Gene Focus

Concise expert focus on a gene at the heart of pharmacogenomic research

Pharmacogenetic relevance of *MTHFR* polymorphisms

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The 5,10-methylenetetrahydrofolate reductase (MTHFR) is a key enzyme for intracellular folate homeostasis and metabolism. Two common *MTHFR* polymorphisms, C677T and A1298C, which lead to an altered amino acid sequence, have been associated with a decreased enzyme activity and susceptibility to cancer suggesting that these genetic variants may modulate the risk of several malignancies. C667T, and to a lesser extent A1298C polymorphisms, are also reported to influence the cytotoxic effect of fluoropyrimidines and antifolates providing support for their pharmacogenetic role in predicting the efficacy and the toxicity in cancer and rheumatoid arthritis patients. A combined polymorphisms and haplotype analysis may result in a more effective approach than a single polymorphism one. Moreover gene–nutrient/environmental and gene–racial/ethnic interactions have been shown to affect the impact of these *MTHFR* genetic variants. Further well-designed studies are needed to clarify the role of *MTHFR* polymorphisms to derive dose adjustment recommendations on the basis of the patient's genotype.

C677T & A1298C *MTHTR* polymorphisms

The 5,10-methylenetetrahydrofolate reductase (MTHFR) is a key enzyme for intracellular folate homeostasis and metabolism. It catalyzes the irreversible conversion of 5,10-methylentetrahydrofolate required for purine and thymidine synthesis, to 5-methyltetrahydrofolate, the primary methyl donor for the remethylation of homocysteine to methionine, which is indispensable for nucleic acid methylation **(Figure 1)** [1].

Two relatively common nonsynonymous genetic variants, which lead to altered amino acid processes, have been described for the *MTHFR* gene: C677T (rs1801133), causing an alanine to valine replacement at codon 222 (Ala222Val) [2], and A1298C (rs1801131), causing a glutamic acid to alanine substitution at codon 429 (Glu429Ala) [3].

The C677T variant has been associated with a decreased activity of MTHFR, an increased level of homocysteine and altered distribution of folate [2]. The A1298C polymorphism has also been related to reduced MTHFR activity, but its effects have been considered to be less potent then those of the C677T variant [3]. The frequency of both polymorphisms varies significantly between regions and ethnic groups [4–6]; these genetic variants occur frequently among Caucasian and Asian populations, with a prevalence of approximately 25–40%, while they are quite rare in African populations. Haplotype analyses have shown that the two polymorphic sites are in linkage disequilibrium (LD) [7,8]. While the genotype polymorphic at both loci (677TT/1298CC) is very rare, suggesting that it may result in a severely adverse phenotype, the compound heterozygous (i.e., 677CT/1298AC) is quite common, with a prevalence of approximately 15–23% in Caucasians.

