

Involvement of the *ABCB1* C3435T Variant but Not the *MTHFR* C677T or *MTHFR* A1298C Variant in High-Dose Methotrexate-Induced Toxicity in Pediatric Acute Lymphoblastic Leukemia Patients in China

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Purpose: It remains unclear whether the *MTHFR* C677T, *MTHFR* A1298C and *ABCB1* C3435T genetic variants are associated with methotrexate (MTX) elimination delay and high-dose MTX (HD-MTX) toxicities in the treatment of pediatric acute lymphoblastic leukemia (ALL). The aim of our study was to analyze the potential predictive role of *MTHFR* C677T, *MTHFR* A1298C and *ABCB1* C3435T in toxicities and the relationship between these variants and MTX elimination delay during HD-MTX therapy in pediatric ALL patients.

Patients and Methods: We conducted a retrospective study on ALL patients receiving HD-MTX treatment with available *MTHFR* C677T, *MTHFR* A1298C and *ABCB1* C3435T genotype and 44-h plasma MTX levels. Logistic regression analyses and chi-square tests were used to assess the relationship between the variants and HD-MTX toxicities and MTX elimination delay.

Results: Genotype frequencies were in Hardy-Weinberg equilibrium. MTX elimination delay did not significantly differ between *MTHFR* C677T and *MTHFR* A1298C or *ABCB1* C3435T. Leukopenia ($P=0.028$), neutropenia ($P=0.034$) and oral mucositis ($P=0.023$) were 6.444-fold, 4.978-fold and 9.643-fold increased, respectively, in *ABCB1* C3435T homozygous genotype (TT) patients compared to wild-type (CC) patients. No significant association was found between the toxicities investigated and *MTHFR* C677T or *MTHFR* A1298C.

Conclusion: This study showed that the *ABCB1* C3435T homozygous allele genotype (TT) is associated with increased MTX-related toxicities (leukopenia, neutropenia and oral mucositis). These results may help to distinguish pediatric ALL patients with a relatively high risk of MTX-related toxicities before HD-MTX infusion and optimize MTX treatment.

Keywords: high-dose methotrexate toxicities, *MTHFR* C677T and *MTHFR* A1298C genetic variants, *ABCB1* C3435T genetic variant, MTX elimination delay, acute lymphoblastic leukemia

Introduction

Acute lymphoblastic leukemia (ALL) is a common malignancy in children.¹ Methotrexate (MTX), which can induce a low level of folate, affects the intracellular folate pool and influences the activity of the enzyme methylenetetrahydrofolate reductase (*MTHFR*), is an important drug for treatment of children with ALL.^{2–5} Although high-dose MTX (HD-MTX) with enhanced antineoplastic activity has achieved remarkable clinical success in treatment of children with ALL, unpredictable toxicities continue to challenge its clinical use.⁶ Our previous study identified clinical predictors from among patient characteristics (eg, age, sex, body surface area, subtype of leukemia, complete blood counts and liver function tests) for the occurrence of severe toxicities during HD-MTX therapy in children with ALL.³ Some studies have

also shown that chemotherapeutic agent-related toxicities vary with polymorphism frequency.^{7,8} Overall, the identification of genetic predictors for MTX-induced toxicities would be valuable for selecting patients who may benefit from personalized treatment strategies.

MTHFR gene polymorphism is critical in intracellular folate homeostasis and metabolism.⁹ There are two well-characterized *MTHFR* polymorphisms: (1) a transition from a *C* to a *T* at nucleotide 677 (*C677T*, rs1801133), which results in an increased level of homocysteine, decreasing enzymatic activity and altering folate distribution,¹⁰ and (2) a transition from an *A* to *C* nucleobase at position 1298 (*A1298C*, rs1801131), which results in reduced *MTHFR* activity.¹¹ Several published clinical reports conclude that risks of HD-MTX toxicities in treatment of children with ALL are associated with *MTHFR C677T* or *MTHFR A1298C*.^{1,5,8,12–20} However, other studies have reported the opposite conclusions, namely, that there is no significant relationship between *MTHFR C677T* or *MTHFR A1298C* and HD-MTX toxicities.^{4,21–24} Therefore, the potential predictive role of *MTHFR C677T* and *MTHFR A1298C* genetic variants in HD-MTX toxicities remains unclear. Moreover, HD-MTX toxicities vary among different races and could significantly affect its clinical use.¹⁸

The membrane transport protein P-glycoprotein (P-gp), also known as multidrug resistance protein 1 (MDR1), is encoded by the ATP-binding cassette subfamily B1 (*ABCB1*) gene and facilitates entry and exit of many molecules into the cell, particularly influencing the metabolism and clearance of drugs,^{25,26} such as MTX.¹ One of the well-characterized polymorphisms that occurs in the *ABCB1* gene is the transition from a *C* to a *T* at nucleotide 3435 in exon 26 (*C3435T*).²⁶ However, most studies to date have investigated influences of *ABCB1* gene SNPs on risk of ALL development,^{27,28} whereas little is known about whether they affect HD-MTX toxicities in the treatment of children with ALL.¹

In addition, whether serum MTX levels are associated with *MTHFR C677T* and *MTHFR A1298C* as well as *ABCB1 C3435T* in HD-MTX treatment of children with ALL has yet to be clearly determined.

