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ORIGINAL RESEARCH

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

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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 Folinate (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/m^2 of MTX, and intermediate- and high-risk children received 5 g/m^2 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 μmol/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: x^2=1.880, P=0.391; *A1298C* variant: x^2=0.046, P=0.977; *C3435T* variant: x^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 (> 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 ≤ 1 μmol/L, and 20 patients had a 44-h plasma MTX level > 1 μmol/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* *CT* *TT*	18 (17.0) 43 (40.6) 45 (42.4)	15 (83.3) 34 (79.1) 37 (82.2)	3 (16.7) 9 (20.9) 8 (17.8)	0.897
MTHFR 1298A > C	*AA* *AC* *CC*	84 (79.2) 21 (19.8) 1 (0.9)	68 (81.0) 17 (81.0) 1 (100.0)	16 (19.0) 4 (19.0) 0 (0.0)	1.000
ABCB1 3345 C > T	*CC* *CT* *TT*	47 (44.3) 49 (46.2) 10 (9.4)	38 (80.9) 40 (81.6) 8 (80.0)	9 (19.1) 9 (18.4) 2 (20.0)	1.000

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 1)

Polymorphism	Genotype	Non-Toxicity n (%)	Toxicity n (%)	OR	P	95% CI
MTHFR 677C > T		Leucopenia (< grade 1)	Leucopenia (≥ grade 1)			
	CC *CT* *TT*	8 (44.4) 25 (58.1) 20 (44.4)	10 (55.6) 18 (41.9) 25 (55.6)	Reference 0.576 1.000	 0.330 1.000	 0.190–1.747 0.333–3.004
		Neutropenia (< grade 1)	Neutropenia (≥ grade 1)			
	CC *CT* *TT*	10 (55.6) 25 (58.1) 26 (57.8)	8 (44.4) 18 (41.9) 19 (42.2)	Reference 0.900 0.913	 0.852 0.872	 0.297–2.730 0.303–2.750
		Elevated AST (< grade 1)	Elevated AST (≥ grade 1)			
	CC *CT* *TT*	7 (38.9) 18 (41.9) 16 (35.6)	11 (61.1) 25 (58.1) 29 (64.4)	Reference 0.884 1.153	 0.830 0.804	 0.287–2.722 0.374–3.561
		Vomiting (< grade 1)	Vomiting (≥ grade 1)			
	CC *CT* *TT*	18 (100) 40 (93.0) 41 (91.1)	0 (0) 3 (7.0) 4 (8.9)	Reference NE NE	 0.998 0.998	 NE NE
		Oral mucositis (< grade 1)	Oral mucositis (≥ grade 1)			
	CC *CT* *TT*	18 (100) 38 (88.4) 42 (93.3)	0 (0) 5 (11.6) 3 (6.7)	Reference NE NE	 0.998 0.998	 NE NE

(*Continued*)

Table 4 (Continued).

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

	The Number of Wild Homozygous Genes				P
	0	1	2	3	
Leucopenia (≥ grade 1)					
Yes, n=76	5	39	29	3	0.551
No, n=30	0	18	11	1	
Neutropenia (≥ grade 1)					
Yes, n=53	3	30	19	1	0.729
No, n=53	2	27	21	3	
Depressed hemoglobin (≥ grade 1)					
Yes, n=97	5	55	33	4	0.114
No, n=9	0	2	7	0	
Depressed PLT (≥ grade 1)					
Yes, n=9	0	3	6	0	0.230
No, n=97	5	54	34	4	
Elevated ALT (≥ grade 1)					
Yes, n=68	4	35	27	2	0.750
No, n=38	1	22	13	2	
Elevated AST (≥ grade 1)					
Yes, n=65	3	35	25	2	1.000
No, n=41	2	22	15	2	
Vomiting (≥ grade 1)					
Yes, n=7	0	5	2	0	0.840
No, n=99	5	25	38	4	
Oral mucositis (≥ 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] Poland[37] 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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The authors report no conflicts of interest in this work.

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