ORIGINAL ARTICLE

Associations between the C677T and A1298C polymorphisms of MTHFR and the toxicity of methotrexate in childhood malignancies: a meta-analysis

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As a common chemotherapy drug, methotrexate (MTX) has achieved remarkable clinical success. However, high inter-individual variability and unpredictable toxicity continue to challenge its use in clinical practices. Some studies suggest this variation is associated with a methylenetetrahydrofolate reductase (MTHFR) gene polymorphism, but results remain unclear. In this meta-analysis, we include 14 studies that focus on MTHFR C677T and A1298C polymorphisms in pediatric patients with malignancy. We found significant associations of the MTHFR C677T polymorphism with hepatotoxicity (grade $\geqslant 2$; CC vs CT/TT: risk ratio (RR): 0.82, 95% confidence interval (CI): 0.67–0.99; $P = 0.04$), hematological toxicity (grade 3–4; CC vs CT/TT: RR: 0.65, 95% CI: 0.44–0.97; $P = 0.03$) in a dominant genetic model and mucositis (grade $\geqslant 3$) in all models (CC vs CT/TT: RR: 0.18, 95% CI: 0.04–0.87; $P = 0.03$; CC/CT vs TT: RR: 0.10, 95% CI: 0.03–0.32; $P \leqslant 0.0001$; CC vs TT: RR: 0.10, 95% CI: 0.02–0.50; $P = 0.005$). No significant association was found with the MTHFR A1298C polymorphism. For children with malignancy, genotyping of the MTHFR C677T polymorphism is expected to be a useful tool in reducing toxicity and improving outcome in personalized MTX therapy.

INTRODUCTION

A folate antagonist, methotrexate (MTX) has been widely used for the treatment of a variety of adult and childhood cancers, including acute lymphoblastic leukemia (ALL) and lymphoma, and rheumatoid arthritis.[1,2] Although MTX has achieved remarkable clinical success, highly variable individual responses and unpredictable toxicity continue to challenge its clinical use.[3] Intravenous infusion of MTX, especially high-dose MTX followed by leucovorin rescue is associated with several toxic responses in many patients, including myelosuppression, oral mucositis, hepatotoxicity, renal toxicity, and both transient and persistent neurotoxicity. Toxicity can result in the interruption or discontinuation of chemotherapy, thereby increasing relapse risk; it also affects patient quality of life.[4] Studies on the key regulators in the MTX pathway seek to find a reliable biomarker to predict risk of MTX toxicities.

In the MTX pathway, the polymorphism of folate regulation genes is involved in the pharmacodynamics of MTX.[5] Numerous pharmacogenetic studies have reported that single-nucleotide polymorphisms alter the activity or expression of folate pathway enzymes, which may influence the response and toxicity of MTX in various malignancies and autoimmune diseases.[6–8] Among these key genes, the methylenetetrahydrofolate reductase (MTHFR) gene polymorphism has become a research focus due to its critical role in intracellular folate homeostasis and metabolism. Two well-characterized polymorphisms, MTHFR C677T (rs1801133) and MTHFR A1298C (rs1801131) have been reported in enzyme activity reduction.[9] In MTHFR C677T, transition from a C to a T nucleobase encodes proteins with enzymatic activity that is decreased significantly, by about 30–40%, leading to an increased

level of homocysteine and an altered distribution of folate.[10] In MTHFR A1298C, a milder decrease in MTHFR activity is caused by a transition from an A to a C nucleobase.[11]

As high-dose MTX is more easily associated with toxic responses and dosage for malignancy is higher than those for rheumatoid arthritis, people with malignant cancer are more easily affected by toxicities than those with rheumatoid arthritis. Besides, in the chemotherapy regimens, MTX dosage for pediatric patients are usually higher than those for adults;[12] and in the real clinical practice, the MTX response of children is often different from adults,[3] which leads us to paying more attention to pediatric patients with malignant cancers.

So far, there exists three systematic reviews/meta-analyses[13–15] (Table 1) and one umbrella review or systematic review of systematic reviews.[16] Obviously, studies included in the three systematic reviews/meta-analyses were published before 2012 and the literature databases searched were limited, besides, Campbell et al.[16] just synthesized the findings of all the three systematic reviews without including new studies, which means that the studies published nearly 5 years (2012–2016) and some studies in other databases need to be included and reanalyzed, so as to observe whether the results change.

What is more, only one meta-analysis focused on pediatric patients alone. In that study, Lopez-Lopez et al.[14] focused on ALL and found no association between MTHFR C677T or A1298C polymorphisms and individual toxicity. However, five recent cohort studies using a total 601 subjects have found significant associations: one study in non-Hodgkin's lymphoma (NHL) was conducted by D'Angelo et al.;[17] one in osteosarcoma was

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Table 1. Characteristics of systematics reviews/meta-analyses

Author (year)	Polymorphism	Patients	Search strategy
Yang et al.[13]	MTHFR C677T and A1298C	ALL (P+A)	PubMed+Embase+China National Knowledge Infrastructure (before 21 September 2011)
Lopez-Lopez et al.[14]	MTHFR C677T and A1298C	ALL (P < 18 y)	PubMed+Reference (before November 2011)
Hagleitner et al.[15]	MTHFR C677T	ALL+NHL+Osteosarco (P+A)	PubMed (before December 2010)

Abbreviations: A, adults; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin's lymphoma; P, pediatrics.
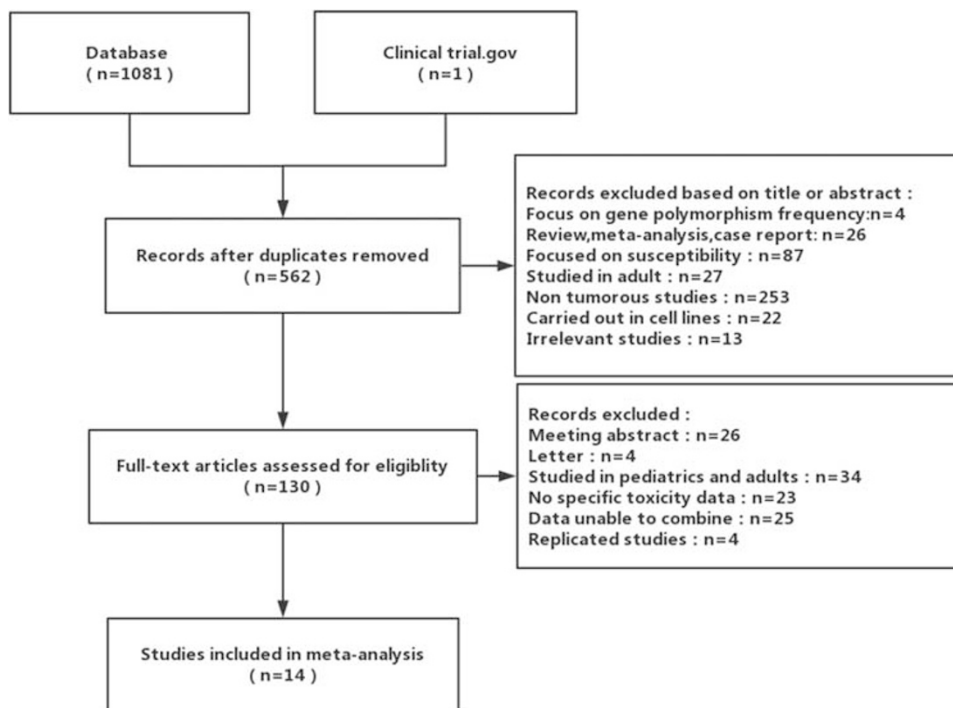