To answer these clinical questions, we performed a retrospective study to analyze the predictive role of the *MTHFR C677T*, *MTHFR A1298C* and *ABCB1 C3435T* genetic variants in HD-MTX toxicities (hematological toxicities, transient liver dysfunctions, vomiting and oral mucositis) during HD-MTX therapy in children with ALL. In addition, this study aimed to analyze the relationship between *MTHFR C677T*, *MTHFR A1298C* and *ABCB1 C3435T* and MTX elimination delay.

Patients and Methods

Patients

We conducted a noninterventional retrospective study between May 2015 and May 2020 in children with ALL who were treated with HD-MTX and had adequate medical records available at the Affiliated Hospital of Qingdao University, China. Children with ALL (aged ≤ 14 years) who received HD-MTX treatment and for whom *MTHFR C677T*, *MTHFR A1298C* and *ABCB1 C3435T* genotype analyses were performed and 44-h plasma MTX levels were determined were enrolled in this study. This study was conducted on the basis of the ethical principles of the Declaration of Helsinki and approved by the Institute Medical Ethics Committee of the Affiliated Hospital of Qingdao University.

HD-MTX Administration and Calcium Folate (CF) Rescue Regimen

All included patients were treated according to the 2015 Chinese children's leukemia cooperation group-ALL protocol (CCCG-ALL-2015). Consolidation chemotherapy consisted of four cycles of HD-MTX; low-risk children received 3 g/m² of MTX, and intermediate- and high-risk children received 5 g/m² of MTX. The 1/10 of the full dose was intravenously transfused within 0.5 h, and the remainder was infused during the following 23.5 h. At 42 h after the beginning of MTX administration, CF rescue was started every 6 h for 3 to 5 doses. The rescue regimen was given on the basis of 44-h plasma MTX levels, as we previously reported.³ MTX elimination delay was defined as a 44-h plasma MTX level > 1 $\mu\text{mol/L}$. As the next MTX dose was adjusted based on the previous 44-h plasma MTX level, we analyzed only the data for the first dose of MTX.

Determination of Plasma MTX Level and Genotyping

Serum levels of MTX for children with ALL monitored by the enzyme multiplied immunoassay technique (EMIT) at our hospital from May 2015 to May 2020 were acquired via the Viva-E[®] system (Siemens Healthcare, Forchheim, Germany).

The *MTHFR* (C677T and A1298C) and *ABCB1* (C3435T) genotypes for children with ALL monitored at our hospital were acquired via a fluorescence detector (TL998A, Xi'an Tianlong Technology Co., Ltd., Xi'an, China) using chromosome fluorescence in situ hybridization. Serum levels of MTX and *MTHFR* (C677T and A1298C) and *ABCB1* (C3435T) genotypes were retrospectively collected.

Data Collection and Toxicities

The clinical data collected by retrospective chart review before the first MTX infusion included the subtype of leukemia, white blood cell count (WBC), neutrophil count (NEUT), hemoglobin (Hb) level, platelet (PLT) count, aspartate transferase (AST) level, and alanine transferase (ALT) level. The 44-h plasma MTX level, WBC, NEUT, Hb, and PLT counts; and AST and ALT levels were also collected after MTX infusion. Hematological toxicity, transient liver dysfunction, vomiting and oral mucositis were assessed on the basis of the National Cancer Institute Common Toxicity Criteria version 5.0 (NCI-CTCAE version 5.0). Hematological toxicity included reductions in WBC, NEUT, Hb, and PLT counts. Hepatic toxicity included increases in AST and ALT.

Statistical Analysis

Allele frequencies of polymorphisms were tested for Hardy–Weinberg equilibrium using the chi-square test. All data were analyzed with SPSS statistical software (version 19.0; SPSS Inc., Chicago, Illinois, USA). Toxicities are represented by a toxicity event that did occur =1 or a toxicity event that did not occur = 0 during the HD-MTX course. Statistical associations between toxicities and *MTHFR* C677T, *MTHFR* A1298C and *ABCB1* C3435T were analyzed using logistic regression and are presented as odds ratios (ORs) and 95% confidence intervals (CIs). The correlation between polymorphisms and MTX elimination delay was assessed using the chi-square test. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Patient Characteristics

A total of 106 children with newly diagnosed ALL who received the first HD-MTX treatment and underwent *MTHFR* C677T, *MTHFR* A1298C and *ABCB1* C3435T genotype analyses and plasma MTX level determination at 44 h after the start of treatment were enrolled in this study. The genotype frequencies were in Hardy-Weinberg equilibrium (C677T variant: $\chi^2=1.880$, $P=0.391$; A1298C variant: $\chi^2=0.046$, $P=0.977$; C3435T variant: $\chi^2=0.293$, $P=0.864$). The remaining patient characteristics and frequencies of genetic polymorphisms are summarized in Table 1. All toxicities categorized by differential grade are listed in Table 2. Toxicities such as depressed PLT, vomiting and oral mucositis were rare, whereas toxicities such as leukopenia, anemia, neutropenia, elevated ALT and elevated AST were common. Anemia was the most common mild drug-induced toxicity ($> \text{grade } 1$) (69.8%), followed by leukopenia (50.0%) and neutropenia (42.5%) (Table 2).

