

# MTHFR polymorphisms in childhood acute lymphoblastic leukemia: influence on methotrexate therapy

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**Abstract:** Methotrexate (MTX) is an important component in the therapy used to treat childhood acute lymphoblastic leukemia (ALL). Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme for MTX pharmacokinetics. Two single-nucleotide polymorphisms in *MTHFR* gene, C677T and A1298C, affecting MTHFR activity, have been widely studied as potential markers of MTX toxicity and/or outcome in pediatric ALL. In this review, we show that the majority of published reports do not find association or present opposite effect. Therefore, *MTHFR* C677T and A1298C polymorphisms do not seem to be good markers of MTX-related toxicity and/or outcome in pediatric ALL. The efforts should be focused on other genes, such as transporter genes or microRNA-related genes.

**Keywords:** MTHFR, methotrexate, toxicity, outcome, C677T, A1298C

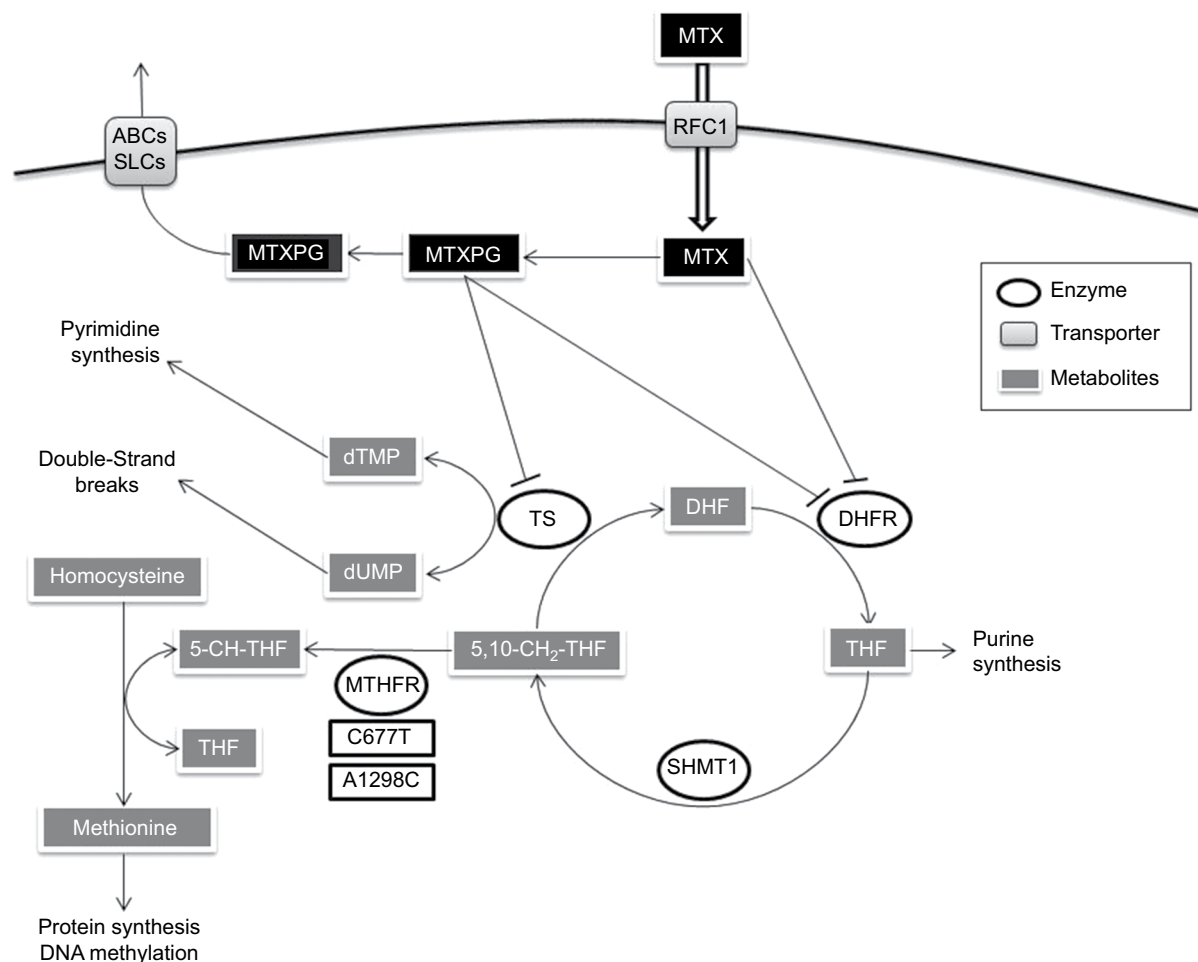
## Introduction

Acute lymphoblastic leukemia (ALL) is the most common type of cancer in children, representing ~30% of all childhood malignancies.<sup>1,2</sup> Survival rates have increased dramatically over the last years because of chemotherapy progress, with expected cure rates higher than 80%.<sup>3</sup> Methotrexate (MTX) is an important drug used in the treatment protocols for ALL. However, MTX can cause toxicity, leading to a dose reduction or treatment interruption, which could compromise the survival.

