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Pharmacogenomics and variations in the risk of toxicity during the consolidation/maintenance phases of the treatment of pediatric B-cell leukemia patients from an admixed population in the Brazilian Amazon

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ABSTRACT

The treatment of Acute Lymphoblastic Leukemia (ALL) in children has a high clinical success rate, although toxicological complications are frequent, and often result in the interruption of the treatment. Various studies have shown that toxicities resulting from the treatment are influenced by pharmacogenetic variants. Most of this research has focused on relatively homogeneous populations, and the influence of these variants in highly admixed populations, such as that of Brazil, is still poorly understood. The present study investigated the association between pharmacogenetic variants and severe toxicities in pediatric B-cell ALL patients from an admixed population of the Brazilian Amazon. The rs2306283 (of *SLCO1B1)* mutant allele increased the risk of neurotoxicity threefold, and the homozygous mutant rs9895420 (of *ABCC3*) genotype was associated with a fivefold increase in protection against severe gastrointestinal toxicity. This indicates that the rs2306283 and rs9895420 polymorphisms may be relevant to the prediction of severe toxicity in pediatric ALL patients.

1. Introduction

Acute Lymphoblastic Leukemia (ALL) is the most common childhood cancer, representing approximately 30% of all malignant pediatric neoplasia [1,2]. In about 85% of children with ALL, the leukemia starts in the B cells (B-cell ALL). Recent advances in the chemotherapy of childhood ALL, based on a cocktail of chemotherapeutic drugs that includes inhibitors of tyrosine kinases, have resulted in survival rates of > 90% [3]. Despite the clinical success of this treatment, around 20% of the children present serious toxicological complications that require a reduction in the dosage or even the complete interruption of the treatment [4].

The drugs 6-mercaptopurine (6-MP) and Methotrexate (MTX) are the key components of this cocktail, and are administrated during both the consolidation and the maintenance phases of the ALL treatment. Various studies have shown that toxicities resulting from the treatment are influenced by pharmacogenetic variants [5–7]. Most of this research has focused on relatively homogeneous populations and the influence of these variants in highly admixed populations, such as that of Brazil, is still poorly understood [8]. In populations of Hispanic ethnicity, which mix European, African, and native American ancestries, children tend to have worse results in the treatment of ALL in comparison with European, American, and Asian populations, and require more intensive treatment. This disparity may be accounted for, at least in part,

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Table 1
Characteristics of the patients that participated in the present study.

Variable	n (%)
Number of patients	121
Sex	
Male	72 (59.5)
Female	49 (40.5)
Mean age at diagnosis (years, SD ±)	5.29 ± 3.32
Age at diagnosis (years)	
< 10	103 (85.1)
≥10	18 (14.9)
Leukometry at diagnosis (μL)	
< 50,000	94 (77.7)
≥50,000	27 (22.3)
Risk group	
Standard	50 (41.3)
Medium	11 (9.1)
High	60 (49.6)
Mean genetic ancestry (SD ±)	
European	0.45 ± 0.10
Amerindian	0.33 ± 0.11
African	0.22 ± 0.07
Toxicity (3rd–4th degree) during consolidation	
Any	55 (45.5)
Gastrointestinal (total)	38 (31.4)
Mucositis	28 (23.1)
Central Nervous System (total)	21 (17.4)
Headache	7 (5.8)
Peripheral neuropathy	5 (4.1)
Convulsions	7 (5.8)
Toxicity (3rd–4th degree) during maintenance	
Any	24 (19.8)
Gastrointestinal (total)	22 (18.1)
Hepatotoxicity	16 (13.2)
Central Nervous System (total)	5 (4.1)

Abbreviations: SD, Standard Deviation.

by the genetic risk factors associated with the native American ancestry of Hispanic populations [9].

These findings are especially relevant to the highly admixed populations found in Brazil, in particular those of the Amazon basin which, like the Hispanic populations, have been formed through the miscegenation of native American, African, and European ancestral groups. Perhaps most relevantly, the proportion of native American ancestry is higher than in other regions of Brazil [10,11], which may contribute to the relatively poor results of the treatment of ALL in this population. The present study investigated the associations between the gene variants related to the 6-MP and MTX pathways with severe (3–4th degree) toxicities in childhood patients with B-cell ALL from a miscegenated population from the Brazilian Amazon region.