Correlation Between Genotype and MTX Elimination Delay

The correlation between genotype and plasma MTX level was statistically analyzed to determine whether different genotypes are associated with 44-h plasma MTX levels. Eighty-six patients had a 44-h plasma MTX level $\leq 1 \mu\text{mol/L}$, and 20 patients had a 44-h plasma MTX level $> 1 \mu\text{mol/L}$ (MTX elimination delay). There was no significant difference in the number of patients with MTX elimination delay between the low-risk group and the intermediate- and high-risk groups; thus, they were analyzed together. The correlation between genotype and MTX elimination delay is shown in Table 3. MTX elimination delay did not significantly differ between *MTHFR* C677T and *MTHFR* A1298C or among *ABCB1* C3435T genetic variants ($P > 0.05$).

Correlation Between Genotype and High-Dose MTX-Induced Toxicity

As there was a difference in the number of patients developing anemia, depressed PLT and elevated ALT between the low-risk group and the intermediate- and high-risk group, they were analyzed separately in line with the degree of risk.

Table 1 Patient Main Characteristics and Frequencies of Genetic Polymorphisms

Characteristic	Number of Patients (%)
Gender	
Male	57 (53.8)
Female	49 (46.2)
Subtype of tumor	
B	96 (90.6)
T	10 (9.4)
Degree of risk	
Low-risk	64 (60.4)
Intermediate- and high-risk	42 (39.6)
Age (years)	
<1	1 (0.9)
1–9	93 (87.7)
≥10	12 (11.3)
MTHFR	
677C > T	
CC	18 (17.0)
CT	43 (40.6)
TT	45 (42.4)
T allele	133 (62.7)
1298A > C	
AA	84 (79.2)
AC	21 (19.8)
CC	1 (0.9)
C allele	23 (10.8)
ABCB1	
3345 C > T	
CC	47 (44.3)
CT	49 (46.2)
TT	10 (9.4)
T allele	69 (32.5)

Notes: Data are presented as numbers (%).

Table 2 Number of Courses per Grade of Adverse Events (n=106)

Adverse events	Grade n (%)				
	0	1	2	3	4
Leucopenia	30 (28.3)	23 (21.7)	24 (22.6)	27 (25.5)	2 (1.9)
Anemia	9 (8.5)	23 (21.7)	58 (54.7)	16 (15.1)	0 (0)
Neutropenia	53 (50.0)	8 (7.5)	20 (18.9)	22 (20.8)	3 (2.8)
Depressed PLT	97 (91.5)	7 (6.6)	1 (0.9)	0 (0)	1 (0.9)
Elevated ALT	38 (35.8)	43 (40.6)	15 (14.2)	9 (8.5)	1 (0.9)
Elevated AST	41 (38.7)	46 (43.4)	11 (10.4)	8 (7.5)	0 (0)
Vomiting	99 (93.4)	6 (5.7)	1 (0.9)	0 (0)	0 (0)
Oral mucositis	98 (92.5)	6 (5.7)	1 (0.9)	1 (0.9)	0 (0)

Notes: Data are presented as numbers (%).

Abbreviations: PLT, platelet count; ALT, alanine transferase; AST, aspartate transferase.

Table 3 The Correlation Between Genotyping and MTX Elimination Delay

Polymorphism	Genotype	No. of patients (%)	44-hour plasma MTX level (μmol/L)		P value
			≤1 (n = 85, %)	>1 (n = 21, %)	
MTHFR 677C > T	CC	18 (17.0)	15 (83.3)	3 (16.7)	0.897
	CT	43 (40.6)	34 (79.1)	9 (20.9)	
	TT	45 (42.4)	37 (82.2)	8 (17.8)	
MTHFR 1298A > C	AA	84 (79.2)	68 (81.0)	16 (19.0)	1.000
	AC	21 (19.8)	17 (81.0)	4 (19.0)	
	CC	1 (0.9)	1 (100.0)	0 (0.0)	
ABCB1 3345 C > T	CC	47 (44.3)	38 (80.9)	9 (19.1)	1.000
	CT	49 (46.2)	40 (81.6)	9 (18.4)	
	TT	10 (9.4)	8 (80.0)	2 (20.0)	

Notes: Data are presented as numbers (%). P<0.05.

The *C677T*, *A1298C* and *C3435T* genetic variants displayed no correlations with toxicities (anemia, depressed PLT and elevated ALT) in the low-risk group or in the intermediate- and high-risk group (Tables S1 and S2).

No significant difference in the number of patients developing other kinds of toxicities (leukopenia, neutropenia, elevated AST, vomiting and oral mucositis) in the groups was found; thus, the groups were analyzed together. The correlation between genotype and toxicities (leukopenia, neutropenia, elevated AST, vomiting and oral mucositis) is shown in Table 4, with

Table 4 Analysis of Genotyping and Methotrexate-Induced Toxicity (≥ Grade I)

