Gene-Nutrient Interactions in One-Carbon Metabolism

Simonetta Friso[1,*] and Sang-Woon Choi[2]

[1]*Department of Clinical and Experimental Medicine, University of Verona School of Medicine, Verona, Italy and* [2]*Vitamins and Carcinogenesis Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA*

Abstract: Advances in molecular biology greatly contributed, in the past decades, to a deeper understanding of the role of gene function in disease development. Environmental as well as nutritional factors are now well acknowledged to interact with the individual genetic background for the development of several diseases, including cancer, cardiovascular disease, and neurodegenerative diseases. The precise mechanisms of such gene-nutrient interactions, however, are not fully elucidated yet. Many micronutrients and vitamins are crucial in regulating mechanisms of DNA metabolism. Indeed, folate has been most extensively investigated for its unique function as mediator for the transfer of one-carbon moieties for nucleotide synthesis/repair and biological methylation. Cell culture, animal, and human studies, clearly demonstrated that folate deficiency induces disruption of DNA synthesis/repair pathways as well as DNA methylation anomalies. Remarkably, a gene-nutrient interaction between folate status and a polymorphism in methylenetetrahydrofolate reductase gene has been reported to modulate genomic DNA methylation. This observation suggests that the interaction between a nutritional status and a mutant genotype may modulate gene expression through DNA methylation, especially when such polymorphism affects a key enzyme in one-carbon metabolism and limits the methyl supply. DNA methylation, both genome-wide and gene-specific, is of particular interest for the study of aging, cancer, and other pathologic conditions, because it affects gene expression without permanent alterations in the DNA sequence such as mutations or allele deletions. Understanding the patterns of DNA methylation through the interaction with nutrients is a critical issue, not only to provide pathophysiological explanations of a disease state, but also to identify individuals at-risk to conduct targeted diet-based interventions.

Key Words: Gene-nutrient interaction, one-carbon metabolism, DNA methylation, epigenetics, MTHFR, 677C>T, 1298A>C, folate, B vitamins.

INTRODUCTION

During the past decades, research in molecular biology greatly contributed to the advance of knowledge in the role of genes in several diseases and, at the same time, highlighted the importance of environmental/nutritional factors in regulating the genome machinery [1]. A number of vitamins and micronutrients are co-factors in metabolic pathways that regulate nucleic acids synthesis and/or repair systems as well as the expression of genes [2]. Deficiency of such nutrients may also result in disruption of genomic integrity and alteration of DNA methylation, a major epigenetic feature of DNA that regulates phenomena related to gene transcription [3], thus linking nutrition with modulation of gene expression. The field of gene-nutrient interactions affecting methylation of DNA, therefore, appears a fascinating model to explain the different response to environmental/diet exposure at a molecular level, although the precise nature and magnitude of such gene-nutrient interactions have not been clarified yet.

In this regard, folate, a water-soluble B vitamin involved in one-carbon metabolism, has gained increasing interest for its essential role in the synthesis, repair and methylation of DNA, all of which are central mechanisms for maintaining the adequate regulation of genome function. Studies in animal models and *in vitro* cell culture systems have demonstrated that folate deficiency induces DNA strand breaks, impairment of DNA repair, increased mutagenesis and aberrant DNA methylation status [4, 5]. The importance of folate metabolism is related to its function in providing one-carbon units for nucleic acids bases synthesis as well as for the synthesis of S-adenosymethionine (S-AdoMet), the universal methyl donor for several biological methylation reactions [6].

DNA methylation is the main epigenetic feature in eukaryotic genomes and occurs at the carbon 5' position of cytosine within the CpG dinucleotides [7] in a complex reaction that involves the cytosine base out of the intact double helix [8]. Typically, DNA methylation occurs in CpG dinucleotide rich regions, the so-called 'CpG islands' that, in contrast to the overall genome, are highly represented in promoter regions or first exons of genes [7]. Because of the strong correlation between DNA methylation in promoter regions and transcriptional repression [7, 9], DNA methylation appears to be a fundamental as well as potentially reversible mechanism for epigenetic control of gene expression [10, 11]. Therefore, an evaluation of genomic DNA methylation status is important for the study of cell growth regulation, tissue specific differentiation [12] and carcinogenesis [11, 13].

*Address correspondence to this author at the Department of Clinical and Experimental Medicine, University of Verona School of Medicine, Policlinico Giambattista Rossi, P. le L.A. Scuro, 10, 37134 Verona, Italy. Phone & Fax: +39-045-580111; E-mail: simonetta.friso@univr.it

1389-2002/05 $50.00+.00 **© 2005 Bentham Science Publishers Ltd.**

Several studies highlighted the role of gene-nutrient relationships in folate metabolism by exploring the effect of the interaction between folate status and a polymorphism in the gene of 5, 10-methylenetetrahydrofolate reductase (*MTHFR*) in determining total plasma levels of homocysteine (tHcy), an important metabolite in one-carbon metabolism and a risk factor for cardiovascular disease [14-16]. MTHFR is a key enzyme in one-carbon metabolism, because its substrates provide one-carbon units for thymidine and purine synthesis and its metabolic products are responsible for the availability of methyl groups for several biological methylation reactions including that of DNA. A transition from cytosine to thymine at the 677 position of the *MTHFR* gene causes enzyme thermolability and reduced activity [17], therefore, perturbations in the function of *MTHFR* such as those associated with the presence of the *677C>T* polymorphic site, are critical for altering nucleic acid metabolic pathways.

Recently, a folate-*MTHFR 677C>T* interaction has been described to affect appropriate levels and patterns of DNA methylation [18, 19]. The altered DNA methylation and potential consequent abnormal modulation of gene expression due to an interaction between folate status and the *MTHFR 677C>T*, certainly opened a new fascinating area within that of gene-nutrient interactions. Therefore, it is of considerable interest to identify the factors that determine the patterns of DNA methylation not only to provide evidences for the mechanisms of several pathological conditions but also to identify safe chemoprevention strategies by modifying a nutritional status in subjects with an at-risk gene-nutrient interaction condition [20, 21].

This review will focus on the most recent knowledge about the effects of nutrients, and more specifically of folate, on gene expression and integrity, with an emphasis on gene-nutrient interactions between folate and the *MTHFR 677C>T* polymorphism on the modulation of DNA methylation.

TWO MAJOR FOLATE FUNCTIONS IN ONE CARBON METABOLISM: DNA METHYLATION AND SYNTHESIS/REPAIR

An indirect evidence of the fundamental role of micronutrients and vitamins in DNA metabolic pathways is that altered dietary intake or tissue/plasma levels of several nutrients are well known to be related with a more elevated risk of developing cancer disease [20-22]. Nevertheless, there is no clear validation for optimal dietary ranges able to protect against DNA damage and aberrant regulation of gene functions, probably because, from the studies so far conducted mostly *in vitro* or using animal models, there is no conclusive evidence on the mechanisms through which nutrients exert their function in maintenance of genomic stability [1, 2].

The great majority of data explaining an interaction of nutrients and DNA metabolism refer to dietary folate and/or methyl group supply, because these dietary elements are directly involved in DNA methylation and synthesis *via* one-carbon metabolism [22]. The main biochemical function known for all co-enzymatic forms of folate is to transfer one-carbon moieties (Fig. (**1**)). The synthesis of S-AdoMet pertains precisely to the scope of this function. S-AdoMet is a compound that serves as universal methyl donor for a sizeable number of methylation reactions, including the methylation of nucleic acids [23]. Methionine is restored from homocysteine by methionine synthase (MS), a vitamin B_{12} dependent enzyme, in a reaction where 5-methyltetrahydrofolate (5-methylTHF) serves both as a cofactor and as a substrate (Fig. (**1**)). The reduced availability of 5-methylTHF, the main circulating form of folate, induces the reduction of S-AdoMet biosynthesis, thus functioning as a limiting factor for the availability of methyl groups for methylation reactions. Not only dietary folate depletion has been proven to decrease genomic DNA methylation in both humans [24, 25] and animal models [26] but also, dietary folate supplementation can restore the DNA methylation status [25].

