PRECISION MEDICINE

original reports abstract

Polygenic Pharmacogenomic Markers as Predictors of Toxicity Phenotypes in the Treatment of Acute Lymphoblastic Leukemia: A Single-Center Study

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PURPOSE Acute lymphoblastic leukemia (ALL) is the most prevalent cause of childhood cancer and requires a long course of therapy consisting of three primary phases with interval intensification blocks. Although these phases are necessary to achieve remission, the primary chemotherapeutic agents have potentially serious toxicities, which may lead to delays or discontinuations of therapy. The purpose of this study was to perform a comprehensive pharmacogenomic evaluation of common antileukemic agents and develop a polygenic toxicity risk score predictive of the most common toxicities observed during ALL treatment.

METHODS This cross-sectional study included 75 patients with pediatric ALL treated between 2012 and 2020 at the University of Florida. Toxicity data were collected within 100 days of initiation of therapy using CTCAE v4.0 for toxicity grading. For pharmacogenomic evaluation, single-nucleotide polymorphisms (SNPs) and genes were selected from previous reports or PharmGKB database. 116 unique SNPs were evaluated for incidence of various toxicities. A multivariable multi-SNP modeling for up to 3-SNP combination was performed to develop a polygenic toxicity risk score of prognostic value.

RESULTS We identified several SNPs predictive of toxicity phenotypes in univariate analysis. Further multivariable SNP-SNP combination analysis suggest that susceptibility to chemotherapy-induced toxicities is likely multigenic in nature. For 3-SNPscore models, patients with high scores experienced increased risk of GI (P = 2.07E-05, 3 SNPs: TYMS-rs151264360/FPGS-rs1544105/GSTM1-GSTM5-rs3754446), neurologic (P = .0005, 3 SNPs: DCTD-rs6829021/SLC28A3-rs17343066/CTPS1-rs12067645), endocrine (P = 4.77E-08, 3 SNPs: AKR1C3-rs1937840/TYMS-rs2853539/CTH-rs648743), and heme toxicities (P = .053, 3 SNPs: CYP3A5-rs776746/ABCB1-rs4148737/CTPS1-rs12067645).

CONCLUSION Our results imply that instead of a single-SNP approach, SNP-SNP combinations in multiple genes in drug pathways increases the robustness of prediction of toxicity. These results further provide promising SNP models that can help establish clinically relevant biomarkers allowing for greater individualization of cancer therapy to maximize efficacy and minimize toxicity for each patient.

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the leading cause of childhood cancer, representing approximately 25% of all cancers in patients younger than 15 years.[1] Cure rates have improved from 10% in the 1960s to more than 90% in contemporary clinical trials.[2,3] Standard treatment regimens consist of prolonged cytotoxic therapy administered over three primary phases—remission induction, consolidation, and maintenance—with interval intensification blocks.[4] This approach, although effective, produces a myriad of chronic health consequences for survivors.[5] Interpatient variation in pharmacogenomics can result in unpredictable variability in occurrence of adverse events and toxicity as well as discrepancies in therapeutic efficacy. Dose-limiting toxicities lead to modifications in treatment regimens and dosing schedules. Data have shown these delays and omissions can affect long-term outcomes in patients with pediatric cancer[6]. Studies in leukemia cohorts have uncovered a need for less toxic approaches without compromising efficacy.

Pharmacogenomic biomarkers are an evolving area that may help identify patient-specific factors affecting responses to chemotherapeutic agents. Inherited variation in genes involved in drug metabolism and transport have been described in a multitude of modern drugs.[7-11] Genetic polymorphisms can influence the gene expression and/or activity, thereby affecting drug pharmacokinetics and causing interindividual variation in drug levels, which can alter toxicity phenotype and therapeutic efficacy.[12] To

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Patients with acute lymphoblastic leukemia (ALL) are treated with intensive chemotherapy that results in severe toxicities, which can sometimes result in delays or discontinuations of therapy. The objective of this study was to comprehensively evaluate pharmacogenomics of antileukemic agents and establish a polygenic toxicity risk score predictive of the common toxicities observed during ALL treatment.

Knowledge Generated

We took a pharmacological pathway–based pharmacogenomics approach and identified several single-nucleotide polymorphisms (SNPs) associated with individual toxicities. A multi-SNP predictor modeling approach established a 3-SNP combination score with significant association with specific toxicities.

Relevance

Multi-SNP combination approach is more robust and takes into account multiple SNPs predictive of toxicity and thus holds significant clinical relevance. Promising SNP models can help establish clinically relevant biomarkers that can be used preemptively and monitor risk of toxicity and accordingly design interventions to reduce toxicity and improve quality of life in patients with ALL.

date, the most understood example of this in pediatric cancer is thiopurine S-methyltransferase (TPMT) and nudix hydrolase 15 (NUDT15) activities.[13] First described as early as the 1980s,[14] a total of 21 TPMT genetic polymorphisms have since been described and are associated with decreased levels of TPMT enzyme activity and/or thiopurine drug-induced toxicity.[15] This knowledge led to standardized practices evaluating TPMT and NUDT15 genetic polymorphisms in patients to tailor dosing before the initiation of purines on the basis of these genetic variations.[12,16,17] Numerous efforts have been made to replicate these findings in other key genes.[18,19]

Herein, we sought to describe single-nucleotide polymorphism (SNP) variants as pharmacogenomic biomarkers predictive of treatment toxicity phenotypes in a cohort of children with ALL. Characterization of such variations could establish clinically relevant predictors, allowing for personalized leukemia therapy tailored toward optimizing drug efficacy while lessening toxicities. The objective of this study was to identify SNPs in target genes associated with drug metabolism or transport that could predict undue toxicity from antileukemic agents in children with ALL.

