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Review

C677T and A1298C MTHFR polymorphisms, a challenge for antifolate and fluoropyrimidine-based therapy personalisation

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ABSTRACT

Pharmacogenetics represents an exciting, new promising tool for the individualisation of therapy. Several genetic polymorphisms and haplotypes have been considered in an attempt to optimise therapy with specific drugs but, up to now, their clinical applications remain limited.

5,10-Methylenetetrahydrofolate reductase (MTHFR), a key enzyme of one-carbon metabolism, catalyses the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Two common non-synonymous variants, the C677T (Ala222Val) and A1298C (Glu429Ala), were described for the *MTHFR* gene and associated with a decreased enzymatic activity and an alteration of intracellular folate distribution. Other MTHFR polymorphisms with marginal impact on enzymatic activity were also reported.

Several published clinical studies have investigated the potential predictive role of C677T and A1298C genetic variants on toxicity and efficacy of antifolate and fluoropyrimidine agents, such as methotrexate (MTX), 5-fluorouracil (5-FU) and raltitrexed. Many of these studies show significant associations with MTHFR variants, but others report neither association nor opposite results. A significant interaction between MTHFR polymorphisms and nutrient/environmental factors (i.e. folate status) as well as the ethnicity was reported. Finally, a haplotype approach and the combined analysis of multiple folate pathway gene variants seem to provide a more comprehensive strategy compared to single-locus investigations.

The aim of this review is to critically analyse the available data on the importance of MTHFR polymorphisms in modulating the clinical outcome of antifolate and fluoropyrimidine therapies.

1. Introduction

Pharmacogenetics represents a new promising tool for the individualisation of therapy. Several genetic polymorphisms and haplotypes involved in drug metabolism, transport and mechanism of action have been investigated as markers for optimising treatment outcome. The non-synonymous C677T and A1298C variants in the 5,10-*methylenetetrahydrofolate*

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reductase (MTHFR) gene are among the most studied genetic polymorphisms for identifying predictors of response to antifolates and fluoropyrimidines. MTHFR is a key enzyme for intracellular folate homeostasis and metabolism. It catalyses 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[1] (Fig. 1). An alteration in reduced folate pools, derived from inherited changes in MTHFR activity, may have a significant effect on the response of malignant and non-malignant cells to antifolates and fluoropyrimidines, whose activity depends on cellular composition of folate.

The C677T variant (Ala222Val, rs1801133) has been associated with a decreased activity of MTHFR, an increased level of homocysteine and an altered distribution of folate.[2–4] The A1298C variant (Glu 429Ala, rs1801131) has also been related to a reduced MTHFR activity, but at a lower degree compared to C677T.[5–7]

The frequency of both polymorphisms varies by geographical origin[8,9]: these genetic variants occur frequently among Caucasian, Asian, Hispanic and Latino/Mexico–American populations, with a prevalence of about 25–45%, while they

