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ORIGINAL ARTICLE

Susceptibility to thiopurine toxicity by *TPMT* and *NUDT15* variants in Colombian children with acute lymphoblastic leukemia

Asociación de variantes genéticas en *TPMT* y *NUDT15* con los eventos de toxicidad durante la terapia de mantenimiento en niños colombianos con Leucemia Linfoide Aguda

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Palabras clave:
leucemia linfoide aguda; efectos adversos; farmacogenética; niños; tiopurina; pruebas de farmacogenómica

Abstract

Objective:

This study aimed to correlate the genetic profile of the *NUDT15* and *TPMT* genes with the side effects of the treatment of pediatric patients with acute lymphoid leukemia who were undergoing maintenance therapy at a tertiary care hospital in 2017.

Methods:

This was an analytical, longitudinal, observational study in which the genotypes of the genes of interest were determined by PCR allelic discrimination with TaqMan® probes in patients receiving chemotherapy during the maintenance phase in the Pediatric Hematology and Oncology Unit in 2017. Sociodemographic and clinical data corresponding to the first six months of their maintenance chemotherapy were collected, and the correlation between the genotypes obtained and the development of side effects during the maintenance phase of chemotherapy in these patients was evaluated.

Results:

Seventy pediatric patients were included in the study. Genetic analyses were carried out of these for *NUDT15* and *TPMT* (rs1800462 and rs1800460) on 68 patients, while for the rs1142345 polymorphism, typing was achieved in 42 patients. 4/68 patients were heterozygous for NUDT15, and the same number of patients were heterozygous for rs1800462 and rs1142345, while for rs1800460, 6 heterozygous patients were identified. No statistically significant association was identified between the genetic variants and the outcomes of interest.

Conflicts of interest:
All authors do not have any possible conflicts of interest

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Conclusion:

Studies with a larger population size are needed and the evaluation of other genetic variants that may influence the development of side effects during maintenance chemotherapy.

Resumen

Objetivo:

la finalidad de este estudio fue evaluar las asociaciones entre los perfiles de los genes *NUDT15* y *TPMT* con los efectos adversos del tratamiento de mantenimiento en pacientes pediátricos con Leucemia Linfoblástica Aguda atendidos en un hospital de referencia durante el 2017.

Métodos:

Este fue un estudio observacional analítico, de corte longitudinal en el que los genotipos de los genes de interés fueron determinados mediante PCR de discriminación alélica con sondas TaqMan® en pacientes que estaban recibiendo quimioterapia de mantenimiento en la Unidad de Oncohematología Pediátrica durante el 2017. Los datos clínicos y sociodemográficos correspondientes a los primeros 6 meses de sus tratamientos de mantenimiento fueron colectados, y se evaluó la correlación entre los genotipos identificados y el desarrollo de efectos secundarios en estos pacientes.

Resultados:

setenta pacientes fueron incluidos en el estudio, de estos, los análisis genéticos para NUDT15 y TPMT (rs1800462 and rs1800460) fueron realizados en 68 pacientes, en tanto que para el polimorfismo rs1142345 se logró la tipificación en 42 pacientes. 4/68 pacientes fueron heterocigotos para NUDT15 y el mismo número de pacientes fueron heterocigotos para rs1800462 and rs1142345, mientras que para rs1800460, 6 pacientes heterocigotos fueron identificados. No se identificaron asociaciones estadísticamente significantes entre las variants genéticas y los resultados clínicos de interés.

Conclusiones:

Estos hallazgos resaltan la importancia de realizar estudios de este tipo con un mayor número de sujetos de estudio, así como plantean la necesidad de evaluar otras variantes genéticas que podrían tener algún impacto en el desarrollo de efectos secundarios durante la quimioterapia de mantenimiento.

Remark

1) Why was this study conducted?

Several studies carried out mainly in the United States and Asia have shown associations between the variants of the TPMT and NUDT15 genes with the toxicity outcomes in patients with acute lymphoid leukemia, the information referring to this topic is scarce in our country, so we decided to evaluate this association in our population which represents a sample with genetic admixture and in whom pharmacogenetic differences related to ancestry have been described.

2) What were the most relevant results of the study?

No statistically significant associations were identified between genetic variants in TPMT and NUDT15 with toxicity outcomes evaluated (myelotoxicity and liver toxicity, as well as the dose reduction or interruption of 6-MP).

3) What do these results contribute?

Our results point to the need to carry out national studies aimed at the search for "autochthonous biomarkers" that allow the identification of pediatric cancer patients who present a higher risk of developing toxicity outcomes during their treatments.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy in childhood, accounting for 25% of all malignancies in children under 15 years of age [1]. It is classified into two large groups, according to the cell of origin. 85% of the cases correspond to ALL with B cell precursors and 15% with T cell [2,3]. It occurs more frequently in children with Hispanic and Caucasian ancestry than in those of African ancestry [4,5]. A few environmental risk factors have been associated with ALL in children (exposure to radiation and certain chemicals); however, these associations explain only a minority of cases [6].

