Biological and clinical implications of the MTHFR C677T polymorphism

Per Magne Ueland, Steinar Hustad, Jørn Schneede, Helga Refsum and Stein Emil Vollset

The enzyme methylenetetrahydrofolate reductase (MTHFR) directs folate species either to DNA synthesis or to homocysteine (Hcy) remethylation. The common MTHFR C677T polymorphism affects the activity of the enzyme and hence folate distribution. Under conditions of impaired folate status, the homozygous *TT* genotype has been regarded as harmful because it is associated with a high concentration of plasma total Hcy, increased risk of neural tube defects and colorectal neoplasias, and can also predispose individuals to adverse effects from drugs with antifolate effects. The MTHFR C677T polymorphism shows no consistent correlation with cardiovascular risk and longevity but, in combination with positive folate balance, the *TT* genotype is associated with decreased risk of colorectal neoplasias. Because of the high prevalence of this polymorphism in most populations, the *TT* variant might represent an ancestral genetic adaptation to living constraints (tissue injury or unbalanced vitamin intake) that has become a determinant of disease profiles in modern times.

Per Magne Ueland*
Steinar Hustad
Jørn Schneede
Helga Refsum
Stein Emil Vollset
LOCUS for homocysteine and related vitamins, Armauer Hanssens hus, University of Bergen, 5021 Bergen, Norway.
*e-mail: per.ueland@ikb.uib.no

The flavin adenine dinucleotide (FAD)-dependent enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR, EC 1.5.1.20) catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which serves as a methyl donor in the remethylation of homocysteine (Hcy) to methionine. The enzyme resides at a metabolic branch point directing the folate pool towards Hcy remethylation at the expense of DNA and RNA biosynthesis[1] (Figs 1 and 2).

The enzyme MTHFR received much interest after the pivotal discovery of Kang *et al.*[2], who reported that a thermolabile enzyme variant was associated with increased cardiovascular risk and an increased concentration of plasma total Hcy (tHcy). Plasma tHcy has now been identified as a risk factor for occlusive disease in the coronary, cerebral and peripheral arteries and for venous thrombosis[3], and has also been related to the occurrence of neural tube and other birth defects[4] and pregnancy complications[5]. tHcy concentrations are elevated in some diseases and are also determined by genetic and physiological factors, lifestyle and intake of B-vitamins[3] such as folates, cobalamin, vitamin B6 and riboflavin[6]. The effects of vitamins are explained by their functions as co-factors or co-substrates in Hcy metabolism (Fig. 1).

In 1995, Frosst and colleagues reported on the C677T polymorphism in the *MTHFR* gene. The phenotype of this genetic variant is characterized by reduced catalytic activity and thermolability *in vitro*, and elevated tHcy under conditions of impaired folate status[7]. Later studies confirmed that this polymorphism is a common genetic determinant of plasma tHcy in the general population[8]. Its prevalence is related to ethnicity: the frequency of homozygosity for the *T* allele (*TT* genotype) is ~10% in caucasians, ~20% in some Italian populations and only a few per cent in Afro–Americans[9].

The first studies investigating the clinical relevance of the MTHFR C677T polymorphism were based on the simplistic view that this base transition caused a defective enzyme leading to elevated tHcy. Accordingly, most studies focused on conditions known to be related to elevated tHcy such as vascular disease and pregnancy complications. However, the combined evidence from a large series of case-control studies showed that an increased concentration of tHcy attributable to the *TT* genotype conferred essentially no cardiovascular risk enhancement[8,10].

This article reviews three aspects of the MTHFR C677T polymorphism: first, the metabolic effects, including altered folate distribution and tHcy concentrations, and the interrelationship between the concentration of tHcy and lifestyle factors and diseases; second, its association with risk of diseases, with emphasis on cardiovascular diseases, colorectal cancer, birth defects and pregnancy complications; and finally, the possible consequences of this polymorphism for drug therapy.