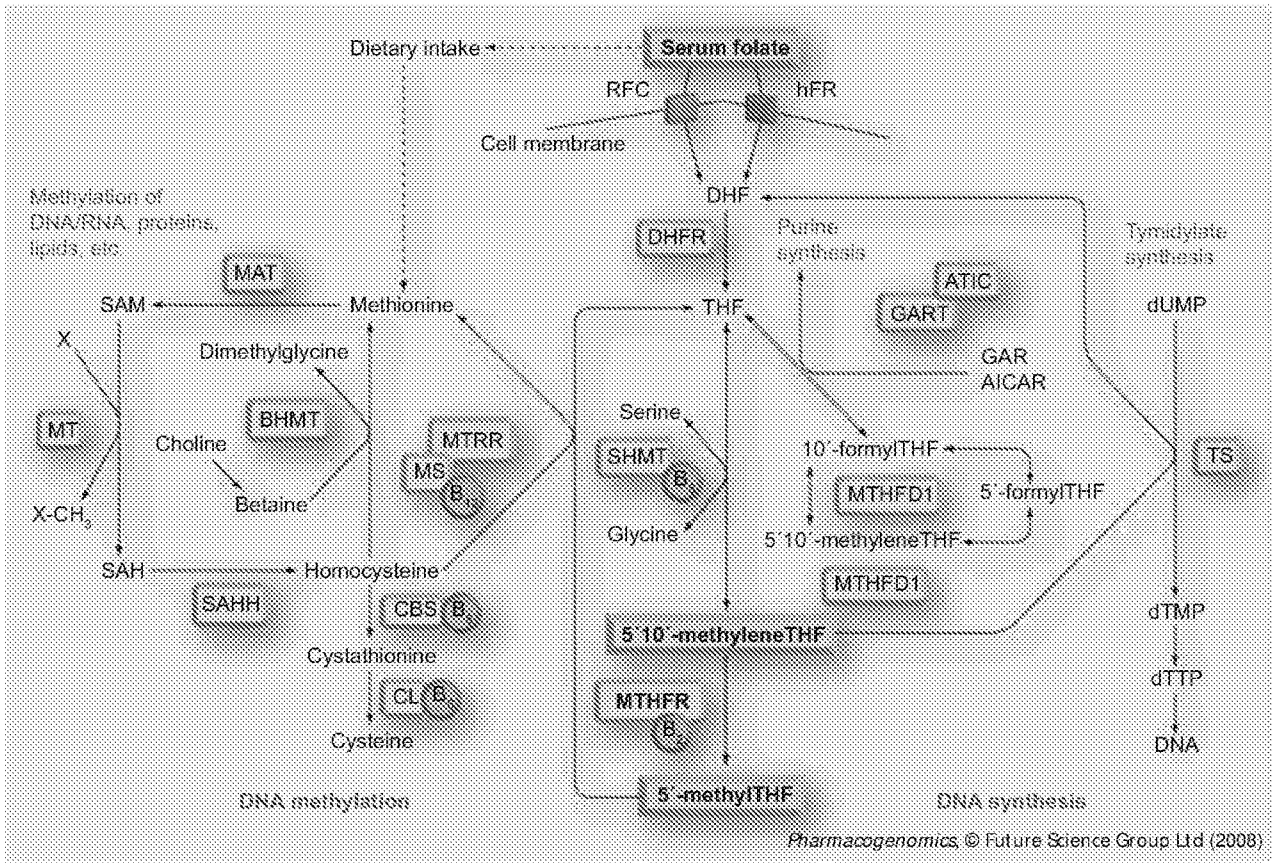
Recent analysis has indicated a significant functional interaction between the C677T and A1298C polymorphisms with a combinatorial and synergic effect on MTHFR enzyme activity

and its metabolic role [8]. Moreover the folate status, modifying the stability of the polymorphic enzyme, has been shown to have a relevant influence on the phenotypic effects of the *MTHFR* genetic variants; in condition of high intracellular folate, the folate molecules appear to be able to hold the variant MTHFR protein in the appropriate and fully functional 3D structure, thus stabilizing the thermolabile form and counteracting the reduction in enzyme activity [8].

C677T & A1298C in disease susceptibility

Many studies have demonstrated the involvement of *MTHFR* polymorphisms, mainly the C677T variant, in cancer susceptibility: colorectal cancer [9,10], lymphoproliferative diseases [11,12], gastric cancer [13], esophageal squamous cell carcinoma [14], prostate cancer [14], breast cancer, particularly in women in premenopausal status [15], and gynecological tumors [16,17]; preliminary evidence has also been reported for

Figure 1. Simplified overview of folate metabolism and related pathways.



In the schematic illustration, evidence of the crucial role of MTHFR in regulating the fine equilibrium between DNA synthesis and DNA methylation can be observed.
Transporters: hFR: Human folate receptor; RFC: Reduced folate carrier.
Enzymes (denoted as ovals): ATIC: 5-aminoimidazole-4-carboxamide ribonucleotide formyltranferase/IMP cyclohydrolase; BHMT: Beatine-homocysteine-*S*-methyltransferase; CBS: Cystathionine-β-synthase; CL: Cystathionine lyase; DHFR: Dihydrofolate reductase; GART: Glycinamide ribonucleotide formyltransferase; MAT: Methionine adenosyltransferase; MS: Methionine synthase; MT: Methyltransferase; MTHFR: 5,10-methylenetetrahydrofolate reductase; MTHFD1: Methylenetetrahydrofolate dehydrogenase 1; MTRR: Methionine synthase reductase; SAHH: *S*-adenosylhomocysteine hydrolase; SHMT: Serine hydroxymethyltransferase; TS: Thymidylate synthase.
Metabolites: AICAR: 5-aminoimidazole-4-carboxamide ribonucleotide; CH_3: Methyl group; DHF: Dihydrofolate; dTMP: Deoxythymidine 5´-monophosphate; dTTP: Deoxythymidine triphosphate; dUMP: Deoxyuridine 5´-monophosphate; GAR: Glycinamide ribonucleotide; SAH: *S*-adenosylhomocysteine; SAM: *S*-adenosylmethionine; THF: Tetrahydrofolate; X: Various substrates for methylation.
Vitamins B_2, B_6 and B_{12} are cofactors in the pathway.