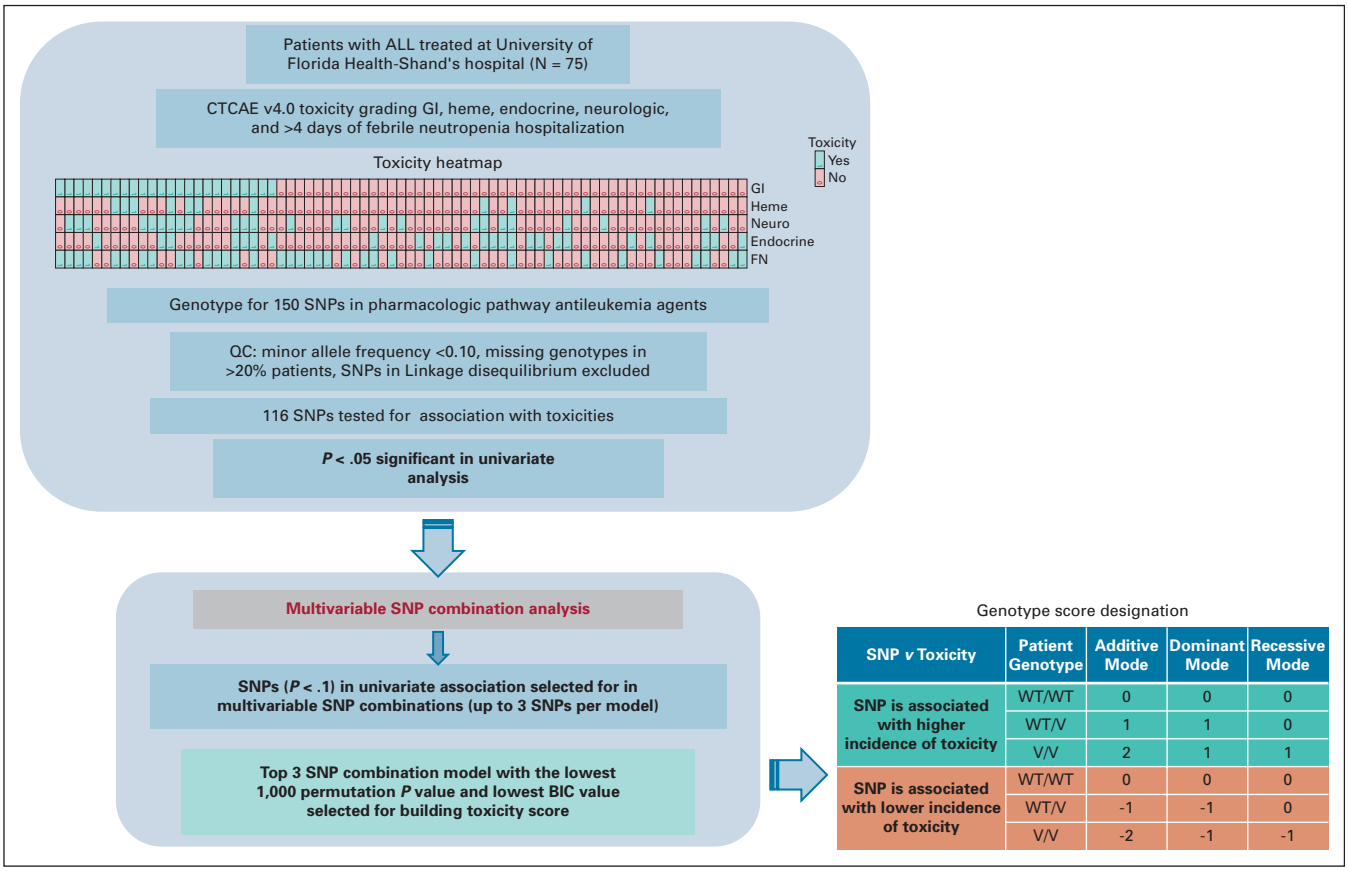
METHODS

Study Design and Patients

This was a cross-sectional study of subjects treated at a tertiary academic center. Overall study design is shown in Figure 1. Patients age 3 months to 26 years with a diagnosis of de novo or secondary ALL who received induction and consolidation therapy between May 2012 and December 2019 at the University of Florida were eligible for enrollment, regardless of disease risk category, sex, or racial or ethnic background, for enrollment. Study enrollment occurred between February 2019 and May 2020 at the University of Florida. Patients who met eligibility criteria were excluded if they declined participation, or if they were unable to provide an adequate blood specimen. The study protocol was approved by the University of Florida Institutional Review Board (IRB#201802623). All patients provided written informed consent before participating in the study and received treatment according to standard-of-care options at the discretion of their treating physician. To be eligible for assessment, patients were required to have received induction and consolidation chemotherapy at the University of Florida. A total of 75 patients treated between 2012 and 2020 were included in the study.

Comprehensive Pharmacogenomic Evaluation

A peripheral blood sample (5-10 mL) for pharmacogenomic testing was collected at a single time point during routine follow-up care. Blood samples were stored in a malignant hematology biorepository for subsequent genomic studies. Genomic DNA was isolated from the samples for further genotyping. 150 SNPs in key candidate genes involved in cellular transport and metabolism of cytarabine, vincristine, methotrexate, daunorubicin/doxorubicin, and mercaptopurine/thioguanine were analyzed. Sequenom genotyping that uses matrix-assisted laser desorption/ionization-time of flight–based chemistry was performed at University of Minnesota, Biomedical Genomics Center. Genes involved in pharmacological chemotherapy agents were obtained from PharmGKB.[20] Literature search as well as information from PharmGKB[20] was used to select the SNPs. After excluding 27 SNPs with minor-allele frequency (MAF) < 0.1, 1 SNP missing genotypes in $>20\%$ of the samples, and 6 SNPs that occurred in linkage disequilibrium (LD) with at least one another SNP, a total of 116 unique SNPs (listed in Appendix Table A1 [Supplementary Table 1]) were included in the study. Toxicities were documented in real time by the



SNP *v* Toxicity	Patient Genotype	Additive Mode	Dominant Mode	Recessive Mode
SNP is associated with higher incidence of toxicity	WT/WT	0	0	0
	WT/V	1	1	0
	V/V	2	1	1
SNP is associated with lower incidence of toxicity	WT/WT	0	0	0
	WT/V	-1	-1	0
	V/V	-2	-1	-1