Polymorphism	Genotype	Non-Toxicity n (%)	Toxicity n (%)	OR	P	95% CI
MTHFR 677C > T		Leucopenia (< grade I)	Leucopenia (≥ grade I)			
	CC	8 (44.4)	10 (55.6)	Reference		
	CT	25 (58.1)	18 (41.9)	0.576	0.330	0.190–1.747
	TT	20 (44.4)	25 (55.6)	1.000	1.000	0.333–3.004
		Neutropenia (< grade I)	Neutropenia (≥ grade I)			
	CC	10 (55.6)	8 (44.4)	Reference		
	CT	25 (58.1)	18 (41.9)	0.900	0.852	0.297–2.730
	TT	26 (57.8)	19 (42.2)	0.913	0.872	0.303–2.750
		Elevated AST (< grade I)	Elevated AST (≥ grade I)			
	CC	7 (38.9)	11 (61.1)	Reference		
	CT	18 (41.9)	25 (58.1)	0.884	0.830	0.287–2.722
	TT	16 (35.6)	29 (64.4)	1.153	0.804	0.374–3.561
		Vomiting (< grade I)	Vomiting (≥ grade I)			
	CC	18 (100)	0 (0)	Reference		
	CT	40 (93.0)	3 (7.0)	NE	0.998	NE
	TT	41 (91.1)	4 (8.9)	NE	0.998	NE
		Oral mucositis (< grade I)	Oral mucositis (≥ grade I)			
	CC	18 (100)	0 (0)	Reference		
	CT	38 (88.4)	5 (11.6)	NE	0.998	NE
	TT	42 (93.3)	3 (6.7)	NE	0.998	NE

(Continued)

Table 4 (Continued).

Polymorphism	Genotype	Non-Toxicity n (%)	Toxicity n (%)	OR	P	95% CI
<i>MTHFR</i> 1298A > C		Leucopenia (< grade I)	Leucopenia (≥ grade I)			
	AA	40 (47.6)	44 (52.4)	Reference		
	AC	12 (57.1)	9 (42.9)	0.682	0.436	0.260–1.789
	CC	1 (100)	0 (0)	NE	1.000	NE
		Neutropenia (< grade I)	Neutropenia (≥ grade I)			
	AA	46 (54.8)	38 (45.2)	Reference		
	AC	14 (66.7)	7 (33.3)	0.605	0.327	0.222–1.652
	CC	1 (100)	0 (0)	NE	1.000	NE
		Elevated AST (< grade I)	Elevated AST (≥ grade I)			
	AA	32 (38.1)	52 (61.9)	Reference		
	AC	9 (42.9)	12 (57.1)	0.821	0.689	0.311–2.164
	CC	0 (0)	1 (100)	NE	1.000	NE
		Vomiting (< grade I)	Vomiting (≥ grade I)			
	AA	77 (91.7)	7 (8.3)	Reference		
	AC	21 (100)	0 (0)	NE	0.998	NE
	CC	1 (100)	0 (0)	NE	1.000	NE
		Oral mucositis (< grade I)	Oral mucositis (≥ grade I)			
	AA	77 (91.7)	7 (8.3)	Reference		
	AC	20 (95.2)	1 (4.8)	0.550	0.586	0.064–4.732
	CC	1 (100)	0 (0)	NE	1.000	NE
<i>ABCB1</i> 3345 C > T		Leucopenia (< grade I)	Leucopenia (≥ grade I)			
	CC	29 (61.7)	18 (38.3)	Reference		
	CT	22 (44.9)	27 (55.1)	1.977	0.101	0.876–4.463
	TT	2 (20.0)	8 (80.0)	6.444	0.028*	1.229–33.803
		Neutropenia (< grade I)	Neutropenia (≥ grade I)			
	CC	32 (68.1)	15 (31.9)	Reference		
	CT	26 (53.1)	23 (46.9)	1.887	0.134	0.822–4.333
	TT	3 (30.0)	7 (70.0)	4.978	0.034*	1.127–21.978
		Elevated AST (< grade I)	Elevated AST (≥ grade I)			
	CC	19 (40.4)	28 (59.6)	Reference		
	CT	17 (34.7)	32 (65.3)	1.277	0.562	0.558–2.923
	TT	5 (50.0)	5 (50.0)	0.679	0.579	0.172–2.670
		Vomiting (< grade I)	Vomiting (≥ grade I)			
	CC	45 (95.7)	2 (4.3)	Reference		
	CT	45 (91.8)	4 (8.2)	2.000	0.437	0.349–11.474
	TT	9 (90.0)	1 (10.0)	2.500	0.473	0.204–30.605
		Oral mucositis (< grade I)	Oral mucositis (≥ grade I)			
	CC	45 (95.7)	2 (4.3)	Reference		
	CT	46 (93.9)	3 (6.1)	1.467	0.682	0.234–9.201
	TT	7 (70.0)	3 (30.0)	9.643	0.023*	1.360–68.349

Notes: OR values were computed considering the number of *MTHFR* 677CC, *MTHFR* 1298AA or *ABCB1* 3345CC cases as a reference. *P<0.05.

Abbreviations: AST, aspartate transferase; OR, odds ratio; CI, confidence interval; NE, not estimable.

leukopenia being 6.444-fold increased in patients with the *ABCB1* C3435T homozygous genotype (TT) compared to patients with the wild-type genotype (CC) (P=0.028). Furthermore, neutropenia was 4.978-fold increased in patients with the homozygous TT genotype compared to wild-type (CC) patients (P=0.034), and oral mucositis was 9.643-fold increased in the former (P=0.023). Conversely, no significant association between the toxicities investigated and the *MTHFR* C677T or *MTHFR* A1298C polymorphisms was found ($P > 0.05$).

Correlation Between the Number of Wild-Type Homozygous Genes and MTX-Induced Toxicity

To analyze the relationship between the combination of three genotypes and MTX-induced toxicity, analysis of the number of wild-type homozygous genes and MTX-induced toxicity is shown in Table 5. There were 5 patients with 0 homozygosity for wild-type genotypes, 57 patients with 1 homozygosity for wild-type genotypes, 40 patients with 2 homozygosities for wild-type genotypes and 4 patients with 3 homozygosities for wild-type genotypes. Overall, there was no significant difference between the number of wild-type genes and MTX-induced toxicity (\geq grade 1) ($P > 0.05$).

