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ORIGINAL ARTICLE

A systematic review and meta-analysis of *MTHFR* polymorphisms in methotrexate toxicity prediction in pediatric acute lymphoblastic leukemia

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Methotrexate (MTX) is an important component of therapy used to treat childhood acute lymphoblastic leukemia (ALL). Two single-nucleotide polymorphisms (SNPs) in the methylenetetrahydrofolate reductase (*MTHFR*) gene, C677T and A1298C, affect MTHFR activity. A large body of studies has investigated the potential role of *MTHFR* SNPs in MTX toxicity in pediatric ALL. However, the results are controversial. In this review and meta-analysis, we critically evaluate the relationship between the C677T and A1298C polymorphisms of *MTHFR* and MTX toxicity in pediatric ALL. The majority of published reports do not find associations between *MTHFR* polymorphisms and toxicity in pediatric ALL. When associations are reported, often the results are contradictory to each other. The meta-analysis confirms a lack of association. In conclusion, *MTHFR*, C677T and A1298C polymorphisms do not seem to be good markers of MTX-related toxicity in pediatric ALL.

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer, accounting for 30% of all pediatric malignancies.[1,2] During the last 20 years, survival rates for ALL have improved dramatically due to advances in specific chemotherapy for childhood ALL, with expected cure rates higher than 80%.[3]

Methotrexate (MTX) is an important component of the therapy for childhood ALL. Despite clinical success, treatment with MTX often causes toxicity, requiring a dose reduction or cessation of treatment. An accurate predictor of the adverse effects of MTX treatment would therefore be very useful in ALL.[4]

MTX enters the cell via active transport mediated by the reduced folate carrier (RFC1).[5] Then, MTX acts by inhibiting mainly two enzymes. MTX inhibits dihydrofolate reductase, inhibiting the folic acid cycle and affecting other important enzymes, such as methylenetetrahydrofolate reductase (MTHFR) and serine hydromethyl transferase (SHMT1). On the other hand, the conversion of MTX to its polyglutamated forms results in the inhibition of thymidylate synthase. These combined mechanisms interfere with nucleic acid synthesis, favoring cell death.[6] Effective cellular levels of MTX are reduced by different transporters that pump MTX out of the organism. These transporters include ABC transporters, such as the multidrug resistance protein (ABCB1) and the breast cancer resistance protein (ABCG2),[7] and organic anion transporters, such as SLCO1B1[8,9] (Figure 1).

MTHFR is a key enzyme for intracellular folate homeostasis and metabolism. MTHFR catalyzes the irreversible conversion of 5,10-methylentetrahydrofolate (5,10-CH2-THF), required for purine and thymidine synthesis, to 5-methyltetrahydrofolate (5-CH-THF), which is required for protein synthesis and nucleic acid methylation. Alterations in reduced folate pools, as a consequence of changes in MTHFR activity, may have a significant effect on the responsiveness of malignant and non-malignant cells to MTX. Accordingly, it has been proposed that an impaired conversion of 5,10-CH2-THF to 5-CH-THF and the subsequent modification in the intracellular folates pool could increase the toxic effect of MTX.[10]

In this context, the non-synonymous single-nucleotide polymorphisms (SNPs) C677T (causing Ala222Val) and A1298C (causing Glu429Ala) in the *MTHFR* gene have been widely studied. The *MTHFR* 677T allele encodes proteins with decreased enzymatic activity, in comparison with the normal allele 677C. People with the 677CT and 677TT genotype exhibit 60% and 30%, respectively, of the normal MTHFR activity.[11,12] In the *MTHFR* A1298C polymorphism, the 1298C allele is responsible for a milder decrease in MTHFR activity with respect to the normal allele 1298A. The 1298CC homozygous individuals have 60% of the normal activity.[13]

A large body of published studies has investigated the potential role of *MTHFR* polymorphisms in susceptibility, toxicity and response to MTX in pediatric ALL, with conflicting results. Possible reasons for these discrepancies are differences in treatment protocols among studies, small or non-homogeneous populations, ethnic differences and the use of different criteria defining toxicity.

In this study, we have performed a critical review of the published articles on the relationship between genetic variants of *MTHFR* and the toxicity of MTX in pediatric ALL. Then, we undertook a meta-analysis on all eligible studies, separating them by toxicity criteria, to determine the role of the *MTHFR* C677T and A1298C polymorphisms on MTX toxicity in this pediatric ALL patients.