FIG 1. Overall study design. In table, 0 = no toxicity and 1 = toxicity as per description in the methods section. FN, Febrile Neutropenia; GI, gastrointestinal; Heme, hematological toxicity; Neuro, neurological toxicity; QC, quality control; SNP, single-nucleotide polymorphism; V, variant; WT, wildtype.

primary treatment team according to standard-of-care practices and additionally confirmed by physicians on the study team and included the following: hepatic injury defined by increase in serum total bilirubin >3.0 times the upper normal limit; hematologic impairment including severe neutropenia, thrombocytopenia, and anemia leading to treatment delays; number of hospitalizations for febrile neutropenia (FN); thromboembolic events requiring medical intervention; pancreatitis requiring medical intervention; neurotoxicity defined by detailed neurologic examination and alterations in function; and glucose resistance defined by insulin dependence. Overall, CTCAE v4.0 was used for toxicity grading, and all toxicity events during the first 100 days of therapy that include gastrointestinal (GI), hematologic, neurologic, endocrine toxicities, and prolonged hospitalization (>4 days) because of FN were included in the analysis. Logistic regression models were used to test the association between the 116 SNPs in additive, dominant, and recessive modes of inheritance with all different types of toxicities. Odds ratio (OR) and 95% CI were calculated for each test. $P < .05$ was considered significant. In this exploratory study, no adjustment for multiple testing was done. For multivariable SNP combination analysis, SNPs with univariate association $P < .1$ were selected for each toxicity, and then SNP combinations (up to 3 SNPs per model) were tested for association with each toxicity. The combination model with the 1,000 permutation $P < .05$ and lowest Bayesian information criterion (BIC) value was selected to build a 3-SNP score after considering the mode of inheritance and the direction of association with the toxicity for the individual genotypes. 3-SNP score was generated by summation of genotype scores for the individual SNPs passing the top model. Patients were further classified into three groups: 3SNP_Tox score group of ">0," "0," or "<0." Chi-square and the Cochran-Armitage trend tests were used to test for the association between the toxicity risk score groups and the incidence of each of the evaluated toxicities.

RESULTS

Demographics and Clinical Comparisons

Table 1 summarizes demographics and incidence of toxicities. Twenty-five (33%) patients had neurologic and endocrinologic toxicities (grade 1-3). Twenty-four patients had GI toxicities (grades 2-4), 11 (15%) patients had hematologic toxicities (grade 2-4), and 36 patients were hospitalized at least one time >4 days because of FN.

Univariate Analysis of SNP Association With Toxicity Risk

Table 2 provides summary of the univariate analysis. At $P < .05$, 5 unique SNPs were found significantly associated with GI toxicity, 5 SNPs were associated with

TABLE 1. Characteristics Summary for 75 Patients Included in the Study

Characteristics	Patients
Age, years, median (range)	9.9 (1.2-25)
Sex, No. (%)	
Male	42 (56)
Female	32 (42.7)
Unknown	1 (1.3)
Race, No. (%)	
Black/African American	9 (12)
Caucasian/White	33 (44)
Hispanic	18 (24)
Asian	2 (2.7)
Other or unknown	13 (17.3)
Diagnosis, No. (%)	
B-cell ALL	12 (16)
Standard-risk B-cell ALL	29 (38.6)
High- or very high-risk B-cell ALL	26 (34.6)
T-cell ALL	6 (8)
Other	2 (2.6)
GI toxicity, No. (%)	
Grade ≥ 2	24 (32)
Grade < 2	51 (68)
Hematologic toxicity, No. (%)	
Grade ≥ 2	11 (15)
Grade < 2	64 (85)
Febrile neutropenia, No. (%)	
Hospitalization > 4 days	36 (48)
<4 days	39 (52)
Neurologic toxicity, No. (%)	
Grade 1-3	25 (33.3)
No toxicity	50 (66.6)
Endocrine toxicity, No. (%)	
Grade 1-3	25 (33.3)
No toxicity	50 (66.6)

Abbreviation: GI, gastrointestinal.

hematologic toxicities, 9 SNPs were associated with neurologic toxicity, 6 SNPs were associated with endocrine toxicities, and 8 SNPs were found associated with prolonged hospitalization because of FN. For GI toxicities, variant alleles for SNPs rs151264360 in TYMS, rs3740065 in ABCC2, and rs1544105 in folylpolyglutamate synthetase (FPGS) were found associated with reduced risk of toxicity (OR < 1), while rs11853372 in SLC28A1 and rs4880 in SOD2 were found associated with higher risk of toxicity (OR > 1). For hematologic toxicities, SNPs in CDA (rs1048977), CTPS1 (rs12067645), SLIT1 (rs2784917), and CBR3 (rs1056892) were associated with higher risk of toxicity, while ABCB1 SNP rs4148737 was associated with lower risk of toxicity. Higher risk of neurologic toxicity was associated with variant alleles of rs12067645 (CTPS1), rs1979277 (SHMT1), rs4673 (CYBA), rs11598702 (NT5C2), rs6829021 deoxycytidine deaminase (DCTD), and rs5760410 (ADPRA2A), while rs2228100 (ALDH3A1), rs1883112 (NCF4), and rs1937840 (AKR1C3) were found associated with lower risk of neurologic toxicity. With respect to endocrine toxicity, variant alleles of 3 SNPs (rs1051740 in EPHX1, rs2853539 in TYMs, and rs1544105 in FPGS) were found associated with higher risk of toxicity, while rs1937840 in AKR1C3 and rs2838958 in SLC19A1 were associated with lower risk of toxicity. We also tested length of hospitalization for FN for association with polymorphism in genes important in metabolism of chemotherapy. rs12404655 in CDA and rs4673 in CYBA were associated with higher risk of > 4 days of hospitalization, whereas six other SNPs were predictive of lower risk (rs1051266 in SLC19A1, rs4715354 in GSTA5, rs2853539 in TYMS, rs10948059 in GNMT, rs1053129 in DHFR, and rs2413739 in PACSIN2).

