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TPMT and MTHFR Genotype is not Associated With Altered Risk of Thioguanine-Related Sinusoidal Obstruction Syndrome in Pediatric Acute Lymphoblastic Leukemia: A Report from the Children's Oncology Group

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Abstract

Sinusoidal obstruction syndrome is a complication of therapy for pediatric ALL and may be modified by thiopurine methyltransferase activity as well as by MTHFR genotype. We assessed TPMT * 3A, * 3B, * 3C, and MTHFR C677T and A1298C germline genetic polymorphisms among 351 patients enrolled in the thioguanine treatment arm of CCG-1952 clinical trial. TPMT and MTHFR C677T genotypes were not associated with SOS risk. The combination of MTHFR and TPMT variant genotypes was not associated with SOS risk. These suggest that germline genetic variation in TPMT and MTHFR do not significantly alter SOS risk in patients exposed to thioguanine.

Keywords

acute lymphoblastic leukemia; MTHFR; SOS; thioguanine; TPMT; toxicity

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Authors' contributions: L.W. performed laboratory studies, interpreted data, and wrote the manuscript. M.V. performed statistical analyses, interpreted data, and wrote the manuscript. T.M. performed laboratory studies and analyzed data. S.C. performed laboratory studies and analyzed data. M.D. performed statistical analysis, interpreted data, and wrote the manuscript. L.S. designed the study, interpreted data, and wrote the manuscript. R.A. conceived the project, designed the study, interpreted data, and wrote the manuscript.

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INTRODUCTION

Thiopurine-related sinusoidal obstruction syndrome (SOS) is a recognized complication of pediatric ALL therapy, observed most commonly in association with oral thioguanine (TG) [1,2]. The Children's Cancer Group 1952 clinical trial (CCG-1952) studied substitution of oral TG for mercaptopurine (MP); SOS was an anticipated side effect occurring in approximately 20% of patients randomized to oral TG [3]. Little is currently known regarding prospective identification of patients who may be at highest risk for development of thiopurine-related SOS.

Previous work noted an association between TPMT activity and risk of SOS among patients enrolled on United Kingdom Medical Research Council trial ALL97. TPMT activity was significantly lower among children who developed SOS and the frequency of TPMT*3 variant alleles was increased in cases although the difference did not reach statistical significance [4]. Smaller studies have given conflicting results [2,5,6], while a study in adult patients undergoing allogeneic hematopoietic stem cell transplant (HSCT) noted an increased incidence of SOS among patients homozygous for the MTHFR A1298C C allele [7]. MTHFR catalyzes the formation of 5-methyltetrahydrofolate, which is the methyl donor when homocysteine is remethylated to methionine. TPMT functions to make homocysteine available for this reaction [8]. In this way, MTHFR polymorphisms may affect TPMT activity.

Given these background data, we sought to address the role of germline genetic variation in TPMT and MTHFR in a large cohort of pediatric patients. We hypothesized that TPMT and MTHFR genotype may be associated with altered risk for hepatic SOS among patients undergoing therapy for pediatric ALL on CCG- 1952.

METHODS

CCG-1952 enrolled 2,027 eligible pediatric patients with standard risk ALL between 5/1996 and 2/2000. Outcomes have been reported previously [3,9]. Eligible patients were randomized using a 2×2 factorial design comparing post-induction intrathecal methotrexate against intrathecal triple therapy (methotrexate, cytarabine, hydrocortisone) and mercaptopurine (MP) versus TG as the maintenance thiopurine. Patients were scheduled to receive the following doses of thiopurine drugs: during consolidation (days 1–27), interim maintenance 1, 2 (days 0–49), maintenance (12-week cycles) MP 75 mg/m² daily or TG 60 mg/m² daily, decreased to 50 mg/m² per day in 2/1998; during delayed intensification 1, 2 (days 28–41) TG 60 mg/m² daily. Only patients on the thiopurine treatment arm were included in the analysis. The Children's Hospital of Philadelphia Institutional Review Board approved this research.