Metabolic effects

Hcy and folate

Several reports have shown consistently that the *T* allele is associated with a high concentration of plasma tHcy. The effect on the concentration of tHcy is most pronounced in homozygous *TT* subjects with low folate concentrations[8]. This is envisaged by a steeper slope of the curve of the inverse relationship between plasma tHcy and serum folate in subjects with the *TT* compared with the *CC* genotype[11], which suggests that the C677T transition confers increased folate responsiveness. In line with this, folic acid at daily doses of 0.5–2.0 mg caused a marked decrease in tHcy in *TT* subjects who obtained the same tHcy concentration as subjects with the *CC* genotype[1,12,13].

The concentration of erythrocyte folate varies according to genotype, but the variations are related to the folate assay used[14]. This is explained by differential detection by different assays of various intracellular folate species, the distribution of which is related to the *MTHFR* genotype. The latter observation was made by Bagley and Selhub[15], who reported that in the homozygous *TT* subjects,

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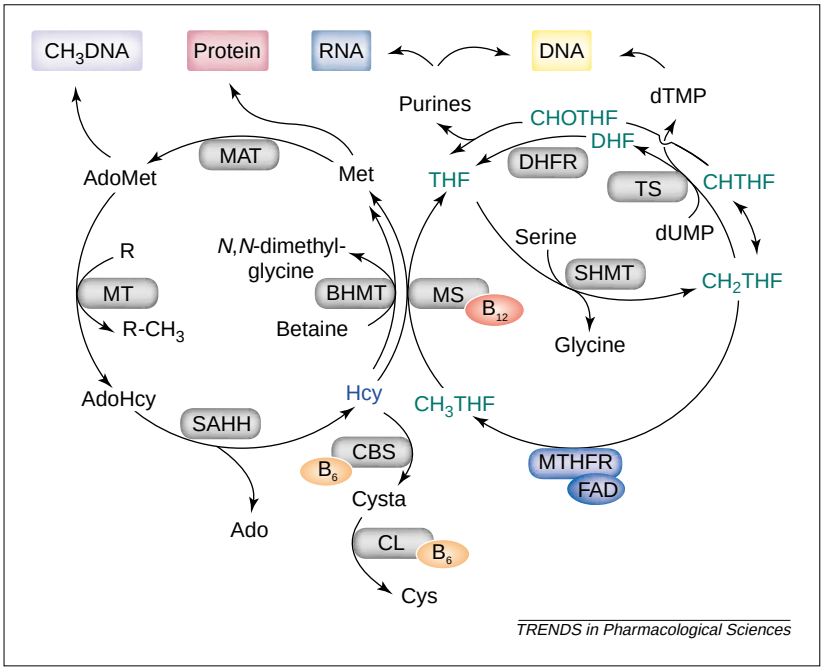

Fig. 1. Homocysteine (Hcy) formation, remethylation and trans-sulfuration, and the enzymes and B-vitamins involved. Hcy is formed from *S*-adenosylhomocysteine (AdoHcy). Remethylation to methionine (Met) is in most tissues catalyzed by the ubiquitous methionine synthase (MS), which requires cobalamin (B_{12}) as a cofactor and 5-methyltetrahydrofolate (CH_3THF) as a substrate. CH_3THF is formed by the action of the flavine adenine dinucleotide (FAD)-dependent enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR), which resides at a crucial metabolic locus directing the folate pool (green) to Hcy remethylation at the expense of folate used for DNA and RNA biosynthesis. Abbreviations: Ado, adenosine; AdoMet, *S*-adenosylmethionine; BHMT, betaine-homocysteine *S*-methyltransferase; CBS, cystathionine β-synthase; CH_2THF, 5,10-methylenetetrahydrofolate; CH_3DNA, methylated DNA; CHOTHF, formyltetrahydrofolate; CHTHF, methenyltetrahydrofolate; CL, cystathionine lyase; Cys, cysteine; Cysta, cystathionine; DHF, dihydrofolate; DHFR, dihydrofolate reductase; dTMP, deoxythymdine 5′-monophosphate; dUMP, deoxyuridine 5′-monophosphate; MAT, methionine adenosyltransferase; MT, methyltransferase; SAHH, *S*-adenosylhomocysteine hydrolase; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate; TS, thymidylate synthase.