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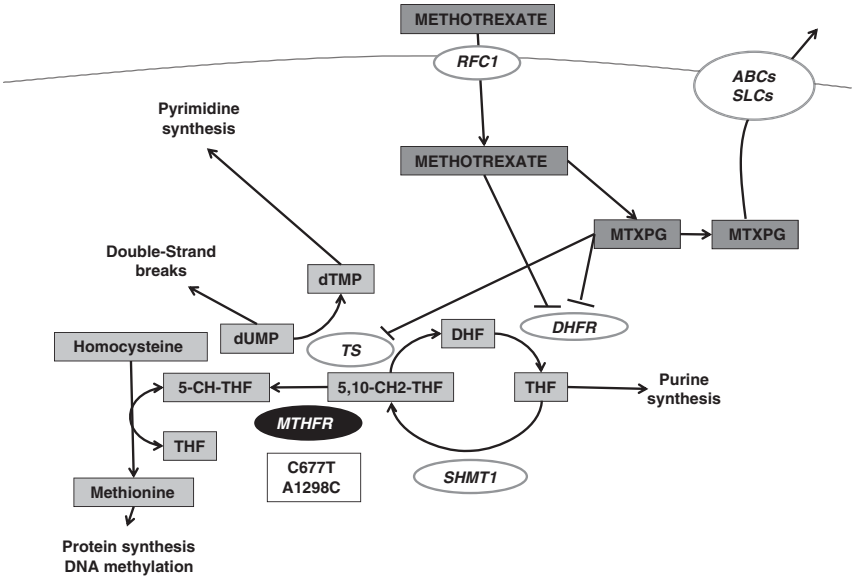

Figure 1. MTX pathway. MTX and its metabolites are indicated. Enzymes and transporters are encircled. *MTHFR* is in black.

MATERIALS AND METHODS

Search strategy

We performed an exhaustive search to identify studies that examined the association between the C677T and A1298C polymorphisms of *MTHFR* and MTX toxicity in pediatric ALL patients. We used the keywords and subject terms 'MTHFR and acute leukemia', and 'MTHFR and polymorphism(s) and toxicity' to search Pubmed (www.ncbi.nlm.nih.gov/pubmed) for articles published through November 2011. All references within the identified studies were then reviewed to possibly identify additional works.

Meta-analysis

Inclusion and exclusion criteria. The inclusion criteria for meta-analysis required that each trial be an independent association study, that the article supplied enough information on toxicity by genotype, that it studied short-term toxic effects and that the population was composed only of pediatric patients (<18 y). An article was excluded from meta-analysis if the study did not provide enough information, was performed on adult patients, the diagnosis was not mainly ALL or was a case study.

Data extraction

For each article included in the study, we gathered ethnicity of study population, patients number, age and diagnosis, MTX dose, *MTHFR* C677T and A1298C genotype data and toxicity types.

Statistical analysis

Statistical analysis was performed using R software using the meta library (R version 2.11.0, the R Foundation for Statistical Computing). We used a recessive model, assuming a recessive effect of the minor allele of each *MTHFR* SNP, that was consistent with previous results and allowed inclusion of the maximum number of studies. For the C677T SNP, we compared individuals having the TT homozygous genotype with all others (CC + CT), and for the A1298C SNP, we compared CC homozygous individuals with all others (AA + AC). The overall pooled relative risk and corresponding 95% confidence interval of toxicity to MTX were estimated using Mantel–Haenszel's method with random effect model. The random effects model assumes different underlying effects, considering both within- and between-study variation, offering an advantage as it accommodates diversity between studies and provides a more conservative estimate of the assessed effect.

Heterogeneity of the studies was assessed using the Cochrane *Q* test with a *P*-value below 0.05, below which heterogeneity was considered statistically significant. The heterogeneity was also quantified using the I^2 statistic, which is independent of the number of studies in the meta-analysis. This statistic quantifies the effect of heterogeneity, providing a measure of the degree of inconsistency in the study's results. The I^2 statistic has a value between 0 and 100% and describes the percentage of total variation across studies that is due to between-studies heterogeneity rather than chance. A higher I^2 value denotes a greater degree of heterogeneity (customary interpretations of the I^2 value are 0–25% no heterogeneity, 25–50% moderate heterogeneity, 50–75% large heterogeneity and 75–100% extreme heterogeneity). Sensitivity analysis leaving out one study at the time was also performed when possible: outlying studies were identified and excluded and the I^2 estimates for these different sets of studies examined.

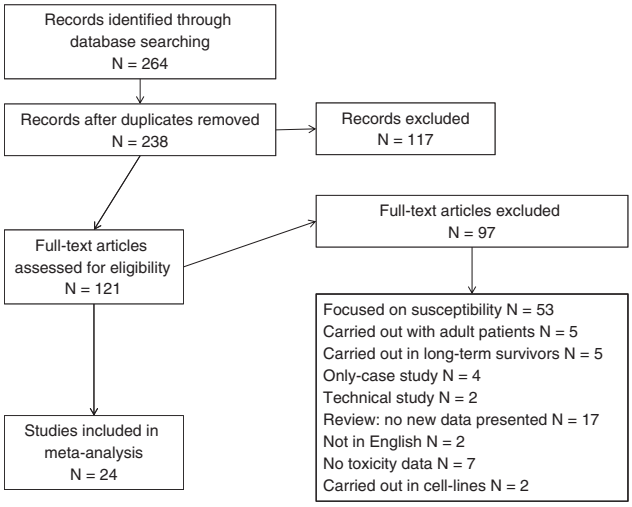

Figure 2. Flow diagram of study selection.