Folate-derived one–carbon groups are essential for the *de novo* synthesis of thymidylate, as well as for the purine synthesis. Since the fidelity of DNA synthesis is critically dependent on the correct balance and availability of deoxynucleotides [22], disruptions in intracellular nucleotide pools induced by folate deficiency [27] or pharmacologic inhibition of folate metabolism [28], results in inappropriate uracil incorporation into DNA [29, 30], because in mammalian cells the *de novo* synthesis of thymidylate from deoxyuridylate is a rate-limiting step for DNA synthesis [30, 31].

The fact that folate derived one-carbon groups are essential for the synthesis of purines and pyrimidines [32] highlights the importance of folate also in mechanisms of DNA repair. Although the biochemical and molecular basis for the relationship between folate and DNA repair are not completely elucidated yet, it has been demonstrated that folate depletion induces an impairment of DNA repair in a rat model [4] as well as in a lymphocyte cell culture system [33].

MTHFR POLYMORPHISMS

Methylenetetrahydrofolate reductase (MTHFR) is considered a key enzyme in one-carbon metabolism as it catalyses the irreversible conversion of 5, 10-methylenetetrahydrofolate to 5-methylTHF (Fig. (**1**)) [6]. In 1988, Kang *et al.*, identified a thermolabile variant of MTHFR with reduced activity [34]. The thermolabile MTHFR has been subsequently found to be due to a common missense mutation, a C to T nucleotide transition at *MTHFR* base pair *677*, which results in an alanine to valine substitution at 222 position in MTHFR amino acid sequence [17]. The prevalence of the valine-valine substitution is rather common, with a frequency of homozygous individuals as high as 20% in North American and European populations [17, 35, 36]. Individuals who are homozygous mutant for the rare variant (*TT*) have about 30% of normal enzyme activity, whereas heterozygotes (*CT*) have a mean MTHFR activity of about 65% compared to wild-type subjects (*CC*) [17]. With respect to the carriers of the common variant (*CC*), the heterozygotes (*CT*) have 10% and *TT* homozygotes 18% lower red cell folate levels [37].

The biological significance of the *MTHFR 677C>T* mutation is predominantly related to the reduced availability of 5-methylTHF. Consistent with this concept, is also the recent observation that the distribution of different co-

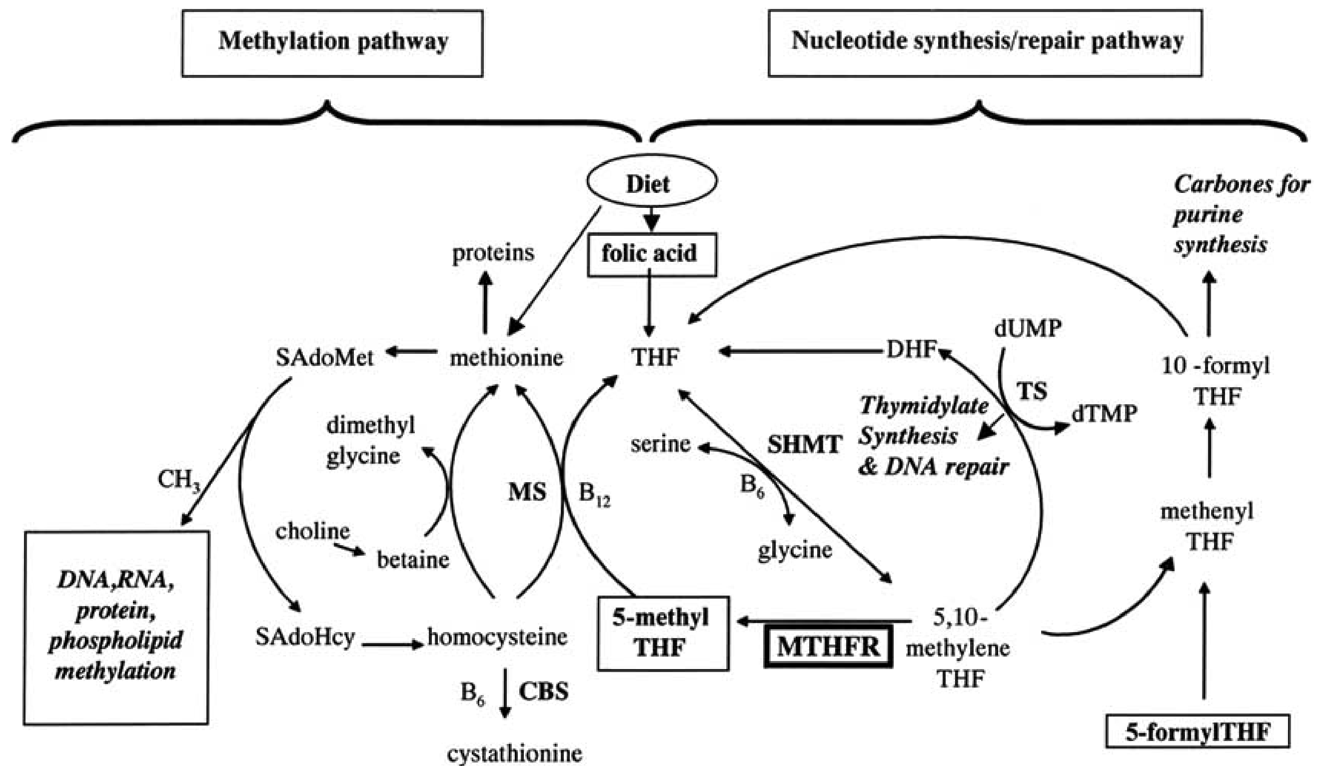

Fig. (1). Folate and MTHFR gene in one carbon metabolism.

MTHFR = 5, 10-methylenetetrahydrofolate reductase; SHMT = serine hydroxymethyltransferase; TS = thymidylate synthase; CBS = cysthationine synthase; MS = methionine synthase.

enzymatic forms of folate is altered in *MTHFR TT* homozygotes [38]. The red blood cells (RBC) of *TT* homozygous mutants show variable amounts of formylated tetrahydrofolate polyglutammates at the expenses of methylated tetrahydrofolates. In contrast, cells from the *CC* wild-type individuals contain exclusively methylated tetrahydrofolate derivatives [19, 38].

A second common mutation in the *MTHFR* gene at base pair 1298 resulting in an adenine to cytosine substitution has been described [39]. Although individuals homozygous for the polymorphism show reduced *MTHFR* activity by a 39%, the *1298 A>C* mutation, differently from the *677C>T*, does not confer thermolability [40]. Moreover, neither the homozygous nor the heterozygous state is associated *per se* with higher total plasma homocysteine (tHcy) levels or a lower plasma folate concentration-phenomena that are evident for the *677C>T* homozygosity condition [40]. However, there appears to be an interaction between these two common mutations. When compared with heterozygous for either the *677C>T* or the *1298A>C* mutations, the combined heterozygosity for the *1298A>C* and *677C>T* is associated with reduced MTHFR specific activity, higher tHcy, and decreased plasma folate levels. Thus, combined heterozygosity for both *MTHFR* mutations seems to have features similar to those observed in homozygotes for the *677C>T* mutation taken alone [40].

These two polymorphisms are usually not present in the same allele (*i.e.*, in "*cis*") [40, 41] but studies have shown that very rare *MTHFR* alleles have both polymorphisms [42, 43].

INTERACTION OF FOLATE AND *MTHFR GENE*: A PROTOTYPE OF GENE-NUTRIENT INTERACTION

Folate and MTHFR Interaction in One-Carbon Metabolism

Under low folate status conditions, the less 5-methylTHF available for the impaired activity of the MTHFR mutant enzyme, diminish the conversion of homocysteine to methionine, resulting in increased tHcy concentrations in subjects with the 677 *TT* genotype [17, 36, 44]. The higher level of this sulfur-containing amino acid appears, therefore, as an indicator of altered one-carbon metabolism [27, 29].

