that this polymorphism is not associated with a risk for head and neck cancer, but these authors also found an interaction between alcohol abuse and the *MTRR* A66G polymorphism in a Japanese population. However, a study by Zhang et al. (2005) showed that individuals with the homozygous wild genotype (*MTRR* 66AA) are at a lower risk for head and neck cancer, confirming that the A allele is protective.

6.1.4. Lung cancer

The role of folate gene variants in the development of lung cancer has also been studied. In one study involving a large non-Hispanic white population, significant gene–diet interaction was observed between the *TS 3′UTR* (thymidylate synthase 3′UTR) polymorphism and alcohol consumption, and between *TSER* (thymidylate synthase promoter enhancer region) and vitamin B_{12} (Shi et al., 2005a). However, the underlying biological relevance of these *TS* polymorphisms and their mechanisms of gene–diet interactions warrant further study. Similarly, the association between the *MTR A2756G* and *MTRR A66G* polymorphisms and lung cancer (Shi et al., 2005b) and evidence of gene–diet interactions between the *MTHFR C677T* polymorphism and dietary intake of vitamin B_6, vitamin B_{12}, and methionine in women has also been studied (Shi et al., 2005a).

6.1.5. Acute lymphoblastic leukemia

A number of studies have been carried out to determine the role of folate gene polymorphisms in the development of ALL. However, these studies have shown contradictory results. For example; five studies involving MTHFR *677CT*, found no significant difference in susceptibility to ALL (Chiusolo et al., 2004; Kim et al., 2009; Skibola et al., 1999; Thirumaran et al., 2005, and Oh et al., 2007) whereas four studies found a decreased susceptibility to ALL, two of these studies involved Brazilian population and the other two West-European (de Jonge et al., 2009; Franco et al., 2001; Gemmati et al., 2004; Zanrosso et al., 2006).

Similarly, for *MTHFR 677TT*, one study (Kim et al., 2009) found an increased susceptibility to ALL in a Korean population. Four studies (Chiusolo et al., 2004; Thirumaran et al., 2005, 2007; Zanrosso et al., 2006) found no significant difference in susceptibility to ALL. Three studies (Franco et al., 2001; Gemmati et al., 2004; Skibola et al., 1999) found a decreased susceptibility to ALL, one of these involved Brazilian population and the other two studied West-European. For *MTHFR 677CT + TT*, two studies (Thirumaran et al., 2005, 2007) found no significant difference in susceptibility to ALL. Three studies (Chatzidakis et al., 2006; Franco et al., 2001; Zanrosso et al., 2006) found a decreased susceptibility to ALL; one study involved a Greek population, the other two studied populations were Brazilian.

In the case of *MTHFR 1298AC*, there are numerous studies having a varied results for the modulation of relative risk vis a vis with the three genotypes. While two studies (Alcasabas et al., 2008; Zanrosso et al., 2006) found an increased susceptibility to ALL, both concerned exclusively of childhood ALL; other studies (Chiusolo et al., 2004; Franco et al., 2001; Thirumaran et al., 2005, 2007) found no significant difference in susceptibility to ALL, one of these concerned exclusively of childhood ALL and two studies (Kamel et al., 2007; Skibola et al., 1999) found a decreased susceptibility to ALL.

For *MTRR*, a study involving Korean population has shown that MTRR A66G polymorphism has no significant association with the development of ALL. However another study involving a different population found a decreased susceptibility to leukemia for MTRR A66G polymorphism (Gast et al., 2007), while as a British study (Skibola et al., 2002) found no significant difference in the susceptibility to ALL for MTR A2756G. Another (Italian) study (Gemmati et al., 2004) found a decreased susceptibility to ALL for MTR A2756G.

For TSER polymorphism, studies have shown inconsistent results, two Western European studies (de Jonge et al., 2009; Skibola et al., 2002) found a decreased susceptibility to ALL; one of them (de Jonge et al., 2009) concerned exclusively of childhood ALL. Results from several other studies have found that there is no significant association between TSER polymorphism and ALL (Lauten et al., 2003; Lightfoot et al., 2010; Nazki et al., 2012; Rahimi et al., 2010).

6.1.6. Gastric cancer

The association between folate gene polymorphisms especially MTHFR polymorphisms and gastric cancer have been studied. The association with MTHFR polymorphism was first investigated by Shen et al. (2001). In recent years several studies have been carried out to determine the association between gastric cancer and MTHFR polymorphisms; however, the results of these studies have been quite inconsistent. Of the published studies, some studies especially in China (Gao et al., 2002; Miao et al., 2002; Mu et al., 2004; Shen et al., 2001, 2005; Stolzenberg-Solomon et al., 2003; Wang et al., 2007), Italy (Boccia et al., 2007; Graziano et al., 2006), and Mexico (Lacasana-Navarro et al., 2006), found that the MTHFR 677TT genotype was a strong risk factor for gastric cancer, others no association (Gotze et al., 2007; Kim et al., 2005; Vollset et al., 2007; Zeybek et al., 2006; Zhang et al., 2007), and few suggested a decreased risk (Cui et al., 2010; Galvan-Portillo et al., 2009).