One of the major milestones in the therapy of children with ALL was the development of an intensive regimen of 8 drugs, eight weeks of induction and consolidation, which formed the basis of the BFM (Berlin-Frankfurt-Münster) regimen, which is the backbone of current therapies for ALL. In addition, the chemotherapy strategy also includes intensification and maintenance therapy [6]. Since the introduction of this therapeutic regimen, multiple collaborative clinical trials have been developed that have resulted in important advances on the survival of patients with ALL, reaching overall survival rates up to 90% [6,7].

Despite the improvements in the survival rate for most pediatric patients, relapses occur between 15% and 20% and are an important cause of morbidity and mortality in pediatric patients with ALL [8,9]. At the same time, in the majority of multicenter trials of first-line treatment for ALL in children, up to 5% die due to the treatment toxic side effects[10,11]. Treatment-related deaths occur during recurrent and prolonged episodes of neutropenia and lymphopenia due to cytotoxic and immunosuppressive drugs or because leukemia itself could inhibit bone marrow recovery after induction therapy, especially in those patients classified as slow responders [10,12].

Technological progress has allowed the identification of genomic variations that determine characteristic pharmacogenomic patterns, responsible for the individual differences observed in response to treatment in terms of effectiveness or toxicity. Currently, the *TPMT* genotype is the main pharmacogenetic pattern with implications for treatment protocols for patients with ALL

with treatment based on thiopurines [13]. 6- mercaptopurine (6-MP) is specifically important in therapy maintenance, the longest phase during treatment. In patients with homozygous variants in *TPMT*, an 85%-90% dose reduction is required [14-17]. Other genes involved in thiopurine metabolism have been evaluated for their behavior in ALL [15]; Among these, the *NUDT15* gene has recently emerged as an important prediction candidate for toxicity [18].

Yang *et al.* [19], through a study of GWAS and its respective replication cohort, identified two loci related to the dose intensity of 6-MP: rs1142345 in the *TPMT* gene and rs116855232 in *NUDT15*. This last variant was common among Asians and Hispanics but infrequent among Europeans, and not observed in Africans, thus contributing to differences in mercaptopurine tolerance related to ancestry. Also, this study showed that heterozygous patients for *TPMT* or *NUDT15* variants required a 50% decrease in the 6-MP dose compared to those with wild type genotype [19]. Based on these results, the same group of researchers, subsequently replicated these results in cohorts in Guatemala, Singapore, and Japan, adding functional genomics studies where they concluded that the presence of SNPs resulted in a loss of nucleotide diphosphatase activity, and patients with these defective *NUDT15* alleles exhibited high levels of active thiopurine metabolites, thus increasing their toxicity [20]. Scientific interest in this gene has increased in recent years. It has led to its association with age related reductions in mercaptopurine dose[21], association with myelosuppression [22], development of severe toxicity [23], and 6-MP intolerance in specific population groups [24], making it an important candidate for evaluation in pharmacogenetic studies related to 6-MP tolerance, although with less support in the literature than the *TPMT*.

To date, there are no published reports of pharmacogenomic approaches to the treatment response and chemotherapy toxicity for ALL in the pediatric population of Colombia. This becomes even more important when pharmacogenetic differences related to ancestry have been described [25-28]. It has been suggested that pharmacogenetic studies should be carried out independently or collaboratively in different Latin American countries to better understand these relationships [29]. This study aimed to evaluate the association between the genetic profile of the *NUDT15* and *TPMT* genes and the side effects during maintenance phase of treatment in a sample of Colombian pediatric patients with acute lymphoid leukemia.

Materials and Methods

Design

This was an observational, longitudinal study carried out at a university-affiliated pediatric cancer center in Bogota, Colombia, that receives patients from rural and urban areas, and it is a national referral hospital for childhood cancer care.

Patients and data collection

Patients from 1 to 18 years of age diagnosed with acute lymphoblastic leukemia who were admitted to the Pediatric Hematology and Oncology Unit and who were in the maintenance therapy of BFM ALLIC 2009 chemotherapy protocol during 2017 were enrolled in this study. Patients younger than 1-year-old or those with Down syndrome as comorbidity were excluded from this study. For this study, a convenience sampling was carried out, including consecutively all the patients who met the selection criteria and agreed to participate. The characteristics and clinical results obtained with this protocol in our setting have been previously published [,30]. The study was conducted in accordance with the national policy for clinical research and followed the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. This research protocol was approved by the ethics committees of the participating university and hospital. (Act 015-184-16 of 08/25/16 and CEI-38-16 of 08/11/16). Patients' blood samples and clinical data were collected after obtaining parental consent. Additionally, for each patient from 14 years of age, their assent to participate in this study was obtained.