RESULTS

The original search provided 264 records. After eliminating duplications, 238 records remained. Of these, 117 were discarded after reviewing the abstracts because they clearly did not meet the required criteria for inclusion. The full texts of the remaining 121 studies were examined in detail. Of these, we identified 24 studies that investigated *MTHFR* SNPs and MTX-related toxicity in pediatric ALL patients for meta-analysis (Figure 2). All 24 studied the C677T polymorphism (Table 1) and 16 of these also studied the A1298C polymorphism (Table 2).

Table 1. List of 24 studies that analyzed association between the *MTHFR* C677T polymorphism and MTX toxicity in pediatric ALL, grouped according to the level of association between the SNP and MTX toxicity

MTHFR C677T				
Patient population	*MTX dose*	*Population*	*Association with toxicity*	*Reference*
15 ALL or LBL[A]	High	Japanese	NA	Shimasaki *et al.*[18]
24 ALL or LBL[A]	Low	Japanese	NA	Horinouchi *et al.*[27]
35 ALL[C]	High	Cretan	NA	Karathanasis *et al.*[19]
46 ALL[C]	High	Greek	NA	Chatzidakis *et al.*[29]
53 ALL[C]	High	Various	NA	Kishi *et al.*[36]
76 ALL[C]	High	Thai	NA	Pakakasama *et al.*[20]
81 ALL[B]	High	European	NA	Huang *et al.*[14]
115 ALL[A]	High	Spanish	NA	Lopez-Lopez *et al.*[15]
167 ALL[C]	High	European	NA	Erculj *et al.*[22]
240 ALL[A]	High	North American	NA	Kishi *et al.*[34]
201 ALL[C]	Low	French-Canadian	NA	Krajinovic *et al.*[33]
520 ALL[B]	Low	Various	NA	Aplenc *et al.*[21]
37 ALL or NHL[C]	High	Turkish	−T	Kantar *et al.*[16]
88 ALL[C]	High	European	−T	van Kooten *et al.*[30]
186 ALL[B]	Low	European	−T	Costea *et al.*[28]
20 ALL or LBL[A]	Low	Japanese	+T	Shimasaki *et al.*[37]
26 ALL or ML[A]	High	Japanese	+T	Imanishi *et al.*[4]
40 ALL[C]	High	Egyptian	+T	Tantawy *et al.*[26]
40 ALL[A]	High	Egyptian	+T	EL-Khodary *et al.*[32]
64 ALL or ML[A]	High	European	+T	Faganel Kotnik *et al.*[23]
141 ALL[C]	High	Spanish	+T	Salazar *et al.*[25]
151 ALL[C]	High	European	+T	D'Angelo *et al.*[35]
181 ALL[B]	High	Chinese	+T	Liu *et al.*[17]
557 ALL[C]	High	Various	+T	Sepe *et al.*[31]

Abbreviations: ALL, acute lymphoblastic leukemia; LBL, lymphoblastic lymphoma; ML, malignant lymphoma; MTX, methotrexate; NA, no association between the SNP and toxicity; NHL, non-Hodgkin's lymphoma; +T, SNP is associated with increased toxicity; –T, SNP is associated with decreased toxicity.
Type of sample: A, normal; B, tumor; C, unknown. High MTX dose = 1.5–5 g m^{-2}; Low MTX dose = 15–30 mg m^{-2}.

Table 2. Association of *MTHFR* A1298C polymorphism and toxicity in pediatric ALL

MTHFR A1298C				
Patient population	*MTX dose*	*Population*	*Association with toxicity*	*Reference*
40 ALL[C]	High	Egyptian	NA	Tantawy *et al.*[26]
115 ALL[A]	High	Spanish	NA	Lopez-Lopez *et al.*[15]
151 ALL[C]	High	European	NA	D'Angelo *et al.*[35]
167 ALL[C]	High	European	NA	Erculj *et al.*[22]
186 ALL[B]	Low	European	NA	Costea *et al.*[28]
201 ALL[B]	Low	French-Canadian	NA	Krajinovic *et al.*[33]
240 ALL[A]	High	North American	NA	Kishi *et al.*[34]
520 ALL[C]	Low	Various	NA	Aplenc *et al.*[21]
64 ALL or ML[A]	High	European	−T	Faganel Kotnik *et al.*[23]
76 ALL[C]	High	Thai	−T	Pakakasama *et al.*[20]
81 ALL[C]	High	European	−T	Huang *et al.*[14]
88 ALL[C]	High	European	−T	van Kooten *et al.*[30]
181 ALL[B]	High	Chinese	−T	Liu *et al.*[17]
35 ALL[C]	High	Cretan	+T	Karathanasis *et al.*[19]
37 ALL or NHL[C]	High	Turkish	+T	Kantar *et al.*[16]
141 ALL[C]	High	Spanish	+T	Salazar *et al.*[25]

Abbreviations: ALL, acute lymphoblastic leukemia; ML, malignant lymphoma; MTX, methotrexate; NA, no association between the SNP and toxicity; NHL, non-Hodgkin's lymphoma; +T, SNP is associated with increased toxicity; –T, SNP is associated with decreased toxicity.
Type of sample: A, normal; B, tumor; C, unknown. High MTX dose = 1.5–5 g m^{-2}; Low MTX dose = 15–30 mg m^{-2}.