Development of Pharmacogenomics Toxicity Risk Score Models

To enhance the clinical utility of the pharmacogenomic discoveries, we performed a multi-SNP predictor modeling to create a pharmacogenomics toxicity risk score as described in Methods (Appendix Table A2 [Supplementary Table 2] provides the list of SNPs included in the modeling as explained above). The best 3-SNP predictor model with lowest BIC and 1,000 permutations test *P* value of < .05 was selected for each toxicity. Toxicity score was created by adding the genotype scores of the 3 SNPs in the top model with consideration of a direction of association with toxicity (negative for lower toxicity and positive for higher toxicity) as well as mode of inheritance. Overall, higher score meant higher incidence of toxicity.

The top 3-SNP model predictor of GI toxicity included rs151264360 in TYMS (TTAAAG > del), rs1544105 in FPGS (C>T), and rs3754446 GSTM5 (A>C). GI toxicity score was created as shown in Figure 2A. Patients were classified into three groups: score <0, score = 0, and score >0. As shown in the bar plot, patients within >0 score group had higher incidence of GI toxicity compared with those in score <0 or = 0 (GI toxicity incidence: 8% *v* 30% *v* 79% in score <0, = 0 or >0, respectively; *P* = 2.07E-05). The Cochran-Armitage trend test, which tests if the trend of incidence of toxicity increases by increasing score, was also significant for GI toxicity (*P* = 3.89E-06). For hematologic toxicity, the top model consisted of CYP3A5-rs776746 (C>T), ABCB1-rs4148737 (T>C), and CTPS1-rs12067645 (G>A). Figure 2B shows the genotype scores for SNPs used to create the toxicity score. As shown in the bar plot, none of the patients with score <0 experienced toxicity, whereas for patients with score >0 around 24%, patients experienced significant hematologic toxicity (heme toxicity incidence: 0% *v* 12% *v* 24% in score <0, = 0, or >0, respectively; Fisher's exact *P* = .053;

TABLE 2. Results From Univariate Analysis of SNPs *v* Toxicity After Treatment

SNP	Gene Symbol	Gene Name	MOI	OR	95% CI	P
					GI toxicity	
rs151264360	*TYMS*	Thymidylate synthase	A[a](D)	0.229	(0.09 to 0.6)	.0026
rs3740065	*ABCC2*	ATP-binding cassette subfamily C member 2 (ABCC2)	A[a](D)	0.179	(0.04 to 0.80)	.0248
rs1544105	*FPGS*	Folylpolyglutamate synthase	R	0.308	(0.11 to 0.88)	.0272
rs4880	*SOD2*	Superoxide dismutase 2	R	1.915	(1.05 to 3.49)	.0343
rs11853372	*SLC28A1*	Solute carrier family 28 member 1	R	3.162	(1.03 to 9.75)	.0451
					Hematologic toxicity	
rs1048977	*CDA*	Cytidine deaminase	A[a](R)	3.232	(1.1 to 9.42)	.0318
rs4148737	*ABCB1*	ABCB1	D	0.488	(0.25 to 0.95)	.0341
rs12067645	*CTPS1*	CTPS1	A	3.575	(1.06 to 11.9)	.0389
rs2784917	*SLIT1*	Slit guidance ligand 1	A	3.409	(1.03 to 11.3)	.0450
rs1056892	*CBR3*	Carbonyl reductase 3	R	2.332	(1.01 to 5.37)	.0468
					Neurologic toxicity	
rs2228100	*ALDH3A1*	Aldehyde dehydrogenase 3 family member A1	A[a](D)	0.309	(0.14 to 0.7)	.0048
rs12067645	*CTPS1*	CTPS1	D[a](A)	2.062	(1.21 to 3.49)	.0070
rs1979277	*SHMT1*	Serine hydroxymethyltransferase	D	1.871	(1.13 to 3.09)	.0144
rs4673	*CYBA*	Cytochrome b-245 alpha chain	D	2.291	(1.17 to 4.47)	.0149
rs11598702	*NT5C2*	5'-nucleotidase, cytosolic II	A	2.349	(1.07 to 5.13)	.0321
rs1937840	*AKR1C3*	Aldo-keto reductase family 1 member C3	A	0.478	(0.24 to 0.95)	.0350
rs6829021	*DCTD*	dCMP deaminase	R	3.179	(1.02 to 9.81)	.0444
rs5760410	*ADPRA2A*	Adenosine receptor 2a	D	1.984	(1.01 to 3.88)	.0451
rs1883112	*NCF4*	Neutrophil cytosolic factor 4	A	0.466	(0.22 to 0.99)	.0469
					Endocrine toxicity	
rs1937840	*AKR1C3*	Aldo-keto reductase family 1 member C3	A[a](D)	0.289	(0.13 to 0.62)	.0015
rs1051740	*EPHX1*	Epoxide hydrolase	A[a](R)	2.747	(1.29 to 5.84)	.0086
rs2853539	*TYMS*	Thymidylate Synthase	D	2.126	(1.2 to 3.74)	.0088
rs1544105	*FPGS*	Folylpolyglutamate synthase	A[a](R)	2.173	(1.1 to 4.3)	.0255
rs3768142	*MTR*	5-methyltetrahydrofolate-homocysteine methyltransferase	A[a](D)	0.391	(0.16 to 0.94)	.0372
rs2838958	*SLC19A1*	Solute carrier family 19 member 1	D	0.585	(0.35 to 0.99)	.0445
					Febrile neutropenia	
rs1051266	*SLC19A1*	Solute carrier family 19 member 1	A[a](D)	0.418	(0.20 to 0.84)	.0157
rs12404655	*CDA*	Cytidine deaminase	A[a](D)	3.432	(1.22 to 9.64)	.0193
rs4715354	*GSTA5*	Glutathione S-transferase alpha 5	R	0.524	(0.30 to 0.90)	.0200
rs2853539	*TYMS*	Thymidylate synthase	D	0.571	(0.35 to 0.92)	.0222
rs4673	*CYBA*	Cytochrome b-245 alpha chain	D	1.799	(1.06 to 3.04)	.0277
rs10948059	*GNMT*	Glycine N-methyltransferase	D[a](A)	0.589	(0.35 to 0.97)	.0368
rs2413739	*PACSIN2*	Protein kinase C and casein kinase substrate in neurons 2	D	0.604	(0.37 to 0.97)	.0392
rs1053129	*DHFR*	Dihydrofolate reductase	A	0.436	(0.19 to 0.986)	.0460

Abbreviations: ABCB1, ATP-binding cassette subfamily B member 1; A, additive; CTPS1, CTP synthase; DCTD, deoxycytidine deaminase; D, dominant; FPGS, folylpolyglutamate synthetase; MOI, mode of inheritance; OR, odds ratio; R, recessive.