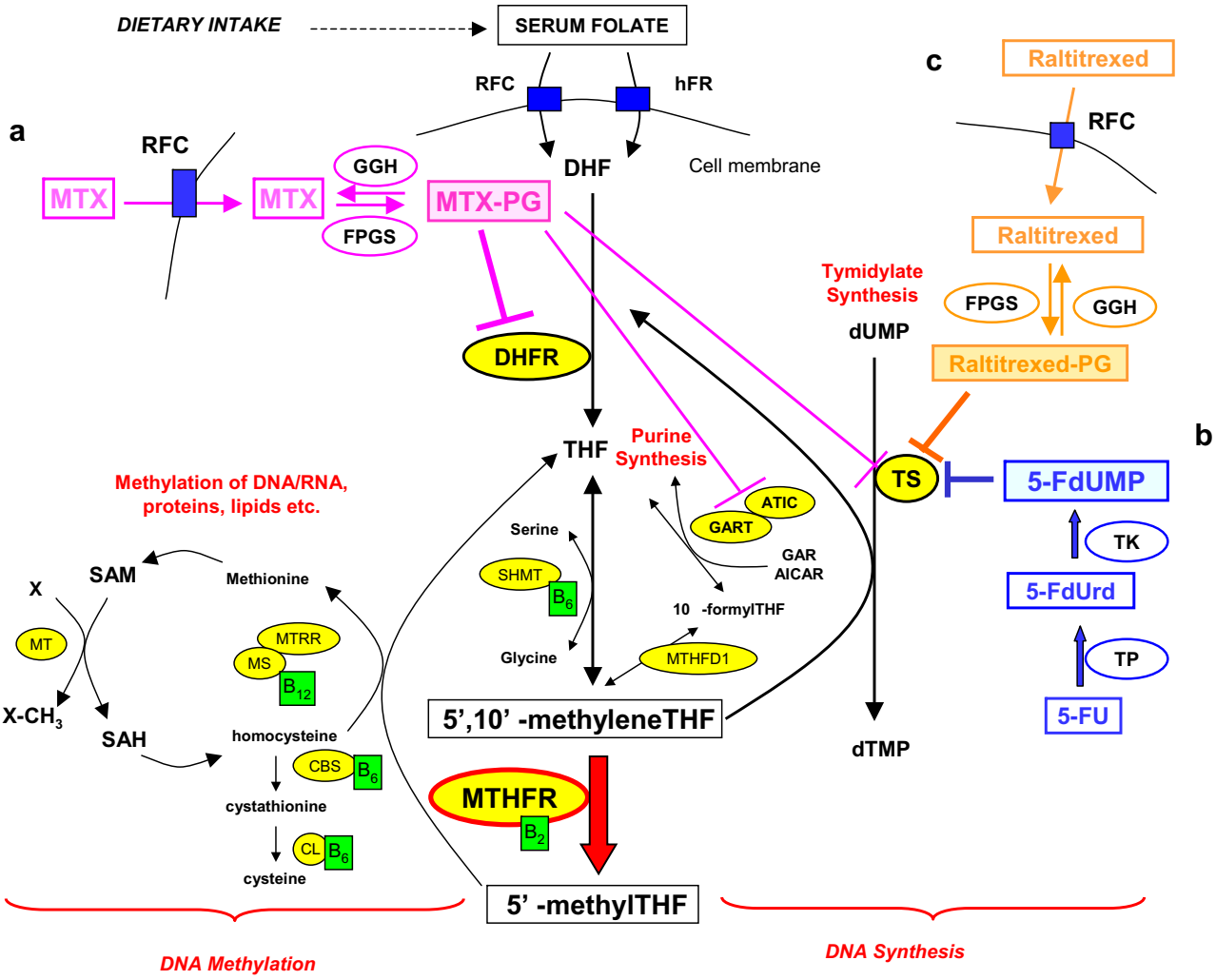

Fig. 1 – A simplified view of the folate metabolism pathway and of the targets of the major fluoropymiridine and antifolate agents. Transporters: hFR, human folate receptor; RFC, reduced folate carrier. Enzymes (denoted as ovals): ATIC, 5-aminoimidazole-4-carboxamide ribonucleotide formyltranferase/IMP cyclohydrolase; CBS, cystathionine-β-synthase; CL, cystathionine lyase; DHFR, dihydrofolate reductase; FPGS, folylpolyglutamyl synthase; GART, glycinamide ribonucleotide formyltransferase; GGH, γ-glutamyl hydrolase; MS, methionine synthase; MT, methyltransferase; MTHFR, 5,10-methylene-tetrahydrofolate reductase; MTHFD1, methylenetetrahydrofolate dehydrogenase 1; MTRR, methionine synthase reductase; TK, thymidine kinase; TP, thymidine phosphorylase; TS, thymidylate synthase. Metabolites: AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; CH_3, methyl group; DHF, dihydrofolate; dTMP, deoxythymidine 5′-monophosphate; dUMP, deoxyuridine 5′-monophosphate; GAR, glycinamide ribonucleotide; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; THF, tetrahydrofolate; X, various substrates for methylation. Vitamins B2, B6 and B12 are cofactors in the pathway. Drug: 5-FdUMP, 5-fluoro-2deoxyuridine-5′-monophospate; 5-FdUrd, 5-fluoro-2-deoxyuridine; 5-FU, 5-fluorouracil; MTX, methotrexate; Drug-PG, drug-polyglutamated.

Table 1 – Allele frequency (%) of MTHFR C677T and A1298C polymorphisms in the main ethnic/racial groups.

	European/ Caucasian	Hispanic or Latino/ Mexico–American	African–American	Sub-Saharan African	Asian			
					Indian[a]	Chinese	Japanese	Korean
MTHFR C677T								
Allele C	63.0–76.3	52.5–69.5	85.4–91.7	89.0–89.2	90.5	48.9–66.7	59.7–64.4	59.8
Allele T	23.7–37.0	30.5–47.5	8.3–14.6	10.8–11.0	9.5	33.3–51.1	35.6–40.3	41.2
MTHFR A1298C								
Allele A	62.9–70.8	76.1–85.0	81.2–88.1	89.2–89.8	73.0	79.8–85.4	81.4–82.2	45.0
Allele C	29.2–37.1	15.0–23.9	11.9–18.8	10.2–10.8	27.0	14.6–20.2	17.8–18.6	25.0

Data from http://www.ncbi.nlm.nih.gov/SNP/.
a [55].

are rarer in African populations (see Table 1 for details). Haplotype analyses showed that these two polymorphic sites are in high linkage disequilibrium (LD).[10–15] In several studies, no individuals with homozygous variant allele at both loci (677TT/1298CC) were found, suggesting that such genotype could result in a severely impaired phenotype.[10,12,14–18] However, heterozygote patients (i.e. 677CT/1298AC) are quite common (about 15–23% in Caucasians).[6,10,14,16,18] Recent analyses have indicated that the resulting effect of these two variants on the MTHFR function is synergic.[5,14] A large-scale epidemiological study has evidenced that the haplotype-based approach (considering the combined effect of C677T and A1298C polymorphisms) is more predictive than the single MTHFR polymorphism for total plasma homocysteine levels.[14] A possible model of the MTHFR enzyme dimer, arranged in different configurations based on a specific combination of the 677 and 1298 variants,[14] was also proposed. It has been consistently demonstrated that the physiopathological consequences of MTHFR genetic variants, especially the C677T polymorphism, are significantly affected by demographic and environmental factors such as folate status, age, smoking and alcohol intake, all parameters that may additionally alter the fine equilibrium of one-carbon metabolism.[19–21] In particular, the phenotypic effects of the C677T and A1298C polymorphisms are affected by folate status.[14,22] It has been suggested that the stability of the polymorphic enzyme is significantly modified by folate levels. In the condition of high intracellular folate, the folate molecule may be able to hold the variant MTHFR protein in the appropriate and fully functional three-dimensional structure, thus stabilising the thermolabile form and counteracting the reduction in enzyme activity.[14]

The cytotoxic effect of commonly used therapeutic agents like antifolates and fluoropyrimidines is dependent upon a competitive interaction with folate metabolism. On this ground the C677T and A1298C MTHFR polymorphisms could have an important role in modulating the clinical toxicity and efficacy of methotrexate (MTX), 5-fluorouracil (5-FU) and raltitrexed. In this review we critically investigated the involvement of genetic variants of MTHFR, both as single-locus and as haplotypes, in the cytotoxic effect of treatment with antifolates and fluoropyrimidines.

The aim was to provide new insights for a personalised therapy based on the human genotype.

2. C677T and A1298C polymorphisms and therapy outcome

2.1. *MTX-based therapy*

A large body of published studies have investigated the potential role of MTHFR polymorphisms on toxicity and response to MTX-based cancer chemotherapy and anti-inflammatory therapy (Table 2). MTX is an antifolate agent whose uptake into the cells is mainly controlled by the reduced folate carrier (RFC). The main intracellular target of this drug is dihydrofolate reductase (DHFR), whose inhibition results in the accumulation of dihydrofolate and the depletion of cellular folates. Within cells, MTX is rapidly converted into a polyglutamated form by folylpolyglutamyl synthase (FPGS), a process that can be reversed by the enzyme γ-glutamyl hydrolase (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 thymidylate synthase (TS), glycinamide ribonucleotide formyltransferase (GART) and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC). MTHFR is also involved in MTX activity by modulating the intracellular pool of folates[23,24] (Fig. 1a), even if the biochemical effect of MTX on intracellular folates still remains to be precisely delineated and the implication of an inherited alteration of MTHF activity is not completely defined. It can be hypothesised that the combined effect of MTX treatment and decreased activity of MTHFR, associated with C677T and A1298C, may lead to alterations in intracellular reduced folate pools, a severe low folate state and an increased homocysteine concentration that may interfere with both the antitumour activity of the drug and its related toxicity.[15,25–29]

MTX has been widely employed as an antineoplastic agent, alone or in combination with other drugs, for the treatment of a number of solid tumours and haematologic malignancies. Its potential anti-inflammatory and autoimmune effects make this antifolate compound one of the most common drugs used in rheumatic and other inflammatory conditions as well as in haemopoietic cell transplantation to prevent graft-versus-host disease (GVHD).

Table 2 – MTHFR polymorphisms and MTX therapy outcome.