In general, the 24 studies could be categorized according to the level of association between *MTHFR* SNPs and MTX toxicity: those that found no association, those that found an association between *MTHFR* SNP and a significant increase in toxicity and those that found an association between *MTHFR* SNP and a significant decrease in toxicity. No population, even grouped by ethnicity or geography, was overrepresented in any of the three groups of studies. Additionally, both high- and low-dose MTX were found in all three studied groups. We did not find either differences between the reports that used normal or tumor

Table 3. Types of toxicities analyzed and the findings in each study of the associations between the *MTHFR* C677T polymorphism and MTX toxicity

	MTHFR C677T								
Reference	*Hematologic toxicity*					*MTX plasma levels*	*Mucositis*	*Hepatic toxicity*	*Other*
	Anemia	*Leuko-penia*	*Neutro-penia*	*Thrombo-cytopenia*	*Myelo-suppression*				
Shimasaki *et al.*[18]					NA		NA	NA	NA
Horinouchi *et al.*[27]								NA	
Karathanasis *et al.*[19]	NA	NA		NA			NA	NA	
Chatzidakis *et al.*[29]	NA	NA						NA	
Kishi *et al.*[36]									NA
Pakakasama *et al.*[20]					NA		NA		NA
Huang *et al.*[14]	NA	NA		NA		NA	NA	NA	NA
Lopez-Lopez *et al.*[15]						NA			
Erculj *et al.*[22]	NA	NA		NA			NA	NA	NA
Kishi *et al.*[34]									NA
Krajinovic *et al.*[33]									NA
Aplenc *et al.*[21]							NA	NA	NA
Kantar *et al.*[16]	NA	NA		−T		NA		NA	NA
van Kooten *et al.*[30]		NA	−T	NA				NA	NA
Costea *et al.*[28]		−T	NA	NA				NA	
Shimasaki *et al.*[37]									+T
Imanishi *et al.*[4]						+T		NA	
Tantawy *et al.*[26]			+T				+T	+T	+T
EL-Khodary *et al.*[32]	+T	+T		+T				+T	+T/NA
Faganel Kotnik *et al.*[23]		NA		NA			+T		NA
Salazar *et al.*[25]	NA	NA		+T			NA	NA	+T
D'Angelo *et al.*[35]					NA				+T
Liu *et al.*[17]	NA		NA	+T	NA	NA	NA	NA	NA
Sepe *et al.*[31]								+T	NA

Abbreviations: MTX, methotrexate; NA, no association between the SNP and toxicity; +T, SNP is associated with increased toxicity; –T, SNP is associated with decreased toxicity.

samples (Tables 1 and 2). As different studies analyzed toxicity according to different criteria, we performed in-depth analysis for each toxicity criterion (Tables 3 and 4).

MTHFR C677T polymorphism and toxicity in pediatric ALL

We could not perform a meta-analysis for treatment interruption, MTX clearance, diarrhea, hyperbilirubinemia and renal toxicity due to lack of data. We did perform meta-analysis for MTX plasma levels, mucositis, hepatic toxicity, neutropenia, thrombocytopenia, anemia and leukopenia.

MTX plasma levels. MTX plasma levels were studied in five works that used high MTX doses[4,14–17] Only two studies provided enough data, from a total of 137 patients, to be included in the meta-analysis.[4,15] We found no statistical association between C677T and MTX plasma levels (Figure 3).

Mucositis. Mucositis was surveyed in 10 studies.[14,17–25] One of them was performed with low MTX doses[21] and the rest with high doses. We performed meta-analysis on four studies[17,19,23,26] with data from a total of 484 observations. No association with mucositis was observed (Figure 3). As the heterogeneity among studies was high, a sensitivity analysis was undertaken and this identified the study by Tantawy *et al.*[26] as an outlier. Removing this data from the meta-analysis reduced the heterogeneity, yet the pooled relative risk remained non-significant.

Hepatic toxicity. We compiled 16 studies that analyzed hepatic toxicity with low[21,27,28] or high MTX doses.[4,14,16–19,22,25,26,29–32] Of these 16 studies, 6 presented enough data to allow meta-analysis[4,17,19,26,27,31] with data from a total of 757 patients. No association between C677T genotypes and hepatic toxicity was observed (Figure 3). As there was a great heterogeneity between studies, a sensitivity analysis was undertaken and this identified the study by Tantawy *et al.*[26] as an outlier. Removing this data from the meta-analysis reduced the heterogeneity, yet the pooled relative risk remained non-significant. We also performed a meta-analysis with the five high dose studies that provided data and we obtained similar results.

Neutropenia. From the four papers that analyzed neutropenia with low[28] or high MTX doses[17,26,30] (Table 1), two provided enough data to be included in the meta-analysis[17,26] with data from 200 patients. No association between C677T SNP and neutropenia was observed (Figure 3).