Figure 1. Flow diagram of study.

conducted by Park and Shin;[5] and three studies were in pediatric ALL (one in 2014 and two in 2015).[18–20] Further, Hagleitner et al.[15] showed that the effect of a germline genetic variant in a metabolizing gene can reasonably be expected to be independent of the type of malignancy present. In light of this information, we performed a meta-analysis in children with a childhood malignancy, including ALL, lymphoma and osteosarcoma, to assess the possible associations between two common MTHFR gene polymorphisms (C677T and A1298C) and MTX-induced toxicities.

MATERIALS AND METHODS

Search strategy

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement.[21] Relevant literature published before 1 August 2016 in the Cochrane Library (Appendix 1), PubMed (Appendix 2), EMBASE (Appendix 3) and Web of Science (Appendix 4) were thoroughly searched, independently by two reviewers, for studies exploring the association between MTHFR polymorphisms and MTX-induced toxicities in children with malignancies. Only English language papers were searched, but no restrictions on the study design were imposed. We also hand-searched ClinicalTrials.gov and references of retrieved studies and reviews. The following keywords and subject terms were used: methotrexate; methylenetetrahydrofolate reductase; MTHFR; methotrexate; MTX; pediatric; children.

Study selection

Two reviewers (CZ and YWL) independently screened the literature search results for relevant papers. Studies in this meta-analysis met the following inclusion criteria: evaluation of the association between MTHFR polymorphism and MTX-induced toxicities was done in children with a malignancy; and sufficient data were provided regarding genotype distributions to calculate the risk ratio (RR) and its corresponding 95% confidence interval (CI). When a single group of subjects was involved in multiple studies, we included only one of the studies.

Data extraction

The following data from eligible studies were extracted in duplicate by two investigators (CZ and YWL) working independently: first author's name, publication year, ethnicity or country of subjects, sample size, disease, age, gender, treatment protocol, MTX dosage, genotype, MTX-induced toxicities, Hardy–Weinberg equilibrium (HWE) values for gene polymorphisms, and toxicity criteria used to define and grade the toxicities noted during chemotherapy. For studies that did not provide graded genotyped toxicity data in their original published paper, the corresponding authors were contacted by e-mail or phone to obtain any available graded genotyped toxicity data. A third investigator (SZW) was consulted to reach a consensus decision where any discrepancy occurred in the data extracted.

Quality assessment

Two independent reviewers (CZ and SZW) applied the Newcastle–Ottawa Quality Assessment Scale to assess the selected studies, including

Table 2. Characteristics of studies included in the meta-analysis

Study	Disease	Age (years)	Sample size		Ethnicity/country	MTX dose (g m^{-2})	Toxicity criteria	HWE
			Male	Female				
Kishi et al.[26]	ALL	0–18	23	30	Caucasian/non-Caucasian	2.5/5	NCI-CTCAE V2.0	√
Imanishi et al.[27]	ALL/ML	10 months–15	26	26	Japan	3	NCI-CTCAE V2.0	—
Shimasaki et al.[28]	ALL/LBL	2–15	11	9	Japan	—	NCI-CTCAE V3.0	√
Tantawy et al.[23]	ALL	3–15	22	18	Egypt	2/5	NCI-CTCAE V2.0	—
Horinouchi et al.[29]	ALL/LBL	—	11	13	Asian/Japan	—	NCI-CTCAE V2.0	—
D'Angelo et al.[24]	ALL	3 months–15	103	47	Caucasian/European	2/5	NCI-CTCAE	—
Karathanasis et al.[25]	ALL	< 18	21	14	Cretan	—	NCI-CTCAE V4.0	√
Liu et al.[4]	ALL	1–15	115	66	China	2/5	NCI-CTCAE V1.0	√
Sepe et al.[30]	ALL	4	381	176	Caucasian/Black/Hispanic/Asian/Other	—	NCI-CTCAE V2.0	√
D'Angelo et al.[17]	NHL	6.75–10	72	23	Caucasian and European	1/3/5	WHO criteria	—
Zgheib et al.[18]	ALL	—	49	68	Caucasian/Lebanese	2.5/5	NCI-CTCAE V4.0	√
Moulik et al.[20]	ALL	7.03 ± 3.36	125	25	North India	—	NCI-CTCAE V4.03	—
Aráoz et al.[19]	ALL	1.1–17.2	107	95	Buenos Aires, Argentina	2/5	WHO criteria	√
Park et al.[5]	Osteosarcoma	7.2–15.7	21	17	Korean	12	NCI-CTCAE V3.0	—

Abbreviations: ALL, acute lymphoblastic leukemia; CTCAE, Common Terminology Criteria for Adverse Events; HWE, Hardy–Weinberg equilibrium; LBL, lymphoblastic lymphoma; ML, malignant lymphoma; MTX, methotrexate; NCI, National Cancer Institute; NHL, non-Hodgkin's lymphoma; NOS, Newcastle–Ottawa Quality Assessment Scale; —, not mentioned; √, genotype distributions were consistent with Hardy–Weinberg equilibrium.