MTX is a folate analog that enters the cell via active transport mediated by the reduced folate carrier (RFC1).<sup>4</sup> Then, MTX inhibits dihydrofolate reductase, arresting the folic acid cycle and affecting other important enzymes such as methylenetetrahydrofolate reductase (MTHFR), an enzyme that interferes with nucleic acid synthesis and favors cell death.<sup>5</sup> Thus, MTHFR is a key enzyme for intracellular folate homeostasis and metabolism, because it catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate, required for purine and thymidine synthesis, to 5-methyltetrahydrofolate, required for protein synthesis and nucleic acid methylation. Subsequently, changes in the activity of MTHFR provoking an impaired conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate could modify folate pools and in turn alter the response of malignant and nonmalignant cells to MTX and influence its toxicity<sup>6</sup> (Figure 1).

In this context, two of the *MTHFR* polymorphisms most widely studied in relation to the toxicity of MTX are C677T (causing Ala222Val) and A1298C (causing Glu429Ala). Both polymorphisms have been associated with reduced enzyme activity. In the case of C677T, 677CT and 677TT individuals exhibit 60% and 30% of the normal MTHFR

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**Figure 1** MTX pathway.

**Note:** MTX and its metabolites are indicated.

**Abbreviations:** ABCs, ABC family transporters; DHF, dihydrofolate; DHFR, dihydrofolate reductase; MTHFR, methylenetetrahydrofolate reductase; MTX, methotrexate; MTXPG, methotrexate polyglutamated forms; RFC1, reduced folate carrier; SHMT1, serine hydromethyl transferase; SLCs, SLC family transporters; THF, tetrahydrofolate; TS, thymidilate synthase; 5-CH-THF, 5-methyltetrahydrofolate; 5,10-CH<sub>2</sub>-THF, 5,10-methylenetetrahydrofolate.

activity, respectively.<sup>7,8</sup> In the case of A1298C, 1298CC individuals show 60% of the normal activity.<sup>9</sup> Therefore, the patients carrying the variant alleles might have a higher intolerance to MTX or an increased risk of progression.<sup>8</sup>

MTX intolerance can manifest through adverse reactions of several organ systems such as hematological (anemia, thrombocytopenia, leukopenia, neutropenia), gastrointestinal (mucositis), hepatobiliary, urogenital, or central nervous system.<sup>10</sup> Also, MTX plasma levels are used as an objective MTX-related toxicity marker.<sup>11</sup> In some patients, the toxic effects are so serious that the dose must be cut down or the treatment paused, which besides the problems related to toxicity, can also have a negative impact on survival.<sup>12</sup>

As we have mentioned herein, variants that alter MTHFR activity may increase the availability of 5,10-methylenetetrahydrofolate and decrease 5-methyltetrahydrofolate. The reduction of this last would lead to DNA hypomethylation, which could invoke carcinogenesis through three different

mechanisms: chromosomal instability, reactivation of elements, and loss of imprinting.<sup>13</sup> The loss of methylation might favor mitotic recombination, leading to loss of heterozygosity as well as promoting karyotypically detectable rearrangements. Intragenomic parasitic DNA and other previously silent transposons may then be transcribed and even “jump” to other parts of the genome, leading to disruption of normal genes. Hypomethylation can likewise affect imprinted genes, which have been shown to contribute to carcinogenesis. Finally, genomic DNA hypomethylation can also increase through all the tumorigenic steps,<sup>14</sup> from the benign proliferations to the invasive cancers.<sup>13</sup> Therefore, decreased MTHFR activity due to 677T or 1298C alleles could induce hypomethylation, and all these modifications can lead to an increase in carcinogenesis.

To date, several groups have investigated the potential role of *MTHFR* polymorphisms in relation to the toxicity of MTX, as well as outcome in pediatric ALL, but the

conclusions remain controversial. Some studies do not find association, whereas others present opposite effect. In consequence, we consider that it would be interesting to clarify these discrepancies.

## Toxicity and *MTHFR* C677T and A1298C polymorphisms

In 2013, our group 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](http://www.ncbi.nlm.nih.gov/pubmed)) for articles published through November 2011. We also carried out a meta-analysis with those articles supplying enough information on toxicity by genotype. The study was performed in a population composed only of pediatric ALL patients for short-term toxic effects including (MTX plasma levels, mucositis, hepatic toxicity, neutropenia, thrombocytopenia, anemia, and leucopenia).

The meta-analysis included 24 studies for C677T and 16 studies for A1298C. For C677T, none of the analysis revealed a statistically significant association with toxicity. In the case of A1298C, only a slight protective effect of 1298CC genotype for leukopenia was observed.<sup>15</sup> Therefore, we concluded that there was no evidence to support the use of either the *MTHFR* C677T or the A1298C single-nucleotide polymorphisms (SNPs) as MTX toxicity markers in pediatric ALL patients, and consequently the MTX dose should not be adjusted based on these variants.