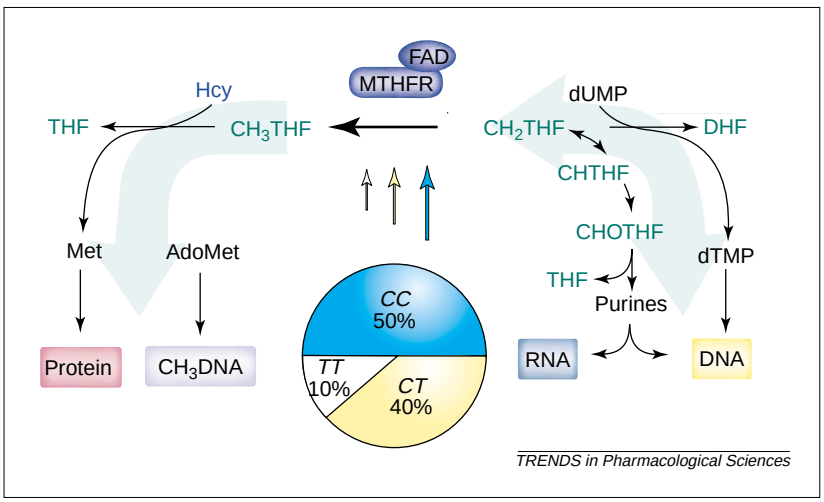

Fig. 2. The 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism affects the distribution between folate species (green) used for DNA and RNA syntheses and 5-methyltetrahydrofolate required for homocysteine (Hcy) remethylation and thereby protein synthesis. The pie chart in the center indicates the genotype prevalence often found in caucasian populations and the size of the associated vertical arrows indicates the MTHFR activity according to the genotype. Abbreviations: AdoMet, *S*-adenosylmethionine; CHOTHF, formyltetrahydrofolate; CHTHF, methenyltetrahydrofolate; CH_2THF, 5,10-methylenetetrahydrofolate; CH_3DNA, methylated DNA; CH_3THF, 5-methyltetrahydrofolate; DHF, dihydrofolate; dTMP, deoxythymidine 5′-monophosphate; dUMP, deoxyuridine 5′-monophosphate; FAD, flavine adenine dinucleotide; Hcy, homocysteine; Met, methionine; THF, tetrahydrofolate. *CC* and *TT* are the homozygous genotypes and *CT* is the heterozygous genotype. Modified, with permission, from Ref. 10.

formylated tetrahydrofolate polyglutamates accumulated at the expense of 5-methyl-tetrahydrofolate species, the prevailing forms in subjects with the *CC* genotype. Thus, the thermolabile enzyme variant causes a retention of the folate species committed to purine and pyrimidine synthesis (Fig. 2).

Vitamins B_2 and B_{12}

Recently, it has been shown that the concentration of riboflavin is inversely related to the plasma concentration of tHcy, and this relationship has been demonstrated in healthy subjects with both low and high concentrations of serum folate. The relationship is genotype dependent and is essentially confined to individuals with the C677T transition[6]. A possible regulation of folate distribution and tHcy concentration by riboflavin status and the C677T polymorphism could be explained by the function of FAD as a cofactor for MTHFR (Ref. 6), and it gains further support from the observation that a mutation in the bacterial *MTHFR* that is homologous to the C677T transition affects FAD dissociation kinetics[16].

Serum cobalamin is an established determinant of, and is inversely related to, plasma tHcy. The negative slope of the relationship between cobalamin and tHcy was found to be more pronounced in subjects with the *TT* genotype than those with *CT* or *CC* genotypes[17]. Notably, the tHcy–cobalamin relationship was maintained after folate supplementation, which makes confounding from folate status unlikely[17]. The mechanism responsible for this interaction is not readily understood but could reflect the propensity of subjects with the *TT* genotype to respond with a large tHcy increment to several factors that cause hyperhomocysteinemia.