[a]SNP is associated with toxicity in at least two different modes of inheritance.

Cochran-Armitage trend test $P = .01$). All 3 SNPs identified in the model for predicting neurologic toxicities belong to the cytarabine metabolic pathway. Top model included rs6829021 (G>A) in DCTD, rs17343066 (G>A) in uptake transporter SLC28A3, and rs12067645 (G>A) in CTPS1 (Fig 2C). As shown in the bar plot (Figs 2C), none of the

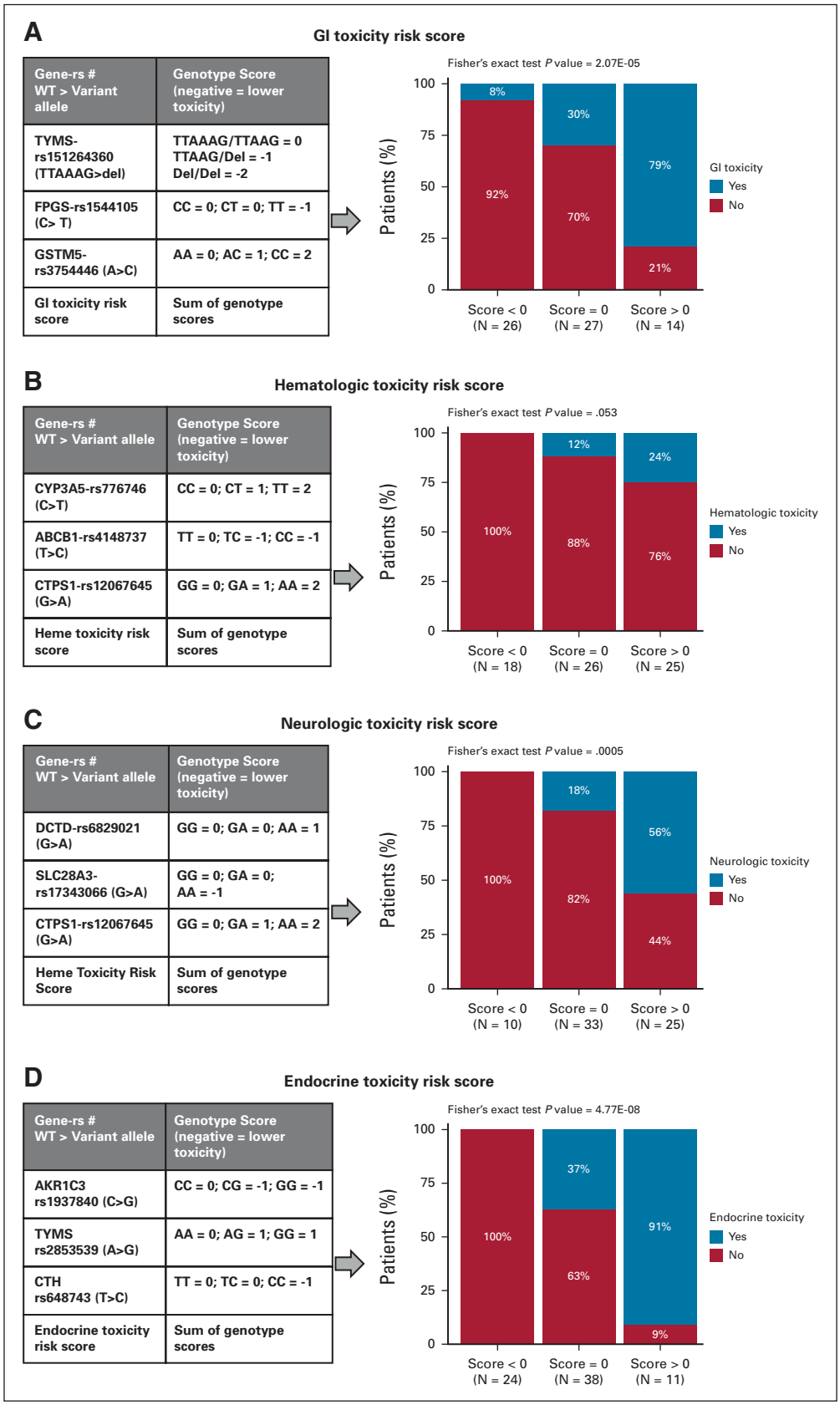


FIG 2. Toxicity incidence by respective composite 3-SNP score groups within patients with ALL. Top model 3 SNPs that were used to create each toxicity SNP score and bar plot of score group versus incidence of toxicity is shown for (A) GI toxicity, n = 67, (B) hematologic toxicity, n = 71, (C) neurologic toxicity, n = 68, (D) and endocrine toxicity, n = 73. Toxicity risk score was computed just for patients with genotype data available for the 3 SNPs in the model. *P* values for Fisher's exact test are listed on top of each bar plot. GI, gastrointestinal; SNP, single-nucleotide polymorphism.

patients with score <0 experienced toxicity, while 56% of patients with score >0 experienced significant neurologic toxicity (neurologic toxicity incidence: 0% *v* 18% *v* 56% in score <0, = 0 or >0, respectively; Fisher's exact *P* = .0005; Cochran-Armitage trend test *P* = 9.27E-05). For endocrine toxicity, top multi-SNP predictor model consisted of AKR1C3-rs1937840(C>G), TYMS-rs2853539(A>G), and cystathionase (CTH)-rs648743(T>C). Again, none of the patients with score <0 experienced any endocrine toxicity compared with 91% of patients with score >0 (endocrine toxicity incidence: 0% *v* 37% *v* 91% in score <0, = 0 or >0, respectively; Fisher's exact *P* = 4.77E-08; Cochran-Armitage trend test *P* = 5.34E-08; Figure 2D). For FN, the score was not generated as none of the 3 SNP-based model's reached permuted *P* < .05.