Thrombocytopenia. A total of 10 studies[14,16,17,19,22,23,25,28,30,32] analyzed thrombocytopenia. One of them was performed with low MTX doses[28] and the rest with high doses. In the meta-analysis, three studies were included[17,19,23] with data from a total of 381 observations. No association between the C677T SNP and thrombocytopenia was observed (Figure 3).

Anemia. From the eight reports that studied anemia[14,16,17,19,22,25,29,32] with high MTX doses, a single study[32] found an association between C677T and increased anemia. In the meta-analysis, we excluded five studies due to lack of data,

Table 4. Types of toxicities analyzed and the findings in each study of the associations between the *MTHFR* A1298C polymorphism and MTX toxicity

	MTHFR A1298C								
Reference	*Hematologic toxicity*					*MTX plasma levels*	*Mucositis*	*Hepatic toxicity*	*Other*
	Anemia	*Leuko-penia*	*Neutro-penia*	*Thrombo-cytopenia*	*Myelo-suppression*				
Tantawy *et al.*[26]			NA				NA	NA	NA
Lopez-Lopez *et al.*[15]						NA			
D'Angelo *et al.*[35]					NA				NA
Erculj *et al.*[22]	NA	NA		NA			NA	NA	NA
Costea *et al.*[28]		NA	NA	NA				NA	
Krajinovic *et al.*[33]									NA
Kishi *et al.*[34]									NA
Aplenc *et al.*[21]							NA	NA	NA
Faganel Kotnik *et al.*[23]		−T		NA			NA		NA
Pakakasama *et al.*[20]					−T		NA		NA
Huang *et al.*[14]	NA	NA				NA	NA	NA	−T/NA
van Kooten *et al.*[30]		NA	NA	−T				NA	NA
Liu *et al.*[17]	NA		NA	NA	NA	NA	NA	NA	−T
Karathanasis *et al.*[19]	NA	NA		NA			NA	+T	
Kantar *et al.*[16]	+T	NA		+T		+T		+T	+T
Salazar *et al.*[25]	NA	NA		+T			NA	NA	+T

Abbreviations: MTX, methotrexate; NA, no association between the SNP and toxicity; +T, SNP is associated with increased toxicity; −T, SNP is associated with decreased toxicity.

leaving two studies with data from 192 patients.[17,19] We observed no association with anemia (Figure 3).

Leukopenia. We found 10 reports that studied leukopenia with low[28] or high MTX doses.[14,16,19,22,23,25,29,30,32] From these 10 studies, 2 provided genotype data from 221 observations.[19,23] No association between C677T and leukopenia in response to MTX treatment in ALL was observed (Figure 3).

MTHFR A1298C polymorphism and toxicity in pediatric ALL

In the 16 studies that analyzed this polymorphism (Table 2), 8 studies with low[21,28,33] or high MTX doses[15,22,26,34,35] found no association between A1298C and any toxic effect. In five studies, with high MTX doses, the authors reported a protective effect of the 1298C allele against various types of MTX toxicity.[14,17,20,23,30] We found three studies, also with high MTX doses, in which this allele was associated with higher MTX toxicity.[16,19,25]

We could not perform a meta-analysis for transfusions, skin toxicity, MTX plasma levels or febrile neutropenia due to lack of data. We did perform meta-analyses for leukopenia, myelosuppression, thrombocytopenia, hepatic toxicity and anemia with high MTX doses and only observed a slight protective effect of the 1298CC genotype for leukopenia in a meta-analysis study with data from only two reports (Figure 4).

DISCUSSION

MTHFR C677T polymorphism and toxicity in pediatric ALL

In the 24 published studies used in this analysis, 12 did not find a significant association between the *MTHFR* 677T low-functional allele and MTX toxicity.[14,15,18–22,27,29,33,34,36] Three studies found an association between the 677T allele and a decrease in toxicity.[16,28,30] Nine studies found an association between this allele and increased toxicity[4,17,23,25,26,31,32,35,37] (Table 1).

Populations studied or MTX doses could not explain the differences in results among studies. We did not find either differences between the reports that used normal or tumor samples, as it was expected considering that mutations or deletions have not been described in *MTHFR* in ALL. As different studies analyzed toxicity according to different criteria, below we analyze the findings from the 24 studies for each toxicity criterion and report results from meta-analysis if enough data was provided to make it possible.

Treatment interruption. Three studies analyzed MTX treatment interruption. An association between the 677T allele and an increase in interruption was reported by Shimasaki *et al.*[37] with low MTX doses; however, this study was carried out with a small and heterogeneous population (20 ALL or lymphoblastic lymphoma) and only 1 patient with the TT genotype was reported. Two larger studies of 88 and 201 ALL patients did not find any association between 677T and MTX treatment interruption with low[33] or high MTX doses.[30] Consequently, the 677TT genotype cannot be considered a good predictor of treatment interruption. The three articles did not provide enough information to carry out a meta-analysis to confirm it.

MTX pharmacokinetics. Although MTX pharmacokinetics are more directly related with membrane transporters, they have been measured in association with MTHFR C677T polymorphism in several studies using high MTX doses with different parameters: MTX plasma levels, MTX clearance and renal toxicity.