Table 3. Methotrexate-induced toxicity of included studies

Study	Hematological toxicity	Hepatotoxicity	Mucositis	Skin toxicity	Seizures	Diarrhea	Kidney toxicity
Kishi et al.[26]	√				√		
Imanishi et al.[27]		√					
Shimasaki et al.[28]	√	√					
Tantawy,[23]	√	√	√			√	
Horinouchi et al.[29]		√					
D'Angelo et al.[24]	√						
Karathanasis et al.[25]	√	√	√				
Liu et al.[4]	√	√	√	√			
Sepe et al.[30]		√					
D'Angelo et al.[17]	√						
Zgheib et al.[18]	√						
Moulik et al.[20]	√	√	√				
Aráoz et al.[19]	√						
Park et al.[5]		√	√				√

Abbreviation: √, mentioned.

case–control studies and cohort studies. Any disagreements that arose between the reviewers were resolved through discussion. A third reviewer (YWL) was available to settle disputes.

Data synthesis and analysis

Where two or more included studies reported the same toxicities, their results were combined in the meta-analysis if no significant heterogeneity was present. Statistical analysis was conducted using Revman 5.3 (Cochrane IMS) and we estimated the overall pooled RRs and their corresponding 95% CIs using Mantel-Haenszel's method with random-effects model or fixed-effects model depending on I^2 (if $I^2 \geqslant 50\%$, a random-effects model was used; otherwise, a fixed-effects model was used). I^2 value statistical methods and Cochrane Q tests were used to assess heterogeneity across the included studies. A P-value below 0.05 for the Cochrane Q test indicates the presence of statistically significant heterogeneity and an I^2 value $> 50\%$ indicates moderate to extreme heterogeneity. For association evaluation, a Z-test was performed to determine the statistical significance of pooled RRs and a P-value < 0.05 was considered to be statistically significant.[22] Pooled RRs were used for the dominant model (C677T: CC vs CT/CT; A1298C: AA vs AC/CC), recessive model (C677T: CC/CT vs TT; A1298C: AA/AC vs CC) and homozygote

models (C677T: CC vs TT; A1298C: AA vs CC). Meta-analyses for the association between genotype, non-hematological toxicity (hepatotoxicity and mucositis) and hematological toxicity were performed. When I^2 was $> 50\%$, heterogeneity analysis was performed subsequently by sensitivity analysis and subgroup analysis if data permitted.

RESULTS

Identification and characteristics of the studies

The literature selection process is summarized in Figure 1. A total of 1082 records were identified through our initial search. After removing 520 overlapping records and screening titles and abstracts, 130 records were considered potentially eligible and full texts were retrieved and read thoroughly for further evaluation. Subsequently, 116 studies were excluded: 26 were meeting abstracts with full data unavailable; four were letters; 34 were conducted using mixed age group (both pediatrics and adults); 23 did not provide sufficient toxicity data corresponding to each genotype for MTX-induced toxicity; and 25 expressed data in the form of mean ± s.d., which could not be combined with

Table 4. Quality of studies included in the meta-analysis

Study	Newcastle–Ottawa Quality Assessment Scale (cohort study)								Score
	Selection				Comparability	Outcome			
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	
Kishi et al.[26]	☆	☆	☆	☆	☆☆	☆	—	—	7☆
Imanishi et al.[27]	☆	☆	☆	☆	☆☆	☆	—	—	7☆
Shimasaki et al.[28]	☆	☆	☆	☆	☆☆	☆	—	—	7☆
Tantawy et al.[23]	☆	☆	☆	☆	☆☆	☆	☆	☆	9☆
Horinouchi et al.[29]	☆	☆	☆	☆	☆☆	☆	—	—	7☆
D'Angelo et al.[24]	☆	☆	☆	☆	☆☆	☆	☆	☆	9☆
Karathanasis et al.[25]	☆	☆	☆	☆	☆☆	☆	—	—	7☆
Liu et al.[4]	☆	☆	☆	☆	☆☆	☆	—	—	7☆
Sepe et al.[30]	☆	☆	☆	☆	☆☆	☆	☆	—	8☆
D'Angelo et al.[17]	☆	☆	☆	☆	☆☆	☆	—	—	7☆
Zgheib et al.[18]	☆	☆	☆	☆	☆☆	☆	—	—	7☆
Moulik et al.[20]	☆	☆	☆	☆	☆☆	☆	—	—	7☆
Aráoz et al.[19]	☆	☆	☆	☆	☆☆	☆	—	—	7☆
Park et al.[5]	☆	☆	☆	☆	☆☆	☆	—	—	7☆

Q1: The exposed cohort was truly or somewhat representative? Q2: The non-exposed cohort was drawn from the same community as the exposed cohort? Q3: Exposure was ascertained by secure record or structured interview? Q4: Outcome of interest was not present at start of study? Q5: On the basis of the design or analysis cohorts had comparability? (controls for the most important factor? controls for any additional factor?) Q6: Outcome was independent blind assessment or record linkage? Q7: Follow-up was long enough for outcomes to occur? Q8: Follow-up of cohorts was adequate? A study can be awarded a maximum of one star for each numbered item within the selection and outcome categories. A maximum of two stars can be given for comparability.

dichotomous variables in this meta-analysis. Four of the remaining studies used the same data, and only the study with largest sample size was included. Thus, a total of 14 studies met the inclusion criteria and were included in our meta-analysis.

The characteristics of the included studies are summarized in Table 2. In these 14 studies, MTX dose ranged from $1\,g\,m^{-2}$ to $12\,g\,m^{-2}$. Eight studies examined both MTHFR C677T and A1298C polymorphisms[4,5,17,18,20,23–25] and six studies included only MTHFR C677T.[19,26–30] With regard to ethnicity, the 14 studies were divided into four ethnic groups: Caucasian,[17–20,24,25] Asian,[4,5,27–29] African[23] and mixed population,[26,30] with sample sizes of six, five, one and two studies, respectively. For disease type, there were nine studies of ALL,[4,18–20,23–26,30] one of non-Hodgkin's lymphoma,[17] one of osteosarcoma[5] and three of both ALL and lymphoma.[27–29] MTX-induced toxicity of the included studies is summarized in Table 3; hematological toxicity, hepatotoxicity and mucositis were the dominant MTX-induced toxicities. Among the 14 studies, 50% mentioned that genotype distributions of samples were consistent with HWE.

Quality of studies

All the 14 included studies were cohort studies; the highest quality score was nine stars[23,24] and the lowest was seven stars[4,5,17–20,25–29] (Table 4). Most of the cohort studies did well in sample selection and comparability, but failed in outcome due to the short or inadequate follow-up.

Association between MTHFR C677T polymorphism and MTX-induced toxicities

All fourteen studies measured the association between the MTHFR C677T polymorphism and MTX-induced toxicities. Detailed data of

dominant, recessive and homozygote models regarding different toxicities are summarized in Table 5.