For MTHFR A1298C polymorphisms fewer studies have been carried out which suggest that there is no significant association between A1298C polymorphism and gastric cancer risk (Boccia et al., 2007; Kim et al., 2005; Miao et al., 2002; Zhang et al., 2007).

Similar studies involving MTR and MTRR polymorphisms have shown no significant association between gastric cancer and these polymorphisms (Stolzenberg-Solomon et al., 2003; Zhang et al., 2007).

6.2. Vascular diseases

Vascular diseases have been commonly associated with traditional risk factors such as systemic arterial hypertension, diabetes mellitus and smoking, but in the last decade other risk factors have also been identified, one of them being homocysteine. Various studies had identified homocysteine as an independent risk factor for coronary artery disease (CAD) (Brustolin et al., 2010). A study between *MTHFR* polymorphism and the severity of CAD in patients undergoing coronary artery bypass surgery showed that homocysteine levels were significantly higher in patients with CAD than in control subjects and the genotype of *MTHFR* C677T was associated with the extent of CAD in patients at high risk for this pathology (Brustolin et al., 2010; Trabetti, 2008).

6.3. Neural tube defects

Neural tube defects (NTD) are among the most common birth defects worldwide. NTD result from failure of neural tube formation or closure during the first 28 days of pregnancy. Studies have supported the use of folic acid in the prevention of the first occurrence of NTD

(Berry et al., 1999). These findings suggest that impaired folic acid metabolism plays a role in NTD. Several studies on different populations have reported that high maternal homocysteine levels are associated with an increased risk for offspring with NTD (Mills et al., 1995; Ratan et al., 2008; Steegers-Theunissen et al., 1994; Wenstrom et al., 2000). However, a case vs control study conducted in Brazil observed no differences in *MTHFR* polymorphism in mothers, children with NTD or controls (Felix et al., 2004). Similarly, another Brazilian study was also unable to identify the association between *MTHFR* polymorphism and NTD, suggesting that this gene does not play a role in NTD in the Brazilian population (Perez et al., 2003).

6.4. Depression

Folate and vitamin B_{12} deficiency, hyperhomocysteinemia, the *677T* allele of the *MTHFR* gene, which cause impaired methylation reactions in the central nervous system, have been associated with depressive disorders (Bjelland et al., 2003). However other studies did not find this association (Almeida et al., 2005; Tiemeier et al., 2002). A prospective study on 732 Korean subjects investigated associations between folate, vitamin B_{12}, homocysteine, and late life depression. The incident depression was predicted by lower folate and vitamin B_{12} levels and higher homocysteine levels. However, no association between incident depression and *MTHFR* genotype was observed (Kim et al., 2008).

6.5. Down's syndrome

Down's syndrome (DS) occurs due to trisomic condition of chromosome 21 which in most cases results due to the failure of chromosomal segregation during maternal meiosis. An elevated risk for DS has been observed in the presence of the *MTR A2756G* allele in combination with elevated homocysteine concentration (Bosco et al., 2003). However, the association of this polymorphism with the risk for DS was not observed by an additional study (Chango et al., 2005). A study conducted in Brazil with mothers of children with DS and control mothers analyzed homocysteine levels and several variants in the folate pathway (*MTHFR C*677CT, *MTHFR A*1298C, *MTRR A*66G, A*MTR* 2756G), showing that homocysteine levels were higher among DS mothers than among controls. However, only the *MTHFR C677T* allele was associated with altered homocysteine levels in the case group. All genotype distributions were similar in the two groups, but the frequency of the 677T allele was significantly higher in the case group. None of the other polymorphisms showed an association with risk for DS when evaluated separately. However, when the presence of the alleles was evaluated as a whole, the mothers of children with DS tended to have a higher number of variant alleles than control mothers (da Silva et al., 2005).

When the effect of plasma homocysteine concentrations on maternal risk for DS was investigated, homocysteine concentrations were found to be significantly higher in DS mothers compared to control. The study also showed that homocysteine concentrations were significantly higher in DS mothers with the *MTHFR* 1298CC genotype compared to the same genotype of control mothers (Biselli et al., 2008).

7. Conclusion

Folate plays an essential role in several metabolic processes including DNA synthesis and methylation. Therefore, changes in folate status may influence the DNA stability and integrity or affect the methylation patterns in some tissues and predispose it to the development of cancers like, CRC. However, very little evidence is currently available to suggest that folate deficiency alone leads to CRC. Polymorphic variants of the enzymes involved in folate metabolism also play an important role in determining the susceptibility of an individual to cancer. The gene–diet interactions may act in concert and lead to malignant transformation of the cell. In this regard, several studies have been carried out to determine the association of reduced folate inputs and functional polymorphisms in folate genes with the development of cancers. Similar gene–diet interaction has been studied in the development of other diseases. However, the data from these studies have shown conflicting results. Besides the current methodologies employed are lacking for specific hypotheses, the functional effects of such complex gene–environment pathways. Therefore, this area of research must be a priority to achieve the proper understanding of the etiology of complex diseases like cancers.

References

Alcasabas, P., et al., 2008. 5,10-Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and the risk of acute lymphoblastic leukemia (ALL) in Filipino children. Pediatr. Blood Cancer 51, 178–182.