By determination of plasma total homocysteine levels, a strong nutrient-gene interaction was demonstrated in the phenotypic expression of this polymorphism in *MTHFR* [36, 44]. Jacques *et al*., first showed that individuals with the thermolabile *MTHFR* variant might have a higher folate requirement for the regulation of plasma homocysteine concentrations, highlighting the presence of an interaction between this common polymorphism and folate in homocysteine metabolism [44]. We subsequently reported that subjects with *677 TT* with inadequate folate status, as indicated by their blood folate levels lower than the median (11.5 nmol/L), had 59% increased tHcy concentrations and an effect at intermediate extent (21%) was also observed in

heterozygous (*677CT*) individuals [36]. On the other hand, at adequate folate status conditions, there was no difference in tHcy concentrations among the three genotypes [36]. These findings contributed to give emphasis to the interdependence between nutrition and genetics, especially since this relationship of the *MTHFR* polymorphism with plasma folate levels was implicated as the likely link between the *MTHFR* polymorphism and many diseases including cardiovascular disease, neural tube defects and cancer. In coronary artery disease, the interaction between *MTHFR* and folate, which defines a higher risk for the disease, is determined by folate levels below a certain specific thresholds, which differ for each *MTHFR 677C>T* genotype [45]. The limited availability of 5-methylTHF in *677 TT* genotypes, particularly under low folate conditions, significantly impairs the ability of the cell to remethylate homocysteine to methionine resulting in homocysteine accumulation. Hustad *et al.* recently showed that also vitamin B_2 concentrations affect this relationship, thus leading to higher tHcy concentrations only in *677 TT* genotypes but not in *677 CC* wild types [46].

Mechanism of Folate and MTHFR Gene Interaction

The presence of such a nutrient-gene interaction between the mutant MTHFR enzyme and folate status is consistent with the study of Guenther *et al.* who evaluated the biochemical structure of the mutant MTHFR and explained its propensity to lose its essential flavin cofactor [47].

The X-ray analysis of a thermolabile MTHFR variant expressed in *Escherichia coli* provided a model for the catalytic domain shared by all MTHFRs, to show that the mutant *MTHFR 677 TT* results in the exposure of binding sites for the flavin adenine dinucleotide (FAD) co-factor which would be otherwise embedded in a barrel-like structure. Such exposure results in a weakened enzyme/FAD complex, and hence loss of activity. The reduced activity of the mutant *E. coli* enzyme is attributable to diminished FAD binding, which affects the equilibrium between the more stable tetramer and the less stable dimeric form of the protein. The presence of adequate 5-methylTHF substrates is associated with conformational changes that strengthen the complex, thereby protecting wild-type and mutant MTHFR against the loss of its flavin co-factor [47].

Differently from the bacterial form, the human enzyme is a dimer and contains a domain that binds allosterically the inhibitor S-AdoMet. A recent study on human MTHFR function, demonstrated that once FAD is dissociated from the enzyme, occurs a rapid conversion of the dimer to monomers which gives less stability to the complex and is considered the phenomenon associated with the genotype-related loss of activity. Since both 5-methylTHF and S-AdoMet have a protective effect on the complex against FAD dissociation, folate depletion can further reduce MTHFR activity by decreasing the levels of 5-methylTHF and S-AdoMet in *MTHFR 677 TT* genotype. These observations also suggest the interaction among FAD, folate and *MTHFR* gene [48].

Yet, the question of whether and in which extent the recently discovered *1298A>C* polymorphism affects plasma homocysteine levels is still incompletely answered since the results of several studies are rather controversial [40-42; 49, 50] and in most studies the *1298A>C* polymorphism showed no effect on fasting or post-load tHcy levels [41]. It is worth of notice that some authors described even lower tHcy levels associated with the *1298CC* genotype [50]. Only one study thus far showed a trend versus higher tHcy in *1298CC* mutants taken alone [49]. The most plausible explanation for the different effect of the two variants on plasma homocysteine level is their specific effect on the enzyme control. The *1298A>C* affects enzyme regulation through S-AdoMet, an allosteric inhibitor of *MTHFR* that is known to bind in the C-terminal regulatory domain, whereas the *677C>T* is more likely to influence more dramatically the enzyme thermostability because of its localization in the N-terminal catalytic domain [47].

Effect of Folate and MTHFR Gene Interaction on Genomic DNA Methylation

Folate depletion has been reported to cause alterations in DNA methylation patterns. DNA methylation is the most important epigenetic phenomenon in mammalian DNA that regulates gene expression and integrity [7, 13, 51].

The mutant *MTHFR 677 TT*, in association with low folate status, affects this epigenetic feature of DNA in a gene-nutrient interaction manner [18, 19]. Evaluating genomic DNA methylation in peripheral lymphocytes, we observed that subjects homozygous mutant for the *MTHFR 677C>T* polymorphism, possess a lower degree of DNA methylation compared to the *CC* wild-type individuals [18, 19]. When analyzed according to folate status, however, only the *TT* subjects with low levels of folate accounted for the diminished DNA methylation compared to the *CC* wild-types (Fig. (**2**)). Moreover, in *TT* subjects DNA methylation status correlated with the methylated proportion of RBC folate and was inversely related to the formylated proportion of RBC folates that are known to be solely represented in *TT* individuals [19]. The results showed also that genomic DNA methylation directly correlate with folate status and inversely with tHcy levels which is consistent with the hypothesis of an indirect effect on cellular methylation reaction through a concomitant increase in levels of S-adenosylhomocysteine (S-AdoHcy), a potent inhibitor of DNA methylation reactions because most methyltransferases bind S-AdoHcy with higher affinity than S-AdoMet [52, 53]. These findings indicate that the *MTHFR 677C>T* polymorphism influences genomic DNA methylation status through an interaction with folate status [19, 54].

Effect of Folate and MTHFR Gene Interaction on Gene-Specific DNA Methylation

About half of human genes have CpG islands in their promoter regions or within initial exons of genes [51, 55]. The patterns of gene-specific DNA methylation define, at a molecular level, the genes to be expressed selectively [56], therefore, alteration in methylation status within promoter regions are regarded as a very important epigenetic mechanism in gene control [57]. In carcinogenesis, hypermethylation of CpG islands in promoter regions is clearly associated with gene transcriptional silencing, which gives to the methylation process an important role as an alternative