The surplus of the whole blood sample taken as part of the usual controls was used for DNA extraction. In addition, patients were followed up monthly for six months in order to identify the occurrence of adverse effects due to treatment (myelosuppression, liver toxicity, or toxic death). During this follow-up, information regarding laboratory (white blood cell (WBC) count and liver function) and clinical data related to adjustments in treatment and the development of complications were collected. For this study, follow-up was carried out in conjunction with follow-up visits during maintenance, which for these patients are carried out on a monthly basis.

Outcomes

The outcomes of interest were side effects as myelotoxicity and liver toxicity and the dose reduction or interruption of 6-MP. As an exploratory analysis, we evaluated other outcomes as febrile neutropenia, relapse and deaths.

DNA extraction

The DNA extraction was carried out using the QIamp DNA Blood Mini Kit™, following the manufacturer's specifications and as previously reported [31,32]. Briefly, 200 µL of whole blood was subjected to the series of steps described in the manufacturer's guide, using the technique of column filtration by microcentrifugation. The DNA quantification was carried out by spectrophotometry in a Nanodrop2000™, and this same equipment was used to measure the 260/280 nm ratio to determine the purity of the genetic material. The genetic material obtained was stored in 1.5 mL Eppendorf™ tubes at a temperature of -20° C until its use in polymerase chain reaction (PCR) assays.

TaqMan® 5' nuclease real-time PCR

The determination of the genotypes of the SNPs (rs1800462, rs1800460, rs1142345, and rs116855232) in the *TPMT* and *NUDT15* genes was carried out in real-time PCR for allelic discrimination by 5' nuclease using commercially available TaqMan® SNP Genotyping Assays (Assay ID: C__12091552_30, C__30634116_20, C_____19567_20 and C_154823200_10, Thermo Fisher Scientific, USA), following the recommendations described in the "TaqMan® Allelic Discrimination Guide". For this, we used a PCR mixture containing 15 ng DNA (3 µL), 1.25 µL of the corresponding TaqMan® probe (20X), 12.5 µL TaqMan® Gene Expression Master Mix and 8.25 µL of nuclease-free ultrapure water for a final volume per reaction of 25 µL. All the tests were carried out in duplicate. The tests were carried out using the CFX96 Touch ™ Real-Time PCR Detection System (Bio-Rad), following the operating conditions suggested by the manufacturer for each probe.

Data analysis

Data are presented as median plus interquartile ranges for the quantitative variables, while their respective frequencies are shown for the qualitative data. The allelic and genotypic frequencies, as well as the Hardy-Weinberg equilibrium, were evaluated using snpReady. *TPMT* typing was carried out following the standards of the Clinical Pharmacogenetic Implementation Consortium [13]. Briefly, *TPMT* haplotypes were determined based on the combinations of SNPs rs1800462, rs1800460 and rs1142345 as follows: CCT = * 1, GCT = * 2, CTC = * 3A, CTT = * 3B and CCC = * 3C. To determine the association between the genetic variants and the outcome of interest (some type of toxicity), the Chi-square statistic was used. The statistical analyses were performed using SPSS for Mac, version 2.0, and a value of p <0.05 was considered statistically significant.