MTX plasma levels were studied in five works. Imanishi *et al.*[4] studied 26 children with ALL or malignant lymphoma and concluded that patients with the 677TT homozygous genotype had higher MTX plasma levels 48 h after infusion. The other four studies found no association between the C677T SNP and MTX plasma levels 48 or 72 h after infusion.[14–17] We did not find either any statistical association between C677T and MTX plasma levels in the meta-analysis.

Two studies analyzed MTX clearance and reported conflicting results. One study of 64 children with ALL or malignant lymphoma[23] found an association between the 677TT homozygous genotype and a decrease in MTX clearance. The

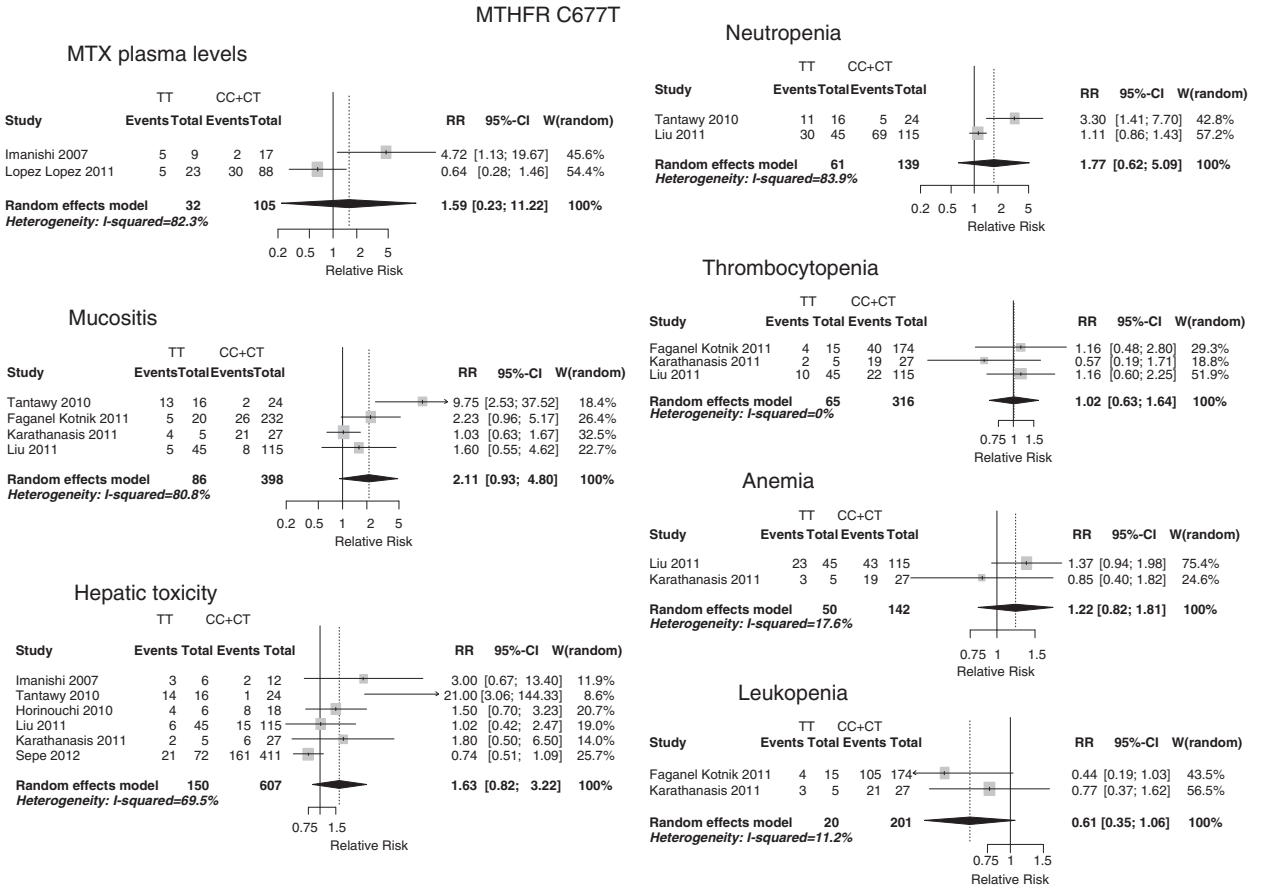

Figure 3. Results of meta-analysis of association between the *MTHFR* C677T SNP and MTX toxicities in the treatment of ALL. No associations were confirmed between genotype and toxicity.

larger study of 240 pediatric ALL patients did not find any association between the C677T SNP and MTX clearance.[34] We could not carry out a meta-analysis for this parameter.

Renal toxicity was reported in three studies. One[32] found an association between the 677T allele and increased renal toxicity. In a combined study of C677T and A1298C, association with increased renal toxicity was also found.[25] Another larger study did not find any association between the 677T allele and increased renal toxicity.[22] Consequently, the published data do not support a clear association between the 677T allele and renal toxicity in response to MTX treatment in ALL. We could not carry out a meta-analysis to confirm it, due to lack of data.