Treatment regimes	Patient population	Race/ethnicity	MTHFR polymorphisms	Main results	Citations
MTX-based treatment	Adult ALL (n = 82)	Caucasian	C677T	No effect on RFS and EFS. In a subset of patients (N = 55) homogenously treated with MTX-based therapy, the 677TT genotype is associated with lower survival and increased toxicity (haematopoietic and hepatic toxicity).	[34]
			A1298C	No effect on RFS, EFS, OS or toxicity.	
MTX-based treatment	ALL (n = 28)	Mexican	C677T	No effect on MTX-induced mucositis.	[60]
MTX-containing combination chemotherapy (n = 68 subgroup)	Adult high-grade NHL (n = 110)	Caucasian	C677T	Association between 677TT genotype and a higher risk of developing toxicity, especially mucositis, hepatic toxicity and thrombocytopaenia. Worse prognosis in term of EFS and risk of adverse events for patients carrying 677T allele.	[11]
			A1298C	Association between 1298CC genotype and higher risk of developing mucositis. No effect on EFS.	
MTX (high-dose)-containing therapy + leucovorin	Paediatric ALL or lymphoblastic lymphoma (n = 15)	Japanese	C677T	No effect on plasma MTX concentration at 48h after infusion and on toxicity.	[46]
MTX (high-dose)-containing therapy + leucovorin	Paediatric ALL (n = 186)	European origin	C677T	Lower rates of haematologic toxicity in patients carrying 677T allele.	[53]
MTX (high-dose)-containing therapy + leucovorin	Paediatric NHL (n = 484)	Caucasian	C677T	No effect on toxicity.	[61]
MTX(high-dose)-containing therapy + leucovorin	Paediatric ALL (n = 53)	European descendent (n = 49) Non-European descendent (n = 14)	C677T, A1298C	No effect on cognitive function following treatment.	[58]
MTX containing chemotherapy	Paediatric ALL (n = 520)	African–American (n = 28) Asian (n = 13) Caucasian (n = 396) Hispanic (n = 58) other (n = 25)	C677T	Higher risk of relapse in patients with at least one 677T allele. No effect on toxicity.	[49]
			A1298C	No association with altered risk of relapse or toxicity.	
MTX	Lymphoblasts from 157 paediatric ALL patients	Western European	C677T A1298C	No effect on *ex vivo* MTX sensitivity. Patients carrying 1298AC genotype manifest decreased *in vitro* MTX sensitivity.	[66]
MTX (high-dose)-containing therapy + leucovorin	Paediatric ALL (n = 201)	French–Canadian origin	C677T/A1298C haplotype	Lower probability of EFS and DFS in patients harbouring 677T/1298A haplotype. No effect on toxicity and OS.	[29]
MTX containing chemotherapy ± leucovorin	ALL (n = 87)	Caucasian	C677T	No effect on pre-disposition to develop bone marrow oedema and aseptic osteonecrosis.	[51]

MTX (high-dose)-containing therapy + leucovorin	Paediatric ALL ($n = 53$)	White ($n = 38$) Non white ($n = 15$)	C677T	Lack of association with homocysteine level or toxicity (neurotoxicity and thrombosis).	[42]
MTX ± carboplatin	Ovarian cancer ($n = 43$)	Caucasian	C677T	Association between 677TT genotype and MTX-induced hyperhomocysteinaemia and higher treatment-related toxicity (mainly thrombocytopaenia and oral mucositis; other significant toxicity reported neutropaenia, anaemia, hepatic toxicity).	[27]
MTX containing chemotherapy	Acute leukaemia ($n = 61$)	Caucasian	C677T	Higher toxicity (with pronounced myelosuppression and hepatic toxicity) in patients carrying 677TT genotype.	[33]
MTX-based therapy	Cryopreserved lymphoblast from 6 paediatric ALL patients who suffered from MTX-related toxicity as neurologic and hepatic toxicity	American (non-specified)	C677T	Association between 677TT genotype and greater *in vitro* MTX sensitivity.	[65]
CMF	Breast cancer ($n = 170$)	Caucasian	C677T	Higher risk of developing severe acute toxicity (mainly myelosuppression) in patients carrying 677TT genotype.	[35]
MTX + cyclosporine GVHD prophylaxis	Patients with haematological disease undergoing allogenic HSCT ($n = 159$)	Japanese	C677T	Lower incidence of grade acute GVHD in recipients with 677TT genotype. No impact of the polymorphism on toxicity and TRM.	[62]
MTX + cyclosporine GVHD prophylaxis	Patients with haematological malignancy or aplastic anaemia undergoing allogenic HSCT ($n = 72$)	Korean	C677T	Increased toxicity (mainly hepatic toxicity and delayed platelet recovery), higher TRM and lower OS in patients with 677TT genotype.	[30]
			A1298C	No effect on clinical outcome (toxicity, TRM, OS).	
MTX + cyclosporine GVHD prophylaxis	Patients with haematological malignancy or disease receiving allogenic HSCT ($n = 84$)	Caucasian	C677T A1298C	No association with hepatic toxicity. Independent predictor for hepatic toxicity with an increased risk for 1298CC genotype.	[12]
MTX + cyclosporine GVHD prophylaxis	CML patients undergoing allogenic HCT ($n = 350$; clinical data available for $n = 304$)	White ($n = 305$) Non-white ($n = 45$)	C677T	Higher efficacy with a decreased risk of acute GVHD in recipients with at least one 677T allele.	[67]
			A1298C	Lower efficacy with an increased risk of acute GVHD in recipients with at least one 1298C allele.	

(*Continued on next page*)

Table 2 – *continued*

Treatment regimes	Patient population	Race/ethnicity	MTHFR polymorphisms	Main results	Citations
MTX + cyclosporine GVHD prophylaxis	CML patients undergoing allogenic HCT (n = 172)	Caucasian	C677T	Higher risk of developing oral mucositis in patients with 677TT genotype and significant trend towards greater mucositis with more copies of the variant 677T allele.	[32]
			A1298C	Significant trend towards decreased mucositis with more copies of the variant 1298C allele but with an inferior impact with respect to C677T polymorphism.	
MTX + cyclosporine GVHD prophylaxis ± folinic acid supplementation	Patients with haematological malignancy/diseases undergoing allogenic HSCT (n = 193)	Caucasian (n = 178) Non-Caucasian (n = 15)	C677T	Significant association between 677T allele and higher efficacy (decreased incidence of acute and chronic GVHD, increased time to onset of first GVHD and time to first GVHD treated with systemic therapy) in HSCT recipients with an HLA-matched related donor.	[25]
MTX + cyclosporine GVHD prophylaxis	CML patients undergoing HCT (n = 133)	White (n = 120) Non-white (n = 13)	C677T	Higher risk of developing oral mucositis in patients with 677TT genotype.	[31]
MTX + cyclosporine GVHD prophylaxis	CML patients undergoing allogenic HCT (n = 336)	Caucasian	C677T	Significant interaction between multivitamin and folate supplement use and C677T polymorphism (increased risk of relapse in patients with 677CC genotype that used multivitamin or folate supplements).	[68]
			A1298C	Significant trend towards decreased risk of relapse with increasing copy numbers of 1298C allele.	
			C677T, A1298C	No effect on OS.	
			C677T/A1298C haplotype	Association between 677CC/1298CC genotype and lowest risk of relapse compared to 677CC/1298AA reference genotype.	
MTX + cyclosporine GVHD prophylaxis	CML patients undergoing bone marrow transplantation (n = 220)	White (n = 196) Non-white (n = 24)	C677T	Higher toxicity (mainly oral mucositis and delayed platelet recovery) in patients with 677TT genotype.	[28]