Genomic DNA was extracted from 351 remission bone marrow aspirate specimens using the Puregene core kit A (Qiagen Inc., Turnberry Lane, Suite 200, Valencia, CA). TPMT genotyping for the *3A, *3B, and *3C variants and MTHFR genotyping for the C677T and A1298C variants was performed by pyrosequencing or on the Taqman platform. Clinical

patient data were prospectively collected from participating centers and obtained from the CCG Group Operations Office in Arcadia, CA, USA.

The primary outcome of interest for this analysis was SOS. Diagnosis of SOS on CCG-1952 required presence of at least two of the following: acute onset of palpable hepatomegaly, noted in 91% of SOS cases, acute thrombocytopenia, with median platelet count 46,000/ml at SOS diagnosis, and ascites on ultrasound, noted in 42/168 (25%) patients imaged [3]. The study chair reviewed each case; there was no central review. All eligible patients with available genotype and SOS data were included in this analysis.

Standard statistics were used to tabulate patient characteristics and genotype frequencies. Logistic regression analysis was used to estimate the risk of SOS by genotype, where the wildtype was chosen as the reference and race, age, and gender were used as covariates. For TPMT *3A, missing genotypes were considered to be wildtype based on expected genotype frequencies. A post-hoc power calculation was performed, based on two independent study groups on a dichotomous primary end point (SOS) with alpha threshold set to 0.05.

RESULTS AND DISCUSSION

Of 2,027 patients in the randomized cohort, genotypes were available on 351/1,017 patients receiving TG. Genotyped patients were generally representative of the overall cohort with respect to age (Table I); differences were observed with regard to gender and race/ethnicity. Not all SNPs were assessed in all patients; this occurred due to inadequate sample quality or quantity. TPMT *3A, *3B, and *3C genotype data was available for 340/351 (96.9%), 291 (82.9%), and 333 (94.9%) patients respectively. MTHFR C677T and A1298C genotype data were available for 253 (72.1%) and 320 (91.2%) patients respectively. All genotypes were in Hardy–Weinberg equilibrium. Genotype frequencies among our cohort were similar to those published for European populations.

SOS was diagnosed in 79/351 (22.5%) patients, comparable to the rate among all eligible patients on the TG arm of CCG-1952. TPMT genotype was not associated with SOS risk [TPMT *3A: odds ratio (OR) ¼ 1.0, 95% confidence interval (95% CI) 0.5–2.0; TPMT *3B: OR ¼ 1.2, 95% CI 0.5–2.8; TPMT *3C: OR ¼ 0.8, 95% CI 0.3–2.1]. MTHFR C677T and A1298C genotypes were not associated with SOS risk [MTHFR C677T: OR ¼ 0.6, 95% CI 0.2–1.6; MTHFR A1298C OR ¼ 2.2, 95% CI 0.9–5.3] (unadjusted estimates, see Table II). Combination of MTHFR and TPMT variant genotypes was not associated with SOS risk [MTHFR C677T þ TPMT: OR ¼ 1.01, 95% CI 0.51–1.99; MTHFR A1298C þ TPMT: OR ¼ 0.88, 95% CI 0.47–1.62]. No genotype covariates (age, sex, race) significantly confounded the SOS risk. A post-hoc power analysis demonstrated that the available sample size had 80% power to detect a difference of 13% in SOS prevalence between MTHFR 1298 CC and AA genotypes.

This is the largest study of TPMT genotype and the only study of MTHFR genotype and TG associated SOS in pediatric ALL patients. In our cohort, no association was noted between TPMT genotype and SOS risk. This is consistent with the results of Lennard and colleagues, who did not find a statistically significant association between TPMT genotype and SOS risk [4]. While those authors noted an association between TPMT activity and SOS risk, it is

unclear whether TPMT genotype reliably correlates with TPMT activity [10]. This may account for a discrepancy between our results and those of Lennard et al. The association between MTHFR A1298C and SOS approached, but did not reach statistical significance. This result is interesting given data from Goekkurt et al. [7] demonstrating an increased risk of SOS in HSCT for patients with the MTHFR A1298C allele/genotype. Additional genotyping in larger sample sets will be necessary to determine definitely whether the MTHFR A1298C allele is associated with SOS in patients receiving TG.