Seven studies (918 subjects in total)[4,5,20,23,27,29,30] provided data on hepatotoxicity (grade $\geqslant 2$); the combined analysis found a significant association between the MTHFR C677T polymorphism and hepatotoxicity (grade $\geqslant 2$) only in the dominant genetic model (CC vs CT/TT: RR: 0.82, 95% CI: 0.67–0.99; $P = 0.04$; Figure 2). A stratified analysis based on ethnicity was conducted to check whether ethnicity influences the association between C677T and risk of grade $\geqslant 2$ hepatotoxicity. The results revealed a statistically significant association between C677T and increased risk in the Asian in recessive model (CC/CT vs TT: RR: 0.72, 95% CI: 0.52–0.99; $P = 0.04$) with no significant heterogeneity (Figure 3). While only one African study was included, the results suggest a statistically significant association in the recessive model of this ethnic group (RR: 0.05, 95% CI: 0.01–0.33; $P = 0.002$) and homozygote model (RR: 0.09, 95% CI: 0.01–0.58; $P = 0.01$). A tendency toward increased risk of hepatotoxicity was also present for ALL disease in the dominant model (CC vs CT/TT: RR: 0.81, 95% CI: 0.65–1.00; $P = 0.05$; Figure 4). For hepatotoxicity, grades 1–4,[25,28] no pattern could be detected in the limited available data from the included studies and further stratified analysis based on ethnicity was not possible.

Seven studies (855 subjects in total)[4,17–20,23,24] were included, which mentioned risk of hematologic toxicity (grade 3–4), showing significant effect in the dominant model (CC vs CT/TT: RR: 0.65, 95% CI: 0.44–0.97; $P = 0.03$; Figure 5). In the other two models, meta-analyses failed because of significant heterogeneity (CC+CT vs TT: $I^2 = 83\%$; CC vs TT: $I^2 = 78\%$). However, two studies, Africans with ALL[23] and Caucasians with ALL[24] showed a statistically significant association between C677T and hematologic toxicity (grade 3–4; Table 5). For grade 1–4 hematologic toxicity, no significant association was detected.

A significant association between C677T and mucositis (grade $\geqslant 3$) was present in all genetic models (CC vs CT/TT: RR: 0.18, 95% CI: 0.04–0.87; $P = 0.03$; CC/CT vs TT: RR: 0.10, 95% CI: 0.03–0.32; $P \leqslant 0.0001$; CC vs TT: RR: 0.10, 95% CI: 0.02–0.50; $P = 0.005$; Figure 6). However, no significant effect was observed in grade 1–4 mucositis toxicity in all genetic models.

Association between MTHFR A1298C polymorphism and MTX-induced toxicities

Seven studies included the MTHFR A1298C polymorphism, two in Asians,[4,5] one in Africans[23] and four in Caucasians.[17,18,20,25] In these studies, five were in ALL,[4,18,20,23,25] one was in NHL[17] and one was in osteosarcoma.[5] The evaluation of associations between the MTHFR A1298C polymorphism and various MTX-induced toxicities in children with malignancy are summarized in Table 5.

Four studies with 381 subjects included analysis of hepatotoxicity (grade $\geqslant 2$)[4,5,20,23] as shown in Table 5. There was no significant association found in any of the three genetic models. Similarly, no significant association was found between the MTHFR A1298C polymorphism and MTX-induced hematological toxicities[4,17,18,23,25] or musositis[4,5,20,23,25] in any of the genetic models. No further stratified analysis based on ethnicity was performed because few studies were available in each comparison. Mild-to-moderate heterogeneity was observed, mainly in the analysis of grade 1–4 mucositis[4,20,25] and hematological toxicities[4,17,18,23,25] under different genetic models. However, Shu-Guang Liu found a significant association between A1298C and grade 3–4 hematologic toxicity in Asians in all except the dominant genetic model[4] (Table 5).

Heterogeneity analysis

Mild-to-moderate between-study heterogeneity was identified during some of the meta-analyses performed for the relationship between MTHFR polymorphisms and MTX toxicity. Therefore, a random-effects model was used and the source of heterogeneity

Table 5. Pooled risk ratios of the association between *MTHFR* C677T and A1298C polymorphisms and methotrexate-induced toxicities in pediatric patients with childhood malignancies