MTX + methylprednisone + higher dose of folic acid supplementation	RA patients (n = 174)	Caucasian	C677T	Significant correlation between 677T allele and higher frequency of remission.	[69]
			A1298C	Trend towards higher remission rate for 1298C allele.	
			C677T/ A1298C haplotype	Patients carrying 677CC/1298AA diplotype show lower probability of remission and increased parameters of disease activity.	
MTX with heterogeneous concomitant medications ± folic acid supplementation	RA patients (n = 218; 91 in training cohort and 127 in validation cohort)	Training cohort (29 African American, 62 Caucasian) validation cohort (35 African American, 92 Caucasian)	C677T	In the training cohort the genetic variant showed a trend towards increased overall toxicity in Caucasian patients. In the validation cohort the polymorphism displayed a significant association with alopecia in African–American patients and an apparent correlation with gastrointestinal toxicity in Caucasian patients.	[70]
MTX ± folic acid supplementation	RA patients (n = 223)	Caucasian (n = 193) African–American (n = 30)	C677T A1298C	No effect on toxicity or response. Association between 1298A allele and higher risk of developing side-effects among Caucasian group population (n = 193). No effect on response.	[57]
MTX	RA patients (n = 34)	Indian	C677T, A1298C	No effect on MTX response (efficacy and toxicity)	[55]
MTX-based therapy ± folic acid	RA patients (n = 227)	Japanese	C677T	Correlation between 677T allele and increased risk of developing overall adverse events. Higher risk of hepatotoxicity (ALT elevation) in patients carrying 677T allele with gene-dose effects.	[37]
			A1298C	Higher efficacy in patients with at least one 1298C allele that are receiving lower doses of MTX. Evidence for gene-dose effects. No impact of the polymorphism on toxicity.	
			C677T/A1298C haplotype	Association in a recessive model between *A haplotype and lower efficacy (administration of higher doses of MTX) and between C* haplotype and lower MTX-related toxicity. In both cases the *A and C* give the lowest P value. («*» any allele)	

(*Continued on next page*)

Table 2 – *continued*

Treatment regimes	Patient population	Race/ethnicity	MTHFR polymorphisms	Main results	Citations
MTX + folic acid	RA patients (n = 205)	White (n = 191) Asian (n = 5) Black (n = 2) others (n = 7)	C677T	Association between 677CC genotype and greater clinical improvement. No effect on toxicity.	[63]
			A1298C	Greater clinical improvement in patients with1298AA genotype. Increased risk of developing adverse drug events (mainly gastrointestinal toxicity) for patients carrying the 1298C allele.	
			C677T/A1298C haplotype	Clinical improvement in patients carrying the 677CC/1298AA haplotype.	
MTX + folic acid	RA patients (n = 150)	Indian	C677T	No effect on toxicity or efficacy.	[48]
MTX containing therapy + folic acid	RA patients (n = 214)	American (non-specified)	C677T	Association between 677TT genotype and increased risk of side-effects in the central nervous system.	[39]
Low dose MTX ± other medications	RA patients (n = 385)	Korean	C677T	Increased MTX-related toxicity in patients with at least one 677T allele; gastrointestinal dysfunction, hair loss and hepatic toxicity were the more frequent adverse events.	[36]
MTX + folic acid ± other medications	RA patients MTX-naïve (n = 45)	American (non-specified)	C677T	Association between lower likelihood of therapeutic response and 677TT genotype. No effect on toxicity.	[54]
			A1298C	Association between toxicity (gastrointestinal and neurologic toxicity as primary side-effects) and 1298C allele. No effect on response.	
MTX	Juvenile idiopathic arthritis (n = 58)	Caucasian	C677T	Correlation between the variant 677T allele and higher toxicity; gastrointestinal dysfunction, hepatic toxicity and hair loss resulted as the more frequent adverse events. No effect on efficacy.	[40]
			A1298C	Lower clinical efficacy in patients in 1298AA genotype. No effect on toxicity.	
MTX-based therapy ± folic acid supplementation	RA patients (n = 93)	Jewish	C677T	No effect on toxicity, plasma homocysteine level and therapeutic response.	[50]
			A1298C	Correlation between 1298CC genotype and lower risk of developing side-effects. No relation between polymorphism and plasma homocysteine level. No evident effect on therapeutic response.	

Low dose MTX	RA patients ($n = 167$)	Japanese	C677T, A1298C	No effect on toxicity or efficacy.	[59]
MTX	RA patients ($n = 106$)	Japanese	C677T	Increased overall MTX toxicity in patients with at least one 677T allele; hepatotoxicity results as the more frequent adverse effect. No effect of the polymorphism on efficacy.	[15]
			A1298C	Higher efficacy in patients with at least one 1298C allele that are receiving lower doses of MTX. No effect on toxicity.	
			C677T/ A1298C haplotype	Association between 677C/1298C haplotype and higher efficacy (administration of lower doses of MTX) and between 677T/1298A haplotype and higher MTX-related toxicity.	
MTX ± folic or folinic acid supplementation	RA patients ($n = 236$)	Caucasian	C677T	Increased risk of developing adverse events (mainly hepatic toxicity) in patients with at least one 677T allele. No effect on efficacy.	[38]
MTX ± folic acid	Psoriasis patients ($n = 203$)	White Caucasian with too few non-Caucasian	C677T	No effect on efficacy or toxicity.	[52]
			A1298C	Protective role of 1298C allelic variant towards hepatic toxicity.	
			C677T/ A1298C haplotype	Association between 677CT/1298AC genotype and lower risk of developing hepatic toxicity in combination with no folate supplementation.	
MTX	Inflammatory bowel disease ($n = 102$)	Caucasian	C677T	No effect on toxicity or response.	[56]
			A1298C	Higher risk of developing adverse events for the 1298CC genotype; the association is particularly strong with nausea and vomiting.	

Abbreviations: ALL, acute lymphoblastic leukaemia; CMF, cyclophosphamide methotrexate and fluorouracil; CML, chronic myelogenous leukaemia; DFS, disease-free survival; EFS, event-free survival; GVHD, graft-versus-host disease; HCT, haemapoietic cell transplantation; HSCT, haemapoietic steam cell transplantation; NLH, non-Hodgkin's lymphoma; OS, overall survival; PFS, progression-free survival; RA, rheumatoid arthritis; RFS, relapse-free survival; TRM, treatment-related mortality; TTP, time to progression.