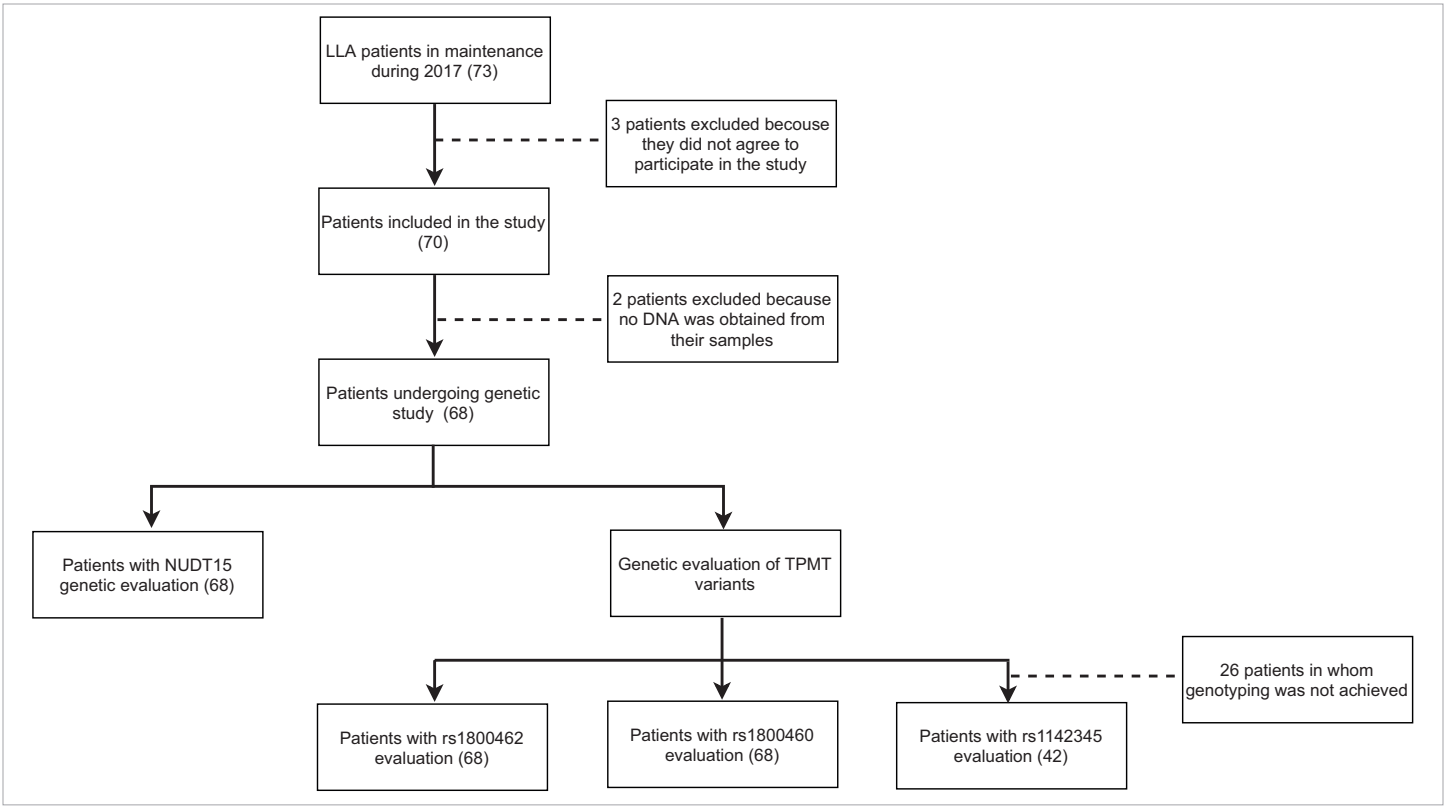

Figure 1. Patients' inclusion flowchart. This diagram shows how the inclusion of patients in the study was carried out and the details of the performance of the genetic studies.

Results

General characteristics of study subjects

A total of 73 patients with ALL diagnosis were in maintenance phase during 2017 in our institution. Of these, 70 patients agreed to participate in the project. From these patients, in two cases, it was not possible to extract DNA for genetic studies, so the statistical analyzes were based on the 68 patients in whom genetic studies were performed. Figure 1 presents a flow diagram showing the selection of patients included in the analysis.

Table 1 summarizes the general characteristics of the study population. A total of 70 patients were included in the study, with a male-to-female ratio of 1:1. The median age was six years when entering the maintenance therapy (IQR: Interquartile range: 7 years). More than 50% of the study population was from Bogota, close to 42% were from small towns and rural areas. Regarding the outcomes evaluated, myelotoxicity in 25% Liver toxicity in 27% and reduction of 6-MP during follow-up was documented in 32.9% of patients. It is important to highlight that in our 6-month follow-up, there were no loss of patients.

Allele and genotype frequencies

Of the 70 patients included in the study, genetic material with adequate quality and quantity was obtained in 68 cases (concentrations from 10 to 129 ng/μL and an average 260/280nm ratio of 1.87; optimal values between 1.8 and 2.0)[33,34]. Although DNA extraction was carried out twice for the other two cases, no genetic material was obtained with the quality conditions required to carrying out the polymerase chain reaction assays. A total of 14 patients carried a susceptibility allele: 4 for *NUDT15*, 4 for rs1800462 (*TPMT*), 6 for rs1800460 (*TPMT*), and 4 for rs1142345 of *TPMT*. Figure 2 shown representative allelic discrimination.

Table 1. General characteristics and clinical follow-up of study population.

Characteristic		Measure	
		n or median	% or IQR
Gender	Female	35	50%
Age (Years old)	6	7	
Lineage	B	65	92.9%
	T	5	7.1%
Risk	Standard	7	10.0%
	Intermediate	34	48.6%
	High	29	41.4%
	6-MP Reduction	23	32.9%
	6-MP Interruption	20	28.6%
	Febrile Neutropenia	8	11.4%
	Relapse	11	15.7%
	Death	2	2.9%
Follow-up 1	ALT (IU/L)	48.7	91.3
	AST (IU/L)	37.0	25.3
	WBC/mm3	3,315	1,370
	Neutrophils/mm3	1,820	1,137.5
	Lymphocytes/mm3	780	690
	Total Bilirubin (mg/dL)	0.5	0.3
Follow-up 2	ALT (IU/L)	42.5	97.5
	AST (IU/L)	33.55	31.13
	WBC/mm3	3,060	2,160
	Neutrophils/mm3	1,721	1,682.5
	Lymphocytes/mm3	630	675
	Total Bilirubin (mg/dL)	0.5	0.3
Follow-up 3	ALT (IU/L)	47.00	98.48
	AST (IU/L)	34	37
	WBC/mm3	3,095	14,87.5
	Neutrophils/mm3	1,615	1,196.5
	Lymphocytes/mm3	742	546
	Total Bilirubin (mg/dL)	0.5	0.4
Follow-up 4	ALT (IU/L)	50.35	76.43
	AST (IU/L)	32.55	23.31
	WBC/mm3	3,400	1,640
	Neutrophils/mm3	1,940	1,276.75
	Lymphocytes/mm3	760	565
	Total Bilirubin (mg/dL)	0.6	0.425
Follow-up 5	ALT (IU/L)	50.8	116.9
	AST (IU/L)	37	42.1
	WBC/mm3	3,360	1700
	Neutrophils/mm3	1,850	1,707.5
	Lymphocytes/mm3	840	465
	Total Bilirubin (mg/dL)	0.6	0.465
Follow-up 6	ALT (IU/L)	65.1	101.9
	AST (IU/L)	39.8	36.2
	WBC/mm3	3370	1,920
	Neutrophils/mm3	1,830	1,674.5
	Lymphocytes/mm3	910	636
	Total Bilirubin (mg/dL)	0.6	0.5