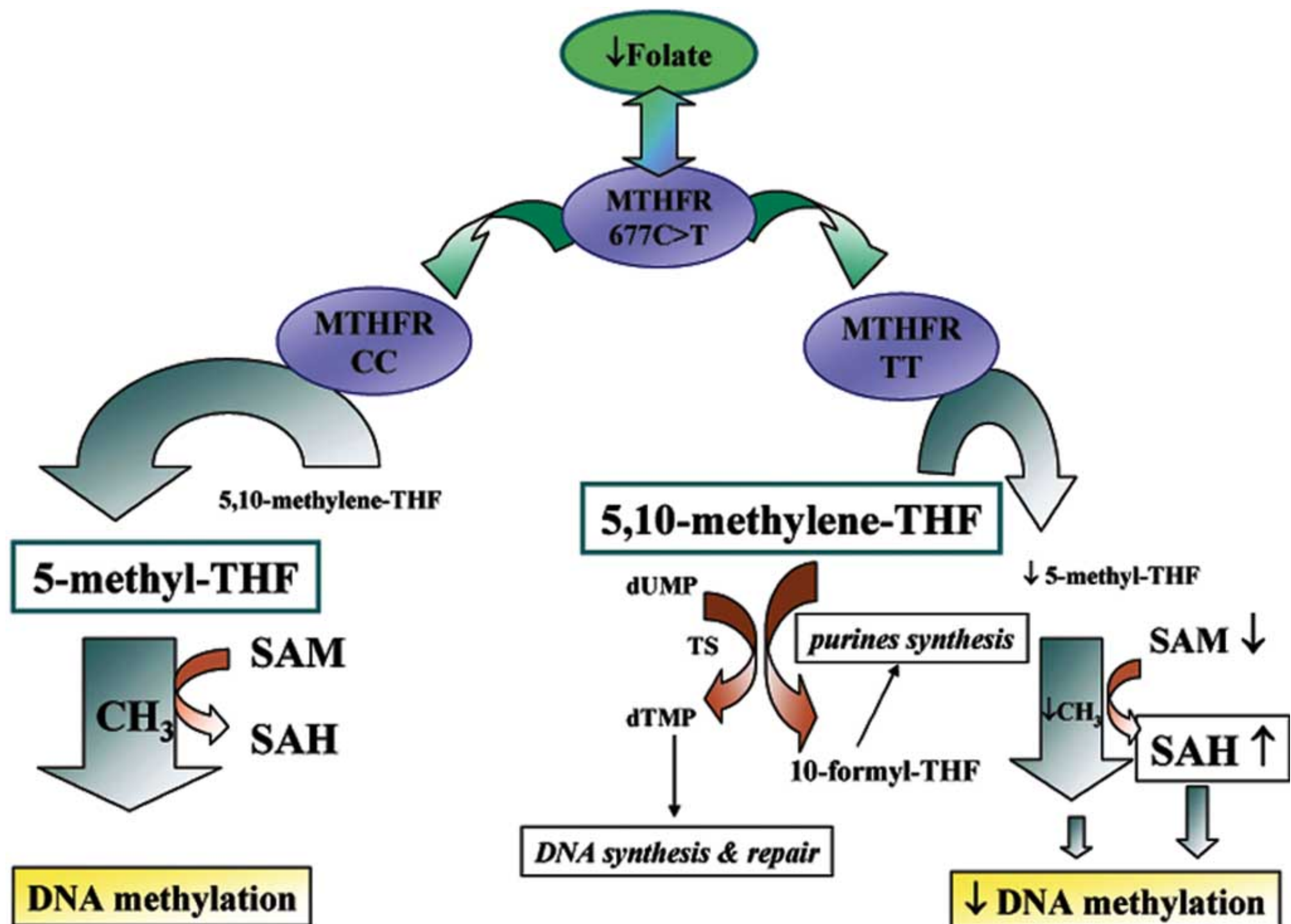

Fig. (2). Scheme illustrating the gene-nutrient interaction between the MTHFR 677C>T polymorphism and folate, and the effects on genomic DNA methylation status.

MTHFR is responsible for the irreversible conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (CH_3-THF). The *MTHFR 677C>T* variant encodes for a thermolabile enzyme with reduced activity. As shown in this figure, under folate deficiency conditions, subjects homozygous for the thermolabile *MTHFR* (*TT*) (right panel), decreased CH_3-THF production that results in lower S-adenosylmethionine levels and consequent lower availability of methyl groups (CH_3) for the methylation reactions including DNA methylation. The reduced availability of CH_3-THF is also reflected by diminished homocysteine remethylation with consequent higher S-adenosylhomocysteine levels (S-AdoHcy), an inhibitor of DNA methylation reaction. The reduced MTHFR activity in homozygous *677 TT* subjects, is thought to cause accumulation of 5, 10-methyleneTHF and therefore increase the availability of one-carbon groups required for the synthesis of dTMP and purines. On the contrary, subjects wild-type for the *677C>T* genotype (*CC*) are not affected by folate deficiency (left panel), since the synthesis of CH_3-THF for methylation reactions is preserved. The MTHFR genotype does not alter the availability of CH_3-THF when folate status is adequate.

The size of the arrows indicates the different entity in enzyme activity and the flux through the folate pools. The size of the font for the metabolic products indicates approximately the relative change in the amount of metabolites among the MTHFR genotypes.

MTHFR = 5, 10-methylenetetrahydrofolate reductase; S-AdoMet = S-adenosylmethionine; S-AdoHcy = S-adenosylhomocysteine; dUMP = deoxyuridylate; dTMP = deoxythymidylate; TS = thymidylate synthase; THF = tetrahydrofolate.

mechanism by which tumor suppressor genes are inactivated without structural DNA anomalies such as mutations or allele deletions. On the other hand, hypomethylation of CpG islands is associated with gene transcriptional activation, which is also an important mechanism by which proto-oncogenes are activated [57]. Data for an active role of promoter methylation in gene silencing are quite convincing, although the exact molecular mechanism by which DNA methylation represses transcription is not yet completely clear. Methylation of promoter-reporter constructs of transfected cells inhibits their expression *in vitro* [58]. DNA demethylating agents such as 5-azadeoxycytidine, a methyltransferase inhibitor, leads to the re-expression of previously methylated and imprinted genes [59]. A germline deletion of the *Dnmt1* gene in homozygous embryos, on which the prototypical mammalian cytosine DNA methyltransferase is

encoded, re-expresses a number of genes, including the normally silent alleles of several imprinted genes and the abundant but normally repressed endogenous retroviral sequences, which are methylated and silent in heterozygous littermates [60].

The mechanism for the CpG island-associated gene silencing seems to involve also the link of specific methylated DNA binding proteins, followed by the recruitment of a silencing complex that includes histone deacetylases [61, 62]. The *de novo* methylation, by itself, has a minimal effect on gene expression. However, methylated DNA conveys methyl-binding proteins to attract histone deacetylases protein complexes. Through the action of methyl-binding proteins and histone deacetylases, the DNA undergoes conformational structure changes that generate a compact chromatin configuration which makes the DNA refractory to nuclease or restriction endonuclease digestion, and leads to the loss of DNase-I-hypersensitive sites resulting, as a consequence, in altered protein assembly function due to permanent inhibition of messenger RNA [63]. Unmethylated CpG islands possess, instead, a nuclease-sensitive chromatin structure that differs from the vastness of the methylated genome [64].

In a rodent model of hepatocellular carcinoma, a choline-deficient diet induced hypomethylation of 5' upstream CpG sites of the *c-myc* gene and causes an over expression of the gene transcript [65]. Interestingly, experimentally induced deficiency of one-carbon nutrients in animals, which showed decreased genomic DNA methylation status, caused a paradoxical, and presumably compensatory, hypermethylation of specific gene loci [66]. Jhaveri *et al*. reported that also *H-cadherin* gene, in folate depletion conditions, showed hypermethylation within 5' sequences and was down regulated in human nasopharyngeal carcinoma KB cells [67].

This phenomenon is of particular interest because the promoter regions of several tumor suppressor genes such as *p16*, p53 and *APC* are frequently hypermethylated in cancer diseases [68-71]. For instance, a highly conserved region of the *p53* tumor suppressor gene appears to be extremely susceptible to the DNA damaging and hypomethylating effects of folate deficiency with molecular effects in decreased levels of *p53* mRNA in a steady state condition [72].

There is accumulating evidence that hypermethylation is involved in carcinogenesis since this phenomenon contribute to suppression of gene transcription [73]. Promoter methylation of *p53* and the mismatch repair gene *hMLH1* induce reduced expression of such genes transcripts in liver [72] and colorectal cancer cells [74].

In other methyl deficient animal studies increased levels of mRNA for *c- fos*, *c-Ha-ras* and *c-myc* were correlated with hypomethylation at specific sites within these genes [75, 76]. These observations suggest that nutrient may affect gene transcription by exon specific DNA methylation.

Based on these observations one might speculate that altered genomic DNA methylation status due to the interaction between folate and *MTHFR* might alter the status of promoter specific DNA methylation in critical genes and alter their expression. However, evidences are few to date.

Effect of Folate and MTHFR Gene Interaction on DNA Synthesis/Repair Pathway

The DNA methylation and DNA synthesis pathways, are highlighted by the common *677C>T* polymorphisms in the *MTHFR* gene, that causes reduced enzyme activity, because the *MTHFR* reaction sits as a pivot between DNA methylation and DNA synthesis, balancing the two pathways to maintain normal homeostasis (Fig. (**1**)). Noteworthy, the unique effects of gene-nutrient interaction between *MTHFR* and folate, are not only evident in respect to DNA methylation, but also in DNA synthesis [32].