This study has several strengths, notably the large sample set derived from CCG-1952. This study had adequate statistical power to detect modest increases in SOS risk. SOS was prospectively identified and monitored during the CCG-1952 trial making it unlikely that patients with SOS were not identified in the clinical trial data.

Despite these strengths, the study has limitations which should be acknowledged. DNA was not available on all patients with SOS. TPMT levels were not prospectively assessed and could not be correlated with genotype. The clinical definition of SOS used on CCG-1952 is less stringent than other clinical definitions [11]. These data thus do not exclude the possibility that TPMT or MTHFR genotypes may play a role in determining the risk of severe SOS.

In summary, our data demonstrate that TPMT and MTHFR genotyping is not predictive of TG associated SOS risk. Additional studies in larger sample sets will be required to develop more effective clinical prediction models.

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TABLE I

Patient Characteristics

Characteristic	CCG-1952	Our cohort	P	Our cohort		P
				SOS	No SOS	
n	2,027	351		79	272	
Age, in years						
1–1.99	165 (8.1%)	29 (8.3%)	NS	8 (10.1%)	21 (7.7%)	NS
2–5.99	1,402 (69.2%)	242 (68.9%)		57 (72.2%)	185 (68.0%)	
6–9.99	460 (22.7%)	80 (22.8%)		14 (17.7%)	66 (24.3%)	
Gender						
Male	1,129 (55.7%)	189 (53.8%)	<0.1	53 (67.1%)	136 (50.0%)	<0.01
Female	898 (44.3%)	162 (46.2%)		26 (32.9%)	136 (50.0%)	
Race/ethnicity						
White	1,376 (68.2%)	263 (74.9%)	<0.01	66 (83.5%)	197 (72.4%)	NS
Black	72 (3.6%)	12 (3.4%)		2 (2.5%)	10 (3.7%)	
Hispanic	444 (22.0%)	52 (14.8%)		8 (10.1%)	44 (16.2%)	
Other	125 (6.2%)	24 (6.8%)		3 (3.8%)	21 (7.7%)	
Immuno-phenotype ^a						
B-cell	1,802 (93.3%)	284 (95.6%)	<0.1	62 (95.4%)	222 (95.7%)	NS
T-cell	130 (6.8%)	13 (4.4%)		3 (4.6%)	10 (4.3%)	

NS, not significant.

^aImmunophenotype has missing data.

TABLE II

SOS Risk by Genotype

SNP	Genotype	SOS	No SOS	OR	95%CI	OR _{adj}	95%CI _{adj}
TPMT*3A ^a	wt/het+het/het	12	42	1.0	0.5–2.0	0.7	0.3–1.6
	wt/wt	64	222				
TPMT*3B	G/A+A/A	8	27	1.2	0.5–2.8	1.0	0.4–2.6
	G/G	50	206				
TPMT*3C	A/G+G/G	6	25	0.8	0.3–2.1	0.7	0.2–1.8
	A/A	68	234				
MTHFR C677T	T/T	6	29	0.6	0.2–1.6	0.9	0.3–2.4
	C/T	23	85	0.8	0.4–1.5	0.9	0.4–1.8
	C/C	28	82				
MTHFR A1298C	C/C	9	18	2.2	0.9–5.3	1.4	0.5–3.8
	A/C	32	106	1.3	0.7–2.3	1.0	0.5–1.9
	A/A	29	126				

TPMT*3A is derived from the TPMT SNPs TPMT 460G>A and TPMT 719 A>G.

TPMT*3B represents TPMT 460G>A. TPMT*3C refers to TPMT 719A>G.

^a Patients where the determination of TPMT*3A genotype was hindered due to one missing value in either 460G>A or 719 A>G were classified as wild type (wt).