DISCUSSION

Although ALL is one of the most successfully treated pediatric malignancies, with the current survival rate >90%, its treatment is related to numerous and sometimes life-threatening toxicities. With the progressive integration of immunotherapy in contemporary clinical trials for leukemia, a greater understanding of toxicities from historical antileukemic agents may allow researchers to tailor future treatment approaches to optimize the balance between standard-of-care chemotherapy and novel agents without sacrificing efficacy. This study focused on the most common chemotherapeutic agents used in the treatment of patients with ALL, and instead of taking a single gene-single drug approach, we performed a comprehensive pharmacogenomics evaluation of key genes implicated in the metabolic pathways of these chemotherapeutic agents. Relationships between SNPs within TPMT and NUDT15 genes and hematologic toxicities have been well established and implemented in standard of care to guide mercaptopurine/thioguanine dosing (Clinical Pharmacogenomics Implementation Consortium Guidelines).[17,21] In our cohort, the NUDT15 and TPMT SNPs occurred at a very low frequency. Herein, we focused on common genetic polymorphisms (minor allele frequency of >0.1) within genes relevant in metabolism of chemotherapeutic agents used in ALL. Our focus was on toxicities observed within 100 days of treatment initiation as this time period is critical in obtaining disease remission. The results show a significant association between SNPs in genes of pharmacologic significance to chemotherapeutic agents with toxicities experienced in patients with pediatric ALL.

To enhance the prediction by co-occurrence of multiple variants within a patient, we performed a multi-SNP predictor modeling to identify the most significant 3-SNP combination that is predictive of a particular toxicity incidence. The top 3-SNP model predictive of GI toxicity included (1) rs151264360 is a 6 bp deletion (TTAAAG > del) in thymidylate synthase (TYMS). TYMS catalyzes methylation of dUMP to dTMP and is targeted by methotrexate. This SNP is also referred to as rs11280056 (as a 9 bp deletion), rs34489327, or rs16430 in many publications, and the deletion has been associated with reduced mRNA stability and TYMS expression. Association of this SNP with toxicity and outcome in patients with rheumatoid arthritis receiving methotrexate and patients with cancer treated with methotrexate or other anticancer agents is summarized in the PharmGKB database.[20] Its association with reduced toxicity in multiple studies in rheumatoid arthritis[22,23] is consistent with our results. (2) rs1544105 (C>T) in FPGS, another gene of relevance to methotrexate. TT genotype of this SNP has been associated with increased response compared with CC and CT genotypes in patients with ALL[24,25]; however, associations with toxicity has not been reported; and (3) rs3754446 (A>C) maps to GSTM1-GSTM5 locus. GST family of genes is involved in metabolism of wide range of drugs and this SNP has previously associated with outcome in patients with AML.[26] For hematologic toxicity, the top 3-SNPs included in the top were the following: (1) rs776746 (C>T, with C allele designated as *3 allele) is the most studied functional SNP in the drug metabolizing enzyme CYP3A5. rs776746 is a splicing SNP, and presence of the C allele (which is more abundant in Caucasian ancestry) results in loss of CYP3A5 expression; (2) rs4148737 (T>C) occurs in a multidrug transporter ABCB1 (also known as PgP1) and has been implicated in efflux of wide range of drugs; and (3) rs12067645 (G>A) is in CTP synthase (CTPS1), which is involved in pyrimidine synthesis and has been associated with cytarabine metabolic pathway. As indicated before, all 3 SNPs in neurologic toxicity mapped to cytarabine metabolic pathway genes and included (1) rs6829021 (G>A) in inactivating enzyme DCTD and (2) rs17343066 (G>A) in uptake transporter SLC28A3. Our group has previously shown this SNP to be associated with intracellular ara-CTP levels in patients with AML.[10] SLC28A3 has also been implication in thiopurine and (3) rs12067645 (G>A) in CTPS1 implicated in pyrimidine synthesis. Endocrine toxicity model included 3-SNPs: (1) rs1937840(C>G), in aldoketoreductase 1C3 (AKR1C3), a member of NAD(P)H oxidoreductase. AKR1C3 has been implicated in multiple malignancies including leukemias and is also involved in the metabolism of anthracyclines. This SNP has been associated with increased response to docetaxel and doxorubicin in breast cancer[27]; (2) rs2853539(A>G) in TYMS, a target of methotrexate. AA genotype for this SNP has been associated with reduced methotrexate response in rheumatoid arthritis previously,[25] and (3) rs648743(T>C) in CTH involved in glutathione synthesis, which has previously been associated with sinusoidal obstruction in transplant patients.[28] Although FN is one of the life-threatening toxicities and we did identify 8 SNPs predictive of patients receiving >4 days of hospitalization because of FN, none of the multi-SNP predictor model passed the permutated *P* value threshold of < .05. So, at this time, we did not create a multi-SNP score for this toxicity. One of the reasons for this might be the limited sample size of the study cohort.

Development of the toxicity score by taking direction of association of the SNP with toxicity risks (positive for higher toxicity risk) and mode of inheritance (additive, dominant, or recessive), we propose a pharmacogenomics-based toxicity score for each type of toxicity. Our results show that each described high multi-gene/SNP-based toxicity risk score is significantly associated with a higher incidence of toxicity.

A limitation of the current study was a limited sample size, warranting validation of these findings in a larger cohort of patients with ALL. Additionally, our cohort, although reflective of patients seen at our center, has ethnicity bias with more patients reflective of Caucasian and Hispanic ethnicity. Nonetheless, this approach demonstrates the advantages of multi-SNP prediction modeling compared with single gene-single SNP evaluations and warrants the need to perform similar analysis in other ethnic and racial groups while considering SNPs more prevalent in the population selected. Although preliminary, the results demonstrate the potential use of pharmacogenomic risk scores in individualizing chemotherapy with a goal of reducing toxicities, avoiding toxicity-related omissions and delays in treatment, and designing future trials to incorporate our current knowledge of antileukemic chemotherapy toxicities with novel treatment approaches.