Almeida, O.P., Flicker, L., Lautenschlager, N.T., Leedman, P., Vasikaran, S., van Bockxmeer, F.M., 2005. Contribution of the MTHFR gene to the causal pathway for depression, anxiety and cognitive impairment in later life. Neurobiol. Aging 26, 251–257.

Andreassi, M.G., et al., 2003. Methylenetetrahydrofolate reductase gene C677T polymorphism, homocysteine, vitamin B_{12}, and DNA damage in coronary artery disease. Hum. Genet. 112, 171–177.

Antony, A.C., 1996. Folate receptors. Annu. Rev. Nutr. 16, 501–521.

Anwar, W., et al., 2001. Hyperhomocysteinemia is related to residual glomerular filtration and folate, but not to methylenetetrahydrofolate-reductase and methionine synthase polymorphisms, in supplemented end-stage renal disease patients undergoing hemodialysis. Clin. Chem. Lab. Med. 39, 747–752.

Bailey, L.B., Gregory III, J.F., 1999. Folate metabolism and requirements. J. Nutr. 129, 779–782.

Berry, R.J., et al., 1999. Prevention of neural-tube defects with folic acid in China. China–U.S. collaborative project for neural tube defect prevention. N. Engl. J. Med. 341, 1485–1490.

Biselli, J.M., Goloni-Bertollo, E.M., Zampieri, B.L., Haddad, R., Eberlin, M.N., Pavarino-Bertelli, E.C., 2008. Genetic polymorphisms involved in folate metabolism and elevated plasma concentrations of homocysteine: maternal risk factors for Down syndrome in Brazil. Genet. Mol. Res. 7, 33–42.

Bjelland, I., Tell, G.S., Vollset, S.E., Refsum, H., Ueland, P.M., 2003. Folate, vitamin B_{12}, homocysteine, and the MTHFR 677C → T polymorphism in anxiety and depression: the Hordaland Homocysteine Study. Arch. Gen. Psychiatry 60, 618–626.

Blom, H.J., Smulders, Y., 2011. Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects. J. Inherit. Metab. Dis. 34, 75–81.

Blount, B.C., et al., 1997. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc. Natl. Acad. Sci. U. S. A. 94, 3290–3295.

Boccia, S., et al., 2007. Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and susceptibility to gastric adenocarcinoma in an Italian population. Biomarkers 12, 635–644.

Bosco, P., et al., 2003. Methionine synthase (MTR) 2756 (A → G) polymorphism, double heterozygosity methionine synthase 2756 AG/methionine synthase reductase (MTRR) 66 AG, and elevated homocysteinemia are three risk factors for having a child with Down syndrome. Am. J. Med. Genet. A 121A, 219–224.

Brustolin, S., Giugliani, R., Félix TM, T.M., 2010. Genetics of homocysteine metabolism and associated disorders. Braz. J. Med. Biol. Res. 43, 1–7.

Chandler, C.J., Wang, T.T., Halsted, C.H., 1986. Pteroylpolyglutamate hydrolase from human jejunal brush borders. Purification and characterization. J. Biol. Chem. 261, 928–933.

Chango, A., et al., 2005. No association between common polymorphisms in genes of folate and homocysteine metabolism and the risk of Down's syndrome among French mothers. Br. J. Nutr. 94, 166–169.

Chatzidakis, K., Goulas, A., thanassiadou-Piperopoulou, F., Fidani, L., Koliouskas, D., Mirtsou, V., 2006. Methylenetetrahydrofolate reductase C677T polymorphism: association with risk for childhood acute lymphoblastic leukemia and response during the initial phase of chemotherapy in Greek patients. Pediatr. Blood Cancer 47, 147–151.

Chen, J., et al., 1996. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. Cancer Res. 56, 4862–4864.

Chen, J., et al., 2001. Influence of a methionine synthase (D919G) polymorphism on plasma homocysteine and folate levels and relation to risk of myocardial infarction. Atherosclerosis 154 (667–72), 35.

Chen, J., Ma, J., Stampfer, M.J., Palomeque, C., Selhub, J., Hunter, D.J., 2002. Linkage disequilibrium between the 677C → T and 1298A → C polymorphisms in human methylenetetrahydrofolate reductase gene and their contributions to risk of colorectal cancer. Pharmacogenetics 12, 339–342.

Chen, J., et al., 2005. One-carbon metabolism, MTHFR polymorphisms, and risk of breast cancer. Cancer Res. 65, 1606–1614.

Chiusolo, P., et al., 2004. Methylenetetrahydrofolate reductase genotypes do not play a role in acute lymphoblastic leukemia pathogenesis in the Italian population. Haematologica 89, 139–144.

Choi, S.W., Mason, J.B., 2002. Folate status: effects on pathways of colorectal carcinogenesis. J. Nutr. 132, 2413S–2418S.

Crider, K.S., Yang, T.P., Berry, R.J., Bailey, L.B., 2012. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv. Nutr. 3, 21–38.