Discussion

MTX is an important drug used in treatment of children with ALL.³ However, drug-related toxicities continue to limit its clinical use.²⁹ Response to MTX largely varies among patients receiving HD-MTX treatment.^{2,30} Research on genetic predictors for MTX-induced toxicities would be helpful for selecting patients who may benefit from personalized treatment strategies. Several SNPs involved in MTX transport and metabolism have been explored as predictors of the

Table 5 Analysis Among the Number of Wild Homozygous Genes and Methotrexate-Induced Toxicity (\geq Grade 1)

	The Number of Wild Homozygous Genes				P
	0	1	2	3	
Leucopenia (\geq grade 1)					
Yes, n=76	5	39	29	3	0.551
No, n=30	0	18	11	1	
Neutropenia (\geq grade 1)					
Yes, n=53	3	30	19	1	0.729
No, n=53	2	27	21	3	
Depressed hemoglobin (\geq grade 1)					
Yes, n=97	5	55	33	4	0.114
No, n=9	0	2	7	0	
Depressed PLT (\geq grade 1)					
Yes, n=9	0	3	6	0	0.230
No, n=97	5	54	34	4	
Elevated ALT (\geq grade 1)					
Yes, n=68	4	35	27	2	0.750
No, n=38	1	22	13	2	
Elevated AST (\geq grade 1)					
Yes, n=65	3	35	25	2	1.000
No, n=41	2	22	15	2	
Vomiting (\geq grade 1)					
Yes, n=7	0	5	2	0	0.840
No, n=99	5	25	38	4	
Oral mucositis (\geq grade 1)					
Yes, n=8	1	5	2	0	0.585
No, n=98	4	52	38	4	

Notes: Data are presented as numbers. $P < 0.05$.

Abbreviations: PLT, platelet count; ALT, alanine transferase; AST, aspartate transferase.

efficacy and toxicity of MTX in treatment of children with ALL.^{31,32} However, the conclusions drawn are inconsistent.⁶ *MTHFR* gene polymorphism is critical for intracellular folate homeostasis and metabolism.⁹ P-gp, encoded by the *ABCB1* gene, may influence metabolism and clearance of MTX.¹ Our retrospective study enrolled 106 children with newly diagnosed ALL who were treated following the CCCG-ALL-2015 protocol at our hospital. The MTX dose was adjusted based on the previous 44-h plasma MTX level; therefore, we analyzed only the data for the first dose of MTX. In this study, we sought to investigate whether the *MTHFR* C677T, *MTHFR* A1298C and *ABCB1* C3435T polymorphisms correlate with MTX elimination delay or MTX-related toxicities in a Chinese cohort.

The allelic frequencies of homozygous *MTHFR* C677T and *MTHFR* A1298C were 42.4 and 0.9%, respectively, whereas that of homozygous *ABCB1* C3435T was 9.4%. The genotype frequencies for each polymorphism were in Hardy-Weinberg equilibrium. *MTHFR* A1298C was predominantly present as homozygous wild-type, but *MTHFR* C677T and *ABCB1* C3435T showed higher allelic frequencies of homozygosity and heterozygosity.

Our study showed that *ABCB1* C3435T was not associated with MTX elimination delay in HD-MTX treatment of children with ALL, which was consistent with some studies^{1,33,34} that concluded that *ABCB1* C3435T was not associated with MTX elimination delay, MTX plasma level or the MTX AUC in HD-MTX treatment of children with ALL. *MTHFR* C677T and *MTHFR* A1298C were also not associated with MTX elimination delay in HD-MTX treatment of children with ALL in our study, consistent with some studies on Chinese populations^{6,29,35} and Korean populations.¹⁸ However, other studies have indicated the opposite conclusions in HD-MTX treatment of children with ALL in Iran,³⁶ Poland³⁷ and Egypt.³⁸ This deviation might be attributed to the difference in racial groups, toxicity criteria, and sample size between the present and previous studies.

The toxicities investigated in our study were found not to be related to the *MTHFR* C677T or *MTHFR* A1298C polymorphism. Although the relationship between *MTHFR* C677T and *MTHFR* A1298C and HD-MTX toxicities has been extensively studied, definitive conclusions remain unavailable.^{2,29} Nevertheless, the majority of published studies found no association between *MTHFR* polymorphisms and risk of HD-MTX toxicities.^{39,40} This discrepancy might be attributed to the difference in racial groups, sample size, treatment regimens used and time points for evaluating HD-MTX toxicities between the present and previous studies. Another important reason may be that there are many other enzymes or transporter proteins that have a combined impact on MTX pharmacokinetic variability in addition to *MTHFR*.²⁹ Therefore, *MTHFR* C677T and *MTHFR* A1298C gene polymorphisms may not be the main factors for HD-MTX toxicities in Chinese patients, though there may be a limited relationship between them.⁶

We also assessed genotype of the most commonly studied *ABCB1* gene polymorphism C3435T, which is associated with decreased exporter activity, and hence, both leukemic blasts and somatic cells are exposed to high doses of chemotherapy. This might lead to an increase in the number and severity of adverse reactions.^{1,41} This study showed that the *ABCB1* C3435T homozygous genotype (TT) was associated with increased neutropenia, which is consistent with a previous study.¹ We also found a strong and significant association between the *ABCB1* C3435T homozygous genotype (TT) and increased rates of leukopenia and oral mucositis. Our results may be explained by the fact that the C3435T mutation in *ABCB1* causes decreased expression of the transporter, leading to higher intracellular levels and hence more toxicity. However, no association was found between *ABCB1* C3435T and HD-MTX toxicities in pediatric patients in some studies.^{34,36,42} These inconsistent results might be attributed to variable treatment protocols, different toxicity criteria, and racial differences. Our data suggest that for patients carrying *ABCB1* C3435T, we should perhaps adopt a precautionary treatment to prevent toxicities in HD-MTX treatment of TT allele pediatric patients.