2.1.1. MTHFR polymorphisms and MTX toxicity

Several clinical studies reported a significant correlation between the C677T variant genotype and an increased risk of developing adverse events (grades 3 and 4 haematologic and non-haematologic toxicity) after treatment with MTX, alone or in combination with other agents, in patients undergoing haematopoietic cell transplantation,[28,30–32] in patients with acute leukaemia,[33,34] with non-Hodgkin's lymphoma,[11] with ovarian[27] and breast[35]cancers and in patients with rheumatoid arthritis[15,36–39] or juvenile idiopathic arthritis.[40] It has been suggested that the biochemical basis of the increased toxicity-related to MTHFR polymorphism has been associated with the increased plasma homocysteine level due to the C677T genetic variant. This reflects an impaired conversion of 5,10-methylentetrahydrofolate to 5-methyltetrahydrofolate, and the modification in the intracellular folates pool could increase the toxic effect of MTX.[27,38,41–43] A recent study in Japanese patients with acute lymphoblastic leukaemia (ALL) or malignant lymphoma[44] has indicated that the C677T polymorphism may affect plasma concentration of MTX. Patients with the 677TT genotype present a significant higher serum concentration of MTX 48 h after starting intravenous infusion[44] compared to patients with other genotypes. The slower elimination and consequent high plasma concentration of MTX are proposed to be associated with the adverse effects of this drug, especially mucositis.[45–47]

At present the real predictive value of MTHFR C677T polymorphism on MTX toxicity is not completely established. Some published studies failed to find an association between increased toxicity and the C677T variant form.[12,42,46,48–63] The non-concordant data reported so far will require prospective and homogeneous studies for final conclusions.

Regarding the role of A1298C polymorphism in modulating the development of severe adverse events after MTX therapy, sparse and quite inconclusive data are available up to date. In tumour settings, there is only one published clinical investigation, carried out on high-grade non-Hodgkin's lymphoma patients receiving chemotherapy containing MTX that has evidenced a significant correlation between 1298CC genotype and a higher risk of developing severe mucositis.[11] In pharmacogenetic analysis of MTX used as GVHD prophylaxis, the 1298C allele seems to be associated with decreased side-effects such as oral mucositis; however, it should be noted that these findings could be influenced by the concomitant presence of the C677T polymorphism.[32] In a recent study by Goekkurt et al. on the same topic, the A1298C polymorphism seemed to be an independent predictor for hepatic toxicity with higher risk for the 1298CC genotype with respect to AC and AA genotypes.[12] In rheumatoid arthritis patients, two investigations reported an association between the A1298C variant and a low rate of MTX-related side-effects.[50,57] On the contrary, Wessel et al. found an association between 1298C allele carriers and higher toxicity.[63] The correlation between 1298C allele and increased toxicity was also reported in a study conducted in a small rheumatoid arthritis MTX-naïve patient group[54] and in an investigation realised in inflammatory bowel disease (IBD) patients.[56] An opposite result was observed in a psoriasis setting where the 1298C variant allele seems to predispose to lower MTX-related hepatic toxicity.[52]

Other investigations performed in the various clinical settings where MTX is employed have not evidenced any effect of the A1298C polymorphisms on MTX toxicity.[15,30,37,49]

2.1.2. MTHFR polymorphisms and MTX efficacy

Concerning the role of C677T polymorphism in modulating the antitumoural effect of MTX, preliminary *in vitro* analysis has evidenced that this genetic variant is associated with a diminished sensitivity to MTX in breast cancer cell lines but not in colon cancer cell lines. These data could suggest that the C677T polymorphism differentially modulates the sensitivity of cancer cells to MTX, depending upon the specific cell type.[64] In view of the importance of MTX in the therapy of adult and paediatric lymphoid malignancies, particularly during the consolidation and maintenance phase of treatment, several studies on the effect of C677T polymorphism on MTX sensitivity and resistance have been conducted in leukaemia patients. A pilot *ex vivo* analysis using cryopreserved lymphoblasts from paediatric ALL patients who had experienced MTX-related toxicity has suggested that the C677T variant may contribute to the enhancement of the *in vitro* MTX sensitivity.[65] This observation, however, could not be confirmed in another *ex vivo* investigation realised on a larger paediatric ALL study.[66] Data from clinical settings appear quite concordant in assigning a negative prognostic value to the C677T genetic variant. A large-scale study in child patients has reported that the C677T polymorphic allele is significantly associated with a moderate increased risk of ALL relapse.[49] In a cohort of adult ALL homogeneously treated with MTX-based maintenance regimen, the 677TT genotype was associated with worse outcome in terms of survival with respect to the 677CC genotype.[34] In agreement with these findings, Gemmati et al. have recently reported a correlation between the 677T allele and a lower probability of event-free survival at 5 years and an increased risk of adverse events in a group of high-grade non-Hodgkin's lymphoma patients receiving combination chemotherapy containing MTX.[11]

The effect of A1298C in modulating the efficacy of MTX therapy in leukaemia patients has been less investigated. Preliminary *ex vivo* analysis on lymphoblasts from paediatric ALL patients evidenced that the 1298AC genotype predisposes to a decreased MTX sensitivity compared to the CC and AA genotypes.[66] This significant role described for the heterozygous genotype is difficult to explain and should be considered only exploratory.

Several studies have investigated the role of C677T polymorphism as a genetic marker of clinical efficacy of MTX used as a member of the protocols for immunosuppression therapy and the prevention of GVHD following haemopoietic cell transplantation. The MTHFR genotype has been investigated both in the host[62,67] and in the donor,[25] and the variant C677T allele was correlated with an increased MTX efficacy. On the contrary, a recent study by Kim et al. found the 677TT genotype to be significantly associated with higher treatment-related mortality and inferior overall survival in respect to 677CT and 677CC genotypes.[30] It should be noted that Robien et al. have suggested that the impact of C677T polymorphism on relapsed risk after haemopoietic cell transplantation may be significantly influenced and modified by folate status. In particular, an increased risk of relapse was

evidenced among patients with the 677CC genotype who took multivitamin or folate supplements. The exploratory nature of these findings does not allow the authors to formulate any specific hypothesis about the underlying biological mechanism.[68]

Pharmacogenetic analysis of the role of A1298C polymorphism in modulating efficacy of MTX therapy in GVHD prophylaxis has issued poor consistent results. In one study the 1298C variant allele appeared to be associated with a diminished risk of relapse without an effect on survival.[68] On the other hand, in another investigation the same polymorphic allele has been reported to correlate with an increased risk of acute GVHD, probably due to a diminished efficacy of MTX therapy.[67]

In a rheumatoid arthritis setting, two pharmacogenetic studies have evidenced an association between the C677T variant genotype and lower clinical improvement and probability of therapeutic response.[54,63] In contrast, a recent investigation has reported a significant association between the 677T polymorphic allele and a higher frequency of remission.[69]

Regarding the A1298C polymorphism, in four clinical studies on patients with arthritis pathology, the 1298C polymorphic allele was associated with an improved clinical efficacy of MTX[15,37,40] and a higher remission rate.[69] An opposite result was reported by Wessel et al. who found a correlation between the 1298AA genotype and clinical improvement in rheumatoid arthritis patients treated with MTX.[63,40]

It must also be taken into account that there are investigations, realised in the various clinical settings where MTX is employed, that could not confirm the role of C677T and A1298C MTHFR polymorphisms in modulating the clinical efficacy of MTX therapy.[38,48,52,55,56,59]

2.1.3. *MTHFR polymorphisms and MTX clinical outcome: critical comment*

From the available data, the most evident association emerging is between the C677T variant form and increased MTX toxicity. Converse correlations between A1298C and toxicity or between A1298C or C677T and MTX efficacy present a pool of heterogeneous and sometimes contrasting results that cannot provide unequivocal conclusions. The discrepant findings observed could be due to: the differences in study design (e.g. retrospective/prospective analyses, low statistical power), the small sample size, the different clinical setting and schedule of treatment (e.g. dosage, coadministration of other therapeutic agents or folate supply compound as leucovorin and folic acid, duration of the therapy), the heterogeneity in pathology and in the clinical and demographic characteristics of patients, the inability to control for confounding and environmental (e.g. folate intake) factors and the different parameters to measure efficacy and toxicity.[11,42,46,48,55,62,63,69] 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.