This table resumes the general clinical characteristics and follow-up data of the patients included in the study. For each laboratory data, the measurement system used has been noted. The data presented in the "Measure" columns corresponds to the n or medians (subcolumn n or median) and in the other subcolumn its percentage or interquartile range (IQR) is evidenced.

Table 2 shows the summary of allelic and genotype frequencies for each of the SNP's tested. All variants were found to be in Hardy-Weinberg equilibrium (HWE). Table 3 summarizes the typing of *TPMT* following the standards of the Clinical Pharmacogenetic Implementation Consortium" in the 42 patients in which the typing of the 3 SNPs was controlled 13. Briefly, *TPMT* haplotypes were determined based on the combinations of SNPs rs1800462, rs1800460 and rs1142345 as follows: CCT = * 1, GCT = * 2, CTC = * 3A, CTT = * 3B and CCC = * 3C.

The allele frequencies obtained in the present study were compared to the frequencies reported in the dbSNP databases for Latin American populations obtained from the ALFA (Allele Frequency aggregator) (Table 3). The database frequency of genotypes and phenotypes

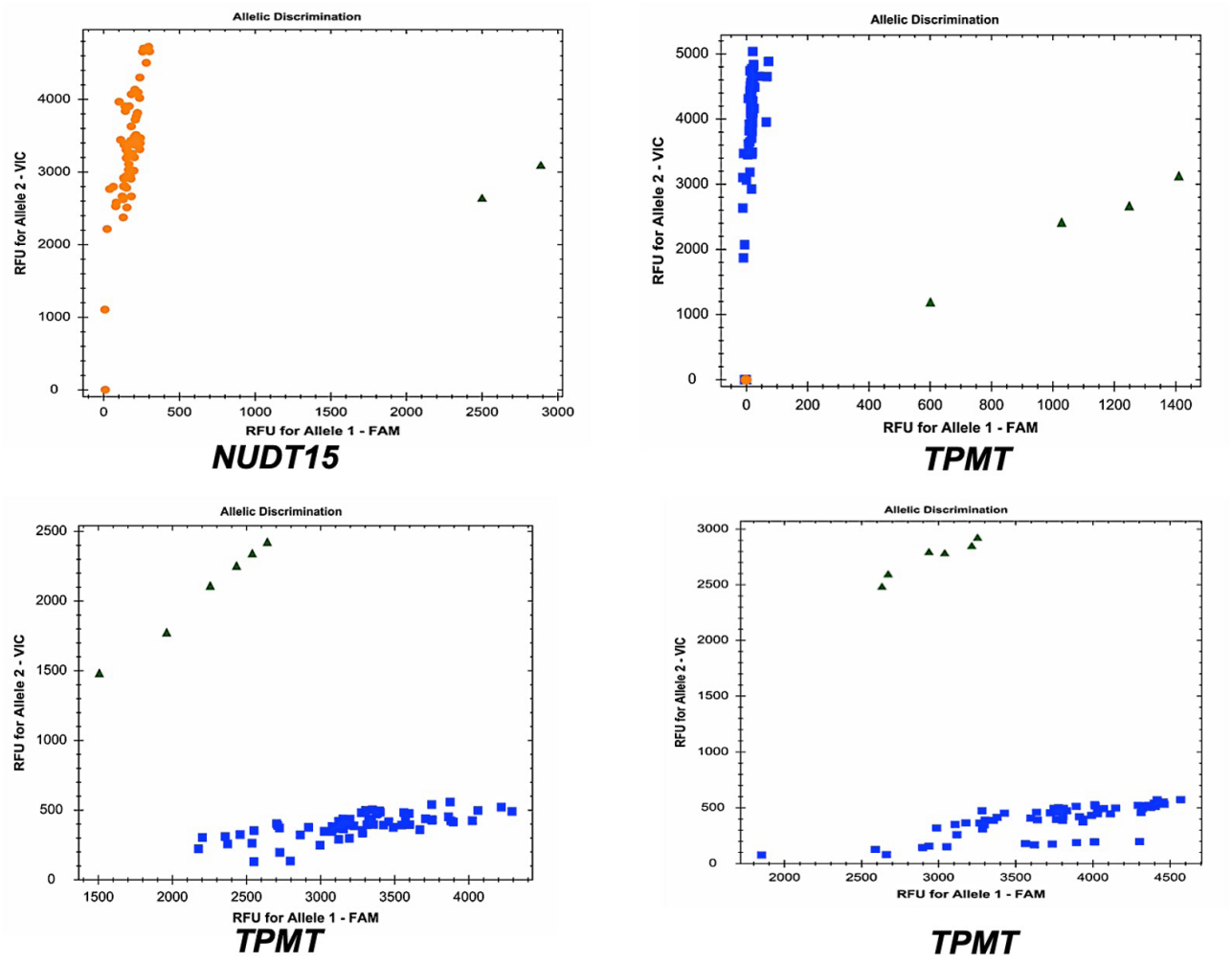

Figure 2. Representative allelic discrimination plots for TaqMan genotyping assays. There were no homozygous patients for the mutated alleles of any of the polymorphisms studied. The graphs represent the groups of samples with the wild type allele aligned towards the X or Y axes according to the probe used for their recognition. The non-aligned dots correspond to the mutated allele.

(dbGap) was developed to archive and distribute the data and results from studies that have investigated the interaction of genotype and phenotype in humans [35]. The results obtained for each SNP analyzed did not show a statistically significant difference from the frequencies obtained in the ALFA project.

Genetics associations

Table 4 summarizes the results of the bivariate analyzes in which the correlation between the genetic variants of the SNPs of interest in *NUDT15* and *TPMT* and the side effects of interest. No association was found between the outcomes of interest and the genetic variant of *NUDT15* or any *TPMT* polymorphisms evaluated individually. Similar analyses were carried out about the typing of *TPMT*, using the homozygosity variable for allele *1 versus carrying *2 or *3A as heterozygous, without evidence of any statistically significant association (supplementary table 1S).