Folate depletion generates an imbalance in nucleotide pools by an accumulation of dUMP at the expenses of dTMP, as reported from results in cell culture studies [27, 77]. The increase in uracil content into DNA under folate depletion condition has been demonstrated both *in vitro* [33, 77-80] and *in vivo* [24, 66]. The crucial role in chromosomal damage of the aberrant uracil incorporation into DNA was first observed by Reidy and colleagues in a folate-deficient cell culture system [81] and subsequently confirmed by the observation that the excision repair mechanisms to remove uracil bases from DNA result in double-strand breaks and induce deletion formation [82] and chromosomal translocations, all phenomena widely described in carcinogenesis [83]. Other factors such as aging have been also demonstrated to enhance the detrimental effects of folate depletion on uracil misincorporation in an animal model [84]. A recent report showed an evidence, in a lymphocyte tissue culture model, regarding the effect of the interaction between folic acid, riboflavin, and the *MTHFR 677C>T* polymorphism on micronuclei formation [85]. The micronuclei assay has been widely used to evaluate the effect of folate on DNA damage, being a valid marker for chromosome breakage and/or loss [86, 87]. The micronuclei levels reported in the study by Kimura *et al*., were 21% higher in *TT* cells than in *CC* cells, and 45% lower in the high folic acid medium than in the low folic acid medium with a highly significant statistically difference [87].

An effect of the common *MTHFR 677C>T* polymorphism on DNA synthesis pathway, was first hypothesized by Ma J. *et al*., in a nested case-control study design within the Physicians' Health Study [88]. From the results of that study, the authors suggested that the reduced colon cancer risk found in carriers of the *MTHFR 677C>T* mutation were due, perhaps, to the increased 5, 10-methylenetetrahydrofolate levels for DNA synthesis in those homozygous for the mutation only under adequate folate status conditions [88]. Subsequently, this hypothesis was tested *in vitro* in a human lymphocytes cell culture system [78]. The *677C>T* polymorphism, however, seems not to affect, by itself, the ability of the cells to limit uracil misincorporation into DNA. A dose-dependent increase in DNA uracil content was only observed during folic acid deficiency in both *677TT* homozygous mutants and controls (*677CC* and *677CT* subjects) [78]. The authors of that cell culture study drew the conclusion that, although the results did not support the hypothesis by Ma, *et al*. that a reduced risk for certain cancers in *677TT* is due to the diversion of folic acid to thymidine synthesis, differences between the *in vivo* and *in vitro* situation as well as short-term exposure to folate

depletion could be the reason for not reaching conclusive results [78].

Considering the major role of both MTHFR enzyme function and folate levels in DNA synthesis/repair system (Fig. (**1**)), more studies are certainly required to test the hypothesis that the DNA synthesis/repair metabolic pathway can be modulated in a gene-nutrient interactive fashion by *MTHFR 677C>T* polymorphism and folate status.

Significance of Folate and MTHFR Gene Interaction

Taking together, all the above described findings highly contributed to enhance the interest in both nutrition and genetics and in their mutual relationships, especially since the correlation of *MTHFR 677C>T* polymorphism with plasma folate levels is considered the link between the *MTHFR* genetic defect and cardiovascular disease, particularly under certain folate conditions, [36, 44, 89, 90] as well as neural tube defects [91-93] and neurodegenerative disorders [94-96].

The *MTHFR 677C>T* polymorphism also provides a paradigm of gene-nutrient interaction in carcinogenesis [5, 20, 22]. The mutant *TT* genotype is associated with a reduced risk for colorectal cancer, however, this protective effect is only observed in individuals with adequate folate status. Among those individuals with abnormal folate status, the protection associated with the polymorphic site vanishes [88], and an even more marked risk toward development of colorectal cancer is reported [5, 22].

OTHER GENE-NUTRIENT INTERACTIONS IN ONE-CARBON METABOLISM

As reported for the common *MTHFR 677 C>T* mutation, folate is likely to interact with other critical genes in one carbon metabolism. However, there are no clear evidences at present, in this regard. Polymorphisms of genes such as serine hydroxymethyltransferase (*SHMT*), thymidylate synthase (*TS*) and cystathionine- -synthase (*C S*), have been reported to significantly alter one carbon metabolism and affect the risk of lymphoid malignancies and colorectal cancer [97, 98].

Polymorphic sites within *SHMT* gene, an enzyme which catalyzes the reversible transfer of formaldehyde from serine to tetrahydrofolate (THF) to generate glycine and 5, 10 methyleneTHF, alters the folate coenzymatic form distribution, because SHMT preferentially supplies one-carbon units for thymidylate biosynthesis, depletes methylenetetrahydrofolate pools for S-AdoMet synthesis by synthesizing serine, and sequesters 5-methyltetrahydrofolate and inhibits S-AdoMet synthesis [99]. A polymorphism within *TS*, an enzyme which catalyzes the transfer of formaldehyde from folate to deoxyuridylate, enhances thymidylate production [100] and diminished activity of C S, a vitamin B_6 containing enzyme, which condenses homocysteine with serine to form cysta-thionine, produces demonstrable impairments in biological methylation [101].

Further evidences also suggest that other nutrients involved in one carbon metabolism might interact with different one-carbon metabolism genes in a similar gene-nutrient interaction fashion. MS and its cofactor vitamin B_{12} may be, in this regard, a good candidate target for further studies to highlight new gene-nutrient interactions within one-carbon metabolism [Fig. (***1***)].

Vitamin B_{12} deficiency is known to be associated with micronuclei formation [2, 31], and reduced transcobalamin II also has been related to chromosomal anomalies [102]. We recently described that low dietary vitamin B_{12} affects one-carbon metabolism also by inducing DNA genomic hypomethylation and increased uracil misincorporaton into the DNA of rat colonic epithelium [103]. In this animal model, we observed that vitamin B_{12} deficiency of a degree that neither produces anemia, illness or death can perturb one-carbon metabolism to a sufficiently large degree to produce anomalies in both biological methylation and DNA synthesis in the colonic mucosa. Since the molecular anomalies produced by vitamin B_{12} depletion arise from inhibition of the same pathways that produce such effects in the setting of folate depletion, we therefore hypothesize that vitamin B_{12} also can have an interaction with one carbon metabolism genes, especially MS enzyme of which needs B_{12} as a coenzyme.

Methionine synthase plays a major role in methyl group metabolism as it catalyses the methylation of homocysteine to methionine with concomitant conversion of 5-methylTHF to tetrahydrofolate (THF) and vitamin B_{12} is a critical coenzyme (Fig. (**1**)). Methionine is then converted to S-AdoMet, universal methyl donor for a number of biological methylation reactions. A polymorphism in the *MS* gene, an A to G transition at bp 2756, leads to the conversion from an aspartic acid residue to a glycine at codon 919 [104], a region believed to be part of a helix involved in the co-factor binding. Although the exact impact of this variant has not been established and the results of most studies are somewhat conflicting, there is some evidence that the *MS 2756A>G* polymorphism may be functional. In some studies, the *2756GG* genotype is associated with lower plasma homocysteine concentrations [105-108] and higher folate levels [106], in others no effects on homocysteine levels have been detected [109-112]. The functional effects of *MS 2756A>G* polymorphism on homocysteine reported so far, seem, unlike the *MTHFR* variants, independent of folate and vitamin B_{12} [107]. While these findings need to be confirmed, some evidences have been described of a gene-nutrient interaction between the common *MS 2756A>G* variant and low cobalamin (vitamin B_{12}) levels for the risk of spina bifida [113]. In a recent report, an interaction was observed also for *MS 2756A>G* polymorphism and risk of colorectal adenoma [114]. Among carriers of the *2756AG* and *2756GG* genotypes, an interaction was observed such that those with lower diet methionine intake and alcohol users showed a higher risk for colorectal adenoma [114]. However, such interaction was found only in women, and no interaction was seen for the *MS 2756A>G* polymorphism and intake of folate, vitamin B_{12}, or vitamin B_6 [114].

CONCLUSIONS

Recent observations from cultured cells, animal models and human studies support clear evidence that gene-nutrient interactions affect DNA methylation, a fundamental epigenetic feature in nucleic acid metabolic pathways that

affects gene expression and genomic integrity [summarized in (Fig. (**1, 2**))]. Several nutrients are well known to be involved in regulation of DNA metabolism, but most compelling data highlight the crucial role of folate as an essential vitamin for DNA synthesis/repair as well as DNA methylation (Fig. (**1**)).