Crott, J.W., Mashiyama, S.T., Ames, B.N., Fenech, M.F., 2001. Methylenetetrahydrofolate reductase C677T polymorphism does not alter folic acid deficiency-induced uracil

incorporation into primary human lymphocyte DNA in vitro. Carcinogenesis 22, 1019–1025.
Cui, L.H., et al., 2010. Methylenetetrahydrofolate reductase C677T polymorphism in patients with gastric and colorectal cancer in a Korean population. BMC Cancer 10, 236.
da Silva, L.R., et al., 2005. Relationship between polymorphisms in genes involved in homocysteine metabolism and maternal risk for Down syndrome in Brazil. Am. J. Med. Genet. A 135, 263–267.
de Jonge, R., et al., 2009. Polymorphisms in folate-related genes and risk of pediatric acute lymphoblastic leukemia. Blood 113, 2284–2289.
Dekou, V., Gudnason, V., Hawe, E., Miller, G.J., Stansbie, D., Humphries, S.E., 2001. Gene-environment and gene–gene interaction in the determination of plasma homocysteine levels in healthy middle-aged men. Thromb. Haemost. 85, 67–74.
Duthie, S.J., Hawdon, A., 1998. DNA instability (strand breakage, uracil misincorporation, and defective repair) is increased by folic acid depletion in human lymphocytes in vitro. FASEB J. 12, 1491–1497.
Duthie, S.J., Grant, G., Narayanan, S., 2000. Increased uracil misincorporation in lymphocytes from folate-deficient rats. Br. J. Cancer 83, 1532–1537.
Duthie, S.J., Narayanan, S., Brand, G.M., Pirie, L., Grant, G., 2002. Impact of folate deficiency on DNA stability. J. Nutr. 132, 2444S–2449S.
Eden, A., Gaudet, F., Waghmare, A., Jaenisch, R., 2003. Chromosomal instability and tumors promoted by DNA hypomethylation. Science 300, 455.
Ericson, U., et al., 2009. Folate intake, methylenetetrahydrofolate reductase polymorphisms, and breast cancer risk in women from the Malmo Diet and Cancer cohort. Cancer Epidemiol. Biomarkers Prev. 18, 1101–1110.
Felix, T.M., Leistner, S., Giugliani, R., 2004. Metabolic effects and the methylenetetrahydrofolate reductase (MTHFR) polymorphism associated with neural tube defects in southern Brazil. Birth Defects Res. A Clin. Mol. Teratol. 70, 459–463.
Fowler, B., 2001. The folate cycle and diseases in humans. Kidney Int. 59, s221–s229.
Franco, R.F., Simoes, B.P., Tone, L.G., Gabellini, S.M., Zago, M.A., Falcão, R.P., 2001. The methylenetetrahydrofolate reductase C677T gene polymorphism decreases the risk of childhood acute lymphocytic leukaemia. Br. J. Haematol. 115, 616–618.
Friedman, G., et al., 1999. A common mutation A1298C in human methylenetetrahydrofolate reductase gene: association with plasma total homocysteine and folate concentrations. J. Nutr. 129, 1656–1661.
Friso, S., 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. U. S. A. 99, 5606–5611.
Frosst, P., et al., 1995. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat. Genet. 10, 111–113.
Galvan-Portillo, M.V., et al., 2009. Gastric cancer in relation to the intake of nutrients involved in one-carbon metabolism among MTHFR 677TT carriers. Eur. J. Nutr. 48, 269–276.
Gao, C., et al., 2002. Polymorphisms of methylenetetrahydrofolate reductase C677T and the risk of stomach cancer. Zhonghua Liu Xing Bing Xue Za Zhi 23, 289–292 (In Chinese).
Gast, A., et al., 2007. Folate metabolic gene polymorphisms and childhood acute lymphoblastic leukemia: a case–control study. Leukemia 21, 320–325.
Gaughan, D.J., et al., 2001. The methionine synthase reductase (MTRR) A66G polymorphism is a novel genetic determinant of plasma homocysteine concentrations. Atherosclerosis 157, 451–456.
Geisel, J., et al., 2001. Genetic defects as important factors for moderate hyperhomocysteinemia. Clin. Chem. Lab. Med. 39, 698–704.
Gemmati, D., et al., 2004. Common gene polymorphisms in the metabolic folate and methylation pathway and the risk of acute lymphoblastic leukemia and non-Hodgkin's lymphoma in adults. Cancer Epidemiol. Biomarkers Prev. 13, 787–794.
Gotze, T., et al., 2007. Gene polymorphisms of folate metabolizing enzymes and the risk of gastric cancer. Cancer Lett. 251, 228–236.