Although no statistically significant associations were identified, two of the four patients heterozygous for *NUDT15* required a decrease in 6-MP during the follow-up period. Concerning the genetic variants of *TPMT*, for rs1800462, two of the four heterozygous

Table 2. Allelic and genotypic frequencies of the polymorphisms of interest.

Gene		Annotation	n	Frequency	HWE (*p* Value)	LatinAmerica1	LatinAmerica2
NUDT15		rs116855232	68		0.802	n= 804	n= 974
	Alleles	C	132	0.97		0.994	0.953
		T	4	0.03		0.006	0.047
	Genotypes	C/C	64	0.94			
		C/T	4	0.06			
		T/T	0	0			
TPMT		rs1800462	68		0.802	n=500	n= 628
	Alleles	C	132	0.970		0.992	0.997
		G	4	0.03		0.008	0.003
	Genotypes	C/C	64	0.94			
		C/G	4	0.06			
		G/G	0	0			
		rs1800460		68	0.703	n= 1,430	n= 7,010
	Alleles	T	6	0.044		0.026	0.045
		C	130	0.956		0.974	0.955
	Genotypes	T/T	0	0			
		T/C	6	0.09			
		C/C	62	0.91			
		rs1142345	42		0.746	n=1232	n= 5,254
	Alleles	C	4	0.0476		0.041	0.052
		T	80	0.9524		0.959	0.948
	Genotypes	C/C	0	0			
		C/T	4	0.10			
		T/T	38	0.90			

This table shows the allelic and genotypic frequencies of the polymorphisms studied. All variants were found to be in Hardy-Weinberg equilibrium (column HWE). Additionally, the allelic frequencies identified in our study were compared with those reported in Latin American population noted in the ALFA project. There were no statistically significant differences between our frequencies and those reported in the ALFA project.

Table 3. TPMT typing

Frequencies		
Haplotypes	*1	78
	*2	2
	*3A	4
Types	*1/*1	36
	*1/*2	2
	*1/*3A	4

Frequencies of TPMT haplotypes based on the standards of the "Clinical Pharmacogenetic Implementation Consortium" are listed. Given that genotyping of rs1142345 was only possible in 42 subjects and that to carry out these classifications, it is necessary to have genotyping of the 3 TPMT loci. These data correspond only to the 42 patients in whom genotyping was completed.

patients presented febrile neutropenia, and the same patients also required discontinuation 6-MP during the first six months of maintenance therapy. In the case of rs1800460, of the six heterozygous patients, three required a dosage decrease, and two were also heterozygous for rs1142345. Additionally, differences in the median for the lab values taken in the first 6 months of maintenance treatment were evaluated: aminotransferases, total bilirubin, leukocytes, and differential cell counts; based on genetic variants, no statistically significant differences were found (Supplementary table 2S).