To analyze the relationship between the combination of three genotypes and MTX-induced toxicity, analysis of the number of wild-type homozygous genes and MTX-induced toxicity was performed, but no relationship was found.

The strengths of our study include unified diagnostic criteria, HD-MTX administration and a CF rescue regimen from a single center. Nonetheless, our study also has certain limitations. First, because this was a retrospective study, we were only able to analyze the potential predictive role of the *MTHFR* C677T, *MTHFR* A1298C and *ABCB1* C3435T genetic variants in toxicities during HD-MTX therapy in children with ALL. We could not analyze other genetic variants that may affect MTX transport and metabolism, such as in *SLCO1B1*, *SHMT1*, and *ABCG2*. Second, the retrospective study was limited to MTX-related toxicities, and other side effects, such as renal dysfunction and neurotoxicity, were not evaluated. Third, our findings are limited to our center, as the study was based on a single center rather than multiple

centers. Therefore, further research is necessary to include a larger study population from multiple centers and investigate the impact of many other genetic variants on HD-MTX toxicities.

Conclusion

Given the cumulative evidence, whether *MTHFR C677T* and *MTHFR A1298C* can be used as predictors of HD-MTX toxicities and MTX elimination delay remains unclear. This study showed the *ABCB1 C3435T* homozygous *TT* genotype was associated with increased MTX-related toxicities (leukopenia, neutropenia and oral mucositis). The current results indicate that *ABCB1 C3435T* can predict some MTX-related toxicities. These findings may help to explain the variability of MTX-related toxicities and distinguish pediatric ALL patients with a relatively high risk of such toxicities before HD-MTX infusion and to optimize MTX treatment.

Data Sharing Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

Ethics Approval

Approval was obtained from the Institute Medical Ethics Committee of the Affiliated Hospital of Qingdao University (number QYFY WZLL 27980). This study was conducted adhere to the ethical principles of the Declaration of Helsinki. The requirement for written informed consent was waived due to the retrospective nature of the study. We declare that we will maintain the confidentiality of the patients' personal information.

Consent for Publication

All authors have agreed for the publication.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Zgheib NK, Akra-Ismael M, Aridi C, et al. Genetic polymorphisms in candidate genes predict increased toxicity with methotrexate therapy in Lebanese children with acute lymphoblastic leukemia. *Pharmacogenetics and Genomics*. 2014;24(8):387–396. doi:10.1097/FPC.0000000000000069
2. Hao Q, Song Y, Fang Q, et al. Effects of genetic polymorphisms on methotrexate levels and toxicity in Chinese patients with acute lymphoblastic leukemia. *Blood Science*. 2023;5(1):32–38. doi:10.1097/BS9.0000000000000142
3. Li X, Sui Z, Jing F, et al. Identifying risk factors for high-dose methotrexate-induced toxicities in children with acute lymphoblastic leukemia. *Cancer Manage Res*. 2019;11:6265–6274. doi:10.2147/CMAR.S207959
4. den Hoed MA, Lopez-Lopez E, te Winkel ML, et al. Genetic and metabolic determinants of methotrexate-induced mucositis in pediatric acute lymphoblastic leukemia. *Pharmacogenomics J*. 2015;15(3):248–254. doi:10.1038/tpj.2014.63
5. D'Angelo V, Ramaglia M, Iannotta A, et al. Methotrexate toxicity and efficacy during the consolidation phase in paediatric acute lymphoblastic leukaemia and MTHFR polymorphisms as pharmacogenetic determinants. *Cancer Chemother Pharmacol*. 2011;68(5):1339–1346. doi:10.1007/s00280-011-1665-1
6. Shen Y, Wang Z, Zhou F, Jin R. The influence of MTHFR genetic polymorphisms on methotrexate therapy in pediatric acute lymphoblastic leukemia. *Open Life Sci*. 2021;16(1):1203–1212. doi:10.1515/biol-2021-0121