lung [18], bladder [19] and hepatic [20] cancer and squamous cell carcinoma of the head and neck [21]. From these epidemiological analyses, it has emerged that the *MTHFR* variants modulate the tumor risk in a site-specific manner decreasing the susceptibility towards such type of malignancies (i.e., colorectal cancer) while increasing the risk of others (i.e., gastric cancer). This opposite effect suggests that each type of cancer may have a peculiar and characteristic underlying etiologic pathway. MTHFR plays a critical role in maintaining the fine equilibrium between DNA methylation and DNA synthesis and repair (**Figure 1**). It can be hypothesized that the biochemical effect of *MTHFR* polymorphisms and, in particular, DNA hypomethylation or uracil misincorporation into DNA, may exert a different impact on tumor susceptibility. DNA hypomethylation linked to the reduced activity of polymorphic MTHFR particularly in a context of low folate status [22], has appeared to be involved in the process of carcinogenesis and could explain the association between *MTHFR* variants and increasing risk of some tumors such as the gastric one [23]. Conversely for other types of malignancies, such as colorectal cancer, a more complex and dichotomous picture, owing to the inhered reduced MTHFR activity, may occur and the protective effect observed could be owing to the enhanced availability of non-methylated folate substrates for *de novo* synthesis of nucleotides that could preserve DNA integrity reducing uracil misincorporation, single- and double-strand breaks, DNA misrepair and genetic instability, all phenomena that possibly favor the development of malignancies [20]. Further mechanistic studies are necessary to confirm this hypothesis and to define the precise etiologic process underlying each type of tumor.

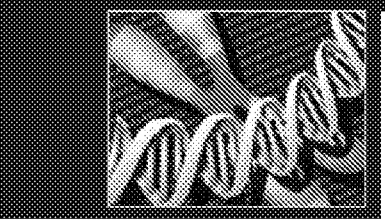
Published data have also provided evidence that the impact of the C677T and A1298C polymorphisms upon cancer susceptibility is deeply influenced by demographic and environmental factors, such as age, smoking, alcohol intake and folate status, all parameters that may additionally affect the fine equilibrium of one-carbon metabolism [13,14,19,24]. For example, the C677T polymorphism has emerged to act as a protective factor when the dietary folate level is adequate and as a risk factor when the folate level is deficient.

Moreover, preliminary results from investigations analyzing the combined effect of more than one polymorphism in *MTHFR* gene or in different genes involved directly or indirectly in the folate pathway and metabolism [11,12,18–21,24,25] has suggested that in association studies these haplotype and polygenic approaches could provide more efficient and informative information than a single polymorphism analysis.

It should be highlighted that in addition to cancer, there are several other diseases that may be influenced by C677T and A1298C polymorphisms such as cardiovascular dysfunctions [26], hypertension [27], thrombosis [28], neuronal tube defects [4], cleft lip/palate [29], preeclampsia [30], Down's syndrome [31] and psychiatric disorders [32].