One of the most important challenges in identifying pharmacogenetic traits will be the need of trials where patients are well characterised and have been uniformly treated and systematically evaluated for toxicities and drug response. Pharmacogenetic analyses will also have to be performed for each therapeutic indication and in different racial and ethnic groups, controlling for the folate status of patients.

2.1.4. *MTHFR polymorphisms and race*

It should be noted that considering the high regional and geographic variability of MTHFR polymorphism prevalence (Table 1), a particularly important cause of inconsistencies among data may be attributable to the racial and ethnic differences among patients included in the clinical trials. In agreement with this observation, Martin et al. have recently reported an interaction between C677T and A1298C genetic variants and race/ethnicity on breast cancer survival, indicating that the demographic origin of patients may be a significant modifier of the effect of MTHFR polymorphisms.[17] A recent pilot study, adopting a retrospective cross-validation approach, has examined the pharmacogenetic association of C677T polymorphism with MTX toxicity in Caucasians and African–Americans with rheumatoid arthritis. The results of this investigation have evidenced that the MTHFR genetic variant has differential effects in these racial groups suggesting that race may significantly interact with the C677T variant to influence the risk of MTX toxicity.[70] A similar race-specific association with MTX-related adverse events in Caucasian and African–American rheumatoid arthritis patients has also been suggested for the A1298C variants.[57] All these preliminary findings call for a better definition of the ethnic and racial differences in the distribution of allele frequency of MTHFR polymorphisms, a variability that could be associated with the difference in therapy outcome; population and epidemiological studies in this direction have recently been started.[9,57,71]

2.1.5. *MTHFR polymorphisms and folate status*

Folate status, a parameter partly determined by the patients' geographical origin and by the different local dietary habits, has been demonstrated to deeply interact with MTHFR polymorphisms in influencing MTX therapy outcome. Robien et al. have evidenced a role of folate status in modulating the association between the C677T polymorphism and the relapsed risk after haemopoietic cell transplantation.[68] Some studies have evidenced that the concomitant administration of folic or folinic acid to patients receiving MTX may reduce the side-effects and the risk of toxicity-related discontinuation of the therapy apparently without a significant diminution of efficacy. In most cases the impact of the MTHFR polymorphisms associated with the MTX-induced adverse events appears to attenuate or disappear in the case of folate supplementation.[31,38,42,50,52,61,69] Additional information on physiological basal blood folate level and folate dietary intake of the patient study population will be useful in order to clarify the role of MTHFR polymorphisms in MTX metabolism.

2.1.6. *C677T/A1298C haplotype and polygenic approach*

Owing to the linkage disequilibrium and the significant functional interaction between the 677 and 1298 polymorphic sites, it is very difficult to discern which genetic variants are responsible for specific clinical associations.

A few studies recently published have started to conduct the simultaneous investigation of both A1298C and C677T

polymorphisms in order to fully understand the impact of these genetic variants avoiding false positive and false negative results.[11,32,67] Preliminary data by Robien et al. have shown that the 677CC/1298CC genotype had a lower risk of relapse compared to the 677CC/1298AA reference genotype in patients undergoing haematopoietic cell transplantation.[68] A study in the rheumatoid arthritis setting has reported an association between the 677C/1298C haplotype and a better efficacy of MTX and between 677T/1298A haplotype and increased toxicity.[15] In the same clinical setting, the 677CC/1298AA diplotype was correlated with a lower probability of remission of rheumatoid arthritis symptoms and with increased parameters of disease activity compared to other diplotypes.[69] A research in paediatric ALL evidenced that the 677T/1298A haplotype decreases the probability of event-free and disease-free survival without influencing the toxicity or overall survival.[29] A pilot study in psoriasis patients has suggested a role of the 677CT/1298AC genotype in pre-disposing to a lower MTX-related hepatic toxicity in the cohort without folic acid supplementation.[52] However, some other studies did not confirm the utility of the haplotype approach.[37,49] Several current research efforts focus on developing haplotype maps across the MTHFR gene; additional studies are necessary to understand the appropriate statistical analysis and sample size requirements for a haplotype-based approach and to clearly define the impact of each diplotype on MTX therapy outcome.

Besides MTHFR, other genes involved in folate homeostasis and in the mechanism of action of MTX can play an additional role; these genes are, for example, RFC, GGH, TS, DHFR, methylenetetrahydrofolate dehydrogenase 1 (MTHFD1), methionine synthase reductase (MTRR), methionine synthase (MS), ATIC and serine-hydroxymethyltransferase (SHMT). Preliminary data suggested that the investigation of the combined effect of polymorphisms in different genes involved in MTX and folate metabolism might constitute a promising approach.[29,39,52–54,58,66,70] This polygenic analysis will require much larger and more collaborative studies.

2.2. *Fluoropyrimidine-based therapy*

2.2.1. *5-Fluorouracil (5-FU)*

Preliminary data from *in vitro* studies with human cancer cells and nude mice xenograft models have suggested that both C677T[64] and A1298C[72] may enhance the chemosensitivity to 5-FU. The critical point for the 5-FU activity is the formation of an inhibitory ternary complex, consisting of its active metabolite 5-fluoro-2-deoxyuridine-5-monophospate (5-FdUMP), TS and 5,10-methylentetrahydrofolate, thereby inhibiting TS activity.[73] It can be hypothesised that MTHFR polymorphisms, by increasing intracellular concentrations of 5,10-methylentetrahydrofolate, may augment the cytotoxic activity (tumour response and/or toxic side-effects) of 5-FU by enhancing the formation and stability of the ternary inhibitory complex (Fig. 1b).[64,74,75]