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PRIOR PRESENTATION

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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APPENDIX

TABLE A1. SNPs With Minor Allele Frequency of >0.1 Tested in the Patient's Cohort

SNP	Gene_function_list
rs1045642	ABCB1/cds-synon
rs1128503	ABCB1/cds-synon
rs2032582	ABCB1/missense
rs4148737	ABCB1/intron
rs35592	ABCC1/intron
rs246240	ABCC1/intron
rs3784864	ABCC1/intron
rs3740065	ABCC2/intron
rs3740066	ABCC2/cds-synon
rs7317112	ABCC4/intron
rs9561778	ABCC4/intron
rs12505410	ABCG2/intron
rs13137622	ABCG2/intron
rs1135989	ACTG1/cds-synon,ACTG1/ncRNA
rs2236624	ADORA2A/intron, ADORA2A-AS1/intron
rs2267076	ADORA2A/intron
rs2298383	ADORA2A/intron
rs1937840	AKR1C3/intron
rs2228100	ALDH3A1/missense
rs2784917	ARHGAP19-SLIT1/intron, SLIT1/intron
rs4948496	ARID5B/intron
rs10821936	ARID5B/intron
rs2372536	ATIC/missense
rs16853826	ATIC/intron
rs1056892	CBR3/missense,CBR3-AS1/intron
rs8133052	CBR3/missense,CBR3-AS1/intron
rs602950	CDA/UTR-5
rs818196	CDA/intron
rs1048977	CDA/cds-synon
rs2072671	CDA/missense
rs3215400	CDA/UTR-5
rs10916819	CDA/Upstream
rs12404655	CDA/intron
rs1044457	CMPK1/ncRNA, CMPK1/UTR-3
rs3088062	CMPK1/ncRNA, CMPK1/UTR-3
rs3925058	CMPK1/nearGene-5
rs4600090	CMPK1/intron
rs17103168	CMPK1/ncRNA, CMPK1/UTR-3
rs648743	CTH/nearGene-5
rs4135385	CTNNB1/intron
rs4364871	CTPS1/intron
rs7533657	CTPS1/Upstream
rs12067645	CTPS1/Upstream
rs12144160	CTPS1/intron
rs1801157	CXCL12/UTR-3
rs4673	CYBA/missense
rs1056836	CY1B1/misense
rs2279343	CYP2B6/missense
rs4802101	CYP2B6/nearGene-5
rs7254579	CYP2B6/Upstream
rs12248560	CYP2C19/nearGene-5
rs1799853	CYP2C9/missense
rs2740574	CYP3A4/nearGene-5
rs776746	CYP3A5/intron,CYP3A5/splice-3
rs4694362	DCK/intron
rs4742	DCTD/cds-synon
rs2037067	TENM3/intron
rs6829021	DCTD/3'
rs9990999	DCTD/intron
rs442767	DHFR/nearGene-5,MSH3/intron
rs1053129	DHFR/UTR-3
rs1643650	DHFR/intron
rs1051740	EPHX1/missense
rs11615	ERCC1/cds-synon
rs3212986	CD3EAP/missense, ERCC1/UTR-3
rs1799793	ERCC2/missense
rs1544105	FPGS/Upstream
rs3824662	GATA3/intron
rs3758149	GGH/nearGene-5
rs10948059	GNMT/nearGene-5
rs3957357	GSTA1/nearGene-5
rs4715354	GSTA5/intron
rs3754446	GSTM5/nearGene-5
rs1695	GSTP1/missense
rs2236225	MTHFD1/missense
rs1476413	MTHFR/intron
rs1801131	MTHFR/missense
rs1801133	MTHFR/missense
rs3768142	MTR/intron
rs1801394	FASTKD3/nearGene-5,MTRR/missense
rs1883112	NCF4/nearGene-5
rs2302254	NME1/nearGene-5,NME1-NME2/UTR-5, NME1-NME2/ncRNA
rs3760468	NME1/nearGene-5,NME1-NME2/nearGene-5
rs3744660	NME1-NME2/intron,NME2/intron
rs5841	DECR2/nearGene-5,NME4/cds-synon

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TABLE A1. SNPs With Minor Allele Frequency of >0.1 Tested in the Patient's Cohort (Continued)

SNP	Gene_function_list
rs1799983	NOS3/missense
rs1143684	NQO2/missense
rs11598702	NT5C2/intron
rs2413739	PACSIN2/intron
rs738409	PNPLA3/missense
rs13058338	RAC2/intron
rs9937	RRM1/cds-synon
rs1561876	STIM1/UTR-3
rs11030918	RRM1/nearGene-5
rs1130609	RRM2/UTR-5,RRM2/missense
rs1979277	SHMT1/missense
rs7853758	SLC28A3/ncRNA,SLC28A3/cds-synon
rs1051266	SLC19A1/missense
rs2838958	SLC19A1/intron
rs9977268	COL18A1/intron
rs11231809	SLC22A11/Upstream
rs714368	SLC22A16/missense
rs11853372	SLC28A1/intron
rs17343066	SLC28A3/intron
rs324148	SLC29A1/intron
rs507964	SLC29A1/nearGene-5
rs693955	SLC29A1/intron
rs2306283	SLCO1B1/missense
rs10841753	SLCO1B1/intron
rs11045879	SLCO1B1/intron
rs4880	SOD2/missense
rs5760410	SPECC1L-ADORA2A/SPECC1L/3'/ADORA2A/5'
rs2853539	C18orf56/nearGene-5,TYMS/intron
rs151264360	ENOSF1/intron, ENOSF1/cds-indel,TYMS/cds-indel
rs25487	XRCC1/missense

(Continued in next column)

TABLE A1. SNPs With Minor Allele Frequency of >0.1 Tested in the Patient's Cohort (Continued)

Abbreviations: CTH, cystathionase; DCTD, deoxycytidine deaminase; FPGS, folylpolyglutamate synthetase; SNP, single-nucleotide polymorphism.