C677T & A1298C as genetic determinants of clinical therapy outcome

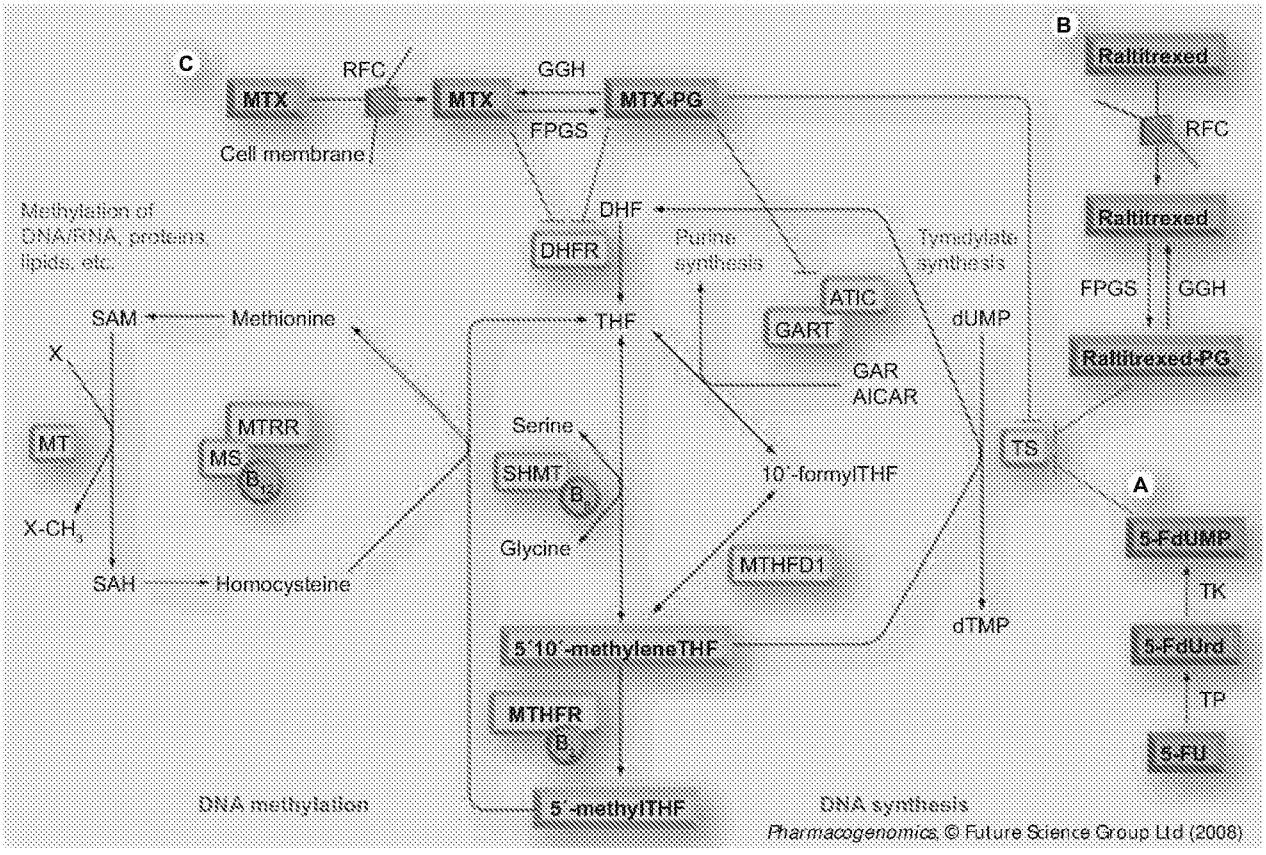
Several studies have investigated the potential role of C667T and A1298C polymorphisms in modulating the therapeutic effect and toxicity of fluoropyrimidines and antifolates, such as 5-fluorouracil (5-FU), raltitrexed and methotrexate (MTX), a commonly used drug whose cytotoxic activity is dependent upon a competitive interaction with folate metabolism (**Figure 2**). Therefore, an alteration in reduced folate pools, derived from inherent changes in MTHFR activity, may have a significant impact on the response of malignant and non-malignant cells to these agents with important clinical consequences as the large interpatient variability demonstrated in the outcome of the therapy. In the last few years, pharmacogenetic analyses have been performed to



examine the contribution of C667T and A1298C polymorphisms to drug response and efficacy of fluoropyrimidines and antifolates in order to optimize patients' treatment.

5-fluorouracil (**Figure 2a**) has been widely used in the treatment of solid malignancies, especially in colorectal carcinoma, both in advanced and adjuvant settings. *In vitro* analyses in human cancer cells and nude mice xenograft models have suggested that both C667T [33] and A1298C [34] may enhance the chemosensitivity to 5-FU. Clinical setting pharmacogenetic studies, performed in advanced colorectal

Figure 2. Mechanism of action of 5-fluorouracil, raltitrexed and methotrexate.