In recent years, several pharmacogenetic analyses have been performed to examine the association between C677T and A1298C and the outcome of patients treated with fluoropyrimidine-based chemotherapy (Table 3). A majority of investigations have been conducted in advanced colorectal cancer patients receiving 5-FU-based therapy mainly as first-line treatment. Some of these studies, considering both tumour material and normal tissue, have reported that C677T genetic variant, but not A1298C, is significantly associated with increased tumour response rate to 5-FU-based therapy.[74–76] The 677TT polymorphic genotype has also been associated with a significantly increased time to progression,[75] while no relevant effects on overall survival[76,77] were reported. The combination of MTHFR C677T genetic variant with the activity or polymorphisms of TS, the key target enzyme of 5-FU, appeared to have a better predictive power on 5-FU-based chemotherapy response compared to the approach that takes into consideration the C677T polymorphism alone.[75,76] Only a few studies have analysed the role of C677T variant on toxic side-effects after fluoropyrimidine therapy in colorectal cancer but, at present, only inconsistent and inconclusive results have been reached.[74,78,79] On the contrary, the 1298CC polymorphic genotype in an advanced colorectal cancer population study has been correlated with an increased risk of developing severe adverse events after 5-FU-based chemotherapy.[79] The 1298CC genotype has also emerged as being associated with a lower specific survival, a parameter that considers only cancer-related death, excluding chemotherapy-related death.[76] Recent work by Zhang et al. has suggested, for the first time, the hypothesis that the MTHFR polymorphisms may be associated with 5-FU-based chemotherapy clinical outcome in a sex-specific manner; in particular, the authors have reported a significant correlation between 1298AA genotype and higher overall survival in female, but not in male patients with advanced colorectal cancer. However, further studies are necessary to validate these findings.[80]

Data from a clinical investigation on advanced gastric cancer patients treated with 5-fluoropyrimidine-based chemotherapy confirmed the correlation between the MTHFR 677TT genotype and an increased response rate and also reported a significant association with a higher treatment-related toxicity.[81]

Wu and colleagues analysed the combined effect of the C677T and A1298C variants in a haplotype approach on oesophageal cancer patients receiving pre-operative chemoradiotherapy containing 5-FU. The individuals with variant allele at both loci present a significant reduced risk of recurrence and better survival compared to those with other haplotypes.[82] This study has also confirmed that the pathway-based approach analysing the combined effect of multiple variant alleles of genes involved in 5-FU and folate metabolism, such as MTHFR and TS, is a useful strategy that might have more informative power than a single-locus investigation in identifying clinical outcome predictors.

Terrazzino et al. have conducted a C677T/A1298C haplotype analysis in patients with rectal cancer treated with preoperative radiotherapy and 5-FU. It was found that the variant MTHFR 677T-1298A haplotype pre-disposes to a worse response and lower tumour regression rate compared to other genotype combinations.[83]

Finally, other recently published studies do not confirm the role of MTHFR polymorphisms as pharmacogenetic determinants of 5-FU therapy outcome in patients with colorectal cancer,[84–88] oesophageal squamous cell carcinoma,[89] gas-

Table 3 – MTHFR polymorphisms and 5-FU therapy outcome.

Treatment regimes	Patient population	Race/ethnicity	MTHFR polymorphisms	Main results	Citations
5-FU ± FA or levamisole	Solid cancer ($n = 683$)	White European	C677T	No relevant effect on toxicity.	[78]
FU-based adjuvant chemotherapy	Stages II and III colorectal cancer ($n = 197$)	White ($n = 112$) African–American ($n = 12$) Asian ($n = 30$) Hispanic ($n = 43$)	C677T, A1298C	No significant effect on TTR.	[84]
5-FU/LV as first-line treatment	Advanced colorectal cancer ($n = 76$)	Caucasian	C677T	No effect on clinical outcome (toxicity, response rate, OS and DFS).	[79]
			A1298C	Higher toxicity in patients with 1298CC genotype. No effect on response rate, OS and DFS.	
FU-based chemotherapy regimens	Metastatic colon cancer ($n = 318$, 177 men 141 women)	White ($n = 234$) Asian ($n = 43$) Black ($n = 15$) Hispanic ($n = 24$) Native American ($n = 2$)	C677T	No effect on OS.	[80]
			A1298C	Higher OS in patients with 1298AA genotype (only in female subgroup).	
First line 5-FU/FA/oxaliplatin palliative chemotherapy as first-line treatment	Metastatic colorectal cancer ($n = 166$)	Caucasian	C677T, A1298C	No effect on clinical outcome (response rate, PFS, toxicity).	[86]
5-FU/irinotecan palliative chemotherapy as first-line treatment	Metastatic colorectal cancer ($n = 146$)	Caucasian	C677T, A1298C	No effect on clinical outcome (response rate, PFS, toxicity).	[87]
5-FU/MTX-based-adjuvant chemotherapy	Breast cancer ($n = 93$)	Hispanic	C677T, A1298C	No effect on DFS.	[92]
Pre-operative 5-FU-based chemoradiotherapy	Rectal cancer ($n = 125$)	Caucasian	C677T/A1298C haplotype	Lower response rate and tumour regression in patients with 677T/1298A haplotype.	[83]
5-FU-containing chemoradiotherapy (in combination with etoposide, cisplatin, FA)	Locally advanced oesophageal squamous cell carcinoma ($n = 68$)	Caucasian	C677T	No effect on response rate and OS.	[89]
5-FU/cisplatin palliative chemotherapy	Advanced gastric cancer ($n = 175$)	Caucasian	C677T	No effect on clinical outcome (response rate, PFS, OS).	[91]
5-FU/LV/oxaliplatin chemotherapy	Advanced colorectal cancer ($n = 54$)	Korean	C677T	No significant role in predicting therapy clinical outcome (response rate, OS).	[88]
5-FU-based chemotherapy (in combination with irinotecan and oxaliplatin) as first-line treatment	Metastatic colorectal cancer ($n = 94$)	Hispanic	C677T A1298C	No effect on clinical outcome (response rate, OS, PFS).	[85]
Pre-operative 5-FU-containing chemoradiotherapy	Oesophageal cancer ($n = 210$)	White ($n = 190$) others ($n = 20$)	C677T	Lower risk of recurrence in patients with genotype 677TT.	[82]
			A1298C	Higher OS in patients with at least one 1298C allele.	
			C677T/A1298C haplotype	Lower risk of recurrence and higher survival in patients with variant alleles at both loci (677T/1298C)	

(*Continued on next page*)

Table 3 – *Continued*

Treatment regimes	Patient population	Race/ethnicity	MTHFR polymorphisms	Main results	Citations
5-FU/LV as first-line treatment	Metastatic colorectal cancer ($n = 139$)	Caucasian	C677T	Higher response rate and longer TTP in patients with 677TT genotype.	[75]
			A1298C	No effect on response rate and TTP.	
Post-operative 5-FU-based chemotherapy ($n = 25$ subgroup)	Gastric cancer ($n = 40$)	Korean	C677T	No effect on survival; no effective markers for tumour sensitivity to therapy.	[90]
5-FU/FA as first-line treatment	Advanced colorectal cancer ($n = 98$)[a]	Caucasian	C677T	Higher response rate in patients with 677TT genotype. No effect on OS.	[76]
			A1298C	Lower specific survival in patients with 1298CC genotype. No effect on response.	
Fluoropymiridine-based chemotherapy	Locally advanced gastric carcinoma ($n = 75$)	Chinese	C677T	Higher response rate and toxicity (mainly vomiting and nausea) in patients with 677TT genotype.	[81]
Fluoropymiridine-based therapy (mainly 5-FU/LV) as first-line treatment	Advanced colorectal cancer ($n = 43$)	Caucasians ($n = 38$) others ($n = 5$)	C677T	Higher response rate in patients with at least one 677T allele. No effect on toxicity.	[74]
5-FU/LV as part of adjuvant therapy	Stage III colon cancer ($n = 51$)	American (non-specified)	C677T	No significant effect on OS. In a subgroup analysis preliminary evidence of a possible role of the 677T allele in pre-disposing to longer survival.	[77]