7. Gregers J, Christensen IJ, Dalhoff K, et al. The association of reduced folate carrier 80G>A polymorphism to outcome in childhood acute lymphoblastic leukemia interacts with chromosome 21 copy number. *Blood*. 2010;115(23):4671–4677. doi:10.1182/blood-2010-01-256958
8. Sepe DM, McWilliams T, Chen J, et al. Germline genetic variation and treatment response on CCG-1891. *Pediatr Blood Cancer*. 2012;58(5):695–700. doi:10.1002/pbc.23192
9. Zhu C, Liu YW, Wang SZ, et al. Associations between the C677T and A1298C polymorphisms of MTHFR and the toxicity of methotrexate in childhood malignancies: a meta-analysis. *Pharmacogenomics J*. 2018;18(3):450–459. doi:10.1038/tpj.2017.34
10. D'Angelo V, Ramaglia M, Iannotta A, et al. Influence of methylenetetrahydrofolate reductase gene polymorphisms on the outcome of pediatric patients with non-Hodgkin lymphoma treated with high-dose methotrexate. *Leukemia Lymphoma*. 2013;54(12):2639–2644. doi:10.3109/10428194.2013.784758
11. Bottiger AK, Hurtig-Wennlof A, Sjostrom M, Yngve A, Nilsson TK. Association of total plasma homocysteine with methylenetetrahydrofolate reductase genotypes 677C>T, 1298A>C, and 1793G>A and the corresponding haplotypes in Swedish children and adolescents. *Int J Mol Med*. 2007;19(4):659–665.
12. Liu SG, Li ZG, Cui L, et al. Effects of methylenetetrahydrofolate reductase gene polymorphisms on toxicities during consolidation therapy in pediatric acute lymphoblastic leukemia in a Chinese population. *Leukemia Lymphoma*. 2011;52(6):1030–1040. doi:10.3109/10428194.2011.563883
13. Tantawy AA, El-Bostany EA, Adly AA, Abou El Asrar M, El-Ghouroury EA, Abdulhaffar EE. Methylene tetrahydrofolate reductase gene polymorphism in Egyptian children with acute lymphoblastic leukemia. *Blood Coagul Fibrinolysis*. 2010;21(1):28–34. doi:10.1097/MBC.0b013e32833135e9
14. Karathanasis NV, Stiakaki E, Goulielmos GN, Kalmanti M. The role of the methylenetetrahydrofolate reductase 677 and 1298 polymorphisms in Cretan children with acute lymphoblastic leukemia. *Genet Test Mol Bioma*. 2011;15(1–2):5–10. doi:10.1089/gtmb.2010.0083
15. Frikha R, Rebai T, Lobna BM, et al. Comprehensive analysis of methylenetetrahydrofolate reductase C677T in younger acute lymphoblastic leukemia patients: a single-center experience. *J Oncol Pharm Pract*. 2019;25(5):1182–1186. doi:10.1177/1078155218818244
16. Ramirez-Pacheco A, Moreno-Guerrero S, Alamillo I, Medina-Sanson A, Lopez B, Moreno-Galvan M. Mexican childhood acute lymphoblastic leukemia: a pilot study of the MDR1 and MTHFR gene polymorphisms and their associations with clinical outcomes. *Genet Test Mol Bioma*. 2016;20(10):597–602. doi:10.1089/gtmb.2015.0287
17. Haase R, Elsner K, Merkel N, et al. High dose methotrexate treatment in childhood ALL: pilot study on the impact of the MTHFR 677C>T and 1298A>C polymorphisms on MTX-related toxicity. *Klin Padiatrie*. 2012;224(3):156–159. doi:10.1055/s-0032-1304623
18. Chae H, Kim M, Choi SH, et al. Influence of plasma methotrexate level and MTHFR genotype in Korean paediatric patients with acute lymphoblastic leukaemia. *J Chemother*. 2020;32(5):251–259. doi:10.1080/1120009X.2020.1764280
19. Faganel Kotnik B, Grabnar I, Bohanec Grabar P, Dolzan V, Jazbec J. Association of genetic polymorphism in the folate metabolic pathway with methotrexate pharmacokinetics and toxicity in childhood acute lymphoblastic leukaemia and malignant lymphoma. *Eur J Clin Pharmacol*. 2011;67(10):993–1006. doi:10.1007/s00228-011-1046-z
20. Mahmoud LB, Mdhaaffar M, Frikha R, et al. Use of MTHFR C677T polymorphism and plasma pharmacokinetics to predict methotrexate toxicity in patients with acute lymphoblastic leukemia. *Adv Clin Exp Med*. 2018;27(8):1061–1068. doi:10.17219/acem/69802
21. Aplenc R, Thompson J, Han P, et al. Methylenetetrahydrofolate reductase polymorphisms and therapy response in pediatric acute lymphoblastic leukemia. *Cancer Res*. 2005;65(6):2482–2487. doi:10.1158/0008-5472.CAN-04-2606
22. Radtke S, Zolk O, Renner B, et al. Germline genetic variations in methotrexate candidate genes are associated with pharmacokinetics, toxicity, and outcome in childhood acute lymphoblastic leukemia. *Blood*. 2013;121(26):5145–5153. doi:10.1182/blood-2013-01-480335
23. Yazicioglu B, Kaya Z, Guntekin Ergun S, et al. Influence of folate-related gene polymorphisms on high-dose methotrexate-related toxicity and prognosis in Turkish children with acute lymphoblastic leukemia. *Turk J Haematol*. 2017;34(2):143–150. doi:10.4274/tjh.2016.0007
24. van Kooten Niekerk PB, Schmiegelow K, Schroeder H. Influence of methylene tetrahydrofolate reductase polymorphisms and coadministration of antimetabolites on toxicity after high dose methotrexate. *Eur J Haematol*. 2008;81(5):391–398. doi:10.1111/j.1600-0609.2008.01128.x
25. Ho GT, Moodie FM, Satsangi J. Multidrug resistance 1 gene (P-glycoprotein 170): an important determinant in gastrointestinal disease? *Gut*. 2003;52(5):759–766. doi:10.1136/gut.52.5.759
26. Maroofi F, Amini S, Roshani D, Ghaderi B, Abdi M. Different frequencies and effects of ABCB1 T3435C polymorphism on clinical and laboratory features of B cell chronic lymphocytic leukemia in kurdish patients. *Tumour Biol*. 2015;36(4):2863–2868. doi:10.1007/s13277-014-2914-9
27. Rao DN, Anuradha C, Vishnupriya S, et al. Association of an MDR1 gene (C3435T) polymorphism with acute leukemia in India. *Asian Pac J Cancer Prev*. 2010;11(4):1063–1066.
28. Pongstaporn W, Pakakasama S, Chaksangchaichote P, Pongtheerat T, Hongeng S, Permitr S. MDR1 C3435T and C1236T polymorphisms: association with high-risk childhood acute lymphoblastic leukemia. *Asian Pac J Cancer Prev*. 2015;16(7):2839–2843. doi:10.7314/APJCP.2015.16.7.2839
29. Tan Y, Kong Q, Li X, et al. Relationship between methylenetetrahydrofolate reductase gene polymorphisms and methotrexate drug metabolism and toxicity. *Transl Pediatr*. 2023;12(1):31–45. doi:10.21037/tp-22-671
30. Li M, Kong XY, Wang SM. Analysis of the frequency distribution of five single-nucleotide polymorphisms of the MTRR gene in a Chinese pediatric population with acute lymphoblastic leukemia. *Pharmacotherapy*. 2022;42(6):442–452. doi:10.1002/phar.2685
31. Li M, Kong XY, Wang SM. Effects of splicing-regulatory polymorphisms in ABCC2, ABCG2, and ABCB1 on methotrexate exposure in Chinese children with acute lymphoblastic leukemia. *Cancer Chemother Pharmacol*. 2023;91(1):77–87. doi:10.1007/s00280-022-04498-0
32. Hu YH, Zhou L, Wang SS, et al. Methotrexate disposition in pediatric patients with acute lymphoblastic leukemia: what have we learnt from the genetic variants of drug transporters. *Curr Pharm Des*. 2019;25(6):627–634. doi:10.2174/1381612825666190329141003
33. Zhang YX, Ma Y, Zhang H, Zhang WP, Yang XY. Genetic polymorphism in MDR1 C3435T is a determinant of methotrexate cerebrospinal fluid concentrations in Chinese children with acute lymphoblastic leukemia. *Int J Clin Pharm Ther*. 2020;58(5):254–260. doi:10.5414/CP203462
34. Lopez-Lopez E, Martin-Guerrero I, Ballesteros J, et al. Polymorphisms of the SLC01B1 gene predict methotrexate-related toxicity in childhood acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2011;57(4):612–619. doi:10.1002/pbc.23074
35. Wang SM, Sun LL, Zeng WX, Wu WS, Zhang GL. Influence of genetic polymorphisms of FPGS, GGH, and MTHFR on serum methotrexate levels in Chinese children with acute lymphoblastic leukemia. *Cancer Chemother Pharmacol*. 2014;74(2):283–289. doi:10.1007/s00280-014-2507-8