Genetic comparison models	Toxicity	Studies	Subjects	Test of association		Test of heterogeneity		Model
				RR (95% CI)	P-value	I² (%)	P-value	
MTHFR C677T								
CC vs CT+TT	Hepatotoxicity (⩾ G2)	7	918	0.82 (0.67, 0.99)*	0.04	0	0.51	Fixed-effect model
	Hepatotoxicity (G1–4)	2	55	0.95 (0.37, 2.40)	0.91	0	0.34	Fixed-effect model
	Mucositis (G1–4)	3	345	0.88 (0.61, 1.26)	0.49	0	0.82	Fixed-effect model
	Mucositis (G⩾3)	2	75	0.18 (0.04, 0.87)*	0.03	0	0.69	Fixed-effect model
	Hematologic toxicity (G1–4)	5	385	0.99 (0.85, 1.15)	0.91	0	0.83	Fixed-effect model
	Hematologic toxicity (G3–4)	7	855	0.65 (0.44, 0.97)*	0.03	78	0.0001	Random-effect model
	Exclude one study not showing dose	6	705	*0.80 (0.59, 1.08)*	*0.14*	*58*	*0.03*	*Random-effect model*
CC+CT vs TT	Hepatotoxicity (⩾ G2)	6	768	0.70 (0.41, 1.20)	0.19	70	0.006	Random-effect model
	Subgroup							
	Asian	*4*	*245*	*0.72 (0.52, 0.99)**	*0.04*	*0*	*0.73*	—
	African	*1*	*40*	*0.05 (0.01, 0.33)**	*0.002*	—	—	—
	Mixed population	*1*	*483*	*1.34 (0.92, 1.96)*	*0.13*	—	—	—
	Hepatotoxicity (G1–4)	2	55	0.67 (0.21, 2.09)	0.49	0	0.49	Fixed-effect model
	Mucositis (G1–4)	2	195	0.75 (0.43, 1.31)	0.31	0	0.48	Fixed-effect model
	Mucositis (G⩾3)	2	75	0.10 (0.03, 0.32)*	< 0.0001	0	0.98	Fixed-effect model
	Hematologic toxicity (G1–4)	4	268	1.00 (0.82, 1.23)	0.97	0	0.74	Fixed-effect model
	Hematologic toxicity (G3–4)	3	351	0.88 (0.34, 2.28)	0.80	83	0.003	Random-effect model
	Tantawy 2010	*African, ALL*	*40*	*0.30 (0.13, 0.71)**	*0.006*	—	—	—
	Liu 2011	*Asian, ALL*	*160*	*0.90 (0.70, 1.16)*	*0.42*	—	—	—
	D'Angelo 2011	*Caucasian, ALL*	*151*	*2.69 (1.04, 6.96)**	*0.04*	—	—	—
CC VS TT	Hepatotoxicity (⩾ G2)	6	453	0.74 (0.44, 1.24)	0.26	51	0.07	Random-effect model
	Subgroup							
	Asian	*4*	*128*	*0.72 (0.44, 1.18)*	*0.20*	*0*	*0.69*	*Fixed-effect model*
	African	*1*	*29*	*0.09 (0.01, 0.58)**	*0.01*	—	—	—
	Mixed population	*1*	*296*	*1.19 (0.80, 1.78)*	*0.39*	—	—	—
	Mucositis (G1–4)	2	102	0.73 (0.38, 1.40)	0.34	0	0.41	Fixed-effect model
	Mucositis (G⩾3)	2	46	0.10 (0.02, 0.50)*	0.005	0	0.86	Fixed-effect model
	Hematologic toxicity (G1–4)	4	105	1.25 (0.64, 2.46)	0.51	0	0.41	Fixed-effect model
	Hematologic toxicity (G3–4)	3	189	0.92 (0.32, 2.66)	0.87	78	0.01	Random-effect model
	Tantawy 2010	*African, ALL*	*29*	*0.22 (0.06, 0.84)**	*0.03*	—	—	—
	Liu 2011	*Asian, ALL*	*80*	*0.94 (0.68, 1.31)*	*0.73*	—	—	—
	D'Angelo 2011	*Caucasian, ALL*	*80*	*2.83 (1.05, 7.65)**	*0.04*	—	—	—
MTHFR A1298C								
AA vs AC+CC	Hepatotoxicity (⩾ G2)	4	381	1.26 (0.88, 1.79)	0.20	0	1	Fixed-effect model
	Mucositis (G1–4)	3	339	0.86 (0.48, 1.55)	0.62	52	0.12	Random-effect model
	Exclude one study with no HWE	*2*	*189*	*1.19 (0.73, 1.94)*	*0.48*	*11*	*0.29*	*Fixed-effect model*
	Mucositis (G⩾3)	2	77	1.18 (0.54, 2.54)	0.68	0	0.47	Fixed-effect model
	Hematologic toxicity (G1–4)	3	306	0.92 (0.80, 1.06)	0.25	0	0.74	Fixed-effect model
	Hematologic toxicity (G3–4)	3	289	1.05 (0.87, 1.27)	0.62	0	0.55	Fixed-effect model
AA+AC vs CC	Hepatotoxicity (⩾ G2)	4	381	1.58 (0.76, 3.26)	0.22	0	0.55	Fixed-effect model
	Mucositis (G1–4)	3	339	1.73 (0.05, 60.56)	0.76	93	< 0.00001	Random-effect model
	Exclude one study with no HWE	*2*	*189*	*0.75 (0.51, 1.09)*	*0.23*	*0*	*0.93*	*Fixed-effect model*
	Mucositis (G⩾3)	2	77	1.19 (0.46, 3.09)	0.73	0	0.47	Fixed-effect model
	Hematologic toxicity (G1–4)	2	189	0.95 (0.44, 2.04)	0.89	59	0.12	Random-effect model
	Liu 2011	*Asian, ALL*	*154*	*0.78 (0.57, 1.06)*	*0.11*	—	—	—
	Karathanasis 2011	*Caucasian, ALL*	*35*	*1.42 (0.52, 3.88)*	*0.49*	—	—	—
	Hematologic toxicity (G3–4)	2	194	1.09 (0.18, 6.68)	0.93	86	0.007	Random-effect model
	Liu 2011	*Asian, ALL*	*154*	*0.67 (0.49, 0.93)**	*0.02*	—	—	—
	Tantawy 2010	*African, ALL*	*40*	*2.00 (0.54, 7.45)*	*0.30*	—	—	—
AA vs CC	Hepatotoxicity (⩾ G2)	4	240	1.66 (0.78, 3.53)	0.19	0	0.59	Fixed-effect model

Table 5. (Continued)

Genetic comparison models	Toxicity	Studies	Subjects	Test of association		Test of heterogeneity		Model
				RR (95% CI)	P-value	I² (%)	P-value	
	Mucositis (G1–4)	3	210	1.42 (0.20, 9.99)	0.72	75	0.02	Random-effect model
	Exclude one study with small sample	2	192	3.16 (0.67, 15.00)	0.15	0	0.34	Fixed-effect model
	Mucositis (G≥3)	2	48	1.13 (0.40, 3.18)	0.82	0	0.64	Fixed-effect model
	Hematologic toxicity (G1–4)	2	132	0.93 (0.43, 2.02)	0.85	57	0.13	Random-effect model
	Liu 2011	Asian, ALL	114	0.76 (0.55, 1.04)	0.09	—	—	—
	Karathanasis 2011	Caucasian, ALL	18	1.43 (0.51, 4.02)	0.50	—	—	—
	Hematologic toxicity (G3–4)	3	218	0.85 (0.42, 1.70)	0.64	55	0.11	Random-effect model
	Tantawy 2010	African, ALL	26	2.19 (0.56, 8.51)	0.26	—	—	—
	Liu 2011	Asian, ALL	114	0.68 (0.49, 0.94)*	0.02	—	—	—
	Moulik 2015	Caucasian, ALL	78	0.71 (0.28, 1.77)	0.46	—	—	—

Abbreviations: ALL, acute lymphoblastic leukemia; CI, confidence interval; HWE, Hardy–Weinberg equilibrium; RR, risk ratio. Statistically significant values (*$P < 0.05$). Entries in italics represent heterogeneity analysis.
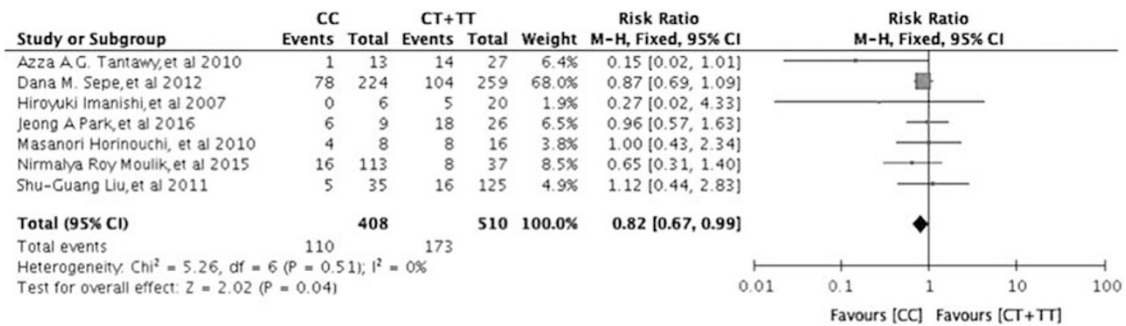