At the same time, Yang et al<sup>16</sup> performed another meta-analysis studying the effect of C677T and A1298C *MTHFR* polymorphisms on MTX-induced myelosuppression, oral mucositis, liver, hematological, gastrointestinal, and neurological toxicities. They only found a slight association between C677T and gastrointestinal toxicity in pediatric ALL. None of the polymorphisms was associated to any of the other studied toxicities, so again in this study, neither C667T nor A1298C were proved to be good toxicity markers for MTX dose reduction.

In spite of all this evidence, new studies are being published every year analyzing the involvement of *MTHFR* C677T and A1298C polymorphisms in MTX toxicity in pediatric ALL treatment. Herein, we have performed an exhaustive search to identify new studies that explore the association between the C677T and A1298C polymorphisms of *MTHFR* and MTX-induced toxicity from November 2011 to November 2016, following the same strategy as before.<sup>15</sup>

Keywords “*MTHFR*” and “acute lymphoblastic leukemia” for PubMed database were used.

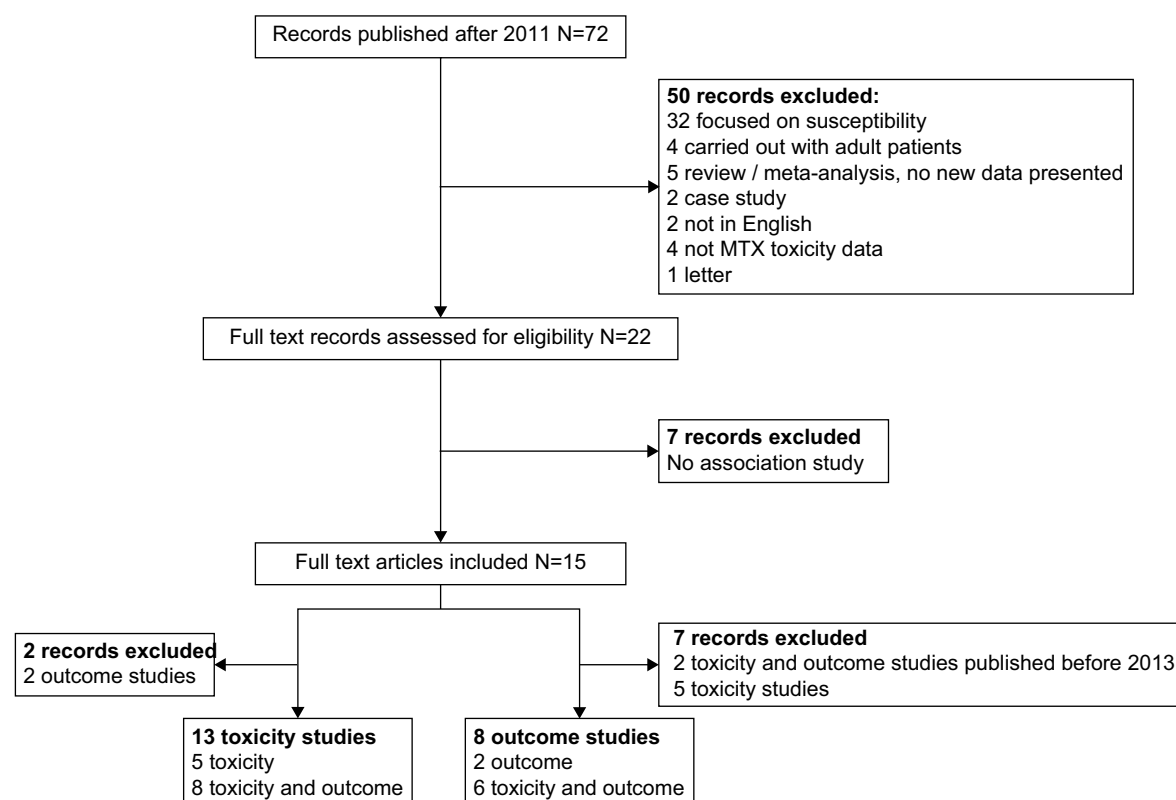
The search provided 186 records, 114 of them were directly discarded because they were published before 2011. From the remaining 72 papers, after abstract screening, 50 were excluded for not analyzing C677T and A1298C polymorphisms in pediatric ALL. For the other 22, full lecture was performed. Seven out of 22 articles were discarded, 4 because they were not focused on the analysis of C677T and A1298C polymorphism and MTX-induced toxicities,<sup>17–20</sup> and other 3 because they analyzed the effect of the C677T/A1298C haplotype in MTX-related toxicity and each genotype could not be evaluated separately.<sup>21–23</sup> We finally reviewed 15 articles that analyzed the *MTHFR* polymorphisms in relation to MTX-induced toxicities in pediatric ALL patients. Of the 15 reviewed papers, 2 were discarded for analyzing only outcome, and 13 studies that analyzed the association with MTX induced toxicities remained. The search and study selection process are shown in Figure 2.