Goyette, P., et al., 1994. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. Nat. Genet. 7, 195–200.
Graziano, F., et al., 2006. Methylenetetrahydrofolate reductase 677C/T gene polymorphism, gastric cancer susceptibility and genomic DNA hypomethylation in an at-risk Italian population. Int. J. Cancer 118, 628–632.
Horie, N., Aiba, H., Oguro, K., Hojo, H., Takeishi, K., 1995. Functional analysis and DNA polymorphism of the tandemly repeated sequences in the 5′-terminal regulatory region of the human gene for thymidylate synthase. Cell Struct. Funct. 20, 191–197.
Hosseini, M., Houshmand, M., Ebrahimi, A., 2011. MTHFR polymorphisms and breast cancer risk. Arch. Med. Sci. 7 (1), 134–137.
Hubner, R.A., Houlston, R.S., 2007. MTHFR C677T and colorectal cancer risk: a meta-analysis of 25 populations. Int. J. Cancer 120, 1027–1035.
Hubner, R.A., Houlston, R.S., 2009. Folate and colorectal cancer prevention. Br. J. Cancer 100, 233–239.
Jacob, R.A., et al., 1998. Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. J. Nutr. 128, 1204–1212.
Kamel, A.M., Moussa, H.S., Ebid, G.T., Bu, R.R., Bhatia, K.G., 2007. Synergistic effect of methyltetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphism as risk modifiers of pediatric acute lymphoblastic leukemia using tissue microarray. J. Egypt. Natl. Cancer Inst. 19, 96–105.
Kaneda, S., Takeishi, K., Ayusawa, D., Shimizu, K., Seno, T., Altman, S., 1987. Role in translation of a triple tandemly repeated sequence in the 5′-untranslated region of human thymidylate synthase mRNA. Nucleic Acids Res. 15, 1259–1270.
Kawakami, K., Watanabe, G., 2003. Identification and functional analysis of single nucleotide polymorphism in the tandem repeat sequence of thymidylate synthase gene. Cancer Res. 63, 6004–6007.
Kawakami, K., Omura, K., Kanehira, E., Watanabe, Y., 1999. Polymorphic tandem repeats in the thymidylate synthase gene is associated with its protein expression in human gastrointestinal cancers. Anticancer Res. 19, 3249–3252.
Kim, Y.I., 2000. Methylenetetrahydrofolate reductase polymorphisms, folate, and cancer risk: a paradigm of gene–nutrient interactions in carcinogenesis. Nutr. Rev. 58, 205–217.
Kim, Y.I., 2007. Folate and colorectal cancer: an evidence-based critical review. Mol. Nutr. Food Res. 51, 267–292.
Kim, J.K., et al., 2005. Polymorphisms of 5,10-methylenetetrahydrofolate reductase and risk of stomach cancer in a Korean population. Anticancer Res. 25, 2249–2252.
Kim, J.M., Stewart, R., Kim, S.W., Yang, S.J., Shin, I.S., Yoon, J.S., 2008. Predictive value of folate, vitamin B_{12} and homocysteine levels in late-life depression. Br. J. Psychiatry 192, 268–274.
Kim, H.N., et al., 2009. Association between polymorphisms of folate-metabolizing enzymes and hematological malignancies. Leuk. Res. 33, 82–87.
Kruszyna, Ł., Lianeri, M., Rydzanicz, M., Gajecka, M., Szyfter, K., Jagodziński, P.P., 2010. Polymorphic variants of folate metabolism genes and the risk of laryngeal cancer. Mol. Biol. Rep. 37, 241–247.
Kureshi, N., Ghaffar, S., Siddiqui, S., Salahuddin, I., Frossard, P.M., 2004. Head and neck cancer susceptibility: a genetic marker in the methylenetetrahydrofolate reductase gene. ORL J. Otorhinolaryngol. Relat. Spec. 66, 241–245.
Lacasana-Navarro, M., Galvan-Portillo, M., Chen, J., López-Cervantes, M., López-Carrillo, L., 2006. Methylenetetrahydrofolate reductase 677C → T polymorphism and gastric cancer susceptibility in Mexico. Eur. J. Cancer 42, 528–533.
Lauten, M., Cario, G., Asgedom, G., Welte, K., Schrappe, M., 2003. Protein expression of the glucocorticoid receptor in childhood acute lymphoblastic leukemia. Haematologica 88, 1253–1258.
Le Marchand, L., Haiman, C.A., Wilkens, L.R., Kolonel, L.N., Henderson, B.E., 2004. MTHFR polymorphisms, diet, HRT, and breast cancer risk: the multiethnic cohort study. Cancer Epidemiol. Biomarkers Prev. 13, 2071–2077.