(A) 5-FU, a fluoropyrimidine compound, is metabolized intracellularly to its active form 5-FdUMP through two consecutive reactions catalyzed by TP and TK. 5-FU carries on this cytotoxic effect by mediating the formation of an inhibitory ternary complex, involving its metabolite 5-FdUMP, TS and 5,10-methylene THF. The formation of this complex inhibits TS activity, with subsequent diminuition of thymidylate levels and consequent suppression of DNA synthesis. Increased 5,10-methylene THF concentration, due to a reduced MTHFR activity, might enhance the formation and stability of this inhibitory complex, thereby augmenting the cytotoxic effect of 5-FU.
(B) Raltitrexed, a quinazoline antifolate agent, is transported intracellularly by RFC, and once in the cells it is extensively polyglutamated by FPGS; this modification is essential to potentiate the drug cellular retention, thus maximizing the direct and specific inhibition of TS, the target of the drug. 5,10-methylene THF competes with raltitrexed for TS inhibition and its level, modulated by MTHFR activity, may alter the cytotoxic effect of the drug.
(C) MTX is an antifolate agent whose uptake into the cells is mainly controlled by RFC1 (also know as SLC19A1). The main intracellular target of MTX is DHFR, the inhibition of which results in accumulation of DHF and depletion of cellular folates. Within cells, MTX is rapidly converted into a polyglutamated form (MTX-PG) by FPGS. This process can be reversed by the enzyme GGH. The larger and more polar polyglutamated form enhances cellular retention of MTX and increases its affinity for other target enzymes of thymidylate and purine biosynthesis pathways such as TS, GART and ATIC. Other enzymes that are indirectly affected by MTX are MTHFR and MTHFD1. The increase of 5,10-methylene THF levels, as a consequence of MTHFR polymorphisms, might alter the mechanism of action of MTX.
5-FdUMP: 5-fluoro-2-deoxyuridine-5'-monophospate; 5-FdUrd: 5-fluoro-2-deoxyuridine; 5-FU: 5-fluorouracil; ATIC: 5-aminoimidazole-4-carboxamide ribonucleotide formyltranferase/IMP cyclohydrolase; DHF: Dihydrofolate; DHFR: Dihydrofolate reductase; FPGS: Folylpolyglutamyl synthase; GAR: Glycinamide ribonucleotide; GART: Glycinamide ribonucleotide formyltransferase; GGH: γ-glutamyl hydrolase; MS: Methionine synthase; MT: Methyltransferase; MTHFR: 5,10-methylenetetrahydrofolate reductase; MTRR: Methionine synthase reductase; MTX: Methotrexate; RFC: Reduced folate carrier; SHMT: Serine hydroxymethyltransferase; THF: Tetrahydrofolate; TK: Thymidine kinase; TP: Thymidine phosphorylase; TS: Thymidylate synthase; X-PG: X-polyglutamated.

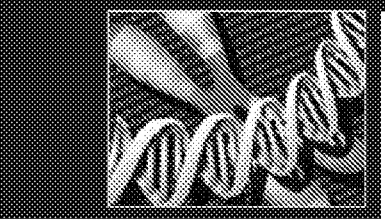
cancer patients receiving 5-FU-based treatment principally as first line treatment, have reported that the C667T genetic variant, but not A1298C, is significantly associated with increased tumor response rate [35–37]. The C677T polymorphism has also been associated with a significantly increased time to progression [37], while no relevant effects on overall survival [36] and toxicity [35,38] have been evidenced. On the other hand, the A1298C polymorphism has been reported to be correlated with an increased risk of developing severe adverse events [39] and with a lower specific survival [36]. The results from clinical analyses conducted in Asian advanced gastric cancer patients treated with 5-fluoropymiridine-based chemotherapy have confirmed the association between the *MTHFR* 677TT genotype and increased response rate and have also indicated a significant correlation with a higher risk to develop severe treatment-related toxicity [40]. However, recently published studies carried out in various tumor sites could not confirm the role of *MTHFR* polymorphisms as pharmacogenetic determinants of 5-FU therapy outcome [41–43]. Unlike previous studies where 5-FU was employed in a monotherapy scheme, the current treatment of cancer patients consists generally of a combination regimen where 5-FU is used in association with other chemotherapeutic drugs (i.e., methotrexate, platinum derivates or irinotecan), the presence of which could modify the impact of *MTHFR* genetic variants on fluoropymiridine therapy outcome, causing the inconsistent results reported in the more recent investigations.

Sparse pharmacogenetics data also exist for other oral fluoropymiridine agents such as uracil/tegafur (UFT) and capecitabine [45], a drug that is converted *in vivo* to 5-FU with a preferential activation in the tumor. The clinical outcome of chemotherapy containing these fluoropymiridines appears to be influenced by *MTHFR* polymorphisms, principally in term of the risk to develop severe toxicity; these findings, however, should be considered only exploratory.

As far as the role of *MTHFR* polymorphisms on raltitrexed treatment is concerned **(Figure 2B)**, up to now only one published study is available. It was realized on patients with solid tumors who had received the drug in combination with irinotecan. This analysis has suggested that the 677TT genotype is associated with a significant reduced toxicity [46]. It can be hypothesized that increased availability of 5,10-methylentetrahydrofolate, as a result of impaired MTHFR activity, could compete with raltitrexed for polyglutamation by folylpolyglutamate synthase and for binding to thymidylate synthase leading to a diminished cytotoxicity of the compound. Further studies are needed to validate these pilot data.