## C677T polymorphism analysis

The 13 selected articles analyzed the C677T polymorphism in relation to MTX-induced toxicities. From them, only 5 found significant associations with toxicity, but with contradictory results: 2 studies related the T variant allele to a decrease in toxicity risk,<sup>24,25</sup> whereas the other 3 associated it to increased risk (Table 1).<sup>26–28</sup>

When we analyzed those studies in depth, we again found discordant results (Table 2). The association study between MTX pharmacokinetics and C677T polymorphism showed that 7 out of 8 studies did not find significant association. Haase et al<sup>25</sup> was the only study in which MTX levels were significantly increased in patients with the *MTHFR* C677T wild type compared to CT/TT genotype variant carriers. However, the number of patients included in this study was very low (n=35). In our previous meta-analysis, only one study found association, but the result was just the opposite.<sup>29</sup> Until now, taking into account our previous meta-analysis and the studies included in this review, a total of 13 studies have been performed about MTX pharmacokinetics, 11 with no association, and 2 with association but with opposite results. Therefore, C677T could not be considered as a good marker for MTX pharmacokinetics.

With regard to hematological toxicity, when anemia was analyzed, only 2 out of 5 studies found association. Haase et al<sup>25</sup> showed that the variant of C677T was associated with a decreased toxicity, whereas Zgheib et al<sup>27</sup> found the opposite effect. The other 3 studies found no association with anemia



**Figure 2** Flow diagram of study selection.

**Abbreviation:** MTX, methotrexate.

**Table 1** List of 13 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

<i>MTHFR</i> C677T				
Patient population	MTX dose	Population	Association with toxicity	Reference, year
21 ALL <sup>C</sup>	High	Indian	NA	Moulik et al, <sup>31</sup> 2016
106 ALL <sup>C</sup>	High	Turkish	NA	Yazıcıoğlu et al, <sup>32</sup> 2016
56 ALL or Lymphoma <sup>A</sup>	High	Japanese	NA	Tsujimoto et al, <sup>47</sup> 2016
53 ALL <sup>A</sup>	Low	Japanese	NA	Suzuki et al, <sup>30</sup> 2015
91 ALL <sup>C</sup>	High	Chinese	NA	Wang et al, <sup>48</sup> 2014
499 ALL <sup>A</sup>	High	German	NA	Radtko et al, <sup>39</sup> 2013
18 ALL <sup>C</sup>	High	Brazilian	NA	de Deus et al, <sup>49</sup> 2012
100 ALL <sup>A</sup>	ND	Korean	NA	Kim et al, <sup>50</sup> 2012
109 ALL <sup>C</sup>	High	Mexican	–T	Ramírez-Pacheco et al, <sup>24</sup> 2016
35 ALL <sup>B</sup>	High	Caucasian	–T	Haase et al, <sup>25</sup> 2012
161 ALL <sup>C</sup>	High	Argentinian	+T	Aráoz et al, <sup>26</sup> 2015
127 ALL <sup>C</sup>	High	Lebanese	+T	Zgheib et al, <sup>27</sup> 2014
103 ALL and NHL <sup>A</sup>	High	Japanese	+T	Fukushima et al, <sup>28</sup> 2013

**Notes:** Type of sample: A, normal; B, tumor; C, unknown. High MTX dose = 1.5–5 g m<sup>-2</sup>; Low MTX dose = 25 mg m<sup>-2</sup>.

**Abbreviations:** *MTHFR*, methylenetetrahydrofolate reductase; SNP, single-nucleotide polymorphism; MTX, methotrexate; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin Lymphoma; ND, no data; NA, no association between SNP and toxicity; +T, SNP is associated with increased toxicity; –T, SNP is associated with decreased toxicity.

(Table 2). Contradictory results were also seen for leukopenia, for which Haase et al<sup>25</sup> showed that the C677T T variant allele was associated with a decreased toxicity, whereas Aráoz et al<sup>26</sup> found that this allele increased the risk of severe leukopenia, but only in carriers who received 2 g/m<sup>2</sup> of MTX. However, Aráoz et al<sup>26</sup> also observed that the 677T allele did not seem to modulate the presence of severe adverse events in patients who received 5 g/m<sup>2</sup> of MTX. The analysis of leukopenia in the other 3 studies showed no association with toxicity (Table 2). Neutropenia was analyzed in 4 studies. Only the study of Aráoz et al<sup>26</sup> found risk 677T allele associated to neutropenia in patients who received low doses of MTX (2 g/m<sup>2</sup>). None of the other 3 studies found association. Again, these results are in line with the results of our previous meta-analysis in which we proposed that C677T polymorphism does not seem to be a good marker for hematological toxicity.

Hepatotoxicity was analyzed in 7 studies, and only the study of Fukushima et al<sup>28</sup> found significant association for 677CT/TT carriers. This study was conducted in a cohort of 103 Japanese patients, that mixed ALL (n=82) and non-Hodgkin lymphoma (NHL) (N=21) patients. In contrast, another study carried out in another Japanese population found no significant association.<sup>30</sup> The other 5 studies performed in other populations found no significant association

**Table 2** Types of toxicities analyzed and the findings in each study of the association between the *MTHFR* C677T polymorphism and MTX toxicity