Lifestyle

As part of the Hordaland Homocysteine Study, subjects with markedly elevated tHcy concentrations (≥40 μmol l^{-1}) were investigated. These individuals represented the upper 0.4% of the tHcy distribution from a general population sample of ~18 000 men and women. More than 70% of these 67 individuals possessed the *TT* genotype combined with folate deficiency. In addition, they were also more frequently heavier smokers, consumed more coffee and had a sedentary lifestyle compared with *CT* or *CC* genotypes[11]. All these factors are established determinants of tHcy (Ref. 18). These findings suggest that an unhealthy lifestyle interacts with the *TT* genotype and provokes a markedly elevated tHcy.

DNA methylation

A recent study by Stern *et al.* showed that the *TT* genotype is associated with lower DNA methylation in peripheral leukocytes compared with the *CC* genotype. A possible reason for this is the reduced availability of 5-methyltetrahydrofolate required for *S*-adenosylmethionine biosynthesis[19]. This

observation is particularly important because it demonstrates that altered folate distribution in subjects with the *TT* genotype has secondary metabolic effects in addition to hyperhomocysteinemia. In addition, DNA methylation has been considered to be an important factor in carcinogenesis[20].

Disease

The relationship between the MTHFR C677T polymorphism and disease involves two aspects. First, the disease might influence tHcy concentrations and there might be effect modification by the MTHFR polymorphism. Second, the genotype might be associated with disease risk, possibly mediated by altered metabolism of folates and Hcy.

Cardiovascular disease

The observation that the MTHFR *TT* genotype confers essentially no cardiovascular disease risk enhancement has been taken as evidence that elevated tHcy concentrations do not cause vascular disease. It also appears to support the view that hyperhomocysteinemia is a mere epiphenomenon related to subclinical nephrosclerosis[21]. However, the original studies and the meta-analyses published so far do not have sufficient statistical power to detect the small risk enhancement related to the mean tHcy increment of 2.6 μmol l^{-1} detected in subjects with the *TT* compared with the *CC* genotype[8,10]. Furthermore, there is evidence to show that elevated tHcy interacts with other risk factors and might provoke the acute vascular events in subjects with an underlying disease or thrombophilia. Notably, the observation that the *TT* genotype is a strong risk factor for cardiovascular disease in Japanese populations highlights the significance of genetic background[10]. Finally, a recent study showed that the *TT* genotype and hyperhomocysteinemia are associated with opposite preclinical modification of carotid artery geometry, which suggests that this genotype might even protect against occlusive vascular disease[22].

Renal failure

Renal disease is associated with increased risk of cardiovascular disease and markedly elevated tHcy (Ref. 23). Recently, renal patients with the *TT* genotype were found to be more susceptible to hyperhomocysteinemia than those with the *CC* genotype[24]. As in healthy subjects, the concentration of serum folate was lower and the regression line relating tHcy to folate was steeper in the patients with the *TT* genotype[25], but patients with this genotype readily responded to high-dose folic acid supplementation – which was evident from a reduction in tHcy – and they more often attained normal tHcy concentrations[26,27].

In renal patients, tHcy predicts mortality and cardiovascular morbidity[23]. The *TT* genotype can negatively affect the clinical outcome in kidney failure[25] and has also been associated with nephropathy in diabetics with a low concentration of folate[28]. Further studies are required to determine whether elevation of the concentration of tHcy attributable to the *TT* genotype confers increased risk of cardiovascular disease.

Congenital abnormalities and pregnancy outcome

The discovery that periconceptional folic acid supplementation markedly reduces the occurrence and recurrence of neural tube defects represents a major achievement of the past century in preventing congenital malformations. Neural tube defects have been related to a low blood concentration of folate and an elevated concentration of tHcy in mothers[4].