Methotrexate **(Figure 2C)** has been widely employed as an antineoplastic agent, alone or in combination with other drugs, for the treatment of a number of solid tumors and hematologic malignancies. Moreover, owing to its potential anti-inflammatory and autoimmune effects, this antifolate compound is one of the most commonly used drugs in rheumatic and other inflammatory conditions as well as in patients with hematological malignancy or disease undergoing hemapoietic cell transplantation to prevent graft-versus-host disease (GVHD). In recent years, a large body of data derived from different clinical settings where this drug is employed, on the potential role of C667T and A1298C polymorphisms as pharmacogenetic determinants of MTX-based-therapy outcome, has been published.

Several clinical trials have investigated the role of C677T polymorphism in modulating the risk of MTX-related adverse events, such as myelosuppression, gastrointestinal and hepatic alterations, mucositis, renal dysfunction and CNS toxicity. In some of these studies, the C677T variant has significantly been associated with an



increased toxicity after treatment with MTX, when used alone or in combination with other agents, in patients undergoing hematopoietic cell transplantation [47–49], in patients with lymphoproliferative malignancies [50–52], ovarian [53] and breast cancer [54] and in patients with arthritic diseases [55–59]. This correlation, however, could not be confirmed in other published researches [5,60–67].

The results obtained so far regarding the effect of C667T polymorphism on response to MTX are quite variable and conflicting. Exploratory *in vitro* analysis has demonstrated that this genetic variant is associated with a diminished sensitivity to MTX in breast cancer cells but not in colon cancer cells, suggesting that the C677T polymorphism differentially modulates the sensitivity of cancer cells to MTX, depending upon the specific cell type [33]. Data from clinical studies performed in adult and pediatric patients with lymphoid malignancies [51,52,60] and in patients with rheumatoid arthritis [63,67] appear quite concordant in assigning a negative prognostic value to the C677T genetic variant. These investigations evaluating different parameters, such as risk of relapse, mortality, event-free survival or therapeutic response, have overall demonstrated a significant association between the C677T polymorphism and worse clinical outcome after MTX-containing therapy. It was only found in one analysis, conducted in rheumatoid arthritis patients, that the 677T allele correlated with higher frequency of remission [68].

A different picture has emerged from investigations on the role of C677T polymorphism as a genetic marker of clinical outcome of MTX in GVHD prophylaxis. In some of these analyses, the variant 677T allele, considering both host [66,69] or donor [70] *MTHFR* genotype, was associated with an increased MTX efficacy. However, in a recent study the 677TT genotype was shown to predispose to higher treatment-related mortality and inferior overall survival [47].

Other investigations, realized in a different clinical setting where MTX is employed, have not evidenced any association between C667T polymorphism and efficacy of the drug [58,62,65].

The effect of A1298C in modulating the outcome of MTX therapy has been less investigated compared with the C677T polymorphism, and the data obtained so far are rather controversial.

In tumor settings to date, only one published study is available, realized in high-grade non-Hodgkin's lymphoma patients receiving MTX-containing chemotherapy, which has evidenced a significant correlation between 1298CC genotype and higher risk to develop severe mucositis [52]. Pharmacogenetic analyses of MTX used as GVHD prophylaxis produced opposite results. The polymorphic 1298C allele was associated with a diminished risk of relapse without effect on survival in one investigation [71]; on the other hand in another study the same variant allele has been correlated with an increased risk of acute GVHD, this is probably owing to a diminished efficacy of MTX therapy [69]. With regard to the impact of the A1298C polymorphism on side effects, the variant 1298CC genotype has been shown to be an independent predictor for higher risk of hepatic toxicity [64], while in another analysis, the 1298C variant allele has appeared to correlate with decreased side effects, such as oral mucositis, a result that, however, could be influenced by the concomitant presence of the C677T variant [49].

Several clinical studies have been conducted in patients with rheumatoid arthritis generating a pool of heterogeneous data, which are not easily summarized. The A1298C polymorphism is reported to be associated with improved efficacy of MTX without an effect on toxicity [57] and with a higher remission rate [68]. However, these results could not be confirmed in other published data that have found a correlation between the 1298AA genotype and clinical improvement after MTX treatment [67]. The role of the A1298C polymorphism in modulating the risk of developing side

effects has controversial results; the variant genotype in different researches was reported to predispose both to a low rate of MTX-related side effects [5,61] and to increased toxicity [63,67].

Sparse data also suggest the implication of the A1298C polymorphism in modulating toxicity and efficacy of MTX used for the treatment of juvenile idiopathic arthritis [56], inflammatory bowel disease [65] and psoriasis [62].