SNPs/genes were selected through literature review or data deposited in PharmGKB.[20]

TABLE A2. List of SNPs Associated With Toxicities at $P < .1$ That Were Included in the 3-SNP Combination Modeling

SNP_MOI	Gene	OR	Lower 95	Upper 95	*P*
GI toxicity					
rs151264360.add	TYMS	0.229	0.088	0.598	.003
rs3740065.add	ABCC2	0.179	0.040	0.804	.025
rs1544105.rec	FPGS	0.308	0.109	0.876	.027
rs4880.rec	SOD2	1.915	1.049	3.495	.034
rs11853372.rec	SLC28A1	3.162	1.026	9.750	.045
rs11045879.add	SLCO1B1	0.287	0.079	1.040	.057
rs9977268.add	SLC19A1	2.571	0.936	7.065	.067
rs2306283.add	SLCO1B1	0.534	0.271	1.053	.070
rs10916819.rec	CDA	1.757	0.953	3.242	.071
rs324148.add	SLC29A1	0.500	0.234	1.067	.073
rs1799793.dom	ERCC2	1.581	0.946	2.643	.081
rs714368.dom	SLC22A16	0.641	0.386	1.063	.085
rs3754446.add	GSTM1-M5	1.933	0.905	4.129	.089
rs12067645.add	CTPS1	2.215	0.882	5.566	.091
Hematologic toxicity					
rs1048977.add	CDA	3.232	1.108	9.428	032
rs4148737.dom	ABCB1	0.488	0.251	0.947	.034
rs12067645.add	CTPS1	3.575	1.067	11.974	.039
rs2784917.add	SLIT1	3.409	1.027	11.310	.045
rs1056892.rec	CBR3	2.332	1.012	5.373	.047
rs776746.add	CYP3A5	2.231	0.967	5.147	.060
rs2228100.dom	ALDH3A1	0.507	0.249	1.029	.060
rs13058338.add	RAC2	3.000	0.860	10.471	.085
rs3957357.add	GSTA1	0.416	0.152	1.140	.088
rs10821936.dom	ARID5B	0.566	0.291	1.101	.094
rs2279343.add	CYP3B6	2.336	0.866	6.299	.094
rs4694362.rec	DCK	1.789	0.902	3.546	.096
Neurologic toxicity					
rs2228100.add	ALDH3A1	0.309	0.136	0.699	.005
rs12067645.add	CTPS1	3.422	1.323	8.851	.011
rs1979277.dom	SHMT1	1.871	1.133	3.089	.014
rs4673.dom	CYBA	2.291	1.175	4.468	.015
rs11598702.add	NT5C2	2.349	1.076	5.130	.032
rs1937840.add	AKR1C3	0.478	0.241	0.949	.035
rs6829021_.rec	DCTD	3.179	1.030	9.815	.044
rs5760410.dom	ADPRA2A	1.984	1.015	3.879	.045
rs1883112.add	NCF4	0.466	0.220	0.990	.047
rs4148737.dom	ABCB1	0.592	0.347	1.009	.054
rs2413739.add	PACSIN2	0.512	0.243	1.076	.077
rs17343066.rec	SLC28A3	0.496	0.225	1.091	.081
rs4742.rec	DCTD	1.958	0.914	4.196	.084
rs3758149.rec	GGH	2.138	0.881	5.189	.093
Endocrine toxicity					
rs1937840.add	AKR1C3	0.289	0.135	0.622	.002

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TABLE A2. List of SNPs Associated With Toxicities at $P < .1$ That Were Included in the 3-SNP Combination Modeling (Continued)

SNP_MOI	Gene	OR	Lower 95	Upper 95	*P*
rs1051740.add	EPHX1	2.747	1.293	5.839	.009
rs2853539.dom	TYMS	2.126	1.209	3.740	.009
rs1544105.add	FPGS	2.173	1.100	4.294	.025
rs3768142_.add	MTR	0.391	0.161	0.946	.037
rs2838958.dom	SLC19A1	0.585	0.347	0.987	.045
rs4148737.rec	ABCB1	1.885	0.948	3.747	.071
rs648743.rec	CTH	0.382	0.133	1.100	.074
rs1801394.rec	MTRR	0.384	0.134	1.103	.076
rs17103168.dom	CMPK1	1.567	0.955	2.572	.076
rs776746.add	CYP3A5	1.855	0.920	3.740	.084
rs3925058.rec	CMPK1	0.504	0.228	1.113	090
rs10916819.rec	CDA	1.689	0.917	3.109	.092
rs11615.add	ERCC1	1.825	0.904	3.684	.093
Febrile neutropenia					
rs1051266.add	SLC19A1	0.418	0.206	0.848	.016
rs12404655.add	CDA	3.432	1.222	9.640	.019
rs4715354.rec	GSTA5	0.524	0.304	0.903	.020
rs2853539.dom	TYMS	0.571	0.353	0.923	.022
rs4673.dom	CYBA	1.799	1.066	3.036	.028
rs10948059.dom	GNMT	0.589	0.358	0.968	.037
rs2413739.dom	PACSIN2	0.604	0.374	0.975	.039
rs1053129.add	DHFR	0.436	0.193	0.986	.046
rs1643650.add	DHFR	0.459	0.210	1.002	.051
rs3768142.dom	MTR	1.589	0.987	2.559	.057
rs4802101.rec	CYP2B6	1.972	0.979	3.971	.057
rs1801131.add	MTHFR	2.184	0.941	5.067	.069
rs11598702.add	NT5C2	1.997	0.942	4.235	.071
rs1801133.add	MTHFR	0.539	0.274	1.062	.074
rs1544105.rec	FPGS	0.602	0.337	1.078	.088
rs2236225.dom	MTHFD1	1.523	0.931	2.491	.094
rs776746.dom	CYP3A5	0.650	0.390	1.084	.099
rs7317112.rec	ABCC4	1.715	0.903	3.259	.099

Abbreviations: add, additive, CTH, cystathionase; DCTD, deoxycytidine deaminase; dom, dominant; GI, gastrointestinal; FPGS, folylpolyglutamate synthetase; MOI, mode of inheritance; rec, recessive; SNP, single-nucleotide polymorphism.