36. Esmaili MA, Kazemi A, Faranoush M, et al. Polymorphisms within methotrexate pathway genes: relationship between plasma methotrexate levels, toxicity experienced and outcome in pediatric acute lymphoblastic leukemia. *Iran J Basic Med Sci.* 2020;23(6):800–809. doi:10.22038/ijbms.2020.41754.9858
37. Cwiklinska M, Czogala M, Kwiecinska K, et al. Polymorphisms of SLC19A1 80 G>A, MTHFR 677 C>T, and tandem ts repeats influence pharmacokinetics, acute liver toxicity, and vomiting in children with acute lymphoblastic leukemia treated with high doses of methotrexate. *Frontiers in Pediatrics.* 2020;8:307. doi:10.3389/fped.2020.00307
38. El-Khodary NM, El-Haggar SM, Eid MA, Ebeid EN. Study of the pharmacokinetic and pharmacogenetic contribution to the toxicity of high-dose methotrexate in children with acute lymphoblastic leukemia. *Med Oncol.* 2012;29(3):2053–2062. doi:10.1007/s12032-011-9997-6
39. Yao P, He X, Zhang R, Tong R, Xiao H. The influence of MTHFR genetic polymorphisms on adverse reactions after methotrexate in patients with hematological malignancies: a meta-analysis. *Hematology.* 2019;24(1):10–19. doi:10.1080/10245332.2018.1500750
40. Frikha R, Jemaa MB, Frikha F, et al. Involvement of C677T MTHFR variant but not A1298C in methotrexate-induced toxicity in acute lymphoblastic leukemia. *J Oncol Pharm Pract.* 2021;27(6):1382–1387. doi:10.1177/1078155220951898
41. Erdelyi DJ, Kamory E, Zalka A, et al. The role of ABC-transporter gene polymorphisms in chemotherapy induced immunosuppression, a retrospective study in childhood acute lymphoblastic leukaemia. *Cell Immunol.* 2006;244(2):121–124. doi:10.1016/j.cellimm.2007.02.007
42. Liu SG, Gao C, Zhang RD, et al. Polymorphisms in methotrexate transporters and their relationship to plasma methotrexate levels, toxicity of high-dose methotrexate, and outcome of pediatric acute lymphoblastic leukemia. *Oncotarget.* 2017;8(23):37761–37772. doi:10.18632/oncotarget.17781

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