Most of the studies on this subject have shown that the *TT* genotype in the mother and baby (but not in the father) is associated with increased risk of neural tube defects. A recent meta-analysis showed that the *TT* genotype in the mother or child confers an overall risk enhancement corresponding to an odds ratio of about 2[9], but a recent large study on 271 baby–mother pairs indicates that the predominant MTHFR-related effect occurs in the developing embryo[29]. A strong nutrient–gene interaction is suggested by the observation that a low concentration of maternal blood folate[30] and no periconceptional vitamin supplementation[31] enhance the risk associated with the *T* allele. The combination of the *TT* genotype and a low concentration of folate increases the concentration of Hcy and impairs methionine formation in the embryo, and it is thought that both factors are involved in the genesis of incomplete neural tube closure. (The relationship between the MTHFR C677T polymorphism and congenital anomalies, including neural tube defects, was the subject of a recent comprehensive review article[9].)

The MTHFR *TT* genotype of the mother has been shown to increase the risk of Down's syndrome by approximately twofold[32,33]. Further risk enhancement has been observed with the combined presence in the mothers of the *TT* genotype and the A66G polymorphism in the gene encoding methionine synthase reductase[33], which is another enzyme involved in the methionine cycle. This observation not only addresses the multigenic origin of Down's syndrome but also emphasizes the importance of folate status in the preconceptional period[33].

Hyperhomocysteinemia and the *TT* genotype have been associated with pre-eclampsia, spontaneous abortion and placental abruption[5]. These conditions are related to impaired function of the placental vascular bed, and it is thought that elevated tHcy might be responsible for the vasculopathy. However, recent publications have questioned the association between the MTHFR C677T polymorphism and placenta-mediated diseases[34–37]. Conceivably, some points raised in the ongoing debate on MTHFR and

cardiovascular disease[10] might also be relevant for the issue of the correlation between pre-eclampsia and MTHFR.

Cancer

Low folate status is a risk factor for colorectal cancer as well as some other forms of cancer[20]. Folate status shows a strong interactive effect with the C677T polymorphism; this has been most convincingly demonstrated for colorectal cancer and its clinical precursor, colorectal adenomas. In folate-replete subjects, the *TT* genotype affords 50% risk reduction, whereas in subjects with low folate status, the *TT* genotype confers no protection or probably risk enhancement[38–41]. One study has suggested that smoking, which is an established risk factor of colorectal neoplasias, might be a particularly strong interactive factor. Furthermore, the study identified two high-risk categories for premalignant colorectal adenomas: smokers with low folate and the *TT* genotype, and smokers with the *CC* genotype and high folate[42]. This observation has implications in relation to the debate of folate fortification.

There is preliminary evidence to suggest that individuals with the *TT* genotype have a decreased risk of adult acute leukemia and increased risk of endometrial cancer, cervical neoplasia and breast cancer[43]. These studies were based on small populations, and there was no assessment of folate status. Risk enhancement in one malignancy versus protection in other malignancies might actually reflect the folate status of the study population.

Published data on the MTHFR C677T polymorphism and cancer risk indicate that the *T* allele protects against cancer in folate-replete subjects but increases the risk under conditions of impaired folate status. The protection might be related to abundant purines and pyrimidines available for DNA synthesis, leading to efficient DNA repair and essentially no uracil incorporation into DNA. The combination of low folate and *TT* genotype impairs Hcy remethylation to methionine; this could thereby cause DNA hypomethylation, which is known to be involved in carcinogenesis[38].

Other diseases

A positive association between the *TT* genotype and inflammatory bowel disease (IBD) has been observed in both an Irish[44] and a Danish[45] population but not in an Italian population[46]. Such differences between study populations could be explained by different folate status, which is in agreement with an elevated concentration of tHcy and a low concentration of folate in the Irish patients. These observations are relevant to the increased risk of colorectal cancer and thromboembolic events in IBD patients[44].

The *TT* genotype has been shown to increase the risk of psychiatric disorders[24] such as dementia[47], schizophrenia[48,49] and depression, but this has been contested in other studies[24,47,50,51]. In addition, investigations of the possible relationship between the C677T polymorphism and diabetic complications have shown conflicting results[24].