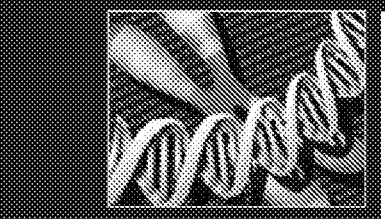
Other clinical studies performed in the different disease setting in which MTX is used have not demonstrated any correlation between the A1298C polymorphism and toxicity or efficacy of MTX-based treatment [47,60].

The presence of all these heterogeneous and quite diverging data for both A1298C and C677T polymorphisms have not yet provided unequivocal evidence for the pharmacogenetic role of these genetic variants in predicting fluoropyrimidine and antifolate treatment outcomes. The discrepant findings observed could be owing to the differences in study design (e.g., retrospective/prospective analyses) and clinical setting (adjuvant, neoadjuvant, first-/second-line palliative chemotherapy), small sample size and relative low statistical power, different schedule of treatment (e.g., dosage, via administration, duration of the therapy, coadministration of other chemotherapeutic agents or folate supply compounds), heterogeneity in pathology (e.g., type and clinical characteristic of the tumor, different hematological or inflammatory disease where these drugs are employed), dissimilarities in clinical and demographic characteristics of patients (e.g., age, sex, race, concomitant diseases, impaired renal and hepatic function), inability to control for confounding and environmental factors (e.g., folate intake) and, finally, different parameters to measure efficacy and toxicity. As a consequence of this heterogeneity in pharmacogenetic investigations, the replication of results in genetic association analysis is generally complicated and comparisons among studies are difficult.

Considering the high regional and geographic variability of the A1298C and C677T polymorphisms frequency, it can be observed that an important reason for these inconsistencies among data may be attributable to the racial and ethnic differences among patients included in the clinical trials. Recent pilot studies in Caucasians and African–Americans with rheumatoid arthritis have shown that the *MTHFR* genetic variants display a differential effect in modulating MTX-related toxicity in these different racial groups, suggesting that race may significantly interact with *MTHFR* polymorphisms to influence the clinical outcome [5,72].

Moreover, another essential factor of variability is introduced by folate status, a parameter determined in part by the geographical origin of patients and the different local dietary habits, and in part by the concomitant administration of folate supplementation during the therapy. As stated in the introduction, folate level has been demonstrated to modify the phenotypic effect of *MTHFR* polymorphisms and to interact with the same genetic variants in influencing therapy outcome [48,58,61,62,68,71].

It should also be highlighted that, owing to LD and the significant functional interaction between the 677 and 1298 polymorphic site, it is very difficult to determine which genetic variants are responsible for many of the associations demonstrated in the published work mentioned previously. Thus, the simultaneous investigation of both polymorphisms with a haplotype approach might be a more effective strategy with respect to a single polymorphism analysis, and it could be more useful to fully understand the real impact of these genetic variants, avoiding false-positive and false-negative results. In accord with this statement, some investigations have started to adopt a haplotype approach to study the genetic markers of therapeutic efficacy and toxicity of fluoropyrimidines [73] and antifolates [57,62,68,71] with encouraging preliminary results.



However, there is also an investigation that could not confirm the better predictive power of the haplotype approach [60]. In addition, studies will be necessary to define the appropriate statistical analysis and sample size requirements.

Moreover, an emerging body of evidence has highlighted the important role of gene–gene interactions within the folate and drug pathways, and how they may correlate with the clinical outcome. Several studies have suggested that the investigation of the combined effect of multiple variant alleles in genes involved in folate metabolism and in fluoropyrimidine- [36,37,39] and antifolate-based [59,62,63,72] therapy could result in an innovative strategy that might have more informative power than a single locus investigation in identifying clinical outcome predictors.

Taking into account all these considerations, in order to clarify the role of *MTHFR* genetic variants as pharmacogenetic determinants of fluoropyrimidines and antifolates, we will need further prospective, large-scale, randomized and well-designed studies where patients are well characterized and have been uniformly treated and systematically evaluated for toxicities and drug response. Furthermore, pharmacogenetic analyses will have to be performed for each therapeutic indication and in different racial and ethnic groups, controlling for the folate status features of studied populations.

In addition, haplotype and polygenic analyses need to be conducted to increase the complexity of these investigations.