<b>MTHFR C677T</b>										
Reference, year	MTX kinetics	Hematologic toxicity				Hepatic toxicity	Mucositis	Renal toxicity	Neurotoxicity	Other
		Anemia	Thrombocytopenia	Leukopenia	Neutropenia					
Moulik et al, <sup>31</sup> 2016	—	NA	NA	—	NA	NA	NA	NA	—	NA <sup>a,b</sup>
Yazicioglu et al, <sup>32</sup> 2016	NA <sup>c</sup>	NA	NA	NA	NA	NA	NA	NA	NA	—
Tsujimoto et al, <sup>47</sup> 2016	—	—	—	—	—	—	—	—	NA	—
Suzuki et al, <sup>30</sup> 2015	—	—	—	NA	—	NA	—	—	—	NA <sup>b</sup>
Wang et al, <sup>48</sup> 2014	NA <sup>d,e</sup>	—	—	—	—	—	—	—	—	—
Radtke et al, <sup>39</sup> 2013	NA <sup>f,g,h</sup>	—	—	—	—	—	NA	—	—	—
de Deus et al, <sup>49</sup> 2012	NA <sup>c</sup>	—	—	—	—	NA	—	NA	—	—
Kim et al, <sup>50</sup> 2012	—	—	—	—	—	NA	—	—	—	NA <sup>b,i</sup>
Ramírez-Pacheco et al, <sup>24</sup> 2016	NA <sup>c</sup>	—	—	NA	—	—	–T	—	—	—
Haase et al, <sup>25</sup> 2012	–T <sup>c</sup>	–T	—	–T	—	—	—	—	—	–T <sup>i</sup>
Aráoz et al, <sup>26</sup> 2015	—	NA	NA	+T	+T	NA	NA	—	—	—
Zgheib et al, <sup>27</sup> 2014	NA <sup>f,g,k</sup>	+T	—	—	NA	—	—	—	—	NA <sup>b</sup>
Fukushima et al, <sup>28</sup> 2013	NA <sup>c</sup>	—	—	—	—	+T	—	—	—	+T <sup>i</sup>

**Notes:** Identifiers: <sup>a</sup>febrile neutropenia; <sup>b</sup>MTX dose reduction; <sup>c</sup>MTX levels; <sup>d</sup>dose corrected MTX level; <sup>e</sup>percent of MTX >40 mmol/L; <sup>f</sup>MTX clearance; <sup>g</sup>MTX AUC; <sup>h</sup>peak MTX plasma level; <sup>i</sup>sepsis; <sup>j</sup>rate of cycles with infection; <sup>k</sup>time to reach MTX level <0.1 mmol/L; <sup>l</sup>MTX administration delay; —, not evaluated.

**Abbreviations:** MTHFR, methylenetetrahydrofolate reductase; SNP, single-nucleotide polymorphism; MTX, methotrexate; NA, no association between SNP and toxicity; +T, SNP is associated with increased toxicity; –T, SNP is associated with decreased toxicity.

either. Once more, these results are in line with the results found by our group in the previous review, in which 13 studies out of 16 were not significant.

Mucositis was analyzed in 5 studies, and only Ramírez-Pacheco et al<sup>24</sup> concluded that CC genotype was associated with a higher risk of developing mucositis in a cohort of 109 children with ALL. The rest of the 4 studies found no association, in line with our meta-analysis results in which 8 out of 10 studies were not significant.

Finally, for thrombocytopenia and renal toxicity with 3 studies and neurotoxicity with 2 studies, none of them found significant association. Once more, these results were in agreement with the previous ones.

In conclusion, after analyzing thoroughly a total of 37 articles and 4,583 patients (24 studies with 3,104 patients from the previous meta-analysis and 13 studies with 1,479 patients from our actual review) and considering that the

majority of studies were not significant, the significant results were often contradictory or with low statistical power, we can conclude that C677T is not a good MTX toxicity marker in pediatric ALL.

## A1298C polymorphism analysis

Of the 13 articles reviewed, 10 analyzed the association of A1298C polymorphism with MTX-induced toxicity (Table 3). From them, only 3 studies found significant association. Moulik et al<sup>31</sup> and Haase et al<sup>25</sup> showed the C variant allele favoring an increase in toxicity, whereas Fukushima et al<sup>28</sup> showed a protective role for the C variant allele. When the different toxicities were considered, almost all the results found no significant associations (Table 4).

Regarding anemia, 3 of 5 studies found no significant results, whereas both Moulik et al<sup>31</sup> and Haase et al<sup>25</sup> found association with an increase in toxicity for patients carrying



**Table 3** List of 10 studies that analyzed association between the *MTHFR* A1298C polymorphism and MTX toxicity in pediatric ALL, grouped according to the level of association between the SNP and MTX toxicity