Le Marchand, L., Wilkens, L.R., Kolonel, L.N., Henderson, B.E., 2005. The MTHFR C677T polymorphism and colorectal cancer: the multiethnic cohort study. Cancer Epidemiol. Biomarkers Prev. 14, 1198–1203.
Leclerc, D., et al., 1996. Human methionine synthase: cDNA cloning and identification of mutations in patients of the cblG complementation group of folate/cobalamin disorders. Hum. Mol. Genet. 5, 1867–1874.
Leclerc, D., et al., 1998. Cloning and mapping of a cDNA for methionine synthase reductase, a flavoprotein defective in patients with homocystinuria. Proc. Natl. Acad. Sci. U. S. A. 95, 3059–3064.
Lewis, S.J., Harbord, R.M., Harris, R., Smith, G.D, 2006. Meta-analyses of observational and genetic association studies of folate intakes or levels and breast cancer risk. J. Natl. Cancer Inst. 98, 1607–1622.
Libbus, B.L., Borman, L.S., Ventrone, C.H., Branda, R.F., 1990. Nutritional folate-deficiency in Chinese hamster ovary cells. Chromosomal abnormalities associated with perturbations in nucleic acid precursors. Cancer Genet. Cytogenet. 46, 231–242.
Lievers, K.J.A., et al., 2001. A second common variant in the methylenetetrahydrofolate reductase (MTHFR) gene and its relationship to MTHFR enzyme activity, homocysteine, and cardiovascular disease risk. J. Mol. Med. 79, 522–528.
Lightfoot, T.J., et al., 2005. Risk of non-Hodgkin lymphoma associated with polymorphisms in folate-metabolizing genes. Cancer Epidemiol. Biomarkers Prev. 14, 2999–3003.
Lightfoot, T.J., et al., 2010. Genetic variation in the folate metabolic pathway and risk of childhood leukemia. Blood 115, 3923–3929.
Lissowska, J., et al., 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, 2696–2703.
Liu, J., Ward, R.L., 2010. Folate and one-carbon metabolism and its impact on aberrant DNA methylation in cancer. Adv. Genet. 71, 80–121.
Ma, J., et al., 1997. Methylenetetrahydrofolate reductase polymorphism, dietary interactions and risk of colorectal cancer. Cancer Res. 57, 1098–1102.
Ma, J., et al., 1999. A polymorphism of the methionine synthase gene: association with plasma folate, vitamin B_{12}, homocyst(e)ine, and colorectal cancer risk. Cancer Epidemiol. Biomarkers Prev. 8, 825–829.
Mandola, M.V., et al., 2003. A novel single nucleotide polymorphism within the 5′ tandem repeat polymorphism of the thymidylate synthase gene abolishes USF-1 binding and alters transcriptional activity. Cancer Res. 63, 2898–2904.
Mandola, M.V., et al., 2004. A 6 bp polymorphism in the thymidylate synthase gene causes message instability and is associated with decreased intratumoral TS mRNA levels. Pharmacogenetics 14, 319–327.
McKinnon, P.J., Caldecott, K.W., 2007. DNA strand break repair and human genetic disease. Annu. Rev. Genomics Hum. Genet. 8, 37–55.
Miao, X., Xing, D., Tan, W., Qi, J., Lu, W., Lin, D., 2002. Susceptibility to gastric cardia adenocarcinoma and genetic polymorphisms in methylenetetrahydrofolate reductase in an at-risk Chinese population. Cancer Epidemiol. Biomarkers Prev. 11, 1454–1458.
Mills, J.L., et al., 1995. Homocysteine metabolism in pregnancies complicated by neural-tube defects. Lancet 345, 149–151.
Molloy, A.M., et al., 1997. Thermolabile variant of 5,10-methylenetetrahydrofolate reductase associated with low red-cell folates: implications for folate intake recommendations. Lancet 349, 1591–1593.
Mu, L.N., et al., 2004. A case–control study on the relationship between methyl-tetrahydrofolic acid reductase 677 gene polymorphism and the risk of stomach cancer. Zhonghua Liu Xing Bing Xue Za Zhi 25, 495–498 (In Chinese).
Narayanan, S., 2001. The Effect of Folic Acid and Genetic Polymorphisms on DNA Stability and Colorectal Cancer. University of Aberdeen, Aberdeen, Scotland.
Nazki, F.H., Masood, A., Banday, M.A., Bhat, A., Ganai, B.A., 2012. Thymidylate synthase enhancer region polymorphism not related to susceptibility to acute lymphoblastic leukemia in the Kashmir population. Genet. Mol. Res. 11, 906–917.
Neumann, A.S., 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, 131–136.