Discussion

To our knowledge, this is the first study in Colombia that evaluated the the NUDT15 and TPMT genes polymorphisms and their correlation with the side effects of chemotherapy in pediatric patients with acute lymphoblastic leukemia. Although we are considered as a Latino population, different degrees of Caucasian, Amerindian and African ancestry are found in different regions of Colombia. Although, our findings vary significantly from previous reports regarding the association between variants and outcomes [16,19,36,37], those studies were carried out in non-Latino population.

The allelic frequency for the *NUDT15* mutation identified in our study (0.03) is lower than previously reported [19,36,38,39], a possible explanation for this finding is that most studies on

Table 4. Association analysis between genotypes and outcomes.

OUTCOMES	NUDT15		TPMT					
					rs1800462		rs1800460	rs1142345
	C/C	C/T	C/C	C/G	T/C	C/C	C/T	T/T
6-MP Reduction								
Yes	21	2	22	1	3	20	2	8
No	43	2	42	3	3	42	2	30
6-MP Interruption								
Yes	19	1	18	2	1	19	0	10
No	45	3	46	2	5	43	4	28
Neutropenia								
Yes	7	1	6	2	0	8	0	5
No	57	3	58	2	6	54	4	33
Relapse								
Yes	8	1	8	1	0	9	0	5
No	56	3	56	3	6	53	4	33
Death								
Yes	1	1	2	0	0	2	0	2
No	63	3	62	4	6	60	4	36

Data are presented as absolute frequency values for each outcome evaluated. No statistically significant association was found between any of the polymorphisms studied and the outcomes of interest.

this polymorphism were done in populations of Asian origin, finding allelic frequencies of up to 0.27. In the sample tested in our study, originated from the central Andean region of Colombia, the caucasian component (European ancestry) could account for near 50-60% of the genetic ancestry [40]. Another reason could be related to our sample size [24,41]. For the particular case of *TPMT* polymorphisms, only one study has previously been conducted in the Colombian population, in which Isaza *et al.* [42], describe frequencies for allele *2 and *3A of 0.004 and 0.035, respectively, which are lower than those found by us (0.02 and 0.05, respectively). When compared to other Latin American results, we found that our frequencies are also slightly different from those described by Garrido *et al.* [43], in pediatric patients with acute lymphoblastic leukemia in Guatemala. They identified a frequency of 0.005 for *2 and 0.0375 for *3A. However, our *2 frequency is similar to that found in Brazil by Boson *et al.* [44], who describe a frequency of 0.022 but a much lower frequency for *3A (0.015). More recently, in Uruguayan pediatric patients who have acute lymphoblastic leukemia, a *3A frequency of 0.05, which is similar to ours, has been described, but with a lower frequency of *2 [37].

Increasing evidence highlights the role of genetic variants of *NUDT15* in the development of side effects of 6-MP toxicity [19,20,36,37]. However, when evaluating the possible associations in our study population, we failed to identify any statistical significance, possibly because of the sample size. Concerning *TPMT*, evidence supports its role in both the pharmacokinetics and the toxic effects of mercaptopurine [13,16]. Our study did not find any association between the allelic frequencies of each polymorphism or in those 42 cases in which we had the complete typing of *TPMT*. Among the possible reasons that would explain this results, for the case of *NUDT15,* could be that the original studies describing such associations were based on GWAS approach [19], in which the representation of the Latino population was low, and this may be due to an effect related to the specific ancestry of the Colombian population [45]. On the other hand, in the case of *TPMT*, an explanation could be that the alleles found in our population (*2 and *3A) correspond to intermediate metabolizers, which usually do not require many adjustments in therapy [13].

In exploratory analyses, we evaluated whether there was an association between the genetic variants studied and the outcomes: relapses or death, reviewed as of October 2018, without showing any correlation, which is consistent with what is described by Lennard *et al.* [46], who, after genotyping *TPMT* in 2,387 patients with acute lymphoblastic leukemia, found no association with event-free survival. Within the limitations of the present study, there are the sample size and selection bias since it is a captive sample. However, patients were included prospectively and consecutively, which allowed better follow-up of the development of the adverse events of interest. On the other hand, a great strength of the present study is the translational approach, based on previous clinical observations and the evaluation of basic biomedical aspects that could answer the search for predictive factors for the development of toxicity in the treatment of pediatric patients with oncological conditions.

Conclusions

In summary, the allelic frequencies of the genes of interest were different from those previously described, even when compared with Latin American studies. We did not find any statistical association with toxicity events for any of the polymorphisms studied. These findings highlight the need for future studies with a larger sample size; either through a multicenter study with pediatric ALL patients or even including other pediatric populations receiving management with thiopurines, such as patients with inflammatory bowel disease or juvenile idiopathic arthritis, which may allow us to have an adecuate sample size on which to identify better inferences of associations with toxicity outcomes, as well as, to evaluate the possibility that other genes may be influencing toxicity and side effects and to examine the effect that ancestry could have on these interactions in populations with a genetic admixture like our population.