Drugs

Several drugs that interfere with folate status increase the concentration of tHcy (Ref. 18). Such drugs include not only the classical antifolate methotrexate, trimethoprim[52], but also sulfasalazine and the antiepileptic drugs phenytoin, carbamazepine and valproic acid.

In rheumatoid patients treated with methotrexate or sulfasalazine[53] and in epileptics receiving anticonvulsant medication[54], the tHcy concentrations are higher in *TT* than in *CC* subjects. In hypercholesterolemic children, treatment with cholestyramine was associated with a significant increase in tHcy concentrations, but only in those children with one or two *T* alleles[55].

L-Dihydroxyphenylalanine (L-dopa) increases Hcy production by serving as a substrate for catechol *O*-methyltransferase[56]. In rats this produces a rise in plasma tHcy, which is superimposed on the hyperhomocysteinemia induced by folate deficiency[56]. The tHcy response to L-dopa treatment is accentuated in patients with the MTHFR *TT* genotype[57]. Thus, for drugs that cause hyperhomocysteinemia either by interference with folate metabolism or by enhanced Hcy production, there is a marked effect modification by the MTHFR C677T polymorphism, and the *TT* genotype enhances the Hcy response. The important question is whether some of the side-effects caused by these drugs are mediated by Hcy or by altered folate distribution, and whether subjects with the *TT* genotype are at increased risk. Some observations suggest that this might be the case. A high concentration of tHcy in rheumatoid patients treated with methotrexate has been associated with adverse effects[53]. Furthermore, a preliminary report suggests that essentially all cancer patients experiencing severe toxicity from the drug combination cyclophosphamide, methotrexate and fluorouracil (CMF) possessed the *TT* genotype[58].

The MTHFR C677T polymorphism might modulate the effect of some drugs. The genotype could even interact with drugs to increase the risk of diseases associated with hyperhomocysteinemia such as cardiovascular disease, birth defects and pregnancy complications. Therefore, because genotyping could be useful for tailoring the dosage of some drugs, in particular antifolate agents, the pharmacogenetics of the MTHFR C677T polymorphism will be an important area for future research.

Concluding remarks and a hypothesis

The C677T transition has been implicated in several diseases. For neural tube defects and some malignant diseases, the *TT* genotype confers increased risk at low folate concentrations. At high folate concentrations, the *TT* genotype affords protection

Box 1. The effect of MTHFR C677T polymorphism on folate distribution under different conditions – a hypothetical model

The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) is responsible for the irreversible conversion of 5,10-methylene-tetrahydrofolate (CH_2THF) to 5-methyl-tetrahydrofolate (CH_3THF). Figure I depicts a model that proposes a modification by the MTHFR C677T polymorphism of the metabolic consequences of folate, cobalamin or riboflavin deficiency. This model integrates data on the metabolic effects, regulation and genetics of the MTHFR polymorphism – with some biological observations – into a unifying hypothesis.

The model explains the propensity towards hyperhomocysteinemia in subjects with the *TT* genotype, whereas folate species used for DNA and RNA synthesis are preserved. A high concentration of total homocysteine can by itself have a procoagulant effect and thereby predispose susceptible individuals to vascular occlusive disease, but folate sparing might also secure cell proliferation and tissue repair, which could be beneficial in subjects who are deficient in folate, riboflavin or cobalamin.

Additional support for the model can be gained from the following observations. One report described a patient with an inborn error of cobalamin metabolism (complementation group CblG) and the *TT* genotype who did not have megaloblastic anemia, which suggests that the *TT* genotype protects the bone marrow[b]. In addition, MTHFR is among the flavo enzymes that are most sensitive to impaired riboflavin status[c]. Thus, riboflavin deficiency could influence the tissue distribution and economy of reduced folate by decreasing MTHFR activity[d]. The FAD dissociation kinetics of the enzyme variant associated with the *T* allele[e] might favor this adaptive process. In experimental animals, riboflavin deficiency causes an increased oxidation state of the folate pool and a reduction in the relative amounts of 5-methyltetra-hydrofolate[c]. Riboflavin deficiency does not seem to be associated with overt megaloblastic anemia[d], and this could be due to a sparing effect on folate species used for DNA and RNA synthesis.