Nijhout, H.F., Reed, M.C., Ulrich, C.M., 2008. Chapter 2 mathematical models of folate mediated one-carbon metabolism. Vitam. Horm. 79, 45–82.
O'Leary, V.B., et al., 2002. MTRR and MTHFR polymorphism: link to Down syndrome? Am. J. Med. Genet. 107, 151–155.
Oh, D., Kim, N.K., Jang, M.J., et al., 2007. Association of the 5,10 methylenetetrahydrofolate reductase (MTHFR C677T and A1298C) polymorphisms in Korean patients with adult acute lymphoblastic leukemia. Anticancer Res. 27, 3419–3424.
Perez, A.B., D'Almeida, V., Vergani, N., Oliveira, A.C., de Lima, F.T., Brunoni, D., 2003. Methylenetetrahydrofolate reductase (MTHFR): incidence of mutations C677T and A1298C in Brazilian population and its correlation with plasma homocysteine levels in spina bifida. Am. J. Med. Genet. A 119A, 20–25.
Rady, P.L., et al., 2002. Genetic polymorphisms of methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) in ethnic populations in Texas; a report of a novel MTHFR polymorphic site, G1793A. Am. J. Med. Genet. 107, 162–168.
Rahimi, Z., et al., 2010. Thymidilate synthase and methionine synthase polymorphisms in children with acute lymphoblastic leukemia in western Iran. Int. J. Hematol. Oncol. Stem Cell Res. 9–12.
Ratan, S.K., et al., 2008. Evaluation of the levels of folate, vitamin B_{12}, homocysteine and fluoride in the parents and the affected neonates with neural tube defect and their matched controls. Pediatr. Surg. Int. 24, 803–808.
Reljic, A., Simundic, A.M., Topic, E., Nikolac, N., Justinic, D., Stefanovic, M., 2007. The methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and cancer risk: the Croatian case–control study. Clin. Biochem. 40, 981–985.
Rozen, R., 1997. Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). Thromb. Haemost. 78, 523–526.
Ryan, B.M., Weir, D.G., 2001. Relevance of folate metabolism in the pathogenesis of colorectal cancer. J. Lab. Clin. Med. 138, 164–176.
Sameer, A.S., Shah, Z.A., Nissar, S., Mudassar, S., Siddiqi, M.A., 2011. MTHFR C677T polymorphism and colorectal cancer predisposition in Kashmiri population. Genet. Mol. Res. 10, 1200–1210.
Shane, B., 1995. In: Baile, L.B. (Ed.), Folate Chemistry and Metabolism. In Folate in Health and Disease. Marcel Dekker, New York, pp. 1–22.
Sharp, L., Little, J., 2004. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. Am. J. Epidemiol. 159, 423–443.
Shen, H., 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, 332–336.
Shen, H., et al., 2005. Methylenetetrahydrofolate reductase polymorphisms/haplotypes and risk of gastric cancer: a case–control analysis in China. Oncol. Rep. 13, 355–360.
Shi, Q., et al., 2005a. Sex differences in risk of lung cancer associated with methylenetetrahydrofolate reductase polymorphisms. Cancer Epidemiol. Biomarkers Prev. 14, 1477–1484.
Shi, Q., Zhang, Z., Neumann, A.S., Li, G., Spitz, M.R., Wei, Q., 2005b. Case–control analysis of thymidylate synthase polymorphisms and risk of lung cancer. Carcinogenesis 26, 649–656.
Shrubsole, M.J., et al., 2004. MTHFR polymorphisms, dietary folate intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. Cancer Epidemiol. Biomarkers Prev. 13, 190–196.
Shrubsole, M.J., et al., 2006. MTR and MTRR polymorphisms, dietary intake, and breast cancer risk. Cancer Epidemiol. Biomarkers Prev. 15, 586–588.
Silaste, M.L., Rantala, M., Sampi, M., Alfthan, G., Aro, A., Kesäniemi, Y.A., 2001. Polymorphisms of key enzymes in homocysteine metabolism affect diet responsiveness of plasma homocysteine in healthy women. J. Nutr. 131, 2643–2647.
Sirotnak, F.M., Tolner, B., 1999. Carrier-mediated membrane transport of folates in mammalian cells. Annu. Rev. Nutr. 19, 91–122.
Skibola, C.F., et al., 1999. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc. Natl. Acad. Sci. U. S. A. 96, 12810–12815.
Skibola, C.F., et al., 2002. Polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and risk of adult acute lymphocytic leukemia. Blood 99, 3786–3791.
Solomon, P.R., Selvam, G.S., Shanmugam, G., 2008. Polymorphism in ADH and MTHFR genes in oral squamous cell carcinoma of Indians. Oral Dis. 14, 633–639.
Steegers-Theunissen, R.P., et al., 1994. Maternal hyperhomocysteinemia: a risk factor for neural-tube defects? Metabolism 43 (1475), 1480.
Stern, L.L., Mason, J.B., Selhub, J., Choi, S.W., 2000. Genomic DNA hypomethylation, a characteristic of most cancers, is present in peripheral leukocytes of individuals who are homozygous for the C677T polymorphism in the methylenetetrahydrofolate reductase gene. Cancer Epidemiol. Biomarkers Prev. 9, 849–853.
Stevens, V.L., et al., 2007. Association of polymorphisms in one-carbon metabolism genes and postmenopausal breast cancer incidence. Cancer Epidemiol. Biomarkers Prev. 16, 1140–1147.
Stolzenberg-Solomon, R.Z., et al., 2003. Esophageal and gastric cardia cancer risk and folate- and vitamin B(12)-related polymorphisms in Linxian, China. Cancer Epidemiol. Biomarkers Prev. 12, 1222–1226.
Suzuki, T., et al., 2007. One-carbon metabolism-related gene polymorphisms and risk of head and neck squamous cell carcinoma: case–control study. Cancer Sci. 98, 1439–1446.