Figure 2. Meta-analysis of association between MTHFR C677T polymorphism and risk of hepatotoxicity (grade ≥2) in pediatric patients with malignancies in dominant model. CI, confidence interval.
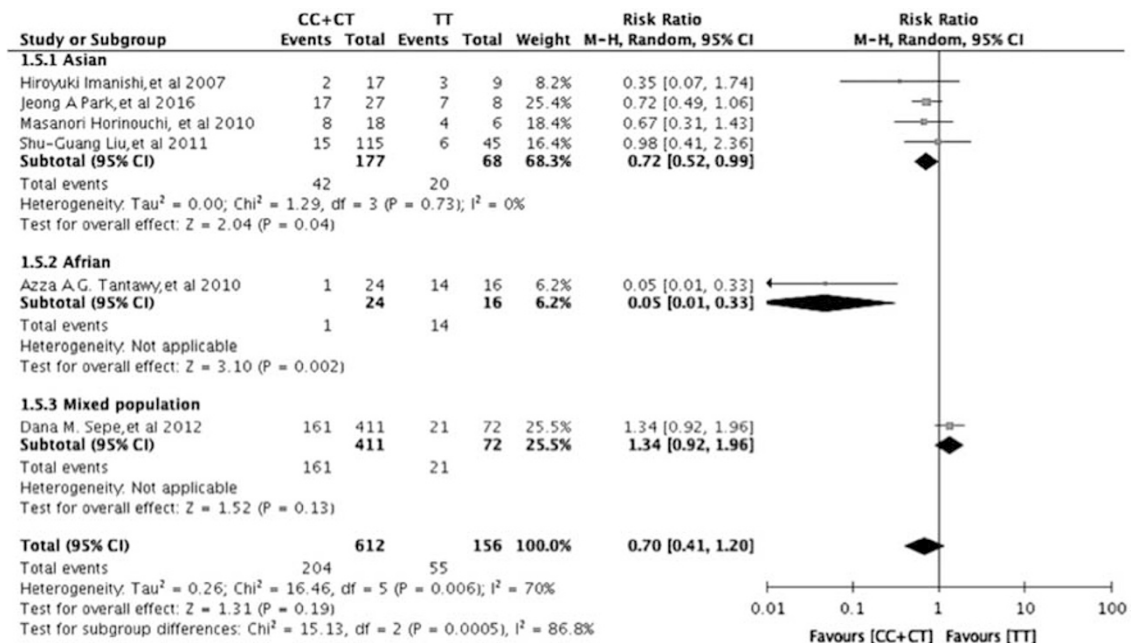

Figure 3. Subgroup analysis of association between MTHFR C677T polymorphism and risk of hepatotoxicity (grade ≥ 2) in pediatric patients with malignancies based on ethnicity in recessive model. CI, confidence interval.
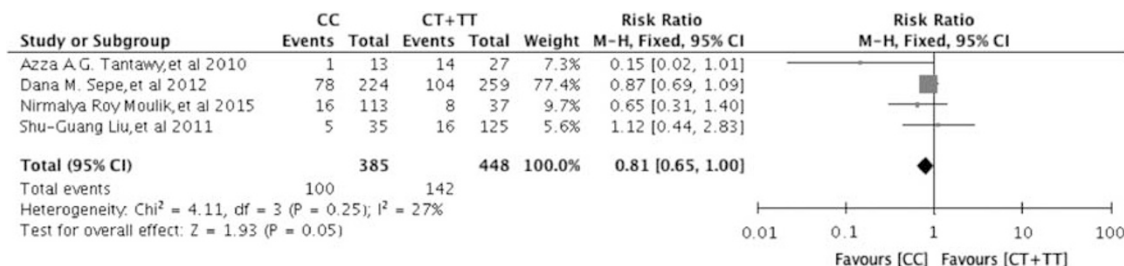
Figure 4. Meta-analysis of association between MTHFR C677T polymorphism and risk of hepatotoxicity (grade ⩾ 2) in pediatric patients with ALL in dominant model. CI, confidence interval.
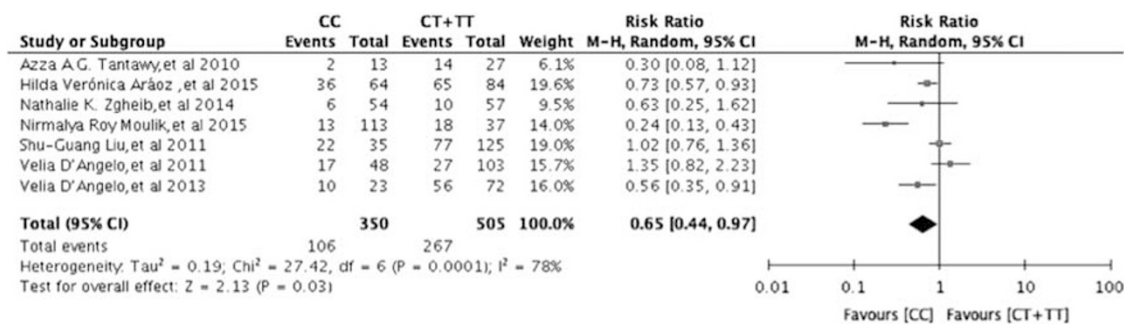