The interaction between folate and *MTHFR 677C>T* polymorphism, which affects DNA methylation status as described above, represents a prototype for gene-nutrient interaction models, suggesting that many disease processes might be affected through altered DNA methylation, a fundamental epigenetic feature in nucleic acid metabolic pathways that affects gene expression and genomic integrity. In addition to such peculiar paradigm of gene-nutrient interaction other evidences also suggest that: 1) folate can interact with *MTHFR* gene and this interaction can affect DNA synthesis/repair system, 2) folate can interact with other one-carbon metabolism-related genes such as SHMT, TS and C S and this interaction can affect certain disease processes such as those related to development of colon cancer and lymphoblastic malignancies and 3) other nutrients such as vitamin B_{12} can also interact with genes in one-carbon metabolism and this interaction can affect DNA methylation as well as DNA synthesis/repair system as described for folate.

Studies regarding new potential modalities of gene-nutrient interactions can give us a more profound understanding for the mechanisms by which nutrients modulate DNA methylation and consequently the expression of genes and, therefore, affect disease processes such as those well recognized to be linked to the pathogenesis of cancer, cardiovascular disease and neurodegenerative disorders. Evidences for the beneficial effects of adequate folate status as well as folate form distribution according to specific *MTHFR 677C>T* genotypes also give us more insights for a strategy for future individual-tailored nutritional intervention strategies.

ACKNOWLEDGEMENTS

This work is supported by grants from: the National Funding of the Ministry of University, Scientific and Technologic Research, and the Ministry of Health of Italy; the Cariverona Foundation, Italy; the Regione Veneto, Ricerca Finalizzata, Venezia, Italy.

This work is also supported in part by National Cancer Institute grant (RO3 CA96004-02, SWC).

This material is based upon work supported by the U.S. Department of Agriculture, under agreement No. 58-1950-4-401. Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Dept of Agriculture.

ABBREVIATIONS

MTHFR = Methylenetetrahydrofolate reductase (enzyme)

MTHFR = Methylenetetrahydrofolate reductase (gene)

S-AdoMet = S-adenosyl-methionine

S-AdoHcy = S-adenosylhomocysteine

tHcy = Total plasma homocysteine

dUMP = Deoxyuridylate

dTMP = Deoxythymidylate

5-methylTHF = 5-methyltetrahydrofolate

THF = Tetrahydrofolate

MS = Methionine synthase (enzyme)

MS = Methionine synthase (gene)

SHMT = Serine hydroxymethyltransfrease (enzyme)

SHMT = Serine hydroxymethyltransfrease (gene)

TS = Thymidylate synthase (enzyme)

TS = Thymidylate synthase (gene)

C S = Cystathionine- -synthase (enzyme)

C S = Cystathionine- -synthase (gene)

REFERENCES

[1] Ames, B.N. (2001) *Mutat. Res.,* 475(**1-2**), 7-20.
[2] Fenech, M. and Ferguson L.R. (2001) *Mutat. Res.,* **475**(1-2), 1-6.
[3] Razin, A. and Riggs, A.D. (1980) *Science,* **210**(4470), 604-610.
[4] Choi, S.W.; Kim, Y.I.; Weitzel, J.N. and Mason JB. (1998) *Gut,* **43**(1), 93-99.
[5] Choi, S.W. and Mason, J.B. (2002) *J. Nutr*., **132**(8 Suppl), 2413S-2418S.
[6] Selhub, J. (1999) *Annu. Rev. Nutr.*, **19**, 217-246.
[7] Robertson, K.D. and Wolffe, A.P. (2000) *Nat. Rev. Genet.,* **1**(1), 11-19.
[8] Klimasauskas, S.; Kumar, S.; Roberts, R.J. and Cheng, X. (1994) *Cell*, **76**(2), 357-369.
[9] Laird P.W. and Jaenisch, R. (1994) *Hum. Mol. Genet.,* 3 Spec No, 1487-1495.
[10] Wolffe, A.P. and Matzke, M.A. (1999) *Science,* **286**(5439), 481-486.
[11] Jones, P.A. and Laird, P.W. (1999) *Nat. Genet.,* **21**(2), 163-167.
[12] Feinberg, A.P. (2001) *Nat. Genet.,* **27**(1), 9-10.
[13] Laird, P.W. (2003) *Nat. Rev. Cancer.,* **3**(4), 253-266.
[14] McCully, K.S. (1969) *Am. J. Pathol.,* **56**(1), 111-128.
[15] Clarke, R.; Daly, L.; Robinson, K.; Naughten, E.; Cahalane, S.; Fowler, B. and Graham, I. (1991) *N. Engl. J. Med.*, **324**(17), 1149-1155.
[16] Welch, G.N. and Loscalzo, J. (1998) *N. Engl. J. Med.,* **338**(15), 1042-1050.
[17] Frosst, P.; Blom, H.J.; Milos, R.; Goyette, P.; Sheppard, C.A.; Matthews, R.G.; Boers, G.J.H.; denHeijer, M.; Kluijtmans, L.A.J.; van den Heuvel, L.P. and Rozen, R. (1995) *Nature Genet.,* **10**(1), 111-113.
[18] Stern, L.L.; Mason, J.B.; Selhub, J. and Choi, S.W. (2000) *Cancer. Epidemiol. Bio. Prev.,* **9**(8), 849-853.
[19] Friso, S.; Choi, S.W.; Girelli, D.; Mason, J.B.; Dolnikowski, G.G.; Bagley, P.J.; Olivieri, O.; Jacques, P.F.; Rosenberg, I.H.; Corrocher, R. and Selhub, J. (2002) *Proc. Natl. Acad. Sci. USA*, **99**(8), 5606-5611.
[20] Kim, Y.I. (1999) *J. Nutr. Biochem.,* **10**, 66-88.
[21] Go, V.L.; Wong, D.A. and Butrum, R. (2001) *J. Nutr.,* **131**(11 Suppl), 3121S-3126S.
[22] Choi, S.W. and Mason, J.B. (2000) *J. Nutr.,* **130**(2), 129-132.
[23] Wagner, C. (1995) In *Folate in health and disease*, (Bailey L.B., Ed.), Marcel Dekker, New York, pp. 23-42.
[24] Jacob, R.A.; Gretz, D.M.; Taylor, P.C.; James, S.J.; Pogribny, I.P.; Miller, B.J.; Henning, S.M. and Swendseid, M.E. (1998) *J. Nutr.,* **128**(7), 1204-1212.
[25] Rampersaud, G.C.; Kauwell, G.P.; Hutson, A.D.; Cerda, J.J. and Bailey, L.B. (2000) *Am. J. Clin. Nutr.,* **72**(4), 998-1003.
[26] Balaghi, M. and Wagner, C. (1993) *Biochem. Biophys. Res. Commun.,* **193**(3), 1184-1190.