<b><i>MTHFR</i> A1298C</b>				
Patient population	MTX dose	Population	Association with toxicity	Reference, year
106 ALL <sup>C</sup>	High	Turkish	NA	Yazicioğlu et al, <sup>32</sup> 2016
53 ALL <sup>A</sup>	Low	Japanese	NA	Suzuki et al, <sup>30</sup> 2015
161 ALL <sup>C</sup>	High	Argentinian	NA	Aráoz et al, <sup>26</sup> 2015
127 ALL <sup>C</sup>	High	Lebanese	NA	Zgheib et al, <sup>27</sup> 2014
499 ALL <sup>A</sup>	High	German	NA	Radtke et al, <sup>39</sup> 2013
18 ALL <sup>C</sup>	High	Brazilian	NA	de Deus et al, <sup>49</sup> 2012
100 ALL <sup>A</sup>	ND	Korean	NA	Kim et al, <sup>50</sup> 2012
103 ALL and NHL <sup>A</sup>	High	Japanese	-T	Fukushima et al, <sup>28</sup> 2013
21 ALL <sup>C</sup>	High	Indian	+T	Moulik et al, <sup>31</sup> 2016
35 ALL <sup>B</sup>	High	Caucasian	+T	Haase et al, <sup>25</sup> 2012

**Notes:** Type of sample: A, normal; B, tumor; C unknown. High MTX dose = 1.5–5 g m<sup>-2</sup>; Low MTX dose = 25 mg m<sup>-2</sup>.

**Abbreviations:** *MTHFR*, methylenetetrahydrofolate reductase; SNP, single-nucleotide polymorphism; MTX, methotrexate; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin Lymphoma; ND, no data; NA, no association between SNP and toxicity; +T, SNP is associated with increased toxicity; -T, SNP is associated with decreased toxicity.

the CC genotype. These studies included a low number of patients (n=21 and n=35, respectively), whereas the studies that did not result in significance were performed in cohorts of 106,<sup>32</sup> 161,<sup>26</sup> and 127<sup>27</sup> patients. Since our previous review found no association in 5 of the 6 studies that analyzed anemia and the one that showed association was performed in a population of 37 patients,<sup>33</sup> we can conclude that A1298C variant is not a good marker for anemia.

Hepatotoxicity was analyzed in 7 studies, only Fukushima et al<sup>28</sup> showed significant results in their cohort of children, which as previously mentioned, mixed 82 ALL patients and 21 NHL patients. In this study, 1298CC genotype patients presented a lower hepatic toxicity risk comparing to the 1298AA genotype patients. The other 6 studies did not find association between the polymorphism and hepatic toxicity. These results agree with our previous meta-analysis where 8 out of 10 studies neither found significant association. Moreover, the 2 studies that showed association in the previous meta-analysis found the opposite effect for the variant C allele that increased hepatic toxicity.<sup>33,34</sup>

For MTX pharmacokinetics and A1298C since November 2011, 6 new studies have been performed, but significant results have not been detected in any of them. These results in combination with the results from our previous meta-analysis, where only one study conducted in a population of 37 patients obtained significant results,<sup>33</sup> give us a total confirmation of no association between the A1298C polymorphism and MTX pharmacokinetics.

None of the other analyzed toxicities (leukopenia, neutropenia, and mucositis with 4 studies; thrombocytopenia and renal toxicity with 3 studies; and neurotoxicity with 1 study) showed statistically significant results (Table 4).

The results from 26 articles and 3,546 patients (16 studies with 2,323 patients from the previous meta-analysis and 10 studies with 1,223 patients from our actual review) makes us reject the *MTHFR* A1298C polymorphism as a MTX toxicity marker.

To sum up, in these 13 new studies analyzing C677T and A1298C and MTX-induced toxicities, we have confirmed our previous results. Most of the studies concluded that there was no association between *MTHFR* polymorphisms and MTX toxicity, and in those few studies with positive associations, opposite effects were often observed. Therefore, we consider that *MTHFR* C677T and A1298C polymorphisms are not good toxicity markers in pediatric ALL, and we think that no further studies are needed in this line.

## Outcome and *MTHFR* C677T and A1298C polymorphisms

In 2014, He et al<sup>35</sup> published the results of a meta-analysis that studied the relationship between the *MTHFR* C677T and A1298C polymorphisms and ALL relapse risk. They conducted a literature search of PubMed ([www.ncbi.nlm.nih.gov/pubmed](http://www.ncbi.nlm.nih.gov/pubmed)) and Web of Knowledge (<http://isiknowledge.com/>) using the following keywords and subject terms: “acute lymphoblastic leukemia and relapse and polymorphism (variant)” and “methylenetetrahydrofolate reductase (*MTHFR*) and acute lymphoblastic leukemia and relapse”, last updated on September 2013. The association between the two SNPs and ALL relapse was evaluated in childhood ALL patients. In He et al<sup>35</sup> work, the meta-analysis for childhood ALL was performed with 6 studies for C677T (n=1,553) and with 3 studies for A1298C (n=711). Significant association was only found for C677T. According to their results, the relapse risk in pediatric ALL was higher for the 677TT genotype than for the CT/CC genotypes.

Simultaneously, Ojha and Gurney<sup>36</sup> published a systematic review on the association between *MTHFR* C677T and overall survival in pediatric ALL (n=909). The search included literature through March 2013, and the review was based on 6 studies, 3 of them already included on He et al<sup>35</sup> meta-analysis. As expected, they reached a similar conclusion, individuals with *MTHFR* 677T variant showed a higher relative risk of pediatric ALL mortality, with greater statistical support for the genotype *MTHFR* 677TT.