Figure 5. Meta-analysis of association between MTHFR C677T polymorphism and risk of hematologic toxicity (grade 3–4) in pediatric patients with malignancies in dominant model. CI, confidence interval.
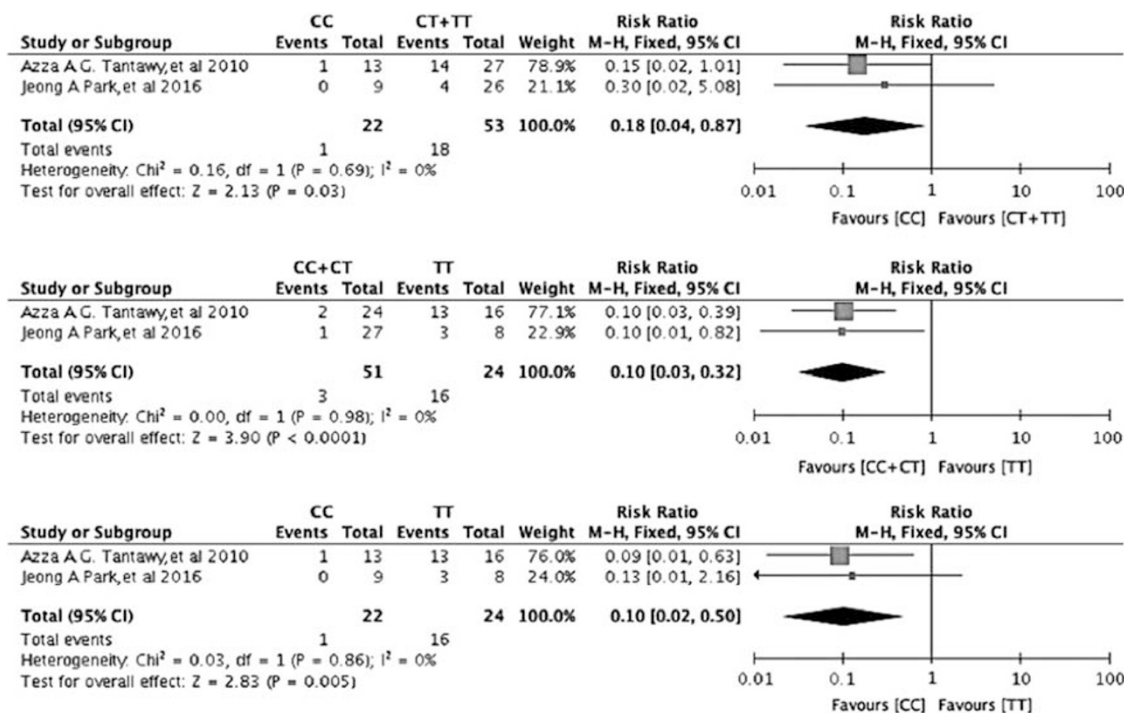

Figure 6. Meta-analysis of association between MTHFR C677T polymorphism and risk of mucositis (grade ⩾ 3) in pediatric patients with malignancies in all three genetic models. CI, confidence interval.

assessed in subgroup analysis according to ethnicity (Table 5). Heterogeneity may also be due to diseases, MTX dose, sample size, HWE and/or the criteria used for toxicity assessment, so that sensitivity analysis was also conducted. One study with relatively small sample[25] or no HWE[20] or no dose[20] was omitted and analyses re-done to test the stability of the final pooled results. Finally, in A1298C, I^2 of all models in grade 1–4 mucositis were dramatically decreased to less than 50%; while in C677T, I^2 of CC

vs CT/TT in grade 3–4 hematologic toxicity was smoothly altered, but still more than 50% (Table 5), which indicated that all the factors above could be the recourses of heterogeneity.

DISCUSSION

As an antifolate chemotherapeutic agent, MTX has been widely used in the treatment of childhood malignancies, such as ALL,

lymphoma and osteosarcoma. Although many studies have analyzed MTHFR polymorphisms and their association with MTX-induced toxicities, definitive conclusions cannot be drawn. Considering the different study designs and low power of individual studies on pediatric patients, this meta-analysis was conducted in an attempt to provide a more comprehensive and reliable conclusion on the possible association, with less random errors and more precise estimates.

To the best of our knowledge, this meta-analysis is the first to evaluate the association between MTHFR C677T and A1298C polymorphisms and risk of MTX-induced toxicity in children with malignancy across a range of ethnic backgrounds. After pooling available data from included studies, the meta-analysis demonstrates that MTHFR C677T polymorphism is significantly associated with increased risk of mucositis (grade ⩾ 3). Moreover, the significant associations between MTHFR C677T polymorphism and increased risk of hepatotoxicity (grade ⩾ 2) and grade 3–4 hematological toxicity were found in dominant model. A subgroup analysis by ethnicity was conducted in recessive and homozygote models, which revealed a significant association between C677T and hepatotoxicity (grade ⩾ 2) in African, Asian. The protective effect of the heterozygous CC/CT genotype was more evident in Africans. However, the study number was limited and sample size was small in our meta-analysis; therefore, further studies are needed to verify the association in different ethnicities.

In the chemotherapy regimens for pediatric patients with childhood malignancy, MTX dosages adopted are typically high (higher than 1 g m^{-2}), which is different from adult patients where a low-dose MTX regimen is also common.[12] In our meta-analysis, toxicity data related to dosage were limited, so a subgroup analysis by MTX dosage to clarify whether MTX dosage influences the associations was not possible. Although Zhao, et al.[3] conducted a meta-analysis in adult patients with hematological malignancies, including six studies with the final sample size of 409, and a subgroup analysis by MTX dosage was conducted to clarify whether MTX dosage may influence the association findings. The result revealed that MTHFR 677T allele increased risk of MTX-induced all-grade (G1–4) and severe (G3–4) hepatic and gastrointestinal toxicity in Caucasian independent of MTX dosage. However, the available data were also insufficient, which means that more new studies are necessary to validate this finding and reach a more robust conclusion. In real clinical practice, the treatment response of children to MTX is often much better than in adults.[3] Some studies show that pediatric ALL patients typically have a much higher completion rate than adult ALL patients, and the 5-year event-free survival rate reaches nearly 80% for children but is only 40% for adults.[1,2] Therefore, the association between MTHFR variants and MTX-induced toxicity may be less obvious in children, which is also suggested by our meta-analysis results.

Our meta-analysis found no significant association between MTHFR A1298C polymorphism and hepatotoxicity, hematological toxicity or mucositis, which is in agreement with results reported in previous studies.[3,31–34] However, a few studies have revealed that the MTHFR A1298C mutation can reduce enzyme activity and leave more substrate for thymidylate synthesis, subsequently resulting in more DNA synthesis and less toxiciy.[4] Furthermore, some studies have shown that the A1298C polymorphism has a protective role.[13,22,35] Overall, the MTHFR A1298C polymorphism does not seem to be a good predictor of MTX toxicity, although it may be a protective factor in children with childhood malignancy.