Consequently, the 677T allele does not seem to be a good marker of MTX pharmacokinetics in ALL treatment.

Gastrointestinal toxicity. Some studies found associations between the *MTHFR* 677T allele and gastrointestinal toxicity parameters: diarrhea and mucositis.

Four studies analyzed diarrhea with high MTX doses. An association between the 677TT homozygous genotype and higher risk of diarrhea was found in a single study of 40 pediatric ALL patients.[26] Three additional studies carried out with 240, 520 and 557 pediatric ALL patients did not find this correlation.[21,31,34] Accordingly, the 677TT genotype cannot be considered a good predictor of severe diarrhea in response to MTX treatment for ALL. Only one of the four articles provided genotype information, so we were unable to confirm this with a meta-analysis.

Mucositis was surveyed in 10 studies. Two studies of 40 and 64 children with ALL found an association between 677TT genotype and higher risk of mucositis with high MTX doses.[23,26] The other eight studies, most of which were larger, studied various ethnic populations and included low[21] and high MTX doses,[14,17–20,22,25] did not find this association. This lack of consistent results across these studies does not support an effect of the 677TT genotype in the risk of mucositis in response to MTX treatment for ALL. Our meta-analysis supported this lack of association.

Hepatic toxicity. We compiled 16 studies that analyzed hepatic toxicity (transaminitis). Three of them found an association between the 677TT genotype and increased hepatic toxicity with high MTX doses.[26,31,32] However, 2 of these studies do not have a very high statistical power, and the other 13 studies that analyzed this parameter found no association between 677TT genotype and hepatic toxicity with low[21,27,28] or high MTX doses;[4,14,16–19,22,25,29,30] therefore, we conclude that the 677TT genotype does not appear to be a good predictor of hepatic toxicity in response to MTX treatment for ALL. This lack of association was confirmed with the meta-analyses performed.

Hyperbilirubinemia, which is also associated with hepatic toxicity, was studied in four reports. One study of thirty-seven patients[16] found that individuals with 677CT or 677TT genotypes had less hyperbilirubinemia with high MTX doses. In all, 3 larger studies of 240, 520 and 557 patients did not find this association with low[21] or high MTX doses.[31,34] None of these articles provided enough information to perform a meta-analysis. We conclude that the 677TT genotype does not appear to be