Herein, we performed a new review of the literature published after the mentioned two reviews, from September 2013 to November 2016, to assess the relationship between

**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, year	MTX kinetics	Hematologic toxicity			Neutropenia			Hepatic toxicity	Mucositis	Renal toxicity	Neurotoxicity	Other
		Anemia	Thrombocytopenia	Leukopenia	Neutropenia							
Yazıcıoğlu et al. <sup>32</sup> 2016	NA <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	—
Suzuki et al. <sup>30</sup> 2015	—	—	—	NA	—	—	—	NA	—	—	—	NA <sup>b</sup>
Aráoz et al. <sup>26</sup> 2015	—	NA	NA	NA	NA	NA	NA	NA	NA	—	—	—
Zgheib et al. <sup>27</sup> 2014	NA <sup>c, d, e</sup>	NA	—	—	—	—	—	—	—	—	—	NA <sup>b</sup>
Radtke et al. <sup>39</sup> 2013	NA <sup>c, d, f</sup>	—	—	—	—	—	—	—	NA	—	—	—
de Deus et al. <sup>49</sup> 2012	NA <sup>a</sup>	—	—	—	—	—	—	NA	—	NA	—	—
Kim et al. <sup>50</sup> 2012	—	—	—	—	—	—	—	NA	—	—	—	NA <sup>b, g</sup>
Fukushima et al. <sup>28</sup> 2013	NA <sup>a</sup>	—	—	—	—	—	—	—	—	—	—	NA <sup>h</sup>
Moulik et al. <sup>31</sup> 2016	—	+T	—	—	—	—	—	—	—	—	—	NA <sup>h</sup>
Haase et al. <sup>25</sup> 2012	NA <sup>a</sup>	+T	NA	—	—	—	NA	NA	NA	NA	—	NA <sup>i</sup>

**Notes:** Identifiers: <sup>a</sup>MTX levels; <sup>b</sup>MTX dose reduction; <sup>c</sup>MTX clearance; <sup>d</sup>MTX AUC; <sup>e</sup>time to reach MTX level <0.1 mmol/L; <sup>f</sup>peak MTX plasma level; <sup>g</sup>sepsis; <sup>h</sup>MTX administration delay; <sup>i</sup>febrile neutropenia; <sup>j</sup>rate of cycles with infection. —, not evaluated.

**Abbreviations:** MTHFR, methylenetetrahydrofolate reductase; SNP, single-nucleotide polymorphism; MTX, methotrexate; NA, no association between the SNP and toxicity; +T, SNP is associated with increased toxicity; —T, SNP is associated with decreased toxicity.

childhood ALL outcome and *MTHFR* C677T and A1298C polymorphisms. Keywords “*MTHFR*” and “acute lymphoblastic leukemia” for PubMed database were used.

The study selection process, previously described for toxicity studies, was the same for outcome in the first steps. Out of the 15 articles selected for complete lecture, 7 were discarded, 2 for being published before 2013 and 5 for not studying outcome. Finally, we analyzed 8 articles that studied the *MTHFR* polymorphisms in relation to pediatric ALL patients outcome (Figure 2).

## C677T polymorphism analysis

From 2013, eight studies that included 1,353 pediatric ALL patients have analyzed the association between the C677T polymorphism and outcome (relapse, death, or secondary malignancy). None of the new eight studies showed significant association between outcome and C677T variant (Table 5). These results differ from the previously mentioned reviews that found association between increased relapse/mortality risk and 677TT genotype. Nevertheless, in the He et al's,<sup>35</sup> meta-analysis, the significant result is based only in two studies (D'Angelo et al<sup>37</sup> and Tantawy et al<sup>38</sup>) from the 6 studies included. Taking all these results in consideration, we cannot affirm that *MTHFR* C677T polymorphism is a good outcome marker for pediatric ALL.

## A1298C polymorphism analysis

In the case of A1298C, six new studies that included 1,104 pediatric ALL patients analyzed the association with outcome (relapse, death, or secondary malignancy). From them, 2 studies showed significant results but with opposite effect. Fukushima et al<sup>28</sup> demonstrated that individuals with AC-CC genotypes presented increased Event Free Survival (EFS) (versus AA genotype), whereas Radtke et al's<sup>39</sup> study found that individuals with AC or CC genotypes showed decreased EFS (versus AA genotype) (Table 6). The other 4 studies showed no association between A1298C and outcome, which is in line with the previously published meta-analysis of He et al<sup>35</sup> and other 3 studies. All these results together suggest that *MTHFR* A1298C polymorphism is not a good predictor for outcome in childhood ALL.

In conclusion, our review indicates that *MTHFR* C677T and A1298C polymorphisms cannot be considered as outcome markers for childhood ALL.