Suzuki, T., et al., 2008. One-carbon metabolism-related gene polymorphisms and risk of breast cancer. Carcinogenesis 29, 356–362.
Tan, L.U., Drury, E.J., Mackenzie, R.E., 1977. Methylenetetrahydrofolate dehydrogenase-methenyltetrahydrofolate cyclohydrolase-formyltetrahydrofolate synthetase. A multifunctional protein from porcine liver. J. Biol. Chem. 252, 1117–1122.
Thirumaran, R.K., et al., 2005. MTHFR genetic polymorphisms and susceptibility to childhood acute lymphoblastic leukemia. Blood 106, 2590–2591.
Tiemeier, H., van Tuijl, H.R., Hofman, A., Meijer, J., Kiliaan, A.J., Breteler, M.M., 2002. Vitamin B_{12}, folate, and homocysteine in depression: the Rotterdam Study. Am. J. Psychiatry 159, 2099–2101.
Trabetti, E., 2008. Homocysteine, MTHFR gene polymorphisms, and cardio-cerebrovascular risk. J. Appl. Genet. 49, 267–282.
Trembath, D., et al., 1999. Analysis of select folate pathway genes, PAX3, and human T in a midwestern neural tube defect population. Teratology 59, 331–341.
Trinh, B.N., Ong, C.N., Coetzee, G.A., Yu, M.C., Laird, P.W., 2002. Thymidylate synthase: a novel genetic determinant of plasma homocysteine and folate levels. Hum. Genet. 111, 299–302.
Ulrich, C.M., Bigler, J., Velicer, C.M., Greene, E.A., Farin, F.M., Potter, J.D., et al., 2000. Searching expressed sequence tag databases: discovery and confirmation of a common polymorphism in the thymidylate synthase gene. Cancer Epidemiol. Biomarkers Prev. 9, 1381–1385.
Ulrich, C.M., Curtin, K., Potter, J.D., Bigler, J., Caan, B., Slattery, M.L., 2005. Polymorphisms in the reduced folate carrier, thymidylate synthase, or methionine synthase and risk of colon cancer. Cancer Epidemiol. Biomarkers Prev. 14, 2509–2516.
Vairaktaris, E., et al., 2006. Methylenetetrahydrofolate reductase polymorphism and minor increase of risk for oral cancer. J. Cancer Res. Clin. Oncol. 132, 219–222.
van der Put, N.M., et al., 1997. Sequence analysis of the coding region of human methionine synthase: relevance to hyperhomocysteinaemia in neural-tube defects and vascular disease. QJM 90, 511–517.
van der Put, N.M., et al., 1998. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am. J. Hum. Genet. 62, 1044–1051.
Vollset, S.E., et al., 2007. The association of gastric cancer risk with plasma folate, cobalamin, and methylenetetrahydrofolate reductase polymorphisms in the European Prospective Investigation into Cancer and Nutrition. Cancer Epidemiol. Biomarkers Prev. 16, 2416–2424.
Wagner, C., 1995. Biochemical role of folate in cellular metabolism. In: Baile, L.B. (Ed.), Folate in Health and Disease. Marcel Dekker, New York, pp. 23–42.
Wang, Y., et al., 2007. Association of MTHFR C677T and SHMT(1) C1420T with susceptibility to ESCC and GCA in a high incident region of northern China. Cancer Causes Control 18, 143–152.
Weinstein, S.J., et al., 2002. Folate intake, serum homocysteine and methylenetetrahydrofolate reductase (MTHFR) C677T genotype are not associated with oral cancer risk in Puerto Rico. J. Nutr. 132, 762–767.
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, 169–172.
Wenstrom, K.D., et al., 2000. Amniotic fluid homocysteine levels, 5,10-methylenetetrahydrofolate reductase genotypes, and neural tube closure sites. Am. J. Med. Genet. 90, 6–11.
Wilcken, D.E., Wilcken, B., 2001. Historical overview and recent persectives. In: Carmel, R., Jacobsen, D.W. (Eds.), Homocysteine in Health and Disease. Cambridge University Press, Cambridge, pp. 1–8.
Wilson, A., et al., 1999. A common variant in methionine synthase reductase combined with low cobalamin (vitamin B_{12}) increases risk for spina bifida. Mol. Genet. Metab. 67, 317–323.
Xu, X., et al., 2007. A functional 19-base pair deletion polymorphism of dihydrofolate reductase (DHFR) and risk of breast cancer in multivitamin users. Am. J. Clin. Nutr. 85, 1098–1102.
Yi, P., Melnyk, S., Pogribna, M., Pogribny, I.P., Hine, R.J., James, S.J., 2000. Increase in plasma homocysteine associated with parallel increases in plasma S-adenosylhomocysteine and lymphocyte DNA hypomethylation. J. Biol. Chem. 275, 29318–29323.
Zanrosso, C.W., et al., 2006. The role of methylenetetrahydrofolate reductase in acute lymphoblastic leukemia in a Brazilian mixed population. Leuk. Res. 30, 477–481.
Zeybek, U., et al., 2006. Methylenetetrahydrofolate reductase C677T polymorphism in patients with gastric and colorectal cancer. Cell Biochem. Funct. 25, 419–422.
Zhang, J., et al., 2004. Association of the thymidylate synthase polymorphisms with esophageal squamous cell carcinoma and gastric cardiac adenocarcinoma. Carcinogenesis 25, 2479–2485.
Zhang, Z., Shi, Q., Liu, Z., Sturgis, E.M, Spitz, M.R., Wei, Q., 2005. Polymorphisms of methionine synthase and methionine synthase reductase and risk of squamous cell carcinoma of the head and neck: a case–control analysis. Cancer Epidemiol. Biomarkers Prev. 14, 1188–1193.
Zhang, F.F., et al., 2007. Genetic polymorphisms in folate metabolism and the risk of stomach cancer. Cancer Epidemiol. Biomarkers Prev. 16, 115–1121.
Zijno, A., et al., 2003. Folate status, metabolic genotype, and biomarkers of genotoxicity in healthy subjects. Carcinogenesis 24, 1097–1103.