Fig. I

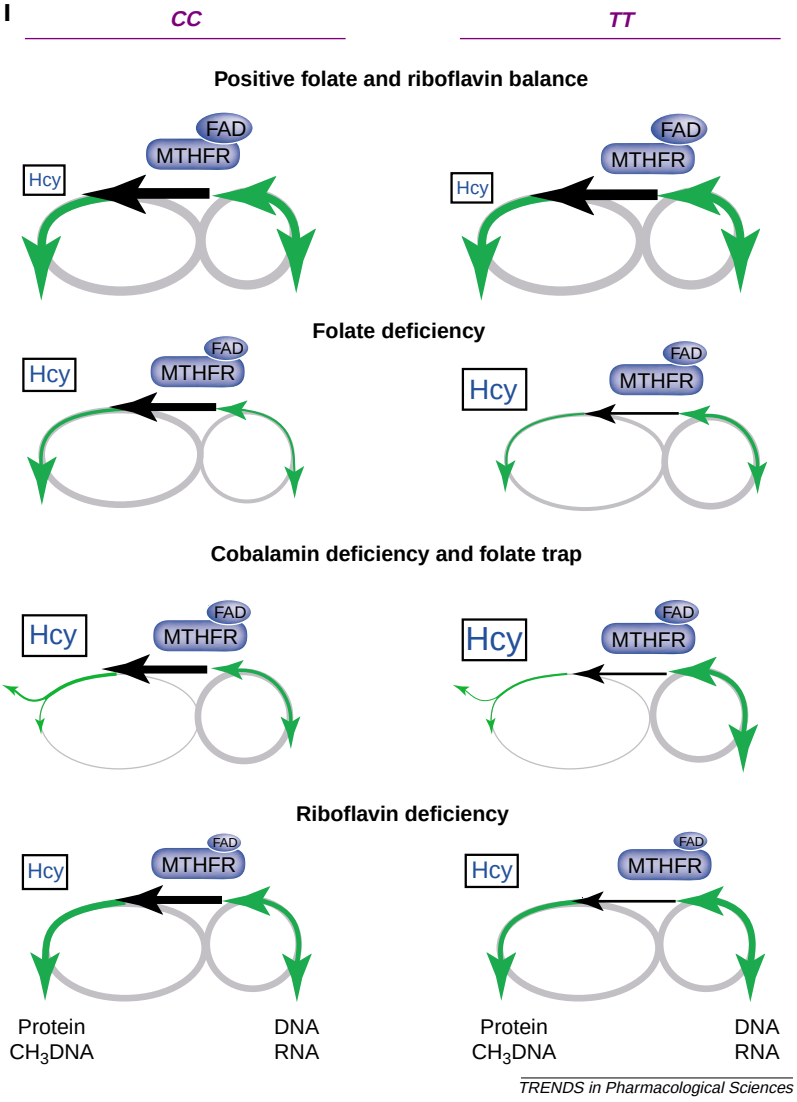

Fig. I. A hypothetical model for the effect of 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism on folate distribution under normal conditions and in folate, cobalamin or riboflavin deficiency. The folate homeostasis in vitamin-replete subjects (upper panel) ensures optimal homocysteine (Hcy) remethylation and low plasma total Hcy (tHcy) concentrations in both genotypes. In folate deficiency (second panel) the amount of all folate species is reduced. In the *TT* genotype, low MTHFR activity decreases 5-methyltetrahydrofolate (CH_3THF) formation and thereby Hcy remethylation, whereas there is a sparing effect on other one-carbon-substituted folates. In cobalamin deficiency (third panel), secondary folate loss can develop as a result of folate trapping[a]. The low MTHFR activity associated with the *TT* genotype could have a protective effect by antagonizing the loss of folate. Finally, the model predicts that riboflavin deficiency might have a similar sparing effect on one-carbon-substituted folates other than CH_3THF (fourth panel), as a result of depletion of the cofactor flavine adenine dinucleotide (FAD) and low MTHFR activity. In subjects with the *TT* genotype, this effect is enhanced because of lower cofactor affinity. The figures on the left- and right-hand sides demonstrate folate balance in subjects with the *CC* and the *TT* genotypes, respectively. The thickness of the black arrows represents the enzyme activity and the metabolic flux through this pathway. The thickness of the green arrows indicates the size of and flux through the folate pools, and the sizes of Hcy boxes represent the plasma tHcy concentrations. The sizes of the FAD circles and the overlap with the MTHFR boxes indicate FAD concentrations and binding to the MTHFR, respectively.

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against colorectal neoplasias. However, despite intensive research, no conclusion has been reached regarding the association between the polymorphism and cardiovascular disease.

Thus, the MTHFR *TT* genotype appears to protect against some diseases and increase the risk of others, which is in accordance with observations showing no correlation between genotype and longevity[24,59]. This polymorphism might therefore influence the disease profile in a population rather than the overall morbidity and mortality.