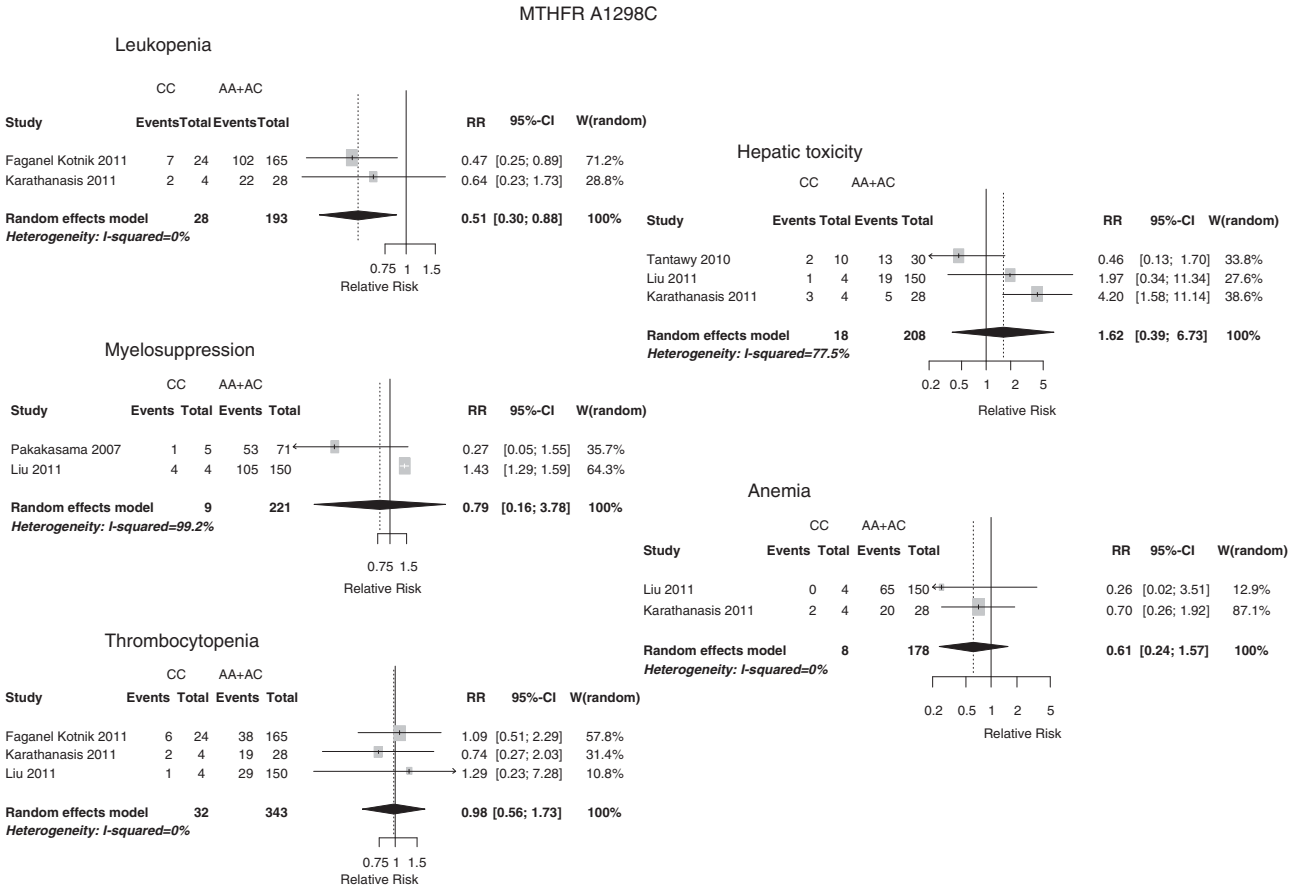

Figure 4. Results of meta-analysis of association between the *MTHFR* A1298C polymorphism and MTX toxicities in treatment of ALL. We observed a slight protective effect of the 1298CC genotype with leukopenia using data from only two reports.

either a good predictor of hyperbilirubinemia in response to MTX treatment for ALL.

Hematologic toxicity. Some studies have found associations between *MTHFR* 677T allele and different hematologic toxicity parameters: neutropenia, thrombocytopenia, anemia and leukopenia.

From the four papers that analyzed neutropenia, only one reported an association between the 677TT genotype and higher risk of neutropenia with high MTX doses.[26] Two larger studies did not find this association with low[28] or high MTX doses.[17] A fourth study reported the opposite effect, finding an association between the 677TT genotype and a lower risk of neutropenia[30] with high MTX doses (Table 1). The controversial reports and the results of our meta-analysis do not support an association between C677T SNP and neutropenia.

A total of 10 studies analyzed thrombocytopenia with low[28] or high MTX doses.[14,16,17,19,22,23,25,30,32] An association between the 677CT and 677TT genotypes and an increased risk of thrombocytopenia was reported in two studies,[17,32] but was only statistically significant for the 677CT genotype. The apparent disadvantage of the heterozygous genotype is difficult to explain from a functional point of view. Furthermore, another study reported a correlation between the 677CT and TT genotypes with decreased risk of thrombocytopenia.[16] In another study that looked at both C677T and A1298C, an association between the combined 677T and 1298C alleles and increased thrombocytopenia was found.[25] An additional six studies did not find any association between C677T SNP and thrombocytopenia.[14,19,22,23,28,30] In conclusion, the available data do not support a clear association between the 677T allele and a higher risk of thrombocytopenia in response to MTX treatment for ALL, which is in agreement with our meta-analysis.

From the eight reports that studied anemia with high MTX doses,[14,16,17,19,22,25,29,32] a single study[32] found an association between C677T and increased anemia. In the meta-analysis, we observed no association with anemia.

We also found 10 reports that studied leukopenia. One[32] found an association between C677T and increased leukopenia with high MTX doses. Another study reported the opposite, finding an association between 677T and decreased leukopenia with low MTX doses.[28] Eight studies did not find any association with high MTX doses.[14,16,19,22,23,25,29,30] These results and our meta-analysis do not support a clear association between 677T allele and leukopenia.

Consequently, MTHFR 677T allele does not seem to be a good marker of hematologic toxicity.

Finally, in our review of the literature, we re-analyzed, when possible, the data provided in the articles. In one case in which the authors reported an association between the 677TT genotype and an increase in global toxicity,[35] we detected a statistical error and drew the opposite conclusion to what the authors proposed.[38]

MTHFR A1298C polymorphism and toxicity in pediatric ALL

In the 16 studies that analyzed this polymorphism (Table 2), 8 studies[15,21,22,26,28,33–35] found no association between A1298C and any toxic effect. In five studies, the authors reported a protective effect of the 1298C allele against various types of MTX

toxicity.[14,17,20,23,30] We found three studies in which this allele was associated with higher MTX toxicity.[16,19,25] In our meta-analyses, we only observed a slight protective effect of the 1298CC genotype for leukopenia in a meta-analysis study with data from only two reports.

According to the published data and the meta-analysis reported, the 1298C allele does not seem to be a good MTX toxicity marker in pediatric ALL patients. If anything, it seems to be more likely a protective factor rather than a toxicity marker.

In summary, considering all the previously discussed combined with the fact that works that could not be included in the meta-analyses due to lack of data, are, in general, those that found no association with toxicity, we conclude that there is no evidence to support the use of either the *MTHFR* C677T or the A1298C SNP as MTX toxicity markers.

Taking into account our conclusion that *MTHFR* C677T and A1298C SNPs do not seem good MTX toxicity markers and that a recent study reported that patients receiving higher doses of MTX have better survival,[25] patients with pediatric ALL might benefit from higher MTX doses in spite of their *MTHFR* genotype.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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