[27] James, S.J.; Basnakian, A.G. and Miller, B.J. (1994) *Cancer Res.,* **54**(19), 5075-5080.
[28] Kasahara, Y.; Nakai, Y.; Miura, D.; Kanatani, H.; Yagi, K.; Hirabayashi, K.; Takahashi, Y. and Izawa, Y. (1993) *Mutat. Res.,* **319**(2), 143-149.
[29] Blount, B.C. and Ames, B.N. (1994) *Anal. Biochem.,* **219**(2), 195-200.
[30] Blount, B.C.; Mack, M.M.; Wehr, C.M.; MacGregor, J.T.; Hiatt, R.A.; Wang, G.; Wickramasinghe, S.N.; Everson, R.B. and Ames, B.N. (1997) *Proc. Natl. Acad. Sci. USA,* **94**(7), 3290-3295.
[31] Fenech, M.; Aitken, C. and Rinaldi, J. (1998) *Carcinogenesis,* **19**(7), 1163-1171.
[32] Shane, B. and Stokstad, E.L. (1985) *Annu. Rev. Nutr.,* **5**, 115-141.
[33] Duthie, S.J. and Hawdon, A. (1998) *FASEB J.,* **12**(14), 1491-1497.
[34] Kang, S.S.; Zhou, J.; Wong, P.W.; Kowalisyn. J. and Strokosch, G. (1988) *Am. J. Hum. Genet.,* **43**(4), 414-421.
[35] Ma, J.; Stampfer, M.J.; Hennekens, C.H.; Frosst, P.; Selhub, J.; Horsford, J.; Malinow, M.R.; Willett, W.C. and Rozen, R. (1996) *Circulation,* **94**(10), 2410-2416.
[36] Girelli, D.; Friso, S.; Trabetti, E.; Olivieri, O.; Russo, C.; Pessotto, R.; Faccini, G.; Pignatti, P.F.; Mazzucco, A. and Corrocher, R. (1998) *Blood,* **91**(11), 4158-4163.
[37] Sharp, L. and Little, J. (2004) *Am. J. Epidemiol.,* **159**(5), 423-443.
[38] Bagley, P.J. and Selhub, J. (1998) *Proc. Natl. Acad. Sci. USA,* **95**(22), 13217-13220.
[39] Viel, A.; Dall'Agnese, L.; Simone, F.; Canzonieri, V.; Capozzi, E.; Visentin, M.C.; Valle, R. and Boiocchi, M. (1997) *Br. J. Cancer,* **75**(8), 1105-1110.
[40] van der Put, N.M.J.; Gabreels, F.; Stevens, E.M.B.; Smeitink, J.A.M.; Trijbels, F.J.M.; Eskes, T.K.A.B.; van den Heuvel, L.P. and Blom, H.J. (1998) *Am. J. Hum. Genet,* **62**(5), 1044-1051.
[41] Friso, S.; Girelli, D.; Trabetti, E.; Stranieri, C.; Olivieri, O.; Tinazzi, E.; Martinelli, N.; Faccini, G.; Pignatti, P.F. and Corrocher, R. (2002) *Clin. Exp. Med*., **2**(1), 7-12.
[42] Weisberg, I.; Tran, P.; Christensen, B.; Sibani, S. and Rozen, R. (1998) *Mol. Genet. Metab.,* **64**(3), 169-172.
[43] Isotalo, P.A.; Wells, G.A. and Donnelly, J.G. (2000) *Am. J. Hum. Genet*., **67**(4), 986-990.
[44] Jacques, P.F.; Bostom, A.G.; Williams, R.R.; Ellison, R.C.; Eckfeldt, J.H.; Rosenberg, I.H.; Selhub, J. and Rozen, R. (1996) *Circulation*, **93**(1), 7-9.
[45] Girelli, D.; Martinelli, N.; Pizzolo, F.; Friso, S.; Olivieri, O.; Stranieri, C.; Trabetti, E.; Faccini, G.; Tinazzi, E.; Pignatti, P.F. and Corrocher R. (2003) *J. Nutr.,* **133**(5), 1281-1285.
[46] Hustad, S.; Ueland, P.M.; Vollset, S.E.; Zhang, Y.; Bjorke-Monsen, A.L. and Schneede, J. (2000) *Clin. Chem.,* **46**(8 Pt 1), 1065-1071.
[47] Guenther, B.D.; Sheppard, C.A.; Tran, P.; Rozen, R.; Matthews, R.G. and Ludwig, M.L. (1999) *Nature. Struct. Biol.*, **6**(4), 359-365.
[48] Lathrop Stern, L.; Shane, B.; Bagley, P.J.; Nadeau, M.; Shih, V.and Selhub, J. (2003) *J. Nutr.,* **133**(9), 2716-2720.
[49] Chango, A.; Boisson, F.; Barbé, F.; Quilliot, D.; Droesch, S.; Pfister, M.; Fillon-Emery, N.; Lambert, D.; Frémont, S.; Rosenblatt, D.S. and Nicolas, J.P. (2000) *Br. J. Nutr.,* **83**(6), 593-596.
[50] Friedman, G.;Goldschmidt, N.; Friedlander, Y.; Ben-Yehuda, A.; Selhub, J.; Babaey, S.; Mendel, M.; Kidron, M.; Bar-On, H. (1999) *J. Nutr.,* **129**(9), 1656-1661.
[51] Bird, A. (1992) *Cell*, **70**(1), 5-8.
[52] Hoffman, D.R.; Cornatzer, W.E.and Duerre, J.A. (1979) *Can. J. Biochem.*, **57**(1), 56-65.
[53] Yi, P.; Melnyk, S.; Pogribna, M.; Pogribny, I.P.; Hine, R.J. and James, S.J. (2000) *J. Biol. Chem.,* **275**(38), 29318-29323.
[54] Friso, S. and Choi, S.W. (2002) *J. Nutr.,* **132**(8 Suppl.), 2382S-2387S.
[55] Antequera, F. and Bird, A. (1993) *Proc. Natl. Acad. Sci. USA,* **90**(24), 11995-11999.
[56] Cedar, H. (1988) *Cell*, **53**(1), 3-4.
[57] Baylin, S.B.; Belinsky, S.A. and Herman, J.G. (2000) *J. Natl. Cancer Inst.,* **92**(18), 1460-1461.
[58] Stein, R.; Razin, A. and Cedar, H. (1982) *Proc. Natl. Acad. Sci. USA,* **79**(11), 3418-3422.
[59] Cameron, E.E.; Bachman, K.E.; Myohanen, S.; Herman, J.G. and Baylin, S.B. (1999) *Nat. Genet.,* **21**(1), 103-107.
[60] Li, E.; Bestor, T.H. and Jaenisch, R. (1992) *Cell***, 69**(6), 915-926.
[61] Jones, P.L.; Veenstra, G.J.; Wade, P.A.; Vermaak, , D.; Kass, S.U.; Landsberger, N.; Strouboulis, J. and Wolffe, A.P. (1998) *Nat. Genet.,* **19**(2), 187-191
[62] Nan, X.; Ng, H.H.; Johnson, C.A.; Laherty, C.D.; Turner, B.M.; Eisenman, R.N. and Bird, A. (1998) *Nature,* **393**(6683), 386-389.
[63] Santini, V.; Kantarjian, H.M. and Issa, J.P. (2001) *Ann. Intern. Med.,* **134**(7), 573-586.
[64] Tazi, J. and Bird, A. (1990) *Cell,* **60**(6), 909-920.
[65] Tsujiuchi, T.; Tsutsumi, M.; Sasaki, Y.; Takahama, M. and Konishi, Y. (1999) *Jpn. J. Cancer Res.,* **90**(9), 909-913.
[66] Pogribny, I.P.; Miller, B.J. and James, S.J. (1997) *Cancer Lett*., **115**(1):31-38.
[67] Jhaveri, M.S.; Wagner, C. and Trepel, J.B. (2001) *Mol. Pharmacol.,* **60**(6), 1288-1295.
[68] Herman, J.G.; Merlo, A.; Mao, L.; Lapidus, R.G.; Issa, J.P.; Davidson, N.E.; Sidransky, D. and Baylin, S.B. (1995) *Cancer Res*., **55**(20), 4525-4530.
[69] Schroeder, M. and Mass, M.J. (1997) *Biochem. Biophys. Res. Commun*., **235**(2), 403-406.
[70] Hiltunen, M.O.; Alhonen, L.; Koistinaho, J.; Myohanen, S.; Paakkonen, M.; Marin, S.; Kosma, V.M. and Janne, J. (1997) *Int. J. Cancer,* **70**(6), 644-648.
[71] Pogribny, I.P. and James, S.J. (2002) *Cancer Lett.,* **187**(1-2), 69-75.
[72] Pogribny, I.P. and James, S.J. (2002) *Cancer Lett.,* **176**(2), 169-174.
[73] Siegfried, Z.; Eden, S.; Mendelsohn, M.; Feng, X.; Tsuberi, B.Z. and Cedar, H. (1999) *Nat. Genet*., **22**(2), 203-206.
[74] Deng, G.; Peng, E.; Gum, J.; Terdiman, J.; Sleisenger, M. and Kim, Y.S. (2002) *Br. J. Cancer,* **86**(4), 574-579.
[75] Zapisek, W.F.; Cronin, G.M.; Lyn-Cook, B.D. and Poirier, L.A. (1992) *Carcinogenesis,* **13**(10), 1869-72.
[76] Dizik, M.; Christman, J.K. and Wainfan, E. (1991) *Carcinogenesis*, **12**(7), 1307-1312.
[77] Melnyk, S.; Pogribna, M.; Miller, B.J.; Basnakian, A.G.; Pogribny, I.P. and James, S.J. (1999) *Cancer Lett.,* **146**(1), 35-44.
[78] Crott, J.W.; Mashiyama, S.T.; Ames, B.N. and Fenech, M.F. (2001) *Carcinogenesis,* **22**(7), 1019-1025.
[79] Koury, M.J.; Horne, D.W.; Brown, Z.A.; Pietenpol, J.A.; Blount, B.C.; Ames, B.N.; Hard, R. and Koury, S.T. (1997) *Blood,* **89**(12), 4617-4623.
[80] Duthie, S.J.; Narayanan, S.; Blum, S.; Pirie, L. and Brand, G.M. (2000) *Nutr. Cancer,* **37**(2), 245-251.
[81] Reidy, J.A. (1987) *Mutat. Res.,* **192**(3), 217-219.
[82] Dianov, G.L.; Timchenko, T.V.; Sinitsina, O.I.; Kuzminov, A.V.; Medvedev, O.A. and Salganik, R.I. (1991) *Mol. Gen. Genet.,* **225**(3), 448-452.
[83] Mitelman, F. (2000) *Mutat. Res.,* **462**(2-3), 247-253.
[84] Choi, S.W.; Friso, S.; Dolnikowski, G.G.; Bagley, P.J.; Edmondson, A.N.; Nadeau, M.R.; Smith, D.E. and Mason, J.B. (2003) *J. Nutr.,* **133**(4), 1206-1212.
[85] Kimura, M.; Umegaki, K.; Higuchi, M.; Thomas, P. and Fenech, M. (2004) *J. Nutr.,* **134**(1), 48-56.
[86] Fenech, M.; Crott, J.; Turner, J. and Brown, S. (1999) *Mutagenesis,* **14**(6), 605-612.
[87] Crott, J.W.; Mashiyama, S.T.; Ames, B.N. and Fenech, M. (2001) *Cancer Epidemiol. Biomarkers. Prev.,* **10**(10), 1089-1096.
[88] Ma, J.; Stampfer, M.J.; Giovannucci, E.; Artigas, C.; Hunter, D.J.; Fuchs, C.; Willett, W.C.; Selhub, J.; Hennekens, C.H. and Rozen, R. (1997) *Cancer Res.,* **57**(6), 1098-1102.
[89] Klerk, M.; Verhoef, P.; Clarke, R.; Blom, H.J.; Kok, F.J.; Schouten, E.G.; MTHFR Studies Collaboration Group. (2002) *JAMA,* **288**(16), 2023-2031.
[90] Ueland, P.M.; Hustad, S.; Schneede, J.; Refsum, H. and Vollset, S.E. (2001) *Trends Pharmacol. Sci.,* **22**(4), 195-201.
[91] van der Put, N.M.; Steegers-Theunissen, R.P.; Frosst, P., Trijbels, F.J.; Eskes, T.K.; van den Heuvel, L.P.; Mariman, E.C.; den Heyer, M.; Rozen, R. and Blom, H.J. (1995) *Lancet,* **346**(8982), 1070-1071.
[92] van der Put, N.M. and Blom, H.J. (2000) *Eur. J. Obstet. Gynecol. Reprod. Biol.,* **92**(1), 57-61.
[93] Schwahn, B. and Rozen, R. (2001) *Am. J. Pharmacogenomics,* **1**(3), 189-201.
[94] Clarke, R.; Smith, A.D.; Jobst, K.A.; Refsum, H.; Sutton, L. and Ueland, P.M. (1998) *Arch. Neurol.*, **55**(11), 1449-1455.
[95] Yoo, J.H.; Choi, G.D. and Kang, S.S. (2000) *Arterioscler. Thromb. Vasc. Biol.,* **20**(8), 1921-1295.