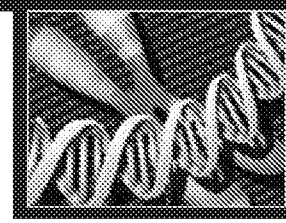
It should be reported that the *MTHFR* polymorphisms, although to a less extent, have also been studied in relation with other medications, such as acetylcholine esterase (ACE) inhibitors [74], anticonvulsants [75], levodopa [76], hormone- (estrogen) replacement therapy [77] and cholestyramine [78]. However, the role of *MTHFR* polymorphisms in this setting remains quite unknown. Preliminary results have suggested that the C677T genetic variant can enhance and worsen the folate deficiency and hyperhomocysteinemia associated with anticonvulsant, levodopa and cholestyramine treatment. The effect of this polymorphism in counteracting the homocysteine-lowering protective effect of hormone-replacement therapy remains, to date, largely uncertain and controversial.

Other *MTHFR* polymorphisms

Other polymorphisms and haplotype have been reported for both intronic and exonic regions of the *MTHFR* gene [101]. The functional phenotypic effect of these polymorphisms on MTHFR activity as well as their impact in disease susceptibility and in modulating the clinical outcome of therapy are, to date, largely unknown. Only few preliminary data, although quite encouraging, are available for the nonsynonymous G1793 to A transition (rs2274976), producing an arginine to glutamine replacement at codon 594 (Arg594Gln), the synonymous 116C to T substitution (rs2066470) at codon 39, which conserved a proline residue (Pro39Pro), the silent 1059T to C conversion (rs2066462) at codon 352 codifying for the amino acid serine (Ser352Ser) and, finally, the synonymous T>C nucleotide change, identified with the reference SNP ID rs4846051, which leads to a silent conversion at residue 435 codifying for the amino acid phenylalanine (Phe435Phe) [5,21,25,79–81].

Future perspective

In conclusion, on the basis of the evidence discussed, the C667T and A1298C polymorphisms could account for a portion of cancer risk and could also influence clinical outcome of MTX- and 5-FU-based cancer and anti-inflammatory therapy. However, the existence of controversial results will require large, randomized, controlled trials and formal Phase I studies based on genetic profile to clarify and unequivocally define the precise role of these polymorphisms, either as single-locus or in a haplotype approach, in modulating the treatment outcome. In addition, further efforts will be necessary to deepen understanding of how gene–nutrient/environmental interactions



and racial/ethnic variation in allele frequency could modify and affect the impact of *MTHFR* polymorphisms.

The most important goal for future research will be the definition of the precise role of *MTHFR* genetic variants on adverse drug reactions and efficacy, in order to develop diagnostics that can be used in a clinical setting. At present, the impact of *MTHFR* C677T and A1298C polymorphism analysis appears promising and feasible commercial kits for diagnosis are now available. Preliminary data resulting from cost–effectiveness analysis have evidenced that the genotype-based approach is both less costly and more effective than the conventional strategy for MTX treatment [55].

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Highlights

- 5,10-methylenetetrahydrofolate reductase (MTHFR) is a key enzyme for intracellular folate homeostasis and metabolism. It catalyzes the irreversible conversion of 5,10-methylentetrahydrofolate, required for purine and thymidine synthesis to 5-methyltetrahydrofolate. MTHFR serves as a methyl donor for remethylation of homocysteine to methionine. It is involved in DNA synthesis, methylation, repair and uracil misincorporation.
- Two common nonsynonymous genetic variants, the *MTHFR* C677T and A1298C, have been described as factors leading to altered amino acid and reduced enzyme activity. Several other polymorphisms and haplotypes have been reported, but their effects need further investigation.
- The C677T and A1298C variants are involved in cancer susceptibility. The polymorphisms are also involved in cardiovascular disease, thrombosis, neural tube defects, cleft lip/palate, pre-eclampsia susceptibility, Down's syndrome and psychiatric disorders.
- The C677T and A1298C variants are involved in the cytotoxic effect of fluoropirimidines and antifolates. Haplotype and polygenic approaches may be more effective than a single polymorphism analysis; both methods are of great utility in fully understanding the real impact of these genetic variants.
- Folate status and geographic origin of studied populations have been reported to significantly modify the epidemiological and pharmacogenetic impact of *MTHFR* polymorphisms.

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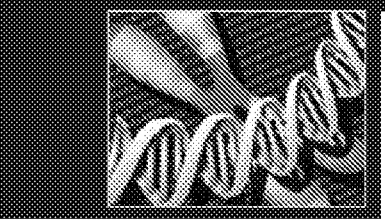

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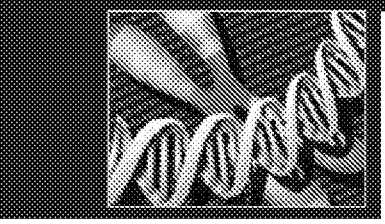

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