## Conclusion

Numerous studies have been performed analyzing the relationship between the C677T and A1298C polymorphisms of *MTHFR* and MTX toxicity and/or outcome in pediatric

**Table 5** List of 8 studies that analyzed association between the *MTHFR* C677T polymorphism and outcome

<b><i>MTHFR</i> C677T</b>					
Patient population	MTX dose	Population	Association with outcome	Analyzed event	Reference, year
140 ALL <sup>A</sup>	High	Argentinian	NA	EFS <sup>a</sup>	Leonardi et al, <sup>51</sup> 2016
109 ALL <sup>C</sup>	High	Mexican	NA	Relapse	Ramírez-Pacheco et al, <sup>24</sup> 2016
106 ALL <sup>C</sup>	High	Turkish	NA	EFS <sup>b</sup>	Yazicioğlu et al, <sup>32</sup> 2016
202 ALL <sup>C</sup>	High	Argentinian	NA	EFS <sup>c</sup>	Aráoz et al, <sup>26</sup> 2015
94 ALL <sup>C</sup> /141 ALL <sup>C</sup>	High	Caucasian/Vietnamese	NA	EFS <sup>a</sup>	Hoang et al, <sup>52</sup> 2015
103 ALL and NHL <sup>A</sup>	High	Japanese	NA	EFS <sup>b</sup>	Fukushima et al, <sup>28</sup> 2013
499 ALL <sup>A</sup>	High	German	NA	EFS <sup>c</sup>	Radtke et al, <sup>39</sup> 2013
53 ALL <sup>A</sup>	Low	Japanese	NA	Relapse	Suzuki et al, <sup>30</sup> 2015

**Notes:** Type of sample: A, normal; C, unknown. High MTX dose = 1.5–5 g m<sup>-2</sup>; Low MTX dose = 25 mg m<sup>-2</sup>. Identifiers: <sup>a</sup>event defined as relapse; <sup>b</sup>event defined as relapse/death; <sup>c</sup>event defined as relapse/death/secondary malignancies.

**Abbreviations:** MTHFR, methylenetetrahydrofolate reductase; SNP, single-nucleotide polymorphism; MTX, methotrexate; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin Lymphoma; NA, no association between the SNP and outcome; EFS, event free survival.

**Table 6** List of 6 studies that analyzed association between the *MTHFR* A1298C polymorphism and outcome

<b><i>MTHFR</i> A1298C</b>					
Patient population	MTX dose	Population	Association with outcome	Analyzed event	Reference, year
106 ALL <sup>C</sup>	High	Turkish	NA	EFS <sup>a</sup>	Yazicioğlu et al, <sup>32</sup> 2016
202 ALL <sup>C</sup>	High	Argentinian	NA	EFS <sup>b</sup>	Aráoz et al, <sup>26</sup> 2015
94 ALL <sup>C</sup> /141 ALL <sup>C</sup>	High	Caucasian/Vietnamese	NA	EFS <sup>c</sup>	Hoang et al, <sup>52</sup> 2015
53 ALL <sup>A</sup>	Low	Japanese	NA	Relapse	Suzuki et al, <sup>30</sup> 2015
499 ALL <sup>A</sup>	High	German	–AS	EFS <sup>c</sup>	Radtke et al, <sup>39</sup> 2013
103 ALL and NHL <sup>A</sup>	High	Japanese	+AS	EFS <sup>b</sup>	Fukushima et al, <sup>28</sup> 2013

**Notes:** Type of sample: A, normal; C, unknown. High MTX dose = 1.5–5 g m<sup>-2</sup>; Low MTX dose = 25 mg m<sup>-2</sup>. Identifiers: <sup>a</sup>event defined as relapse/death; <sup>b</sup>event defined as relapse/death/secondary malignancies; <sup>c</sup>event defined as relapse.

**Abbreviations:** MTHFR, methylenetetrahydrofolate reductase; SNP, single-nucleotide polymorphism; MTX, methotrexate; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin Lymphoma; NA, no association between the SNP and outcome; +AS, SNP is associated to increased outcome; –AS, SNP is associated with decreased outcome; EFS, event free survival.

ALL. The majority of these studies does not find association or present opposite effect. As a result, *MTHFR* C677T and A1298C polymorphisms cannot be considered as toxicity or outcome markers for childhood ALL. Therefore, the efforts should be focused on other genes. For instance, interesting and robust results have been obtained in regards to transporter genes and MTX toxicity. In fact, Treviño et al<sup>40</sup> performed a genome-wide association study in patients with ALL and found rs4149081 and rs11045879 in *SLCO1B1* strongly associated for the first time with MTX clearance, and this association was widely confirmed by subsequent studies.<sup>11,39,41</sup>

As a result, other works have focused their interest on the analysis of polymorphism in MTX transporters, finding several SNPs in genes such as *SLC19A1*, *ABCC4*, or *ABCC2* also associated with MTX levels and other toxicities.<sup>42–44</sup>

Additionally, miRNA-related SNPs interfering with miRNA levels or function may lead to drug resistance/sensitivity. Since miRNA expression can be exogenously controlled by blocking the expression of upregulated miRNAs

or by restoring the expression of downregulated miRNAs, this field seems very promising in pharmacogenetics. Indeed, our group has detected 3 SNPs in miR-5189, miR-595, and miR-453 that might affect *SLC46A1*, *SLC19A1*, *SLCO1A2*, and *ABCC4* MTX transport genes regulation and could affect MTX levels in patients with pediatric B-ALL.<sup>45,46</sup>

## Disclosure

The authors report no conflicts of interest in this work.

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