[96] Postiglione, A.; Milan, G.; Ruocco, A.; Gallotta, G.; Guiotto, G. and Di Minno, G. (2001) *Gerontology,* **47**(6), 324-329.

[97] Skibola, C.F.; Smith, M.T.; Hubbard, A.; Shane, B.; Roberts, A.C.; Law, G.R.; Rollinson, S.; Roman, E.; Cartwright, R.A. and Morgan, G.J. (2002) *Blood,* 99(10), 3786-3791.

[98] Ulrich, C.M.; Bigler, J.; Bostick, R.; Fosdick, L. and Potter, J.D. (2002) *Cancer Res.,* **62**(12), 3361-3364.

[99] Herbig, K.; Chiang, E.P.; Lee, L.R.; Hills, J.; Shane, B. and Stover, P.J. (2002) *J. Biol. Chem.,* **277**(41), 38381-38389.

[100] Kawakami, K.; Omura, K.; Kanehira, E. and Watanabe, Y. (1999) *Anti Cancer Research,* **19**(4B), 3249-3252.

[101] Choumenkovitch, S.F.; Selhub, J.; Bagley, P.J.; Maeda, N.; Nadeau, M.R.; Smith, D.E. and Choi, S.W. (2002) *J. Nutr.,* **132**(8), 2157-2160.

[102] Rana, S.R.; Colman, N.; Goh, K.O.; Herbert, V. and Klemperer, M.R. (1983) *Am. J. Hematol.,* **14**(1), 89-96.

[103] Choi, S.W.; Friso, S.; Ghandour, H.; Bagley, P.J.; Selhub, J. and Mason, J.B. (2004) *J. Nutr.,* **134**(4), 750-755.

[104] Leclerc, D.; Campeau, E.; Goyette, P.; Adjalla, C.E.; Christensen, B.; Ross, M.; Eydoux, P.; Rosenblatt, D.S.; Rozen, R. and Gravel, R.A. (1996) *Hum. Mol. Genet.,* **5**(12), 1867-1874.

[105] Dekou, V.; Gudnason, V.; Hawe, E.; Miller, G.J.; Stansbie, D. and Humphries, S.E. (2001) *Thromb. Haemost.,* **85**(1), 67-74.

[106] Chen, J.; Stampfer, M.J.; Ma, J.; Selhub, J.; Malinow, M.R.; Hennekens, C.H. and Hunter, D.J. (2001) *Atherosclerosis,* **154**(3), 667-672.

[107] Harmon, D.L.; Shields, D.C.; Woodside, J.V.; McMaster, D.; Yarnell, J.W.; Young, I.S.; Peng, K.; Shane, B.; Evans, A.E. and Whitehead, A.S. (1999) *Genet. Epidemiol.,* **17**(4), 298-309.

[108] Tsai, M.Y.; Bignell, M.; Yang, F.; Welge, B.G.; Graham, K.J. and Hanson, N.Q. (2000) *Atherosclerosis,* **149**(1), 131-137.

[109] Morita, H.; Kurihara, H.; Sugiyama, T.; Hamada, C.; Kurihara, Y.; Shindo, T.; Oh-hashi, Y. and Yazaki, Y. (1999) *Arterioscler. Thromb. Vasc. Biol.,* **19**(2), 298-302.

[110] van der Put, N.M.; van der Molen, E.F.; Kluijtmans, L.A.; Heil, S.G.; Trijbels, J.M.; Eskes, T.K.; Van Oppenraaij-Emmerzaal, D.; Banerjee, R. and Blom, H.J. (1997) *QJM*, **90**(8), 511-517.

[111] Jacques, P.F.; Bostom, A.G.; Selhub, J.; Rich, S.; Ellison, R.C.; Eckfeldt, J.H.; Gravel, R.A. and Rozen, R., National Heart, Lung and Blood Institute, National Institutes of Health. (2003) *Atherosclerosis,* **166**(1), 49-55.

[112] Klerk, M.; Lievers, K.J.; Kluijtmans, L.A.; Blom, H.J.; den Heijer, M.; Schouten, E.G.; Kok, F.J. and Verhoef, P. (2003) *Thromb. Res.,* **110**(2-3), 87-91.

[113] Wilson, A.; Platt, R.; Wu, Q.; Leclerc, D.; Christensen, B.; Yang, H.; Gravel, R.A.; Rozen, R. (1999) *Mol. Genet. Metab.,* **67**(4), 317-323.

[114] Goode, E.L.; Potter, J.D.; Bigler, J. and Ulrich, C.M. (2004) *Cancer Epidemiol. Biomarkers. Prev.,* **13**(1), 157-162.