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Supplementary material.

Table 1S. Association analysis between TPMT diplotypes and outcomes.

OUTCOMES	*TPMT* diplotypes		*p* Value
	*1/*1	*1/another	
6-MP Reduction			
Yes	8	2	0.616
No	28	4	
6-MP Interruption			
Yes	10	0	0.308
No	26	6	
Neutropenia			
Yes	5	0	1.000
No	31	6	
Relapse			
Yes	4	1	0.557
No	32	5	
Death			
Yes	2	0	1.000
No	34	6	

Data are presented as absolute frequency values for each outcome evaluated. No statistically significant association was found between any of the TPMT diplotypes studied and the outcomes of interest.

Table 2S. Comparison of laboratory follow-up according to genotypes.

Follow-up		*NUDT15* rs1800462				*TPMT* rs1800460		rs1142345	
		C/C	C/T	C/C	C/G	T/C	C/C	C/T	T/T
1st	ALT (IU/L)	46.20	50.50	49	36	45.15	48.60	49.30	53
	AST (IU/L)	34.20	41.80	36.90	30.30	42.90	35.35	42.90	38.55
	WBC/mm3	3,315	3,780	3,315	3,610	2,200	3,380	2,030	3,310
	Neutrophils/mm3	1,820	1,995	1,820	1,737	980	1,910	1,020	1,980
	Lymphocytes/mm3	783	409	780	773	630	783	600	725
	Total Bilirubin (mg/dL)	0.50	0.60	0.51	0.40	0.60	0.50	0.45	0.52
2nd	ALT (IU/L)	45	74.50	55.40	28.80	26	46	53.15	45
	AST (IU/L)	33.55	33.70	33.55	33.15	37	32.70	42.80	38.25
	WBC/mm3	3,135	2,170	3,020	3,980	2,160	3,225	2,305	3,245
	Neutrophils/mm3	1,835	655	1,691	2,515.50	1,295	1,835	1,440	1,835
	Lymphocytes/mm3	630	1,290	640	475	727	630	583.50	746.50
	Total Bilirubin (mg/dL)	0.50	0.23	0.50	0.45	0.40	0.50	0.40	0.59
3rd	ALT (IU/L)	40	59.10	39.50	89.45	37	54	80.20	54
	AST (IU/L)	34.50	33.10	33.30	38.65	42.20	33.65	42.20	35
	WBC/mm3	3,070	2,865	3,100	2,085	3,100	3,050	3,130	3,055
	Neutrophils/mm3	1,635	1,430	1,645	1,175	1,440	1,635	2,680	1,540
	Lymphocytes/mm3	734	1010	753	470	1030	712	875	768
	Total Bilirubin (mg/dL)	0.50	0.30	0.50	0.60	0.50	0.50	0.50	0.50
4th	ALT (IU/L)	52.40	18.10	52.40	33.70	41.60	52.40	138.30	63
	AST (IU/L)	31.10	32.10	31.20	36.30	47.21	31.20	89.90	30.40
	WBC/mm3	3,400	3,135	3,400	2,980	2,570	3,510	2,615	3,635
	Neutrophils/mm3	1,940	1,720	1,955	860	1,700	1,970	1,735	1,955
	Lymphocytes/mm3	760	740	760	1,305	535	800	535	800
	Total Bilirubin (mg/dL)	0.70	0.40	0.60	0.70	0.45	0.70	0.40	0.70
5th	ALT (IU/L)	54	23.40	55	24	29.10	54	67.10	60.75
	AST (IU/L)	40.70	35.40	42.10	25.50	42.10	38.15	55.10	47.30
	WBC/mm3	3,470	3,060	3,430	3,435	2,615	3,470	2,615	3,740
	Neutrophils/mm3	1,930	1,410	1,905	1,764	1,360	1,930	1,360	1,800
	Lymphocytes/mm3	845	560	830	900	793	850	845	870
	Total Bilirubin (mg/dL)	0.50	0.45	0.50	0.80	0.30	0.60	0.40	0.65
6th	ALT (IU/L)	68.55	38	70.30	35.45	71	66.05	71	70.10
	AST (IU/L)	41.50	30.90	43.95	31.35	53.80	40	63	44.90
	WBC/mm3	3,385	2,655	3,385	3,145	3,200	3,370	2,755	3,650
	Neutrophils/mm3	1,820	1,415	1,790	1,790	1,515	1,800	1,185	1,750
	Lymphocytes/mm3	905	1,060	910	810	681	915.50	680	921
	Total Bilirubin (mg/dL)	0.60	0.50	0.60	0.71	0.56	0.60	0.38	0.60

Data are presented as median values for each laboratory evaluated. When the medians of each laboratory value were compared according to the genotyping of each SNPs, no statistically significant differences were identified.