The C667T polymorphism should not be regarded *a priori* as a genetic defect that causes disease but rather as a genetic variant that could confer some contingent advantages during the reproductive period of life. For example, it is possible that the procoagulant effect of elevated tHcy concentrations in *TT* subjects enhances hemostasis. Furthermore, sufficient folate for DNA synthesis could ensure effective cell proliferation and tissue repair, which could be advantageous under conditions of delivery and tissue injury. The *TT* genotype could conserve folate species used for DNA synthesis in individuals on nutriture characterized by unbalanced or low content of folate, riboflavin or cobalamin (Box 1). These features might have promoted survival or reproduction in ancient times, and the C667T transition might therefore represent an example of ecological genetics of hemostasis, tissue repair or nutrition. Thus, an ancestral genetic adaptation might have turned into a predictor of diseases in modern times.

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FGF and VEGF function in angiogenesis: signalling pathways, biological responses and therapeutic inhibition

Michael J. Cross and Lena Claesson-Welsh

Angiogenic growth factors such as fibroblast growth factors (FGFs) and vascular endothelial growth factors (VEGFs) are currently targets of intense efforts to inhibit deregulated blood vessel formation in diseases such as cancer. FGFs and VEGFs exert their effects via specific binding to cell surface-expressed receptors equipped with tyrosine kinase activity. Activation of the receptor kinase activity allows coupling to downstream signal transduction pathways that regulate proliferation, migration and differentiation of endothelial cells. Inhibitors of FGF and VEGF signalling are currently in clinical trials. In this article, the current knowledge of FGF- and VEGF-induced signal transduction that leads to specific biological responses will be summarized. Furthermore, the manner in which this knowledge is being exploited to regulate angiogenesis will be discussed.

Angiogenesis denotes the formation of new blood vessels from pre-existing vessels. Physiological angiogenesis, which is required for embryonic development, wound healing and the menstrual cycle, is characterized by tight regulation both spatially and temporally. Angiogenic factors, such as fibroblast growth factors (FGFs) and vascular endothelial growth factors (VEGFs), stimulate endothelial cells to secrete several proteases and plasminogen activators, resulting in the degradation of the vessel basement membrane, which in turn allows cells to invade the surrounding matrix. The cells migrate, proliferate and eventually differentiate to form a new, lumen-containing vessel. Finally, the endothelial cells deposit a new basement membrane and secrete growth factors, such as platelet-derived growth factor (PDGF), which attract supporting cells such as pericytes, ensuring the stability of the new vessel[1]. This is a complex process that involves the concerted action of several other factors, such as the angiopoietins and ephrins, that act on specific receptors to regulate vessel stability[2].

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