Abbreviations: DFS, disease-free survival; FA, folinic acid; LV, leucovorin; OS, overall survival; PFS, progression-free survival; Pts; patients; TTP, time to progression; TTR, time to recurrence.

a This study analysed samples obtained from liver metastatic biopsy (all patients) and primary tumour biopsy (53 patients).

tric[90,91] and breast cancer.[92] It must be considered that in all of these inconsistent studies, fluoropyrimidines were used in association with other antineoplastic drugs (e.g. oxaliplatin, cisplatin and irinotecan), while in the analyses that showed positive correlations, 5-FU was generally employed alone or with leucovorin. Probably the drugs used in addition to fluoropyrimidines could influence the impact of MTHFR polymorphisms on 5-FU treatment outcome. Moreover, a difference in clinical setting (adjuvant, neoadjuvant, first/second line palliative chemotherapy) and in the 5-FU administration schedule (bolus and infusion) might affect the associations between MTHFR polymorphisms and fluoropyrimidine activity. Other important issues to be considered are the interpatient variability arising from the individual folate status and the differences in racial/ethnic origin of patients.

2.2.2. *Other fluoropyrimidines*

UFT (uracil plus ftorafur) shows activity as a single agent in a variety of tumours.[93] Preliminary data from a phase I trial, conducted in a small group of patients with advanced solid cancers receiving UFT/leucovorin/irinotecan combination chemotherapy, have suggested a role of MTHFR C677T polymorphism in pre-disposing to an increase in adverse events.[94]

Capecitabine is an oral fluoropyrimidine that is converted *in vivo* to 5-FU by three reactions catalysed by carboxylesterase, cytidine deaminase and thymidine phosphorylase, with a preferential activation in the tumour. This drug has been extensively used both in adjuvant and in metastatic settings for the treatment of several neoplasms such as colorectal and breast cancer.[95] A study realised in 54 patients with advanced colorectal cancer receiving capecitabine showed a protective role on the development of toxicity for the 677TT and 1298AA genotypes and for the 677T/1298A heterozygote haplotype; in this investigation, there was a trend towards shorter overall survival for the 677TT genotype. The MTHFR polymorphisms have also displayed a role in modulating pre-treatment levels of serum folate and plasma homocysteine: 677TT genotype individuals showed a significant lower level of pre-treatment serum folate and higher plasma homocysteine with respect to patients with other genotypes; the same metabolic effects have been evidenced for the 677T/1298A haplotype.[96] Another clinical research conducted in 105 advanced breast cancer patients treated with capecitabine as monotherapy did not confirm the predictive role of MTHFR polymorphisms on capecitabine toxicity.[97]

2.3. *Raltitrexed therapy*

Preliminary clinical results on raltitrexed treatment obtained in patients with solid tumours receiving the drug in combination with irinotecan have suggested that the 677TT genotype is associated with a significantly reduced toxicity.[98] Raltitrexed is a quinazoline antifolate agent that is transported intracellularly by RFC, and once into the cells it is extensively polyglutamated by FPGS; this modification is essential to potentiate the drug cellular retention thus maximising the direct and specific inhibition of TS, the target of the drug (Fig. 1c).[99] The increased availability of 5,10-methylentetrahydrofolate, as a result of impaired MTHFR activity, could compete with raltitrexed for polyglutamation and binding to TS, leading to a diminished cytotoxicity of the compound.[98] Further studies are needed to validate these pilot data.

3. Other MTHFR polymorphisms

Besides the two most common variants C677T and A1298C, other polymorphisms and haplotypes have been identified and described for both the intronic and exonic regions of the MTHFR gene (http://www.ncbi.nlm.nih.gov/SNP/). Some of these genetic variants are relatively frequent even if less prevalent in respect to C677T and A1298C polymorphisms. The impact of the identified genetic variants in modulating the clinical outcome of therapy remains, up to date, largely unknown, and only few preliminary data, although quite encouraging, are available mainly from functional analyses and disease susceptibility studies.[100–105] Exploratory pharmacogenetic data exist for two genetic variants. The synonymous 1317 T > C nucleotide change (rs4846051, Phe435Phe) has been associated with increased MTX toxicity in African–American (frequency of 27–34%) but not in Caucasian (frequency of 1%) rheumatoid arthritis patients.[7,57] The non-synonymous G1793A (rs2274976, Arg594Gln),[18] that has a frequency in Caucasians of about 4–6% has recently been suggested as being involved in influencing the overall survival of Caucasian lung cancer patients receiving a heterogeneous combination of chemoradiotherapy and surgery.[106] The 1793A allele has also been associated with a lower total plasma homocysteine level.[5]

4. Conclusion and future directions

In conclusion, on the basis of the clinical data obtained so far, the potential pharmacogenetic role of C677T and A1298C polymorphisms as genetic determinants of the efficacy and toxicity of antifolate and fluoropyrimidine-based therapy and their interaction with environmental factors remain quite uncertain, and any definitive conclusion should be drawn with extreme caution. The existence of conflicting and opposing results requires further studies, preferably within the context of large randomised controlled trials that will prospectively test the clinical role of these MTHFR variants and their haplotypes. Moreover, polygenic analyses need to be performed, increasing the complexity of these studies.

The real goal of future research will be the detection of a panel of disease-specific genotypes that can be utilised to identify subsets of patients who are pre-disposed to having a better clinical benefit from specific drugs with less toxicity and higher response.

After identification and validation of polygenic determinants of therapy outcome, the final objective will be the translation of these discovered pharmacogenetic markers into widespread clinical practice with the development of pre-treatment standardised genetic tests and molecular diagnostic commercial kits. Such diagnostic tools would lead to a better clinical management of cancer patients, providing an effective tailored therapy with advantages in cost benefits for both patients and society. Preliminary data of cost-effectiveness analysis have recently evidenced that the genotype-based dosage approach, using the MTHFR C677T polymorphism screening to reduce MTX-related toxicity in

Korean rheumatoid arthritis patients, is both less costly and more effective than the conventional strategy for MTX treatment.[36] This analysis has assessed the total expected direct cost (e.g. MTX drug cost, laboratory cost of monitoring the toxicity, prescribing fee, hospitalisation cost and PCR cost) and the probability of continuing MTX treatment because of the absence of severe side-effects, revealing a superiority of the genotype-based approach with respect to the conventional dosage strategy, both in terms of cost and in terms of effectiveness in reducing MTX-related toxicity.

Conflict of interest statement

None declared.

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