2. Materials and methods

2.1. Patients, treatment and toxicity

The present study included 121 patients (Table 1) diagnosed with B-cell ALL. The study adhered to the general principles laid down in the Helsinki Declaration, and was approved by the Ethics Committee for Research from Federal University of Pará (approval number 119,649).

The initial treatment of the patients was based on the protocol of the international Berlin-Frankfurt-Münster study group (ALL IC-BFM 2002) and the patients were divided into standard, medium, and high risk groups [12]. The details of the chemotherapy are provided in Table 1 Supplementary. The toxicity data were collected from the medical records of the patients and were classified based on the Common Toxicity Criteria, version 4.0. The study focused only on the cases classified as 3–4th degree toxicity during the consolidation and maintenance phases of the treatment. We investigated gastrointestinal and central nervous system (CNS) toxicities.

2.2. Genotyping

The samples were genotyped using a QuantStudio™ 12 K Flex Real-Time PCR System, following the protocol published by Applied Biosystems. The genetic ancestry was analyzed based on the set of 61 Ancestry Informative Markers described by Santos et al. [10] and Ramos et al. [13]. The proportion of individuals of European, African, and Amerindian ancestry were estimated using STRUCTURE v2.3.3.

2.3. Statistical analysis

The data were first tested for the Hardy-Weinberg Equilibrium (HWE) in each polymorphism. The influence of the genetic variants on the risk of developing severe toxicity (3–4th degree) was evaluated by applying a multivariate Bayesian logistic regression, which included age, sex and risk group (for the maintenance phase), to control for confounding effects. The effect of each variant was evaluated using recessive, dominant and log-additive models, and the model with the lowest AIC (Akaike Information Criterion) was chosen for the variant association test in the treatment phase. A Bayesian logistic regression was used to control for the effects of rare mutations. All statistical tests were two-tailed and considered significant at $p < 0.05$. The analyses were run in the R program v.3.4.0.

3. Results

We selected 28 polymorphic genes (Table 2 Supplementary). The polymorphisms that had a minor allele frequency (MAF) of less than 1%, a genotyping rate of less than 10% or were not in HWE were excluded from the analysis. This left 12 polymorphisms for the analysis of associations (Table 3 Supplementary). The clinical data are summarized in Table 1. Overall, 72 (59.5%) patients presented at least one episode of 3rd–4th degree toxicity during the consolidation and maintenance phases of the treatment, and seven patients presented toxicity episodes in both treatment phases. During the consolidation phase, 55 (45.5% of the total) patients presented at least one episode of severe toxicity and the most frequent toxicity was mucositis (23.1%). During the maintenance phase, 24 (19.8% of the total) patients suffered some type of severe toxicity, with hepatotoxicity (13.2%) being the most frequent.

Based on the data on genetic ancestry, the study sample was subdivided into multiple clusters (Fig. 1 Supplementary). No significant relationship was found between genetic ancestry and any type of toxicity (Fig. 2 Supplementary, Table 4 Supplementary).

For the analysis of toxicity in the consolidation phase, the standard/medium risk patients (Table 5 Supplementary) and high risk group (Fig. 1, Table 2) were treated separately. Based on the log-additive model the rs2306283 (*SLCO1B1*) mutant G allele was associated with a nearly threefold increase in the risk of severe CNS toxicity (OR = 2.88; 95%CI = 1. 13-7.54; P = 0.029), while the homozygous mutant rs9895420 (*ABCC3*) genotype was associated with a fivefold increase in protection against severe gastrointestinal toxicity (OR = 0.22; 95%CI = 0.05-0.97; P = 0.044), both in the high risk group.

No significant association was found between toxicity and the investigated variants in the maintenance phase (Table 6 Supplementary). Strong, but non-significant trends were identified in the variants of the *MTHFR* gene rs1801122 (Fig. 1, Table 2), *ABCC1* rs28364006, *AMPD1* rs17602729, and *SLCO1B1* rs4149056 (Table 6 Supplementary). The absence of significance in these cases may be related to the small sample size.

4. Discussion

Higher toxicity rates were found in the study patients during both phases of the treatment (Table 1). These rates are higher than those recorded in similar studies of other populations around the world [12,14]. Stary et al. [12] conducted a randomized clinical trial (ALL IC-