Besides the MTX-induced toxicity, MTHFR C677T and A1298C polymorphism also affect other aspects in MTX treatment, such as MTX dosage, event-free survival, relapse and mortality. Thus, it can give us information to optimize the protocol and predict the outcome. Salazar et al.[36] found that children with a favorable MTHFR genotype treated with MTX doses of 5 g m^{-2} develop a similar or, in some cases, less toxicity than those children with an unfavorable genotype that were treated with the classical MTX dose of 3 g m^{-2}. Krajinovic et al.[37] showed that patients with the MTHFR T677A1298 haplotype had a poorer event-free survival. After controlling for known risk factors, Sepe et al.[30] observed that polymorphisms in MTHFR (HR = 1.61, P = 0.034) significantly increased relapse risk. More recently, Moulik et al.[20] indicated that folate deficiency in 26% at baseline was more common in children with MTHFR 677 mutations, and sepsis-related deaths were more frequent in folate-deficient children (P = 0.02) during induction.

Heterogeneity in results was observed during our meta-analysis, some of which persisted even when subgroup analysis by ethnicity and disease type was performed. But sensitivity analysis led to a dramatic decrease in I^2 (Table 5). This indicates that sample size and HWE are also the reasons for heterogeneity. In addition, to provide a relatively conservative estimate of the evaluated association, a random-effects model was used to accommodate diversity between studies. It is also possible that differences in MTX dose, length of follow-up, definition of toxicity grade and folate supplementation may be responsible for the observed heterogeneity; the true cause remains unclear.

Some limitations of our meta-analysis warrant consideration. First, the number of studies and sample sizes were relatively small, leading to potentially insufficient power to detect mild effects. Second, although our meta-analysis was conducted in across ethnicities, the African population was under-represented, with only one study that had a sample size of 40. Therefore, it is possible that our subgroup analysis by ethnicity may be unable to provide robust results. Third, the included studies used different chemotherapy regimens of MTX. Various MTX doses, duration of therapy and combined chemotherapy agents in different chemotherapy protocols may markedly influence MTX toxicity. Fourth, different studies had different toxicity grading scales, which might influence the accuracy of toxicity combination. Therefore, further studies with a larger sample size, multiple centers and more detailed information should be performed to better assess the effect of MTHFR polymorphism on the risk of MTX-induced toxicity in children with childhood malignancy.

CONCLUSION

In conclusion, our meta-analysis of published studies demonstrates that the MTHFR C677T polymorphism is associated with MTX-induced toxicity in children with malignancy, especially in mucositis (⩾ grade 3). The MTHFR A1298C polymorphism was not found to be useful in predicting the risk of MTX-induced toxicity. Therefore, for children with childhood malignancy, genotyping of the MTHFR C677T polymorphism is expected to be a useful tool in reducing toxicity and improving outcome in personalized MTX therapy. However, larger and better-designed studies and further evaluations are needed to verify our findings.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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APPENDIX 1

Appendix 1 Search strategy for Cochrane Library

The following text words were used:

methotrexate OR MTX OR mexate OR amethopterin
The search was performed in title, abstract or keywords

APPENDIX 2

Appendix 2 Search strategy for PubMed

1. For methotrexate the following MeSH headings and text words were used:

methotrexate OR MTX OR mexate OR amethopterin

2. For children, the following MeSH headings and text words were used:

infant OR infan* OR newborn OR newborn* OR new-born* OR baby OR baby* OR babies OR neonat* OR child OR child* OR schoolchild* OR schoolchild OR school child OR school child* OR kid OR kids OR toddler* OR adolescent OR adoles* OR teen* OR boy* OR girl* OR minors OR minors* OR underag* OR under ag* OR juvenil* OR youth* OR kindergar* OR puberty OR puber* OR pubescen* OR prepubescen* OR prepuberty* OR pediatrics OR pediatric* OR paediatric* OR peadiatric* OR schools OR nursery school* OR preschool* OR pre school* OR primary school* OR secondary school* OR elementary school* OR elementary school OR high school* OR highschool* OR school

age OR schoolage OR school age* OR schoolage* OR infancy OR schools, nursery OR infant, newborn

3. For MTHFR, the following MeSH headings and text words were used:

MTHFR OR methylenetetrahydrofolate reductase OR 5,10-methylenetetrahydrofolate reductase OR methylenetetrahydrofolate, reductase OR FADH OR FADH2 OR EC 1.1.1.171. OR NADPH OR NAD* OR NAD*H methylene-THF reductase OR NADPH2 OR methylene tetrahydrofolate reductase OR tetrahydrofolate reductase, methylene

Final search: 1 AND 2 AND 3[* = zero or more characters]

APPENDIX 3

Appendix 3 Search strategy for Embase

1. For methotrexate, the following Emtree terms and text words were used:

   a. Methotrexate/ or methotrexate.mp.
   b. mexate or amethopterin.mp.
   c. MTX.mp
   d. or/1-3

2. For children, the following Emtree terms and text words were used:?

   a. infant/ or infancy/ or newborn/ or baby/ or child/ or preschool child/ or school child/
   b. adolescent/ or juvenile/ or boy/ or girl/ or puberty/ or prepuberty/ or pediatrics/
   c. primary school/ or high school/ or kindergarten/ or nursery school/ or school/
   d. or/1-3
   e. (infant$ or (newborn$ or new born$) or (baby or baby$ or babies) or neonate$).mp.
   f. (child$ or (school child$ or schoolchild$) or (school age$ or schoolage$) or (pre school$ or preschool$)).mp.

   g. (kid or kids or toddler$ or adoles$ or teen$ or boy$ or girl$).mp.
   h. (minors$ or (under ag$ or underage$) or juvenil$ or youth$).mp.
   i. (puber$ or pubescen$ or prepubescen$ or prepubert$).mp.
   j. (pediatric$ or paediatric$ or peadiatric$).mp.
   k. (school or schools or (high school$ or highschool$) or primary school$ or nursery school$ or elementary school or secondary school$ or kindergar$).mp.
   l. or/5-11
   m. 4 or 12

3. For MTHFR, the following Emtree terms and text words were used:

   a. methylenetetrahydrofolate reductase.mp. or Methylenetetrahydrofolate Reductase/
   b. (MTHFR or NADPH2).mp.
   c. (methylene-THF reductase or methylene tetrahydrofolate reductase).mp.
   d. EC 1.1.1.171.mp.
   e. (NADPH or NAD$ or FADH2 or FADH$).mp.
   f. or/1-5

Final search: 1 and 2 and 3 (Methotrexate AND Children AND MTHFR) [mp = title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer name; / = Emtree term; $ = zero or more characters]

APPENDIX 4

Search strategy for Web of Science

The following text words were used:

(Methotrexate OR MTX) AND (child* OR pediatric* OR p*diatric*) AND (MTHFR OR methylenetetrahydrofolate reductase)

The search was performed in subject headings

[* = zero or more characters]